Metabolites from the Marine-Derived Fungi: *Aspergillus* sp. PSU-F154, *Curvularia* sp. PSU-F22, *Fusarium* sp. PSU-F14, *Nigrospora* sp. PSU-F5 and PSU-F18 and *Penicillium* sp. PSU-F40 and PSU-F44

Kongkiat Trisuwan

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Organic Chemistry

Prince of Songkla University

2010

Thesis Title	Metabolites from the Marine-Derived Fungi: *Aspergillus* sp. PSU-F154, *Curvularia* sp. PSU-F22, *Fusarium* sp. PSU-F14, *Nigrospora* sp. PSU-F5 and PSU-F18 and *Penicillium* sp. PSU-F40 and PSU-F44
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(Assoc. Prof. Dr. Krerkchai Thongnoo)
Dean of Graduate School

ชื่อวิทยานิพนธ์ เมทาโบไลต์จากเชื้อราทะเล: *Aspergillus* sp. PSU-F154, *Curvularia* sp. PSU-F22, *Fusarium* sp. PSU-F14, *Nigrospora* sp. PSU-F5 และ PSU-F18 และ *Penicillium* sp. PSU-F40 และ PSU-F44

ผู้เขียน นายก้องเกียรติ ไตรสุวรรณ

สาขาวิชา เคมีอินทรีย์

ปีการศึกษา 2552

บทคัดย่อ

จากการศึกษาเมทาโบไลต์จากเชื้อราทะเลจำนวน 7 ชนิดได้แก่ *Aspergillus* sp. PSU-F154, *Curvularia* sp. PSU-F22, *Fusarium* sp. PSU-F14, *Nigrospora* sp. PSU-F5 และ PSU-F18 และ *Penicillium* sp. PSU-F40 และ PSU-F44 โดยนำส่วนสกัดหยาบเอทิลอะซิเตทจากน้ำเลี้ยงเชื้อและเซลล์ของเชื้อราดังกล่าวมาทำให้บริสุทธิ์ด้วยวิธีทางโครมาโทกราฟี วิเคราะห์โครงสร้างของสารบริสุทธิ์ด้วยข้อมูลทางสเปกโทรสโกปี โดยเฉพาะข้อมูล 1D และ 2D NMR สเปกโทรสโกปี สารบริสุทธิ์ที่แยกได้มีจำนวน 75 สาร โดยจัดเป็นสารใหม่จำนวน 29 สาร ดังนี้ สำหรับสารประกอบ **K47** จากเชื้อรา *Fusarium* sp. PSU-F14 แยกในรูปอนุพันธ์อะซิเตท

-สารใหม่จำนวน 4 สาร **(K5-K8)** และสารที่มีการรายงานโครงสร้างแล้วจำนวน 9 สาร **(K1-K4** และ **K9-K13)** จากส่วนสกัดหยาบน้ำเลี้ยงเชื้อรา *Nigrospora* sp. PSU-F5

-สารใหม่จำนวน 4 สาร **(K15-K18)** และสารที่มีการรายงานโครงสร้างแล้วจำนวน 5 สาร **(K14** และ **K19-K22)** จากส่วนสกัดหยาบน้ำเลี้ยงเชื้อรา *Nigrospora* sp. PSU-F18

-สารใหม่จำนวน 2 สาร **(K23** และ **K24)** และสารที่มีการรายงานโครงสร้างแล้วจำนวน 3 สาร **(K25-K27)** จากส่วนสกัดหยาบน้ำเลี้ยงเชื้อรา *Penicillium* sp. PSU-F44

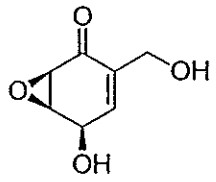
-สารใหม่จำนวน 9 สาร **(K28-K36)** และสารที่มีการรายงานโครงสร้างแล้วจำนวน 3 สาร **(K37-K39)** จากส่วนสกัดหยาบน้ำเลี้ยงเชื้อรา *Penicillium* sp. PSU-F40 และสารที่มีการรายงานโครงสร้างแล้วจำนวน 2 สาร **(K40** และ **K41)** จากส่วนสกัดหยาบเซลล์เชื้อรา

-สารใหม่จำนวน 1 สาร **(K42)** และสารที่มีการรายงานโครงสร้างแล้วจำนวน 7 สาร **(K43-K45** และ **K47-K50)** จากส่วนสกัดหยาบน้ำเลี้ยงเชื้อรา *Fusarium* sp. PSU-F14 และสารที่มีการรายงานโครงสร้างแล้วจำนวน 2 สาร **(K46** และ **K51)** จากส่วนสกัดหยาบเซลล์เชื้อรา

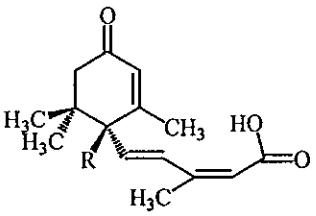
-สารใหม่จำนวน 2 สาร **(K53** และ **K54)** และสารที่มีการรายงานโครงสร้างแล้วจำนวน 5 สาร **(K56-K60)** จากส่วนสกัดหยาบน้ำเลี้ยงเชื้อรา *Curvularia* sp. PSU-F22

และสารใหม่จำนวน 1 สาร **(K52)** และสารที่มีการรายงานโครงสร้างแล้วจำนวน 1 สาร **(K55)** จากส่วนสกัดหยาบเซลล์เชื้อรา

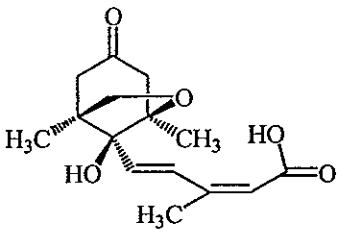
-สารใหม่จำนวน 5 สาร **(K61-K65)** และสารที่มีการรายงานโครงสร้างแล้วจำนวน 9 สาร **(K67-K75)** จากส่วนสกัดหยาบน้ำเลี้ยงเชื้อรา *Aspergillus* sp. PSU-F154 และสารใหม่จำนวน 1 สาร **(K66)** จากส่วนสกัดหยาบเซลล์เชื้อรา



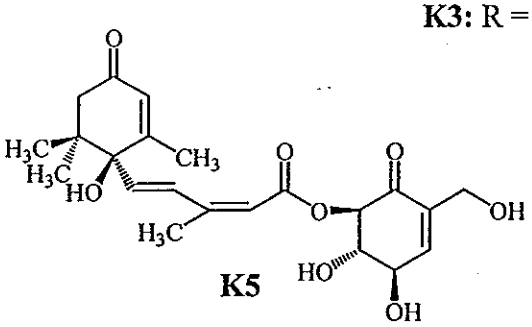
K1



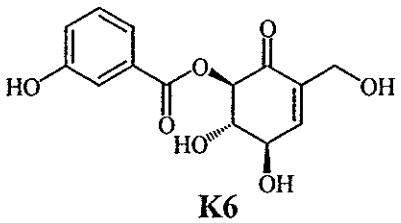
K2: R = OH
K3: R = H



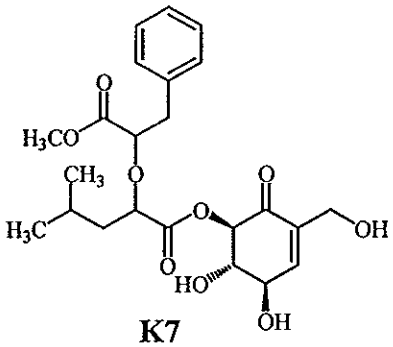
K4



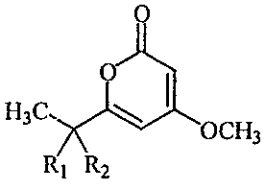
K5



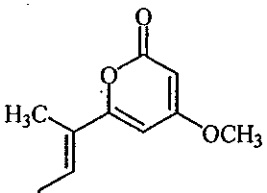
K6



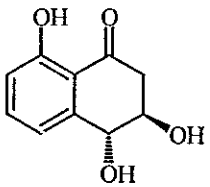
K7



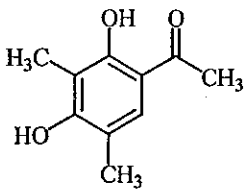
K8 : R_1 = OH, R_2 = H
K18: $R_1 + R_2$ = O



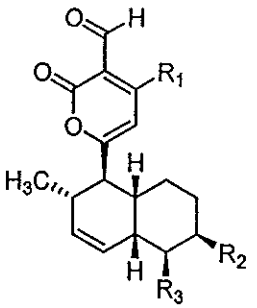
K9 : R = H
K10: R = OH

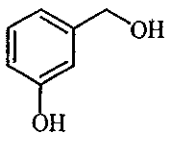


K11



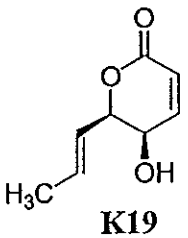
K12



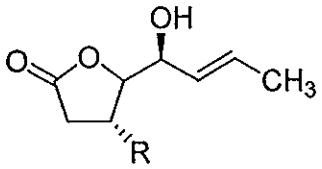


K13

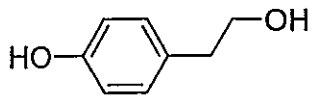
K14: $R_1 = OCH_3$, $R_2 = R_3$ = H
K15: $R_1 = OCH_3$, R_2 = H, R_3 = OH
K16: $R_1 = NHCH_2CH_2OH$, R_2 = H, R_3 = OH
K17: $R_1 = NHCH_2CH_2OH$, R_2 = OH, R_3 = H



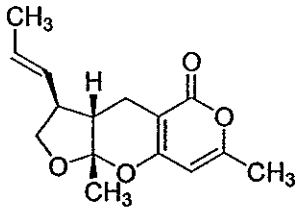
K19



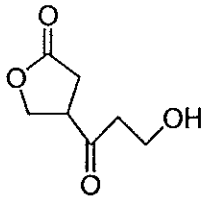
K20: R = H
K21: R = OH



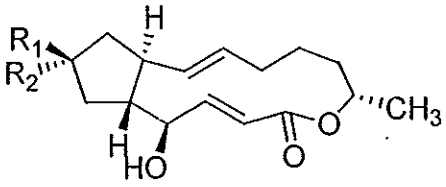
K22



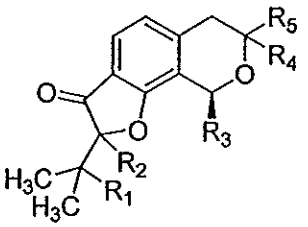
K23

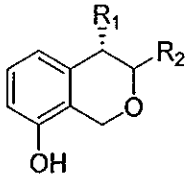


K24



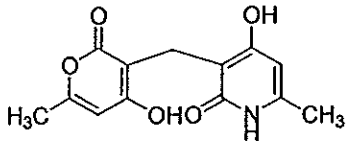
K25: $R_1 = R_2 = H$
K26: $R_1 = OH$, $R_2 = H$
K27: $R_1 + R_2 = O$



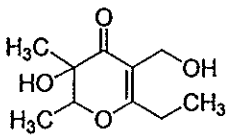


K31: $R_1 = H$, $R_2 = CH_3$
K32: $R_1 = OH$, $R_2 = \alpha$-CH_3

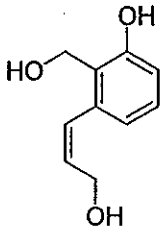
K28: $R_1 + R_2$ = double bond, $R_3 = H$, $R_4 = OCH_3$, $R_5 = CH_3$
K29: $R_1 = R_2 = R_4 = H$, $R_3 = OCH_3$, $R_5 = \alpha$-CH_3
K30: $R_1 = R_2 = R_4 = H$, $R_3 = OH$, $R_5 = \alpha$-CH_3



K33



K34



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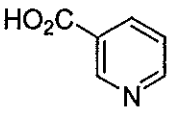
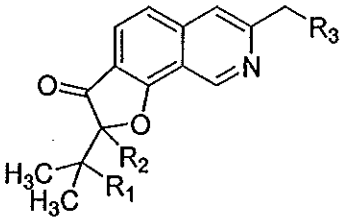
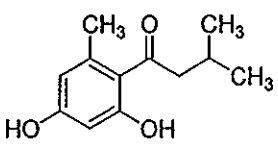


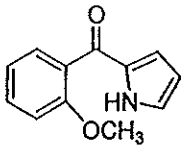
K41



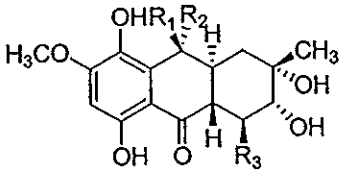
K36: $R_1 = H$, $R_2 = R_3 = OH$
K37: $R_1 + R_2$ = double bond, $R_3 = H$
K38: $R_1 = R_3 = H$, $R_2 = OH$



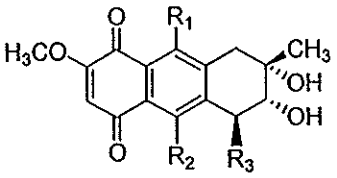
K39



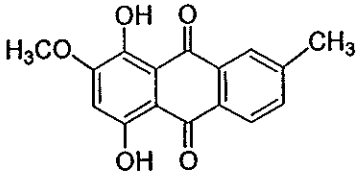
K40



K42: $R_1 = OH, R_2 = R_3 = H$
K43: $R_1 = R_3 = H, R_2 = OH$
K44: $R_1 = H, R_2 = R_3 = OH$



K45: $R_1 = OH, R_2 = R_3 = H$
K46: $R_1 = R_3 = H, R_2 = OH$
K47: $R_1 = R_2 = R_3 = OH$



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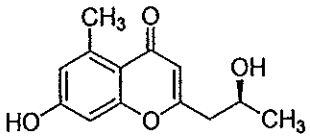
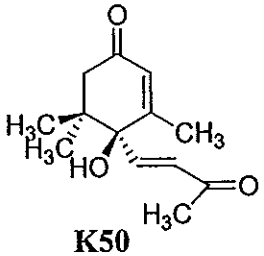


K49



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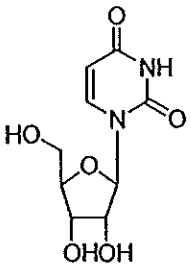

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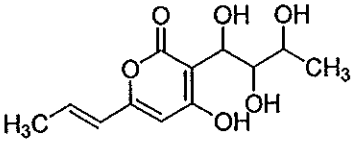

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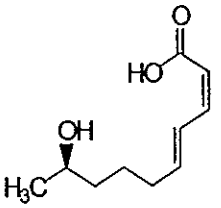

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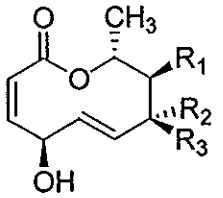
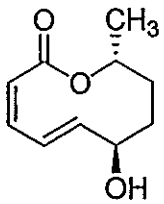
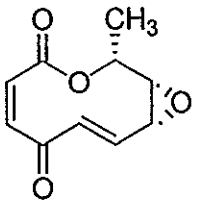
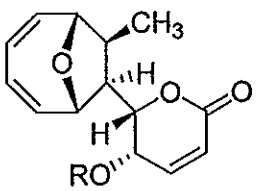






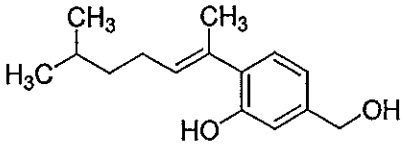


K54: $R_1 = R_3 = OH, R_2 = H$
K55: $R_1 = R_3 = H, R_2 = OH$
K56: $R_1 = R_2 = R_3 = H$

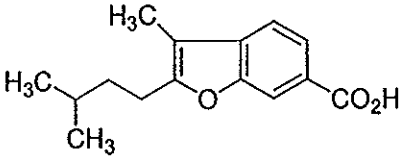
K57

K58

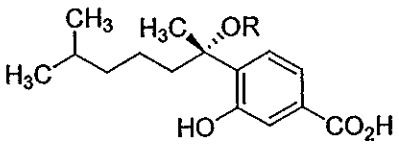
K59: R = Ac
K60: R = H



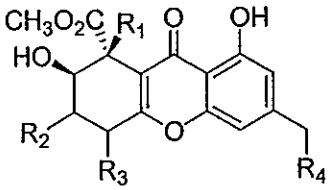
K61



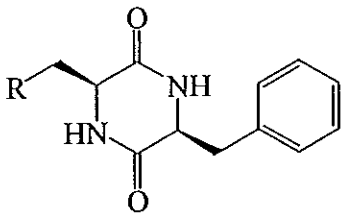
K62



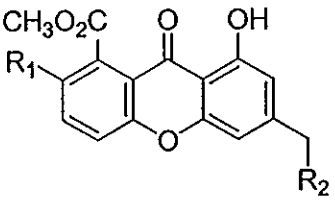
K63: R = CH_3
K67: R = H



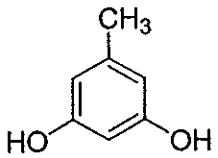
K64: $R_1 = R_4 = H$, $R_2 + R_3$ = double bond
K65: $R_1 = R_4 = OH$, $R_2 = R_3 = H$
K68: $R_1 = H$, $R_2 + R_3$ = double bond, $R_4 = OH$
K69: $R_1 = OH$, $R_2 = R_3 = R_4 = H$



K66: R = 2-substituted phenol
K74: R = 3-substituted indole



K70: $R_1 = R_2 = H$
K71: $R_1 = H$, $R_2 = OH$
K72: $R_1 = OH$, $R_2 = H$
K73: $R_1 = R_2 = OH$



K75

Thesis Title	Metabolites from the Marine-Derived Fungi: *Aspergillus* sp. PSU-F154, *Curvularia* sp. PSU-F22, *Fusarium* sp. PSU-F14, *Nigrospora* sp. PSU-F5 and PSU-F18 and *Penicillium* sp. PSU-F40 and PSU-F44
Author	Mr. Kongkiat Trisuwan
Major Program	Organic Chemistry
Academic Year	2009

ABSTRACT

This work involved chemical investigation of the ethyl acetate extracts from the culture broth and the mycelia of seven marine-derived fungi: *Aspergillus* sp. PSU-F154, *Curvularia* sp. PSU-F22, *Fusarium* sp. PSU-F14, *Nigrospora* sp. PSU-F5 and PSU-F18 and *Penicillium* sp. PSU-F40 and PSU-F44. Each extract was purified by chromatographic techniques. The structures of the isolated compounds were determined by analysis of spectroscopic data, especially 1D and 2D NMR spectroscopic data. This investigation resulted in the isolation of 75 compounds including 29 new compounds as follows. In addition, compound **K47** obtained from *Fusarium* sp. PSU-F14 was isolated as a monoacetate derivative.

- Four new **(K5-K8)** and nine known **(K1-K4** and **K9-K13)** metabolites from the broth extract of *Nigrospora* sp. PSU-F5.

- Four new **(K15-K18)** and five known **(K14** and **K19-K22)** compounds from the broth extract of *Nigrospora* sp. PSU-F18.

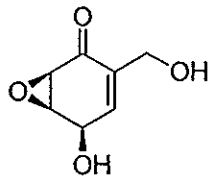
- Two new **(K23** and **K24)** and three known **(K25-K27)** metabolites from the broth extract of *Penicillium* sp. PSU-F44.

- Nine new **(K28-K36)** and three known **(K37-39)** compounds from the broth extract of *Penicillium* sp. PSU-F40, and two known metabolites **(K40** and **K41)** from the mycelial extract.

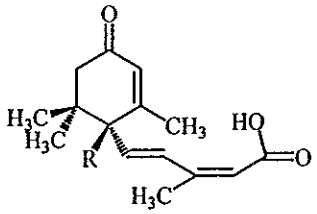
- One new **(K42)** and seven known **(K43-K45** and **K47-K50)** compounds from the broth extract of *Fusarium* sp. PSU-F14 together with two known metabolites **(K46** and **K51)** from the mycelial extract.

- Two new (**K53** and **K54**) and five known (**K56-K60**) compounds from the broth extract of *Curvularia* sp. PSU-F22, and one new (**K52**) and one known (**K55**) metabolites from the mycelial extract.

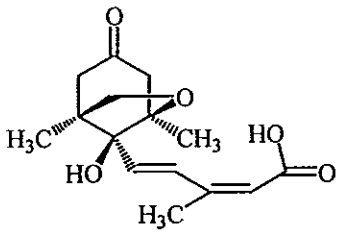
- Five new (**K61-K65**) and nine known (**K67-K75**) compounds from the broth extract of *Aspergillus* sp. PSU-F154 together with one new compound (**K66**) from the mycelial extract.



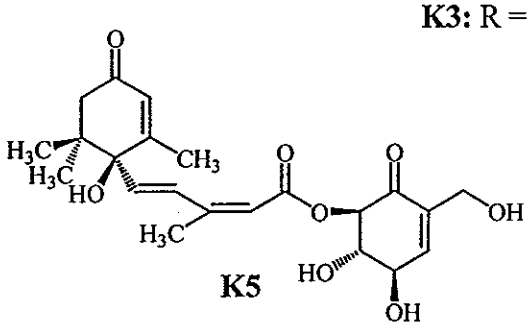
K1



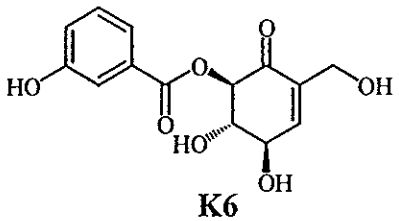
K2: R = OH
K3: R = H



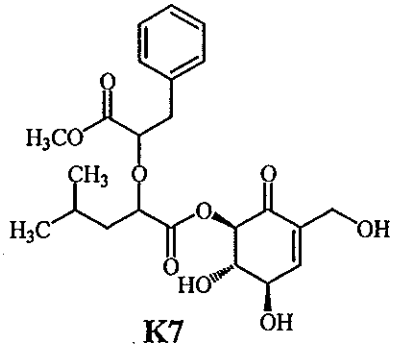
K4



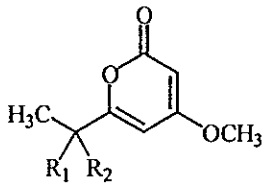
K5



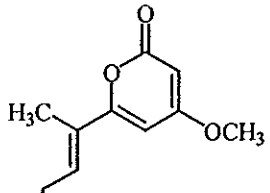
K6



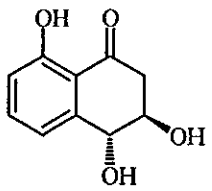
K7



K8 : R_1 = OH, R_2 = H
K18: $R_1 + R_2$ = O



K9 : R = H
K10: R = OH



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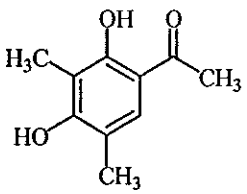
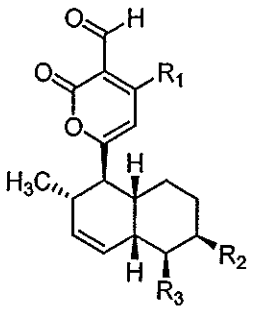
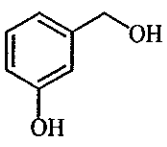


K12



K14: R_1 = OCH_3, R_2 = R_3 = H
K15: R_1 = OCH_3, R_2 = H, R_3 = OH
K16: R_1 = $NHCH_2CH_2OH$, R_2 = H, R_3 = OH
K17: R_1 = $NHCH_2CH_2OH$, R_2 = OH, R_3 = H



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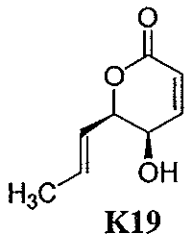
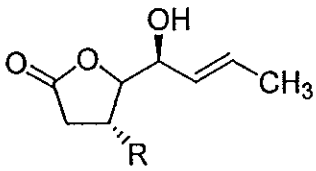
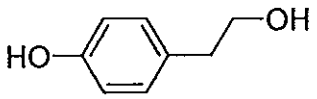


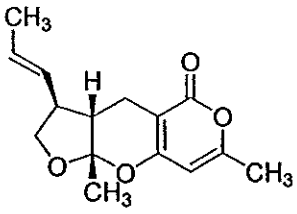
K19



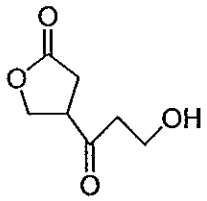
K20: R = H
K21: R = OH



K22



K23



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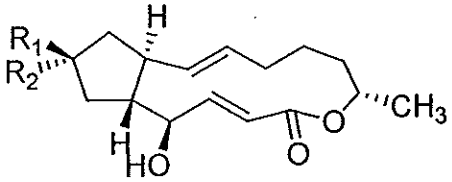
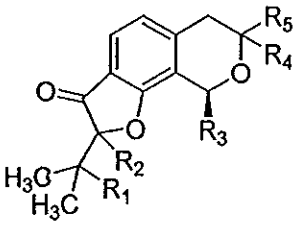
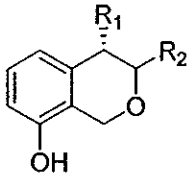


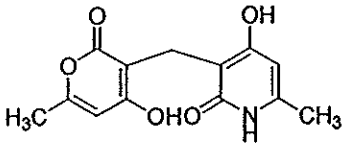
K25: $R_1 = R_2 = H$
K26: $R_1 = OH$, $R_2 = H$
K27: $R_1 + R_2 = O$





K31: $R_1 = H$, $R_2 = CH_3$
K32: $R_1 = OH$, $R_2 = \alpha$-CH_3

K28: $R_1 + R_2$ = double bond, $R_3 = H$, $R_4 = OCH_3$, $R_5 = CH_3$
K29: $R_1 = R_2 = R_4 = H$, $R_3 = OCH_3$, $R_5 = \alpha$-CH_3
K30: $R_1 = R_2 = R_4 = H$, $R_3 = OH$, $R_5 = \alpha$-CH_3



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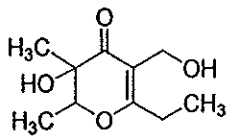

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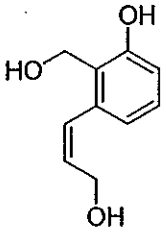

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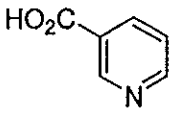

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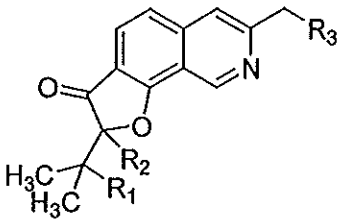
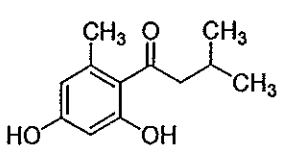
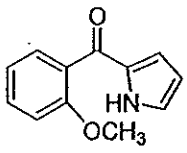


K36: $R_1 = H$, $R_2 = R_3 = OH$
K37: $R_1 + R_2$ = double bond, $R_3 = H$
K38: $R_1 = R_3 = H$, $R_2 = OH$



K39



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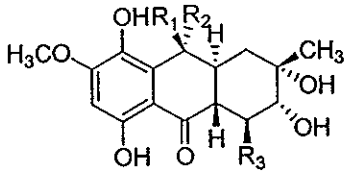
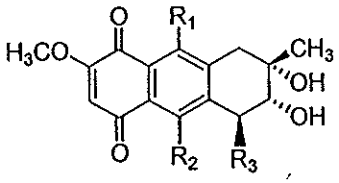
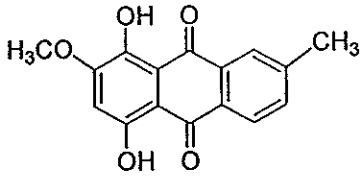


K42: $R_1 = OH, R_2 = R_3 = H$
K43: $R_1 = R_3 = H, R_2 = OH$
K44: $R_1 = H, R_2 = R_3 = OH$



K45: $R_1 = OH, R_2 = R_3 = H$
K46: $R_1 = R_3 = H, R_2 = OH$
K47: $R_1 = R_2 = R_3 = OH$



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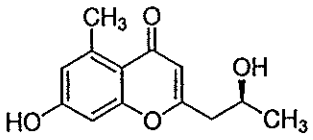

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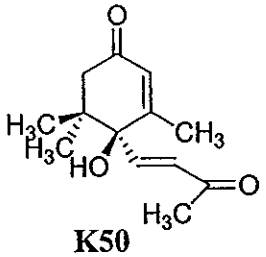

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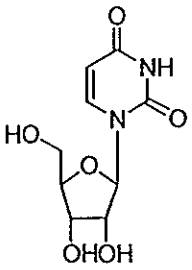

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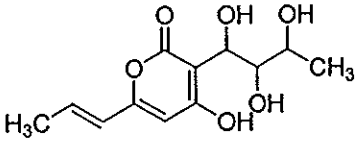

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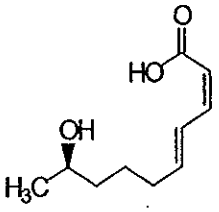

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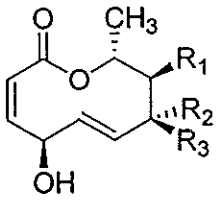
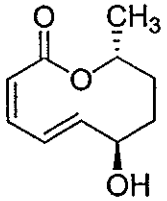


K54: $R_1 = R_3 = OH, R_2 = H$
K55: $R_1 = R_3 = H, R_2 = OH$
K56: $R_1 = R_2 = R_3 = H$



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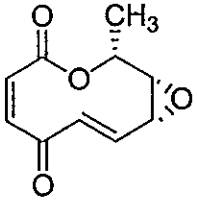
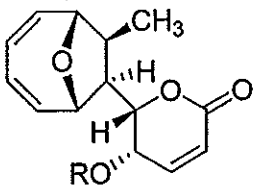
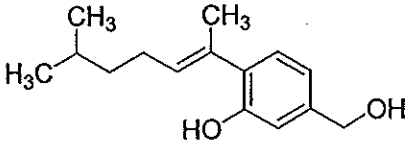


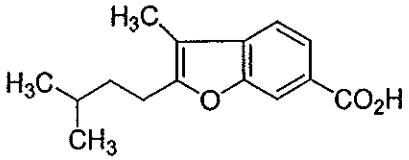
K58



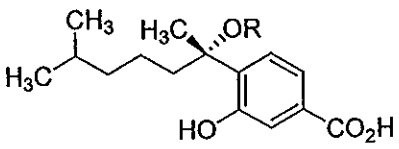
K59: R = Ac
K60: R = H



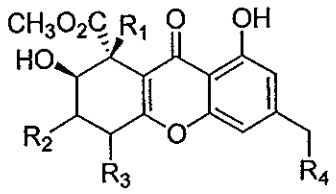
K61



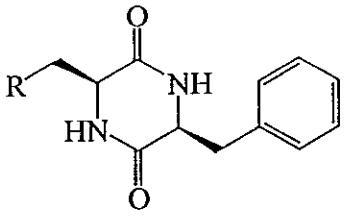
K62



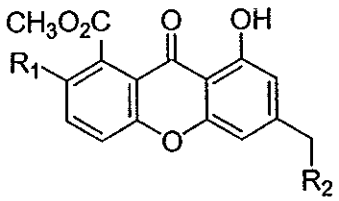
K63: R = CH_3
K67: R = H



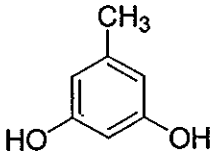
K64: $R_1 = R_4 = H$, $R_2 + R_3$ = double bond
K65: $R_1 = R_4 = OH$, $R_2 = R_3 = H$
K68: $R_1 = H$, $R_2 + R_3$ = double bond, $R_4 = OH$
K69: $R_1 = OH$, $R_2 = R_3 = R_4 = H$



K66: R = 2-substituted phenol
K74: R = 3-substituted indole



K70: $R_1 = R_2 = H$
K71: $R_1 = H$, $R_2 = OH$
K72: $R_1 = OH$, $R_2 = H$
K73: $R_1 = R_2 = OH$



K75

ACKNOWLEDGEMENT

I wish to express my deepest gratitude and sincere appreciation to my supervisor, Professor Dr. Vatcharin Rukachaisirikul, for valuable instruction, expert guidance and excellent suggestion. I would also like to direct my appreciation to her correction of my thesis.

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I am grateful to the staffs of the Department of Chemistry for making this thesis possible and to Assoc. Prof. Dr. Souwalak Phongpaichit, the Department of Microbiology, Faculty of Science, Prince of Songkla University, for the isolation, fermentation and identification of the fungi.

I would like to thank the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0109/2550) of the Thailand Research Fund for a scholarship. The Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and the Graduate School, Prince of Songkla University are acknowledged for partial support.

Finally, none of this would have been possible without love and encouragement of my family, colleagues and friends. I thank them all for their kindness and valuable advice. Everything will be always kept in my mind.

Kongkiat Trisuwan

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Drug-resistant bacteria and fungi have caused many diseases in the present. Consequently, an intensive search for more effective antibacterial, antifungal and other bioactive compounds is needed. The culture broth and mycelia extracts of the marine-derived fungi: *Aspergillus* sp. PSU-F154, *Curvularia* sp. PSU-F22, *Fusarium* sp. PSU-F14, *Nigrospora sp.* PSU-F5 and PSU-F18 and *Penicillium* sp. PSU-F40 and PSU-F44, displayed interesting antibacterial (against *Staphylococcus aureus* and methicilline-resistant *S. aureus*), antifungal (against *Microsporum gypseum*) and antioxidant activities. Therefore, it was of interest in searching for new and known metabolites from these extracts. Seventy five compounds including twenty nine new ones have been isolated and identified. Their biological activities are being evaluated.

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LIST OF ABBREVIATIONS AND SYMBOLS

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
q	=	*quartet*
qn	=	*quintet*
m	=	*multiplet*
brs	=	*broad singlet*
brd	=	*broad doublet*
dd	=	*doublet of doublet*
dt	=	*doublet of triplet*
dq	=	*doublet of quartet*
td	=	*triplet of doublet*
qd	=	*quartet of doublet*
tq	=	*triplet of quartet*
δ	=	chemical shift relative to TMS
J	=	coupling constant
m/z	=	a value of mass divided by charge
R_f	=	retention factor
g	=	gram
mg	=	milligram
mL	=	milliliter
L	=	liter
cm^{-1}	=	reciprocal centimeter (wavenumber)
nm	=	nanometer
ppm	=	part per million
λ_{max}	=	maximum wavelength
ν	=	absorption frequency
ε	=	molar extinction coefficient

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

Hz	=	Hertz
MHz	=	megaHertz
$[\alpha]$	=	specific rotation
c	=	concentration
TLC	=	thin-layer chromatography
UV-S	=	Ultraviolet-short wavelength
FT-IR	=	Fourier Transform Infrared
MS	=	Mass Spectroscopy
EIMS	=	Electron Impact Mass Spectroscopy
LRMS	=	Low Resolution Mass Spectroscopy
HRMS	=	High Resolution Mass Spectroscopy
NMR	=	Nuclear Magnetic Resonance
1D NMR	=	One Dimensional Nuclear Magnetic Resonance
2D NMR	=	Two Dimensional Nuclear Magnetic Resonance
HMQC	=	Heteronuclear Mutilple Quantum Coherence
HMBC	=	Heteronuclear Mutilple Bond Correlation
DEPT	=	Distortionless Enhancement by Polarization Transfer
NOEDIFF	=	Nuclear Overhauser Effect Difference
COSY	=	Correlation Spectroscopy
TMS	=	tetramethylsilane
Acetone-d_6	=	hexadeuteroacetone
ASA	=	anisaldehyde-sulphuric acid in acetic acid solution
$CDCl_3$	=	deuterochloroform
CD_3OD	=	tetradeuteromethanol
$CHCl_3$	=	chloroform
CH_2Cl_2	=	dichloromethane
DMSO-d_6	=	hexadeuterodimethyl sulfoxide
EtOH	=	ethanol

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

EtOAc	=	ethyl acetate
HCl	=	hydrochloric acid
H_2O	=	water
MeOH	=	methanol
$NaHCO_3$	=	sodium hydrogen carbonate
NaOH	=	sodium hydroxide
Na_2SO_4	=	sodium sulfate

PART I

METABOLITES FROM THE MARINE-DERIVED FUNGUS *NIGROSPORA* SP. PSU-F5

CHAPTER 1.1

INTRODUCTION

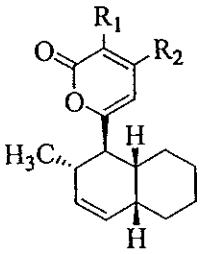
1.1.1 Introduction

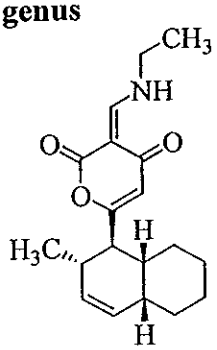
The genus *Nigrospora* is a rich source of biologically active secondary metabolites. Some of them showed interesting biological activities **(Table 1)**. Metabolites isolated from the genus *Nigrospora* reported since the year 2009 are summarized in the **Table 1** based on SciFinder Scholar Database. The marine-derived fungus *Nigrospora* sp. PSU-F5 was isolated from the sea fan *Annella* sp., collected near the Similan Islands, Phangnga Province, Thailand, in the year 2005. This fungus was deposited as PSU-F5 at the Department of Microbiology, Faculty of Science, Prince of Songkla University. The ethyl acetate extract from the culture broth of this fungus exhibited interesting antibacterial activities against *Staphylococcus aureus* ATCC 25923 (SA) and a clinical isolate of methicillin-resistant *S. aureus* (MRSA) with the MIC values of 64 and 128 μg/mL, respectively.

Table 1 Compounds isolated from the *Nigrospora* genus

Scientific name	Compound	Activity	Reference
Nigrospora sp. YB-141	Solanapyrone N, **1** Solanapyrone O, **2** Solanapyrone C, **3** Nigrosporalactone, **4** Phomalactone, **5**	Antifungal	Wu, *et al.*, 2009

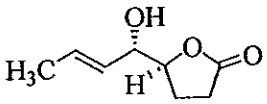
Structures of compounds isolated from the *Nigrospora* genus

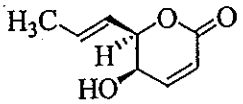




1: $R_1 = CO_2CH_3$, $R_2 = NH_2$: Solanapyrone N **2**: Solanapyrone O

3: $R_1 = CHO$, $R_2 = NHCH_2CH_2OH$: Solanapyrone C





4: Nigrosporalactone **5**: Phomalactone

1.1.2 The objectives

1. To isolate the secondary metabolites from the marine-derived fungus *Nigrospora* sp. PSU-F5.
2. To elucidate the structure of the isolated metabolites.

CHAPTER 1.2

EXPERIMENTAL

1.2.1 Instruments and chemicals

Melting points were determined on an electrothermal melting point apparatus (Electrothermal 9100) and reported without correction. Infrared spectra (IR) were obtianed on a FTS165 FT-IR spectrometer or a Perkin Elmer Spectrum GX FT-IR system and recorded on wavenumber (cm^{-1}). ^{1}H and ^{13}C-Nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded on a FTNMR, Bruker Avance 300 MHz or 500 MHz spectrometers using tetramethylsilane (TMS) as an internal standard. Spectra were recorded as chemical shift parameter (δ) value in ppm down field from TMS (δ 0.00). Ultraviolet spectra (UV) were measured with an UV-160A SHIMADSU spectrophotometer. Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in MeOH solution. Optical rotations were measured in methanol solution or chloroform solution with sodium D line (590 nm) on an AUTOPOLR® Π automatic polarimeter. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for ethyl acetate which was an analytical grade reagent. Thin-layer chromatography (TLC) and precoated TLC plate were performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel (Merck) type 100 (70-230 mesh ASTM), Sephadex LH-20 or reverse phase C_{18} silica gel.

1.2.2 Fermentation and extraction

The flask culture (15 L) of the fungus PSU-F5 was filtered to separate into filtrate and wet mycelia. The filtrate was divided into 30 portions. Each portion was extracted twice with an equal amount of EtOAc (2 x 300 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to obtain a dark brown gum

(800 mg). The mycelial cakes were extracted with MeOH (500 mL). The aqueous MeOH layer was concentrated under reduced pressure. To the extract was added H_2O (50 mL), and the mixture was washed with hexane (500 mL) and extracted twice with an equal amount of EtOAc (2 x 300 mL). The combined EtOAc extracts were dried over anhydrous Na_2SO_4 and then evaporated to dryness under reduced pressure to obtain a brown gum (102 mg). The extracts from the culture filtrate and mycelia were separately subjected to chromatographic fractionation.

1.2.3 Purification of the broth extract

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven fractions as shown in **Table 2.**

Table 2 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
5A	7.1	Brown solid
5B	53.8	Brown solid
5C	307.0	Brown gum
5D	229.3	Brown gum
5E	181.3	Brown gum
5F	9.5	Brown gum
5G	2.4	Brown solid

Fraction 5A displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 5B showed two UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.07 and 0.24. It was

purified by column chromatography over silica gel. Elution was performed initially with 3% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 3**.

Table 3 Subfractions obtained from **fraction 5B** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
5B1	3% MeOH/CH_2Cl_2	4.4	Green gum
5B2	5% MeOH/CH_2Cl_2	4.8	Green gum
5B3	5% MeOH/CH_2Cl_2	4.5	White solid
5B4	7% MeOH/CH_2Cl_2	5.1	White solid
5B5	10% MeOH/CH_2Cl_2	5.7	Yellow solid
5B6	20% MeOH/CH_2Cl_2	2.5	Yellow solid
5B7	20% MeOH/CH_2Cl_2	6.7	Brown solid
5B8	20-50% MeOH/CH_2Cl_2	15.8	Brown solid
5B9	70% MeOH/CH_2Cl_2-100% MeOH	10.0	Brown solid

Subfraction 5B1 displayed a long tail on normal phase TLC under UV-S using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 5B2 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.23, 0.30, 0.35 and 0.46. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Subfraction 5B3 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.14,

0.21, 0.23 and 0.33. Its ^{1}H NMR spectrum contained signals in the high field region. Thus, it was not purified.

Subfraction 5B4 showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.24, 0.29 and 0.33. Because of low quantity, it was not further purified.

Subfraction 5B5 showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.10, 0.19, 0.20 and 0.26. Because of low quantity, it was not further purified.

Subfraction 5B6 showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.28 and the other spot with the R_f value of 0.28 as a red spot after dipping in anisaldehyde reagent and subsequently heating the TLC plate. Attempted purification using various mobile phase systems was not successful. Thus, it was not further investigated.

Subfraction 5B7 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.14, 0.21 and 0.70. Its ^{1}H NMR spectrum displayed broad signals. Thus, it was not further purified.

Subfraction 5B8 showed four UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.17, 0.18, 0.21 and 0.30. Its ^{1}H NMR spectrum showed broad signals. Thus, it was not further purified.

Subfraction 5B9 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.13 and 0.18. Its ^{1}H NMR spectrum displayed broad signals. Thus, it was not further purified.

Fraction 5C showed three UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.31 and 0.79. It was further separated by column chromatography over silica gel. Elution was performed

initially with 5% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 4**.

Table 4 Subfractions obtained from **fraction 5C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
5C1	5% MeOH/CH_2Cl_2	15.6	Colorless gum
5C2	5% MeOH/CH_2Cl_2	25.3	Yellow gum
5C3	5% MeOH/CH_2Cl_2	25.4	Colorless gum
5C4	5% MeOH/CH_2Cl_2	15.4	Yellow gum
5C5	5% MeOH/CH_2Cl_2	17.1	Yellow gum
5C6	7-10% MeOH/CH_2Cl_2	71.3	Yellow gum
5C7	20-50% MeOH/CH_2Cl_2	48.0	Yellow gum
5C8	50% MeOH/CH_2Cl_2-100% MeOH	70.0	Brown solid

Subfraction 5C1 showed three UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.19, 0.38 and 0.50. It was then purified by precoated TLC with 80% dichloromethane in petroleum ether as a mobile phase (6 runs) to afford two bands.

Band1 (K9) was a colorless solid (2.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 80% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.44.

Melting point (°C)	104.3-104.6
UV λ_{max}(nm)(MeOH)(logε)	230 (3.93), 309 (3.91)
FTIR(neat): ν(cm^{-1})	1708 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	6.62 (*qq*, *J* = 6.9, 1.2 Hz, 1H), 5.83 (*d*, *J* = 2.1

	Hz, 1H), 5.38 (*d*, *J* = 2.1 Hz, 1H), 3.74 (*s*, 3H), 1.78 (*s*, 3H), 1.77 (*d*, *J* = 6.9 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	171.49, 164.32, 161.33, 130.20, 126.67, 97.18, 88.02, 55.84, 14.19, 12.01
DEPT135: CH;	130.20, 97.18, 88.02
CH_3;	55.84, 14.19, 12.01

Band 2 (K8) was a colorless solid (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 80% dichloromethane in petroleum ether as a mobile with the R_f value of 0.35.

$[\alpha]_D^{29}$	-110 (*c* 0.01, Acetone)
Melting point (°C)	196.6-196.8
UVλ_{max}(nm)(MeOH)(logε)	206 (3.44), 273 (3.64)
FTIR(neat): ν(cm^{-1})	3365 (O-H stretching), 1697 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	5.95 (*d*, *J* = 2.5 Hz, 1H), 5.36 (*d*, *J* = 2.5 Hz, 1H), 3.73 (*s*, 3H), 3.04 (*q*, *J* = 5.0 Hz, 1H), 1.32 (*d*, *J* = 5.0 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	170.40, 164.55, 163.05, 96.45, 87.37, 60.74, 55.00, 12.94
DEPT135: CH;	96.45, 87.37
CH_3;	55.00, 12.94
EIMS *m/z* (% relative intensity):	170 (97), 149 (100), 125 (95), 83 (50), 71 (77)

Subfraction 5C2 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 5C3 showed four UV-active spots on normal phase TLC using 7% methanol in dichloromethane as a mobile with the R_f values of 0.24, 0.39, 0.64 and 0.79. It was then purified by precoated TLC with 20% ethyl acetate in petroleum ether as a mobile phase (6 runs) to afford three bands.

Band 1 was a white solid (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.63. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Band 2 (K3) was a colorless gum (4.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.33.

$[\alpha]_D^{29}$		+341 (*c* 0.20, MeOH)
UV λ_{max}(nm)(MeOH)(logε)		287 (4.39)
FTIR(neat): ν(cm^{-1})		3556 (O-H stretching), 1704 and 1697 (C=O stretching)
^1H NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (300 MHz):		7.67 (*d*, *J* = 15.9 Hz, 1H), 5.94 (*brs*, 1H), 5.93 (*dd*, *J* = 15.9, 9.3 Hz, 1H), 5.72 (*brs*, 1H), 2.73 (*d*, *J* = 9.3 Hz, 1H), 2.40 (*d*, *J* = 16.8 Hz, 1H), 2.16 (*d*, *J* = 16.8 Hz, 1H), 2.01 (*d*, *J* = 1.2 Hz, 3H), 1.92 (*d*, *J* = 1.2 Hz, 3H), 1.06 (*s*, 3H), 0.98 (*s*, 3H)
^{13}C NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (75 MHz):		199.65, 168.00, 162.00, 150.31, 133.92, 131.44, 125.82, 117.68, 56.37, 47.48, 36.50, 27.85, 27.25, 23.55, 21.08
DEPT135:	CH;	133.92, 131.44, 125.82, 117.68, 56.37
	CH_2;	47.48
	CH_3;	27.85, 27.25, 23.55, 21.08

Band 3 (K10) was a colorless solid (3.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with R_f value of 0.10.

Melting point (°C)	146.5-146.7
UV λ_{max}(nm)(MeOH)(log ε)	282 (3.83), 312 (3.25)
FTIR(neat):υ(cm^{-1})	3385 (O-H stretching), 1690 (C=O stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(500 MHz):	6.58 (*tq*, *J* = 5.5, 1.0 Hz, 1H), 6.10 (*d*, *J* = 2.1 Hz, 1H), 5.51 (*d*, *J* = 2.1 Hz, 1H), 4.35 (*t*, *J* = 5.5 Hz, 2H), 4.06 (*t*, *J* = 5.5 Hz, 1H), 3.89 (*s*, 3H), 1.89 (*q*, *J* = 1.0 Hz, 3H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(125 MHz):	171.15, 162.30, 160.40, 134.57, 126.01, 97.84, 88.13, 58.65, 55.78, 11.62
DEPT 135: CH;	134.57, 97.84, 88.13
CH_2;	58.65
CH_3;	55.78, 11.62

Subfraction 5C4 showed four UV-active spots on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f values of 0.13, 0.25, 0.31 and 0.43. Further purification by column chromatography over Sephadex LH-20 was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 5**.

Table 5 Subfractions obtained from **subfraction 5C4** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
5C41	1.3	Brown gum
5C42	0.8	Brown gum
5C43	3.1	Brown gum
5C44	7.8	Brown gum
5C45	2.5	Brown gum

Subfraction 5C41 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 5C42 displayed unseparated spots under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 5C43 showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.27, 0.40 and 0.50. It was then purified by precoated TLC with 30% acetone in petroleum ether as a mobile phase (3 runs) to afford two bands.

Band 1 was a white solid (0.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% acetone in petroleum ether as a mobile phase with the R_f value of 0.30. Its ^{1}H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 2 (K2) was a colorless gum (1.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% acetone in petroleum ether as a mobile phase with the R_f value of 0.20.

$[\alpha]_D^{29}$	+275 (*c* 0.20, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	280 (3.84)
FTIR(neat):υ(cm^{-1})	3345 (O-H stretching), 1703 and 1694 (C=O stretching)
^{1}H NMR(CD_3OD)(δ_{ppm})(300 MHz):	7.70 (*d*, *J* = 16.2 Hz, 1H), 6.24 (*d*, *J* = 16.2 Hz, 1H), 5.93 (*q*, *J* = 1.2 Hz, 1H), 5.75 (*brs*, 1H), 2.54 (*d*, *J* = 17.1 Hz, 1H), 2.18 (*d*, *J* = 17.1 Hz, 1H), 2.03 (*d*, *J* = 1.2 Hz, 3H), 1.93 (*d*, *J* = 1.2 Hz, 3H), 1.06 (*s*, 3H), 1.03 (*s*, 3H)

^{13}C NMR(CD_3OD)(δ_{ppm})(75 MHz):		199.73, 168.13, 165.21, 149.42, 136.41, 128.06, 126.17, 118.39, 79.23, 49.25, 41.45, 23.25, 22.15, 19.82, 18.22
DEPT 135:	CH;	136.41, 128.06, 126.17, 118.39
	CH_2;	49.25
	CH_3;	23.25, 21.15, 19.82, 18.22

Subfraction 5C44 showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.27 and 0.37. Its ^{1}H NMR spectrum indicated that the major compound was **K2**. Therefore, it was not purified.

Subfraction 5C45 showed four UV-active spots on normal phase TLC using 30% acetone in petroleum ether as a mobile phase with the R_f values of 0.26, 0.33, 0.39 and 0.56. Its ^{1}H NMR spectrum indicated that the major compound was **K2**. Therefore, it was not purified.

Subfraction 5C5 showed three UV-active spots on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f values of 0.25, 0.45 and 0.63. Its ^{1}H NMR spectrum indicated that the major compound was **K2**. Therefore, it was not purified.

Subfraction 5C6 showed four UV-active spots on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f values of 0.15, 0.45, 0.60 and 0.70. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 30% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eigth subfractions as shown in **Table 6**.

Table 6 Subfractions obtained from **subfraction 5C6** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
5C61	30% MeOH/H_2O	9.5	Yellow solid
5C62	50% MeOH/H_2O	5.8	White solid
5C63	60% MeOH/H_2O	7.4	Yellow solid
5C64	70% MeOH/H_2O	14.2	Yellow solid
5C65	80% MeOH/H_2O	6.7	Yellow solid
5C66	90% MeOH/H_2O	6.2	Yellow solid
5C67	100% MeOH	4.0	Yellow solid
5C68	100% MeOH	21.1	Yellow solid

Subfraction 5C61 showed two UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.43 and 0.52. It was then purified by precoated TLC with 50% ethyl acetate in petroleum ether as a mobile phase (10 runs) to afford five bands.

Band 1 (K1) was a colorless gum (1.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 80% ethyl acetate in petroleum ether as a mobile with the R_f value of 0.63.

$[\alpha]_D^{29}$	+110 (*c* 0.92, EtOH)
UV λ_{max}(nm)(MeOH)(log ε)	279 (3.99), 328 (3.56)
FTIR(neat):υ(cm^{-1})	3363 (O-H stretching), 1690 (C=O stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(300 MHz):	6.72 (*brs*, 1H), 4.98 (*brs*, 1H), 4.66 (*d*, *J* = 4.5 Hz, 1H), 4.27 (*d*, *J* = 15.6 Hz, 1H), 4.16 (*d*, *J* = 15.6 Hz, 1H), 3.78 (*t*, *J* = 3.3 Hz, 1H), 3.41 (*d*, *J* = 3.3 Hz, 1H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(75 MHz):	193.51, 138.53, 136.24, 62.42, 58.16, 57.96, 53.24

DEPT 135:	CH;	138.53, 62.42, 57.96, 53.24
	CH_2;	58.16

Band 2 was a white solid (3.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 80% ethyl acetate in petroleum ether as a mobile with the R_f value of 0.49. Its ^1H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 3 was a white solid (0.3 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 100% ethyl acetate as a mobile phase with the R_f value of 0.50. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 4 was a white solid (0.6 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 100% ethyl acetate as a mobile phase with the R_f value of 0.47. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 5 was a white solid (1.0 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 100% ethyl acetate as a mobile phase with the R_f value of 0.10. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 5C62 showed three major UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.04, 0.18 and 0.36. It was then separated by precoated TLC with 50% ethyl acetate in petroleum ether as a mobile phase (13 runs) to afford three bands.

Band 1 (K4) was a colorless solid (0.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 80% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.44.

$[\alpha]_D^{29}$	-162 (*c* 0.01, acetone)
Melting point (°C)	192.8-193.7
UV λ_{max}(nm)(MeOH)(log ε)	282 (4.43)
FTIR(neat):υ(cm^{-1})	3566 (O-H stretching), 1703 and 1697 (C=O stretching), 1651 (C=C stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(500 MHz):	8.04 (*d*, *J* = 16.0 Hz, 1H), 6.44 (*d*, *J* = 16.0 Hz, 1H), 5.78 (*s*, 1H), 3.85 (*dd*, *J* = 8.5, 3.5 Hz, 1H), 3.76 (*d*, *J* = 8.5 Hz, 1H), 2.80 (*d*, *J* = 17.0 Hz, 1H), 2.79 (*dd*, *J* = 17.0, 3.5 Hz, 1H), 2.23 (*dd*, *J* = 17.0, 1.0 Hz, 1H), 2.17 (*dd*, *J* = 17.0, 1.0 Hz, 1H), 2.12 (*s*, 3H), 1.10 (*s*, 3H), 0.94 (*s*, 3H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(125 MHz):	208.22, 166.47, 150.10, 137.27, 127.35, 117.24, 84.14, 81.32, 76.18, 50.91, 50.38, 46.29, 21.08, 20.43, 17.44
DEPT 135: CH;	137.27, 127.35, 117.24
CH_2;	76.18, 50.91, 50.38
CH_3;	21.08, 20.43, 17.44

Band 2 was a white solid (1.2 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 80% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.33. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Therefore, it was not further investigated.

Band 3 was a white solid (2.1 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 80% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.12. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 5C63 showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.21 and 0.30. It was then separated by precoated TLC with 50% ethyl acetate in petroleum ether as a mobile phase (13 runs) to afford four bands.

Band 1 was a colorless gum (1.9 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.23. Its ^{1}H NMR spectrum indicated that the major compound was **K2**. Therefore, it was not investigated.

Band 2 was a white solid (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.19. Its ^{1}H NMR spectrum indicated that the major compound was **K4**. Therefore, it was not investigated.

Band 3 (K5) was a colorless solid (1.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% ethyl acetate as a mobile phase with the R_f value of 0.31.

$[\alpha]_D^{29}$	+10 (*c* 0.06, EtOH)
Melting point (°C)	173.6-173.8
UV λ_{max}(nm)(MeOH)(log ε)	281 (3.41)
FTIR(neat):υ(cm^{-1})	3442 (O-H stretching), 1716 and 1685 (C=O stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(500 MHz):	7.78 (*d*, *J* = 16.0 Hz, 1H), 6.94 (*d*, *J* = 2.0 Hz, 1H), 6.43 (*d*, *J* = 16.0 Hz, 1H), 5.84 (*s*, 1H), 5.83 (*q*, *J* = 1.0 Hz, 1H), 5.32 (*d*, *J* = 11.5 Hz, 1H), 5.06 (*d*, *J* = 4.5 Hz, 1H), 4.96 (*d*, *J* = 4.5 Hz, 1H), 4.56 (*s*, 1H), 4.54 (*m*, 1H), 4.21 (*m*, 2H), 3.90 (*ddd*, *J* = 11.5, 8.0, 4.5 Hz, 1H), 2.57 (*t*, *J* = 17.0 Hz, 1H),

	2.16 (*t*, *J* = 17.0 Hz, 1H), 2.10 (*d*, *J* = 1.0 Hz, 3H), 1.91 (*d*, *J* = 1.0 Hz, 3H), 1.08 (*s*, 3H), 1.05 (*s*, 3H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(125 MHz):	196.74, 192.22, 164.53, 162.34, 150.91, 145.58, 138.10, 137.24, 127.87, 126.52, 117.24, 79.28, 77.08, 76.09, 71.61, 58.02, 49.46, 41.39, 23.80, 22.66, 20.38, 18.37
DEPT 135: CH;	145.58, 138.10, 127.87, 126.52, 117.24, 77.08, 76.09, 71.61
CH_2;	58.02, 49.46
CH_3;	23.80, 22.66, 20.38, 18.37
EIMS *m/z* (% relative intensity):	420 (1), 256 (17), 149 (62), 111 (40), 83 (69), 71 (100)

Band 4 was a white solid (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% ethyl acetate as a mobile with the R_f value of 0.20. Its ^{1}H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Subfraction 5C64 showed two UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.21 and 0.33. Its ^{1}H NMR spectrum indicated that the major compound was **K2**. Therefore, it was not purified.

Subfraction 5C65 showed two UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.16 and 0.26. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not further investigated.

Subfraction 5C66 (K7) was a colorless solid (6.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile with the R_f value of 0.18.

$[\alpha]_D^{29}$	+39 (*c* 0.31, EtOH)
Melting point (°C)	366.2-366.7
UV λ_{max}(nm)(MeOH)(log ε)	282 (2.89)
FTIR(neat):υ(cm^{-1})	3370 (O-H stretching), 1749, 1734 and 1698 (C=O stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(500 MHz):	7.25 (*m*, 5H), 6.93 (*s*, 1H), 5.25 (*d*, *J* = 11.1 Hz, 1H), 4.56 (*d*, *J* = 8.4 Hz, 1H), 4.26 (*d*, *J* = 13.6 Hz, 1H), 4.15 (*d*, *J* = 13.6 Hz, 1H), 3.87 (*dd*, *J* = 11.1, 8.4 Hz, 1H), 3.62 (*dd*, *J* = 6.9, 6.0 Hz, 1H), 3.60 (*s*, 3H), 3.33 (*dd*, *J* = 9.0, 5.4 Hz, 1H), 2.93 (*m*, 2H), 1.88 (*m*, 1H), 1.59 (*ddd*, *J* = 13.5, 8.1, 5.4 Hz, 1H), 1.45 (*ddd*, *J* = 13.5, 9.0, 6.0 Hz, 1H), 0.87 (*d*, *J* = 6.6 Hz, 3H), 0.81 (*d*, *J* = 6.6 Hz, 3H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(125 MHz):	191.73, 174.35, 173.73, 145.71, 138.10, 138.07, 129.52, 127.97, 126.20, 77.59, 75.92, 71.39, 61.32, 58.62, 57.93, 51.04, 43.01, 39.25, 24.25, 22.50, 21.38
DEPT 135: CH;	145.71, 129.52, 127.97, 126.20, 77.59, 75.92, 71.39, 61.32, 58.62, 24.25
CH_2;	57.93, 43.01, 39.25
CH_3;	51.04, 22.50, 21.38
EIMS *m/z* (% relative intensity):	450 (11), 390 (26), 358 (100), 248 (67), 156 (42)

Subfraction 5C67 showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.15. Its ^{1}H NMR spectrum showed signals in the high field region. Thus, it was not further investigated.

Subfraction 5C68 displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 5C7 showed four UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.23, 0.28, 0.51 and 0.57. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 7**.

Table 7 Subfractions obtained from **subfraction 5C7** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
5C71	1.7	Yellow solid
5C72	12.3	Yellow solid
5C73	4.1	Yellow solid
5C74	23.2	Yellow solid

Subfraction 5C71 displayed a long tail under UV-S on normal phase TLC using 6% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 5C72 showed three UV-active spots on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f values of 0.01, 0.28 and 0.35. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 5C73 showed two UV-active spots on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f values of 0.07 and 0.26. Its ^{1}H NMR spectrum displayed broad signals. Thus, it was not further purified.

Subfraction 5C74 showed seven UV-active spots on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f values of 0.01, 0.23, 0.28, 0.33, 0.38, 0.44 and 0.49. Its ^1H NMR spectrum showed broad signals. Therefore, it was not further purified.

Subfraction 5C8 displayed a long tail under UV-S on normal phase TLC using 7% methanol in dichloromethane as a mobile phase. This subfraction was dissolved in a mixture of acetic anhydride (3 mL) and pyridine (1 mL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into water and then extracted with ethyl acetate (3 × 15 mL). The combined ethyl acetate extracts were successively washed with 10% aqueous HCl (2 × 20 mL), 10% aqueous $NaHCO_3$ (3 × 20 mL) and water (3 × 20 mL), and then dried over anhydrous Na_2SO_4. After removal of ethyl acetate solvent, the acetate derivative was obtained as a dark brown gum (45 mg). It was further separated by column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 8.**

Table 8 Subfractions obtained from **subfraction 5C8** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
5C81	1% MeOH/CH_2Cl_2	5.1	Yellow gum
5C82	3% MeOH/CH_2Cl_2	10.3	Yellow gum
5C83	5% MeOH/CH_2Cl_2	4.3	Yellow gum
5C84	5% MeOH/CH_2Cl_2	4.5	Yellow gum
5C85	10% MeOH/CH_2Cl_2-100% MeOH	18.9	Yellow gum

Subfraction 5C81 showed two UV-active spots on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.10 and 0.21. Because of low quantity, it was not further purified.

Subfraction 5C82 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.35, 0.49, 0.63 and 0.74. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 9**.

Table 9 Subfractions obtained from **subfraction 5C82** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
5C821	0.5	White solid
5C822	6.4	Yellow solid
5C823	3.6	White solid
5C824	1.4	Yellow solid

Subfraction 5C821 displayed a long tail under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 5C822 showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.35. Because its ^{1}H NMR spectrum displayed broad signals, it was not further purified.

Subfraction 5C823 showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.04, 0.16, 0.39, 0.69 and 0.74. Its ^{1}H NMR spectrum showed broad signals. Therefore, it was not further purified.

Subfraction 5C824 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.30, 0.34 and 0.60. Its ^1H NMR spectrum displayed broad signals. Thus, it was not further purified.

Subfraction 5C83 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.26, 0.28 and 0.37. Because its ^1H NMR spectrum indicated that broad signals, it was not further purified.

Subfraction 5C84 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.23 and 0.28. Its ^1H NMR spectrum showed broad signals. Therefore, it was not further purified.

Subfraction 5C85 displayed a long tail under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 5D showed five UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.24, 0.29, 0.36 and 0.79. It was further separated by column chromatography over silica gel. Elution was performed initially with 5% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 10**.

Table 10 Subfractions obtained from **fraction 5D** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
5D1	5% MeOH/CH_2Cl_2	5.8	Yellow gum
5D2	5% MeOH/CH_2Cl_2	7.7	Yellow gum

Table 10 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
5D3	7% MeOH/CH_2Cl_2	11.7	Yellow gum
5D4	7-10% MeOH/CH_2Cl_2	123.5	Yellow gum
5D5	10-20% MeOH/CH_2Cl_2	10.9	Yellow gum
5D6	20% MeOH/CH_2Cl_2-100% MeOH	67.0	Yellow gum

Subfraction 5D1 showed two UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.23 and 0.54. It was then separated by precoated TLC with 30% dichloromethane in petroleum ether (5 runs) and 50% dichloromethane in petroleum ether as mobile phases (6 runs), respectively, to afford two bands.

Band 1 was a colorless solid (1.7 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.69. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 2 was a colorless solid (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.15. Its ^1H NMR spectrum indicated that the major compound was **K9**. Therefore, it was not investigated.

Subfraction 5D2 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.12, 0.28, 0.52 and 0.56. It was then separated by precoated TLC with 100% dichloromethane as a mobile phase (10 runs) to afford three bands.

Band 1 was a yellow solid (0.7 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a

mobile phase with the R_f value of 0.46. Its ^1H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 2 was a yellow solid (0.9 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.44. Its ^1H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 3 was a white solid (1.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.12. Its ^1H NMR spectrum indicated that the major compound was **K10**. Therefore, it was not investigated.

Subfraction 5D3 showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.57. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, It was then separated by precoated TLC with 3% methanol in dichloromethane (9 runs) to afford three bands.

Band 1 was a yellow solid (1.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.41. Its ^1H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 2 was a yellow solid (1.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.28. Its ^1H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 3 was a brown solid (5.8 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 3% methanol in dichloromethane as

a mobile phase with the R_f value of 0.35. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 5D4 showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.13 and 0.24. Its ^1H NMR spectrum indicated that the major compound was **K1**. Therefore, it was not investigated.

Subfraction 5D5 showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.04, 0.13 and 0.19. It was then separated by precoated TLC with 3% methanol in dichloromethane as a mobile phase (13 runs) to afford three bands.

Band 1 was a white solid (1.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.34. Its ^1H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 2 was a white solid (3.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.24. Its ^1H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 3 was a white solid (2.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.12. Its ^1H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Subfraction 5D6 showed four UV-active spots on reverse phase TLC using 80% methanol in water as a mobile phase with the R_f values of 0.22, 0.56, 0.85 and 0.88. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 80% methanol in water followed by increasing

amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 11**.

Table 11 Subfractions obtained from **subfraction 5D6** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
5D61	80% MeOH/H_2O	10.5	Brown solid
5D62	80% MeOH/H_2O	6.0	Brown solid
5D63	90% MeOH/H_2O	38.7	Brown solid
5D64	100% MeOH	6.0	White solid

Subfraction 5D61 showed five UV-active spots on normal phase TLC using 7% methanol in dichloromethane as a mobile phase with the R_f values of 0.16, 0.27, 0.38, 0.43 and 0.51. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Subfraction 5D62 showed two UV-active spots on normal phase TLC using 7% methanol in dichloromethane as a mobile phase with the R_f values of 0.14 and 0.27. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Subfraction 5D63 showed two UV-active spots on normal phase TLC using 7% methanol in dichloromethane as a mobile phase with the R_f values of 0.21 and 0.30. Its ^1H NMR spectrum displayed broad signals. Thus, it was not further purified.

Subfraction 5D64 displayed a long tail on normal phase TLC under UV-S using 7% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 5E showed four UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.29, 0.33 and 0.79.

It was further separated by column chromatography over silica gel. Elution was performed initially with 5% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 12.**

Table 12 Subfractions obtained from **fraction 5E** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
5E1	5% MeOH/CH_2Cl_2	6.3	Brown solid
5E2	7% MeOH/CH_2Cl_2	3.9	Brown solid
5E3	7% MeOH/CH_2Cl_2	8.7	Yellow solid
5E4	10% MeOH/CH_2Cl_2	86.0	Yellow gum
5E5	20% MeOH/CH_2Cl_2	8.7	Yellow solid
5E6	20-30% MeOH/CH_2Cl_2	18.3	Colorless gum
5E7	50-70% MeOH/CH_2Cl_2	12.6	Yellow solid
5E8	70% MeOH/CH_2Cl_2-100% MeOH	3.5	Brown solid

Subfraction 5E1 showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.26, 0.64, 0.71 and 0.88. It was then separated by precoated TLC with 100% dichloromethane (9 runs) and 2% methanol in dichloromethane as a mobile phase (5 runs), respectively, to afford three bands.

Band 1 (K12) was a colorless solid (2.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.64.

Melting point (°C)	179.1-180.2
UV λ_{max}(nm)(MeOH)(log ε)	216 (3.64), 280 (3.72), 329 (3.10),
FTIR(neat):υ(cm^{-1})	3430 (O-H stretching), 1680 (C=O stretching)

^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	12.38 (*s*, 1H), 7.31 (*s*, 1H), 2.56 (*s*, 3H), 2.21 (*s*, 3H), 2.14 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	202.66, 161.32, 158.71, 129.81, 114.43, 113.47, 110.23, 26.29, 15.46, 7.37
DEPT 135: CH;	129.81
CH_3;	26.29, 15.46, 7.37

Band 2 was a white solid (1.7 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.29. Its ^{1}H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 3 was a white solid (1.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.10. Its ^{1}H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Subfraction 5E2 showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.17, 0.24, 0.28 and 0.33. It was then separated by precoated TLC with 3% methanol in dichloromethane as a mobile phase (9 runs) to afford a white solid (2.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.28. Its ^{1}H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Subfraction 5E3 showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.17, 0.26 and 0.36. It was then separated by precoated TLC with 3% methanol in dichloromethane as a mobile phase (9 runs) to afford four bands.

Band 1 (K13) was a colorless solid (2.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.31.

Melting point (°C)	72.1-73.0
UV λ_{max}(nm)(MeOH)(log ε)	202 (3.66), 214 (3.56), 272 (3.60)
FTIR(neat):υ(cm^{-1})	3435 (O-H stretching), 1654 (C=C stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(300 MHz):	8.55 (*brs*, 1H), 7.12 (*t*, *J* = 8.0 Hz, 1H), 6.86 (*s*, 1H), 6.79 (*brd*, *J* = 8.0 Hz, 1H), 6.69 (*dd*, *J* = 8.0, 2.0 Hz, 1H), 4.55 (*s*, 2H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(75 MHz):	157.49, 144.10, 129.02, 117.35, 113.63, 113.38, 63.66
DEPT 135: CH;	129.02, 117.35, 113.63, 113.38
CH_2;	63.66

Band 2 (K11) was a colorless gum (1.9 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.24.

$[\alpha]_D^{29}$	-34 (*c* 0.019, MeOH)
Melting point (°C)	191.1-192.0
UV λ_{max}(nm)(MeOH)(log ε)	214 (3.45), 263 (3.65), 328 (2.73)
FTIR(neat):υ(cm^{-1})	3439 (O-H stretching), 1696 (C=O stretching)
^{1}H NMR($CDCl_3$+CD_3OD)(δ_{ppm})(500 MHz):	12.26 (*s*, 1H), 7.54 (*t*, *J* = 8.0 Hz, 1H), 7.27 (*dd*, *J* = 7.5, 0.5 Hz, 1H), 6.91 (*d*, *J* = 8.5 Hz, 1H), 4.64 (*d*, *J* = 8.0 Hz, 1H), 4.00 (*m*, 1H), 3.10 (*dd*, *J* = 17.0, 4.5 Hz, 1H), 2.73 (*dd*, *J* =

	17.0, 11.0 Hz, 1H)
^{13}C NMR($CDCl_3$+CD_3OD)(δ_{ppm})(125 MHz):	202.0, 162.00, 143.00, 137.20, 117.03, 116.93, 115.30, 73.38, 71.00, 44.43
DEPT 135: CH;	137.20, 117.03, 116.93, 73.38, 71.00
CH_2;	44.43

Band 3 was a white solid (1.7 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.17. Its ^{1}H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 4 was a white solid (2.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.10. Its ^{1}H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Subfraction 5E4 showed one UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.39. Its ^{1}H NMR spectrum indicated that the major compound was **K1**. Therefore, it was not investigated.

Subfraction 5E5 showed five UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.14, 0.21, 0.26 and 0.39. It was then separated by precoated TLC with 5% methanol in dichloromethane as a mobile phase (9 runs) to afford three bands.

Band 1 was a white solid (2.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.39. Its ^{1}H NMR spectrum indicated that the major compound was **K1**. Therefore, it was not investigated.

Band 2 was a white solid (1.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.17. Its ^{1}H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Band 3 was a white solid (0.9 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.14. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 5E6 (K6) showed one UV-active spot on normal phase TLC using 10% methanol in dichloromethane as a mobile phase with the R_f value of 0.12.

$[\alpha]_D^{29}$	-2 (*c* 0.35, EtOH)
UV λ_{max}(nm)(MeOH)(log ε)	210 (4.55), 232 (4.34), 270 (4.06)
FTIR(neat):υ(cm^{-1})	3360 (O-H stretching), 1717 and 1696 (C=O stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(300 MHz):	7.56 (*dt*, *J* = 8.1, 1.5 Hz, 1H), 7.55 (*dd*, *J* = 2.7, 1.5 Hz, 1H), 7.35 (*t*, *J* = 8.1 Hz, 1H), 7.12 (*ddd*, *J* = 8.1, 2.7, 1.5 Hz, 1H), 6.99 (*q*, *J* = 1.8 Hz, 1H), 5.50 (*d*, *J* = 11.1 Hz, 1H), 4.64 (*brd*, *J* = 8.1 Hz, 1H), 4.28 (*d*, *J* = 15.3 Hz, H), 4.23 (*d*, *J* = 15.3 Hz, 1H), 4.08 (*dd*, *J* = 11.1, 8.1 Hz, 1H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(75 MHz):	191.94, 165.30, 157.50, 145.72, 137.23, 131.41, 129.50, 120.19, 120.08, 116.30, 78.19, 76.11, 71.49, 58.00
DEPT 135: CH;	145.72, 129.50, 120.19, 120.08, 116.30, 78.19, 76.11, 71.49
CH_2;	58.00

EIMS *m/z* (% relative intensity): 294 (30), 279 (40), 256 (60), 210 (90), 196 (100)

Subfraction 5E7 showed two UV-active spots on normal phase TLC using 10% methanol in dichloromethane as a mobile phase with the R_f values of 0.09 and 0.19. It was then separated by precoated TLC with 8% methanol in dichloromethane as a mobile phase (7 runs) to afford two bands.

Band 1 was a white solid (2.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% methanol in dichloromethane as a mobile phase with the R_f value of 0.15. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Band 2 was a white solid (1.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% methanol in dichloromethane as a mobile phase with the R_f value of 0.10. Its ^{1}H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Subfraction 5E8 displayed a long tail under UV-S on normal phase TLC using 10% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 5F showed four UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.12, 0.20 and 0.29. It was then separated by precoated TLC with 3% methanol in dichloromethane (9 runs) and 5% methanol in dichloromethane as mobile phases (5 runs), respectively, to afford two bands.

Band 1 was a white solid (1.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.64. Because its ^{1}H NMR spectrum showed signals in the high field region, it was not purified.

Band 2 was a white solid (2.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.52. Its ^1H NMR spectrum indicated the presence of many compounds. Because of the minute quantity, it was not further investigated.

Fraction 5G displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

1.2.4 Purification of the EtOAc extract from mycelia

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four fractions as shown in **Table 13**.

Table 13 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
5CE1	19.1	Brown gum
5CE2	55.9	Brown gum
5CE3	17.1	Brown gum
5CE4	7.3	Brown gum

Fraction 5CE1 displayed a long tail under UV-S on normal phase TLC using 100% dichloromethane as a mobile phase. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Fraction 5CE2 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.52, 0.72 0.87 and 0.95. Its ^1H NMR spectrum displayed broad signals. Thus, it was not further purified.

Fraction 5CE3 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.28, 0.41, 0.49 and 0.79. It was then separated by precoated TLC with 100% dichloromethane as mobile phase (5 runs) to afford three bands

Band 1 was a brown gum (5.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.75. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 2 was a brown gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.49. Its ^1H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further investigated.

Band 3 was a brown gum (2.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.28. Its ^1H NMR spectrum displayed broad signals. Thus, it was not further purified.

Fraction 5CE4 displayed a long tail under UV-S on normal phase TLC using 100% dichloromethane as a mobile phase. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

CHAPTER 1.3

RESULTS AND DISCUSSION

Four new compounds **(K5-K8)** were isolated from the broth extract together with nine known ones **(K1-K4** and **K9-K13)**. The structures were identified by spectroscopic methods.

1.3.1 Compound K1

Compound **K1** was obtained as a colorless gum. The UV spectrum exhibited absorption bands at 279 and 328 nm. The IR spectrum showed absorption bands at 3363 cm^{-1} and 1690 cm^{-1} for hydroxyl and carbonyl groups, respectively. The ^{1}H NMR spectrum **(Figure 1) (Table 14)** displayed signals for one olefinic proton (δ 6.72, *brs*, 1H), three oxymethine protons [δ 4.66 (*d*, J = 4.5 Hz, 1H), 3.78 (*t*, J = 3.3 Hz, 1H) and 3.41 (*d*, J = 3.3 Hz, 1H)], one hydroxymethyl group [δ 4.27 (*d*, J = 15.6 Hz, 1H) and 4.16 (*d*, J = 15.6 Hz, 1H)] and one hydroxy proton (δ 4.98, *brs*, 1H). The ^{13}C NMR **(Figure 2) (Table 14)** and DEPT 135 spectra **(Table 14)** displayed seven carbon resonances for two quaternary (δ 193.51 and 136.24), four methine (δ 138.53, 62.42, 57.96 and 53.24) and one oxymethylene (δ 58.16) carbons. In the ^{1}H-^{1}H COSY spectrum **(Table 15)**, the oxymeyhtine proton, H-4 (δ 4.66), was coupled with the olefinic proton, H-3 (δ 6.72), and the oxymethine proton, H-5 (δ 3.78), which was further coupled with the remaining oxymethine proton, H-6 (δ 3.41). In the HMBC spectrum **(Table 15)**, H-3 gave cross peaks with C-1 (δ 193.51) and C-7 (δ 58.16) while H-6 showed a cross peak with C-1. These results indicated that **K1** had a cyclohexenone ring with a C2-C3 double bond and the hydroxymethyl group at C-2 (δ 136.24). The location of the hydroxymethyl group was confirmed by HMBC correlations of H_{ab}-7 (δ 4.27 and 4.16) with C-1, C-2 and C-3 (δ 138.53) and enhancement of H_{ab}-7 signals upon irradiation of H-3. The chemical

shift values of C-5 (δ 57.96) and C-6 (δ 53.24) established an epoxide functionality at these carbons. Irradiation of H-5 in the NOEDIFF experiment **(Table 15)** enhanced signal intensity of H-4 and H-6, indicating their *cis*-relationship. The observed optical rotation of **K1**, $[\alpha]_D^{29}$ +110 (*c* 0.92, EtOH), was similar to that of (4*R*,5*R*,6*R*)-(+)-epoxydon, $[\alpha]_D^{29}$ +98.0 (*c* 1.00, EtOH) (Mehta and Islam, 2004), indicating that all chiral carbons of **K1** possessed the same absolute configuration as those of (4*R*,5*R*,6*R*)-(+)-epoxydon which was previously isolated from the endophytic fungus *Ophiosphaerella herpotricha* (Venkatasubbaiah, *et al.*, 1994).

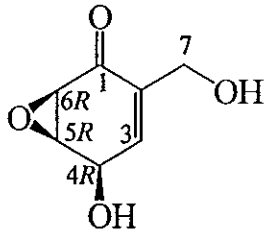


Table 14 The ^{1}H and ^{13}C NMR data of compound **K1** and **(4*R*,5*R*,6*R*)-(+)-Epoxydon** in acetone-d_6

Position	K1		(4*R*,5*R*,6*R*)-(+)-Epoxydon	
	δ_H (*mult*, *J* Hz)	δ_C (C-Type)	δ_H (*mult*, *J* Hz)	δ_C (C-Type)
1	-	193.51 (C)	-	194.50 (C)
2	-	136.24 (C)	-	135.20 (C)
3	6.72 (*brs*)	138.53 (CH)	6.50 (*d*, 1.8)	141.40 (CH)
4	4.66 (*d*, 4.5)	62.42 (CH)	4.91 (*d*, 7.5)	65.50 (CH)
4-OH	4.98 (*brs*)	-	4.77-4.80 (*m*)	-
5	3.78 (*t*, 3.3)	57.96 (CH)	3.80 (*d*, 3.0)	55.00 (CH)
6	3.41 (*d*, 3.3)	53.24 (CH)	3.34 (*d*, 4.2)	54.00 (CH)
7	a: 4.27 (*d*, 15.6)	58.16 (CH_2)	4.06-4.24 (*m*)	59.10 (CH_2)
	b: 4.16 (*d*, 15.6)			
7-OH	-	-	4.06-4.24 (*m*)	-

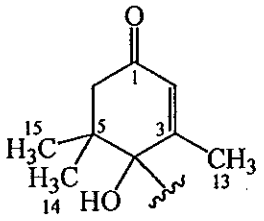
Table 15 The HMBC, COSY and NOEDIFF data of compound **K1** in acetone-d_6

Proton	HMBC	COSY	NOEDIFF
H-3	C-1, C-2, C-4, C-5, C-7	H-4, H_{ab}-7	H-4, H_{ab}-7
H-4	C-2, C-3, C-5, C-6	H-3, H-5	H-3, H-5
H-5	C-3, C-4, C-6	H-4, H-6	H-4, H-6
H-6	C-1, C-2, C-5	H-4, H-5	H-5
H_a-7	C-1, C-2, C-3	H-3	H-3
H_b-7	C-1, C-2, C-3	H-3	H-3

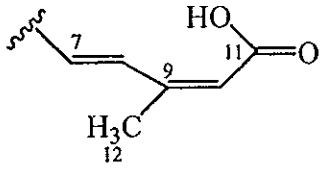
1.3.2 Compound K2

Compound **K2** was obtained as a colorless gum. The UV spectrum showed an absorption band at 280 nm. The IR spectrum exhibited absorption bands at 3345, 1730 and 1694 cm^{-1} for hydroxyl, conjugated carboxylic carbonyl and conjugated ketone carbonyl groups, respectively. The ^1H NMR spectrum **(Figure 3) (Table 16)** consisted of signals for *trans*-olefinic protons [δ 7.70 (*d*, J = 16.2 Hz, 1H) and 6.24 (*d*, J = 16.2 Hz, 1H)], two olefinic protons of two trisubstituted double bonds [δ 5.93 (*q*, J = 1.2 Hz, 1H) and 5.75 (*brs*, 1H)], two nonequivalent methylene protons [δ 2.54 (*d*, J = 17.1 Hz, 1H) and 2.18 (*d*, J = 17.1 Hz, 1H)] and four methyl groups [δ 2.03 (*d*, J = 1.2 Hz, 3H), 1.93 (*d*, J = 1.2 Hz, 3H), 1.06 (*s*, 3H) and 1.03 (*s*, 3H)]. The ^{13}C NMR **(Figure 4) (Table 16)** and DEPT 135 spectra **(Table 16)** displayed fifteen carbon resonances for six quaternary (δ 199.73, 165.21, 168.13, 149.42, 79.23 and 41.45), four methine (δ 136.41, 126.17, 128.06 and 118.39), one methylene (δ 49.25) and four methyl (δ 23.25, 22.15, 19.82 and 18.22) carbons. In the HMBC spectrum **(Table 17)**, the olefinic proton, H-2 (δ 5.93), showed cross peaks with C-4 (δ 79.23), C-6 (δ 49.25) and C-13 (δ 18.22) while the methylene protons, H_{ab}-6 (δ 2.54 and 2.18), were correlated with C-1 (δ 199.73), C-4, C-5 (δ 41.45), C-14 (δ 22.15) and C-15 (δ 23.25). In addition, the methyl protons, H_3-14 (δ 1.06) and H_3-15 (δ 1.03), gave HMBC cross peaks with C-4, C-5 and C-6, thus indicating the attachment of both methyl groups at C-5. The presence of the methyl group at C-3

(δ 165.21) was supported by HMBC correlations of H_3-13 with C-2 (δ 126.17), C-3 and C-4. These results together with the chemical shift of C-4 established substructure 1.



substructure 1



substructure 2

One of the *trans*-olefinic protons, H-8 (δ 7.70), showed HMBC cross peaks with C-7 (δ 136.41), C-9 (δ 149.42), C-10 (δ 118.39) and C-12 (δ 19.82) while the other *trans*-olefinic proton, H-7 (δ 6.24), was correlated with C-8 (δ 128.06), C-9, C-10 and C-12. The attachment of the methyl group at C-9 was confirmed by HMBC correlations of H_3-12 (δ 2.03) with C-8, C-9 and C-10. These data together with HMBC correlations of the remaining olefinic proton, H-10 (δ 5.75), with C-9, C-11 (δ 168.13) and C-12 constructed a 3-methyl-2,4-pentadienoyl unit (substructure 2). Substructures 1 and 2 were joined together by forming a bond between C-4 of substructure 1 with C-7 of substructure 2 on the basis of HMBC correlations of H-7 of substructure 2 with C-3, C-4 and C-5 of substructure 1. Signal enhancement of H_3-12 after irradiation of the H-10 in the NOEDIFF experiment **(Table 17)**, established *Z* configuration for a C9-C10 double bond. The observed optical rotation of **K2**, $[\alpha]_D^{29}$ +275 (*c* 0.20, MeOH), was almost identical to that of (4*S*)-(+)-abscisic acid, $[\alpha]_D^{23}$ +278.3 (*c* 0.21, MeOH) (Kikuzaki, *et al.*, 2004), indicating that they had the same absolute configuration at C-4. Therefore, **K2** was (4*S*)-(+)-abscisic acid which was previously isolated from *Prunus domestica* L. (Ferreres, *et al.*, 1996).

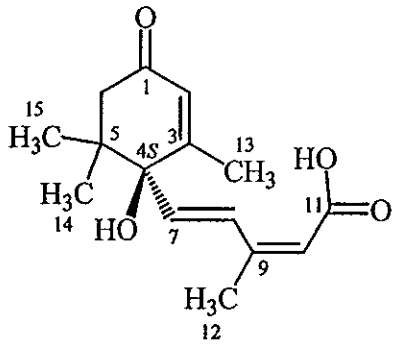


Table 16 The ^{1}H and ^{13}C NMR data of compound **K2** and **(4*S*)-(+)-Abscisic acid** in CD_3OD

Position	K2		(4*S*)-(+)-Abscisic acid	
	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	δ_H (*mult*, J_{Hz})	δ_C (C-Type)
1	-	199.73 (C)	-	198.16 (C)
2	5.93 (*q*, 1.2)	126.17 (CH)	5.98 (*s*)	127.19 (CH)
3	-	165.21 (C)	-	151.51 (C)
4	-	79.23 (C)	-	79.97 (C)
5	-	41.45 (C)	-	41.74 (C)
6	a: 2.54 (*d*, 17.1)	49.25 (CH_2)	a: 2.50 (*d*, 17.1)	49.82 (CH_2)
	b: 2.18 (*d*, 17.1)		b: 2.30 (*d*, 17.1)	
7	6.24 (*d*, 16.2)	136.41 (CH)	6.18 (*d*, 15.9)	136.97 (CH)
8	7.70 (*d*, 16.2)	128.06 (CH)	7.82 (*d*, 15.9)	128.49 (CH)
9	-	149.42 (C)	-	162.80 (C)
10	5.75 (*brs*)	118.39 (CH)	5.78 (*s*)	118.39 (CH)
11	-	168.13 (C)	-	170.58 (C)
12	2.03 (*d*, 1.2)	19.82 (CH_3)	2.05 (*s*)	19.10 (CH_3)
13	1.93 (*d*, 1.2)	18.22 (CH_3)	1.93 (*s*)	19.10 (CH_3)
14	1.06 (*s*)	22.15 (CH_3)	1.12 (*s*)	21.44 (CH_3)
15	1.03 (*s*)	23.25 (CH_3)	1.04 (*s*)	23.20 (CH_3)

Table 17 The HMBC, COSY and NOEDIFF data of compound **K2** in CD_3OD

Proton	HMBC	COSY	NOEDIFF
H-2	C-4, C-6, C-13	H_3-13	H_3-13
H_a-6	C-1, C-4, C-5, C-14, C-15	H_b-6	H_b-6, H_3-14, H_3-15
H_b-6	C-1, C-4, C-5, C-14, C-15	H_a-6	H_a-6, H_3-14, H_3-15
H-7	C-3, C-4, C-5, C-8, C-9, C-10, C-11	H-8	H_3-12
H-8	C-4, C-7, C-9, C-10, C-12	H-7	H_3-13
H-10	C-8, C-9, C-11, C-12	H_3-12	H_3-12
H_3-12	C-8, C-9, C-10	H-10	H-7, H-10

Table 17 Continued

Proton	HMBC	COSY	NOEDIFF
H_3-13	C-2, C-3, C-4	H-2	H-2, H-8
H_3-14	C-4, C-5, C-6, C-15	-	H-6, H-7
H_3-15	C-4, C-5, C-6, C-14	-	H-6, H-8

1.3.3 Compound K3

Compound **K3** was obtained as a colorless gum. Its UV and IR data were similar to those of **K2**. The ^{1}H NMR spectrum **(Figure 5) (Table 18)** was similar to that of **K2** except for an additional signal of a methine proton (δ 2.73, *d*, J = 9.3 Hz, 1H) in **K3**. Comparison of their ^{13}C NMR **(Figure 6) (Table 18)** and DEPT 135 spectra **(Table 18)** indicated that the oxyquaternary carbon in **K2** was replaced by a methine carbon. HMBC correlations **(Table 19)** of the olefinic proton, H-7 (δ 5.93, *dd*, J = 15.9 and 9.3 Hz, 1H), with C-3 (δ 162.00), C-4 (δ 56.37) and C-5 (δ 36.50) revealed that the 4-OH group in **K2** was replaced by a hydrogen atom. The observed optical rotation of **K3**, $[\alpha]_D^{29}$ +341 (*c* 0.20, MeOH), was almost identical to that of (4*S*)-(+)-deoxyabscisic acid, $[\alpha]_D^{29}$ +344 (*c* 0.20, MeOH), indicating that **K3** was (4*S*)-(+)-deoxyabscisic acid, previously isolated from the endophytic fungus *Botrytis cinerea* TB-3-H8 (Todoroki, *et al.*, 1995).

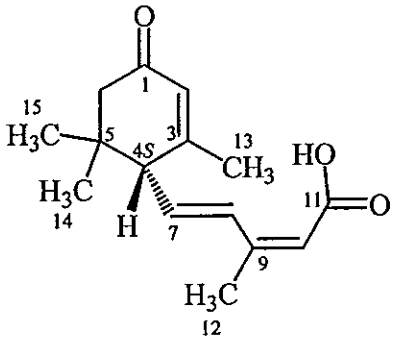


Table 18 The ^{1}H and ^{13}C NMR data of compound **K3** in $CDCl_3$+CD_3OD and **(4*S*)-(+)- Deoxyabscisic acid** in $CDCl_3$

Position	K3		(4*S*)-(+)-Deoxyabscisic acid
	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	δ_H (*mult*, J_{Hz})
1	-	199.65 (C)	-
2	5.94 (*brs*)	125.82 (CH)	5.95 (*s*)
3	-	162.00 (C)	-
4	2.73 (*d*, 9.3)	56.37 (CH)	2.75 (*d*, 9.6)
5	-	36.50 (C)	-
6	a: 2.40 (*d*, 16.8)	47.48 (CH_2)	a: 2.38 (*d*, 16.9)
	b: 2.16 (*d*, 16.8)		b: 2.16 (*d*, 16.9)
7	5.93 (*dd*, 15.9, 9.3)	133.92 (CH)	5.98 (*dd*, 15.7, 9.6)
8	7.67 (*d*, 15.9)	131.44 (CH)	7.70 (*d*, 15.7)
9	-	150.31 (C)	-
10	5.72 (*brs*)	117.68 (CH)	5.74 (*s*)
11	-	168.00 (C)	-
12	2.01 (*d*, 1.2)	21.08 (CH_3)	2.04 (*d*, 0.7)
13	1.92 (*d*, 1.2)	23.55 (CH_3)	1.93 (*d*, 0.7)
14	0.98 (*s*)	27.25 (CH_3)	0.99 (*s*)
15	1.06 (*s*)	27.85 (CH_3)	1.07 (*s*)

Table 19 The HMBC, COSY and NOEDIFF data of compound **K3** in $CDCl_3$+CD_3OD

Proton	HMBC	COSY	NOEDIFF
H-2	C-4, C-6, C-13	H_3-13	H_3-12, H_3-13
H-4	C-2, C-3, C-5, C-7, C-8, C-13	H-7, H_3-13	H-8, H_3-13, H_3-14, H_3-15
H_a-6	C-1, C-4, C-5, C-14, C-15	H_b-6	H_3-14
H_b-6	C-1, C-4, C-5, C-14, C-15	H_a-6	H_3-14, H_3-15
H-7	C-3, C-4, C-9	H-4, H-8	H_3-12, H_3-13
H-8	C-4, C-9, C-10, C-12	H-7	-
H-10	C-8, C-12	H-8, H_3-12	H_3-12

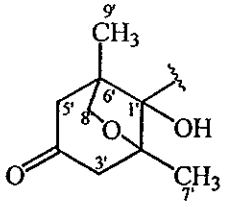
Table 19 Continued

Proton	HMBC	COSY	NOEDIFF
H_3-12	C-8, C-9, C-10	H-10	H-10
H_3-13	C-2, C-3, C-4	H-2, H-4	H-10
H_3-14	C-4, C-5, C-6, C-15	-	H-4, H-7
H_3-15	C-4, C-5, C-6, C-14	-	H-4

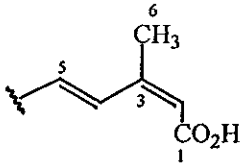
1.3.4 Compound K4

Compound **K4** was obtained as a colorless solid, melting at 192.8-193.7 °C. The UV spectrum showed an absorption band at 282 nm. The IR spectrum exhibited absorption bands at 3566, 1703 and 1697 cm^{-1} for hydroxyl, carboxylic carbonyl and ketone carbonyl groups, respectively. The ^1H NMR spectrum **(Figure 7)** **(Table 20)** showed characteristic signals for a 3-methyl-2,4-pentadienoyl moiety as found in **K2** [δ 8.04 (*d*, *J* = 16.0 Hz, 1H), 6.44 (*d*, *J* = 16.0 Hz, 1H)], 5.78 (*s*, 1H) and 2.12 (*s*, 3H)], two nonequivalent oxymethylene protons [δ 3.85 (*dd*, *J* = 8.5 and 3.5 Hz, 1H) and 3.76 (*d*, *J* = 8.5 Hz, 1H)], two methylene groups [δ 2.79 (*dd*, *J* = 17.0 and 3.5 Hz, 1H)/2.17 (*dd*, *J* = 17.0 and 1.0 Hz, 1H) and 2.80 (*d*, *J* = 17.0 Hz, 1H)/2.23 (*dd*, *J* = 17.0 and 1.0 Hz, 1H)] and two methyl groups [δ 1.10 (*s*, 3H) and 0.94 (*s*, 3H)]. The ^{13}C NMR **(Figure 8)** **(Table 20)** and DEPT 135 spectra **(Table 20)** displayed fifteen carbon resonances; six quaternary, three methine, three methylene and three methyl carbons. The carbonyl carbon resonances at δ 208.22 and 166.74 supported the presence of the ketone and carboxylic functional groups in the IR spectrum. In the HMBC spectrum **(Table 21)**, the methylene protons, H_{ab}-5' (δ 2.79 and 2.17), showed cross peaks with C-1' (δ 81.32), C-4' (δ 208.22), C-6' (δ 46.29), C-8' (δ 76.18) and C-9' (δ 17.44). The methylene protons, H_{ab}-3' (δ 2.80 and 2.23), correlated with C-1', C-2' (δ 84.14) and C-4' and while the oxymethylene protons, H_{ab}-8' (δ 3.85 and 3.76), correlated with C-1', C-2', C-5' (δ 50.38), C-6' and C-9'. Furthermore, the methyl protons, H_3-9' (δ 0.94), gave HMBC cross peaks with C-1', C-5', C-6' and C-8' whereas the methyl protons, H_3-7' (δ 1.10), showed the same cross

peaks with C-1', C-2' and C-3' (δ 50.91). These HMBC correlations together with the chemical shifts of C-1', C-2' and C-8' established substructure 1. The following HMBC correlations confirmed the presence of the 3-methyl-2,4-pentadienoyl unit (substructure 2): H-4 (δ 8.04)/C-2 (δ 117.24), C-3 (δ 150.10) and C-5 (δ 137.27), H-2 (δ 5.78)/C-1 (δ 166.74) and H_3-6/C-3 (δ 150.10).



substructure 1



substructure 2

Bond formation between C-5 of substructure 2 with C-1' of substructure 1 was established by HMBC correlations of both H-4 and H-5 of substructure 2 with C-1' of substructure 1. Irradiation of H_3-6 affected signal intensity of H-2 and H-5, in the NOEDIFF experiment **(Table 21)**. Thus, a C2-C3 double bond had *Z* configuration. The signals of H_3-7' and H_3-9' were enhanced when H-5 was irradiated, indicating that the 3-methyl-2,4-pentadienoyl unit had *cis* relationship to both H_3-7' and H_3-9'. The observed optical rotation of **K4**, $[\alpha]_D^{29}$ -162 (*c* 0.01, acetone), was similar to that of (1'*S*,2'*R*,6'*R*)-(-)-phaseic acid, $[\alpha]_D^{29}$ -176.0 (*c* 0.01, acetone), indicating that all chiral carbons of **K4** possessed the same absolute configuration as those of (1'*S*,2'*R*,6'*R*)-(-)-phaseic acid. Therefore, **K4** was identified as (1'*S*,2'*R*,6'*R*)-(-)-phaseic acid which was previously isolated from the fungus *Folium Fici Microcarpae* (Todoroki, *et al.*, 2000).

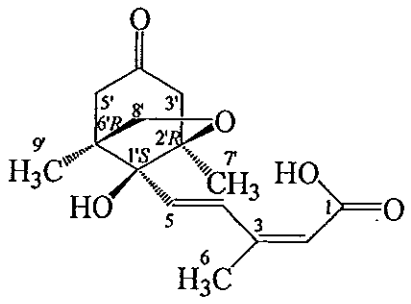


Table 20 The ^{1}H and ^{13}C NMR data of compound **K4** in acetone-d_6 and **(1′*S*,2′*R*,6′*R*)-(-)-Phaseic acid** in CD_3OD

Position	K4		(1′*S*,2′*R*,6′*R*)-(-)-Phaseic acid
	δ_H (*mult*, J Hz)	δ_C (C-Type)	δ_H (*mult*, J Hz)
1	-	166.47 (C)	-
2	5.78 (*s*)	117.24 (CH)	5.79 (*s*)
3	-	150.10 (C)	-
4	8.04 (*d*, 16.0)	127.35 (CH)	8.10 (*d*, 16.0)
5	6.44 (*d*, 16.0)	137.27 (CH)	6.45 (*d*, 16.0)
6	2.12 (*s*)	20.43 (CH_3)	1.94 (*s*)
1′	-	81.32 (C)	-
2′	-	84.14 (C)	-
3′	a: 2.80 (*d*, 17.0)	50.91 (CH_2)	a: 2.80 (*d*, 18.0)
	b: 2.23 (*dd*, 17.0, 1.0)		b: 2.70 (*dd*, 18.0, 2.4)
4′	-	208.22 (C)	-
5′	a: 2.79 (*dd*, 17.0, 3.5)	50.38 (CH_2)	a: 2.47 (*dd*, 18.0, 2.8)
	b: 2.17 (*dd*, 17.0, 1.0)		b: 2.39 (*dd*, 18.0, 2.4)
6′	-	46.29 (C)	-
7′	1.10 (*s*)	21.08 (CH_3)	1.22 (*s*)
8′	a: 3.85 (*dd*, 8.5, 3.5)	76.18 (CH_2)	a: 3.95 (*dd*, 7.7, 2.8)
	b: 3.76 (*d*, 8.5)		b: 3.67 (*d*, 7.7)
9′	0.94 (*s*)	17.44 (CH_3)	1.01 (*s*)

Table 21 The HMBC, COSY and NOEDIFF data of compound **K4** in acetone-d_6

Proton	HMBC	COSY	NOEDIFF
H-2	C-1, C-3, C-4	H-6	H-6
H-4	C-2, C-3, C-5, C-6, C-1'	H-5	-
H-5	C-3, C-4, C-1'	H-4	H-6, H-7', H_a-8', H_3-9'
H-6	C-2, C-3, C-4	-	H-2, H-5
H_a-3′	C-1', C-2', C-4', C-7''	H_b-3', H_b-5'	-

Table 21 Continued

Proton	HMBC	COSY	NOEDIFF
H_b-3′	C-1′, C-2′, C-4′, C-7′	H_a-3′	-
H_a-5′	C-1′, C-4′, C-6′, C-8′, C-9′	H_b-5′, H_a-8′	-
H_b-5′	C-1′, C-4′, C-6′, C-8′, C-9′	H_a-5′	-
H-7′	C-1′, C-2′, C-3′	-	-
H_a-8′	C-1′, C-2′, C-5′,C-6′, C-9′	H_a-5′, H_b-8′	-
H_b-8′	C-1′, C-2′, C-5′,C-6′, C-9′	H_a-8′	-
H_3-9′	C-1′, C-5′, C-6′, C-8′	-	H-5

1.3.5 Compound K5

Compound **K5** with the molecular formula $C_{22}H_{28}O_8$ from EIMS (*m/z* 420) **(Figure 9)** was obtained as a colorless solid, melting at 173.6-173.8 °C. The UV spectrum showed an absorption band at 281 nm. The IR spectrum showed absorption bands at 3442, 1716 and 1685 cm^{-1} for hydroxyl, conjugated ester carbonyl and conjugated ketone carbonyl groups, respectively. The ^1H **(Figure 10) (Table 22)** and ^{13}C NMR spectra **(Figure 11) (Table 22)** consisted of signals for the (+)-epoxydon unit [δ 6.94 (*d*, J = 2.0 Hz, 1H), 5.32 (*d*, J = 11.5 Hz, 1H), 4.54 (*m*, 1H), 4.21 (*m*, 2H) and 3.90 (*ddd*, J = 11.5, 8.0 and 4.5 Hz, 1H)]. The major differences were the downfield shifts of H-6 (δ 5.32 in **K5** and δ 3.41 in **K1**), C-5 (δ 76.09 in **K5** and δ 57.96 in **K1**) and C-6 (δ 77.08 in **K5** and δ 53.24 in **K1**). The chemical shifts of these carbon signals indicated the presence of two oxysubstituents at C-5 and C-6 in **K5**, rather than an epoxide ring as found in **K1**. Irradiation of H-6 in the NOEDIFF experiment **(Table 23)** enhanced only signal intensity of H-4 (δ 4.54), indicating that H-4 and H-6 were *cis*, but *trans* to H-5 (δ 3.90). Since H-5 was coupled with both H-4 and H-6 with large coupling constants of 8.0 and 11.5 Hz, respectively, all protons were assigned to pseudoaxial position. In addition, the remaining ^1H and ^{13}C NMR data of **K5** were almost identical to those of (4*S*)-(+)-abscisic acid (**K2**). The presence of such a unit was confirmed by the following HMBC correlations **(Table 22)**: H-8

(δ 5.84)/C-7 (δ 164.53), C-10 (δ 127.87) and C-21 (δ 20.38); H-10 (δ 7.78)/C-8 (δ 117.24), C-12 (δ 79.28) and C-21; 12-OH (δ 4.56)/C-12, C-13 (δ 162.34) and C-17 (δ 41.39); H-14 (δ 5.83)/C-12, C-16 (δ 49.46) and C-20 (δ 18.37); H_{ab}-16 (δ 2.57 and 2.16)/C-12, C-14 (δ 126.52), C-15 (δ 196.74) and C-17; H_3-18 (δ 1.08) and H_3-19 (δ 1.05)/C-12, C-16 and C-17. The configuration of both C8-C9 and C13-C14 double bonds was determined to be *Z* on the basis of the NOEDIFF enhancement of H_3-21 (δ 2.10) and H_3-20 (δ 1.91) upon irradiation of H-8 (δ 5.84) and H-14 (δ 5.83), respectively. A HMBC correlation of H-6 of the hydroxyepoxydon unit with C-7 of the abscisic acid moiety established an ester linkage between these units. Consequently, compound **K5** was identified as a new epoxydon derivative which could be obtained by condensation of **K2** with the hydroxyepoxydon moiety.

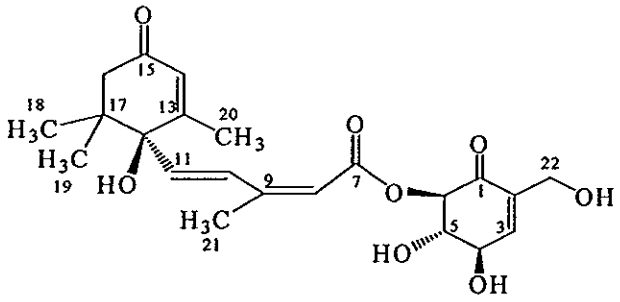


Table 22 The ^1H, ^{13}C NMR and HMBC data of compound **K5** in acetone-d_6

Position	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	HMBC
1	-	192.22 (C)	-
2	-	137.24 (C)	-
3	6.94 (*d*, 2.0)	145.58 (CH)	C-1, C-2, C-5, C-22
4	4.54 (*m*)	71.61 (CH)	-
4-OH	4.96 (*d*, 4.5)	-	-
5	3.90 (*ddd*, 11.5, 8.0, 4.5)	76.09 (CH)	-
5-OH	5.06 (*d*, 4.5)	-	-
6	5.32 (*d*, 11.5)	77.08 (CH)	C-1, C-4, C-5, C-7
7	-	164.53 (C)	-
8	5.84 (*s*)	117.24 (CH)	C-7, C-10, C-21
9	-	150.91 (C)	-
10	7.78 (*d*, 16.0)	127.87 (CH)	C-8, C-12, C-21

Table 22 Continued

Position	δ_H (*mult*, J Hz)	δ_C (C-Type)	HMBC
11	6.43 (*d*, 16.0)	138.10 (CH)	C-9, C-10, C-12, C-13
12-OH	4.56 (*s*)	79.28 (C)	C-12, C-13, C-17
13	-	162.34 (C)	-
14	5.83 (*q*, 1.0)	126.52 (CH)	C-12, C-16, C-20
15	-	196.74 (C)	-
16	a: 2.57 (*d*, 17.0)	49.46 (CH_2)	C-12, C-14, C-15
	b: 2.16 (*d*, 17.0)		
17	-	41.39 (C)	-
18	1.08 (*s*)	22.66 (CH_3)	C-12, C-16, C-17, C-19
19	1.05 (*s*)	23.80 (CH_3)	C-12, C-16, C-17, C-18
20	1.91 (*d*, 1.0)	18.37 (CH_3)	C-13, C-14, C-15
21	2.10 (*d*, 1.0)	20.38 (CH_3)	C-8, C-9, C-10
22	4.21 (*m*)	58.02 (CH_2)	C-2

Table 23 The COSY and NOEDIFF data of compound **K5** in acetone-d_6

Proton	COSY	NOEDIFF
H-3	H-4, H_2-22	H-4
H-4	H-3, 4-OH, H-5	H-3, H-6
4-OH	H-4	-
H-5	H-4, 5-OH, H-6	-
5-OH	H-5	-
H-6	H-5	H-4
H-8	-	H_3-21
H-10	H-11	-
H-11	H-10	-
H-14	H_3-20	H_3-20
H_a-16	H_b-16	-
H_b-16	H_a-16	-
H_3-20	H-14	H-14

Table 23 Continued

Proton	COSY	NOEDIFF
H_3-21	H-8	H-8
H_2-22	H-3	H-3

1.3.6 Compound K6

Compound **K6** with the molecular formula $C_{14}H_{14}O_7$ from EIMS (*m/z* 294) **(Figure 12)** was obtained as a colorless gum. The UV spectrum showed absorption bands at 210, 232 and 270 nm. The IR spectrum exhibited absorption bands at 3360 cm^{-1} for a hydroxyl group, 1717 cm^{-1} for a conjugated ester carbonyl group and 1696 cm^{-1} for a conjugated ketone carbonyl group. The ^1H NMR spectrum **(Figure 13) (Table 24)** showed characteristic signals for four aromatic protons of a 1,3-disubstituted benzene [δ 7.56 (*dt*, J = 8.1 and 1.5 Hz, 1H), 7.55 (*dd*, J = 2.7 and 1.5 Hz, 1H), 7.35 (*t*, J = 8.1 Hz, 1H) and 7.12 (*ddd*, J = 8.1, 2.7 and 1.5 Hz, 1H)] and a hydroxyepoxydon unit as found in **K5** [δ 6.99 (*q*, J = 1.8 Hz, 1H), 5.50 (*d*, J = 11.1 Hz, 1H), 4.64 (*brd*, J = 8.1 Hz, 1H), 4.28 (*d*, J = 15.3 Hz, 1H), 4.23 (*d*, J = 15.3 Hz, 1H) and 4.08 (*dd*, J = 11.1 and 8.1 Hz, 1H)]. The ^{13}C NMR spectrum **(Figure 14) (Table 24)** showed one ketone carbonyl carbon (δ 191.94), one ester carbonyl carbon (δ 165.30), three quaternary carbons (δ 157.50, 137.23 and 131.41), eight methine carbons (δ 145.72, 129.50, 120.80, 120.19, 116.30, 78.19, 76.11 and 71.49) and one methylene carbon (δ 58.00). The substituent at C-10 of the 1,3-disubstituted benzene was a hydroxyl group on the basis of the chemical shift of C-10 (δ 157.50). ^3J HMBC correlations of the aromatic protons, H-9 (δ 7.55) and H-13 (δ 7.56), and the oxymethine proton, H-6 (δ 5.50), of the hydroxyepoxydon unit with the ester carbonyl carbon, C-7 (δ 165.30), constructed an ester linkage between C-8 of the 1,3-disubstituted benzene and C-6 of the hydroxyepoxydon unit. Therefore, **K6** was identified as a condensation product of the hydroxyepoxydon unit and 3-hydroxybenzoic acid.

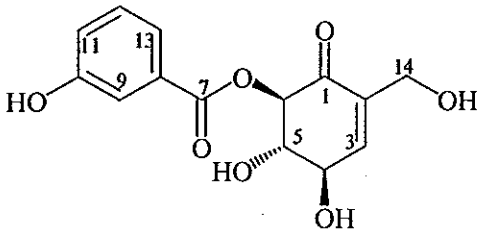


Table 24 The ^{1}H, ^{13}C NMR and HMBC data of compound **K6** in acetone-d_6

Position	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	HMBC
1	-	191.94 (C)	-
2	-	137.23 (C)	-
3	6.99 (*q*, 1.8)	145.72 (CH)	C-1, C-2, C-5, C-14
4	4.64 (*brd*, 8.1)	71.49 (CH)	C-2, C-3, C-5
5	4.08 (*dd*, 11.1, 8.1)	76.11 (CH)	C-1, C-4, C-6
6	5.50 (*d*, 11.1)	78.19 (CH)	C-1, C-4, C-5, C-7
7	-	165.30 (C)	-
8	-	131.41 (C)	-
9	7.55 (*dd*, 2.7, 1.5)	116.30 (CH)	C-7, C-8, C-10, C-11, C-13
10	-	157.50 (C)	-
11	7.12 (*ddd*, 8.1, 2.7, 1.5)	120.19 (CH)	C-9, C-10, C-12, C-13
12	7.35 (*t*, 8.1)	129.50 (CH)	C-7, C-8, C-10, C-11, C-13
13	7.56 (*dt*, 8.1, 1.5)	120.80 (CH)	C-7, C-8, C-9, C-11
14	a: 4.28 (*d*, 15.3)	58.00 (CH_2)	-
	b: 4.23 (*d*, 15.3)		

Table 25 The COSY and NOEDIFF data of compound **K6** in acetone-d_6

Proton	COSY	NOEDIFF
H-3	H-4, H_{ab}-14	H-4, H_{ab}-14
H-4	H-3, H-5	H-3, H-6
H-5	H-4, H-6	-
H-6	H-5	H-4
H-9	H-11, H-13	H-5, H-6

Table 25 Continued

Proton	COSY	NOEDIFF
H-11	H-9, H-12, H-13	-
H-12	H-11, H-13	-
H-13	H-9, H-11, H-12	H-5, H-6
H_a-14	H-3	H-3
H_b-14	H-3	H-3

1.3.7 Compound K7

Compound **K7** with the molecular formula $C_{23}H_{30}O_9$ from EIMS (*m/z* 450) **(Figure 15)** was obtained as a colorless solid, melting at 366.2-366.7 °C. The UV spectrum showed an absorption band at 282 nm. The IR spectrum exhibited absorption bands at 3370 cm^{-1} for a hydroxyl group, 1749 and 1734 cm^{-1} for ester carbonyl groups and 1698 cm^{-1} for a conjugated ketone carbonyl group. Three carbon resonances at δ 191.73, 174.35 and 173.73 suggested the presence of one ketone carbonyl carbon and two ester carbonyl carbons. The ^1H NMR spectrum **(Figure 16)** **(Table 26)** showed characteristic signals of five aromatic protons of a monosubstituted benzene (δ 7.25, *m*, 5H), one 1-substituted-3-methylbutoxyl group [δ 3.33 (*dd*, J = 9.0 and 5.4 Hz, 1H), 1.88 (*m*, 1H), 1.59 (*ddd*, J = 13.5, 8.1 and 5.4 Hz, 1H), 1.45 (*ddd*, J = 13.5, 9.0 and 6.0 Hz, 1H), 1.88 (*m* ,1H), 0.87 (*d*, J = 6.6 Hz, 3H) and 0.81 (*d*, J = 6.6 Hz, 3H)], one oxymethine proton (δ 3.62, *dd*, J = 6.9 and 6.0 Hz, 1H), one methoxyl group (δ 3.60, *s*, 3H) and one methylene group (δ 2.93, *m*, 2H). The ^1H-^1H COSY correlations **(Table 27)** were fully consistent with the presence of the 1-substituted-3-methylbutoxyl unit. The methine proton, H-10 (δ 1.88), was coupled with the methyl protons, H_3-11 (δ 0.87) and H_3-12 (δ 0.81), and methylene protons, H_{ab}-9 (δ 1.59 and 1.45), which gave cross peaks with the oxymethine proton, H-8 (δ 3.33). Furthermore, H_{ab}-9 and H-8 showed cross peaks in the HMBC spectrum **(Table 27)** with the ester carbonyl carbon, C-7 (δ 174.35), thus jointing C-8 (δ 58.62) of the 1-substituted-3-methylbutoxyl unit with C-7 to form a 2-oxy-4-methylpentanoyl fragment. The oxymethine proton, H-13 (δ 3.62), showed a

cross peak in the ^{1}H-^{1}H COSY spectrum with H_2-16 (δ 2.93). In addition, H-13 (δ 3.62) showed HMBC cross peaks with C-8 of the pentanoyl unit, C-17 (δ 138.10) of the monosubstituted benzene and the ester carbonyl carbon, C-14 (δ 173.73). This carbonyl carbon further gave a HMBC correlation with the methoxy protons (δ 3.60). These data established a methyl 2-oxy-3-phenylpropanoate moiety of which C-13 (δ 61.32) formed an ether linkage with C-8 of the pentanoyl moiety. Irradiation of H-8 enhanced signal intensity of H-13, thus indicating that they were located at the same side of the molecule. The typical ^{1}H resonances at δ 6.93 (*s*, 1H), 5.25 (*d*, J = 11.1 Hz, 1H), 4.56 (*d*, J = 8.4 Hz, 1H), 4.26 (*d*, J = 13.6 Hz, 1H), 4.15 (*d*, J = 13.6 Hz, 1H) and 3.87 (*dd*, J = 11.1 and 8.4 Hz, 1H) and the characteristic ^{13}C resonances at δ 191.73, 145.71, 138.07, 77.59, 75.92, 71.39 and 57.93 together with J values indicated that **K7** had a hydroxyepoxydon moiety. The ester linkage between the pentanoyl and hydroxyepoxydon fragments was established by a HMBC correlation of H-6 (δ 5.25) with C-7. However, the relative configuration at C-8 and C-13 could not be assigned on the basis of NOEDIFF results due to a lack of correlations between protons of the ester moiety and those of the hydroxyepoxydon fragment. Consequently, **K7** was identified as a new epoxydon derivative.

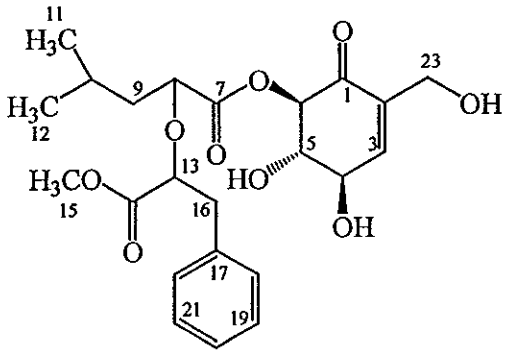


Table 26 The ^{1}H, ^{13}C NMR and HMBC data of compound **K7** in acetone-d_6

Position	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	HMBC
1	-	191.73 (C)	-
2	-	138.07 (C)	-
3	6.93 (*s*)	145.71 (CH)	C-1, C-2, C-5, C-23

Table 26 Continued

Position	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	HMBC
4	4.56 (*d*, 8.4)	71.39 (CH)	C-3
5	3.87 (*dd*, 11.1, 8.4)	75.92 (CH)	C-4, C-6
6	5.25 (*d*, 11.1)	77.59 (CH)	C-1, C-4, C-5, C-7
7	-	174.35 (C)	-
8	3.33 (*dd*, 9.0, 5.4)	58.62 (CH)	C-7, C-9, C-10, C-13
9	a: 1.59 (*ddd*, 13.5, 8.1, 5.4)	43.01 (CH_2)	C-7, C-9, C-10, C-11,
	b: 1.45 (*ddd*, 13.5, 9.0, 6.0)		C-12
10	1.88 (*m*)	24.25 (CH)	C-11, C-12
11	0.87 (*d*, 6.6)	22.50 (CH_3)	C-9, C-10, C-12
12	0.81 (*d*, 6.6)	21.38 (CH_3)	C-9, C-10, C-11
13	3.62 (*dd*, 6.9, 6.0)	61.32 (CH)	C-8, C-14, C-16, C-17
14	-	173.73 (C)	-
15	3.60 (*s*)	51.04 (CH_3)	C-14
16	2.93 (*m*)	39.25 (CH_2)	C-13, C-14, C-17, C-18,
			C-22
17	-	138.10 (C)	-
18,22	7.25 (*m*)	129.52 (CH)	C-16, C-20
19,21	7.25 (*m*)	127.97 (CH)	C-17, C-18, C-20, C-22
20	7.25 (*m*)	126.20 (CH)	C-18, C-19, C-21, C-22
23	a: 4.26 (*d*, 13.6)	57.93 (CH_2)	-
	b: 4.15 (*d*, 13.6)		

Table 27 The COSY and NOEDIFF data of compound **K7** in acetone-d_6

Proton	COSY	NOEDIFF
H-3	H-4, H_{ab}-23	H-4
H-4	H-3, H-5	H-3, H-6
H-5	H-4, H-6	-
H-6	H-5	H-4
H-8	H_{ab}-9	H_{ab}-9, H_3-13

Table 27 Continued

Proton	COSY	NOEDIFF
H_a-9	H-8, H_b-9, H-10	-
H_b-9	H-8, H_a-9, H-10	-
H-10	H_{ab}-9, H_3-11, H_3-12	-
H_3-11	H-10	-
H_3-12	H-10	-
H-13	H_2-16	-
H_2-16	H-13	-
H-18, H-22	H-19, H-20	-
H-19, H-21	H-18, H-20	-
H-20	H-18, H-19	-
H_a-23	H_b-23	-
H_b-23	H_a-23	-

1.3.8 Compound K9

Compound **K9** was obtained as a colorless solid, melting at 104.3-104.6 °C. The UV spectrum showed absorption bands at 230 and 309 nm while a carbonyl absorption band was found at 1708 cm^{-1}, in the IR spectrum. The ^1H NMR spectrum **(Figure 21) (Table 28)** consisted of signals for two olefinic protons of two trisubstituted double bonds [δ 5.83 (*d*, J = 2.1 Hz, 1H) and 5.38 (*d*, J = 2.1 Hz, 1H)], one 1-methyl-1-propenyl unit [δ 6.62 (*qq*, J = 6.9 and 1.2 Hz, 1H), 1.78 (*s*, 3H) and 1.77 (*d*, J = 6.9 Hz, 3H)] and one methoxyl group (3.74, *s*, 3H). The ^{13}C NMR **(Figure 22) (Table 28)** and DEPT 135 spectra **(Table 28)** showed ten carbon resonances; one ester carbonyl carbon (δ 164.32), three quaternary carbons (δ 171.49, 161.33, and 126.67), three methine carbons (δ 130.20, 97.18 and 88.02), one methoxy carbon (δ 55.84) and two methyl carbons (δ 14.19 and 12.01). The olefinic proton of the trisubstituted double bond, H-2 (δ 5.38), correlated with C-1 (δ 164.32), C-3 (δ 171.49) and C-4 (δ 97.18) in the HMBC spectrum **(Table 29)**. The remaining olefinic proton of the other trisubstituted double bond, H-4 (δ 5.83), showed cross

peaks in the HMBC spectrum with C-2 (δ 88.02), C-3 and C-5 (δ 161.33). These results together with a HMBC correlation of the methoxy protons, H_3-10 (δ 3.74), with C-3 indicated that **K9** had a pyrone ring with the methoxyl group at C-3. The 1-methyl-1-propenyl unit was linked at C-5 on the basis of a HMBC correlation of the olefinic proton, H-7 (δ 6.62), with C-5 of the pyrone ring. The configuration of the double bond in the 1-methyl-1-propenyl unit was *E* on the basis of the NOEDIFF enhancement **(Table 29)** of H_3-9 (δ 1.78) signal upon irradiation of H_3-8 (δ 1.77). Therefore, **K9** was identified as pestalopyrone which was previously isolated from the endophytic fungus *Pestalotiopsis microspora* (Lee, *et al.*, 1995).

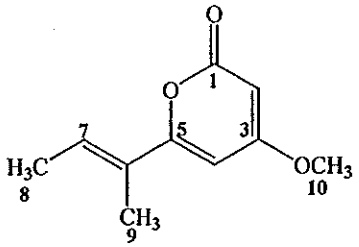


Table 28 The ^1H and ^{13}C NMR data of compound **K9** in $CDCl_3$ and **Pestalopyrone** in CD_3OD

Position	**K9**		**Pestalopyrone**	
	δ_H (*mult*, J Hz)	δ_C (C-Type)	δ_H (*mult*, J Hz)	δ_C (C-Type)
1	-	164.32 (C)	-	174.1 (C)
2	5.38 (*d*, 2.1)	88.02 (CH)	6.12 (*d*, 1.0)	98.8 (CH)
3	-	171.49 (C)	-	162.6 (C)
4	5.83 (*d*, 2.1)	97.18 (CH)	5.55 (*d*, 1.0)	88.5 (CH)
5	-	161.33 (C)	-	166.9 (C)
6	-	126.67 (C)	-	128.3 (C)
7	6.62 (*qq*, 6.9, 1.2)	130.20 (CH)	6.63 (*qq*, 7.2, 1.2)	131.0 (CH)
8	1.77 (*d*, 6.9)	14.19 (CH_3)	1.85 (*d*, 7.5)	12.0 (CH_3)
9	1.78 (*s*)	12.01 (CH_3)	1.87 (*d*, 1.0)	14.3 (CH_3)
10	3.74 (*s*)	55.84 (CH_3)	3.70 (*s*)	56.9 (CH_3)

Table 29 The HMBC, COSY and NOEDIFF data of compound **K9** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-2	C-1, C-3, C-4	H-4	H_3-10
H-4	C-2, C-3, C-5, C-6	H-2	H-9
H-7	C-5, C-8, C-9	H_3-8	H_3-8
H_3-8	C-6, C-7	H-7	H-7, H_3-9
H_3-9	C-5, C-6, C-7	-	H-4, H_3-8
H_3-10	C-3	-	H-2

1.3.9 Compound K10

Compound **K10** was obtained as a colorless solid, melting at 146.5-146.7 °C. Its UV spectrum was similar to that of **K9** while the IR spectrum showed an additional absorption band of a hydroxyl group at 3385 cm^{-1}. Its ^1H NMR spectrum **(Figure 23) (Table 30)** was similar to that of **K9** except for the replacement of one of the methyl signals in **K9** with signals of a hydroxymethyl group [δ 4.53 (*t*, J = 5.5 Hz, 2H) and δ 4.06 (*t*, J = 5.5 Hz, 1H)]. The presence of one methyl carbon (δ 11.62) and one oxymethylene carbon (δ 58.65) in the ^{13}C NMR **(Figure 24) (Table 30)** and DEPT 135 spectra **(Table 30)** supported above conclusion. HMBC correlations of H_2-8 (δ 4.35) with C-6 (δ 126.10) and C-7 (δ 134.57) together with signal enhancement of both H-4 (δ 6.10) and H_2-8 upon irradiation of H_3-9 (δ 1.89) in the NOEDIFF experiment established the attachment of the hydroxymethyl group at C-7, not at C-6. Consequently, **K10** was assigned as hydroxypestalopyrone which was previously isolated from the endophytic fungus *Pestalotiopsis microspora* (Lee, *et al.*, 1995).

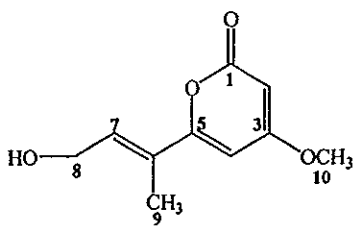


Table 30 The ^{1}H and ^{13}C NMR data of compound **K10** in acetone-d_6 and **Hydroxypestalopyrone** in CD_3OD

Position	K10		Hydroxypestalopyrone
	δ_H (*mult*, J Hz)	δ_C (C-Type)	δ_H (*mult*, J Hz)
1	-	162.30 (C)	-
2	5.51 (*d*, 2.1)	88.13 (CH)	6.21 (*d*, 2.0)
3	-	171.15 (C)	-
4	6.10 (*d*, 2.1)	97.84 (CH)	5.60 (*d*, 2.0)
5	-	160.40 (C)	-
6	-	126.01 (C)	-
7	6.58 (*tq*, 5.5, 1.0)	134.57 (CH)	6.58 (*qq*, 6.5, 1.5)
8	4.35 (*t*, 5.5)	58.65 (CH_2)	4.30 (*d*, 6.5)
8-OH	4.06 (*t*, 5.5)	-	-
9	1.89 (*d*, 1.0)	11.62 (CH_3)	1.88 (*d*, 1.0)
10	3.89 (*s*)	55.78 (CH_3)	3.86 (*s*)

Table 31 The HMBC, COSY and NOEDIFF data of compound **K10** in acetone-d_6

Proton	HMBC	COSY	NOEDIFF
H-2	C-1, C-3, C-4	H-4	H_3-10
H-4	C-2, C-5, C-6	H-2	H_3-9
H-7	C-5, C-8, C-9	H_2-8, H_3-9	H_2-8
H_2-8	C-6, C-7	H-7, 8-OH	H-7, H_3-9
8-OH	-	H_2-8	-
H_3-9	C-5, C-6, C-7	H-7	H-4, H_3-8
H_3-10	C-3	-	H-2

1.3.10 Compound K8

Compound **K8** with the molecular formula $C_8H_{10}O_4$ from EIMS (*m/z* 170) **(Figure 18)** was obtained as a colorless solid, melting at 196.6-196.8 °C. Its

UV spectrum was similar to that of compound **K9** while the IR spectrum showed an additional absorption band for a hydroxyl group at 3365 cm^{-1}. Its ^1H NMR spectrum **(Figure 19) (Table 32)** was similar to that of compound **K9** except for the replacement of the 2-substituted-2-butenyl signals in **K9** with signals for a 1-hydroxyethyl unit in **K8**. The attachment of this fragment at C-5 (δ 164.55) was confirmed by a HMBC correlation **(Table 32)** of the methine proton, H-6 (δ 3.04), with C-5. Consequently, compound **K8** was identified as a new pyrone derivative.

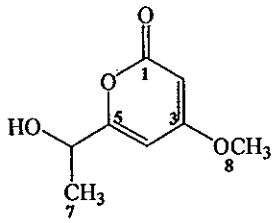


Table 32 The NMR data of compound **K8** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	HMBC	COSY
1	-	163.05 (C)	-	-
2	5.95 (*d*, 2.5)	96.45 (CH)	C-1, C-4, C-5	H-4
3	-	170.40 (C)	-	-
4	5.36 (*d*, 2.5)	87.37 (CH)	C-2, C-3, C-5	H-2
5	-	164.55 (C)	-	-
6	3.04 (*q*, 5.0)	60.74 (CH)	C-5, C-7	H_3-7
7	1.32 (*d*, 5.0)	12.94 (CH_3)	C-6	H-6
8	3.73 (*s*)	55.00 (CH_3)	C-3	

1.3.11 Compound K11

Compound **K11** was obtained as a colorless solid, melting at 191.1-192.0 °C. The UV spectrum displayed absorption bands at 214, 263 and 328 nm, indicating the presence of an aromatic chromophore. The IR spectrum showed absorption bands at 3439 cm^{-1} for a hydroxyl group and 1696 cm^{-1} for a carbonyl group. The ^1H NMR spectrum **(Figure 25) (Table 33)** exhibited characteristic signals for one chelated hydroxy proton (δ 12.26, *s*, 1H), three aromatic protons of a 1,2,3-

trisubstituted benzene [δ 7.54 (*t*, *J* = 8.0 Hz, 1H), 7.27 (*dd*, *J* = 7.5 and 0.5 Hz, 1H) and 6.91 (*d*, *J* = 8.5 Hz, 1H)], two oxymethine protons [δ 4.64 (*d*, *J* = 8.0 Hz, 1H) and 4.00 (*m*, 1H)] and two nonequivalent methylene protons [δ 3.10 (*dd*, *J* = 17.0 and 4.5 Hz, 1H) and 2.73 (*dd*, *J* = 17.0 and 11.0 Hz, 1H)]. The chelated hydroxyl group was placed at C-8 (δ 162.00), an *ortho* position of a carbonyl group. The chelated hydroxy proton showed a cross peak in the HMBC spectrum **(Table 34)** with C-7 (δ 116.93) which exhibited a HMQC cross peak **(Table 33)** with the aromatic proton, H-7 (δ 6.91). Thus, the remaining aromatic protons resonating at δ 7.54 and δ 7.27 were attributed to H-6 and H-5, respectively. In the ^{1}H-^{1}H COSY spectrum **(Table 34)**, the oxymethine proton, H-3 (δ 4.00), was coupled with the oxymethine proton, H-4 (δ 4.64), and the methylene protons, H_{ab}-2 (δ 3.10 and 2.73). HMBC cross peaks between H-4 with C-5 (δ 117.03) and C-4a (δ 143.00) and those of H_{ab}-2 with C-1 (δ 202.00) and C-8a (δ 115.30) constructed a naphthalenone skeleton bearing three hydroxyl groups at C-3, C-4 and C-8. Irradiation of H-3 in the NOEDIFF experiment **(Table 34)**, did not affect signal intensity of H-4 indicating their *trans*-relationship. In addition, H-3 and H-4 were placed at pseudoaxial position as they were coupled with a large coupling constant of 8.0 Hz. The observed optical rotation of **K11**, $[\alpha]_D^{29}$ -34 (*c* 0.019, MeOH), was similar to that of (3*R*,4*R*)-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone, $[\alpha]_D^{29}$ -42 (*c* 0.02, MeOH), indicating that they possessed the same absolute configuration at C-3 and C-4. Therefore, **K11** was assigned as (3*R*,4*R*)-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone which was previously isolated from the endophytic fungus *Hypoxylon mamatum* (Borgschulte, *et al.*, 1991).

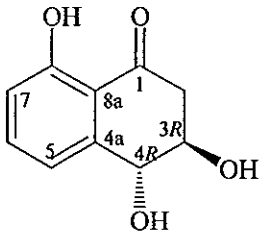


Table 33 The ^{1}H and ^{13}C NMR data of compound **K11** in $CDCl_3$+CD_3OD and **(3*R*,4*R*)-3,4-Dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone** in CD_3OD

Position	K11		(3*R*,4*R*)-3,4-Dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone	
	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	δ_H (*mult*, J_{Hz})	δ_C (C-Type)
1	-	202.00 (C)	-	204.30 (C)
2	a: 3.10 (*dd*, 17.0, 4.5) b: 2.73 (*dd*, 17.0, 11.0)	44.43 (CH_2)	a: 3.12 (*dd*, 17.2, 4.0) b:2.74 (*dd*, 17.2, 8.1)	44.40 (CH_2)
3	4.00 (*m*)	71.00 (CH)	4.12 (*ddd*, 8.1, 6.8, 4.0)	71.10 (CH)
4	4.64 (*d*, 8.0)	73.38 (CH)	4.65 (*d*, 6.8)	73.30 (CH)
4a	-	143.00 (C)	-	140.00 (C)
5	7.27 (*dd*, 7.5, 0.5)	117.03 (CH)	7.17 (*d*, 7.6)	120.00 (CH)
6	7.54 (*t*, 8.0)	137.20 (CH)	7.57 (*dd*, 8.4, 7.6)	137.90 (CH)
7	6.91 (*d*, 8.5)	116.93 (CH)	6.91 (*d*, 8.4)	117.70 (CH)
8-OH	12.26 (*s*)	162.00 (C)	-	163.22 (C)
8a	-	115.30 (C)	-	114.50 (C)

Table 34 The HMBC, COSY and NOEDIFF data of compound **K11** in $CDCl_3$+CD_3OD

Proton	HMBC	COSY	NOEDIFF
H_a-2	C-1, C-3, C-4, C-8a	H_b-2, H-3	H_b-2
H_b-2	C-1, C-3, C-4, C-8a	H_a-2, H-3	H_a-2, H-3
H-3	C-4	H_{ab}-2, H-4	H_b-2
H-4	C-2, C-3, C-4a, C-5, C-8a	H-3	-
H-5	C-4, C-7, C-8a	H-6, H-7	H-6
H-6	C-4a, C-8	H-5, H-7	H-5, H-7
H-7	C-5, C-8a	H-5, H-6	H-6
8-OH	C-7, C-8, C-8a	-	-

1.3.12 Compound K12

Compound **K12** was obtained as a colorless solid, melting at 179.1-180.2 °C. The UV spectrum showed absorption bands at 216, 280 and 329 nm, indicating the presence of an aromatic chromophore. The IR spectrum exhibited absorption bands for hydroxyl (3430 cm^{-1}) and ketone carbonyl (1680 cm^{-1}) groups. The ^1H NMR spectrum **(Figure 27) (Table 35)** displayed signals for one chelated hydroxy proton (δ 12.83, *s*, 1H), one aromatic proton (δ 7.31, *s*, 1H) and three methyl groups [δ 2.56 (*s*, 3H), 2.21 (*s*, 3H) and 2.14 (*s*, 3H)]. The chelated hydroxyl group was placed at C-2 (δ 161.32), an *ortho* position of a ketone carbonyl group. The chelated hydroxy proton gave HMBC correlations **(Table 36)** with C-1 (δ 113.47), C-2 and C-3 (δ 110.23). The methyl group resonating at δ 2.14 (H_3-9) was attached at C-3 on the basis of HMBC correlations with of H_3-9 with C-2, C-3 and C-4 (δ 158.71). ^3J HMBC cross peaks of H_3-10 (δ 2.21) with C-4 and C-6 (δ 129.81) revealed the location of this methyl group at C-5. The HMQC correlation **(Table 35)** of C-6 with the aromatic proton (δ 7.31) together with the chemical shift of C-4 established the attachment of the aromatic proton and a hydroxyl group at C-6 and C-4, respectively. The remaining methyl group resonating at δ 2.56 (H_3-8) was linked with a ketone carbonyl carbon, C-7 (δ 202.66), due to a HMBC correlation of H_3-8 with C-7. Signal enhancement of both H_3-8 and H_3-10 upon irradiation of H-6 supported the assigned location. Therefore, **K12** was clavatol which was previously isolated from the lichen *Trichoderma pseudokoningii* Rifai (Astudillo, *et al.*, 2000).

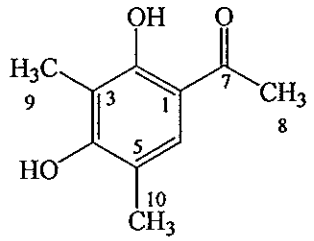


Table 35 The ^{1}H and ^{13}C NMR data of compound **K12** and **Clavatol** in $CDCl_3$

Position	K12		Clavatol	
	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	δ_H (*mult*, J_{Hz})	δ_C (C-Type)
1	-	113.47 (C)	-	110.12 (C)
2-OH	12.83 (*s*)	161.32 (C)	12.86 (*s*)	158.22 (C)
3	-	110.23 (C)	-	113.31 (C)
4	-	158.71 (C)	-	161.22 (C)
5	-	114.43 (C)	-	114.44 (C)
6	7.31 (*s*)	129.81 (CH)	7.35 (*s*)	129.83 (CH)
7	-	202.66 (C)	-	202.78 (C)
8	2.56 (*s*)	26.29 (CH_3)	2.54 (*s*)	26.31 (CH_3)
9	2.14 (*s*)	7.37 (CH_3)	2.11 (*s*)	7.43 (CH_3)
10	2.21 (*s*)	15.46 (CH_3)	2.19 (*d*, 0.8)	15.57 (CH_3)

Table 36 The HMBC and NOEDIFF data of compound **K12** in $CDCl_3$

Proton	HMBC	NOEDIFF
2-OH	C-1, C-2, C-3	-
H-6	C-2, C-4, C-7, C-10	H-8, H_3-10
H_3-8	C-1, C-7	H-6
H_3-9	C-2, C-3, C-4	-
H_3-10	C-4, C-5, C-6	H-6

1.3.13 Compound K13

Compound **K13** was obtained as a colorless solid, melting at 72.1-73.0 °C. The UV spectrum showed absorption bands at 202, 214 and 272 nm, indicating the presence of an aromatic chromophore. The IR spectrum displayed an absorption band at 3455 cm^{-1} for a hydroxyl group. The ^{1}H NMR spectrum **(Figure 29) (Table 37)** exhibited characteristic signals for four aromatic protons of a 1,3-disubstituted benzene [δ 7.12 (*t*, J = 8.0 Hz, 1H), 6.79 (*brd*, J = 8.0 Hz, 1H), 6.69 (*dd*,

J = 8.0 and 2.0 Hz, 1H) and 6.86 (*s*, 1H)] and one hydroxylmethyl group (δ 4.55, *s*, 2H). These data together with the chemical shift of C-1 (δ 157.49) established the substituents of the 1,3-disubstituted benzene to be a hydroxyl group and the hydroxylmethyl group. HMBC **(Table 38)** and NOEDIFF **(Table 38)** data supported the assigned structure. Therefore, **K13** was 3-(hydroxymethyl)phenol which was previously isolated from the endophytic fungus *Penicillium novae-zeelandiae* (Alfaro, *et al.*, 2003).

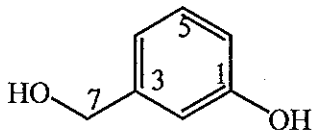


Table 37 The ^{1}H and ^{13}C NMR data of compound **K13** and **3-(Hydroxymethyl)phenol** in acetone-d_6

Position	**K13**		**3-(Hydroxymethyl)phenol**	
	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	δ_H (*mult*, J_{Hz})	δ_C (C-Type)
1-OH	8.55 (*brs*)	157.49 (C)	8.30 (*brs*)	158.70 (C)
2	6.86 (*s*)	113.38 (CH)	6.89 (*s*)	114.60 (CH)
3	-	144.10 (C)	-	145.50 (C)
4	6.79 (*brd*, 8.0)	117.35 (CH)	6.72 (*dd*, 7.8, 3.0)	118.80 (CH)
5	7.12 (*t*, 8.0)	129.02 (CH)	7.15 (*t*, 7.8)	130.40 (CH)
6	6.69 (*dd*, 8.0, 2.0)	113.63 (CH)	6.82 (*brd*, 7.8)	114.90 (CH)
7	4.55 (*s*)	63.66 (CH_2)	4.58 (*s*)	65.10 (CH_2)
7-OH	-	-	4.20 (*brs*)	-

Table 38 The HMBC and NOEDIFF data of compound **K13** in acetone-d_6

Proton	HMBC	NOEDIFF
H-2	C-1, C-4, C-6, C-7	H_2-7
H-4	C-2, C-5, C-7	H_2-7
H-5	C-1, C-3, C-4, C-6	-
H-6	C-1, C-2, C-4	-
H_2-7	C-2, C-3, C-4	H-2, H-4

PART II

METABOLITES FROM THE MARINE-DERIVED FUNGUS *NIGROSPORA* SP. PSU-F18

CHAPTER 2.1

INTRODUCTION

2.1.1 Introduction

The marine-derived fungus *Nigrospora* sp. PSU-F18 was isolated together with *Nigrospora* sp. PSU-F5 in the year 2006. This fungus was deposited as PSU-F18 at the Department of Microbiology, Faculty of Science, Prince of Songkla University. The ethyl acetate extracts from the culture broth and mycelia of this fungus showed antibacterial activity against SA and MRSA with the equal MIC values of 128 μg/mL.

2.1.2 The objectives

1. To isolate the secondary metabolites from the marine-derived fungus *Nigrospora* sp. PSU-F18.

2. To elucidate the structure of the isolated metabolites.

CHAPTER 2.2

EXPERIMENTAL

2.2.1 Fermentation and extraction

The fermentation and the extraction of the culture broth (15 L) and mycelia were performed using the same procedure as those of *Nigrospora* sp. PSU-F5 to afford an orange gum (4.4 g) and a brown gum (720 mg) from the culture broth and mycelia, respectively. Each extract was subjected to chromatographic separation.

2.2.2 Purification of the broth extract

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five fractions as shown in **Table 39.**

Table 39 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
18A	44.2	Brown solid
18B	253.2	Yellow gum
18C	4,104	Orange gum
18D	31.9	Yellow gum
18E	7.3	Yellow gum

Fraction 18A showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.45 and 0.55. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Fraction 18B showed five UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.31, 0.46, 0.61 and 0.76. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 40.**

Table 40 Subfractions obtained from **fraction 18B** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18B1	2% MeOH/CH_2Cl_2	15.0	Colorless gum
18B2	2% MeOH/CH_2Cl_2	21.0	Yellow gum
18B3	2% MeOH/CH_2Cl_2	42.0	Yellow gum
18B4	2% MeOH/CH_2Cl_2	22.0	Yellow gum
18B5	4% MeOH/CH_2Cl_2	20.0	Yellow gum
18B6	4% MeOH/CH_2Cl_2	15.0	Yellow gum
18B7	7% MeOH/CH_2Cl_2-100% MeOH	89.0	Yellow gum

Subfraction 18B1 showed two UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.24 and 0.31. Its ^1H NMR spectrum displayed signals in the high field region. Therefore, it was not purified.

Subfraction 18B2 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.47, 0.52, 0.62, 0.71 and 0.83 and one gray spot with the R_f value of 0.43 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated that the major compound was **K14**. Therefore, it was not purified.

Subfraction 18B3 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.35 and one gray spot with the R_f value of 0.47 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated that it contained a mixture of **K14** and **K19**. Further investigation was then not carried out.

Subfraction 18B4 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.31 and one gray spot with the R_f value of 0.47 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated that it contained a mixture of **K14** and **K15**. Further investigation was then not performed.

Subfraction 18B5 showed two pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.25 and 0.31. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18B6 showed four pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.26, 0.28 and 0.31. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18B7 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 18C showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.22, 0.31, 0.58 and 0.68. It was further separated by column chromatography over silica gel. Elution was performed initially with 100% dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 41.**

Table 41 Subfractions obtained from **fraction 18C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C1	100% CH_2Cl_2	5.3	Colorless gum
18C2	100% CH_2Cl_2	7.1	Yellow gum
18C3	2% MeOH/CH_2Cl_2	13.0	Yellow gum
18C4	2% MeOH/CH_2Cl_2	4.5	Yellow gum
18C5	4% MeOH/CH_2Cl_2	420.9	Yellow gum
18C6	7% MeOH/CH_2Cl_2	3,200	Yellow gum
18C7	7% MeOH/CH_2Cl_2	53.4	Brown gum
18C8	10-50% MeOH/CH_2Cl_2	43.0	Brown gum
18C9	50% MeOH/CH_2Cl_2-100% MeOH	299.2	Brown gum

Subfraction 18C1 showed two pale UV-active spots on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.67 and 0.95. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18C2 showed three pale UV-active spots on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.37, 0.50 and 0.67. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18C3 showed five UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.21, 0.37, 0.42, 0.50 and 0.76. It was further separated by column chromatography over silica gel. Elution was performed with 20% ethyl acetate in petroleum ether. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 42.**

Table 42 Subfractions obtained from **subfraction 18C3** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
18C3A	5.7	Yellow gum
18C3B	0.9	Yellow gum
18C3C	0.7	Colorless gum
18C3D	2.5	Colorless gum
18C3E	1.3	Yellow solid

Subfraction 18C3A showed none of major spots under UV-S on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 18C3B showed two UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.19 and 0.51. Because of the minute quantity, it was not further investigated.

Subfraction 18C3C showed three UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.17, 0.18 and 0.51. Its ^{1}H NMR spectrum indicated that the major compound was **K18.** Therefore, it was not purified.

Subfraction 18C3D (K18) showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.17.

UV λ_{max}(nm)(MeOH)(log ε)	229 (3.19), 254 (2.32), 304 (2.63)
FTIR(neat):υ(cm^{-1})	1706 and 1698 (C=O stretching), 1636 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	6.67 (*d*, *J* = 2.4 Hz, 1H), 5.72 (*d*, *J* = 2.4 Hz, 1H), 2.53 (*s*, 3H), 3.87 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	192.00, 169.80, 161.50, 154.50, 103.90, 93.20, 56.00, 26.10
DEPT 135: CH;	103.90, 93.20
CH_3;	56.00, 26.10
EIMS *m/z* (% relative intensity):	168 (77), 125 (100), 69 (90)

Subfraction 18C3E showed four UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.10, 0.86, 0.88 and 0.95. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Subfraction 18C4 showed four UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.07, 0.20, 0.25 and 0.33. Its ^{1}H NMR spectrum indicated that the major compound was **K14**. Therefore, it was not purified.

Subfraction 18C5 showed four UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.15, 0.20, 0.25 and 0.37. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 20% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 43.**

Table 43 Subfractions obtained from **subfraction 18C5** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C5A	20% EtOAc/Petrol	9.0	Yellow gum
18C5B	30% EtOAc/Petrol	3.0	Yellow gum
18C5C	30% EtOAc/Petrol	3.1	Yellow gum
18C5D	50% EtOAc/Petrol	3.2	Yellow gum
18C5E	80% EtOAc/Petrol	22.0	Yellow gum
18C5F	100% EtOAc	315.0	Yellow gum
18C5G	100% EtOAc-100% MeOH	49.7	Yellow solid

Subfraction 18C5A showed none of UV-active spots on normal phase TLC using 5% ethyl acetate in petroleum ether as a mobile phase but showed many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not purified.

Subfraction 18C5B showed three UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.19, 0.30 and 0.35 and one purple spot with the R_f value of 0.05 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction 18C5C showed two UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.21 and 0.30 and one purple spot with the R_f value of 0.05 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated that the major compound was **K14**. Further investigation was then not performed.

Subfraction 18C5D showed three UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.14, 0.16 and 0.19. Because of low quantity, it was not further investigated.

Subfraction 18C5E showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.36 and 0.39. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 30% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 44.**

Table 44 Subfractions obtained from **subfraction 18C5E** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C5E1	30% EtOAc/Petrol	3.7	Colorless gum
18C5E2	30% EtOAc/Petrol	0.8	Colorless gum
18C5E3	40% EtOAc/Petrol	10.7	Colorless gum
18C5E4	50-80% EtOAc/Petrol	3.1	Yellow gum
18C5E5	90% EtOAc/Petrol-100% MeOH	3.0	Yellow gum

Subfraction 18C5E1 showed two UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.49 and 0.98 and one gray spot with the R_f value of 0.78 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 18C5E2 showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.46 and one gray spot with the R_f value of 0.39 after dipping the TLC plate in anisaldehyde

reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction 18C5E3 (K14) showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.31.

$[\alpha]_D^{23}$	- 55 (*c* 0.22, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	207 (4.43), 227 (4.39), 319 (4.41)
FTIR(neat):υ(cm^{-1})	1728 and 1686 (C=O stretching), 1640 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	10.15 (*s*, 1H), 6.17 (*s*, 1H), 5.68 (*ddd*, *J* = 9.9, 5.1, 2.7 Hz, 1H), 5.44 (*dt*, *J* = 9.9, 1.8 Hz, 1H), 4.08 (*s*, 3H), 2.63 (*m*, 1H), 2.48 (*dd*, *J* = 11.4, 9.9 Hz, 1H), 2.32 (*m*, 1H), 2.16 (*m*, 1H), 1.75 (*m*, 1H), 1.71 (*m*, 1H), 1.46 (*m*, 1H), 1.42 (*m*, 2H), 1.22 (*m*, 1H), 1.20 (*m*, 1H), 1.13 (*m*, 1H), 0.95 (*d*, *J* = 6.9 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	186.70, 176.70, 173.61, 162.38, 131.55, 130.05, 101.84, 95.85, 57.72, 47.98, 36.79, 36.04, 35.18, 29.75, 28.39, 25.88, 21.04, 20.31
DEPT 135: CH;	131.55, 130.05, 95.85, 47.98, 36.79, 36.04, 35.18
CH_2;	29.75, 28.39, 25.88, 21.04
CH_3;	57.72, 20.31

Subfraction 18C5E4 showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.31 and one gray spot with the R_f value of 0.21 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K14**. Further investigation was then not carried out.

Subfraction 18C5E5 displayed a long tail under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 18C5F showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.45 and two spots with the R_f values of 0.12 (purple) and 0.33 (gray) after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Further purification by column chromatography over silica gel was performed. Elution was performed with 20% acetone in petroleum ether. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 45.**

Table 45 Subfractions obtained from **subfraction 18C5F** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
18C5F1	4.0	Yellow gum
18C5F2	11.7	Yellow gum
18C5F3	7.6	Yellow gum
18C5F4	240.9	Yellow gum
18C5F5	4.9	Yellow gum
18C5F6	2.5	Yellow gum
18C5F7	20.6	Yellow gum

Subfraction 18C5F1 showed none of UV-active spots on normal phase TLC using 20% acetone in petroleum ether as a mobile phase but showed many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not purified.

Subfraction 18C5F2 showed one UV-active spot on normal phase TLC using 20% acetone in petroleum ether as a mobile phase with the R_f value of 0.31. Its ^1H

NMR spectrum indicated the presence of **K14**. Further investigation was then not carried out.

Subfraction 18C5F3 showed one UV-active spot on normal phase TLC using 20% acetone in petroleum ether as a mobile phase with the R_f value of 0.31 and two purple spots with the R_f values of 0.27 and 0.29 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K14**. Further investigation was then not carried out.

Subfraction 18C5F4 (K20) showed one gray spot on normal phase TLC using 20% acetone in petroleum ether as a mobile phase with the R_f value of 0.16 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate.

$[\alpha]_D^{25}$	+ 49 (*c* 0.760, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	205 (2.81)
FTIR(neat):υ(cm^{-1})	3350 (O-H stretching), 1766 (C=O stretching), 1634 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	5.64 (*dq*, *J* = 15.6, 6.6 Hz, 1H), 5.32 (*dd*, *J* = 15.6, 6.9 Hz, 1H), 4.28 (*q*, *J* = 6.9 Hz, 1H), 3.92 (*t*, *J* = 6.3 Hz, 1H), 2.36 (*m*, 2H), 2.03 (*m*, 1H), 1.91 (*m*, 1H), 1.54 (*d*, *J* = 6.6 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	178.05, 129.66, 128.35, 83.08, 74.13, 28.39, 23.45, 17.70
DEPT 135: CH;	129.66, 128.35, 83.08, 74.13
CH_2;	28.39, 23.45
CH_3;	17.70

Subfraction 18C5F5 showed two spots on normal phase TLC using 20% acetone in petroleum ether as a mobile phase with the R_f values of 0.14 (yellow) and 0.16 (gray)

after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K20**. Further investigation was then not carried out.

Subfraction 18C5F6 showed one UV-active spot on normal phase TLC using 20% acetone in petroleum ether as a mobile phase with the R_f value of 0.25 and two spots with the R_f values of 0.14 (yellow) and 0.16 (gray) after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K20**. Further investigation was then not carried out.

Subfraction 18C5F7 showed four UV-active spots on normal phase TLC using 20% acetone in petroleum ether as a mobile phase with the R_f values of 0.12, 0.26, 0.33 and 0.35 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not further investigated.

Subfraction 18C5G showed two UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.12 and 0.14 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 18C6 showed two UV-active spots on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase with the R_f values 0.05 and 0.10. Further purification by column chromatography over Sephadex LH-20 was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 46.**

Table 46 Subfractions obtained from **subfraction 18C6** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
18C6A	2.9	Yellow gum
18C6B	8.0	Yellow gum
18C6C	34.8	Yellow gum
18C6D	3,074	Yellow gum
18C6E	29.0	Yellow gum
18C6F	2.2	Yellow gum

Subfraction 18C6A displayed a long tail under UV-S on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 18C6B showed four UV-active spots on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.12, 0.16, 0.21 and 0.33. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 18C6C showed two UV-active spots on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.19 and 0.63 and two spots with the R_f values of 0.16 (purple) and 0.28 (gray) after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was further separated by column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 47**.

Table 47 Subfractions obtained from **subfraction 18C6C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C6C1	1% MeOH/CH_2Cl_2	5.6	Yellow gum
18C6C2	1% MeOH/CH_2Cl_2	4.3	Yellow gum
18C6C3	3% MeOH/CH_2Cl_2	2.5	Yellow gum
18C6C4	3% MeOH/CH_2Cl_2	8.0	Yellow gum
18C6C5	5-50% MeOH/CH_2Cl_2	3.7	Yellow gum
18C6C6	70% MeOH/CH_2Cl_2-100% MeOH	10.5	Yellow gum

Subfraction 18C6C1 showed one pale UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.60 and one gray spot with the R_f value of 0.35 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18C6C2 showed one gray spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.35 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated the presence of **K20**. Further investigation was then not carried out.

Subfraction 18C6C3 showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.24 and two gray spots with the R_f values of 0.14 and 0.33 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K20**. Further investigation was then not performed.

Subfraction 18C6C4 (K15) showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.17.

$[\alpha]_D^{29}$	- 254 (*c* 0.10, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	207 (3.83), 228 (3.75), 273 (3.84), 320 (2.65)
FTIR(neat):υ(cm^{-1})	3390 (O-H stretching), 1719 and 1698 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	10.15 (*s*, 1H), 6.14 (*s*, 1H), 6.06 (*ddd*, *J* = 9.9, 5.1, 2.4 Hz, 1H), 5.63 (*d*, *J* = 9.9 Hz, 1H), 4.08 (*s*, 3H), 3.50 (*dt*, *J* = 10.2, 4.5 Hz, 1H), 2.65 (*m*, 1H), 2.37 (*m*, 1H), 2.33 (*m*, 1H), 2.06 (*m*, 1H), 2.00 (*m*, 1H), 1.63 (*m*, 1H), 1.40 (*m*, 3H), 1.28 (*m*, 1H), 0.98 (*d*, *J* = 7.2 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	186.63, 175.47, 173.49, 162.16, 132.07, 128.47, 101.95, 95.89, 73.63, 57.77, 48.99, 44.12, 36.82, 35.36, 35.12, 27.92, 20.24, 20.13
DEPT 135: CH;	132.07, 128.47, 95.89, 73.63, 48.99, 44.12 36.82, 35.12
CH_2;	35.36, 27.92, 20.13
CH_3;	57.77, 20.24
EIMS *m/z* (% relative intensity):	318 (9), 290 (100), 153 (97), 71 (62)

Subfraction 18C6C5 showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.31 and 0.41. Because the ^{1}H NMR spectrum indicated that it contained a mixture of **K14** and **K20**, further purification was not performed.

Subfraction 18C6C6 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 18C6D showed two UV-active spots on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.41 and 0.62. Because the ^1H NMR spectrum indicated that it contained a mixture of **K19** and **K20**, further investigation was then not carried out.

Subfraction 18C6E showed one UV-active spot on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase with the R_f value of 0.41 and and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was further separated by column chromatography over silica gel. Elution was performed with 15% ethyl acetate in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 48**.

Table 48 Subfractions obtained from **subfraction 18C6E** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
18C6E1	3.0	Colorless gum
18C6E2	11.5	Colorless gum
18C6E3	4.9	Colorless gum
18C6E4	8.3	Yellow gum

Subfraction 18C6E1 showed none of UV-active spots on normal phase TLC using 15% ethyl acetate in dichloromethane as a mobile phase. Therefore, it was not further investigated.

Subfraction 18C6E2 showed one UV-active spot on normal phase TLC using 20% acetone in dichloromethane as a mobile phase with the R_f value of 0.36 and one gray spot with the R_f value of 0.40 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was then separated by precoated TLC with 20% acetone in dichloromethane as a mobile phase (6 runs) to afford two bands.

Band 1 was a colorless gum (2.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% acetone in dichloromethane as a mobile phase with the R_f value of 0.36. Its ^1H NMR spectrum indicated that it was **K20.** Further investigation was then not performed.

Band 2 (K19) was a colorless gum (8.3 mg). Its chromatogram showed one gray spot on normal phase TLC using 20% acetone in dichloromethane as a mobile phase with the R_f value of 0.36 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate.

$[\alpha]_D^{23}$	+163 (*c* 0.60, EtOH)
UV λ_{max}(nm)(MeOH)(log ε)	206 (3.57)
FTIR(neat):υ(cm^{-1})	3400 (O-H stretching), 1723 (C=O stretching) 1629 (C=C stretching)
^1H NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (300 MHz):	6.99 (*dd*, *J* = 9.6, 5.4 Hz, 1H), 6.09 (*d*, *J* = 9.6 Hz, 1H), 5.97 (*dqd*, *J* = 15.3, 6.6, 0.9 Hz, 1H), 5.77 (*ddq*, *J* = 15.3, 7.5, 1.5 Hz, 1H), 4.97 (*dd*, *J* = 7.5, 3.3, 1H), 4.14 (*dd*, *J* = 5.4, 3.3 Hz, 1H), 1.80 (*ddd*, *J* = 6.6, 1.5, 0.9 Hz, 3H)
^{13}C NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (75 MHz):	164.10, 145.34, 132.59, 124.22, 122.13, 81.69, 62.83, 17.86
DEPT 135: CH;	145.34, 132.59, 124.22, 122.13, 81.69, 62.83
CH_3;	17.83

Subfraction 18C6E3 showed one UV-active spot on normal phase TLC using 20% acetone in dichloromethane as a mobile phase with the R_f value of 0.36 and one gray spot with the R_f value of 0.40 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because the ^1H NMR spectrum indicated that it consisted of a mixture of **K19** and **K20**, further investigation was not carried out.

Subfraction 18C6E4 showed five UV-active spots on normal phase TLC using 20% acetone in dichloromethane as a mobile phase with the R_f values of 0.18, 0.25, 0.40, 0.50 and 0.51. Because of low quantity, it was not further investigated.

Subfraction 18C6F displayed a long tail under UV-S on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 18C7 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.25, 0.35 and 0.55. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 49**.

Table 49 Subfractions obtained from **subfraction 18C7** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C7A	50% MeOH/H_2O	0.8	Yellow gum
18C7B	50% MeOH/H_2O	35.2	Yellow gum
18C7C	60-70% MeOH/H_2O	7.0	Yellow gum
18C7D	80% MeOH/H_2O-100% MeOH	6.2	Yellow gum

Subfraction 18C7A showed two purple spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07 and 0.12 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further purified.

Subfraction 18C7B showed six UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.10,

0.12, 0.17, 0.20 and 0.27 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was then separated by column chromatography over silica gel. Elution was performed initially with 3% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 50.**

Table 50 Subfractions obtained from **subfraction 18C7B** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C7B1	3% MeOH/CH_2Cl_2	4.8	Yellow gum
18C7B2	3% MeOH/CH_2Cl_2	3.4	Yellow gum
18C7B3	3% MeOH/CH_2Cl_2	10.3	Yellow gum
18C7B4	5% MeOH/CH_2Cl_2-100% MeOH	14.3	Yellow gum

Subfraction 18C7B1 showed three UV-active spots on normal phase TLC using 60% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.21, 0.79 and 0.88. Because the ^1H NMR spectrum indicated the presence of a mixture of **K19** and **K20**, further purification was not performed.

Subfraction 18C7B2 showed two UV-active spots on normal phase TLC using 60% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.24 and 0.33 and two gray spots with the R_f values of 0.79 and 0.86 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not further investigated.

Subfraction 18C7B3 showed three UV-active spots on normal phase TLC using 60% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.30, 0.51 and 0.65 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was then purified by precoated TLC with 60% ethyl acetate in petroleum ether as a mobile phase (8 runs) to afford three bands.

Band 1 (K16) was a colorless gum (1.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 60% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.30.

$[\alpha]_D^{29}$	- 232 (*c* 0.10, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	208 (4.15), 237 (4.43), 280 (4.10), 316 (4.02),
FTIR(neat):υ(cm^{-1})	3390 (O-H stretching), 1719 and 1692 (C=O stretching), 1652 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	10.85 (*brs*, 1H), 9.98 (*s*, 1H), 6.04 (*ddd*, *J* = 10.2, 4.8, 2.4 Hz, 1H), 5.97 (*s*, 1H), 5.62 (*d*, *J* = 10.2 Hz, 1H), 3.89 (*t*, *J* = 5.4 Hz, 2H), 3.54 (*q*, *J* = 5.4 Hz, 2H), 3.46 (*m*, 1H), 2.63 (*m*, 1H), 2.41 (*m*, 1H), 2.24 (*dd*, *J* = 12.0, 9.9 Hz, 1H), 2.02 (*m*, 1H), 1.96 (*m*, 1H), 1.46 (*m*, 3H), 1.32 (*m*, 1H), 1.30 (*m*, 1H), 0.98 (*d*, *J* = 6.9 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	191.32, 175.00, 171.50, 160.50, 132.47, 128.25, 96.06, 94.87, 73.79, 61.06, 48.48, 44.91, 44.16, 36.28, 35.40, 34.58, 27.87, 20.21, 20.14
DEPT 135: CH;	132.47, 128.25, 96.06, 73.79, 48.48, 44.16, 36.28, 34.58
CH_2;	61.06, 44.91, 35.40, 27.87, 20.14
CH_3;	20.21
EIMS *m/z* (% relative intensity):	347 (59), 257 (37), 110 (100), 105 (11)

Band 2 (K22) was a colorless gum (1.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 60% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.53.

UV λ_{max}(nm)(MeOH)(log ε)	204 (3.26), 222 (3.34), 278 (2.78)
FTIR(neat):υ(cm^{-1})	3391 (O-H stretching), 1655 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	7.11 (*d*, *J* = 9.0 Hz, 2H), 6.79 (*d*, *J* = 9.0 Hz, 2H), 3.83 (*t*, *J* = 6.0 Hz, 2H), 2.81 (*t*, *J* = 6.0 Hz, 2H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	154.22, 130.55, 130.15, 115.45, 63.80, 38.27
DEPT 135: CH;	130.15, 115.45
CH_2;	63.80, 38.27

Band 3 (K21) was a colorless gum (2.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 60% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.58.

$[\alpha]_D^{25}$	+ 8 (*c* 0.32, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	203 (3.14), 219 (3.09)
FTIR(neat):υ(cm^{-1})	3440 (O-H stretching), 1750 (C=O stretching), 1630 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	5.91 (*dqd*, *J* = 15.3, 6.3, 0.6 Hz, 1H), 5.68 (*ddq*, *J* = 15.3, 7.2, 1.5 Hz, 1H), 4.67 (*ddd*, *J* = 6.6, 5.1, 3.0 Hz, 1H), 4.58 (*dd*, *J* = 7.2, 4.2 Hz, 1H), 4.37 (*dd*, *J* = 5.1, 4.2 Hz, 1H), 2.82 (*dd*, *J* = 17.7, 6.6 Hz, 1H), 2.63 (*dd*, *J* = 17.7, 3.0 Hz, 1H), 1.76 (*ddd*, *J* = 6.3, 1.5, 0.6 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	175.02, 131.10, 128.16, 84.51, 71.70, 69.18, 39.16, 17.84
DEPT 135: CH;	131.10, 128.16, 84.51, 71.70, 69.18
CH_2;	39.16
CH_3;	17.84

Subfraction 18C7B4 showed one UV-active spot on normal phase TLC using 60% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.30 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Therefore, it was not further purified.

Subfraction 18C8 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.15 and 0.53 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was further separated by column chromatography over silica gel. Elution was performed initially with 50% ethyl acetate in dichloromethane and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford ten subfractions as shown in **Table 51.**

Table 51 Subfractions obtained from **subfraction 18C8** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C8A	50% EtOAc/CH_2Cl_2	2.0	Colorless gum
18C8B	50% EtOAc/CH_2Cl_2	3.6	Yellow gum
18C8C	50% EtOAc/CH_2Cl_2	2.0	Yellow gum
18C8D	60% EtOAc/CH_2Cl_2	1.7	Yellow gum
18C8E	70% EtOAc/CH_2Cl_2	1.7	Yellow gum
18C8F	70% EtOAc/CH_2Cl_2	5.1	Yellow gum
18C8G	80% EtOAc/CH_2Cl_2-100% EtOAc	4.1	Yellow gum
18C8H	100% EtOAc-2% MeOH/EtOAc	4.1	Yellow gum
18C8I	5-50% MeOH/EtOAc	8.1	Yellow gum
18C8J	50% MeOH/EtOAc-100% MeOH	10.0	Yellow gum

Subfraction 18C8A showed four UV-active spots on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.08, 0.35, 0.55 and 0.95. Because of the minute quantity, it was not further purified.

Subfraction 18C8B showed one gray spot on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase with the R_f value of 0.43 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because the ^{1}H NMR spectrum indicated that it contained a mixture of **K19** and **K20**, further investigation was not performed.

Subfraction 18C8C displayed a long tail under UV-S on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 18C8D showed three UV-active spots on normal phase TLC using 50% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.51, 0.52 and 0.65. Because of the minute quantity, it was not further purified.

Subfraction 18C8E showed two UV-active spots on normal phase TLC using 50% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.41 and 0.51 and one gray spot with the R_f value of 0.37 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction 18C8F showed two UV-active spots on normal phase TLC using 50% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.36 and 0.41 and two gray spots with the R_f values of 0.24 and 0.29 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that it consisted of a mixture of **K14** and **K20**. Further investigation was then not performed.

Subfraction 18C8G showed two UV-active spots on normal phase TLC using 50% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.56 and 0.68. It was then purified by precoated TLC with 2% methanol in dichloromethane as a mobile phase (9 runs) to afford two bands.

Band 1 (K17) was a yellow gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.36.

$[\alpha]_D^{29}$	- 202 (*c* 0.10, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	205 (3.69), 235 (3.85), 281 (3.41) 314 (3.42),
FTIR(neat):υ(cm^{-1})	3395 (O-H stretching), 1706 and 1689 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	10.87 (*brt*, J = 1.5 Hz, 1H), 10.00 (*s*, 1H), 5.98 (*s*, 1H), 5.64 (*m*, 1H), 5.49 (*dt*, J = 10.0, 1.5 Hz, 1H), 4.10 (*m*, 1H), 3.90 (*t*, J = 5.5 Hz, 2H), 3.52 (*q*, J = 5.5 Hz, 2H), 2.59 (*m*, 2H), 2.29 (*m*, 2H), 1.93 (*tm*, J = 13.0 Hz, 1H), 1.81 (*dm*, J = 14.0 Hz, 1H), 1.55 (*m*, 2H), 1.36 (*td*, J = 14.0, 2.5 Hz, 1H), 1.28 (*m*, 1H), 0.97 (*d*, J = 7.0 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	191.36, 175.00, 172.50, 160.61, 131.15, 130.95, 95.57, 94.90, 65.64, 61.18, 46.84, 44.83, 36.12, 35.25, 34.70, 30.37, 27.72, 21.50, 20.27
DEPT 135: CH;	131.15, 130.95, 95.57, 94.90, 65.64, 46.84, 35.25, 34.70, 30.37
CH_2;	61.18, 44.83, 36.12, 27.72, 21.50
CH_3;	20.27
EIMS *m/z* (% relative intensity):	347 (24), 257 (35), 110 (100), 105 (31)

Band 2 was a yellow gum (0.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.14. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Subfraction 18C8H showed three UV-active spots on normal phase TLC using 50% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.43, 0.56 and 0.72. It was then purified by precoated TLC with 80% ethyl acetate in petroleum ether as a mobile phase (9 runs) to afford two bands.

Band 1 was a yellow gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% ethyl acetate in dichloromethane as a mobile phase with the R_f value of 0.36. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Band 2 was a yellow gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% ethyl acetate in dichloromethane as a mobile phase with the R_f value of 0.34. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Subfraction 18C8I showed five UV-active spots on normal phase TLC using 50% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.04, 0.17, 0.29, 0.34 and 0.51 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 18C8J displayed a long tail under UV-S on normal phase TLC using 50% ethyl acetate in dichloromethane as a mobile phase. Thus, it was not purified.

Subfraction 18C9 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.33 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently

heating the plate. Further purification by column chromatography over Sephadex LH-20 was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 52**.

Table 52 Subfractions obtained from **subfraction 18C9** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
18C9A	20.1	Yellow gum
18C9B	18.5	Yellow gum
18C9C	54.7	Yellow gum
18C9D	144.4	Yellow gum
18C9E	31.3	Yellow gum
18C9F	13.1	Yellow gum

Subfraction 18C9A displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not purified

Subfraction 18C9B displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 18C9C showed two UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.31 and 0.40. This subfraction was subjected to acetylation reaction. After work up, the reaction mixture was obtained a dark brown gum (30.0 mg). It was further separated by column chromatography over silica gel. Elution was performed initially with 100% dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 53**.

Table 53 Subfractions obtained from **subfraction 18C9C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C9C1	100% CH_2Cl_2	2.1	Brown gum
18C9C2	1% MeOH/CH_2Cl_2	0.8	Brown gum
18C9C3	3% MeOH/CH_2Cl_2	5.7	Brown gum
18C9C4	5-50% MeOH/CH_2Cl_2	7.5	Brown gum
18C9C5	70% MeOH/CH_2Cl_2-100% MeOH	9.6	Brown gum

Subfraction 18C9C1 showed three pale UV-active spots on normal phase TLC using 80% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.40, 0.52 and 0.62. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18C9C2 showed five UV-active spots on normal phase TLC using 80% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.19, 0.33, 0.40, 0.52 and 0.62. Because of the minute quantity, it was not further investigated.

Subfraction 18C9C3 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.43, 0.50, 0.63 and 0.73. It was then purified by precoated TLC with 80% dichloromethane in petroleum ether as a mobile phase (5 runs) to afford three bands.

Band 1 was a brown gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 80% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.63. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Band 2 was a brown solid (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 80% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.45. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Band 3 was a brown solid (1.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 80% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.10. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Subfraction 18C9C4 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.33, 0.38 and 0.43. Therefore, it was not further investigated.

Subfraction 18C9C5 displayed a long tail under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 18C9D showed six UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.31, 0.40, 0.42, 0.74, 0.81 and 0.93. It was further separated by column chromatography over silica gel. Elution was performed initially with 5% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 54**.

Table 54 Subfractions obtained from **subfraction 18C9D** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C9D1	5% MeOH/CH_2Cl_2	5.0	Brown gum
18C9D2	5% MeOH/CH_2Cl_2	9.1	Brown gum
18C9D3	5% MeOH/CH_2Cl_2	3.0	Brown gum

Table 54 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
18C9D4	7% MeOH/CH_2Cl_2	9.0	Brown gum
18C9D5	10-20% MeOH/CH_2Cl_2	8.2	Brown gum
18C9D6	40% MeOH/CH_2Cl_2-100% MeOH	99.0	Brown gum

Subfraction 18C9D1 showed two pale UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.46 and 0.67. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18C9D2 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.60, 0.57, 0.80 and 0.83 and one gray spot with the R_f value of 0.70 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated that the major compound was **K20**. Further investigation was then not performed.

Subfraction 18C9D3 showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.32, 0.38, 0.47 and 0.62. Because of the minute quantity, it was not further investigated.

Subfraction 18C9D4 showed six UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.20, 0.25, 0.33, 0.48, 0.50 and 0.58. Thus, it was not further purified.

Subfraction 18C9D5 showed six UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.10, 0.13, 0.15, 0.25, 0.35 and 0.38. Thus, it was not further purified.

Subfraction 18C9D6 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. This subfraction (50.0 mg) was subjected to acetylation reaction. After work up, the acetate derivative was obtained as a brown gum (30.0 mg). It was further separated by column chromatography over silica gel. Elution was performed initially with 100% dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 55.**

Table 55 Subfractions obtained from **subfraction 18C9D6** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C9D6A	100% CH_2Cl_2- 2% MeOH/CH_2Cl_2	4.1	Yellow gum
18C9D6B	4-10% MeOH/CH_2Cl_2	2.2	Yellow gum
18C9D6C	20-50% MeOH/CH_2Cl_2	6.9	Yellow gum
18C9D6D	70% MeOH/CH_2Cl_2- 100% MeOH	14.6	Brown solid

Subfraction 18C9D6A showed many UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Therefore, it was not further purified.

Subfraction 18C9D6B showed one gray spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.17 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated the presence of an acetate derivative of **K14.** Further investigation was then not carried out.

Subfraction 18C9D6C showed four pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12,

0.19, 0.29 and 0.41. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18C9D6D displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 18C9E showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.30, 0.41 and 0.88. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 56.**

Table 56 Subfractions obtained from **subfraction 18C9E** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C9E1	50% MeOH/H_2O	15.8	Yellow gum
18C9E2	50% MeOH/H_2O	5.0	Yellow gum
18C9E3	60% MeOH/H_2O-100% MeOH	9.0	Brown solid

Subfraction 18C9E1 showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.25 and 0.35. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 20% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 57.**

Table 57 Subfractions obtained from **subfraction 18C9E1** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18C9E1A	20% MeOH/H_2O	11.4	Yellow gum
18C9E1B	20% MeOH/H_2O	1.2	Yellow gum
18C9E1C	40-60% MeOH/H_2O	2.0	Yellow gum
18C9E1D	70% MeOH/H_2O-100% MeOH	1.0	Yellow gum

Subfraction 18C9E1A showed three pale UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.15 and 0.19. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18C9E1B showed two UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.09 and 0.16. Because of the minute quantity, it was not further purified.

Subfraction 18C9E1C showed five UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.02, 0.07, 0.12, 0.16 and 0.21. Because of the minute quantity, it was not further purified.

Subfraction 18C9E1D displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 18C9E2 showed one UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.42. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Subfraction 18C9E3 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 18C9F displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 18D showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.22 and 0.37. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 58**.

Table 58 Subfractions obtained from **fraction 18D** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
18D1	2% MeOH/CH_2Cl_2	5.5	Yellow gum
18D2	4-6% MeOH/CH_2Cl_2	6.3	Yellow gum
18D3	10% MeOH/CH_2Cl_2-100% MeOH	17.6	Brown gum

Subfraction 18D1 showed five UV-active spots on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.02, 0.07, 0.17, 0.51 and 0.95. It was then purified by precoated TLC with 50% dichloromethane in petroleum ether (3 runs) and 100% dichloromethane as mobile phases (5 runs), respectively, to afford four bands.

Band 1 was a yellow gum (1.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.44. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Band 2 was a yellow gum (0.8 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.82. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 3 was a yellow gum (0.7 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.58. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 4 was a yellow gum (1.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.39. Its ^1H NMR spectrum indicated that it contained a mixture of **K19** and **K20**. Further investigation was then not carried out.

Subfraction 18D2 showed four pale UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.06, 0.12, 0.23 and 0.28. Its ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 18D3 showed three UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.23, 0.25 and 0.37. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

2.2.3 Purification of the EtOAc extract from mycelia

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four fractions as shown in **Table 59**.

Table 59 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
18CE1	230.8	Brown gum
18CE2	313.3	Brown gum
18CE3	134.6	Brown gum
18CE4	40.5	Brown gum

Fraction 18CE1 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 18CE2 showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.23, 0.36 and 0.57. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 18CE3 showed many UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum indicated that it contained a mixture of **K19** and **K20**. Further investigation was then not carried out.

Fraction 18CE4 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

CHAPTER 2.3

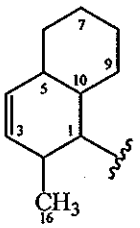
RESULTS AND DISCUSSION

Three new solanapyrones **(K15-K17)** and one pyrone derivative **(K18)** were isolated from the broth extract together with five known ones **(K14** and **K19-K22)**. The structures were identified by spectroscopic methods.

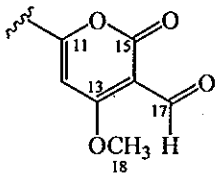
2.3.1 Compound K14

Compound **K14** was obtained as a colorless gum. The UV spectrum showed absorption bands at 207, 227 and 319 nm. The IR spectrum exhibited absorption bands at 1728 cm^{-1} and 1686 cm^{-1} for ester and aldehyde carbonyl groups, respectively. The ^1H NMR spectrum **(Figure 31) (Table 60)** consisted of signals for one aldehyde proton (δ 10.15, *s*, 1H), three olefinic protons [δ 6.17 (*s*, 1H), 5.68 (*ddd*, J = 9.9, 5.1 and 2.7 Hz, 1H) and 5.44 (*dt*, J = 9.9 and 1.8 Hz, 1H)], four methine protons [δ 2.63 (*m*, 1H), 2.48 (*dd*, J = 11.4 and 9.9 Hz, 1H), 2.32 (*m*, 1H) and 2.16 (*m*, 1H)], four methylene groups [δ 1.75 (*m*, 1H)/1.13 (*m*, 1H), 1.71 (*m*, 1H)/1.20 (*m*, 1H), 1.46 (*m*, 1H)/1.22 (*m*, 1H) and 1.42 (*m*, 2H)], one methoxyl group (δ 4.08, *s*, 3H) and one methyl group (δ 0.95, *d*, J = 6.9 Hz, 3H). The aldehyde carbon at δ 186.70 (C-17) in the ^{13}C NMR spectrum **(Figure 32) (Table 60)** supported the presence of aldehyde functional group. In the ^1H-^1H COSY spectrum **(Table 61)**, the methine proton, H-2 (δ 2.63), was coupled with the methine proton, H-1 (δ 2.43), the olefinic proton, H-3 (δ 5.44) and the methyl protons, H_3-16 (δ 0.95). Furthermore, the olefinic proton, H-4 (δ 5.68), was coupled with H-3 and the methine proton, H-5 (δ 2.16), which was coupled with the methylene protons, H_{ab}-6 (δ 1.75 and 1.13), and the methine proton, H-10 (δ 2.32). The methylene protons, H_{ab}-7 (δ 1.71 and 1.20), showed cross peaks in the ^1H-^1H COSY spectrum with H_{ab}-6 and the methylene protons, H_2-8 (δ 1.42) which were further coupled with the remaining methylene protons, H_{ab}-9 (δ 1.46 and 1.22).

The methine proton, H-10 (δ 2.32), was coupled with H-1 and H-5. These data together with the HMBC correlations **(Table 61)** indicated that **K14** had a decalin skeleton with a C3 (δ 130.05)-C4 (δ 131.55) double bond and a methyl group attached at C-2 (δ 35.18) (substructure 1). The location of the methyl group was confirmed by HMBC correlations of H_3-16 (δ 0.95) with C-1 (δ 47.98), C-2 and C-3. The remaining olefinic proton, H-12 (δ 6.17), showed cross peaks in HMBC spectrum with C-11 (δ 176.70), C-13 (δ 173.61), C-14 (δ 101.84) and C-17. The aldehyde proton, H-17 (δ 10.15), was correlated with C-12 (δ 95.85), C-13, C-14 and C-15 (δ 162.38) while the methoxy protons, H_3-18 (δ 4.08), showed a HMBC cross peak with C-13. These results together with the chemical shifts of these carbons revealed the presence of a pyrone ring with the methoxyl and the formyl groups at C-13 and C-14, respectively (substructure 2). The assigned location of these substituents was confirmed by signal enhancement of both H-12 and H-17 upon irradiation of H_3-18, in the NOEDIFF experiment **(Table 61)**.



Substructure 1



Substructure 2

The linkage between C-1 of the decaline unit and C-11 of the pyrone ring was established according to the HMBC correlations of H-1 of the decalin unit with the C-11 and C-12 of the pyrone ring. The relative stereochemistry of **K14** was established by the NOEDIFF results. Irradiation of H-2 (δ 2.63) affected signal intensity of H-10 (δ 2.32) and H-12 (δ 6.17), indicating their location at the same side of the molecule and pseudoaxial orientations for H-2 and H-10. When H-5 (δ 2.16) was irradiated, the signal intensity of H-10 was enhanced, thus suggesting a *cis* ring fusion of the decalin moiety. The observed optical rotation of **K14**, $[\alpha]_D^{23}$ -55 (*c* 0.22, $CHCl_3$), was similar to that of (1*R*,2*S*,5*R*,10*R*)-solanapyrone A, $[\alpha]_D^{20}$ -58 (*c* 0.22, $CHCl_3$). These data revealed that they had identical absolute configuration. Thus, **K14**

was assigned as (1*R*,2*S*,5*R*,10*R*)-solanapyrone A which was previously isolated from the fungus *Ascochyta rabiei* (Alam, *et al.*, 1989).

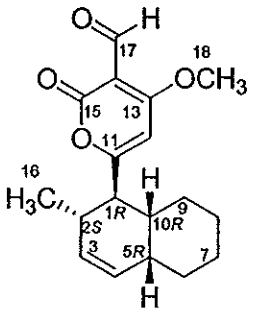


Table 60 The ^{1}H and ^{13}C NMR data of compound **K14** and **(1*R*,2*S*,5*R*,10*R*)-Solanapyrone A** in $CDCl_3$

Position	K14		(1*R*,2*S*,5*R*,10*R*)-Solanapyrone A	
	δ_H (*mult*, J Hz)	δ_C (C-Type)	δ_H (*mult*, J Hz)	δ_C (C-Type)
1	2.48 (*dd*, 11.4, 9.9)	47.98 (CH)	2.48 (*dd*, 11.6, 10.0)	48.0 (CH)
2	2.63 (*m*)	35.18 (CH)	2.63 (*m*)	35.2 (CH)
3	5.44 (*dt*, 9.9, 1.8)	130.05 (CH)	5.44 (*ddd*, 10.0, 2.0, 1.8)	130.0 (CH)
4	5.68 (*ddd*, 9.9, 5.1, 2.7)	131.55 (CH)	5.67 (*ddd*, 10.0, 5.0, 2.5)	131.5 (CH)
5	2.16 (*m*)	36.79 (CH)	2.15 (*m*)	36.8 (CH)
6	a: 1.75 (*m*) b: 1.13 (*m*)	29.75 (CH_2)	a: 1.74 (*m*) b: 1.11 (*m*)	29.7 (CH_2)
7	a: 1.71 (*m*) b: 1.20 (*m*)	25.88 (CH_2)	a: 1.71 (*m*) b: 1.25 (*m*)	25.9 (CH_2)
8	1.42 (*m*)	28.39 (CH_2)	a: 1.18 (*m*) b: 1.47 (*m*)	21.0 (CH_2)
9	a: 1.46 (*m*) b: 1.22 (*m*)	21.04 (CH_2)	a: 1.48 (*m*) b: 1.41 (*m*)	28.4 (CH_2)
10	2.32 (*m*)	36.04 (CH)	2.32 (*m*)	36.0 (CH)
11	-	176.70 (C)	-	173.6 (C)

Table 60 Continued

Position	K14		(1*R*,2*S*,5*R*,10*R*)-Solanapyrone A	
	δ_H (*mult*, J_{Hz})	δ_C (C-Type)	δ_H (*mult*, J_{Hz})	δ_C (C-Type)
12	6.17 (*s*)	95.85 (CH)	6.15 (*s*)	95.8 (CH)
13	-	173.61 (C)	-	176.4 (C)
14	-	101.84 (C)	-	101.8 (C)
15	-	162.38 (C)	-	162.4 (C)
16	0.95 (*d*, 6.9)	20.31 (CH_3)	0.95 (*d*, 6.8)	20.3 (CH_3)
17	10.15 (*s*)	186.70 (CH)	10.15 (*s*)	186.4 (C)
18	4.08 (*s*)	57.72 (CH_3)	4.10 (*s*)	57.7 (CH_3)

Table 61 The HMBC, COSY and NOEDIFF data of compound **K14** in $CDCl_3$

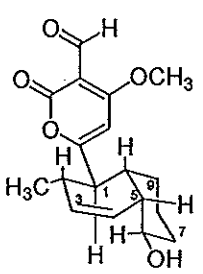
Proton	HMBC	COSY	NOEDIFF
H-1	C-2, C-5, C-10, C-11, C-12, C-16	H-2, H-10	H-12, H_3-16
H-2	C-1, C-3, C-4, C-16	H-1, H-3, H-4, H_3-16	H-3, H-10, H-12, H_3-16
H-3	C-1, C-2, C-4, C-5, C-16	H-2, H-4, H-5	H-2, H-4, H_3-16
H-4	C-2, C-3, C-5, C-10	H-2, H-3, H-5	H-3, H-5, H_a-6
H-5	C-1, C-3, C-4, C-6, C-10	H-3, H-4, H_{ab}-6, H-10	H-4, H_{ab}-6, H-10
H_a-6	C-5, C-7, C-8, C-10	H-5, H_b-6, H_{ab}-7	-
H_b-6	C-5, C-7, C-8, C-10	H-5, H_a-6, H_{ab}-7	-
H_a-7	C-5, C-8, C-9	H_{ab}-6, H_b-7, H-8	-
H_b-7	C-5, C-8, C-9	H_{ab}-6, H_a-7, H-8	-
H_2-8	C-5, C-7, C-9	H_ab-7, H_{ab}-9	-
H_a-9	C-1, C-5, C-7, C-8	H-8	-
H_b-9	C-1, C-5, C-7, C-8	H-8	-
H-10	C-1, C-5, C-8, C-9	H-1, H-5, H_{ab}-9	H-2, H-5, H-12
H-12	C-1, C-11, C-13, C-14, C-17	-	H-1, H-2, H-10, H_3-18

Table 61 Continued

Proton	HMBC	COSY	NOEDIFF
H_3-16	C-1, C-2, C-3	H-2	H-1, H-2
H-17	C-12, C-13, C-14, C-15	-	H_3-18
H_3-18	C-13	-	H-9, H-12, H-17

2.3.2 Compound K15

Compound **K15** with the molecular formula $C_{18}H_{22}O_5$ from EIMS (*m/z* 318) **(Figure 33)** was obtained as a colorless gum. Its UV spectrum was similar to that of **K14** while the IR spectrum showed an additional absorption band of a hydroxyl group at 3390 cm^{-1}. Its ^1H NMR spectrum **(Figure 34) (Table 62)** was similar to that of **K14** except for the replacement of signals for nonequivalent methylene protons in **K14** with signal of a hydroxymethine proton (δ 3.50, *td*, *J* = 10.2 and 4.5 Hz, 1H). The presence of one oxymethine carbon (δ 73.63) and three methylene carbons (δ 20.13, 27.92 and 35.36) in the ^{13}C NMR **(Figure 35) (Table 62)** and DEPT 135 spectra **(Table 62)** supported above conclusion. This oxymethine proton showed cross peaks with the methine proton, H-5 (δ 2.06), and the methylene protons, H_{ab}-7 (δ 2.00 and 1.28), in the ^1H-^1H COSY spectrum **(Table 63)**. These data together with the ^3J HMBC correlations of this proton with C-4 (δ 128.47) and C-8 (δ 27.92) supported the location of a hydroxyl group at C-6 (δ 73.63). Irradiation of H-6 (δ 3.50) in the NOEDIFF spectrum **(Table 63)** did not affect signal intensity of H-5, indicating their *trans*-relationship. In addition, H-5 and H-6 were placed at pseudoaxial and axial positions, respectively, as they were coupled with a large coupling constant of 10.2 Hz. Therefore, **K15** was determined as a 6-hydroxy derivative of **K14**, a new solanapyrone derivative.



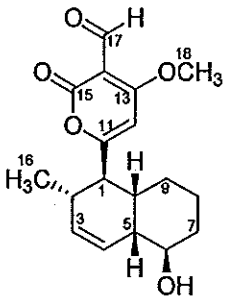


Table 62 The ^{1}H, ^{13}C NMR and HMBC data of compound **K15** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
1	2.33 (*m*)	48.99 (CH)	C-5, C-11, C-12, C-16
2	2.65 (*m*)	35.12 (CH)	C-3, C-16
3	5.63 (*d*, 9.9)	132.07 (CH)	C-1, C-2, C-5, C-16
4	6.06 (*ddd*, 9.9, 5.1, 2.4)	128.47 (CH)	C-2, C-5, C-10
5	2.06 (*m*)	44.12 (CH)	C-1, C-3, C-4, C-6, C-10
6	3.50 (*td*, 10.2, 4.5)	73.63 (CH)	C-4, C-8
7	a: 2.00 (*m*)	35.36 (CH_2)	C-5, C-6, C-8, C-9
	b: 1.28 (*m*)		
8	1.40 (*m*)	27.92 (CH_2)	C-6, C-10
9	a: 1.63 (*m*)	20.13 (CH_2)	C-5, C-8, C-10
	b: 1.40 (*m*)		
10	2.37 (*m*)	36.82 (CH)	C-2, C-5, C-8
11	-	175.47 (C)	-
12	6.14 (*s*)	95.89 (CH)	C-1, C-11, C-13, C-14, C-17
13	-	173.49 (C)	-
14	-	101.95 (C)	-
15	-	162.16 (C)	-
16	0.98 (*d*, 7.2)	20.24 (CH_3)	C-1, C-2, C-3
17	10.15 (*s*)	186.63 (CH)	C-13, C-14, C-15
18	4.08 (*s*)	57.77 (CH_3)	C-13

Table 63 The COSY and NOEDIFF data of compound **K15** in $CDCl_3$

Proton	COSY	NOEDIFF
H-1	H-2, H-10	H-12, H_3-16
H-2	H-1, H-3, H-4, H_3-16	H-3, H-10, H-12, H_3-16
H-3	H-2, H-4, H-5	H-2, H-4, H_3-16
H-4	H-2, H-3, H-5	H-3, H-5
H-5	H-3, H-4, H-6, H-10	H-4, H-10

Table 63 Continued

Proton	COSY	NOEDIFF
H-6	H-5, H_{ab}-7	H_{ab}-7
H_a-7	H-6, H_2-8	H-6
H_b-7	H-6, H_2-8	H-6
H_2-8	H_{ab}-7, H_{ab}-9	-
H_a-9	H_2-8, H-10	-
H_b-9	H_2-8, H-10	-
H-10	H-1, H-5, H_{ab}-9	H-5
H-12	-	H-1, H_3-18
H_3-16	H-2	H-1, H-2, H-3
H-17	-	H_3-18
H_3-18	-	H-12, H-17

2.3.3 Compound K16

Compound **K16** with the molecular formula $C_{19}H_{25}NO_5$ from EIMS (*m/z* 347) **(Figure 36)** was obtained as a colorless gum. The UV and IR spectra were almost identical to those of **K15**. Its ^{1}H NMR spectrum **(Figure 37) (Table 64)** was similar to that of **K15** except for the replacement of the methoxyl signal in **K15** with signals for an aminohydroxyethyl moiety [δ 10.85 (*brs*, 18-NH), 3.89 (*t*, J = 5.4 Hz, 2H) and 3.54 (*q*, J = 5.4 Hz, 2H)]. The attachment of this unit at C-13 (δ 171.50) was confirmed by a HMBC correlation of H_2-18 (δ 3.54) with C-13 **(Table 65)**. Consequently, **K16** was identified as an aminoalcohol derivative of **K15**.

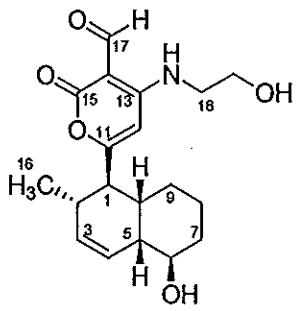


Table 64 The ^{1}H, ^{13}C NMR and HMBC data of compound **K16** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1	2.24 (*dd*, 12.0, 9.9)	48.48 (CH)	C-2, C-5, C-10, C-11, C-12, C-16
2	2.63 (*m*)	34.58 (CH)	
3	5.62 (*d*, 10.2)	132.47 (CH)	C-1, C-2, C-5, C-16
4	6.04 (*ddd*, 10.2, 4.8, 2.4)	128.25 (CH)	C-2, C-5, C-10
5	2.02 (*m*)	44.16 (CH)	C-1, C-3, C-4, C-6, C-10
6	3.46 (*m*)	73.79 (CH)	-
7	a: 1.96 (*m*)	35.40 (CH_2)	C-6, C-8, C-9
	b: 1.30 (*m*)		
8	1.46 (*m*)	27.87 (CH_2)	C-7, C-9
9	a: 1.46 (*m*)	20.14 (CH_2)	C-7, C-8
	b: 1.32 (*m*)		
10	2.41 (*m*)	36.28 (CH)	C-1, C-5
11	-	175.00 (C)	-
12	5.97 (*s*)	96.06 (CH)	C-1, C-11, C-13, C-14, C-17
13	-	171.50 (C)	-
14	-	94.87 (C)	-
15	-	160.50 (C)	-
16	0.98 (*d*, 6.9)	20.21 (CH_3)	C-1, C-2, C-3
17	9.98 (*s*)	191.32 (CH)	C-12, C-14, C-15
18	3.54 (*q*, 5.4)	44.91 (CH_2)	C-13, C-19
18-NH	10.85 (*brs*)	-	-
19	3.89 (*t*, 5.4)	61.09 (CH_2)	C-18

Table 65 The COSY and NOEDIFF data of compound **K16** in $CDCl_3$

Proton	COSY	NOEDIFF
H-1	H-2, H-10	H-12, H_3-16
H-2	H-1, H-3, H_3-16	H-3, H-10, H-12

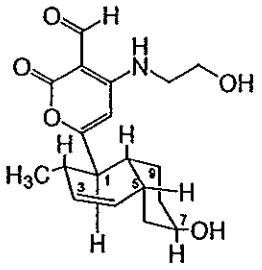
Table 65 Continued

Proton	COSY	NOEDIFF
H-3	H-2, H-4, H-5	H-2, H-4, H_3-16
H-5	H-3, H-4, H-6, H-10	H-4, H-10
H-6	H-5, H_{ab}-7	-
H_a-7	H-6, H_b-7, H_2-8	-
H_b-7	H-6, H_a-7, H_2-8	-
H_2-8	H-7, H_{ab}-9	-
H_a-9	H_2-8, H-10	-
H_b-9	H_2-8, H-10	-
H-10	H-1, H-5, H_{ab}-9	H-5, H-12
H-12	-	H-1, H_2-18
H_3-16	H-2	H-1, H-2, H-3
H_2-18	H_2-19	H_2-19
H_2-19	H_2-18	H_2-18

2.3.4 Compound K17

Compound **K17** was obtained as a colorless gum with the molecular formula identical to that of **K16**. Its UV, IR and ^{1}H NMR spectra **(Figure 40) (Table 66)** were almost identical to those of **K16**. Furthermore, compounds **K16** and **K17** consisted of the same number and types of carbons **(Figure 41) (Table 66)**. The differences were found in the ^{1}H-^{1}H COSY spectrum **(Table 67)**. A hydroxymethine proton (δ 4.10, *m*, H-7) was correlated with H_2-6 [δ 1.81 (*dm*, J = 14.0 Hz, H_a-6) and 1.36 (*td*, J = 14.0 and 2.5 Hz, H_b-6)] and H_2-8 (δ 1.55, *m*), but not with H-5 as found in **K16**. These data established the attachment of the hydroxymethine proton at C-7 (δ 65.64), not at C-6. Irradiation of H-5 (δ 2.59, *m*) affected signal intensity of H-10 (δ 2.29, *m*), but not H-7, in the NOEDIFF experiment **(Table 67)**, established a *cis* ring fusion of the decalin skeleton and the presence of 7-OH at axial position. The appearance of H-5 at much lower field and that of C-5 at much higher field than those observed in compounds **K15** and **K16** due to Van der Waals repulsion (a 1,3-diaxial

interaction) supported the assigned location of 7-OH. Thus, **K17** differed from **K16** in the location of the hydroxyl group in the decalin skeleton. Consequently, **K17** was identified as a new solanapyrone derivative.



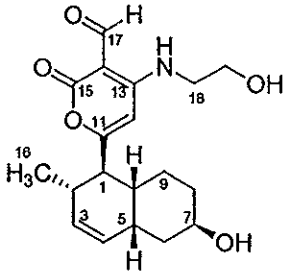


Table 66 The ^{1}H, ^{13}C NMR and HMBC data of compound **K17** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1	2.29 (*m*)	46.84 (CH)	C-10, C-11, C-16
2	2.59 (*m*)	34.70 (CH)	C-1
3	5.49 (*dt*, 10.0, 1.5)	130.95 (CH)	C-1, C-2, C-5, C-16
4	5.64 (*m*)	131.15 (CH)	C-3
5	2.59 (*m*)	30.37 (CH)	C-1
6	a: 1.81 (*dm*, 14.0)	36.12 (CH_2)	C-5
	b: 1.36 (*td*, 14.0, 2.5)		
7	4.10 (*m*)	65.64 (CH)	-
8	1.55 (*m*)	27.72 (CH_2)	-
9	a: 1.93 (*tm*, 13.0)	21.50 (CH_2)	C-1
	b: 1.28 (*m*)		
10	2.29 (*m*)	35.25 (CH)	C-1
11	-	175.00 (C)	-
12	5.98 (*s*)	95.57 (CH)	C-1, C-13, C-14
13	-	172.50 (C)	-
14	-	94.90 (C)	-
15	-	160.61 (C)	-
16	0.97 (*d*, 7.0)	20.27 (CH_3)	C-1, C-2, C-3
17	10.00 (*s*)	191.36 (CH)	C-13, C-14, C-15
18	3.52 (*q*, 5.5)	44.83 (CH_2)	C-19

Table 66 Continued

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
18-NH	10.87 (*brt*, 1.5)	-	-
19	3.90 (*t*, 5.5)	61.18 (CH_2)	C-18

Table 67 The COSY and NOEDIFF data of compound **K17** in $CDCl_3$

Proton	COSY	NOEDIFF
H-1	H-2, H-10	H-12, H_3-16
H-2	H-1, H-3, H_3-16	-
H-3	H-2, H-4	-
H-4	H-3, H-5	-
H-5	H-4, H_b-6, H-10	H-10
H_a-6	H_b-6, H-7	-
H_b-6	H-5, H_a-6	-
H-7	H_a-6, H_2-8	-
H_2-8	H-7, H_a-9	-
H_a-9	H_2-8, H_b-9, H-10	-
H_b-9	H_2-8, H_a-9, H-10	-
H-10	H-1, H-5, H_{ab}-9	H-5, H-12
H-12	-	H-1, H-10, H_2-18
H_3-16	H-2	-
H_2-18	H_2-19	H-12, H_2-19
H_2-19	H_2-18	H_2-18

2.3.5 Compound K18

Compound **K18** with the molecular formula $C_8H_8O_4$ from EIMS (*m/z* 168) **(Figure 42)** was obtained as a colorless gum. Its UV spectrum was similar to that of **K9** while the IR spectrum showed an additional absorption band of a conjugated ketone carbonyl at 1698 cm^{-1}. Its ^1H NMR spectrum **(Figure 43) (Table 68)** was similar to that of **K9** except that signals for the 1-methyl-1-propenyl unit in

K9 were replaced with a methyl signal of an acetyl group (δ 2.53, *s*, 3H). The presence of the acetyl substituent was supported by signals of ketone carbonyl and methyl carbons at δ 192.00 and 26.10, respectively, in the ^{13}C NMR spectrum **(Figure 44) (Table 68)**. The attachment of this unit at C-6 (δ 154.50) was confirmed by a HMBC correlation of H_3-8 (δ 2.53) with C-6. Consequently, **K18** was assigned as a new pyrone derivative.

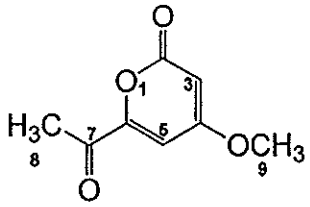


Table 68 The NMR data of compound **K18** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC	NOEDIFF
2	-	161.50 (C)	-	-
3	5.72 (*d*, 2.4)	93.20 (CH)	C-2, C-4, C-5	H_3-9
4	-	169.80 (C)	-	-
5	6.67 (*d*, 2.4)	103.90 (CH)	C-3, C-4, C-6, C-7	-
6	-	154.50 (C)	-	-
7	-	192.00 (C)	-	-
8	2.53 (*s*)	26.10 (CH_3)	C-6, C-7	-
9	3.87 (*s*)	56.00 (CH_3)	C-4	H-3

2.3.6 Compound K19

Compound **K19** was obtained as a colorless gum. The UV spectrum showed an absorption band at 206 nm while hydroxyl and carbonyl absorption bands were observed at 3400 and 1723 cm^{-1}, respectively, in the IR spectrum. The ^1H NMR spectrum **(Figure 45) (Table 69)** consisted of signals for a *trans*-1-propenyl unit [δ 5.97 (*dqd*, J = 15.3, 6.6 and 0.9 Hz, 1H), 5.77 (*ddq*, J = 15.3, 7.5 and 1.5 Hz, 1H) and 1.80 (*ddd*, J = 6.6, 1.5 and 0.9 Hz, 3H)], *cis*-olefinic protons [δ 6.99 (*dd*, J = 9.6 and

5.4 Hz, 1H) and 6.09 (*d*, *J* = 9.6 Hz, 1H)] and two oxymethine protons [δ 4.97 (*dd*, *J* = 7.5 and 3.3 Hz, 1H) and 4.14 (*dd*, *J* = 5.4 and 3.3 Hz ,1H)]. The ^{13}C NMR **(Figure 46) (Table 69)** and DEPT 135 spectra **(Table 69)** displayed eight carbon resonances for one ester carbonyl (δ 164.10), six methine (δ 145.34, 132.59, 124.22, 122.13, 81.69 and 62.83) and one methyl (δ 17.86) carbons. In the ^{1}H-^{1}H COSY spectrum **(Table 70)**, the oxymethine proton, H-5 (δ 4.14), was coupled with the oxymethine proton, H-6 (δ 4.97), and the olefinic proton, H-4 (δ 6.99), which gave cross peak with the *cis*-olefinic proton, H-3 (δ 6.09). H-3 and H-6 showed a HMBC cross peak with the ester carbonyl carbon, C-2 (δ 164.10). These results indicated that **K19** had a 5,6-dihydropyrone ring. The *trans*-1-propenyl unit was linked at C-6 (δ 81.69) of the dihydropyrone ring on the basis of a HMBC correlation of the *trans*-olefinic proton, H-7 (δ 5.77), with C-6. Irradiation of H-5 in the NOEDIFF spectrum **(Table 70)**, affected signal intensity of H-6, indicating their *cis*-relationship. The observed optical rotation of **K19**, $[\alpha]_D^{23}$ +163 (*c* 0.60, EtOH), was similar to that of (5*R*,6*R*)-(+)-phomalactone, $[\alpha]_D^{20}$ +172 (*c* 0.60, EtOH), indicating that they had the same absolute configuration. Therefore, **K19** was determined as (5*R*,6*R*)-(+)-phomalactone which was previously isolated from the fungus *Nigrospora sacchari* (Yang, *et al.*, 1997).

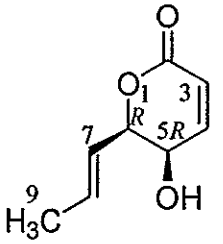


Table 69 The ^{1}H and ^{13}C NMR data of compound **K19** in $CDCl_3$+CD_3OD and **(5*R*,6*R*)-(+)-Phomalactone** in $CDCl_3$

Position	K19		(5*R*,6*R*)-(+)-Phomalactone
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})
2	-	164.10 (C)	-
3	6.09 (*d*, 9.6)	122.13 (CH)	6.13 (*d*, 9.7)
4	6.99 (*dd*, 9.6, 5.4)	145.34 (CH)	6.99 (*dd*, 9.7, 5.3)
5	4.14 (*dd*, 5.4, 3.3)	62.83 (CH)	4.20 (*dd*, 5.3, 3.1)

Table 69 Continued

Position	K19		(5*R*,6*R*)-(+)-Phomalactone
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})
6	4.97 (*dd*, 7.5, 3.3)	81.69 (CH)	4.83 (*dd*, 7.0, 3.1)
7	5.77 (*ddq*, 15.3, 7.5, 1.5)	124.22 (CH)	5.76 (*m*)
8	5.97 (*dqd*, 15.3, 6.6, 0.9)	132.59 (CH)	6.01 (*m*)
9	1.80 (*ddd*, 6.6, 1.5, 0.9)	17.86 (CH_3)	1.81 (*dd*, 6.5, 1.2)

Table 70 The HMBC, COSY and NOEDIFF of compound **K19** in $CDCl_3$+CD_3OD

Proton	HMBC	COSY	NOEDIFF
H-3	C-2, C-4, C-5	H-4	H-4
H-4	C-2, C-3, C-5, C-6, C-7	H-3, H-5	H-3, H-5
H-5	C-3, C-4, C-6	H-4, H-6	H-4, H-6
H-6	C-2, C-4, C-5, C-7, C-8	H-5, H-7, H_3-9	H-5, H-7, H-8
H-7	C-6, C-8, C-9	H-6, H-8, H_3-9	H-6, H_3-9
H-8	C-6, C-7, C-9	H-6, H-7, H_3-9	H-6, H_3-9
H_3-9	C-5, C-6, C-7, C-8	H-6, H-7, H-8	H-7, H-8

2.3.7 Compound K20

Compound **K20** was obtained as a colorless gum. It exhibited an UV absorption band at 205 nm. The IR spectrum showed absorption bands at 3350 cm^{-1} and 1766 cm^{-1} for hydroxyl and ester carbonyl groups, respectively. The ^1H NMR spectrum **(Figure 47) (Table 71)** consisted of signals for a *trans*-1-hydroxy-2-butenyl unit [δ 5.64 (*dq*, J = 15.6 and 6.6 Hz, 1H), 5.32 (*dd*, J = 15.6 and 6.9 Hz, 1H), 3.92 (*t*, J = 6.9 Hz, 1H) and 1.54 (*d*, J = 6.6 Hz, 3H)], one oxymethine proton (δ 4.28, *q*, J = 6.9 Hz, 1H) and two sets of methylene protons [δ 2.36 (*m*, 2H) and 2.03 (*m*, 1H)/1.91 (*m*, 1H)]. In the ^1H-^1H COSY spectrum **(Table 72)**, the methylene protons, H_{ab}-4 (δ 2.03 and 1.91), showed cross peaks with the methylene protons, H_2-3 (δ 2.36), and the oxymethine proton, H-5 (δ 4.28). H_2-3 and H-5 were correlated with the same

ester carbonyl carbon, C-2 (δ 178.05), in the HMBC spectrum **(Table 72)**, thus constructing a γ-lactone ring. The *trans*-1-hydroxy-2-butenyl unit was located at C-5 (δ 83.08) on the basis of a HMBC correlation of the oxymethine proton, H-6 (δ 3.92), of the hydroxybutenyl unit with C-5 of the lactone ring. The observed optical rotation of **K20**, $[\alpha]_D^{25}$ +49 (*c* 0.760, $CHCl_3$), was almost identical to that of 5-(*S*)-[1-(1(*S*)-hydroxybut-2-enyl)]dihydrofuran-2-one, $[\alpha]_D^{25}$ +51.2 (*c* 0.760, $CHCl_3$), indicating that they had the same absolute configuration at C-5 and C-6. Therefore, **K20** was identified as 5-(*S*)-[1-(1(*S*)-hydroxybut-2-enyl)]dihydrofuran-2-one which was previously isolated from the fungus *Nigrospora sacchari* (Fukushima, *et al.*, 1998).

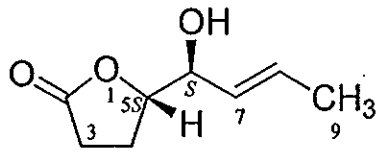


Table 71 The ^1H and ^{13}C NMR data of compound **K20** and **5-(*S*)-[1-(1(*S*)-Hydroxybut-2-enyl)]dihydrofuran-2-one** in $CDCl_3$

Position	K20		5-(*S*)-[1-(1(*S*)-Hydroxybut-2-enyl)]dihydrofuran-2-one	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
2	-	178.05 (C)	-	177.1 (C)
3	2.36 (*m*)	28.39 (CH_2)	a: 2.61 (*ddd*, 17.9, 9.9, 6.0) b: 2.52 (*ddd*, 17.9, 9.4, 8.4)	28.6 (CH_2)
4	a: 2.03 (*m*) b: 1.91 (*m*)	23.45 (CH_2)	a: 2.23 (*dddd*, 13.1, 9.4, 7.2, 6.0) b: 2.08 (*dddd*, 13.1, 9.9, 8.4, 7.2)	23.9 (CH_2)
5	4.28 (*q*, 6.9)	83.08 (CH)	4.44 (*td*, 7.2, 5.5)	82.8 (CH)
6	3.92 (*t*, 6.9)	74.13 (CH)	4.09 (*brt*, 6.0)	75.1 (CH)

Table 71 Continued

Position	K20		5-(*S*)-[1-(1(*S*)-Hydroxybut-2-enyl)]dihydrofuran-2-one	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
7	5.32 (*dd*, 15.6, 6.9)	128.35 (CH)	5.52 (*ddq*, 15.4, 7.2, 1.7)	128.0 (CH)
8	5.64 (*dq*, 15.6, 6.6)	129.66 (CH)	5.86 (*dqd*, 15.4, 6.5, 0.9)	131.1 (CH)
9	1.54 (*d*, 6.6)	17.70 (CH_3)	1.74 (*dd*, 6.5, 1.7)	18.0 (CH_3)

Table 72 The HMBC, COSY and NOEDIFF data of compound **K20** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H_2-3	C-2, C-4, C-5	H_{ab}-4	-
H_a-4	C-2, C-3, C-5, C-6	H_2-3, H-5	H-5, H-6
H_b-4	C-2, C-3, C-5, C-6	H_2-3, H-5	H-5, H-6
H-5	C-2, C-3, C-4, C-7	H_{ab}-4, H-6	H_{ab}-4, H-6
H-6	C-4, C-5, C-7, C-8	H-5, H-7	H_{ab}-4, H-5, H-8
H-7	C-5, C-6, C-8, C-9	H-6, H-8	H-5, H_3-9
H-8	C-5, C-6, C-7, C-9	H-7, H_3-9	H_{ab}-4, H-5, H-6, H_3-9
H_3-9	C-5, C-6, C-7, C-8	H-8	H-7, H-8

2.3.8 Compound K21

Compound **K21** was obtained as a colorless gum. Its UV and IR data were almost identical to those of **K20**. The ^{1}H NMR spectrum **(Figure 49) (Table 73)** was similar to that of **K20** except for the replacement of signals for methylene protons in **K20** with signal of a hydroxymethine proton (δ 4.67, *ddd*, J = 6.6, 5.1 and 3.0 Hz, 1H). The presence of one methylene carbon (δ 39.16) and one oxymethine carbon (δ 69.18) of the γ-lactone unit in the ^{13}C NMR **(Figure 50) (Table 73)** and DEPT 135

spectra **(Table 73)** supported above conclusion. This methine proton was attributed to H-4 according to its ^{1}H-^{1}H COSY correlation with H_{ab}-3 (δ 2.82 and 2.63) and H-5 (δ 4.37). Irradiation of H-4 (δ 4.67) in the NOEDIFF spectrum **(Table 74)** did affect signal intensity of H-5, indicating their *cis*-relationship. The observed optical rotation of **K21**, $[\alpha]_D^{25}$ +8, *c* 0.32, $CHCl_3$), was similar to that of (4*R*,5*S*,6*S*)-musacin F, $[\alpha]_D^{20}$ +10.28, *c* 0.32, $CHCl_3$), suggesting that they had the same absolute configuration. Therefore, **K21** was identified as (4*R*,5*S*,6*S*)-musacin F which was previously isolated from the fungus *Streptomyces griseoviridis* DSM 7429 (Grabley, *et al.*, 1994).

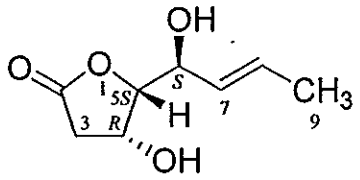


Table 73 The ^{1}H and ^{13}C NMR data of compound **K21** and **(4*R*,5*S*,6*S*)-Musacin F** in $CDCl_3$

Position	K21		(4*R*,5*S*,6*S*)-Musacin F	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
2	-	175.02 (C)	-	179.1 (C)
3	a: 2.63 (*dd*, 17.7, 3.0) b: 2.82 (*dd*, 17.7, 6.6)	39.16 (CH_2)	a: 2.30 (*dd*, 18.0, 1.5) b: 2.89 (*dd*, 18.0, 7.2)	39.5 (CH)
4	4.67 (*ddd*, 6.6, 5.1, 3.0)	69.18 (CH)	4.45 (*ddd*, 7.0, 1.5, 1.5)	68.0 (CH)
5	4.37 (*dd*, 5.1, 4.2)	84.51 (CH)	4.29 (*dd*, 3.0, 1.5)	92.4 (CH)
6	4.58 (*dd*, 7.2, 4.2)	71.70 (CH)	4.24 (*m*)	72.7 (CH)
7	5.68 (*ddq*, 15.3, 7.2, 1.5)	128.16 (CH)	5.53 (*ddq*, 15.5, 6.0, 1.5)	130.0 (CH)
8	5.91 (*dqd*, 15.3, 6.3, 0.6)	131.10 (CH)	5.86 (*dqd*, 15.5, 6.5, 1.5)	130.0 (CH)
9	1.76 (*ddd*, 6.3, 1.5, 0.6)	17.84 (CH_3)	1.74 (*ddd*, 6.5, 1.5, 1.5)	18.1 (CH_3)

Table 74 The HMBC, COSY and NOEDIFF data of compound **K21** in $CDCl_3$

Proton	HMBC	COSY	NOE
H_a-3	C-2, C-4, C-5	H_b-3, H-4	H-4
H_b-3	C-2, C-4, C-5	H_a-3, H-4	H-4
H-4	C-5	H_{ab}-3, H-5	H_{ab}-3, H-5
H-5	C-2, C-4, C-7	H-4, H-6	H-4, H-6
H-6	C-7, C-8	H-5, H-7	H-4
H-7	C-8, C-9	H-6, H-8, H_3-9	H-6, H_3-9
H-8	C-6, C-7, C-9	H-6, H-7, H_3-9	H-6, H_3-9
H_3-9	C-7, C-8	H-6, H-7, H-8	H-7, H-8

2.3.9 Compound K22

Compound **K22** was obtained as a colorless gum. The UV spectrum showed absorption bands at 204, 222 and 278 nm, indicating the presence of an aromatic chromophore. The IR spectrum displayed an absorption band at 3391 cm^{-1} for a hydroxyl group. The ^1H NMR spectrum **(Figure 51) (Table 75)** exhibited characteristic signals of four aromatic protons of a 1,4-disubstituted benzene [δ 7.11 (*d*, J = 9.0 Hz, 2H) and 6.79 (*d*, J = 9.0 Hz, 2H)] and a hydroxyethyl group [δ 3.83 (*t*, J = 6.0 Hz, 2H) and 2.81 (*d*, J = 6.0 Hz, 2H)]. These data together with the chemical shift of C-1 (δ 154.22) established the other substituent of the 1,4-disubstituted benzene to be a hydroxyl group. HMBC data **(Table 75)** supported the assigned structure. Therefore, **K22** was identified as tyrosol which was previously isolated from the fungus *Gloeophyllum* sp. (Rasser, *et al.*, 2000).

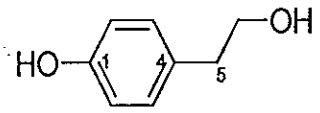


Table 75 The NMR data of compound **K22** in $CDCl_3$ and **Tyrosol** in CD_3OD

Position	K22				Tyrosol
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC	COSY	δ_H (*mult.*, J_{Hz})
1	-	154.22 (C)	-	-	-
2	6.79 (*d*, 9.0)	115.45 (CH)	C-1, C-3	H-3	6.70 (*d*, 8.4)
3	7.11 (*d*, 9.0)	130.15 (CH)	C-1, C-2, C-5	H-2	6.99 (*d*, 8.4)
4	-	130.55 (C)	-	-	-
5	2.81 (*t*, 6.0)	38.27 (CH_2)	C-3, C-6	H-6	2.79 (*t*, 6.8)
6	3.83 (*t*, 6.0)	63.80 (CH_2)	C-4, C-5	H-5	3.70 (*t*, 6.8)

PART III

METABOLITES FROM THE MARINE-DERIVED FUNGUS *PENICILLIUM* SP. PSU-F44

CHAPTER 3.1

INTRODUCTION

3.1.1 Introduction

The fungi in the genus *Penicillium* have produced a variety of compounds, for example, polyketide-terpenoid (Stierle, *et al.*, 2008), alkaloid, (Ge, *et al.*, 2008), lactone (Li, *et al.*, 2007) and quinone derivatives (Li, *et al.*, 2006). Some of these exhibit a wide range of biological and pharmacological activities, e.g., cytotoxic penicidones (Ge, *et al.*, 2008), antifungal botryodiplodin (Cabedo, *et al.*, 2007), antibacterial rugulotrosins (Stewart, *et al.*, 2004) and antioxidant pennicitrinones (Lu, *et al.*, 2008). Metabolites isolated from the genus *Penicillium* reported since the year 2009 are summarized in **Table 76** based on SciFinder Scholar Database. Investigation of bioactive compounds from these fungi is therefore of interest. The EtOAc extract from the culture broth of *Penicillium* sp. PSU-F44 isolated from a sea fan *Annella* sp., exhibited interesting antibacterial activity against SA, MRSA and a clinical isolate of *Microsporum gypseum* (MG) SH-MU-4 with MIC values of 64, 320, and 160 μg/mL, respectively.

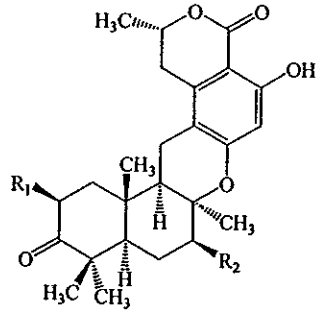
Table 76 Compounds isolated from the *Penicillium* genus

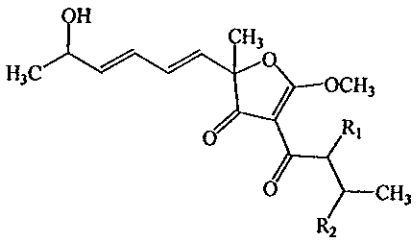
Scientific name	Compound	Activity	Reference
P. cecidicola FKI-3765-1	Pentacecilide A, **6** Pentacecilide B, **7** Pentacecilide C, **8**	Inhibitor of lipid droplet formation in mouse macrophages	Yamazaki, *et al.*, 2009
P. daleae K.M. Zalessky	Penicilliol A, **9** Penicilliol B, **10**	Inhibitor of eukaryotic Y-family DNA polymerases	Kimura, *et al.*, 2009

Table 76 Continued

Scientific name	Compound	Activity	Reference
Penicillium sp. NBRC 103941	JBIR-12, **11**	Antioxidant	Izumikawa, *et al.*, 2009
Penicillium sp. strain HLKG-44	Brefeldin A formylate, **12** Brefeldin A, **13** Ergosterol, **14**	Cytotoxic	Zhang, *et al.*, 2009

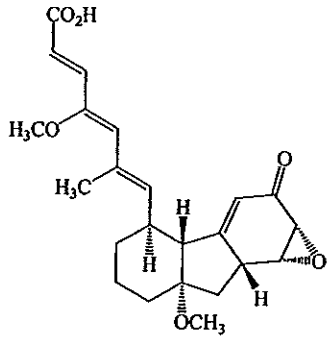
Structures of compounds isolated from the *Penicillium* genus

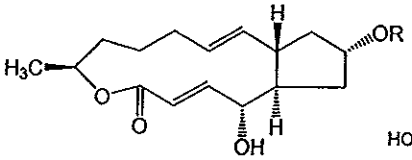


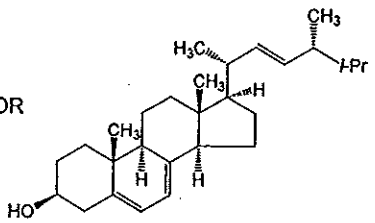


6: $R_1 = R_2 = H$: Pentacecilide A
7: $R_1 = OAc$, $R_2 = H$: Pentacecilide B
8: $R_1 = OAc$, $R_2 = OH$: Pentacecilide C

9: $R_1 + R_2$ = double bond : Penicilliol A
10: $R_1 = H$, $R_2 = OH$: Penicilliol B







11: JBIR-12

12: R = CHO :Brefeldin A formylate
13: R = H :Brefeldin A

14: Ergosterol

3.1.2 The objectives

1. To isolate the secondary metabolites from the marine-derived fungus *Penicillium* sp. PSU-F44.

2. To elucidate the structure of the isolated metabolites.

CHAPTER 3.2

EXPERIMENTAL

3.2.1 Fermentation and extraction

The fermentation and the extraction of the culture broth (15 L) and mycelia were performed using the same procedure as those of *Nigrospora* sp. PSU-F5 to afford a brown gum (1.80 g) and a dark brown gum (320 mg) from the culture broth and mycelia, respectively. Each extract was subjected to chromatographic separation.

3.2.2 Purification of the broth extract

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six fractions as shown in **Table 77.**

Table 77 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
44A	24.7	Brown gum
44B	150.8	Brown gum
44C	700.0	Brown gum
44D	501.2	Brown gum
44E	111.7	Brown gum
44F	286.2	Colorless gum

Fraction 44A displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 44B showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.24, 0.46, 0.72, 0.80, 0.86 and 0.94. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 78.**

Table 78 Subfractions obtained from **fraction 44B** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44B1	50% MeOH/H_2O	16.7	Brown gum
44B2	50% MeOH/H_2O	19.5	Brown gum
44B3	50% MeOH/H_2O	13.6	Brown gum
44B4	50% MeOH/H_2O	18.5	Brown gum
44B5	70% MeOH/H_2O	13.6	Brown gum
44B6	70% MeOH/H_2O-100% MeOH	61.9	Brown gum

Subfraction 44B1 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44B2 showed three pale UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.02, 0.07 and 0.16. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44B3 showed three pale UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.03, 0.06 and 0.08. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44B4 showed three pale UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.14 and 0.25. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44B5 showed none of UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase but showed many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not purified.

Subfraction 44B6 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 44C showed ten UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.11, 0.21, 0.28, 0.32, 0.37, 0.48, 0.56, 0.67, 0.81 and 0.96. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 79**.

Table 79 Subfractions obtained from **fraction 44C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44C1	2% MeOH/CH_2Cl_2	16.7	Brown gum
44C2	2% MeOH/CH_2Cl_2	53.9	Brown gum

Table 79 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
44C3	4% MeOH/CH_2Cl_2	47.9	Brown gum
44C4	4-10% MeOH/CH_2Cl_2	23.4	Brown gum
44C5	10-20% MeOH/CH_2Cl_2	18.5	Brown gum
44C6	30-50% MeOH/CH_2Cl_2	54.5	Brown gum
44C7	70-100% MeOH	126.1	Brown gum
44C8	100% MeOH	157.2	Yellow solid

Subfraction 44C1 showed five UV-active spots on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.24, 0.48, 0.55, 0.62 and 0.83. It was further separated by column chromatography over silica gel. Elution was performed initially with 70% dichloromethane in petroleum ether and gradually enriched with dichloromethane and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 80.**

Table 80 Subfractions obtained from **subfraction 44C1** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44C1A	70% CH_2Cl_2/Petrol	5.0	Yellow gum
44C1B	70-80% CH_2Cl_2/Petrol	4.0	Yellow gum
44C1C	80% CH_2Cl_2/Petrol	3.2	Yellow gum
44C1D	80% CH_2Cl_2/Petrol-100% MeOH	4.0	Yellow gum

Subfraction 44C1A showed three pale UV-active spots on normal phase TLC using 30% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.30, 0.40 and 0.50. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44C1B showed four pale UV-active spots on normal phase TLC using 30% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.31, 0.48, 0.51 and 0.53. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated

Subfraction 44C1C showed two UV-active spots on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.18 and 0.39. It was then purified by precoated TLC with 50% dichloromethane in petroleum ether as a mobile phase (7 runs) to afford two bands.

Band 1 (K23) was a colorless gum (2.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.18.

$[\alpha]_D^{29}$	+51 (*c* 0.065, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	244 (3.51), 269 (3.50)
FTIR(neat):υ(cm^{-1})	3423 (O-H stretching), 1713 (C=O stretching), 1653 (C=C stretching)
^1H NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (300 MHz):	5.75 (*s*, 1H), 5.52 (*dq*, *J* = 15.0, 6.5 Hz, 1H), 5.22 (*ddq*, *J* = 15.0, 9.0, 1.5 Hz, 1H), 4.16 (*t*, *J* = 9.0 Hz, 1H), 3.17 (*t*, *J* = 9.0 Hz, 1H), 2.61 (*d*, *J* = 18.0 Hz, 1H), 2.55 (*m*, 1H), 2.47 (*dd*, *J* = 18.0, 6.5 Hz, 1H), 2.21 (*s*, 3H), 2.18 (*m*, 1H), 1.67 (*dd*, *J* = 6.5, 1.5 Hz, 3H), 1.52 (*s*, 3H)
^{13}C NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (75 MHz):	163.09, 160.55, 129.76, 128.54, 109.22, 100.48, 94.10, 72.50, 45.93, 44.65, 22.34, 19.63, 17.84, 17.79
DEPT 135: CH;	129.76, 128.54, 100.48, 44.65, 45.93
CH_2;	72.50, 17.84
CH_3;	22.34, 19.63, 17.79
EIMS *m/z* (% relative intensity):	262 (4), 223 (15), 139 (100), 115 (34)

Band 2 was a colorless gum (0.6 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.38. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44C1D showed three UV-active spots on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.18, 0.30 and 0.49. Its ^1H NMR spectrum indicated that the major compound was **K23**. Further investigation was then not performed.

Subfraction 44C2 showed five UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.13, 0.26, 0.39, 0.45 and 0.53. It was further separated by column chromatography over silica gel. Elution was performed initially with 20% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 81**.

Table 81 Subfractions obtained from **subfraction 44C2** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44C2A	20% EtOAc/Petrol	11.0	Yellow gum
44C2B	20-40% EtOAc/Petrol	13.5	Yellow gum
44C2C	40-60% EtOAc/Petrol	14.9	Yellow gum
44C2D	80% EtOAc/Petrol-100% EtOAc	12.2	Yellow gum
44C2E	100% EtOAc-100% MeOH	4.0	Yellow gum

Subfraction 44C2A showed three UV-active spots on normal phase TLC using 10% acetone in petroleum ether as a mobile phase with the R_f values of 0.23, 0.33 and

0.57. It was then purified by precoated TLC with 10% acetone in petroleum ether as a mobile phase (2 runs) to afford four bands.

Band 1 was a colorless gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% acetone in petroleum ether as a mobile phase with the R_f value of 0.49. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Band 2 was a colorless gum (1.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% acetone in petroleum ether as a mobile phase with the R_f value of 0.36. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Band 3 was a colorless gum (2.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% acetone in petroleum ether as a mobile phase with the R_f value of 0.29. Its ^1H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Band 4 (K25) was a colorless gum (2.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% acetone in petroleum ether as a mobile phase with the R_f value of 0.22.

$[\alpha]_D^{25}$	+28 (*c* 0.09, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	205 (3.75)
FTIR(neat):υ(cm^{-1})	3389 (O-H stretching), 1705 (C=O stretching), 1641 (C=C stretching)
^1H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	7.33 (*dd*, *J* = 15.5, 3.0 Hz, 1H), 5.90 (*dd*, *J* = 15.5, 2.0 Hz, 1H), 5.73 (*ddd*, *J* = 15.0, 10.0, 4.5 Hz, 1H), 5.19 (*dd*, *J* = 15.0, 9.5 Hz, 1H), 4.91 (*m*, 1H), 4.08 (*brd*, *J* = 9.5 Hz, 1H), 2.25 (*qn*, *J* = 9.0 Hz, 1H), 2.01 (*m*,

	2H), 1.85 (*m*, 3H), 1.74 (*m*, 1H), 1.62 (*m*, 3H), 1.58 (*m*, 1H), 1.53 (*m*, 1H), 1.39 (*m*, 1H), 1.26 (*d*, J = 6.0 Hz, 3H), 0.94 (*m*, 1H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	166.27, 151.86, 136.32, 130.33, 117.38, 76.06, 71.65, 54.06, 46.93, 35.15, 34.12, 31.92, 31.85, 26.78, 25.24, 20.86
DEPT 135: CH;	151.86, 136.32, 130.33, 117.38, 76.06, 71.65, 54.06, 46.93
CH_2;	35.15, 34.12, 31.92, 31.85, 26.78, 25.24
CH_3;	20.86

Subfraction 44C2B showed four UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.05, 0.14, 0.21 and 0.26 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 44C2C showed six UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.31, 0.38, 0.40, 0.47, 0.52 and 0.60 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 44C2D showed six UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.13, 0.21, 0.23, 0.25, 0.40 and 0.47 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 44C2E displayed a long tail under UV-S on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 44C3 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.27, 0.32, 0.37 and 0.60. It was further separated by column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 82.**

Table 82 Subfractions obtained from **subfraction 44C3** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44C3A	1% MeOH/CH_2Cl_2	3.7	Yellow gum
44C3B	2-4% MeOH/CH_2Cl_2	5.0	Yellow gum
44C3C	4% MeOH/CH_2Cl_2	7.3	Yellow gum
44C3D	4-10% MeOH/CH_2Cl_2	17.4	Yellow gum
44C3E	10% MeOH/CH_2Cl_2-100% MeOH	13.5	Yellow gum

Subfraction 44C3A showed none of UV-active spots under UV-S on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 44C3B showed four UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.15, 0.30, 0.36 and 0.45. Because of low quantity, it was not further investigated.

Subfraction 44C3C showed two UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.05 and 0.53. It was then purified by precoated TLC with 20% acetone in petroleum ether as a mobile phase (4 runs) to afford two bands.

Band 1 (K27) was a colorless gum (3.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% acetone in petroleum ether as a mobile phase with the R_f value of 0.20.

$[\alpha]_D^{29}$	+80 (*c* 0.26, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	208 (4.36)
FTIR(neat):υ(cm^{-1})	3424 (O-H stretching), 1710 and 1698 (C=O stretching), 1649 (C=C stretching)
^{1}H NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (300 MHz):	7.39 (*dd*, *J* = 15.6, 3.6 Hz, 1H), 5.96 (*dd*, *J* = 15.6, 1.8 Hz, 1H), 5.81 (*ddd*, *J* = 15.0, 9.6, 5.4 Hz, 1H), 5.21 (*dd*, *J* = 15.0, 9.0 Hz, 1H), 4.91 (*m*, 1H), 4.21 (*brd*, *J* = 8.1 Hz, 1H), 2.83 (*dd*, *J* = 15.6, 7.8 Hz, 1H), 2.69 (*m*, 1H), 2.53 (*dd*, *J* = 18.6, 8.4 Hz, 1H), 2.22 (*dd*, *J* = 15.6, 9.0 Hz, 1H), 2.10 (*m*, 3H), 1.89 (*m*, 1H), 1.75 (*m*, 2H), 1.55 (*m*, 1H), 1.28 (*d*, *J* = 6.3 Hz, 3H), 1.06 (*m*, 1H)
^{13}C NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (75 MHz):	215.90, 166.08, 150.55, 135.16, 132.28, 118.35, 76.55, 71.72, 49.88, 46.56, 44.87, 42.48, 34.31, 31.57, 26.40, 20.66
DEPT 135: CH;	150.55, 135.16, 132.28, 118.35, 76.55, 71.72, 49.88, 42.4
CH_2;	46.56, 44.87, 34.31, 31.57, 26.40,
CH_3;	20.66

Band 2 was a colorless gum (0.9 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 20% acetone in petroleum ether as a mobile phase with the R_f value of 0.13. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44C3D showed four UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.02,

0.15, 0.50 and 0.53. Its ^{1}H NMR spectrum indicated that the major compound was **K27**. Further investigation was then not performed.

Subfraction 44C3E showed three UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.06, 0.16 and 0.47. Its ^{1}H NMR spectrum indicated that the major compound was **K27**. Further investigation was then not performed.

Subfraction 44C4 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.15, 0.29 and 0.37. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 83**.

Table 83 Subfractions obtained from **subfraction 44C4** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44C4A	50% MeOH/H_2O	4.0	Colorless gum
44C4B	50% MeOH/H_2O	5.2	Colorless gum
44C4C	50% MeOH/H_2O	3.7	Colorless gum
44C4D	70% MeOH/H_2O	3.0	Colorless gum
44C4E	70% MeOH/H_2O	2.1	Colorless gum
44C4F	70% MeOH/H_2O-100% MeOH	4.9	Colorless gum

Subfraction 44C4A showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.11, 0.16, 0.23, 0.29 and 0.34. Because of low quantity, it was not further investigated.

Subfraction 44C4B showed six UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.11, 0.23, 0.29, 0.31 and 0.34. Because of low quantity, it was not further investigated.

Subfraction 44C4C showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.23, 0.29, 0.32 and 0.34. Because of low quantity, it was not further investigated.

Subfraction 44C4D showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.13, 0.33 and 0.34. Because of the minute quantity, it was not further investigated.

Subfraction 44C4E showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.23. Its ^1H NMR spectrum indicated the presence of **K27**. Further investigation was then not carried out.

Subfraction 44C4F displayed a long tail under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44C5 showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.10, 0.27 and 0.39. Its ^1H NMR spectrum indicated that the major compound was **K26**. Further investigation was then not performed.

Subfraction 44C6 showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.20 and 0.30. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 84**.

Table 84 Subfractions obtained from **subfraction 44C6** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44C6A	4% MeOH/CH_2Cl_2	5.2	Yellow gum
44C6B	4% MeOH/CH_2Cl_2	4.4	Yellow gum
44C6C	4-10% MeOH/CH_2Cl_2	4.1	Yellow gum
44C6D	10-20% MeOH/CH_2Cl_2	9.5	Yellow gum
44C6E	20-40% MeOH/CH_2Cl_2	16.7	Yellow gum
44C6F	40% MeOH/CH_2Cl_2-100% MeOH	12.1	Yellow gum

Subfraction 44C6A showed none of UV-active spots under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44C6B showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.39, 0.48 and 0.50. Because of low quantity, it was not further investigated.

Subfraction 44C6C showed four UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.14, 0.19 and 0.26. Because of low quantity, it was not further investigated.

Subfraction 44C6D showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.02, 0.09 and 0.19 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not purified.

Subfraction 44C6E showed five UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.02, 0.09, 0.19, 0.37 and 0.38. It was further separated by column chromatography over

Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four fractions as shown in **Table 85.**

Table 85 Subfractions obtained from **subfraction 44C6E** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
44C6E1	3.1	Yellow gum
44C6E2	7.5	Yellow gum
44C6E3	4.4	Yellow gum
44C6E4	1.5	Yellow gum

Subfraction 44C6E1 showed three pale UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.50, 0.59 and 0.85. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44C6E2 showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.16 and 0.33. It was then purified by precoated TLC with 4% methanol in dichloromethane as a mobile phase (4 runs) to afford two bands.

Band 1 was a yellow gum (3.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.33. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a yellow gum (3.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.16. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 44C6E3 showed three pale UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.33 and 0.39. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44C6E4 displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44C6F showed none of UV-active spots under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44C7 showed four UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.16, 0.29, 0.34 and 0.54. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four fractions as shown in **Table 86.**

Table 86 Subfractions obtained from **subfraction 44C7** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
44C7A	20.1	Brown gum
44C7B	42.1	Brown gum
44C7C	47.4	Brown gum
44C7D	23.1	Brown gum

Subfraction 44C7A displayed a long tail under UV-S on normal phase TLC using 10% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44C7B showed three UV-active spots on normal phase TLC using 10% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.13 and 0.23 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated the presence of sugar as a major compound. Thus, it was not further investigated.

Subfraction 44C7C showed six UV-active spots on normal phase TLC using 10% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.29, 0.32, 0.41, 0.53 and 0.59. Its ^{1}H NMR spectrum indicated the presence of sugar as a major compound. Thus, it was not further investigated.

Subfraction 44C7D showed none of UV-active spots under UV-S on normal phase TLC using 10% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44C8 showed two UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.18 and 0.22. Its ^{1}H NMR spectrum indicated that the major compound was **K26**. Further investigation was then not performed.

Fraction 44D showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.11, 0.21, 0.27, 0.35 and 0.46. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 87**.

Table 87 Subfractions obtained from **fraction 44D** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44D1	2% MeOH/CH_2Cl_2	9.2	Yellow gum
44D2	2% MeOH/CH_2Cl_2	5.7	Yellow gum
44D3	2-4% MeOH/CH_2Cl_2	141.2	Brown gum
44D4	4-10% MeOH/CH_2Cl_2	35.5	Brown gum
44D5	10-20% MeOH/CH_2Cl_2	144	Brown gum
44D6	20% MeOH/CH_2Cl_2-100% MeOH	152.6	Brown gum

Subfraction 44D1 showed five UV-active spots on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.08, 0.20, 0.30, 0.50 and 0.70 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not purified.

Subfraction 44D2 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.19, 0.25, 0.34 and 0.47. It was then purified by precoated TLC with 100% dichloromethane as a mobile phase (6 runs) to afford three bands.

Band 1 was a colorless gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.48. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a colorless gum (1.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.34. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was a colorless gum (0.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.19. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 44D3 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.25, 0.33, 0.50 and 0.55. It was further separated by column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 88.**

Table 88 Subfractions obtained from **subfraction 44D3** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44D3A	1% $MeOH/CH_2Cl_2$	9.7	Brown gum
44D3B	1% $MeOH/CH_2Cl_2$	68.7	Brown gum
44D3C	3-5% $MeOH/CH_2Cl_2$	28.1	Brown gum
44D3D	5% $MeOH/CH_2Cl_2$-100% MeOH	30.0	Brown gum

Subfraction 44D3A showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.34, 0.41, 0.53 and 0.61 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not purified.

Subfraction 44D3B showed two UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.30 and 0.32. Its ^1H NMR spectrum indicated the presence of sugar as a major compound. Thus, it was not further investigated.

Subfraction 44D3C showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.17, 0.32 and 0.48. It was further separated by column chromatography over silica gel. Elution was performed initially with 40% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 89.**

Table 89 Subfractions obtained from **subfraction 44D3C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44D3C1	40% EtOAc/Petrol	5.3	Brown gum
44D3C2	40-60% EtOAc/Petrol	6.6	Brown gum
44D3C3	60% EtOAc/Petrol	4.5	Brown gum
44D3C4	80% EtOAc/Petrol	1.3	Brown gum
44D3C5	80% EtOAc/Petrol	5.0	Yellow gum
44D3C6	100% EtOAc	1.6	Yellow gum
44D3C7	100% EtOAc-100% MeOH	2.3	Colorless gum

Subfraction 44D3C1 showed four UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.48, 0.58, 0.73 and 0.87. Because of low quantity, it was not further investigated.

Subfraction 44D3C2 showed two UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.33 and 0.40. It was then purified by precoated TLC with 20% ethyl acetate in petroleum ether as a mobile phase (10 runs) to afford two bands.

Band 1 was a yellow gum (2.9 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.15. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a colorless gum (1.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.25. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 44D3C3 showed two UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.28 and 0.31. It was then purified by precoated TLC with 20% ethyl acetate in petroleum ether as a mobile phase (10 runs) to afford two bands.

Band 1 was a yellow gum (1.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.28. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a yellow gum (1.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.32. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 44D3C4 showed three UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.23, 0.30 and 0.50. Because of the minute quantity, it was not further investigated.

Subfraction 44D3C5 showed five UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.24, 0.28, 0.37, 0.42 and 0.51. Because of the minute quantity, it was not further investigated.

Subfraction 44D3C6 showed three UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.03, 0.08 and 0.15. Because of the minute quantity, it was not further investigated.

Subfraction 44D3C7 (K24) showed one spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.15 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate.

$[\alpha]_D^{29}$	+23 (*c* 0.12, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	211 (2.23)
FTIR(neat):υ(cm^{-1})	3414 (O-H stretching), 1769 and 1715 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	4.50 (*t*, *J* = 9.0 Hz, 1H), 4.44 (*t*, *J* = 9.0 Hz, 1H), 3.93 (*t*, *J* = 5.5 Hz, 2H), 3.63 (*qn*, *J* = 8.0 Hz, 1H), 2.84 (*dd*, *J* = 17.5, 7.5 Hz, 1H), 2.75 (*t*, *J* = 5.5 Hz, 2H), 2.71 (*dd*, *J* = 17.5, 9.5 Hz, 1H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	206.91, 174.76, 67.97, 57.51, 46.92, 43.95, 30.00
DEPT 135: CH;	46.92
CH_2;	67.97, 57.51, 43.95, 30.00
EIMS *m/z* (% relative intensity):	140 (4), 113 (8), 86 (78), 73 (100)

Subfraction 44D3D showed four pale UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.12, 0.17 and 0.41. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44D4 showed seven UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.13, 0.23, 0.33, 0.35, 0.38, 0.55 and 0.70. It was further separated by column chromatography over silica gel. Elution was performed initially with 4% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 90**.

Table 90 Subfractions obtained from **subfraction 44D4** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44D4A	4% MeOH/CH_2Cl_2	2.0	Brown gum
44D4B	4% MeOH/CH_2Cl_2	0.9	Brown gum
44D4C	4% MeOH/CH_2Cl_2	7.0	Brown gum
44D4D	4-7% MeOH/CH_2Cl_2	6.6	Brown gum
44D4E	7% MeOH/CH_2Cl_2	1.4	Brown gum
44D4F	7% MeOH/CH_2Cl_2-100% MeOH	15.3	Brown gum

Subfraction 44D4A showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.66, 0.76 and 0.88. Because of the minute quantity, it was not further investigated.

Subfraction 44D4B showed none of UV-active spots under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44D4C showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.16 and 0.45 and one orange spot with the R_f value of 0.15 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was then purified by precoated TLC with 40% ethyl acetate in petroleum ether as a mobile phase (7 runs) to afford two bands.

Band 1 was a yellow gum (3.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.16. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a yellow gum (3.0 mg). Its chromatogram showed one orange spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.15 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated the presence of **K24**. Further investigation was then not carried out.

Subfraction 44D4D showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.31 and 0.36 and two purple spots with the R_f values of 0.45 and 0.57 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction 44D4E showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.27 and 0.36. Because of the minute quantity, it was not further investigated.

Subfraction 44D4F displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44D5 showed four UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.18, 0.30, 0.55 and 0.70. It was further separated by column chromatography over silica gel. Elution was performed initially with 4% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 91**.

Table 91 Subfractions obtained from **subfraction 44D5** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44D5A	4% MeOH/CH_2Cl_2	4.5	Brown gum

Table 91 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
44D5B	4% MeOH/CH_2Cl_2	18.5	Brown gum
44D5C	4-10% MeOH/CH_2Cl_2	37.1	Brown gum
44D5D	10% MeOH/CH_2Cl_2-100% MeOH	77.0	Brown gum

Subfraction 44D5A showed one pale UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.18. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44D5B showed five UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.19, 0.31, 0.33 and 0.71. Its ^1H NMR spectrum indicated that the major compound was **K26.** Further investigation was then not performed.

Subfraction 44D5C showed six UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.07, 0.19, 0.31, 0.33, 0.64 and 0.76. It was further separated by column chromatography over silica gel. Elution was performed initially with 50% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 92.**

Table 92 Subfractions obtained from **subfraction 44D5C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44D5C1	50% EtOAc/Petrol	3.9	Colorless gum
44D5C2	50-70% EtOAc/Petrol	2.1	White solid

Table 92 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
44D5C3	70% EtOAc/Petrol-100% EtOAc	7.9	White solid
44D5C4	100% EtOAc-5% MeOH/EtOAc	6.1	Brown gum
44D5C5	5-20% MeOH/EtOAc	9.3	Brown gum
44D5C6	30% MeOH/EtOAc-100% MeOH	6.7	Brown gum

Subfraction 44D5C1 displayed a long tail under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 44D5C2 showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.33 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not purified.

Subfraction 44D5C3 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.02, 0.10 and 0.32. Its ^{1}H NMR spectrum indicated that the major compound was **K26**. Further investigation was then not performed.

Subfraction 44D5C4 showed two UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.02 and 0.32. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 44D5C5 showed two UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.05 and 0.58. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 44D5C6 showed two UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.05 and 0.25. Its ^1H NMR spectrum indicated that the major compound was **K26.** Further investigation was then not performed.

Subfraction 44D5D showed two UV-active spots on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.07 and 0.76. It was further separated by column chromatography over silica gel. Elution was performed initially with 70% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 93.**

Table 93 Subfractions obtained from **subfraction 44D5D** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44D5D1	70% EtOAc/Petrol	4.0	Brown gum
44D5D2	70% EtOAc/Petrol	5.4	Brown gum
44D5D3	100% EtOAc	7.6	Brown gum
44D5D4	100% EtOAc- 7% MeOH/EtOAc	50.0	Brown gum
44D5D5	7% MeOH/EtOAc- 100% MeOH	3.2	Brown gum

Subfraction 44D5D1 showed none of UV-active spots under UV-S on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 44D5D2 showed one UV-active spot on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.32 and

many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not purified.

Subfraction 44D5D3 showed three UV-active spots on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.03, 0.05 and 0.09. Its ^{1}H NMR spectrum indicated that the major compound was **K26**. Further investigation was then not performed.

Subfraction 44D5D4 showed four UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.28, 0.86 and 0.88. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three fractions as shown in **Table 94**.

Table 94 Subfractions obtained from **subfraction 44D5D4** by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
44D5D41	30.9	Yellow gum
44D5D42	9.1	Yellow gum
44D5D43	9.8	Brown gum

Subfraction 44D5D41 showed three UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.19 and 0.27. Its ^{1}H NMR spectrum indicated that the major compound was **K24**. Further investigation was then not performed.

Subfraction 44D5D42 showed five UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.22, 0.31, 0.45 and 0.57. Its ^{1}H NMR spectrum indicated that the major compound was **K24**. Further investigation was then not performed.

Subfraction 44D5D43 showed none of UV-active spots under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44D5D5 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44D6 showed two UV-active spots on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f values of 0.03 and 0.18. It was further separated by column chromatography over silica gel. Elution was performed initially with 6% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 95.**

Table 95 Subfractions obtained from **subfraction 44D6** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44D6A	6-10% MeOH/CH_2Cl_2	10.3	Yellow gum
44D6B	10% MeOH/CH_2Cl_2	6.2	Yellow gum
44D6C	20-40% MeOH/CH_2Cl_2	35.3	Yellow gum
44D6D	40% MeOH/CH_2Cl_2-100% MeOH	30.5	Yellow gum
44D6E	100% MeOH	30.1	Yellow gum

Subfraction 44D6A showed two pale UV-active spots on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f values of 0.13 and 0.15. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44C6B showed three UV-active spots on normal phase TLC using 10% acetone in dichloromethane as a mobile phase with the R_f values of 0.07, 0.13 and 0.23. It was then purified by precoated TLC with 10% acetone in dichloromethane as a mobile phase (8 runs) to afford two bands.

Band 1 was a colorless solid (1.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% acetone in dichloromethane as a mobile phase with the R_f value of 0.19. Its ^{1}H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Band 2 was a colorless solid (1.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% acetone in dichloromethane as a mobile phase with the R_f value of 0.10. Its ^{1}H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Subfraction 44D6C showed five UV-active spots on normal phase TLC using 10% acetone in dichloromethane as a mobile phase with the R_f values of 0.07, 0.23, 0.30, 0.35 and 0.50. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 96**.

Table 96 Subfractions obtained from **subfraction 44D6C** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44D6C1	50% MeOH/H_2O	10.0	Yellow gum
44D6C2	50-70% MeOH/H_2O	6.4	Yellow gum
44D6C3	70% MeOH/H_2O-100% MeOH	15.6	Yellow gum

Subfraction 44D6C1 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed high field signals. Thus, it was not purified.

Subfraction 44D6C2 showed four UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.19, 0.26 and 0.39. Because of low quantity, it was not further investigated.

Subfraction 44D6C3 showed five pale UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.09, 0.12, 0.19 and 0.23. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44D6D showed two UV-active spots on normal phase TLC using 10% acetone in dichloromethane as a mobile phase with the R_f values of 0.12 and 0.24. Its ^{1}H NMR spectrum indicated that the major compound was **K24**. Further investigation was then not performed.

Subfraction 44D6E displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Fraction 44E showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.39, 0.41 and 0.42. It was further separated by column chromatography over silica gel. Elution was performed initially with 4% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 97**.

Table 97 Subfractions obtained from **fraction 44E** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44E1	4% MeOH/CH_2Cl_2	3.7	Brown gum
44E2	4% MeOH/CH_2Cl_2	9.2	Brown gum
44E3	6-10% MeOH/CH_2Cl_2	74.1	Brown solid
44E4	10% MeOH/CH_2Cl_2-100% MeOH	12.3	Brown gum

Subfraction 44E1 displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44E2 showed three pale UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.23, 0.36 and 0.53. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44E3 showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.16. Its ^1H NMR spectrum indicated the presence of **K26**. Further investigation was then not carried out.

Subfraction 44E4 displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 44F (K26) showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.16.

$[\alpha]_D^{29}$	+89 (*c* 0.20, MeOH)
UVλ_{max}(nm)(MeOH)(log ε)	204 (3.87)

FTIR(neat):υ(cm^{-1})	3392 (O-H stretching), 1698 (C=O stretching), 1647 (C=C stretching)
^{1}H NMR(DMSO-d_6)(δ_{ppm})(300 MHz):	7.34 (*dd*, *J* = 15.3, 2.7 Hz, 1H), 5.70 (*dd*, *J* = 15.3, 1.8 Hz, 1H), 5.66 (*ddd*, *J* = 15.3, 10.2, 4.5 Hz, 1H), 5.19 (*dd*, *J* = 15.3, 9.6 Hz, 1H), 5.16 (*d*, *J* = 5.7 Hz, 1H), 4.70 (*sextet*, *J* = 6.3 Hz, 1H), 4.55 (*d*, *J* = 3.6 Hz, 1H), 4.03 (*m*, 1H), 3.92 (*m*, 1H), 2.31 (*qn*, *J* = 8.4 Hz, 1H), 1.97 (*m*, 1H), 1.92 (*m*, 1H), 1.84 (*m*, 1H), 1.78 (*m*, 1H), 1.76 (*m*, 1H), 1.70 (*m*, 2H), 1.65 (*m*, 1H), 1.45 (*m*, 1H), 1.30 (*m*, 1H), 1.18 (*d*, *J* = 6.3 Hz, 3H), 0.74 (*m*, 1H)
^{13}C NMR(DMSO-d_6)(δ_{ppm})(75 MHz) :	166.19, 154.87, 137.58, 129.71, 116.71, 74.80, 71.39, 71.01, 52.17, 43.78, 43.49, 41.35, 33.85, 31.92, 26.94, 21.17
DEPT 135: CH;	154.87, 137.58, 129.71, 116.71, 74.80, 71.39, 71.01, 52.17, 43.78
CH_2;	43.49, 41.35, 33.85, 31.92, 26.94
CH_3;	21.17

3.2.3 Purification of the EtOAc extract from mycelia

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven fractions as shown in **Table98**.

Table 98 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
44CE1	52.6	Brown gum
44CE2	32.0	Brown gum
44CE3	75.7	Brown gum
44CE4	112.3	Brown gum
44CE5	20.6	Brown gum
44CE6	12.8	Brown gum
44CE7	8.6	Brown gum

Fraction 44CE1 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 44CE2 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.65 and 0.85. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 44CE3 showed seven UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.13, 0.28, 0.33, 0.35, 0.50, 0.58 and 0.65. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 99**.

Table 99 Subfractions obtained from **fraction 44CE3** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44CE3A	2% MeOH/CH_2Cl_2	18.3	Brown gum
44CE3B	2% MeOH/CH_2Cl_2	15.2	Brown gum
44CE3C	2-4% MeOH/CH_2Cl_2	5.5	Brown gum
44CE3D	4-10% MeOH/CH_2Cl_2	14.9	Brown gum
44CE3E	10% MeOH/CH_2Cl_2-100% MeOH	18.0	Brown gum

Subfraction 44CE3A showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.15, 0.24, 0.40, 0.71 and 0.80. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction 44CE3B showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.04, 0.22, 0.40 and 0.53. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction 44CE3C showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.11, 0.22 and 0.44. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction 44CE3D showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.04 and 0.22. Its ^1H NMR spectrum indicated that the major compound was **K26**. Further investigation was then not performed.

Subfraction 44CE3E displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Fraction 44CE4 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.28, 0.33, 0.45 and 0.68. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 100.**

Table 100 Subfractions obtained from **fraction 44CE4** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44CE4A	2% MeOH/CH_2Cl_2	4.3	Yellow gum
44CE4B	2% MeOH/CH_2Cl_2	14.8	Yellow gum
44CE4C	2% MeOH/CH_2Cl_2	12.3	Yellow gum
44CE4D	2% MeOH/CH_2Cl_2	4.6	Yellow gum
44CE4E	2% MeOH/CH_2Cl_2	2.3	Yellow gum
44CE4F	4-10% MeOH/CH_2Cl_2	34.0	White solid
44CE4G	10% MeOH/CH_2Cl_2-100% MeOH	23.0	Brown solid

Subfraction 44CE4A showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.50 and 0.67 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Therefore, it was not further investigated.

Subfraction 44CE4B showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.35 and

0.48. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (5 runs) to afford two bands.

Band 1 was a yellow gum (3.7 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.48. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Band 2 was a colorless gum (7.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.35 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 44CE4C showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.28 and 0.42. Because the ^1H NMR spectrum indicated the presence of **K26** as a major component, further purification was not performed.

Subfraction 44CE4D showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.17 and 0.19 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 44CE4E showed none of UV-active spots under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 44CE4F showed one UV-active spot on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f value of 0.23. Its ^1H

NMR spectrum indicated that the major compound was **K26**. Further investigation was then not performed.

Subfraction 44CE4G displayed a long tail under UV-S on normal phase TLC using 6% methanol in dichloromethane as a mobile phase. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Fraction 44CE5 showed six UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.15, 0.18, 0.35, 0.45 and 0.75. It was further separated by column chromatography over silica gel. Elution was performed initially with 4% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 101.**

Table 101 Subfractions obtained from **fraction 44CE5** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
44CE5A	4% MeOH/CH_2Cl_2	2.3	Yellow gum
44CE5B	4% MeOH/CH_2Cl_2	1.4	Yellow gum
44CE5C	4-8% MeOH/CH_2Cl_2	5.2	Yellow gum
44CE5D	10% MeOH/CH_2Cl_2-100% MeOH	10.2	Yellow gum

Subfraction 44CE5A showed four pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.29, 0.38, 0.59 and 0.63. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44CE5B showed one pale UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.18. Because

the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44CE5C showed three pale UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.18, 0.23 and 0.36. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 44CE5D displayed a long tail under UV-S on normal phase TLC using 6% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 44CE6 showed three pale UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.18 and 0.30. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Fraction 44CE7 displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

CHAPTER 3.3

RESULTS AND DISCUSSION

One new pyrone **(K23)** and one new lactone **(K24)** were isolated from the broth extract together with three known ones **(K25-K27)**. The structures were identified by spectroscopic methods.

3.3.1 Compound K23

Compound **K23** with the molecular formula $C_{15}H_{18}O_4$ from EIMS (*m/z* 262) **(Figure 53)**, was obtained as a colorless gum. The IR spectrum displayed absorption bands at 1713 and 1653 cm^{-1} for ester carbonyl and double bond functional groups, respectively. The UV spectrum exhibited maximum absorption bands at 244 and 269 nm, indicating that **K23** had a conjugated chromophore. The ^1H NMR spectrum **(Figure 54) (Table 102)** consisted of signals for a *trans*-propenyl unit [δ 5.52 (*dq*, J = 15.0 and 6.5 Hz, 1H), 5.22 (*ddq*, J = 15.0, 9.0 and 1.5 Hz, 1H) and 1.67 (*dd*, J = 6.5 and 1.5 Hz, 3H)], one olefinic proton of a trisubstituted double bond (δ 5.75, *s*, 1H), two nonequivalent oxymethylene protons [δ 4.16 (*t*, J = 9.0 Hz, 1H) and 3.71 (*t*, J = 9.0 Hz, 1H)], two nonequivalent methylene protons [δ 2.61 (*d*, J = 18.0 Hz, 1H) and 2.47 (*dd*, J = 18.0 and 6.5 Hz, 1H)], two methine protons [δ 2.55 (*m*, 1H) and 2.18 (*m*, 1H)] and two methyl groups [δ 2.21 (*s*, 3H) and 1.52 (*s*, 3H)]. In the ^1H-^1H COSY spectrum **(Table 103)**, the oxymethylene protons, H_{ab}-6 (δ 4.16 and 3.71), showed cross peaks with the methine proton, H-7 (δ 2.55), which was coupled with the other methine proton, H-7a (δ 2.18). Furthermore, H-7a was coupled with the nonequivalent methylene protons, H_{ab}-8 (δ 2.61 and 2.47). A tetrahydrofuran unit having a methylene group attached at C-7a was established on the basis of the ^3J HMBC correlations **(Table 102)** of H_{ab}-6 with C-4a (δ 109.22). The methyl protons, H_3-11 (δ 1.52), showed HMBC cross peaks with C-4a and C-7a (δ 45.93), thus connecting the methyl group at C-4a. The *trans*-propenyl unit was attached at C-7

(δ 44.65) of the tetrahydrofuran ring according to the ^{1}H-^{1}H COSY correlation between H-12 (δ 5.22) and H-7 as well as a ^{3}J HMBC correlation of H-13 with C-7. Moreover, the ^{1}H and ^{13}C NMR spectra of **K23** revealed the presence of a pyrone ring with the methyl and the oxysubstituent groups at C-2 (δ 160.55) and C-3a (δ 160.55), respectively. This conclusion was confirmed by following HMBC correlations: H-3 (δ 5.75)/C-2 (δ 160.55), C-3a (δ 160.55), C-8a (δ 94.10) and C-10 (δ 19.63) and H_3-10 (δ 2.21)/C-2, C-3 (δ 100.48), as well as the chemical shift of C-3a. The bond connection between the methylene carbon, C-8 (δ 17.84), with C-8a of the pyrone ring was established according to the HMBC correlations of H_{ab}-8 with C-8a and C-9 (δ 163.09). The chemical shift of C-4a and the molecular formula constructed an ether linkage between C-3a and C-4a to form a hydropyran unit. The spatial arrangement of H-7a, H_3-11 and the propenyl moiety is all *cis* on the basis of the NOEDIFF enhancement **(Table 103)** of H_3-11 and H-12 upon irradiation of H-7. Consequently, **K23** was identified as a new tricyclic pyrone derivative.

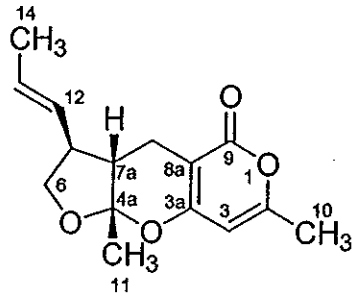


Table 102 The ^{1}H, ^{13}C NMR and HMBC data of compound **K23** in $CDCl_3$+CD_3OD

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
2	-	160.55 (C)	-
3	5.75 (*s*)	100.48 (CH)	C-2, C-3a, C-8a, C-10
3a	-	160.55 (C)	-
4a	-	109.22 (C)	-
6	a: 4.16 (*t*, 9.0)	72.50 (CH_2)	C-4a, C-7, C-7a, C-12
	b: 3.71 (*t*, 9.0)		
7	2.55 (*m*)	44.65 (CH)	-

Table 102 Continued

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
7a	2.18 (*m*)	45.93 (CH)	C-7, C-8, C-8a, C-12
8	a: 2.61 (*d*, 18.0)	17.84 (CH_2)	C-4a, C-7, C-7a, C-8a, C-9
	b: 2.47 (*dd*, 18.0, 6.5)		
8a	-	94.10 (C)	-
9	-	163.09 (C)	-
10	2.21 (*s*)	19.63 (CH_3)	C-2, C-3
11	1.52 (*s*)	22.34 (CH_3)	C-4a, C-7a
12	5.22 (*ddq*, 15.0, 9.0, 1.5)	128.54 (CH)	C-14
13	5.52 (*dq*, 15.0, 6.5)	129.76 (CH)	C-7, C-14
14	1.67 (*dd*, 6.5, 1.5)	17.79 (CH_3)	C-12, C-13

Table 103 The COSY and NOEDIFF data of compound **K23** in $CDCl_3$+CD_3OD

Proton	COSY	NOEDIFF
H-3	-	H_3-10
H_a-6	H_b-6, H-7	H_b-6, H-7
H_b-6	H_a-6, H-7	H_a-6, H-12
H-7	H_{ab}-6, H-7a, H-12	-
H-7a	H-7, H_{ab}-8	H_a-8, H_3-11, H-12
H_a-8	H-7a, H_b-8	-
H_b-8	H-7a, H_a-8	-
H_3-10	-	H-3
H_3-11	-	-
H-12	H-7, H-13, H_3-14	-
H-13	H-15, H_3-14	H-7, H_3-14
H_3-14	H-12, H-13	-

3.3.2 Compound K24

Compound **K24** was obtained as a colorless gum whose molecular formula was assigned as $C_7H_{10}O_4$ by EIMS (*m/z* 158) **(Figure 56)**. The IR spectrum showed absorption bands for a hydroxyl (3414 cm^{-1}), a γ-lactone carbonyl (1769 cm^{-1}) and a ketone carbonyl (1715 cm^{-1}). The ^1H NMR spectrum **(Figure 57) (Table 104)** consisted of signals for a 1-oxo-3-hydroxypropyl unit [δ 3.93 (*t*, J = 5.5 Hz, 2H) and 2.75 (*t*, J = 5.5 Hz, 2H)], one methine proton (δ 3.63, *qn*, J = 8.0 Hz, 1H), two nonequivalent oxymethylene protons [δ 4.50 (*t*, J = 9.0 Hz, 1H) and 4.44 (*t*, J = 9.0 Hz, 1H)] and two nonequivalent methylene protons [δ 2.84 (*dd*, J = 17.5 and 7.5 Hz, 1H) and 2.71 (*dd*, J = 17.5 and 9.5 Hz, 1H)]. The methine proton, H-4 (δ 3.63), showed ^1H-^1H COSY cross peaks **(Table 104)** with the methylene protons, H_{ab}-3 (δ 2.84 and 2.71), and the oxymethylene protons, H_{ab}-5 (δ 4.50 and 4.44). Both H_{ab}-3 and H_{ab}-5 were correlated with the same lactone carbonyl carbon, C-2 (δ 174.8), in the HMBC spectrum **(Table 104)**, thus constructing a γ-lactone unit. The 1-oxo-3-hydroxypropyl unit was located at C-4 (δ 46.9) of the lactone ring on the basis of a HMBC correlation between H_2-7 (δ 2.75) of the 1-oxo-3-hydroxypropyl unit and C-4. Therefore, **K24** was identified as a new γ-lactone derivative.

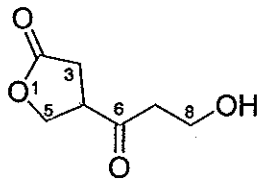


Table 104 The NMR data of compound **K24** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC	COSY
2	-	174.76 (C)	-	-
3	a: 2.84 (*dd*, 17.5, 7.5)	30.00 (CH_2)	C-2, C-4, C-5,	H_b-3, H-4
	b: 2.71 (*dd*, 17.5, 9.5)		C-6	H_a-3, H-4
4	3.63 (*qn*, 8.0)	46.92 (CH)	-	H_{ab}-3, H_{ab}-5
5	a: 4.50 (*t*, 9.0)	67.97 (CH_2)	C-2, C-3, C-4,	H_b-5, H-4
	b: 4.44 (*t*, 9.0)		C-6	H_a-5, H-4

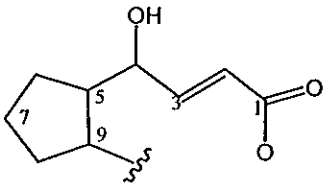
Table 104 Continued

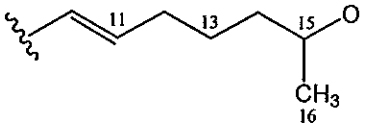
Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC	COSY
6	-	206.91 (C)	-	-
7	2.75 (*t*, 5.5)	43.95 (CH_2)	C-8	H_2-8
8	3.93 (*t*, 5.5)	57.51 (CH_2)	C-6	H_2-7

3.3.3 Compound K25

Compound **K25** was obtained as a colorless gum. The UV spectrum showed an absorption band at 205 nm. The IR spectrum displayed absorption bands at 3389, 1705 and 1641 for hydroxyl, ester carbonyl and double bond functional groups, respectively. The ^{1}H NMR spectrum **(Figure 59) (Table 105)** consisted of signals for two sets of *trans*-olefinic protons [δ 7.33 (*dd*, J = 15.5 and 3.0 Hz, 1H)/ 5.90 (*dd*, J = 15.5 and 2.0 Hz, 1H) and 5.73 (*ddd*, J = 15.0, 10.0 and 4.5 Hz, 1H)/5.19 (*dd*, J = 15.0 and 9.5 Hz, 1H)], two oxymethine protons [δ 4.91 (*m*, 1H) and 4.08 (*brd*, J = 9.5 Hz, 1H)], two methine protons [δ 2.25 (*qn*, J = 9.0 Hz, 1H) and 1.58 (*m*, 1H)], six sets of methylene protons [δ 2.01 (*m*, 2H), 1.62 (*m*, 2H), 1.85 (*m* ,1H)/1.62 (*m*, 1H), 1.85 (*m*, 1H)/1.38 (*m*, 1H), 1.85 (*m*, 1H)/0.94 (*m*, 1H) and 1.74 (*m*, 1H)/1.53 (*m*, 1H)] and one methyl group (δ 1.26, *d*, J = 6.0 Hz, 3H). The ^{13}C NMR **(Figure 60) (Table 105)** and DEPT 135 spectra **(Table 105)** showed sixteen resonances for one quaternary (δ 166.27), eight methine (δ 151.86, 136.32, 130.33, 117.38, 76.06, 71.65, 54.06 and 46.93), six methylene (δ 35.15, 34.12, 31.92, 31.85, 26.78 and 25.24) and one methyl (δ 20.86) carbons. In the ^{1}H-^{1}H COSY spectrum **(Table 106)**, the methylene protons, H_2-7 (δ 1.62), were coupled with two sets of the methylene protons, H_2-6 (δ2.01) and H_{ab}-8 (δ 1.85 and 1.38). H_2-6 and H_{ab}-8 showed cross peaks with the methine protons, H-5 (δ 1.58) and H-9 (δ 2.25), respectively. Furthermore, H-5 showed a cross peak with H-9, to form a cyclopentane ring. One of the *trans*-olefinic protons, H-3 (δ7.33), was coupled with the hydroxymethine proton, H-4 (δ 4.08), which was further coupled with H-5. In addition, H-3 showed a

^3J HMBC correlation **(Table 105)** with the ester carbonyl carbon, C-1 (δ 166.27). These results constructed substructure 1.





substructure 1 substructure 2

The *trans*-olefinic proton, H-11 (δ 5.73), showed a cross peak in the ^{1}H-^{1}H COSY spectrum with the methylene protons, H_{ab}-12 (δ 1.85 and 1.62), which were coupled with the methylene protons, H_{ab}-13 (δ 1.85 and 0.94). Furthermore, the remaining methylene protons, H_{ab}-14 (δ 1.74 and 1.53), were coupled with H_{ab}-13 and the oxymethine proton, H-15 (δ 4.91), which was further coupled with the methyl protons, H_3-16 (δ 1.26). Thus, substructure 2 was established. In the HMBC spectrum, H-10 and H-15 of substructure 2 showed a cross peak with C-9 (δ 46.93) and C-1 of substructure 1, respectively. These data constructed a bicyclic lactone. The relative configuration of **K25** was established by the NOEDIFF experiment **(Table 106)**. Irradiation of H-5, did not affect signal intensity of both H-4 and H-9, indicating their *trans*-relationship. The observed optical rotation of **K25**, $[\alpha]_D^{25}$ +28 (*c* 0.08, $CHCl_3$), was almost identical to that of (4*R*,5*R*,9*S*,15*S*)-(+)-brefeldin C, $[\alpha]_D^{25}$ +30 (*c* 0.08, $CHCl_3$), indicating that they had the same absolute configuration at all chiral carbons. Therefore, **K25** was assigned as (4*R*,5*R*,9*S*,15*S*)-(+)-brefeldin C which was previously isolated from the fungus *Eupenicillium brefeldianum* (Archambaud, *et al.*, 2005).

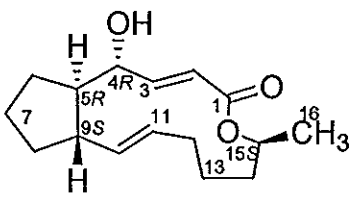


Table 105 The ^{1}H, ^{13}C NMR and HMBC data of compound **K25** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
1	-	166.27 (C)	-
2	5.90 (*dd*, 15.5, 2.0)	117.38 (CH)	C-1, C-4
3	7.33 (*dd*, 15.5, 3.0)	151.86 (CH)	C-1, C-2, C-4
4	4.08 (*brd*, 9.5)	76.06 (CH)	C-5
5	1.58 (*m*)	54.06 (CH)	C-4, C-7, C-8, C-9, C-10
6	2.01 (*m*)	31.92 (CH_2)	C-4, C-5, C-7, C-8, C-9
7	1.62 (*m*)	25.24 (CH_2)	-
8	a: 1.85 (*m*)	35.15 (CH_2)	C-6, C-7, C-9, C-10
	b: 1.38 (*m*)		
9	2.25 (*qn*, 9.0)	46.93 (CH)	C-4, C-5, C-8, C-10, C-11
10	5.19 (*dd*, 15.0, 9.5)	136.32 (CH)	C-5, C-9, C-8, C-12
11	5.73 (*ddd*, 15.0, 10.0, 4.5)	130.33 (CH)	C-9, C-12, C-13
12	a: 1.85 (*m*)	31.85 (CH_2)	C-10, C-11, C-13, C-14
	b: 1.62 (*m*)		
13	a: 1.85 (*m*)	26.78 (CH_2)	C-14, C-15
	b: 0.94 (*m*)		
14	a: 1.74 (*m*)	34.12 (CH_2)	C-12, C-13, C-15
	b: 1.53 (*m*)		
15	4.91 (*m*)	71.65 (CH)	C-1, C-13
16	1.26 (*d*, 6.0)	20.86 (CH_3)	C-14, C-15

Table 106 The COSY and NOEDIFF data of compound **K25** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H-3, H-4	-
H-3	H-2, H-4	H-4, H-9, H-11
H-4	H-2, H-3, H-5	H-3, H-9
H-5	H-4, H_2-6, H-9	-
H_2-6	H-5, H_2-7	-

Table 106 Continued

Proton	COSY	NOEDIFF
H_2-7	H_2-6, H_{ab}-8	-
H_a-8	H_2-7, H-9	-
H_b-8	H_2-7, H-9	-
H-9	H-5, H_{ab}-8, H-10	H-3, H-4, H-11,
H-10	H-9, H-11	-
H-11	H-10, H_{ab}-12	H-3, H-9
H_a-12	H-11, H_b-12, H_{ab}-13	-
H_b-12	H-11, H_a-12, H_{ab}-13	-
H_a-13	H_{ab}-12, H_{ab}-14	-
H_b-13	H_{ab}-12, H_{ab}-14	-
H_a-14	H_{ab}-13, H_b-14, H-15	-
H_b-14	H_{ab}-13, H_a-14, H-15	-
H-15	H_{ab}-14, H_3-16	H_3-16
H_3-16	H-15	H-15

3.3.4 Compound K26

Compound **K26** was obtained as a colorless gum. Its UV and IR spectra were almost identical to those of **K25**. Their ^{1}H NMR spectra **(Figure 61)** **(Table 107)** were similar to those of **K25** except for the replacement of signal for one methylene group in **K25** with signal of a hydroxymethine proton (δ 4.03, *m*, 1H). The ^{1}H-^{1}H COSY correlations **(Table 108)** of this hydroxymethine proton, H-7 (δ 4.03), with H_{ab}-6, (δ 1.84 and 1.65), and H_{ab}-8, (δ 1.97 and 1.30), established the attachment of a hydroxyl group at C-7 (δ 71.01). Irradiation of H-7 in the NOEDIFF spectrum **(Table 108)** affected signal intensity of H-9, indicating their *cis*-relationship. The observed optical rotation of **K26**, $[\alpha]_D^{29}$ +89.7 (*c* 0.2, MeOH), was almost identical to that of (4*R*,5*R*,7*S*,9*S*,15*S*)-(+)-brefeldin A, $[\alpha]_D^{29}$ +89 (*c* 0.2, MeOH), indicating that they had the same absolute configurations at all chiral carbons. Consequently, **K26**

was assigned as (4*R*,5*R*,7*S*,9*S*,15*S*)-(+)-brefeldin A which was previously isolated from the fungus *Eupenicillium brefeldianum* (Shibazaki, *et al.*, 2003).

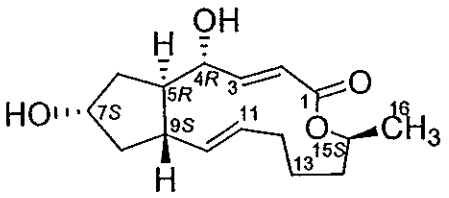


Table 107 The ^{1}H and ^{13}C NMR data of compound **K26** and **(4*R*,5*R*,7*S*,9*S*,15*S*)-(+)-Brefeldin A** in DMSO-d_6

Position	K26		(4*R*,5*R*,7*S*,9*S*,15*S*)-(+)-Brefeldin A	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	-	166.19 (C)	-	165.6 (C)
2	5.70 (*dd*, 15.3, 1.8)	116.71 (CH)	5.71	116.2 (CH)
3	7.34 (*dd*, 15.3, 2.7)	154.87 (CH)	7.34	154.3 (CH)
4	3.92 (*m*)	74.80 (CH)	3.93	74.3 (CH)
4-OH	5.16 (*d*, 5.7)	-	5.08	-
5	1.70 (*m*)	52.17 (CH)	1.71	51.7 (CH)
6	a: 1.84 (*m*)	41.35 (CH_2)	a: 1.83	40.9 (CH_2)
	b: 1.65 (*m*)		b: 1.64	
7	4.03 (*m*)	71.01 (CH)	4.04	70.5 (CH)
7-OH	4.55 (*d*, 3.6)	-	-	-
8	a: 1.97 (*m*)	43.49 (CH_2)	a: 1.97	43.1 (CH_2)
	b: 1.30 (*m*)		b: 1.30	
9	2.31 (*qn*, 8.4)	43.78 (CH)	2.31	43.3 (CH)
10	5.19 (*dd*, 15.3, 9.6)	137.58 (CH)	5.19	137.1 (CH)
11	5.66 (*ddd*, 15.3, 10.2, 4.5)	129.71 (CH)	5.66	126.2 (CH)
12	a: 1.92 (*m*)	31.92 (CH_2)	a: 1.92	31.4 (CH_2)
	b: 1.76 (*m*)		b: 1.77	

Table 107 Continued

Position	K26		(4*R*,5*R*,7*S*,9*S*,15*S*)-(+)-Brefeldin A	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
13	a: 1.78 (*m*)	26.94 (CH_2)	a: 1.77	26.4 (CH_2)
	b: 0.74 (*m*)		b: 0.75	
14	a: 1.70 (*m*)	33.85 (CH_2)	a: 1.72	33.4 (CH_2)
	b: 1.45 (m)		b: 1.48	
15	4.70 (*sextet*, 6.3)	71.39 (CH)	4.70	70.8 (CH)
16	1.18 (*d*, 6.3)	21.17 (CH_3)	1.18	20.7 (CH_3)

Table 108 The HMBC, COSY and NOEDIFF data of compound **K26** in DMSO-d_6

Proton	HMBC	COSY	NOEDIFF
H-2	C-1, C-3, C-4, C-5, C-15	H-3, H-4	H-4, 4-OH, H-9
H-3	C-1, C-2, C-4, C-5	H-2, H-4	H-4, H-9, H-11
H-4	C-2, C-3, C-5, C-6	H-2, H-3, 4-OH, H-5	H-3 , 4-OH, H-9
4-OH	-	H-4	-
H-5	C-4, C-6, C-9, C-10	H-4, H_{ab}-6, H-9	-
H_a-6	C-4, C-5, C-7, C-8, C-9	H-5, H_b-6, H-7	-
H_b-6	C-4, C-5, C-7, C-8, C-9	H-5, H_a-6, H-7	-
H-7	C-5, C-9	H_{ab}-6, 7-OH, H_{ab}-8	7-OH, H-9
7-OH	-	H-7	-
H_a-8	C-5, C-6, C-7, C-9, C-10	H-7, H_b-8, H-9	-
H_b-8	C-5, C-6, C-7, C-9, C-10	H-7, H_a-8, H-9	-
H-9	C-4, C-5, C-8, C-10, C-11	H-5, H_{ab}-8, H-10	H-3, H-4, H-7, H-11
H-10	C-9, C-11, C-12, C-13	H-9, H-11	H-3, H-4, H-9
H-11	C-9, C-12, C-13	H-10, H_{ab}-12	4-OH, H-9
H_a-12	C-11, C-13, C-15	H-11, H_b-12, H_{ab}-13	-
H_b-12	C-11, C-13, C-15	H-11, H_a-12, H_{ab}-13	-
H_a-13	C-11, C-12, C-14, C-15	H_{ab}-12, H_b-13, H_{ab}-14	-
H_b-13	C-11, C-12, C-14, C-15	H_{ab}-12, H_a-13, H_{ab}-14	-

Table 108 Continued

Proton	HMBC	COSY	NOEDIFF
H_a-14	C-12, C-13, C-15	H_{ab}-13, H_b-14, H-15	-
H_b-14	C-12, C-13, C-15	H_{ab}-13, H_a-14, H-15	-
H-15	C-1, C-13, C-14, C-15	H_{ab}-14, H_3-16	H_3-16
H-16	C-14, C-15	H-15	H-15

3.3.5 Compound K27

Compound **K27** was obtained as a colorless gum. Its UV spectrum was similar to that of **K25** while the IR spectrum showed an additional absorption band of a ketone carbonyl group at 1698 cm^{-1}. Its ^1H NMR **(Figure 63) (Table 109)** and ^{13}C NMR **(Figure 64) (Table 109)** spectra were similar to those of **K25** except for the replacement of signal for one methylene carbon in **K25** with signal of a ketone carbonyl carbon at δ 215.90. In the HMBC spectrum **(Table 110)**, the methylene protons, H_{ab}-6 (δ 2.83 and 2.22), and H_{ab}-8 (δ 2.53 and 2.10), were correlated with this carbonyl carbon. These established the location of the ketone group at C-7. Consequently, **K27** was assigned as (4*R*,5*R*,9*S*,15*S*)-7-oxobrefeldin A (Hutchinson, *et al.*, 1981).

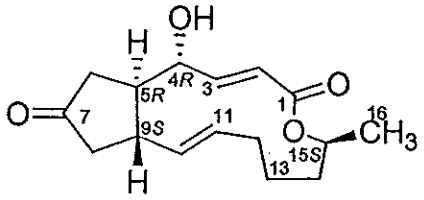


Table 109 The ^1H and ^{13}C NMR data of compound **K27** in $CDCl_3$+CD_3OD and **(4*R*,5*R*,9*S*,15*S*)-7-Oxobrefeldin A** in CD_3OD

Position	**K27**		**(4*R*,5*R*,9*S*,15*S*)-7-Oxobrefeldin A**
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})
1	-	166.08 (C)	-
2	5.96 (*dd*, 15.6, 1.8)	118.35 (CH)	6.56 (*dd*, 15.5, 2.0)
3	7.39 (*dd*, 15.6, 3.6)	150.55 (CH)	7.71 (*dd*, 15.5, 3.3)

Table 109 Continued

Position	K27		(4*R*,5*R*,9*S*,15*S*)-7-Oxobrefeldin A
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})
4	4.21 (*brd*, 8.1)	76.55 (CH)	4.48 (*ddd*, 9.4, 3.3, 2.0)
5	2.10 (*m*)	49.88 (CH)	2.33 (*dddd*, 10.4, 9.7, 9.4, 7.9)
6	a: 2.83 (*dd*, 15.6, 7.8)	44.87 (CH_2)	a: 3.31 (*ddd*, 18.1, 7.9, 1.4)
	b: 2.22 (*dd*, 15.6, 9.0)		b: 2.52 (*ddd*, 18.1, 10.4, 1.4)
7	-	215.90 (C)	-
8	a: 2.53 (*dd*, 18.6, 8.4)	46.56 (CH_2)	a: 2.62 (*ddd*, 18.1, 8.1, 1.4)
	b: 2.10 (*m*)		b: 2.25 (*ddd*, 18.1, 11.1, 1.4)
9	2.69 (*m*)	42.48 (CH)	2.81 (*dddd*, 11.1, 9.7, 9.1, 8.1)
10	5.21 (*dd*, 15.0, 9.0)	135.16 (CH)	5.25 (*dd*, 15.3, 9.1)
11	5.81 (*ddd*, 15.0, 9.6, 5.4)	132.28 (CH)	5.84 (*ddd*, 15.3, 10.1, 5.1)
12	a: 2.10 (*m*)	31.57 (CH_2)	a: 1.98 (*dddd*, 12.5. 10.1, 5.1, 2.6)
	b: 1.89 (*m*)		b: 1.81 (*dddd*, 12.5, 12.2, 10.1, 2.6)
13	a: 1.75 (*m*)	26.40 (CH_2)	a: 1.70 (*dddd*, 14.3, 6.1, 2.6, 0.1)
	b: 1.06 (*m*)		b: 1.06 (*ddddd*, 14.3, 12.2, 7.3, 2.6, 1.3)
14	a: 1.75 (*m*)	34.31 (CH_2)	a: 1.61 (*ddd*, 14.2, 7.3, 0.1)
	b: 1.55 (*m*)		b: 1.50 (*dddd*, 14.2, 8.3, 6.1, 1.3)
15	4.91 (*m*)	71.72 (CH)	4.98 (*m*)
16	1.28 (*d*, 6.3)	20.66 (CH_3)	1.24 (*d*, 6.3)

Table 110 The HMBC, COSY and NOEDIFF data of compound **K27** in $CDCl_3$+CD_3OD

Proton	HMBC	COSY	NOEDIFF
H-2	C-1, C-3, C-4	H-3, H-4	
H-3	C-1, C-2, C-4, C-5	H-2, H-4	H-4, H-9, H-11

Table 110 Continued

Proton	HMBC	COSY	NOEDIFF
H-4	C-3, C-5, C-9	H-2, H-3, H-5	H-3 , H-5, H-9
H-5	C-6, C-9	H-4, H_{ab}-6, H-9	-
H_a-6	C-4, C-7, C-9	H-5, H_b-6	-
H_b-6	C-4, C-7, C-9	H-5, H_a-6	-
H_a-8	C-4, C-5, C-6, C-7, C-9	Hb-8, H-9	-
H_b-8	C-4, C-5, C-6, C-7, C-9	H_a-8, H-9	-
H-9	C-5, C-8, C-10, C-11	H-5, H_{ab}-8, H-10	H-3, H-4, H-11
H-10	C-5, C-8, C-9, C-12	H-9, H-11	-
H-11	C-9, C-12	H-10, H_{ab}-12	-
H_a-12	C-10, C-11, C-13, C-14	H-11, H_b-12, H_{ab}-13	-
H_b-12	C-10, C-11, C-13, C-14	H-11, H_a-12, H_{ab}-13	-
H_a-13	C-15	H_{ab}-12, H_b-13, H_{ab}-14	-
H_b-13	C-15	H_{ab}-12, H_a-13, H_{ab}-14	-
H_a-14	C-12, C-13, C-15	H_{ab}-13, H_b-14, H-15	-
H_b-14	C-12, C-13, C-15	H_{ab}-13, H_a-14, H-15	-
H-15	C-1	H_{ab}-14, H_3-16	H_3-16
H_3-16	C-14, C-15	H-15	H-15

PART IV

METABOLITES FROM THE MARINE-DERIVED FUNGUS *PENICILLIUM* SP. PSU-F40

CHAPTER 4.1

INTRODUCTION

4.1.1 Introduction

The marine-derived fungus *Penicillium* sp. PSU-F40 was isolated from the sea fan *Annella* sp. This fungus was deposited as PSU-F40 at the Department of Microbiology, Faculty of Science, Prince of Songkla University.

4.1.2 The objectives

1. To isolate the secondary metabolites from the marine-derived fungus *Penicillium* sp. PSU-F40.
2. To elucidate the structure of the isolated metabolites.

CHAPTER 4.2

EXPERIMENTAL

4.2.1 Fermentation and extraction

The fermentation and the extraction of the culture broth (15 L) and mycelia were performed using the same procedure as those of *Nigrospora* sp. PSU-F5 to afford a dark brown gum (1.28 g) and a brown gum (543 mg) from the culture broth and mycelia, respectively. Each extract was subjected to chromatographic separation.

4.2.2 Purification of the broth extract

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six fractions as shown in **Table 111.**

Table 111 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
40A	161.7	Brown gum
40B	325.0	Brown gum
40C	395.5	Brown gum
40D	314.0	Brown gum
40E	43.5	Brown gum
40F	27.6	Brown gum

Fraction 40A displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase and its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Fraction 40B showed nine UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.02, 0.07, 0.15, 0.22, 0.27, 0.34, 0.46, 0.56 and 0.68. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 112.**

Table 112 Subfractions obtained from **fraction 40B** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40B1	2% MeOH/CH_2Cl_2	12.8	Yellow gum
40B2	2% MeOH/CH_2Cl_2	19.0	Yellow gum
40B3	2% MeOH/CH_2Cl_2	17.1	Yellow gum
40B4	2-4% MeOH/CH_2Cl_2	28.6	Yellow gum
40B5	4-6% MeOH/CH_2Cl_2	59.2	Brown gum
40B6	6-10% MeOH/CH_2Cl_2	56.4	Brown gum
40B7	10-30% MeOH/CH_2Cl_2	74.0	Brown gum
40B8	30% MeOH/CH_2Cl_2-100% MeOH	40.1	Brown gum

Subfraction 40B1 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.62, 0.71, 0.79 and 0.83. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction 40B2 showed five UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.19, 0.26, 0.31, 0.38 and 0.40. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Subfraction 40B3 showed six pale UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.19, 0.31, 0.40, 0.55, 0.61 and 0.63. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40B4 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.23 and 0.31. It was further separated by column chromatography over silica gel. Elution was performed initially with 40% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 113**.

Table 113 Subfractions obtained from **subfraction 40B4** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40B4A	40% EtOAc/Petrol	6.7	Colorless gum
40B4B	40% EtOAc/Petrol	3.7	Colorless gum
40B4C	40% EtOAc/Petrol	2.2	Colorless gum
40B4D	60% EtOAc/Petrol	3.5	Yellow gum
40B4E	60% EtOAc/Petrol-100% MeOH	10.0	Yellow gum

Subfraction 40B4A displayed a long tail under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40B4B showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.38, 0.41 and 0.57. Because of low quantity, it was not further investigated.

Subfraction 40B4C showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.24 and 0.36. Because of the minute quantity, it was not further investigated.

Subfraction 40B4D showed three UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.32, 0.34 and 0.36. Because of the minute quantity, it was not further investigated.

Subfraction 40B4E displayed a long tail under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Because ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 40B5 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.32, 0.38 and 0.55. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 114.**

Table 114 Subfractions obtained from **subfraction 40B5** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
40B5A	3.7	Yellow gum
40B5B	7.4	Yellow gum
40B5C	8.8	Yellow gum
40B5D	7.9	Yellow gum
40B5E	8.3	Yellow gum
40B5F	21.7	Brown gum

Subfraction 40B5A showed one pale UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.15. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40B5B showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.09, 0.14 and 0.36. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford two subfractions as shown in **Table 115.**

Table 115 Subfractions obtained from **subfraction 40B5B** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40B5B1	1.6	Yellow gum
40B5B2	5.8	Yellow gum

Subfraction 40B5B1 showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.09. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40B5B2 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.14 and 0.26. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (4 runs) to afford two bands.

Band 1 (K34) was a colorless gum (3.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.36.

$[\alpha]_D^{29}$	-135 (*c* 0.16, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	203 (3.32), 269 (3.67)
FTIR(neat):υ(cm^{-1})	3409 (O-H stretching), 1687 (C=O stretching), 1640 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	4.52 (*q*, *J* = 6.6 Hz, 1H), 4.38 (*d*, *J* = 12.6 Hz, 1H), 4.32 (*d*, *J* = 12.6 Hz, 1H), 2.45 (*dq*, *J* = 15.3, 7.5 Hz, 1H), 2.40 (*dq*, *J* = 15.3, 7.5 Hz, 1H), 1.40 (*s*, 3H), 1.31 (*d*, *J* = 6.6 Hz, 3H), 1.18 (*t*, *J* = 7.5 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	196.20, 175.58, 110.07, 81.96, 71.33, 56.32, 25.59, 24.11, 12.30, 11.44
DEPT 135: CH;	81.96
CH_2;	56.32, 25.59
CH_3;	24.11, 12.30, 11.44
EIMS *m/z* (% relative intensity):	200 (11), 167 (6), 129 (76), 111 (100), 72 (62)

Band 2 was a yellow gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.14. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40B5C showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.13 and 0.25. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram

were combined and evaporated to dryness under reduced pressure to afford two subfractions as shown in **Table 116.**

Table 116 Subfractions obtained from **subfraction 40B5C** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40B5C1	3.6	Yellow gum
40B5C2	5.2	Yellow gum

Subfraction 40B5C1 showed two pale UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.11 and 0.34. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40B5C2 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.20, 0.29 and 0.34. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (5 runs) to afford two bands.

Band 1 was a yellow gum (1.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.33. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a yellow gum (3.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.19. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40B5D showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.06, 0.15, 0.22, 0.26 and 0.36. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford two subfractions as shown in **Table 117**.

Table 117 Subfractions obtained from **subfraction 40B5D** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40B5D1	1.3	Yellow gum
40B5D2	5.8	Yellow gum

Subfraction 40B5D1 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.17 and 0.27. Because of the minute quantity, it was not further investigated.

Subfraction 40B5D2 showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07 and 0.17. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (6 runs) to afford two bands.

Band 1 (K39) was a colorless gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.17.

UV λ_{max}(nm)(MeOH)(log ε)	205 (3.85), 212 (3.82), 302 (3.41)
FTIR(neat):υ(cm^{-1})	3398 (O-H stretching), 1675 (C=O stretching), 1620 (C=C stretching)

^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	6.08 (*s*, 1H), 6.00 (*s*, 1H), 2.58 (*d*, *J* = 7.0 Hz, 2H), 2.37 (*s*, 3H), 2.23 (*m*, 1H), 0.96 (*d*, *J* = 6.5 Hz, 3H), 0.94 (*d*, *J* = 7.0 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	200.76, 155.19, 154.35, 143.00, 130.34, 107.73, 93.41, 45.48, 23.66, 22,51, 22.48, 19.33
DEPT 135: CH;	107.73, 93.41, 23,66
CH_2;	45.48
CH_3;	22.51, 22.48, 19.33

Band 2 was a yellow gum (3.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.07. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40B5E showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.13 and 0.21. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Subfraction 40B5F displayed a long tail under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 40B6 showed three UV-active spots on reverse phase TLC using 60% methanol in water as a mobile phase with the R_f values of 0.39, 0.53 and 0.65. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 60% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 118**.

Table 118 Subfractions obtained from **subfraction 40B6** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
40B6A	6.6	Yellow gum
40B6B	3.7	Yellow gum
40B6C	8.1	Yellow gum
40B6D	20.1	Yellow gum
40B6E	7.1	Yellow gum
40B6F	10.0	Yellow gum

Subfraction 40B6A displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not further purified.

Subfraction 40B6B showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.50 and 0.66. Because of low quantity, it was not further investigated.

Subfraction 40B6C showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.09 and 0.14. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 50% methanol in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 119**.

Table 119 Subfractions obtained from **subfraction 40B6C** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40B6C1	1.3	Yellow gum
40B6C2	5.1	Yellow gum
40B6C3	2.4	Yellow gum

Subfraction 40B6C1 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not further purified.

Subfraction 40B6C2 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.12 and 0.14. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40B6C3 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05 and 0.14. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40B6D showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.03, 0.09, 0.13 and 0.28. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 120**.

Table 120 Subfractions obtained from **subfraction 40B6D** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40B6D1	3.2	Yellow gum
40B6D2	14.2	Yellow gum
40B6D3	3.6	Yellow gum

Subfraction 40B6D1 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.12, 0.16 and 0.18. Because of the minute quantity, it was not further investigated.

Subfraction 40B6D2 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.20 and 0.27. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 50% methanol in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 121.**

Table 121 Subfractions obtained from **subfraction 40B6D2** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40B6D2A	3.6	Yellow gum
40B6D2B	8.4	Yellow gum
40B6D2C	2.1	Yellow gum

Subfraction 40B6D2A showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.10 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40B6D2B showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.10 and 0.20, and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40B6D2C showed none of major spots under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 40B6E showed three pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.02, 0.09 and 0.28. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40B6F showed two pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.06 and 0.08. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40B7 showed five UV-active spots on reverse phase TLC using 60% methanol in water as a mobile phase with the R_f values of 0.13, 0.22, 0.31, 0.32 and 0.34. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 122**.

Table 122 Subfractions obtained from **subfraction 40B7** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40B7A	50% MeOH/H_2O	23.3	Yellow gum
40B7B	50% MeOH/H_2O	15.7	Yellow gum
40B7C	50-60% MeOH/H_2O	4.5	Yellow gum
40B7D	60-80% MeOH/H_2O	15.6	Yellow gum
40B7E	80% MeOH/H_2O-100% MeOH	12.7	Yellow gum

Subfraction 40B7A showed four UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.57, 0.58, 0.71 and

0.77. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Subfraction 40B7B showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.37, 0.46 and 0.53. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 123**.

Table 123 Subfractions obtained from **subfraction 40B7B** by column chromatograph over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
40B7B1	2.0	Yellow gum
40B7B2	10.5	Yellow gum
40B7B3	2.0	Yellow gum

Subfraction 40B7B1 showed two UV-active spots on reverse phase TLC using 55% methanol in water as a mobile phase with the R_f values of 0.37 and 0.44. Because of the minute quantity, it was not further investigated.

Subfraction 40B7B2 displayed a long tail under UV-S on reverse phase TLC using 55% methanol in water as a mobile phase. This subfraction was subjected to acetylation reaction. After work up, the reaction mixture was obtained as a yellow gum (6.0 mg) and showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07 and 0.14. It was then purified by precoated TLC with 2% methanol in dichloromethane as a mobile phase (8 runs) to afford two bands.

Band 1 was a yellow gum (2.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a

mobile phase with the R_f value of 0.15. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 2 was a yellow gum (1.4 mg). Its chromatogram showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.08 and 0.12. Because of the minute quantity, it was not further investigated.

Subfraction 40B7B3 showed two pale UV-active spots on reverse phase TLC using 55% methanol in water as a mobile phase with the R_f values of 0.53 and 0.55. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40B7C showed one pale UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.23. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40B7D showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.22 and 0.30. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Subfraction 40B7E showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.10 and 0.40. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Subfraction 40B8 displayed a long tail under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase and its ^1H NMR spectrum showed broad signals. Thus, it was not further purified.

Fraction 40C showed seven UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.17, 0.29,

0.66, 0.68, 0.78, 0.85 and 0.88. It was further separated by column chromatography over silica gel. Elution was performed initially with 100% dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford ten subfractions as shown in **Table 124**.

Table 124 Subfractions obtained from **fraction 40C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40C1	100% CH_2Cl_2	30.5	Yellow gum
40C2	100% CH_2Cl_2	8.2	Yellow gum
40C3	2% MeOH/CH_2Cl_2	15.0	Yellow gum
40C4	2% MeOH/CH_2Cl_2	10.0	Yellow gum
40C5	2% MeOH/CH_2Cl_2	101.9	Yellow gum
40C6	4% MeOH/CH_2Cl_2	54.9	Yellow gum
40C7	7% MeOH/CH_2Cl_2	21.5	Yellow gum
40C8	7% MeOH/CH_2Cl_2	22.7	Yellow gum
40C9	7-10% MeOH/CH_2Cl_2	8.0	Yellow gum
40C10	10% MeOH/CH_2Cl_2-100% MeOH	83.2	brown gum

Subfraction 40C1 showed four pale UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.05, 0.07, 0.12 and 0.16. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40C2 showed two UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.63 and 0.69. It was then purified by precoated TLC with 70% dichloromethane in petroleum ether as a mobile phase (4 runs) to afford three bands.

Band 1 (K29) was a colorless gum (1.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.70.

$[\alpha]_D^{29}$	-57 (*c* 0.08, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	207 (3.60), 240 (3.01), 256 (2.97), 307 (2.70)
FTIR(neat):υ(cm^{-1})	1700 (C=O stretching), 1650 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	7.43 (*d*, *J* = 8.0 Hz, 1H), 6.72 (*d*, *J* = 8.0 Hz, 1H), 5.61 (*s*, 1H), 4.35 (*d*, *J* = 4.0 Hz, 1H), 4.25 (*m*, 1H), 3.45 (*s*, 3H), 2.68 (*dd*, *J* = 17.0 and 3.5 Hz, 1H), 2.62 (*dd*, *J* = 17.0, 11.0 Hz, 1H), 2.28 (*m*, 1H), 1.32 (*d*, *J* = 6.5 Hz, 3H), 1.11 (*d*, *J* = 7.0 Hz, 3H), 0.80 (*d*, *J* = 7.0 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	202.00, 171.01, 145.39, 123.37, 121.97, 120.14, 119.96, 94.71, 90.23, 62.61, 55.78, 35.87, 31.14, 21.12, 18.99, 15.78
DEPT 135: CH;	123.37, 121.97, 94.71, 90.23, 62.61, 31.14
CH_2;	35.87
CH_3;	55.78, 21.12, 18.99, 15.78
EIMS *m/z* (% relative intensity):	276 (13), 245 (100), 189 (98), 161 (11), 115 (8)

Band 2 was a colorless gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.58. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 (K30) was a colorless gum (0.9 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.16.

$[\alpha]_D^{29}$	-55 (*c* 0.08, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	207 (3.63), 241 (3.04), 256 (2.99), 307 (2.73)
FTIR(neat):υ(cm^{-1})	3409 (O-H stretching), 1683 (C=O stretching), 1647 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	7.46 (*d*, *J* = 8.5 Hz, 1H), 6.76 (*d*, *J* = 8.5 Hz, 1H), 6.18 (*s*, 1H), 4.46 (*d*, *J* = 3.5 Hz, 1H), 4.40 (*m*, 1H), 2.72 (*dd*, *J* = 16.5, 2.5 Hz, 1H), 2.62 (*dd*, *J* = 16.5, 10.0 Hz, 1H), 2.29 (*m*, 1H), 1.33 (*d*, *J* = 6.0 Hz, 3H), 1.08 (*d*, *J* = 6.5 Hz, 3H), 0.81 (*d*, *J* = 6.5 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	201.95, 172.48, 146.01, 124.53, 122.49, 121.46, 121.01, 91.04, 88.83, 63.58, 36.94, 32.00, 21.93, 19.53, 16.70
DEPT 135: CH;	124.53, 122.49, 91.04, 88.83, 63.58, 32.00
CH_2;	36.94
CH_3;	21.93, 19.53, 16.70
EIMS *m/z* (% relative intensity):	262 (9), 205 (29), 189 (100), 162 (25), 115 (10)

Subfraction 40C3 showed none of major spots under UV-S on normal phase TLC using 100% dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 40C4 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.02, 0.07, 0.37 and 0.76 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40C5 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.19, 0.26, 0.35 and 0.37. It was further separated by column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed

by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 125.**

Table 125 Subfractions obtained from **subfraction 40C5** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40C5A	1% MeOH/CH_2Cl_2	4.9	Yellow gum
40C5B	1% MeOH/CH_2Cl_2	0.3	Yellow gum
40C5C	1% MeOH/CH_2Cl_2	4.0	Yellow gum
40C5D	1% MeOH/CH_2Cl_2	18.7	Yellow gum
40C5E	2% MeOH/CH_2Cl_2	5.7	Yellow gum
40C5F	2% MeOH/CH_2Cl_2	32.3	Yellow gum
40C5G	2% MeOH/CH_2Cl_2	7.6	Yellow gum
40C5H	4% MeOH/CH_2Cl_2	6.6	Yellow gum
40C5I	4% MeOH/CH_2Cl_2-100% MeOH	13.4	Yellow gum

Subfraction 40C5A showed four UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.74, 0.81, 0.86 and 0.88. Because of low quantity, it was not further investigated.

Subfraction 40C5B showed three UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.33, 0.40 and 0.52. Because of the minute quantity, it was not further investigated.

Subfraction 40C5C showed five UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.09, 0.14, 0.36, 0.50 and 0.55. Because of low quantity, it was not further investigated.

Subfraction 40C5D showed five UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.21, 0.36, 0.43, 0.48 and 0.60. It was further separated by column chromatography over silica gel. Elution was performed initially with 20% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 126**.

Table 126 Subfractions obtained from **subfraction 40C5D** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40C5D1	20% EtOAc/Petrol	3.2	Yellow gum
40C5D2	20% EtOAc/Petrol	2.9	Yellow gum
40C5D3	20% EtOAc/Petrol	3.3	Yellow gum
40C5D4	20% EtOAc/Petrol	1.5	Yellow gum
40C5D5	30% EtOAc/Petrol	3.1	Yellow gum
40C5D6	40% EtOAc/Petrol-100% MeOH	4.2	Yellow gum

Subfraction 40C5D1 showed five UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.27, 0.37, 0.46, 0.51 and 0.70. Because of the minute quantity, it was not further investigated.

Subfraction 40C5D2 showed two UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.72 and 0.76. Its ^1H NMR spectrum indicated that the major compound was **K38**. Further investigation was then not performed.

Subfraction 40C5D3 showed two UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.58 and

0.76. Its ^{1}H NMR spectrum indicated that the major compound was **K36**. Further investigation was then not performed.

Subfraction 40C5D4 showed two UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.58 and 0.60. Because of the minute quantity, it was not further investigated.

Subfraction 40C5D5 showed four UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.37, 0.46, 0.53 and 0.56. Because of the minute quantity, it was not further investigated.

Subfraction 40C5D6 displayed a long tail under UV-S on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40C5E showed none of major spots under UV-S on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40C5F showed four UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.14, 0.19, 0.37 and 0.40. It was further separated by column chromatography over silica gel. Elution was performed initially with 40% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 127**.

Table 127 Subfractions obtained from **subfraction 40C5F** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40C5F1	40% EtOAc/Petrol	8.1	Yellow gum
40C5F2	40% EtOAc/Petrol	5.8	Yellow gum

Table 127 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
40C5F3	40% EtOAc/Petrol	2.9	Yellow gum
40C5F4	40% EtOAc/Petrol	3.2	Yellow gum
40C5F5	60% EtOAc/Petrol	4.6	Yellow gum
40C5F6	100% EtOAc-100% MeOH	5.6	Yellow gum

Subfraction 40C5F1 showed four pale UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.23, 0.34, 0.35 and 0.39. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40C5F2 showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.58 and 0.60. Its ^1H NMR spectrum indicated that the major compound was **K37**. Further investigation was then not performed.

Subfraction 40C5F3 showed three pale UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.37, 0.41 and 0.58. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40C5F4 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.25, 0.34 and 0.51. Because of the minute quantity, it was not further investigated.

Subfraction 40C5F5 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.14, 0.18 and 0.53. Because of low quantity, it was not further investigated.

Subfraction 40C5F6 displayed a long tail under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40C5G showed four UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.07, 0.12, 0.19 and 0.32. Because of low quantity, it was not further investigated.

Subfraction 40C5H showed two UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values 0.02 and 0.32. Its ^{1}H NMR spectrum indicated that the major compound was **K37**. Further investigation was then not performed.

Subfraction 40C5I displayed a long tail under UV-S on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40C6 showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.16, 0.18, 0.19, 0.34, 0.35 and 0.42. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 128**.

Table 128 Subfractions obtained from **subfraction 40C6** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40C6A	2% MeOH/CH_2Cl_2	7.1	Yellow gum
40C6B	2% MeOH/CH_2Cl_2	5.4	Yellow gum
40C6C	2% MeOH/CH_2Cl_2	26.0	Yellow gum
40C6D	2-4% MeOH/CH_2Cl_2	5.7	Yellow gum

Table 128 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
40C6E	4-7% MeOH/CH_2Cl_2	2.8	Yellow gum
40C6F	10% MeOH/CH_2Cl_2-100% MeOH	7.3	Brown gum

Subfraction 40C6A showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.23, 0.42, 0.46 and 0.63. Because of low quantity, it was not further investigated.

Subfraction 40C6B showed four pale UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values 0.21, 0.30, 0.37 and 0.39. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40C6C showed six UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.16, 0.21, 0.33, 0.39, 0.51 and 0.81. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 129**.

Table 129 Subfractions obtained from **subfraction 40C6C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40C6C1	2% MeOH/CH_2Cl_2	1.7	Yellow gum
40C6C2	2% MeOH/CH_2Cl_2	2.5	Yellow gum
40C6C3	2% MeOH/CH_2Cl_2	3.2	Yellow gum

Table 129 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
40C6C4	2-4% MeOH/CH_2Cl_2	8.2	Yellow gum
40C6C5	4% MeOH/CH_2Cl_2-100% MeOH	7.6	Brown gum

Subfraction 40C6C1 showed none of major spots under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 40C6C2 showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.70. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40C6C3 showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.16, 0.23, 0.33, 0.39 and 0.51. Because of the minute quantity, it was not further investigated.

Subfraction 40C6C4 showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.16 and 0.33. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (7 runs) to afford two bands.

Band 1 was a colorless gum (5.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.40. Its ^{1}H NMR spectrum displayed signals in the high field region, it was not purified.

Band 2 was a colorless gum (3.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.36. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40C6C5 displayed a long tail under UV-S on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40C7 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.16, 0.23, 0.30 and 0.42. It was further separated by column chromatography over silica gel. Elution was performed initially with 40% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 130.**

Table 130 Subfractions obtained from **subfraction 40C7** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40C7A	40% EtOAc/Petrol	3.5	Yellow gum
40C7B	40% EtOAc/Petrol	2.0	Yellow gum
40C7C	40-60% EtOAc/Petrol	5.1	Yellow gum
40C7D	60-80% EtOAc/Petrol	5.6	Yellow gum
40C7E	80% EtOAc/Petrol-100% MeOH	5.0	Yellow gum

Subfraction 40C7A showed none of major spots under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40C7B showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.15, 0.27 and 0.48. Its ^1H NMR spectrum indicated that the major compound was **K29**. Further investigation was then not performed.

Subfraction 40C7C showed five UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.15, 0.27, 0.36, 0.41 and 0.48. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not purified.

Subfraction 40C7D showed four UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.13, 0.19, 0.23 and 0.45. Because of low quantity, it was not further investigated.

Subfraction 40C7E displayed a long tail under UV-S on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40C8 showed four UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.43, 0.50, 0.60 and 0.70. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 131**.

Table 131 Subfractions obtained from **subfraction 40C8** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
40C8A	8.1	Yellow gum
40C8B	7.2	Yellow gum
40C8C	7.3	Yellow gum

Subfraction 40C8A showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.15 and 0.27. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 132.**

Table 132 Subfractions obtained from **subfraction 40C8A** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40C8A1	2.6	Yellow gum
40C8A2	0.5	Yellow gum
40C8A3	4.1	Colorless gum

Subfraction 40C8A1 showed none of major spots under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 40C8A2 showed one pale UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.14. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated

Subfraction 40C8A3 (K31) showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.29.

$[\alpha]_D^{29}$	-26 (*c* 0.21, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	251 (3.04), 277 (3.01), 284 (2.97)
FTIR(neat):υ(cm^{-1})	3351 (O-H stretching), 1657 (C=C stretching)

^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	7.11 (*t*, *J* = 7.8 Hz, 1H), 6.74 (*d*, *J* = 7.8 Hz, 1H), 6.71 (*d*, *J* = 7.8 Hz, 1H), 4.85 (*d*, *J* = 13.2 Hz, 1H), 4.79 (*d*, *J* = 13.2 Hz, 1H), 3.92 (*dq*, *J* = 10.0, 6.0 Hz, 1H), 2.75 (*dd*, *J* = 12.5, 2.4 Hz, 1H), 2.74 (*dd*, *J* = 12.5, 10.0 Hz, 1H), 1.24 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	156.23, 137.72, 129.02, 124.91, 122.49, 114.96, 69.10, 58.49, 41.81, 23.43
DEPT 135: CH;	129.02, 122.49, 114.96, 69.10
CH_2;	58.49, 41.81
CH_3;	23.43
EIMS *m/z* (% relative intensity):	164 (14), 120 (100), 91 (78), 77 (17), 65 (14)

Subfraction 40C8B showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.20 and 0.27. Its ^{1}H NMR spectrum indicated that the major compound was **K30**. Further investigation was then not performed.

Subfraction 40C8C showed four pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.02, 0.13, 0.17 and 0.24. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40C9 showed six UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.13, 0.28, 0.30, 0.47, 0.72 and 0.84. Because of low quantity, it was not further investigated.

Subfraction 40C10 showed six UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.09, 0.16, 0.21, 0.30, 0.40 and 0.46. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Fraction 40D showed nine UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.17, 0.27, 0.54, 0.59, 0.63, 0.73, 0.78 and 0.85. It was further separated by column chromatography over silica gel. Elution was performed initially with 100% dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 133.**

Table 133 Subfractions obtained from **fraction 40D** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40D1	100% CH_2Cl_2	4.9	Brown gum
40D2	100% CH_2Cl_2	9.6	Brown gum
40D3	100% CH_2Cl_2	4.0	Brown gum
40D4	2% MeOH/CH_2Cl_2	39.5	Yellow gum
40D5	2% MeOH/CH_2Cl_2	15.2	Brown gum
40D6	4% MeOH/CH_2Cl_2	106.5	Brown gum
40D7	4-7% MeOH/CH_2Cl_2	33.7	Brown gum
40D8	7-10% MeOH/CH_2Cl_2	40.5	Brown gum
40D9	10% MeOH/CH_2Cl_2-100% MeOH	58.4	Brown solid

Subfraction 40D1 displayed a long tail under UV-S on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40D2 showed three UV-active spots on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.09, 0.73 and 0.76. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified

Subfraction 40D3 showed four UV-active spots on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.51, 0.53, 0.65 and 0.74. Because of low quantity, it was not further investigated.

Subfraction 40D4 showed five UV-active spots on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.07, 0.19, 0.23, 0.49 and 0.74. It was further separated by column chromatography over silica gel. Elution was performed initially with 70% dichloromethane in petroleum ether and gradually enriched with dichloromethane and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 134.**

Table 134 Subfractions obtained from **subfraction 40D4** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40D4A	70% CH_2Cl_2/Petrol	2.9	Colorless gum
40D4B	70-80% CH_2Cl_2/Petrol	7.6	Colorless gum
40D4C	80% CH_2Cl_2/Petrol-100% CH_2Cl_2	5.1	Colorless gum
40D4D	2-5% MeOH/CH_2Cl_2	2.1	Yellow gum
40D4E	7% MeOH/CH_2Cl_2-100% MeOH	18.1	Yellow gum

Subfraction 40D4A showed one pale UV-active spot on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.44. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40D4B showed six UV-active spots on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.05,

0.15, 0.19, 0.24, 0.52 and 0.66. Because of low quantity, it was not further investigated.

Subfraction 40D4C showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.14, 0.21, 0.26 and 0.57. Its ^1H NMR spectrum indicated that the major compound was **K28**. Further investigation was then not performed.

Subfraction 40D4D showed two UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.16 and 0.23. Because of the minute quantity, it was not further investigated.

Subfraction 40D4E showed three pale UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.04, 0.07 and 0.12. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40D5 showed two UV-active spots on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.28 and 0.39. Its ^1H NMR spectrum indicated that the major compound was **K28**. Further investigation was then not performed.

Subfraction 40D6 showed seven UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.08, 0.13, 0.21, 0.29, 0.40, 0.67 and 0.73. It was further separated by column chromatography over silica gel. Elution was performed initially with 100% dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 135.**

Table 135 Subfractions obtained from **subfraction 40D6** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40D6A	100% CH_2Cl_2	7.0	Yellow gum
40D6B	1% MeOH/CH_2Cl_2	8.0	Yellow gum
40D6C	2% MeOH/CH_2Cl_2	46.2	Yellow gum
40D6D	2-5% MeOH/CH_2Cl_2	23.3	Yellow gum
40D6E	10-60% MeOH/CH_2Cl_2	5.2	Yellow gum
40D6F	60% MeOH/CH_2Cl_2-100% MeOH	14.3	Brown gum

Subfraction 40D6A showed three pale UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.62, 0.79 and 0.83. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40D6B showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.26, 0.31 and 0.83. It was then purified by precoated TLC with 10% ethyl acetate in petroleum ether as a mobile phase (4 runs) to afford two bands.

Band 1 (K28) was a colorless gum (3.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.50.

$[\alpha]_D^{29}$	+12 (*c* 0.16, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	213 (3.68), 253 (3.28), 269 (3.15), 324 (2.92)
FTIR(neat):υ(cm^{-1})	1695 (C=O stretching), 1647 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	7.46 (*d*, *J* = 7.8 Hz, 1H), 6.77 (*d*, *J* = 7.8 Hz,

	1H), 4.86 (*d*, *J* = 16.2 Hz, 1H), 4.66 (*d*, *J* = 16.2 Hz, 1H), 3.27 (*s*, 3H), 2.94 (*d*, *J* = 16.2 Hz, 1H), 2.85 (*d*, *J* = 16.2 Hz, 1H), 2.29 (*s*, 3H), 2.02 (*s*, 3H), 1.46 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	183.43, 160.37, 145.28, 140.72, 131.54, 123.03, 121.91, 121.19, 118.25, 97.43, 57.75, 49.00, 39.30, 22.99, 20.15, 17.40
DEPT 135: CH;	123.03, 121.91
CH_2;	57.75, 39.30
CH_3;	49.00, 22.99, 20.15, 17.40
EIMS *m/z* (% relative intensity):	274 (13), 242 (36), 200 (100), 149 (13), 115 (7)

Band 2 was a yellow gum (2.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.21. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D6C showed six UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.30, 0.46, 0.53, 0.58, 0.63 and 0.72. It was further separated by column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 136.**

Table 136 Subfractions obtained from **subfraction 40D6C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40D6C1	1% MeOH/CH_2Cl_2	2.9	Yellow gum
40D6C2	1% MeOH/CH_2Cl_2	7.3	Yellow gum

Table 136 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
40D6C3	1% MeOH/CH_2Cl_2	16.1	Yellow gum
40D6C4	1% MeOH/CH_2Cl_2	3.6	Yellow gum
40D6C5	1% MeOH/CH_2Cl_2	4.5	Yellow gum
40D6C6	2% MeOH/CH_2Cl_2-100% MeOH	10.9	Brown gum

Subfraction 40D6C1 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.46, 0.53, 0.67 and 0.76. Because of the minute quantity, it was not further investigated.

Subfraction 40D6C2 showed three UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.28, 0.31 and 0.42. It was then purified by precoated TLC with 100% dichloromethane as a mobile phase (5 runs) to afford two bands.

Band 1 (K37) was a colorless gum (3.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.42.

UV λ_{max}(nm)(MeOH)(log ε)	210 (2.86), 240 (2,48), 253 (2.51), 270 (2.63), 293 (2.55), 352 (2.56)
FTIR(neat):υ(cm^{-1})	1695 (C=O stretching), 1644 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	9.56 (*s*, 1H), 7.83 (*d*, *J* = 8.7 Hz, 1H), 7.38 (*d*, *J* = 8.7 Hz, 1H), 7.56 (*s*, 1H), 2.76 (*s*, 3H), 2.45 (*s*, 3H), 2.26 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	182.21, 164.04, 156.68, 146.21, 145.60, 141.36, 133.75, 124.23, 120.55, 119.55, 119.36, 114.62, 24.65, 20.40, 17.50

DEPT 135: CH; 146.21, 124.23, 120.55, 119.55

CH_3; 24.65, 20.40, 17.50

Band 2 was a colorless gum (2.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.31. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D6C3 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.16, 0.35, 0.42 and 0.51. It was further separated by column chromatography over silica gel. Elution was performed with 100% dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 137.**

Table 137 Subfractions obtained from **subfraction 40D6C3** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
40D6C3A	1.9	Yellow gum
40D6C3B	2.1	Yellow gum
40D6C3C	1.8	Yellow gum
40D6C3D	8.6	Yellow gum

Subfraction 40D6C3A showed five pale UV-active spots on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.35, 0.39, 0.46, 0.65 and 0.72. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40D6C3B showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.31. Because the

^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D6C3C showed two UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.28 and 0.51. Because of the minute quantity, it was not further investigated.

Subfraction 40D6C3D showed two UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.57 and 0.69. It was then purified by precoated TLC with 40% ethyl acetate in petroleum ether as a mobile phase (3 runs) to afford two bands.

Band 1 was a yellow gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.69. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a yellow gum (5.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.57. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D6C4 showed six UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.19, 0.28, 0.37, 0.51, 0.56 and 0.81. Because of low quantity, it was not further investigated.

Subfraction 40D6C5 showed four UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.19, 0.30, 0.42 and 0.51. Because of low quantity, it was not further investigated.

Subfraction 40D6C6 showed four pale UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.12, 0.16, 0.35 and 0.39. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40D6D showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.26, 0.31 and 0.83. It was further separated by column chromatography over silica gel. Elution was performed with 40% ethyl acetate in petroleum ether. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 138.**

Table 138 Subfractions obtained from **subfraction 40D6D** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
40D6D1	3.9	Colorless gum
40D6D2	9.7	Colorless gum
40D6D3	4.3	Yellow gum
40D6D4	4.0	Yellow gum

Subfraction 40D6D1 showed three UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.34, 0.72 and 0.86. Because of low quantity, it was not further investigated.

Subfraction 40D6D2 (K38) showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.39.

$[\alpha]_D^{29}$	-10 (*c* 0.50, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	210 (3.69), 253 (3.67), 352 (3.16)

FTIR(neat):υ(cm^{-1})	3368 (O-H stretching), 1697 (C=O stretching), 1644 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	9.48 (*s*, 1H), 7.53 (*d*, *J* = 8.7 Hz, 1H), 7.13 (*s*, 1H), 6.98 (*d*, *J* = 8.7 Hz, 1H), 2.50 (*s*, 3H), 2.43 (*m*, 1H), 1.24 (*d*, *J* = 7.0 Hz, 3H), 0.99 (*d*, *J* = 7.0 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	198.48, 171.50, 156.71, 146.50, 141.97, 124.27, 119.38, 115.64, 114.61, 110.03, 33.93, 23.70, 15.94, 15.67
DEPT 135: CH;	146.50, 124.27, 119.38, 33.93
CH_3;	23.70, 15.94, 15.67

Subfraction 40D6D3 showed five UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.16, 0.18, 0.20, 0.22 and 0.32. Because of low quantity, it was not further investigated.

Subfraction 40D6D4 showed five UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.11, 0.18, 0.24, 0.28 and 0.33. Because of low quantity, it was not further investigated.

Subfraction 40D6E showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.19, 0.23 and 0.30. Because of low quantity, it was not further investigated.

Subfraction 40D6F displayed a long tail under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Thus, it was not investigate.

Subfraction 40D7 showed four UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.14, 0.21, 0.55 and 0.51. It was further separated by column chromatography over silica gel. Elution was performed initially with 30% ethyl acetate in petroleum ether and

gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 139.**

Table 139 Subfractions obtained from **subfraction 40D7** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40D7A	30% EtOAc/Petrol	6.0	Yellow gum
40D7B	30% EtOAc/Petrol	3.1	Yellow gum
40D7C	30% EtOAc/Petrol	5.4	Yellow gum
40D7D	30% EtOAc/Petrol	5.0	Yellow gum
40D7E	50% EtOAc/Petrol	8.9	Yellow gum
40D7F	70% EtOAc/Petrol-100% MeOH	5.1	Yellow gum

Subfraction 40D7A showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.08 and 0.80 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40D7B showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.65 and 0.70 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction 40D7C showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.20 and 0.24 and many spots after dipping the TLC plate in anisaldehyde reagent and

subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40D7D showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.15 and 0.24. It was then purified by precoated TLC with 20% ethyl acetate in petroleum ether as a mobile phase (5 runs) to afford two bands.

Band 1 (K32) was a colorless gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.18.

$[\alpha]_D^{29}$	-12 (*c* 0.13, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	205 (3.56), 215 (3.35), 268 (2.63), 275 (2.61)
FTIR(neat):υ(cm^{-1})	3402 (O-H stretching), 1653 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	7.07 (*t*, *J* = 8.0 Hz, 1H), 6.73 (*d*, *J* = 8.0 Hz, 1H), 6.60 (*d*, *J* = 8.0 Hz, 1H), 5.06 (*d*, *J* = 12.0 Hz, 1H), 5.01 (*d*, *J* = 12.0 Hz, 1H), 4.95 (*d*, *J* = 2.0 Hz, 1H), 3.85 (*qd*, *J* = 6.5, 2.0 Hz, 1H), 1.26 (*d*, *J* = 6.5 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	150.36, 140.68, 129.25, 126.12, 114.55, 113.99, 88.52, 71.07, 69.96, 18.58
DEPT 135: CH;	129.25, 114.55, 113.99, 88.52, 69.96
CH_2;	71.07
CH_3;	18.58
EIMS *m/z* (% relative intensity):	180 (2), 136 (23), 135 (100), 107 (23), 77 (20)

Band 2 was a colorless gum (2.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.15. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D7E showed five UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.10, 0.15, 0.20, 0.28 and 0.32 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40D7F displayed a long tail under UV-S on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction 40D8 showed seven UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.29, 0.32, 0.42, 0.50, 0.67 and 0.78. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 140**.

Table 140 Subfractions obtained from **subfraction 40D8** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40D8A	2% MeOH/CH_2Cl_2	2.1	Yellow gum
40D8B	2% MeOH/CH_2Cl_2	3.0	Yellow gum
40D8C	2% MeOH/CH_2Cl_2	2.2	Yellow gum
40D8D	4% MeOH/CH_2Cl_2-100% MeOH	30.0	Yellow gum

Subfraction 40D8A showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.21, 0.23 and 0.58. Because of the minute quantity, it was not further investigated.

Subfraction 40D8B showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.26 and 0.36. Because of the minute quantity, it was not further investigated.

Subfraction 40D8C showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.16, 0.23 and 0.31. Because of the minute quantity, it was not further investigated.

Subfraction 40D8D showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.21 and 0.52. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 141.**

Table 141 Subfractions obtained from **subfraction 40D8D** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40D8D1	50% MeOH/H_2O	14.3	Yellow gum
40D8D2	50% MeOH/H_2O	2.9	Yellow gum
40D8D3	50% MeOH/H_2O	2.8	Yellow gum
40D8D4	70% MeOH/H_2O-100% MeOH	9.3	Yellow gum

Subfraction 40D8D1 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.17 and 0.21. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram

were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 142.**

Table 142 Subfractions obtained from **subfraction 40D8D1** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40D8D1A	3.7	Yellow gum
40D8D1B	7.2	Colorless gum
40D8D1C	3.3	Yellow gum

Subfraction 40D8D1A showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.14. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D8D1B (K35) showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.17.

UV λ_{max}(nm)(MeOH)(log ε)	206 (3.10), 250 (2.49), 289 (2.19)
FTIR(neat):υ(cm^{-1})	3328 (O-H stretching), 1638 (C=C stretching)
^{1}H NMR($CDCl_3$+CD_3OD)(δ_{ppm})(300 MHz):	7.14 (*t*, *J* = 7.8 Hz, 1H), 6.81 (*d*, *J* = 7.8 Hz, 1H), 6.64 (*d*, *J* = 11.4 Hz, 1H), 6.59 (*d*, *J* = 7.8 Hz, 1H), 5.97 (*dt*, *J* = 11.4, 6.9 Hz, 1H), 4.78 (*s*, 2H), 4.07 (*dd*, *J* = 6.9, 0.6 Hz, 2H)
^{13}CNMR($CDCl_3$+CD_3OD)(δ_{ppm})(75 MHz):	156.26, 136.29, 131.88, 129.95, 128.45, 123.58, 120.92, 115.30, 59.10, 58.58

DEPT 135: CH;	131.88, 129.95, 128.45, 120.92, 115.30
CH_2;	59.10, 58.58
EIMS *m/z* (% relative intensity):	180 (12), 162 (78), 133 (100), 105 (40), 77 (36)

Subfraction 40D8D1C showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.21. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D8D2 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12 and 0.17. Its ^1H NMR spectrum indicated that the major compound was **K38**. Further investigation was then not performed.

Subfraction 40D8D3 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.21 and 0.29. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D8D4 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.17, 0.61 and 0.65. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 143**.

Table 143 Subfractions obtained from **subfraction 40D8D4** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40D8D4A	2.2	Yellow gum
40D8D4B	4.4	Colorless gum
40D8D4C	2.5	Yellow gum

Subfraction 40D8D4A showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.17 and 0.56. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D8D4B (K36) showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.17.

$[\alpha]_D^{29}$	-21 (*c* 0.50, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	210 (3.72), 252 (3.69), 353 (3.19)
FTIR(neat):υ(cm^{-1})	3245 (O-H stretching), 1692 (C=O stretching), 1641 (C=C stretching)
^{1}H NMR($CDCl_3$+CD_3OD)(δ_{ppm})(300 MHz):	9.48 (*s*, 1H), 7.79 (*s*, 1H), 7.72 (*d*, *J* = 8.4 Hz, 1H), 7.36 (*d*, *J* = 8.4 Hz, 1H), 4.89 (*s*, 2H), 2.36 (*m*, 1H), 1.19 (*brs*, 3H), 0.97 (*brs*, 3H)
^{13}C NMR($CDCl_3$+CD_3OD)(δ_{ppm})(75 MHz):	198.97, 171.93, 159.25, 146.61, 142.70, 124.53, 120.34, 117.36, 115.88, 110.13, 64.54, 34.17, 15.96, 15.45

DEPT 135:	CH;	146.61, 124.53, 120.34, 117.36, 34.17
	CH_2;	64.54
	CH_3;	15.96, 15.45
EIMS *m/z* (% relative intensity):		273 (9), 202 (100), 145 (29), 117 (13), 89 (7)

Subfraction 40D8D4C showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.17 and 0.60. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D9 showed seven UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.15, 0.18, 0.25, 0.33, 0.45 and 0.65. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 144.**

Table 144 Subfractions obtained from **subfraction 40D9** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40D9A	2% MeOH/CH_2Cl_2	4.4	Yellow gum
40D9B	2-4% MeOH/CH_2Cl_2	1.3	Yellow gum
40D9C	4% MeOH/CH_2Cl_2	4.7	Yellow gum
40D9D	4-7% MeOH/CH_2Cl_2	6.3	Brown gum
40D9E	7% MeOH/CH_2Cl_2	14.1	Brown gum
40D9F	7% MeOH/CH_2Cl_2-100% MeOH	21.1	Brown solid

Subfraction 40D9A showed four pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.48, 0.54, 0.91 and 0.98. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40D9B showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.21. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40D9C showed five pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.09, 0.14, 0.18 and 0.20. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40D9D showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.27, 0.36 and 0.95. Because of low quantity, it was not further investigated.

Subfraction 40D9E showed one pale UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.06. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40D9F displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Fraction 40E showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.12, 0.22, 0.44, 0.49 and 0.85. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing

the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 145.**

Table 145 Subfractions obtained from **fraction 40E** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40E1	2% MeOH/CH_2Cl_2	3.9	Yellow solid
40E2	2% MeOH/CH_2Cl_2	1.0	Yellow solid
40E3	2% MeOH/CH_2Cl_2	4.5	Yellow solid
40E4	2% MeOH/CH_2Cl_2	2.6	Colorless gum
40E5	2% MeOH/CH_2Cl_2	2.5	Brown solid
40E6	4% MeOH/CH_2Cl_2	5.9	Brown solid
40E7	8% MeOH/CH_2Cl_2	2.1	Brown solid
40E8	10% MeOH/CH_2Cl_2-100% MeOH	4.4	Brown solid

Subfraction 40E1 showed seven UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.09, 0.21, 0.36, 0.40, 0.62, 0.69 and 0.83. Because of low quantity, it was not further investigated.

Subfraction 40E2 showed two UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.36 and 0.43. Because of the minute quantity, it was not further investigated.

Subfraction 40E3 showed two UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.50 and 0.66. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40E4 (K33) showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.25.

UV λ_{max}(nm)(MeOH)(log ε)	209 (3.84), 224 (3.64), 247 (3.43), 361 (3.31)
FTIR(neat):υ(cm^{-1})	3399 (O-H stretching), 1683 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	6.02 (*s*, 1H), 5.94 (*s*, 1H), 3.64 (*d*, *J* = 9.6 Hz, 1H), 3.54 (*d*, *J* = 9.6 Hz, 1H), 2.30 (*s*, 3H), 2.22 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	169.85, 169.07, 166.94, 166.45, 160.72, 143.11, 108.18, 103.11, 102.76, 102.40, 19.69, 18.99, 18.25
DEPT 135: CH;	103.11, 102.76
CH_2;	18.25
CH_3;	19.69, 18.99
EIMS *m/z* (% relative intensity):	263 (70), 220 (19), 178 (45), 150 (100), 125 (18)

Subfraction 40E5 showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.16 and 0.21. Because of the minute quantity, it was not further investigated.

Subfraction 40E6 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.14 and 0.23. Because of low quantity, it was not further investigated.

Subfraction 40E7 showed one pale UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.18. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40E8 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 40F showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.19, 0.32, 0.46, 0.58 and 0.66. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 146**.

Table 146 Subfractions obtained from **fraction 40F** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40F1	2% MeOH/CH_2Cl_2	1.8	Yellow gum
40F2	4% MeOH/CH_2Cl_2	1.9	Yellow gum
40F3	4-8% MeOH/CH_2Cl_2	3.9	Yellow gum
40F4	10% MeOH/CH_2Cl_2	4.2	Yellow gum
40F5	10% MeOH/CH_2Cl_2	1.3	Yellow gum
40F6	20% MeOH/CH_2Cl_2. 100% MeOH	13.8	Brown gum

Subfraction 40F1 showed five pale UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.14, 0.19, 0.36, 0.43 and 0.72. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40F2 showed four UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.14, 0.19, 0.35 and 0.43. Because of the minute quantity, it was not further investigated.

Subfraction 40F3 showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.14, 0.19, 0.24 and 0.33. Because of low quantity, it was not further investigated.

Subfraction 40F4 showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07 and 0.21. Because of low quantity, it was not further investigated.

Subfraction 40F5 showed three pale UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.16, 0.17 and 0.18. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40F6 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.46, 0.59 and 0.93. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

4.2.3 Purification of the EtOAc extract from mycelia

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six fractions as shown in **Table147.**

Table 147 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
40CE1	181.7	Brown gum
40CE2	181.0	Brown gum

Table 147 Continued

Fraction	Weight (mg)	Physical appearance
40CE3	92.6	Brown gum
40CE4	19.5	Brown gum
40CE5	24.8	Brown gum
40CE6	40.7	Brown gum

Fraction 40CE1 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 40CE2 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.09, 0.19, 0.23 and 0.30. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 40CE3 showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.15, 0.22, 0.35, 0.60 and 0.86. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 148**.

Table 148 Subfractions obtained from **fraction 40CE3** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40CE3A	50% MeOH/H_2O	34.7	Yellow gum
40CE3B	50% MeOH/H_2O	5.9	Yellow gum
40CE3C	50% MeOH/H_2O	8.7	Yellow gum

Table 148 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
40CE3D	60% MeOH/H_2O	6.8	Yellow gum
40CE3E	60% MeOH/H_2O	8.2	Yellow gum
40CE3F	80% MeOH/H_2O	5.1	Yellow gum
40CE3G	80% MeOH/H_2O - 100% MeOH	20.3	Yellow gum

Subfraction 40CE3A showed six UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.05, 0.15, 0.22, 0.35, 0.60 and 0.86. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 149**.

Table 149 Subfractions obtained from **subfraction 40CE3A** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
40CE3A1	24.1	Yellow gum
40CE3A2	8.3	Yellow gum
40CE3A3	1.5	Yellow gum

Subfraction 40CE3A1 showed five UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.21, 0.29, 0.36 and 0.93. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 150**.

Table 150 Subfractions obtained from **subfraction 40CE3A1** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40CE3A1A	2% MeOH/CH_2Cl_2	3.8	Yellow gum
40CE3A1B	5% MeOH/CH_2Cl_2	3.0	Yellow gum
40CE3A1C	5% MeOH/CH_2Cl_2	1.2	Yellow gum
40CE3A1D	5-7% MeOH/CH_2Cl_2	1.1	Yellow gum
40CE3A1E	7-15% MeOH/CH_2Cl_2	3.9	Yellow gum
40CE3A1F	30% MeOH/CH_2Cl_2-100% MeOH	10.0	Yellow gum

Subfraction 40CE3A1A showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.35 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40CE3A1B showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.09 and 0.19 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction 40CE3A1C showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.20 and 0.25 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction 40CE3A1D showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.20, 0.39

and 0.45 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction 40CE3A1E showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.13, 0.23 and 0.27 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40CE3A1F displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 40CE3A2 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05 and 0.78 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40CE3A3 showed none of major spots under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 40CE3B showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.17, 0.26 and 0.37. It was then purified by precoated TLC with 3% methanol in dichloromethane as a mobile phase (7 runs) to afford three bands.

Band 1 (K41) was a colorless gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.37.

UV λ_{max}(nm)(MeOH)(log ε)	206 (3.63), 262 (3.12), 328 (2.51)
FTIR(neat):υ(cm^{-1})	3370 (O-H stretching), 1716 (C=O stretching), 1674 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	9.01 (*brs*, 1H), 8.20 (*dt*, *J* = 8.0, 2.0 Hz, 1H), 7.44 (*dd*, *J* = 8.0, 5.0 Hz, 1H), 8.75 (*brs*, 1H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	167.00, 152.64, 148.18, 135.66, 129.15, 123.68
DEPT 135: CH;	152.64, 148.18, 135.66, 123.68

Band 2 was a colorless gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.28. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was a colorless gum (1.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.17. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40CE3C showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.26, 0.37 and 0.41. It was then purified by precoated TLC with 3% methanol in dichloromethane as a mobile phase (7 runs) to afford two bands.

Band 1 (K40) was a colorless gum (1.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.31.

UV λ_{max}(nm)(MeOH)(log ε)	207 (3.39), 238 (3.13), 284 (3.15), 327 (3.16)

FTIR(neat):υ(cm^{-1})	1695 (C=O stretching), 1647 (C=C stretching)
^{1}H NMR($CDCl_3$+CD_3OD)(δ_{ppm})(300MHz):	10.25 (*brs*, 1H), 8.25 (*dd*, *J* = 8.1, 1.2 Hz, 1H), 7.59 (*td*, *J* = 8.1, 1.2 Hz, 1H), 7.48 (*d*, *J* = 8.1 Hz, 1H), 7.32 (*td*, *J* = 8.1, 1.2 Hz, 1H), 7.01 (*dd*, *J* = 2.7, 1.2 Hz, 1H), 6.76 (*dd*, *J* = 3.6, 1.2 Hz, 1H), 6.33 (*dd*, *J* = 3.6, 2.7 Hz, 1H), 3.88 (*s*, 3H)
^{13}C NMR($CDCl_3$+CD_3OD)(δ_{ppm})(75 MHz):	176.00, 169.50, 139.30, 132.70, 125.98, 124.30, 122.80, 122.76, 117.70, 112.31, 110.42, 52.91
DEPT 135: CH;	132.70, 125.98, 124.30, 122.76, 117.70, 112.31, 110.42
CH_3;	52.91

Band 2 was a colorless gum (3.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.28. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 40CE3D showed five UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.17, 0.28, 0.55, 0.60 and 0.66 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40CE3E showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.21 and 0.55. It was then purified by precoated TLC with 20% ethyl acetate in petroleum ether as a mobile phase (5 runs) to afford three bands.

Band 1 was a colorless gum (1.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.52. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a colorless gum (1.8 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.33. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was a colorless gum (3.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.28. Its ^1H NMR spectrum indicated the presence of **K38**. Further investigation was then not carried out.

Subfraction 40CE3F showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.62 and 0.80 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 40CE3G showed none of major spots under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 40CE4 showed five UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.16, 0.26, 0.33 and 0.51. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 151.**

Table 151 Subfractions obtained from **fraction 40CE4** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40CE4A	2% MeOH/CH_2Cl_2	4.4	Yellow gum
40CE4B	2% MeOH/CH_2Cl_2	3.0	Yellow gum
40CE4C	4% MeOH/CH_2Cl_2	3.5	Yellow gum
40CE4D	7% MeOH/CH_2Cl_2-100% MeOH	7.8	Yellow gum

Subfraction 40CE4A showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.31, 0.36, 045, 0.93 and 0.95. Because of low quantity, it was not further investigated.

Subfraction 40CE4B showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.21. Its ^1H NMR spectrum indicated the presence of **K40**. Further investigation was then not carried out.

Subfraction 40CE4C showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.09, 0.14, 0.21, 0.24 and 0.62. Because of low quantity, it was not further investigated.

Subfraction 40CE4D showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.19 and 0.26. Because of low quantity, it was not further investigated.

Fraction 40CE5 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.13 and 0.19. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram

were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 152.**

Table 152 Subfractions obtained from **fraction 40CE5** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
40CE5	1.5	Brown gum
40CE5	8.8	Brown gum
40CE5	12.4	Brown gum
40CE5	1.3	Brown gum

Subfraction 40CE5A displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 40CE5B showed four pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.06, 0.13, 0.20 and 0.28. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40CE5C showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.06, 0.11, 0.17, 0.24, 0.28 and 0.35. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 153.**

Table 153 Subfractions obtained from **subfraction 40CE5C** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40CE5C1	2% MeOH/CH_2Cl_2	2.3	Yellow gum
40CE5C2	2-7% MeOH/CH_2Cl_2	3.9	Yellow gum
40CE5C3	10% MeOH/CH_2Cl_2-100% MeOH	5.4	Yellow gum

Subfraction 40CE5C1 showed three pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.48, 0.63 and 0.75. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40CE5C2 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.18 and 0.32. Its ^1H NMR spectrum indicated that the major compound was **K40**. Further investigation was then not performed.

Subfraction 40CE5C3 showed four pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.03, 0.10, 0.30 and 0.62. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 40CE5D displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 40CE6 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.18 and 0.39. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by

increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 154.**

Table 154 Subfractions obtained from **fraction 40CE6** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
40CE6A	50% MeOH/H_2O	10.3	Yellow gum
40CE6B	50% MeOH/H_2O	3.7	Yellow gum
40CE6C	50-60% MeOH/H_2O	7.5	Yellow gum
40CE6D	70% MeOH/H_2O-100% MeOH	16.2	Yellow gum

Subfraction 40CE6A showed none of major spots under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not further investigated.

Subfraction 40CE6B showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.20, 0.26 and 0.87. Because of low quantity, it was not further investigated.

Subfraction 40CE6C showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.22 and 0.31. Its ^1H NMR spectrum indicated that the major compound was **K40.** Further investigation was then not performed.

Subfraction 40CE6D showed five pale UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.15,

0.24, 0.33 and 0.51. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

CHAPTER 4.3

RESULTS AND DISCUSSION

Five new isochromans **(K28-K32)**, one new pyrone **(K33)**, one new pyranone **(K34)**, one new phenol **(K35)** and one new isoquinoline **(K36)** together with three known compounds **(K37-K39)** were isolated from the broth extract. Furthermore, two known metabolites **(K40-K41)** were obtained from the mycelial extract. The structures were identified by spectroscopic methods.

4.3.1 Compound K28

Compound **K28** was obtained as a colorless gum. The molecular formula $C_{16}H_{18}O_4$ was assigned by EIMS of M^+-CH_3OH **(Figure 65)** and the presence of 16 carbon resonances for 16 carbons in the ^{13}C NMR spectrum **(Figure 67)**. The UV spectrum displayed absorption bands for an aromatic chromophore at 213, 253, 269, and 324 nm. The IR spectrum showed absorption bands for conjugated ketone carbonyl and double bond functional groups at 1695 and 1647 cm^{-1}, respectively. The ^1H NMR spectrum **(Figure 66)** **(Table 155)** consisted of signals for two *ortho*-coupled aromatic protons [δ 7.46 (*d*, J = 7.8 Hz, 1H) and 6.77 (*d*, J = 7.8 Hz, 1H)], two sets of nonequivalent methylene protons [δ 4.86 (*d*, J = 16.2 Hz, 1H)/4.66 (*d*, J = 16.2 Hz, 1H) and 2.94 (*d*, J = 16.2 Hz, 1H)/2.85 (*d*, J = 16.2 Hz, 1H)], one methoxyl group (δ 3.27, *s*, 3H) and three methyl groups [δ 2.29 (*s*, 3H), 2.02 (*s*, 3H) and 1.46 (*s*, 3H)]. The ^{13}C NMR and DEPT 135 spectra **(Table 155)** showed one conjugated ketone carbonyl (δ 183.43), six aromatic quaternary (δ 160.37, 145.28, 140.72, 131.54, 121.91 and 118.25), two aromatic methine (δ 123.03 and 121.91), one dioxygenated quaternary (δ 97.43), two methylene (δ 57.75 and 39.30), one methoxy (δ 49.00) and three methyl (δ 22.99, 20.15 and 17.40) carbons. The HMBC correlations **(Table 155)** of the methyl protons, H_3-13 (δ2.29)

and H_3-14 (δ 2.02) with C-2 (δ 145.28), C-3 (δ 183.43) and C-12 (δ 131.54) as well as the chemical shifts of these carbons established a 2-oxy-3-methyl-2-butenoyl moiety. One of the *ortho*-coupled aromatic protons resonating at δ 7.46 was assigned as H-4 on the basis of its ^3J HMBC correlation with C-3 of the butenoyl unit. This conclusion was supported by its downfield appearance due to an anisotropic effect of the adjacent ketone carbonyl group. The other *ortho*-coupled proton (δ 6.77) was then attributed to H-5. The nonequivalent methylene protons, H_{ab}-6 (δ 2.94 and 2.85), gave HMBC cross peaks with C-5 (δ 123.03), C-5a (δ 140.72), C-7 (δ 97.43) and C-9a (δ 118.25) while the methoxy (δ 3.27) and the methyl (δ 1.46) protons showed cross peaks with the dioxygenated carbon, C-7. These results attached a 2-methoxy-2-oxypropyl unit at C-5a of the aromatic ring. In addition, the oxymethylene protons, H_{ab}-9 (δ 4.86 and 4.66), displayed the same correlations with C-5a, C-9a and C-9b (δ 160.37), thus linking the oxymethylene unit at C-9a. An isochroman unit having the methoxyl and methyl groups at C-7 was established on the basis of the ^3J HMBC correlations of H_{ab}-9 with C-7. An ether linkage between C-2 and C-9b was formed to construct a 3-oxobenzofuran unit according to the chemical shifts of C-2 and C-9b as well as the mass data. Therefore, **K28** was assigned as a new isochroman derivative.

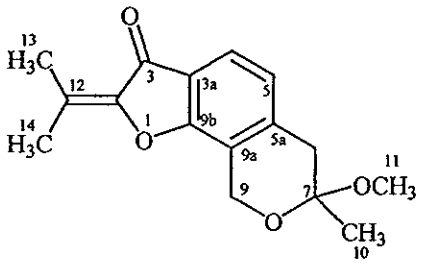


Table 155 The ^{1}H, ^{13}C NMR and HMBC data of compound **K28** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
2	-	145.28 (C)	-
3	-	183.43 (C)	-
3a	-	121.19 (C)	-
4	7.46 (*d*, 7.8)	121.91 (CH)	C-3, C-5a, C-9b
5	6.77 (*d*, 7.8)	123.03 (CH)	C-3a, C-4, C-5a, C-6, C-9a

Table 155 Continued

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
5a	-	140.72 (C)	-
6	a: 2.94 (*d*, 16.2)	39.30 (CH_2)	C-5, C-5a, C-7, C-9a
	b: 2.85 (*d*, 16.2)		C-5, C-5a, C-7, C-9a
7	-	97.43 (C)	-
9	a: 4.86 (*d*, 16.2)	57.75 (CH_2)	C-5a, C-7, C-9a, C-9b
	b: 4.66 (*d*, 16.2)		C-5a, C-7, C-9a, C-9b
9a	-	118.25 (C)	-
9b		160.37 (C)	-
10	1.46 (*s*)	22.99 (CH_3)	C-6, C-7
11	3.27 (*s*)	49.00 (CH_3)	C-7
12	-	131.54 (C)	-
13	2.29 (*s*)	17.40 (CH_3)	C-2, C-3, C-12, C-14
14	2.02 (*s*)	20.15 (CH_3)	C-2, C-3, C-12, C-13

Table 156 The COSY and NOEDIFF data of compound **K28** in $CDCl_3$

Proton	COSY	NOEDIFF
H-4	H-5	H-5
H-5	H-4	H-4
H_a-6	H_b-6	H_b-6
H_b-6	H_a-6	H_a-6, H_3-10
H_a-9	H_b-9	H_b-9
H_b-9	H_a-9	H_a-9, H_3-11
H_3-10	-	H_b-6, H_3-11
H_3-11	-	H_b-9, H_3-10
H_3-13	-	H_3-14
H_3-14	-	H_3-13

4.3.2 Compound K29

Compound **K29** was obtained as a colorless gum. The molecular formula $C_{16}H_{20}O_4$ from EIMS (*m/z* 276) **(Figure 68)** suggested that **K29** was a dihydro analogue of **K28**. This conclusion was supported by the replacement of signals for two olefinic carbons, C-2 (δ 145.28) and C-12 (δ 131.54), in **K28**, with those for two methine carbons (δ 90.23 and 31.14) in **K29 (Table 157)**. The presence of the saturated ketone carbonyl functionality was further confirmed by the appearance of the ketone carbonyl carbon in **K29** at δ 202.00 which resonated at much lower field than that observed in **K28**. Furthermore, the dioxyquaternary carbon, C-7, and the oxymethylene carbon, C-9, in **K28** were replaced by one oxymethine carbon (δ 62.61) and one dioxymethine carbon (δ 94.71) in **K29**. These data were in agreement with the ^{1}H NMR data **(Figure 69) (Table 157)** of which signals for the nonequivalent oxymethylene protons (H_{ab}-9) in **K28** were replaced by those for one dioxymethine proton, H-9 (δ 5.61, *s*) and one oxymethine proton, H-7 (δ 4.25, *m*). In the ^{1}H-^{1}H COSY spectrum **(Table 158)**, H-7 showed cross peaks with H_{ab}-6 (δ 2.68 and 2.62) and the methyl protons, H_3-10 (δ 1.32, *d*, J = 6.5 Hz), indicating the presence of a [-CH_2CH(O-)CH_3] unit. The methoxy protons, H_3-11 (δ 3.45), gave a ^3J HMBC cross peak with C-9 (δ 94.71) while H-9 (δ 5.61) displayed the same correlation with C-7 (δ 62.61). Consequently, an isochroman moiety having the methyl and the methoxyl groups at C-7 and C-9, respectively, was established. As H_b-6 was coupled with H-7 with a large coupling constant of 11.0 Hz, both of them were located at pseudoaxial position. Irradiation of H_3-11 (9-OCH_3) in the NOEDIFF experiment **(Table 158)** enhanced signal intensity of H-7, indicating *trans* relationship between pseudoequatorial H_3-10 (7-CH_3) and pseudoaxial 9-OCH_3. However, the NOEDIFF data were inadequate to identify the relative configuration at C-2. Therefore, **K29** was identified as a new chroman derivative.

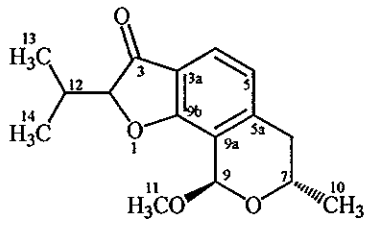


Table 157 The ^{1}H, ^{13}C NMR and HMBC data of compound **K29** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
2	4.35 (*d*, 4.0)	90.23 (CH)	C-3, C-9b, C-12, C-13, C-14
3	-	202.00 (C)	-
3a	-	120.14 (C)	-
4	7.43 (*d*, 8.0)	123.37 (CH)	C-3, C-3a, C-5a, C-9b
5	6.72 (*d*, 8.0)	121.97 (CH)	C-3a, C-5a, C-6, C-9a
5a	-	145.39 (C)	-
6	a: 2.68 (*dd*, 17.0, 3.5)	35.87 (CH_2)	C-5, C-5a, C-7, C-9a, C-10
	b: 2.62 (*dd*, 17.0, 11.0)		C-5, C-5a, C-7, C-9a, C-10
7	4.25 (*m*)	62.61 (CH)	-
9	5.61 (*s*)	94.71 (CH)	C-5a, C-7, C-9a, C-9b, C-11
9a	-	119.96 (C)	-
9b	-	171.01 (C)	-
10	1.32 (*d*, 6.5)	21.12 (CH_3)	C-6, C-7
11	3.45 (*s*)	55.78 (CH_3)	C-9
12	2.28 (*m*)	31.14 (CH)	C-2, C-13, C-14
13	0.80 (*d*, 7.0)	15.78 (CH_3)	C-2, C-12, C-14
14	1.11 (*d*, 7.0)	18.99 (CH_3)	C-2, C-12, C-13

Table 158 The COSY and NOEDIFF data of compound **K29** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H-12	H-12, H_3-13, H_3-14
H-4	H-5	H-5
H-5	H-4	H-4, H_a-6
H_a-6	H_b-6, H-7	H-5, H-7, H_3-10
H_b-6	H_a-6, H-7	H-7, H_3-10
H-7	H_{ab}-6, H_3-10	H_{ab}-6, H_3-10
H-9	-	H_3-11
H_3-10	H-7	H_{ab}-6, H-7

Table 158 Continued

Proton	COSY	NOEDIFF
H_3-11	-	H-9
H-12	H-2, H_3-13, H_3-14	H-2, H_3-13, H_3-14
H_3-13	H_3-12	H-12, H_3-14
H_3-14	H_3-12	H-12, H_3-13

4.3.3 Compound K30

Compound **K30** with the molecular formula $C_{15}H_{18}O_4$ from EIMS (*m/z* 262) **(Figure 71)** was obtained as a colorless gum. Its UV spectrum was similar to that of **K29** while the IR spectrum displayed an additional absorption band for a hydroxyl group at 3409 cm^{-1}. The ^1H NMR spectrum of **K30 (Figure 72) (Table 159)** was similar to that of **K29** except for the absence of the methoxyl signal. These data together with mass information revealed the replacement of the methoxyl group in **K29** with a hydroxyl group in **K30**. The DEPT 135 spectrum **(Table 159)** showed one dioxygenated carbon (δ 88.83) and three methyl carbons (δ 21.93, 19.53 and 16.70), supporting the above conclusion. The location of 7-CH_3 was at pseudoequatorial, identical to that in **K29**, on the basis of a large coupling constant of 10.0 Hz between H_b-6 and H-7. Furthermore, compounds **K29** and **K30** gave almost identical optical rotation, indicating that they would have the same absolute configuration. These data revealed that 9-OCH_3 in **K29** was replaced by a hydroxyl group in **K30**. Therefore, **K30** was assigned as a 9-hydroxy derivative of **K29**.

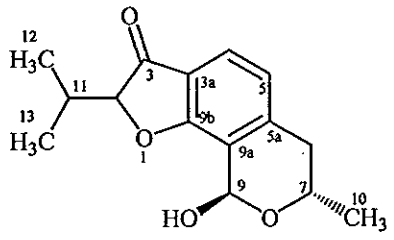


Table 159 The ^{1}H, ^{13}C NMR and HMBC data of compound **K30** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
2	4.46 (*d*, 3.5)	91.04 (CH)	C-3, C-9b, C-11, C-12, C-13
3	-	201.95 (C)	-
3a	-	121.46 (C)	-
4	7.46 (*d*, 8.5)	124.53 (CH)	C-3, C-5a, C-9b
5	6.76 (*d*, 8.5)	122.49 (CH)	C-3a, C-6, C-9a
5a	-	146.01 (C)	-
6	a: 2.72 (*dd*, 16.5, 2.5)	36.94 (CH_2)	C-5, C-5a, C-7, C-9a, C-10
	b: 2.62 (*dd*, 16.5, 10.0)		C-5, C-5a, C-7, C-9a, C-10
7	4.40 (*m*)	63.58 (CH)	-
9	6.18 (*s*)	88.83 (CH)	C-5a, C-7
9a	-	121.01 (C)	-
9b	-	172.48 (C)	-
10	1.33 (*d*, 6.0)	21.93 (CH_3)	C-6, C-7
11	2.29 (*m*)	32.00 (CH)	C-2, C-12, C-13
12	1.08 (*d*, 6.5)	19.53 (CH_3)	C-2, C-11, C-13
13	0.81 (*d*, 6.5)	16.70 (CH_3)	C-2, C-11, C-12

Table 160 The COSY and NOEDIFF data of compound **K30** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H-11	-
H-4	H-5	H-5
H-5	H-4	H-4
H_a-6	H_b-6, H-7	-
H_b-6	H_a-6, H-7	-
H-7	H_{ab}-6, H_3-10	-
H_3-10	H-7	-
H-11	H-2, H_3-12, H_3-13	H_3-12, H_3-13
H_3-12	H-11	H-11
H_3-13	H-11	H-11

4.3.4 Compound K31

Compound **K31**, a colorless gum, has the molecular formula $C_{10}H_{12}O_2$ established by EIMS (*m/z* 164) **(Figure 74)**. The UV spectrum exhibited absorption bands at 251, 277 and 284 nm for a benzene chromophore. The IR spectrum showed an absorption band at 3351 cm^{-1} for a hydroxyl group. The ^1H NMR **(Figure 75)** **(Table 161)** and ^1H-^1H COSY spectra **(Table 162)** displayed signals for three aromatic protons of a 1,2,3-trisubstitued benzene [δ 7.11 (*t*, J = 7.8 Hz, 1H), 6.74 (*d*, J = 7.8 Hz, 1H) and 6.71 (*d*, J = 7.8 Hz, 1H)], the 1-substituted 2-oxypropyl unit [δ 3.92 (*dq*, J = 10.0 and 6.0 Hz, 1H), 2.75 (*dd*, J = 12.5 and 2.4 Hz, 1H), 2.74 (*dd*, J = 12.5 and 10.0 Hz, 1H) and 1.24 (*d*, J = 6.0 Hz, 3H)] and one set of nonequivalent oxymethylene protons [δ 4.85 (*d*, J = 13.2 Hz, 1H) and 4.79 (*d*, J = 13.2 Hz, 1H)]. The aromatic protons resonating at δ 6.74, 7.11 and 6.71 were attributed to H-2, H-3 and H-4, respectively, based on HMBC correlations. The nonequivalent methylene protons, H_{ab}-5 (δ 2.75 and 2.74), of the oxypropyl unit showed the HMBC correlations **(Table 161)** with C-4 (δ 122.49), C-4a (δ 137.72) and C-8a (δ 124.91), linking above unit at C-4a of the aromatic ring. Irradiation of H_{ab}-5 enhanced signal intensity of H-4 (δ 6.71), supporting the assignment. The remaining oxymethylene group was attached at C-8a as H_{ab}-8 (δ 4.85 and 4.79) were correlated with C-1 (δ 156.23), C-4a and C-8a in the HMBC spectrum. These results together with the HMBC correlations of H_{ab}-8 with C-6 (δ 69.10) established an isochroman skeleton. The substituent at C-1 was a hydroxyl group due to the chemical shift of C-1. Therefore, **K31** was determined as a new isochroman derivative. It is worth to note that the oxymethine proton, H-6 (δ 3.92, *dq*, J = 10.0 and 6.0 Hz), was located at psuedoaxial position due to a coupling constant of 10.0 Hz between H_b-5 and H-6.

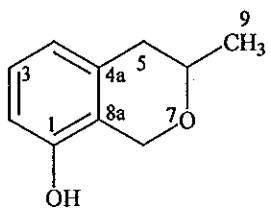


Table 161 The ^{1}H, ^{13}C NMR and HMBC data of compound **K31** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
1	-	156.23 (C)	-
2	6.74 (*d*, 7.8)	114.96 (CH)	C-1, C-4, C-8a
3	7.11 (*t*, 7.8)	129.02 (CH)	C-1, C-4a
4	6.71 (*d*, 7.8)	122.49 (CH)	C-2, C-5, C-8a
4a	-	137.72 (C)	-
5	a: 2.75 (*dd*, 12.5, 2.4)	41.81 (CH_2)	C-4, C-4a, C-6, C-8a, C-9
	b: 2.74 (*dd*, 12.5, 10.0)		C-4, C-4a, C-6, C-8a, C-9
6	3.92 (*dq*, 10.0, 6.0)	69.10 (CH)	C-4a
8	a: 4.85 (*d*, 13.2)	58.49 (CH_2)	C-1, C-4a, C-8a
	b: 4.79 (*d*, 13.2)		C-1, C-4a, C-8a
8a	-	124.91 (C)	-
9	1.24 (*d*, 6.0)	23.43 (CH_3)	C-5, C-6

Table 162 The COSY and NOEDIFF data of compound **K31** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H-3	H-3
H-3	H-2, H-4	H-2, H-4
H-4	H-3	H-3, H_2-5
H_a-5	H-6	H-4, H-6, H_3-9
H_b-5	H-6	H-4, H-6, H_3-9
H-6	H_{ab}-5, H_3-9	H_{ab}-5, H_{ab}-8, H_3-9
H_a-8	H_b-8	H-6
H_b-8	H_a-8	H-6
H_3-9	H-6	H_{ab}-5, H-6

4.3.5 Compound K32

Compound **K32** was isolated as a colorless gum whose molecular formula was determined by EIMS as $C_{10}H_{12}O_3$ (*m/z* 180) **(Figure 77)**, with 16 mass unit higher than **K31**. Its UV and IR spectra were almost identical to those of **K31**. Its ^{1}H NMR spectrum **(Figure 78) (Table 163)** was similar to that of **K31** except for the replacement of signals for H_{ab}-5 in **K31** with signal of a hydroxymethine proton (δ 4.95, *d*, *J* = 2.0 Hz, 1H) in **K32**. This was consistent with the molecular formula and the presence of two oxymethine carbons (δ 88.52 and 69.96) in the ^{13}C NMR **(Figure 79) (Table 163)** and DEPT 135 **(Table 163)** spectra. These data implied that **K32** was a 5-hydroxy derivative of **K31**. A small coupling constant between H-5 and H-6 in **K32** indicated the replacement of H_b-5 in **K31** with a hydroxyl group in **K32**. Therefore, **K32** was determined as a 5-hydroxy derivative of **K31**.

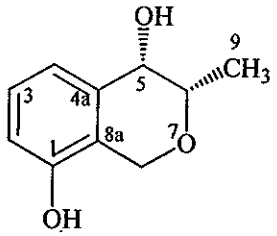


Table 163 The ^{1}H, ^{13}C NMR and HMBC data of compound **K32** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
1	-	150.36 (C)	-
2	6.60 (*d*, 8.0)	114.55 (CH)	C-1, C-4, C-8, C-8a
3	7.07 (*t*, 8.0)	129.25 (CH)	C-1, C-4a
4	6.73 (*d*, 8.0)	113.99 (CH)	C-2, C-5, C-8a
4a	-	140.86 (C)	-
5	4.95 (*d*, 2.0)	88.52 (CH)	-
6	3.85 (*qd*, 6.5, 2.0)	69.96 (CH)	-
8	a: 5.06 (*d*, 12.0)	71.07 (CH_2)	C-4a, C-6, C-8a
	b: 5.01 (*d*, 12.0)		C-4a, C-6, C-8a
8a	-	126.12 (C)	-
9	1.26 (*d*, 6.5)	18.58 (CH_3)	C-5, C-6

Table 164 The COSY and NOEDIFF data of compound **K32** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H-3	H-3
H-3	H-2, H-4	H-2, H-4
H-4	H-3	H-3
H-5	H-6	H-6
H-6	H-5, H_3-9	H-5, H_3-9
H_3-9	H-6	H-6

4.3.6 Compound K33

Compound **K33** was isolated as a colorless gum. The molecular formula was established by analysis of its EIMS as $C_{13}H_{13}NO_5$ (*m/z* 263) **(Figure 80)**. The UV spectrum displayed characteristic absorption bands for a pyrone chromophore at 224, 247 and 361 nm (Trisuwan, *et al.*, 2009). The IR spectrum showed absorption bands at 3399 and 1683 cm^{-1} for hydroxyl and conjugated carbonyl groups, respectively. The ^1H NMR spectrum **(Figure 81) (Table 165)** showed two olefinic protons of trisubstituted double bonds [δ 6.02 (*s*, 1H) and 5.94 (*s*, 1H)], one set of nonequivalent methylene protons [δ 3.64 (*d*, J = 9.6 Hz, 1H) and 3.54 (*d*, J = 9.6 Hz, 1H)] and two methyl groups [δ 2.30 (*s*, 3H) and 2.22 (*s*, 3H)]. The ^{13}C NMR **(Figure 82) (Table 165)** and DEPT 135 **(Table 165)** spectra exhibited one pyridinone carbonyl (δ 169.85) (Yang and Pan, 2007), one pyrone carbonyl (δ 166.45) (Trisuwan, *et al.*, 2009), six quaternary (δ 169.07, 166.94, 160.72, 143.11, 108.18 and 102.40), two methine (δ 103.11 and 102.76), one methylene (δ 18.25) and two methyl (δ 19.69 and 18.99) carbons. The olefinic proton at δ 5.94 was attributed to H-5 of the pyrone moiety on the basis of its chemical shift as well as its HMBC cross peaks with C-3 (δ 102.40), C-4 (δ 169.07) and C-6 (δ 160.72). The methyl protons, H_3-7 (δ 2.22), gave the same correlations with C-5 (δ 102.76) and C-6. In addition, the methylene protons, H_{ab}-8 (δ 3.64 and 3.54), gave the HMBC cross peaks with C-2 (δ 166.45) and C-3. These results together with the chemical shift of C-4 indicated the attachment of

the methylene group, a hydroxyl group and the methyl group at C-3, C-4 and C-6 of the pyrone ring, respectively. The chemical shifts of the remaining carbons indicated the presence of a pyridinone unit (Yang and Pan., 2007). The olefinic proton at δ 6.02 was assigned as H-5′ due to its HMBC correlations with C-3′ (δ 108.18), C-4′ (δ 166.94) and C-6′ (δ 143.11). The methyl protons, H_3-7′ (δ 2.30), gave the HMBC correlations with C-5′ (δ 103.11) and C-6′. These data revealed that the pyridinone unit carried hydroxyl and methyl groups at C-4′ and C-6′, respectively. The HMBC correlations from H_{ab}-8 of the pyrone moiety to C-2′ (δ 169.85) and C-3′ of the pyridinone established the methylene linkage between C-3 of the pyrone and C-3′ of the pyridinone. Consequently, **K33** was assigned as a new pyrone derivative. The appearance of the methylene protons (H_{ab}-8) as nonequivalent protons in the ^{1}H NMR spectrum might be due to the formation of H-bond between either the amino nitrogen or carbonyl oxygen of the pyridinone moiety and the hydroxy hydrogen of the pyrone unit which would prevent bond rotation.

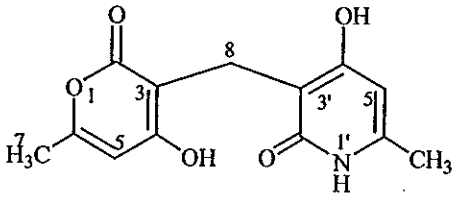


Table 165 The ^{1}H, ^{13}C NMR and HMBC data of compound **K33** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
2	-	166.45 (C)	-
3	-	102.40 (C)	-
4	-	169.07 (C)	-
5	5.94 (*s*)	102.76 (CH)	C-3, C-4, C-6, C-7
6	-	160.72 (C)	-
7	2.22 (*s*)	19.69 (CH_3)	C-5, C-6
8	a: 3.64 (*d*, 9.6)	18.25 (CH_2)	C-2, C-3, C-2′, C-3′
	b: 3.54 (*d*, 9.6)		C-2, C-3, C-2′, C-3
2′	-	169.85 (C)	-
3′	-	108.18 (C)	-

Table 165 Continued

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
4′	-	166.94 (C)	-
5′	6.02 (*s*)	103.11 (CH)	C-3′, C-4′, C-6′, C-7′
6′	-	143.11 (C)	-
7′	2.30 (*s*)	18.99 (CH_3)	C-5′, C-6′

Table 166 The COSY and NOEDIFF data of compound **K33** in $CDCl_3$

Proton	COSY	NOEDIFF
H-5	-	H_3-7
H_3-7	-	H-5
H_a-8	H_b-8	-
H_b-8	H_a-8	-
H-5′	-	H_3-7′
H_3-7′	-	H-5′

4.3.7 Compound K34

Compound **K34** with the molecular formula $C_{10}H_{16}O_4$ from EIMS (*m/z* 200) **(Figure 83)** was obtained as a colorless gum. The UV spectrum showed an absorption band at 269 nm. The IR spectrum displayed absorption bands at 3409 and 1687 cm^{-1} for hydroxyl and conjugated carbonyl functional groups, respectively. The ^1H NMR **(Figure 84) (Table 167)** and ^1H-^1H COSY **(Table 168)** spectra revealed the presence of signals for one ethyl group [δ 2.45 (*dq*, J = 15.3 and 7.5 Hz, 1H), 2.40 (*dq*, J = 15.3 and 7.5 Hz, 1H) and 1.18 (*t*, J = 7.5 Hz, 3H)], one 1-oxyethyl group [δ 4.52 (*q*, J = 6.6 Hz, 1H) and 1.31 (*d*, J = 6.6 Hz, 3H)], one hydroxymethyl group [δ 4.38 (*d*, J = 12.6 Hz, 1H) and 4.32 (*d*, J = 12.6 Hz, 1H)] and one methyl group (δ 1.40, *s*, 3H). The ^{13}C NMR **(Figure 85) (Table 167)** and DEPT 135 **(Table 167)** spectra displayed ten carbon resonances for one ketone carbonyl (δ 196.20), three

quaternary (δ 175.58, 110.07 and 71.33), one methine (δ 81.96), two methylene (δ 56.32 and 25.59) and three methyl (δ 24.11, 12.30 and 11.44) carbons. The oxymethine proton of the 1-oxyethyl unit, H-5 (δ 4.52), gave the HMBC correlations **(Table 167)** with C-1 (δ 196.20), C-3 (δ 175.58) and C-6 (δ 71.33) while H_3-7 (δ 1.40) gave the same correlations with C-1, C-5 (δ 81.96) and C-6. In addition, the hydroxymethyl protons, H_{ab}-9 (δ 4.38 and 4.32), gave the HMBC cross peaks with C-1, C-2 (δ 110.07) and C-3. These results together with the chemical shifts of C-3, C-5 and C-6 established a dihydropyran-4-one unit with two methyl groups at C-5 and C-6, a hydroxyl group at C-6 and the hydroxymethyl substituent at C-2. Consequently, the remaining ethyl group was located at C-3. The HMBC correlations of the methylene protons, H_{ab}-10 (δ 2.45 and 2.40), of the ethyl group with C-2 and C-3 confirmed this assignment. The relative configuration between H-5 and H_3-7 was not assigned as either *cis* or *trans* relationship gave signal enhancement of H-5 upon irradiation of H_3-7. Therefore, **K34** was determined as a new dihydropyran-4-one derivative.

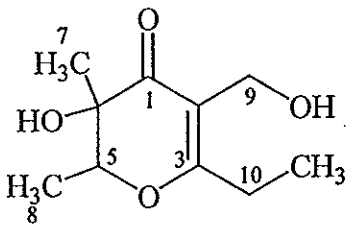


Table 167 The ^1H, ^{13}C NMR and HMBC data of compound **K34** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
1	-	196.20 (C)	-
2	-	110.07 (C)	-
3	-	175.58 (C)	-
5	4.52 (*q*, 6.6)	81.96 (CH)	C-1, C-3, C-6, C-7, C-8
6	-	71.33 (C)	-
7	1.40 (*s*)	24.11 (CH_3)	C-1, C-5, C-6
8	1.31 (*d*, 6.6)	12.30 (CH_3)	C-5, C-6

Table 167 Continued

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
9	a: 4.38 (*d*, 12.6)	56.32 (CH_2)	C-1, C-2, C-3
	b: 4.32 (*d*, 12.6)		C-1, C-2, C-3
10	a: 2.45 (*dq*, 15.3, 7.5)	25.59 (CH_2)	C-2, C-3, C-11
	b: 2.40 (*dq*, 15.3, 7.5)		C-2, C-3, C-11
11	1.18 (*t*, 7.5)	11.44 (CH_3)	C-3, C-10

Table 168 The COSY and NOEDIFF data of compound **K34** in $CDCl_3$

Proton	COSY	NOEDIFF
H-5	H_3-8	H_3-7, H_3-8
H_3-7	-	H-5
H_3-8	H-5	H-5
Ha-9	-	H_{ab}-10
Hb-9	-	H_{ab}-10
H_a-10	H_b-10, H_3-11	H_{ab}-9, H_3-11
H_b-10	H_a-10, H_3-11	H_{ab}-9, H_3-11
H_3-11	H_{ab}-10	H_{ab}-10

4.3.8 Compound K35

Compound **K35** with the molecular formula $C_{10}H_{12}O_3$ from EIMS (*m/z* 180) **(Figure 86)** was obtained as a colorless gum. The UV spectrum displayed absorption bands at 250 and 289 nm for a benzene chromophore. The IR spectrum showed absorption bands at 3328 and 1638 cm^{-1} for hydroxyl and double bond functional groups, respectively. The ^1H NMR **(Figure 87) (Table 169)** and ^1H-^1H COSY spectra **(Table 170)** showed characteristic signals for three aromatic protons of a 1,2,3-trisubstituted benzene [δ 7.14 (*t*, J = 7.8 Hz, 1H), 6.81 (*d*, J = 7.8 Hz, 1H) and 6.59 (*d*, J = 7.8 Hz, 1H)], a (*Z*)-3-hydroxyl-1-propenyl unit [δ 6.64 (*d*, J = 11.4 Hz, 1H), 5.97 (*dt*, J = 11.4 and 6.9 Hz, 1H) and 4.07 (*dd*, J = 6.9 and 0.6 Hz, 2H)] and one

hydroxymethyl group (δ 4.78, *s*, 2H). The aromatic protons resonating at δ 6.59, 7.14 and 6.81 were assigned as H-4, H-5 and H-6, respectively. The (Z)-3-hydroxyl-1-propenyl unit was located at C-3 (δ 136.29) based on the HMBC correlations **(Table 169)** of the olefinic proton, H-8 (δ 6.64), with C-3 and C-4 (δ 120.92). The hydroxymethyl protons, H_2-7 (δ 4.78), showed HMBC cross peaks with C-1 (δ 156.26), C-2 (δ 123.58) and C-3, thus connecting the hydroxymethyl group at C-2. The substituent at C-1 of the benzene ring was a hydroxyl group on the basis of its chemical shift. Signal enhancement of H_2-7 and H-4 (δ 6.59) upon irradiation of H-8 in the NOEDIFF experiment **(Table 170)** supported the assigned location of these substituents. Therefore, **K35** was characterized as a new phenol derivative.

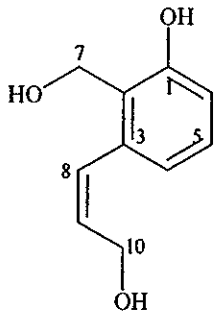


Table 169 The ^{1}H, ^{13}C NMR and HMBC data of compound **K35** in $CDCl_3$+CD_3OD

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
1	-	156.26 (C)	-
2	-	123.58 (C)	-
3	-	136.29 (C)	-
4	6.59 (*d*, 7.8)	120.92 (CH)	C-3, C-6, C-8
5	7.14 (*t*, 7.8)	128.45 (CH)	C-1, C-2, C-3, C-6
6	6.81 (*d*, 7.8)	115.30 (CH)	C-1, C-4
7	4.78 (*s*)	59.10 (CH_2)	C-1, C-2, C-3
8	6.64 (*d*, 11.4)	129.95 (CH)	C-3, C-4
9	5.97 (*dt*, 11.4, 6.9)	131.88 (CH)	C-3, C-10
10	4.07 (*dd*, 6.9, 0.6)	58.58 (CH_2)	C-8, C-9

Table 170 The COSY and NOEDIFF data of compound **K35** in $CDCl_3$+CD_3OD

Proton	COSY	NOEDIFF
H-4	H-5	H-5
H-5	H-4, H-6	H-4, H-6
H-6	H-5	H-5
H_2-7	-	H-8
H-8	H-9	H_2-7, H-9
H-9	H-8, H_2-10	H-8, H_2-10
H_2-10	H-9	H-4, H-9

4.3.9 Compound K37

Compound **K37** was obtained as a colorless gum. The UV spectrum exhibited absorption bands at λ_{max} 210, 240, 253, 270, 293 and 352 nm, while the IR spectrum showed absorption bands at 1695 and 1644 cm^{-1} for conjugated ketone carbonyl and double bond functional groups, respectively. The ^1H NMR spectrum **(Figure 92) (Table 171)** consisted of signals for four aromatic protons [δ 9.56 (*s*, 1H), 7.83 (*d*, J = 7.8 Hz, 1H), 7.56 (*s*, 1H) and 7.38 (*d*, J = 8.7 Hz, 1H)] and three methyl groups [δ 2.76 (*s*, 3H), 2.45 (*s*, 3H) and 2.26 (*s*, 3H)]. The ^{13}C NMR **(Figure 93) (Table 171)** and DEPT 135 **(Table 171)** spectra displayed fifteen carbon resonances for eight quaternary (δ 182.21, 164.04, 156.68, 141.36, 145.60, 133.75, 119.39 and 114.62), four methine (δ 146.21, 124.23, 120.55 and 119.55) and three methyl (δ 24.65, 20.40 and 17.50) carbons. One of *ortho*-coupled aromatic protons, H-4 (δ 7.83), showed HMBC correlations **(Table 172)** with C-3a (δ 119.36), C-5a (δ 141.36) and C-9b (δ 164.04), while the other one, H-5 (δ 7.38), was correlated with C-3a, C-5a, C-6 (δ 119.55) and C-9a (δ 114.62). The singlet aromatic proton, H-6 (δ 7.56), showed HMBC cross peaks with C-5 (δ 120.55), C-7 (δ 156.68), C-9a and C-10 (δ 24.65). In addition, the remaining singlet aromatic proton, H-9 (δ 9.56), was correlated with C-5a, C-7 and C-9b. These data together with HMBC correlations and the chemical shifts of C-7 and C-9 (δ 146.21) indicated that **K37** had an isoquinoline

skeleton with a methyl group at C-7 and other substituents at C-3a and C-9b. The location of H_3-10 was confirmed by its HMBC correlations with C-6 and C-7 of the isoquinoline skeleton. HMBC cross peaks of H_3-12 (δ 2.45) and H_3-13 (δ 2.26) with C-2 (δ 145.60) and C-11 (δ 133.75) and the chemical shift of the carbonyl carbon, C-3 (δ 182.21), established the presence of 2-oxy-3-methyl-2-butenoyl moiety, as found in **K28**. This unit was fused with the isoquinoline fragment to form a 2-methylethylidene-furoisoquinoline-3-one on the basis of the chemical shift C-9b and a HMBC correlation of H-4/C-3. Consequently, **K37** was identified as TMC-120B which was previously isolated from the fungus *Aspergillus ustus* TC 1118 (Kohno, *et al.*, 1999).

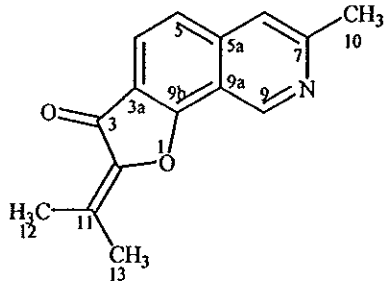


Table 171 The ^{1}H and ^{13}C NMR data of compound **K37** and **TMC-120B** in $CDCl_3$

Position	K37		TMC-120B	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
2	-	145.60 (C)	-	145.6 (C)
3	-	182.21 (C)	-	182.1 (C)
3a	-	119.36 (C)	-	119.3 (C)
4	7.83 (*d*, 8.7)	124.23 (CH)	7.80 (*d*, 8.5)	124.1 (CH)
5	7.38 (*d*, 8.7)	120.55 (CH)	7.35 (*d*, 8.5)	120.5 (CH)
5a	-	141.36 (C)	-	141.3 (C)
6	7.56 (*s*)	119.55 (CH)	7.52 (*s*)	119.5 (CH)
7	-	156.68 (C)	-	156.7 (C)
9	9.56 (*s*)	146.21 (CH)	9.52 (*s*)	146.2 (CH)
9a	-	114.62 (C)	-	114.6 (C)
9b	-	164.04 (C)	-	164.0 (C)

Table 171 Continued

Position	K37		TMC-120B	
	δ_H (*mult*, J_{Hz})	δ_C (C-type)	δ_H (*mul.*, J_{Hz})	δ_C (C-type)
10	2.76 (*s*)	24.65 (CH_3)	2.74 (*s*)	24.7 (CH_3)
11	-	133.75 (C)	-	133.7 (C)
12	2.45 (*s*)	17.50 (CH_3)	2.43 (*d*, 0.7)	17.5 (CH_3)
13	2.26 (*s*)	20.40 (CH_3)	2.25 (*d*, 0.7)	20.4 (CH_3)

Table 172 The HMBC, COSY and NOEDIFF data of compound **K37** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-4	C-3, C-3a, C-5a, C-9b	H-5	H-5
H-5	C-3a, C-4, C-5a, C-6, C-9a	H-4	H-4, H-6
H-6	C-5, C-7, C-9a, C-10	-	H-5, H_3-10
H-9	C-5a, C-7, C-9b	-	H_3-10, H_3-13
H_3-10	C-6, C-7	-	H-6, H-9
H_3-12	C-2, C-11, C-13	-	H_3-13
H_3-13	C-2, C-11, C-12	-	H-9, H_3-12

4.3.10 Compound K38

Compound **K38** was obtained as a colorless gum. Its UV spectrum was similar to that of **K37** while the IR spectrum showed an additional absorption band of a hydroxyl group at 3368 cm^{-1}. Its ^1H NMR spectrum **(Figure 94)** **(Table 173)** was similar to that of **K37** except for the replacement of two methyl singlets in **K37** with two methyl doublets [δ 1.24 (*d*, J = 7.0 Hz, 3H) and 0.99 (*d*, J = 7.0 Hz, 3H)]. In addition, the ^1H NMR spectrum displayed an additional signal of a methine proton (δ 2.43, *m*). In the ^1H-^1H COSY spectrum **(Table 174)**, this additional methine proton was coupled with CH_3-12 (δ 0.99) and CH_3-13 (δ 1.24). CH_3-12 and CH_3-13 showed HMBC correlations **(Table 174)** with C-2 (δ 110.03) and C-11

(δ33.93). A HMQC correlation of this methine proton with C-11 indicated that it was H-11. The chemical shift of C-2 suggested the presence of a hydroxyl substituent at this carbon. The carbonyl carbon, C-3 (δ 198.48), in **K38** was shifted to lower field when compared with that (δ 182.21) in **K37**, thus supporting the assigned structure. Therefore, **K38** was determined as TMC-120C which was previously isolated from the fungus *Aspergillus ustus* TC 1118 (Kohno, *et al.*, 1999).

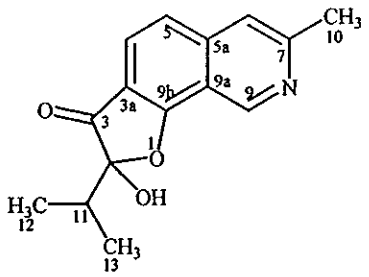


Table 173 The ^{1}H and ^{13}C NMR data of compound **K38** and **TMC-120C** in $CDCl_3$

Position	K38		TMC-120C	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
2	-	110.03 (C)	-	110.3 (C)
3	-	198.48 (C)	-	198.5 (C)
3a	-	115.64 (C)	-	115.6 (C)
4	7.53 (*d*, 8.7)	124.27 (CH)	7.53 (*d*, 8.3)	124.3 (CH)
5	6.98 (*d*, 8.7)	119.38 (CH)	6.99 (*d*, 8.3)	119.4 (CH)
5a	-	141.97 (C)	-	142.0 (C)
6	7.13 (*s*)	119.38 (CH)	7.14 (*s*)	119.4 (CH)
7	-	156.71 (C)	-	156.8 (C)
9	9.48 (*s*)	146.50 (CH)	9.49 (*s*)	146.5 (CH)
9a	-	114.61 (C)	-	114.6 (C)
9b	-	171.50 (C)	-	171.5 (C)
10	2.50 (*s*)	23.70 (CH_3)	2.51 (*s*)	23.8 (CH_3)
11	2.43 (*m*)	33.93 (CH)	2.42 (*m*)	33.9 (CH)
12	0.99 (*d*, 7.0)	15.94 (CH_3)	0.99 (*d*, 6.3)	16.0 (CH_3)
13	1.24 (*d*, 7.0)	15.67 (CH_3)	1.24 (*d*, 6.6)	15.6 (CH_3)

Table 174 The HMBC, COSY and NOEDIFF data of compound **K38** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-4	C-3, C-3a, C-5a, C-9b	H-5	H-5
H-5	C-3a, C-4, C-5a, C-6, C-9a	H-4	H-4, H-6
H-6	C-5, C-7, C-9a, C-10	H_3-10	H-5, H_3-10
H-9	C-5a, C-7, C-9a, C-9b	-	H_3-10, H_3-12, H_3-13
H_3-10	C-6, C-7	-	H-6, H-9
H-11	C-2, C-3	H_3-12, H_3-13	H_3-12, H_3-13
H_3-12	C-2, C-11, C-13	H-11	H-9, H-11, H_3-13
H_3-13	C-2, C-11, C-12	H-11	H-9, H-11, H_3-12

4.3.11 Compound K36

Compound **K36** with the molecular formula $C_{15}H_{15}NO_4$ from EIMS (*m/z* 273) **(Figure 89)** was obtained as a colorless gum. Its UV and IR spectra were almost identical to those of **K38**. The ^{1}H NMR spectrum **(Figure 90) (Table 172)** was similar to that of **K38** except for the replacement of the downfield methyl signal in **K38** with the signal of a hydroxymethyl group (δ 4.89, *s*, 2H). The presence of two methyl (δ 15.96 and 15.45) and one oxymethylene (δ 64.54) carbons in the ^{13}C NMR **(Figure 91) (Table 175)** and DEPT 135 **(Table 175)** spectra supported the ^{1}H NMR data. The HMBC correlations **(Table 175)** of the hydroxymethyl protons, H_2-10 (δ 4.89), with C-6 (δ 117.36) and C-7 (δ 159.25) together with signal enhancement of H_2-10 upon irradiation of H-6 (δ 7.79) established the linkage of the hydroxymethyl group at C-7. Consequently, **K36** was identified as a 10-hydroxy derivative of **K38**.

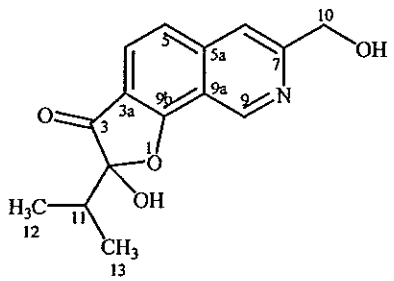


Table 175 The ^{1}H, ^{13}C NMR and HMBC data of compound **K36** in $CDCl_3$+CD_3OD

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
2	-	110.13 (C)	-
3	-	198.97 (C)	-
3a	-	115.88 (C)	-
4	7.72 (*d*, 8.4)	124.53 (CH)	C-3, C-3a, C-5a, C-9b
5	7.36 (*d*, 8.4)	120.34 (CH)	C-3a, C-4, C-5a, C-6, C-9a
5a	-	142.70 (C)	-
6	7.79 (*s*)	117.36 (CH)	C-7, C-9a, C-10
7	-	159.25 (C)	-
9	9.48 (*s*)	146.61 (CH)	C-5a, C-7, C-9a
9a	-	115.88 (C)	-
9b	-	171.93 (C)	-
10	4.89 (*s*)	64.54 (CH_2)	C-6, C-7
11	2.36 (*m*)	34.17 (CH)	C-2
12	0.97 (*brs*)	15.96 (CH_3)	C-2, C-11, C-13
13	1.19 (*brs*)	15.45 (CH_3)	C-2, C-11, C-12

Table 176 The COSY and NOEDIFF data of compound **K36** in $CDCl_3$+CD_3OD

Proton	COSY	NOEDIFF
H-4	H-5	H-5
H-5	H-4	H-4
H-6	H_2-10	H-5, H_2-10
H_2-10	H-6	-
H-11	H_3-12, H_3-13	H_3-12, H_3-13
H_3-12	H-11	H-11
H_3-13	H-11	H-11

4.3.12 Compound K39

Compound **K39** was obtained as a colorless gum. The UV spectrum displayed an aromatic chromophore at 212 and 302 nm. The IR spectrum exhibited absorption bands at 3398, 1675 and 1620 cm^{-1} for hydroxyl, conjugated ketone carbonyl and double bond functional groups, respectively. The ^1H NMR spectrum **(Figure 96) (Table 177)** showed characteristic signals for two aromatic protons [δ 6.08 (*s*, 1H) and 6.00 (*s*, 1H)], a 1-oxo-3-methylbutyl unit [δ 2.58 (*d*, J = 7.0 Hz, 2H), 2.23 (*m*, 1H), 0.96 (*d*, J = 6.5 Hz, 3H) and 0.90 (*d*, J = 7.0 Hz, 3H)] and one methyl group (δ 2.37, *s*, 3H). The aromatic proton at δ 6.00 was assigned as H-3 which gave the HMBC correlations **(Table 177)** with C-1 (δ 130.34), C-2 (δ 143.00) and C-4 (δ 155.19). H_3-12 (δ 2.37) showed the HMBC correlations with C-1, C-2 and C-3 (δ 93.41) while the 1-oxo-3-methylbutyl moiety displayed a ^3J HMBC cross peak of the methylene protons, H_2-8 (δ 2.58), with C-1. These data indicated the attachment of the methyl and 1-oxo-3-methylbutyl units at C-2 and C-1, respectively. In addition, the other aromatic proton (δ 6.08) was attributed to H-5 on the basis of its HMBC correlations with C-1, C-6 (δ 154.35) and C-7 (δ 200.76). The chemical shifts of C-4 and C-6 indicated the presence of hydroxyl groups at these carbons. Consequently, **K39** was identified as 1-(2,4-dihydroxy-6-methylphenyl)-3-methyl-1-butanone (Sakai, *et al.*, 1998).

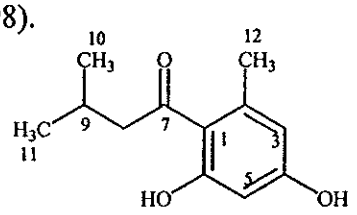


Table 177 The ^1H, ^{13}C NMR and HMBC data of compound **K39** in $CDCl_3$

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
1	-	130.34 (C)	-
2	-	143.00 (C)	-
3	6.00 (*s*)	93.41 (CH)	C-1, C-2, C-4, C-12
4	-	155.19 (C)	-

Table 177 Continued

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC
5	6.08 (*s*)	107.73 (CH)	C-1, C-4, C-6, C-7
6	-	154.35 (C)	-
7	-	200.76 (C)	-
8	2.58 (*d*, 7.0)	45.48 (CH_2)	C-1, C-7, C-10, C-11
9	2.23 (*m*)	23.66 (CH)	C-8, C-10, C-11
10	0.94 (*d*, 7.0)	22.51 (CH_3)	C-8, C-9, C-11
11	0.96 (*d*, 6.5)	22.48 (CH_3)	C-8, C-9, C-12
12	2.37 (*s*)	19.33 (CH_3)	C-1, C-2, C-3

Table 178 The COSY and NOEDIFF data of compound **K39** in $CDCl_3$

Proton	COSY	NOEDIFF
H-3	-	H_3-12
H-5	-	-
H_2-8	H-9	H-9, H_3-10, H_3-11
H-9	H_2-8, H_3-10, H_3-11	H_2-8
H_3-10	H-9	H_2-8
H_3-11	H-9	H_2-8
H_3-12	-	H-3

4.3.13 Compound K40

Compound **K40** was obtained as a colorless gum. The UV spectrum showed absorption bands at 238, 284 and 327 nm. The IR spectrum displayed absorption bands at 1695 and 1647 cm^{-1} for conjugated ketone carbonyl and double bond functional groups, respectively. The ^1H NMR spectrum **(Figure 98) (Table 179)** showed characteristic signals for four aromatic protons of a 1,2-disubstituted benzene [δ 8.25 (*dd*, J = 8.1 and 1.2 Hz, 1H), 7.59 (*td*, J = 8.1 and 1.2 Hz, 1H), 7.48 (*d*, J = 8.1 Hz, 1H) and 7.32 (*td*, J = 8.1 and 1.2 Hz, 1H)], a 2-substituted pyrrole [δ 7.01 (*dd*, J =

2.7 and 1.2 Hz, 1H), 6.76 (*dd*, J = 3.6 and 1.2 Hz, 1H) and 6.33 (*dd*, J = 3.6 and 2.7 Hz, 1H)] and one methoxyl group (δ 3.88, *s*, 3H). The ^{13}C NMR **(Figure 99)** **(Table 179)** and DEPT 135 **(Table 179)** spectra showed four quaternary (δ 176.00, 169.50, 139.30 and 122.80), seven methine (δ 132.70, 125.98, 124.30, 122.76, 117.70, 112.31 and 110.42) and one methoxy (δ 52.91) carbons. The aromatic proton resonating at the lowest field (δ 8.25) was attached at C-6, *ortho* to the ketone carbonyl group. This was confirmed by a HMBC cross peak between H-6 and the carbonyl carbon (C-7, δ 176.00). The remaining aromatic protons at δ 7.48, 7.59 and 7.32 were then assigned as H-3, H-4 and H-5 according to multiplicity and coupling constants. The methoxy group at δ 3.88 was linked at C-2 (δ 169.50) due to its HMBC cross peak with C-2. Consequently, the 2-substituted pyrrole ring was connected with the carbonyl carbon to form a ketone functionality. This was supported by a HMBC cross peak between H-9 (δ 6.33) and C-7. Therefore, **K40** was identified as 2-(2-methoxybenzoyl)pyrrole (Ghigo, *et al.*, 2005).

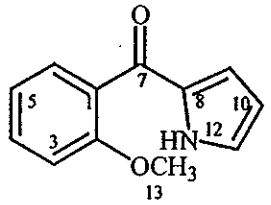


Table 179 The NMR data of compound **K40** in $CDCl_3$+CD_3OD

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC	COSY
1	-	139.30 (C)	-	-
2	-	169.50 (C)	-	-
3	7.48 (*d*, 8.1)	117.70 (CH)	C-5	H-4
4	7.59 (*td*, 8.1, 1.2)	132.70 (CH)	C-5, C-6	H-3, H-5, H-6
5	7.32 (*td*, 8.1, 1.2)	124.30 (CH)	C-3, C-4	H-3, H-4, H-6
6	8.25 (*dd*, 8.1, 1.2)	125.98 (CH)	C-1, C-4, C-5, C-7	H-4, H-5
7	-	176.00 (C)	-	-
8	-	122.80 (C)	-	-
9	6.33 (*dd*, 3.6, 2.7)	110.42 (CH)	C-7, C-10, C-11	H-10, H-11

Table 179 Continued

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC	COSY
10	6.76 (*dd*, 3.6, 1.2)	112.31 (CH)	C-9, C-11	H-9, H-11
11	7.01 (*dd*, 2.7, 1.2)	122.76 (CH)	C-8, C-9, C-10	H-9, H-10
12-NH	10.25 (*brs*)	-	-	-
13	3.88 (*s*)	52.91 (CH_3)	C-2	-

4.3.14 Compound K41

Compound **K41** was obtained as a colorless gum. It exhibited UV absorption bands at 262 and 328 nm. The IR spectrum displayed absorption bands at 3370, 1716 and 1674 cm^{-1} for hydroxyl, carboxylic carbonyl and double bond functional groups, respectively. The ^1H NMR spectrum **(Figure 100) (Table 180)** showed characteristic signals for a 2-substituted pyridine [δ 9.01 (*brs*, 1H), 8.75 (*brs*, 1H), 8.20 (*dt*, J = 8.0 and 2.0 Hz, 1H) and 7.44 (*dd*, J = 8.0 and 5.0 Hz, 1H)]. The ^{13}C NMR **(Figure 101) (Table 180)** and DEPT 135 **(Table 180)** spectra showed one carbonyl carbon of a carboxylic acid (δ 167.00), one quaternary (δ 129.15) and four methine (δ 152.64, 148.18, 135.66 and 123.68) carbons. These data supported the presence of the 2-substituted pyridine and carboxylic functional groups in the IR spectrum. The carboxyl group was located at C-3 (δ 129.15) on the basis of the multiplicity and coupling constants in the ^1H NMR spectrum as well as a HMBC correlation **(Table 180)** of H-4 (δ 8.20) with C-7 (δ 167.00). Consequently, **K41** was nicotinic acid (Soman, *et al.*, 1999).

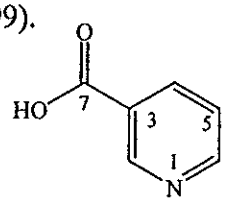


Table 180 The NMR data of compound **K41** in $CDCl_3$+CD_3OD

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC	COSY
2	9.01 (*brs*)	148.18 (CH)	C-3, C-4, C-6	-
3	-	129.15 (C)	-	-

Table 180 Continued

Position	δ_H (*mult*, J_{Hz})	δ_C (C-type)	HMBC	COSY
4	8.20 (*dt*, 8.0, 2.0)	135.66 (CH)	C-2, C-6, C-7	H-2, H-5, H-6
5	7.44 (*dd*, 8.0, 5.0)	123.68 (CH)	C-3, C-6	H-4, H-6
6	8.75 (*brs*)	152.64 (CH)	C-4	H-4, H-5
7	-	167.00 (C)	-	-

CHAPTER 5.1

INTRODUCTION

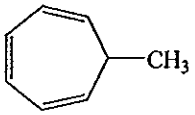
5.1.1 Introduction

Various types of compounds were isolated from the genus *Fusarium*. Some of them showed interesting biological activities, e.g., antimicrobial (Tsuchinari, *et al.*, 2007), antibacterial (Ivanova, *et al.*, 2002) and pytotoxic (Tanaka, *et al.*, 1996) activities. Chemical constituents isolated from the genus *Fusarium* reported since the year 2009 are summarized in **Table 181** according to SciFinder Scholar Database. The EtOAc extract from the culture broth of *Fusarium* sp. PSU-F14 isolated from a sea fan *Annella* sp., exhibited interesting antibacterial activity against SA and MRSA with the equal MIC values of 160 μg/mL.

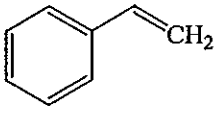
Table 181 Compounds isolated from the *Fusarium* genus

Scientific name	Compound	Activity	Reference
F. oxysporum	7-Methyl-1,3,5-cyclooctatriene, **15** Styrene, **16**	-	Beck, *et al.*, 2009

Structures of compounds isolated from the *Fusarium* genus



15: 7-Methyl-1,3,5-cyclooctatriene



16: Styrene

5.1.2 The objectives

1. To isolate the secondary metabolites from the marine-derived fungus *Fusarium* sp. PSU-F14.

2. To elucidate the structure of the isolated metabolites.

CHAPTER 5.2

EXPERIMENTAL

5.2.1 Fermentation and extraction

The fermentation and the extraction of the culture broth (15 L) and mycelia were performed using the same procedure as those of *Nigrospora* sp. PSU-F5 to afford a dark red solid (3.7 g) and a brown solid (560 mg) from the culture broth and mycelia, respectively. Each extract was subjected to chromatographic separation.

5.2.2 Purification of the broth extract

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven fractions as shown in **Table 182.**

Table 182 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
14A	75.8	Red solid
14B	258.2	Red solid
14C	286.2	Red solid
14D	702.5	Red solid
14E	367.3	Red solid
14F	46.0	Red solid
14G	2,000	Red solid

Fraction 14A displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 14B showed five UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.14, 0.19, 0.24 and 0.33. It was further separated by column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eleven subfractions as shown in **Table 183.**

Table 183 Subfractions obtained from **fraction 14B** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
B1	1% MeOH/CH_2Cl_2	17.6	Red solid
B2	1% MeOH/CH_2Cl_2	3.7	Red solid
B3	1% MeOH/CH_2Cl_2	5.8	Red solid
B4	1% MeOH/CH_2Cl_2	5.4	Red solid
B5	3% MeOH/CH_2Cl_2	1.9	Red solid
B6	3% MeOH/CH_2Cl_2	22.4	Red solid
B7	5% MeOH/CH_2Cl_2	20.3	Red solid
B8	5% MeOH/CH_2Cl_2	14.3	Red solid
B9	7% MeOH/CH_2Cl_2	3.2	Red solid
B10	7-20% MeOH/CH_2Cl_2	84.7	Red solid
B11	30% MeOH/CH_2Cl_2-100% MeOH	63.0	Red solid

Subfraction B1 showed five pale UV-active spots on normal phase TLC using 50% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.08, 0.18, 0.40, 0.53 and 0.93. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction B2 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.65, 0.70, 0.79 and 0.93. Because of low quantity, it was not further investigated.

Subfraction B3 showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.39 and 0.63. It was then purified by precoated TLC with 20% ethyl acetate in petroleum ether as a mobile phase (4 runs) to afford two bands.

Band 1 (K50) was a colorless gum (1.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.29.

$[\alpha]_D^{25}$	+133 (*c* 0.42, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	203 (3.18), 229 (3.05)
FTIR(neat):υ(cm^{-1})	3319 (O-H stretching), 1706 and 1692 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	6.76 (*d*, *J* = 15.5 Hz, 1H), 6.40 (*d*, *J* = 15.5 Hz, 1H), 5.89 (*q*, *J* = 1.5 Hz, 1H), 2.44 (*d*, *J* = 17.0 Hz, 1H), 2.37 (*d*, *J* = 17.0 Hz, 1H), 2.24 (*s*, 3H), 1.82 (*d*, *J* = 1.5 Hz, 3H), 1.04 (*s*, 3H), 0.96 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	197.19, 196.77, 160.09, 144.87, 130.37, 127.85, 79.32, 49.58, 41.41, 28.42, 24.34, 22.93, 18.64
DEPT 135: CH;	144.87, 130.37, 127.85
CH_2;	49.58
CH_3;	28.42, 24.34, 22.93, 18.64

Band 2 was a yellow gum (3.0 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.50. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction B4 showed three pale UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.30, 0.33 and 0.37. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction B5 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction B6 showed three pale UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.19 and 0.26. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction B7 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.33, 0.47 and 0.54. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 184**.

Table 184 Subfractions obtained from **subfraction B7** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
B71	4.5	White solid
B72	1.7	Yellow gum
B73	3.3	Yellow gum

Table 184 Continued

Subfraction	Weight (mg)	Physical appearance
B74	5.0	Colorless gum
B75	5.1	Purple solid

Subfraction B71 showed none of major spots under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction B72 showed one UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.44. Its ^{1}H NMR spectrum indicated the presence of **K43**. Further investigation was then not carried out.

Subfraction B73 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.35 and 0.44. Its ^{1}H NMR spectrum indicated the presence of a mixture of **K43** and **K49**. Further investigation was then not carried out.

Subfraction B74 (K49) showed one UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.35.

$[\alpha]_D^{25}$	+37 (*c* 0.89, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	211 (3.32), 221 (3.31), 240 (3.22), 247 (3.23), 290 (3.07)
FTIR(neat):υ(cm^{-1})	3310 (O-H stretching), 1686 (C=O stretching)
^{1}H NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (300 MHz):	6.60 (*brs*, 1H), 6.57 (*brs*, 1H), 6.04 (*s*, 1H), 4.23 (*m*, 1H), 2.70 (*s*, 3H), 2.65 (*d*, J = 5.7 Hz, 2H), 1.31 (*d*, J = 6.3 Hz, 3H)
^{13}C NMR($CDCl_3$+CD_3OD)(δ_{ppm}) (75 MHz):	180.34, 164.60, 160.81, 159.81, 142.64, 116.89, 115.07, 111.80, 100.77, 65.39, 43.31, 23.12, 22.79

DEPT 135:	CH;	116.89, 111.80, 100.77, 65.39
	CH_2;	43.31
	CH_3;	23.12, 22.79

Subfraction B75 displayed a long tail under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction B8 showed four UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.31, 0.44, 0.53 and 0.61. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 185.**

Table 185 Subfractions obtained from **subfraction B8** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
B81	5.0	White solid
B82	1.5	Yellow gum
B83	3.5	Yellow gum
B84	4.0	Red solid

Subfraction B81 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.57 and 0.65. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B82 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.33 and 0.61. Because of the minute quantity, it was not further investigated.

Subfraction B83 showed one UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.44. Its ^1H NMR spectrum indicated the presence of **K43**. Further investigation was then not carried out.

Subfraction B84 displayed a long tail under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction B9 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.26, 0.51 and 0.70. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction B10 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.14, 0.35 and 0.61. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 40% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 186**.

Table 186 Subfractions obtained from **subfraction B10** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
B10A	40% MeOH/H_2O	22.3	Yellow gum
B10B	50-60% MeOH/H_2O	33.6	Purple gum
B10C	80% MeOH/H_2O-100% MeOH	28.1	Brown gum

Subfraction B10A showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.20. Its ^1H

NMR spectrum indicated the presence of many compounds. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 30% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 187**.

Table 187 Subfractions obtained from **subfraction B10A** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
B10A1	8.0	White solid
B10A2	7.7	Yellow gum
B10A3	4.3	Red solid

Subfraction B10A1 displayed a long tail under UV-S on reverse phase TLC using 30% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction B10A2 showed one UV-active spot on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f value of 0.24. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B10A3 showed two UV-active spots on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f values of 0.24 and 0.30. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B10B showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.05 and 0.20. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Subfraction B10C displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction B11 showed five UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.14, 0.25, 0.51, 0.69 and 0.81. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Fraction 14C showed six UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.09, 0.19, 0.22, 0.33, 0.35 and 0.47. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 188.**

Table 188 Subfractions obtained from **fraction 14C** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
C1	2% MeOH/CH_2Cl_2	9.6	Red solid
C2	2-3% MeOH/CH_2Cl_2	16.2	Red solid
C3	3% MeOH/CH_2Cl_2	16.1	Red solid
C4	5% MeOH/CH_2Cl_2	99.9	Red solid
C5	5% MeOH/CH_2Cl_2	30.9	Red solid
C6	7-10% MeOH/CH_2Cl_2	50.0	Red solid
C7	10% MeOH/CH_2Cl_2-100% MeOH	46.2	Red solid

Subfraction C1 showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.39, 0.51, 0.58, 0.67, 0.72 and 0.77. Because of low quantity, it was not further investigated.

Subfraction C2 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.24, 0.29 and 0.34. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 189**.

Table 189 Subfractions obtained from **subfraction C2** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
C21	3.2	Red gum
C22	3.0	Yellow solid
C23	3.1	Red solid
C24	1.7	Purple solid
C25	4.1	Red gum

Subfraction C21 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.42, 0.47 and 0.52. Because of low quantity, it was not further investigated.

Subfraction C22 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.33 and 0.42. It was then purified by precoated TLC with 50% ethyl acetate in petroleum ether as a mobile phase (10 runs) to afford two bands.

Band 1 was a yellow gum (1.5 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.58. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 2 (K45) was a yellow gum (1.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.50.

$[\alpha]_D^{25}$	-10 (*c* 0.01, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	217 (2.74), 249 (2.23), 290 (1.90)
FTIR(neat):υ(cm^{-1})	3329 (O-H stretching), 1692 and 1681 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	12.19 (*s*, 1H), 7.40 (*s*, 1H), 6.10 (*s*, 1H), 3.90 (*s*, 3H), 3.90 (*d*, *J* = 1.8 Hz, 1H), 3.10 (*m*, 2H), 3.03 (*d*, *J* = 18.5 Hz, 1H), 2.87 (*d*, *J* = 18.5 Hz, 1H), 1.36 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	184.65, 184.08, 160.43, 160.25, 143.62, 129.99, 129.50, 119.59, 112.00, 110.34, 71.95, 70.71, 56.56, 35.83, 34.34, 25.51
DEPT 135: CH;	119.59, 110.34, 71.95
CH_2;	35.83, 34.34
CH_3;	56.56, 25.51

Subfraction C23 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.26 and 0.33. Its ^{1}H NMR spectrum indicated that the major compound was **K45**. Further investigation was then not performed.

Subfraction C24 showed one UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.26. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C25 displayed a long tail under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction C3 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.17, 0.19 and 0.25. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Subfraction C4 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.13 and 0.19. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 190.**

Table 190 Subfractions obtained from **subfraction C4** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
C41	3.8	Yellow gum
C42	16.0	Yellow gum
C43	57.1	Red gum
C44	11.9	Red gum
C45	7.8	Red solid

Subfraction C41 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.12, 0.17 and 0.24. Because of low quantity, it was not further investigated.

Subfraction C42 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.56 and 0.65. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 40% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 191.**

Table 191 Subfractions obtained from **subfraction C42** by column chromatography over sreverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
C421	3.0	Yellow gum
C422	2.4	Yellow gum
C423	10.3	Yellow gum

Subfraction C421 showed one UV-active spot on reverse phase TLC using 40% methanol in water as a mobile phase with the R_f value of 0.45. Its ^1H NMR spectrum indicated the presence of **K44**. Further investigation was then not carried out.

Subfraction C422 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.34 and 0.45. Its ^1H NMR spectrum indicated that the major compound was **K44**. Further investigation was then not performed.

Subfraction C423 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.32 and 0.48. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 40% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 192**.

Table 192 Subfractions obtained from **subfraction C423** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
C423A	3.1	Colorless gum
C423B	4.0	Colorless gum
C423C	3.0	Yellow gum

Subfraction C423A (K42) showed one UV-active spot on reverse phase TLC using 40% methanol in water as a mobile phase with the R_f value of 0.45.

$[\alpha]_D^{29}$	-18 (*c* 0.92, acetone)
UV λ_{max}(nm)(MeOH)(log ε)	209 (4.17), 242 (3.91), 280 (3.61), 353 (3.42)
FTIR(neat):υ(cm^{-1})	3417 (O-H stretching), 1687 (C=O stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(300 MHz):	12.76 (*s*, 1H), 6.44 (*s*, 1H), 5.00 (*t*, *J* = 3.5 Hz ,1H), 4.15 (*d*, *J* = 3.5 Hz, 1H), 3.93 (*s*, 3H), 3.81 (*d*, *J* = 6.5 Hz, 1H), 3.47 (*ddd*, *J* = 11.0, 6.5, 4.5 Hz, 1H), 3.21 (*s*, 1H), 2.82 (*td*, *J* = 12.0, 3.5 Hz, 1H), 2.38 (*dt*, *J* = 12.5, 4.0 Hz, 1H), 2.16 (*tt*, *J* = 11.5, 3.5 Hz, 1H), 1.87 (*t*, *J* = 13.5 Hz, 1H), 1.76 (*dd*, *J* = 13.5, 3.5 Hz, 1H), 1.56 (*q*, *J* = 12.5 Hz, 1H), 1.29 (*s*, 3H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(75 MHz):	203.00, 158.72, 155.00, 136.00, 129.00, 107.80, 98.77, 74.08, 70.48, 62.42, 55.65, 41.45, 40.23, 38.45, 29.61, 26.66
DEPT 135: CH;	98.77, 74.08, 62.42, 41.45, 38.45
CH_2;	40.23, 29.61
CH_3;	55.65, 26.66
EIMS *m/z* (% relative intensity):	324 (14), 304 (27), 288 (100), 218 (43), 175 (18)

Subfraction C423B (K44) showed one UV-active spot on reverse phase TLC using 40% methanol in water as a mobile phase with the R_f value of 0.38.

$[\alpha]_D^{29}$	-93 (*c* 0.15, MeOH)

UV λ_{max}(nm)(MeOH)(log ε)	208 (3.03), 243 (2.90), 283 (2.82), 357 (2.65)
FTIR(neat):υ(cm^{-1})	3379 (O-H stretching), 1689 (C=O stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(300 MHz):	12.25 (*s*, 1H), 6.47 (*s*, 1H), 4.90 (*d*, *J* = 9.0 Hz, 1H), 4.41 (*d*, *J* = 3.0 Hz, 1H), 4.06 (*td*, *J* = 9.0, 3.0 Hz, 1H), 3.92 (*s*, 3H), 3.33 (*d*, *J* = 9.0 Hz, 1H), 2.45 (*t*, *J* = 9.0 Hz, 1H), 2.37 (*brd*, *J* = 12.0 Hz, 3H), 1.45 (*brd*, *J* = 12.0 Hz, 1H), 1.29 (*s*, 3H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(75 MHz):	204.65, 158.91, 156.51, 137.92, 127.51, 108.08, 99.08, 78.04, 72.16, 71.34, 70.25, 55.65, 51.63, 39.47, 39.41, 26.67
DEPT 135: CH;	99.08, 72.16, 71.34, 78.04, 51.63, 39.41
CH_2;	39.47
CH_3;	55.65, 26.67

Subfraction C423C showed one UV-active spot on reverse phase TLC using 40% methanol in water as a mobile phase with the R_f value of 0.38. Its ^{1}H NMR spectrum indicated that the major compound was **K44**. Further investigation was then not performed.

Subfraction C43 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.30, 0.43 and 0.56. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 193.**

Table 193 Subfractions obtained from **subfraction C43** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
C431	3.1	Yellow gum
C432	6.2	Red gum
C433	35.4	Colorless gum
C434	3.0	Colorless gum

Subfraction C431 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction C432 showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.46 and 0.59. It was then purified by precoated TLC with 3% methanol in dichloromethane as a mobile phase (7 runs) to afford three bands.

Band 1 was a yellow gum (1.5 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.59. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 2 was a yellow gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.46. Its ^{1}H NMR spectrum indicated the presence of **K43**. Further investigation was then not carried out.

Band 3 was a yellow gum (1.5 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.07. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction C433 (K43) showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.38.

$[\alpha]_D^{29}$	-8 (*c* 0.16, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	207 (3.13), 245 (2.93), 287 (2.82), 359 (2.65)
FTIR(neat):υ(cm^{-1})	3351 (O-H stretching), 1685 (C=O stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(300 MHz):	12.72 (*s*, 1H), 6.44 (*s*, 1H), 4.83 (*d*, *J* = 10.2 Hz, 1H), 3.90 (*s*, 3H), 3.49 (*brd*, *J* = 12.3 Hz, 1H), 2.47 (*dd*, *J* = 12.3, 3.9 Hz, 1H), 2.43 (*dd*, *J* = 12.6, 5.1 Hz, 1H), 2.32 (*m*, 1H), 2.25 (*dm*, *J* = 12.9 Hz, 1H), 1.64 (*q*, *J* = 12.3 Hz, 1H), 1.34 (*dd*, *J* = 12.6, 11.7 Hz, 1H), 1.28 (*s*, 3H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(75 MHz):	202.51, 158.62, 155.75, 137.78, 127.41, 107.94, 98.99, 73.73, 72.47, 69.85, 55.51, 46.07, 40.92, 40.75, 29.41, 26.49
DEPT 135: CH;	98.99, 73.73, 72.47, 46.07, 40.92
CH_2;	40.75, 29.41
CH_3;	55.51, 26.49

Subfraction C434 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction C44 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.33 and 0.42. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 45% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 194.**

Table 194 Subfractions obtained from **subfraction C44** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
C441	2.1	Yellow gum
C442	6.5	Red gum
C443	1.9	Red solid

Subfraction C441 showed one UV-active spot on reverse phase TLC using 45% methanol in water as a mobile phase with the R_f value of 0.37. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction C442 showed one UV-active spot on reverse phase TLC using 45% methanol in water as a mobile phase with the R_f value of 0.34. Its ^1H NMR spectrum indicated that the major compound was **K49**. Further investigation was then not performed.

Subfraction C443 displayed a long tail under UV-S on reverse phase TLC using 45% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction C45 displayed a long tail under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction C5 showed five UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.08, 0.18, 0.38, 0.50 and 0.58. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 195**.

Table 195 Subfractions obtained from **subfraction C5** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
C51	1.3	Yellow gum
C52	19.0	Red gum
C53	2.4	Red solid
C54	6.3	Red solid

Subfraction C51 displayed a long tail under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction C52 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.22, 0.38 and 0.50. Its ^1H NMR spectrum indicated that the major compound was **K43**. Further investigation was then not performed.

Subfraction C53 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.38 and 0.40. Its ^1H NMR spectrum indicated that the major compound was **K43**. Further investigation was then not performed.

Subfraction C54 showed one pale UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.38. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction C6 displayed a long tail under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction C7 showed five pale UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.20, 0.28, 0.35, 0.72

and 0.83. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Fraction 14D showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.33 and 0.43. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 196**.

Table 196 Subfractions obtained from **fraction 14D** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
D1	2% MeOH/CH_2Cl_2	4.2	Yellow solid
D2	2% MeOH/CH_2Cl_2	8.3	Red gum
D3	2% MeOH/CH_2Cl_2	6.8	Red gum
D4	2% MeOH/CH_2Cl_2	2.0	Red gum
D5	4% MeOH/CH_2Cl_2	65.3	Red solid
D6	7-10% MeOH/CH_2Cl_2	271.3	Red solid
D7	20% MeOH/CH_2Cl_2-100% MeOH	285.5	Red solid

Subfraction D1 showed two pale UV-active spots on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f values of 0.85 and 0.90. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction D2 (K48) showed one UV-active spot on normal phase TLC using 70% dichloromethane in petroleum ether as a mobile phase with the R_f value of 0.85.

UVλ_{max}(nm)(MeOH)(log ε)	209 (2.75), 218 (2.72), 253 (2.43), 281 (2.12)
FTIR(neat):υ(cm^{-1})	3338 (O-H stretching), 1686 and 1681 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	13.70 (*s*, 1H), 13.40 (*s*, 1H), 8.71 (*d*, *J* = 7.8 Hz, 1H), 8.08 (*s*, 1H), 7.56 (*d*, *J* = 7.8 Hz, 1H), 6.64 (*s*, 1H), 3.90 (*s*, 3H), 2.48 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	188.00, 184.67, 160.65, 157.50, 150.00, 145.50, 135.53, 133.50, 131.50, 127.31. 127.06, 111.27, 106.09, 56.65, 21.94
DEPT 135: CH;	135.53, 127.31, 127.06, 106.09
CH_3;	56.65, 21.94

Subfraction D3 showed three pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.50, 0.63 and 0.78. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction D4 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.40. Because of low quantity, it was not further investigated.

Subfraction D5 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.13, 0.18 and 0.26. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 197.**

Table 197 Subfractions obtained from **subfraction D5** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
D51	9.4	Red gum
D52	5.0	Red gum
D53	14.7	Red gum
D54	13.5	Red gum
D55	9.7	Red gum

Subfraction D51 showed five UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.26, 0.33, 0.41, 0.55 and 0.69. Its ^1H NMR spectrum indicated that the major compound was **K44.** Further investigation was then not performed.

Subfraction D52 showed one UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.26. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction D53 showed three pale UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.21, 0.26 and 0.29. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction D54 showed five pale UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.21, 0.28, 0.31, 0.42 and 0.45. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction D55 showed one UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.21. Because the

^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction D6 showed four UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.33, 0.38, 0.52 and 0.60. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 198.**

Table 198 Subfractions obtained from **subfraction D6** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
D61	3.6	Yellow gum
D62	25.6	Yellow gum
D63	23.8	Yellow gum
D64	180.4	Red gum
D65	35.0	Red solid

Subfraction D61 showed none of major spots under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction D62 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.53 and 0.60. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 30% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 199.**

Table 199 Subfractions obtained from **subfraction D62** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
D621	13.2	Yellow gum
D622	8.6	Yellow gum
D623	3.8	Yellow gum

Subfraction D621 showed one UV-active spot on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f value of 0.47. Its ^1H NMR spectrum indicated the presence of **K44**. Further investigation was then not carried out.

Subfraction D622 showed two UV-active spots on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f values of 0.45 and 0.47. Its ^1H NMR spectrum indicated the presence of **K44**. Further investigation was then not carried out.

Subfraction D623 displayed a long tail under UV-S on reverse phase TLC using 30% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction D63 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.55 and 0.62. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 30% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 200**.

Table 200 Subfractions obtained from **subfraction D63** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
D631	3.1	Yellow gum
D632	15.3	Yellow gum
D633	5.0	Yellow gum

Subfraction D631 showed one UV-active spot on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f value of 0.29. Its ^{1}H NMR spectrum indicated the presence of **K42.** Further investigation was then not carried out.

Subfraction D632 showed one UV-active spot on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f value of 0.22. Its ^{1}H NMR spectrum indicated that the major compound was **K44.** Further investigation was then not performed.

Subfraction D633 showed three UV-active spots on reverse phase TLC using 30% methanol in water as a mobile phase with the R_f values of 0.03, 0.13 and 0.22. Its ^{1}H NMR spectrum indicated that the major compound was **K44.** Further investigation was then not performed.

Subfraction D64 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.28, 0.42 and 0.53. Its ^{1}H NMR spectrum indicated that the major compounds was **K44.** Further investigation was then not performed.

Subfraction D65 showed five UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.05, 0.08, 0.18, 0.22 and 0.28. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction D7 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.03 and 0.34. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 201**.

Table 201 Subfractions obtained from **subfraction D7** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
D71	49.0	Red solid
D72	57.7	Red solid
D73	88.4	Red solid

Subfraction D71 showed six UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.49, 0.57, 0.66, 0.69, 0.81 and 0.86. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction D72 showed four pale UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.47, 0.49, 0.56 and 0.69. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction D73 showed three pale UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.09, 0.18 and 0.33. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Fraction 14E showed five UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.16, 0.31, 0.41, 0.57 and

0.81. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 202**.

Table 202 Subfractions obtained from **fraction 14E** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
E1	2% MeOH/CH_2Cl_2	6.7	Red solid
E2	2% MeOH/CH_2Cl_2	2.6	Red solid
E3	2% MeOH/CH_2Cl_2	6.3	Red solid
E4	2% MeOH/CH_2Cl_2	5.7	Red solid
E5	3% MeOH/CH_2Cl_2	13.2	Red solid
E6	3% MeOH/CH_2Cl_2	26.7	Red solid
E7	5% MeOH/CH_2Cl_2	32.5	Red gum
E8	7-20% MeOH/CH_2Cl_2	34.4	Red gum
E9	40% MeOH/CH_2Cl_2-100% MeOH	127.3	Red gum

Subfraction E1 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.65 and 0.75. Its ^1H NMR spectrum indicated that the major compound was **K48**. Further investigation was then not performed.

Subfraction E2 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction E3 showed two pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.47 and

0.74. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction E4 showed two pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.35 and 0.45. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction E5 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.23, 0.29 and 0.35. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 70% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 203**.

Table 203 Subfractions obtained from **subfraction E5** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
E51	7.6	Red gum
E52	1.0	Red gum
E53	1.7	Red gum
E54	3.1	Red solid

Subfraction E51 showed one pale UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.45. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction E52 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.25. Its ^{1}H

NMR spectrum indicated that the major compound was **K48**. Further investigation was then not performed.

Subfraction E53 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.25. The ^1H NMR spectrum indicated the presence of many compounds. Further investigation was then not performed.

Subfraction E54 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.27, 0.32, and 0.33. Because of the minute quantity, it was not further investigated.

Subfraction E6 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.17, 0.24 and 0.29. The ^1H NMR spectrum indicated the presence of many compounds. Further investigation was then not performed.

Subfraction E7 showed three pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.12 and 0.16. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction E8 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.23, 0.29 and 0.35. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 204**.

Table 204 Subfractions obtained from **subfraction E8** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
E81	7.6	Red gum
E82	1.0	Red gum
E83	1.7	Red gum
E84	3.1	Red solid

Subfraction E81 showed one UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.73. Its ^1H NMR spectrum indicated that the major compound was **K44**. Further investigation was then not performed.

Subfraction E82 showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.40 and 0.58. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 40% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 205**.

Table 205 Subfractions obtained from **subfraction E82** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
E821	2.6	Red gum
E822	4.9	Red gum
E823	6.3	Red gum

Subfraction E821 showed one UV-active spot on reverse phase TLC using 40% methanol in water as a mobile phase with the R_f value of 0.39. Its ^1H NMR

spectrum indicated that the major compound was **K44**. Further investigation was then not performed.

Subfraction E822 showed one UV-active spot on reverse phase TLC using 40% methanol in water as a mobile phase with the R_f value of 0.27. Its ^1H NMR spectrum indicated that the major compound was **K43**. Further investigation was then not performed.

Subfraction E823 showed two UV-active spots on reverse phase TLC using 40% methanol in water as a mobile phase with the R_f values of 0.23 and 0.27. Its ^1H NMR spectrum indicated that the major compound was **K43**. Further investigation was then not performed.

Subfraction E83 showed one UV-active spot on reverse phase TLC using 40% methanol in water as a mobile phase with the R_f value of 0.42. The ^1H NMR spectrum indicated the presence of many compounds. Further investigation was then not performed.

Subfraction E84 displayed a long tail under UV-S on reverse phase TLC using 40% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction E9 showed three pale UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.30, 0.33 and 0.40. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Fraction 14F displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Its ^1H NMR spectrum displayed high field signals. Thus, it was not investigated.

Fraction 14G displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. This subfraction (200 mg) was

subjected to acetylation reaction. After work up, the reaction mixture was obtained as a red gum (196.3 mg) and showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.26, 0.41, 0.62, 0.71 and 0.78. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 206.**

Table 206 Subfractions obtained from **fraction 14G** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
14AC1	2% MeOH/CH_2Cl_2	5.0	Red gum
14AC2	2% MeOH/CH_2Cl_2	140.3	Red gum
14AC3	2% MeOH/CH_2Cl_2	13.3	Red gum
14AC4	2% MeOH/CH_2Cl_2	14.2	Red gum
14AC5	4-8% MeOH/CH_2Cl_2	9.5	Red gum
14AC6	20% MeOH/CH_2Cl_2-100% MeOH	10.7	Brown solid

Subfraction 14AC1 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.57 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction 14AC2 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.31, 0.43, 0.64 and 0.76. It was further separated by column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with

similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 207.**

Table 207 Subfractions obtained from **subfraction 14AC2** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
14AC21	2% MeOH/CH_2Cl_2	118.1	Red gum
14AC22	2% MeOH/CH_2Cl_2	5.7	Red gum
14AC23	2% MeOH/CH_2Cl_2	10.0	Red gum
14AC24	4-10% MeOH/CH_2Cl_2	7.6	Red gum
14AC25	20% MeOH/CH_2Cl_2-100% MeOH	4.4	Red gum

Subfraction 14AC21 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.37, 0.52 and 0.59. It was further separated by column chromatography over silica gel. Elution was performed with 1% methanol in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 208.**

Table 208 Subfractions obtained from **subfraction 14AC21** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
14AC211	2.4	Red gum
14AC212	63.4	Red gum
14AC213	34.8	Red gum
14AC214	9.8	Red gum
14AC215	2.4	Red gum

Subfraction 14AC211 showed none of major spots under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 14AC212 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.50, 0.59 and 0.74. It was further separated by column chromatography over silica gel. Elution was performed with 0.5% methanol in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 209**.

Table 209 Subfractions obtained from **subfraction 14AC212** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
14AC212A	6.6	Red gum
14AC212B	35.3	Red gum
14AC212C	14.4	Red gum
14AC212D	3.5	Red gum

Subfraction 14AC212A displayed a long tail under UV-S on normal phase TLC using 0.5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 14AC212B showed three UV-active spots on normal phase TLC using 0.5% methanol in dichloromethane as a mobile phase with the R_f values of 0.29, 0.34 and 0.36. Its ^{1}H NMR spectrum indicated that the major compound was **K43**. Further investigation was then not performed.

Subfraction 14AC212C showed two UV-active spots on normal phase TLC using 0.5% methanol in dichloromethane as a mobile phase with the R_f values of 0.29 and

0.36. Its ^{1}H NMR spectrum indicated that the major compound was **K43**. Further investigation was then not performed.

Subfraction 14AC212D displayed a long tail under UV-S on normal phase TLC using 0.5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 14AC213 showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.36, 0.43, 0.50, 0.59 and 0.74. It was further separated by column chromatography over silica gel. Elution was performed with 0.5% methanol in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 210**.

Table 210 Subfractions obtained from **subfraction 14AC213** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
14AC213A	1.6	Red gum
14AC213B	22.6	Red gum
14AC213C	3.6	Red gum
14AC213D	7.7	Red gum

Subfraction 14AC213A showed one pale UV-active spot on normal phase TLC using 0.5% methanol in dichloromethane as a mobile phase with the R_f value of 0.85. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 14AC213B showed three UV-active spots on normal phase TLC using 0.5% methanol in dichloromethane as a mobile phase with the R_f values of 0.53, 0.66 and 0.76. It was further separated by column chromatography over silica gel. Elution was performed with 2% acetone in dichloromethane. Fractions with similar

chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 211.**

Table 211 Subfractions obtained from **subfraction 14AC213B** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
14ACA	3.1	Red gum
14ACB	7.3	Red gum
14ACC	4.7	Red gum
14ACD	5.6	Red gum

Subfraction 14ACA showed four UV-active spots on normal phase TLC using 2% acetone in dichloromethane as a mobile phase with the R_f values of 0.35, 0.40, 0.45 and 0.59. Because of the minute quantity, it was not further investigated.

Subfraction 14ACB showed one UV-active spot on normal phase TLC using 2% acetone in dichloromethane as a mobile phase with the R_f value of 0.33. Its ^{1}H NMR spectrum indicated it was an acetate derivative of **K43.**

$[\alpha]_D^{29}$	-19 (*c* 0.15, MeOH)
UVλ_{max}(nm)(MeOH)(log ε)	208 (2.75), 216 (2.73), 242 (2.53), 280 (2.34), 353 (2.11)
FTIR(neat):υ(cm^{-1})	3310 (O-H stretching), 1670 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	12.63 (*s*, 1H), 6.49 (*s*, 1H), 6.02 (*d*, *J* = 9.3 Hz, 1H), 4.78 (*dd*, *J* = 12.9, 6.4 Hz, 1H), 3.85 (*s*, 3H), 2.48 (*m*, 1H), 2.43 (*m*, 1H), 2.37 (*m*, 1H), 2.22 (*s*, 3H), 2.18 (*s*, 3H), 2.13 (*s*, 3H), 2.01 (*dd*, *J* = 13.8, 3.0 Hz, 1H), 1.85 (*brd*, *J* = 12.9 Hz, 1H), 1.42 (*dm*, *J* = 13.8 Hz, 1H), 1.26 (*s*, 3H)

^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	200.27, 170.80, 169.94, 168.59, 162.65, 158.35, 130.60, 130.00, 109.31, 100.48, 76.18, 69.82, 69.73, 56.37, 44.91, 38.29, 40.40, 26.91, 25.50, 21.02, 20.93, 20.51
DEPT 135: CH;	100.48, 76.18, 69.73, 44.91, 38.29
CH_2;	40.40, 25.50
CH_3;	56.37, 26.91, 21.02, 20.93, 20.51

Subfraction 14ACC showed one UV-active spot on normal phase TLC using 2% acetone in dichloromethane as a mobile phase with the R_f value of 0.33. Its ^1H NMR spectrum indicated that the major compound was an acetate derivative of **K43**. Further investigation was then not performed.

Subfraction 14ACD showed two UV-active spots on normal phase TLC using 2% acetone in dichloromethane as a mobile phase with the R_f values of 0.35 and 0.53. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 14AC213C showed two UV-active spots on normal phase TLC using 0.5% methanol in dichloromethane as a mobile phase with the R_f values of 0.53 and 0.59. Because of low quantity, it was not further investigated.

Subfraction 14AC213D showed two UV-active spots on normal phase TLC using 0.5% methanol in dichloromethane as a mobile phase with the R_f values of 0.32 and 0.44. Its ^1H NMR spectrum indicated that the major compound was **K47**. Further investigation was then not performed.

Subfraction 14AC214 showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.30 and 0.42. Its ^1H NMR spectrum indicated that the major compound was **K47**. Further investigation was then not performed.

Subfraction 14AC215 showed three pale UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.23 and 0.33. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 14AC22 showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.37. The ^1H NMR spectrum indicated the presence of many compounds. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (5 runs) to afford one band. It was a red gum (2.7 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.26. Its ^1H NMR spectrum indicated the presence of **K47**. Further investigation was then not carried out.

Subfraction 14AC23 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.20, 0.31 and 0.37. Its ^1H NMR spectrum indicated the presence of **K47**. Further investigation was then not carried out.

Subfraction 14AC24 showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.19 and 0.31. Its ^1H NMR spectrum indicated that the major compound was **K47**. Further investigation was then not performed.

Subfraction 14AC25 displayed a long tail under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 14AC3 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.31, 0.43 and 0.50. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (7 runs) to afford three bands.

Band 1 was a red gum (3.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.42. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 2 was a red gum (3.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.37. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was a red gum (4.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.29. Its ^1H NMR spectrum indicated the presence of **K47**. Further investigation was then not carried out.

Subfraction 14AC4 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.23 and 0.43. Its ^1H NMR spectrum indicated that the major compound was **K47**. Further investigation was then not performed.

Subfraction 14AC5 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.23 and 0.43. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (6 runs) to afford two bands.

Band 1 was a red gum (2.6 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.42. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 2 (K47) was a red gum (4.7 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.26.

$[\alpha]_D^{29}$	-39 (*c* 0.80, acetone)
UV λ_{max}(nm)(MeOH)(log ε)	204 (3.41), 226 (3.50), 301 (2.80)
FTIR(neat):υ(cm^{-1})	3415 (O-H stretching), 1680 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	13.51 (*s*, 1H), 12.63 (*s*, 1H), 6.22 (*s*, 1H), 5.25 (*d*, *J* = 7.2 Hz, 1H), 5.18 (*d*, *J* = 7.2 Hz, 1H), 3.96 (*s*, 3H), 3.17 (*d*, *J* = 18.6 Hz, 1H), 2.83 (*d*, *J* = 18.6 Hz, 1H), 2.23 (*s*, 3H), 1.40 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	184.77, 178.16, 170.47, 160.96, 160.80, 159.55, 138.43, 135.22, 110.36, 109.67, 108.61, 78.06, 70.76, 68.68, 56.88, 36.00, 25.99, 20.99
DEPT 135: CH;	109.67, 78.06, 68.68
CH_2;	36.00
CH_3;	56.88, 25.99, 20.99

Subfraction 14AC6 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

5.2.3 Purification of the EtOAc extract from mycelia

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six fractions as shown in **Table212.**

Table 212 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
14CE1	70.3	Brown solid
14CE2	80.1	Brown solid
14CE3	232.3	Brown solid
14CE4	76.8	Brown solid
14CE5	63.1	Brown solid
14CE6	34.4	Brown solid

Fraction 14CE1 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 14CE2 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.34 and 0.43. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 14CE3 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.12, 0.16 and 0.28. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane, followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 213**.

Table 213 Subfractions obtained from **fraction 14CE3** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
V1	2% MeOH/CH_2Cl_2	7.5	Yellow gum
V2	2% MeOH/CH_2Cl_2	17.0	Orange gum
V3	4% MeOH/CH_2Cl_2	10.5	Red gum
V4	4% MeOH/CH_2Cl_2	12.6	Red gum
V5	6-8% MeOH/CH_2Cl_2	44.2	Red gum
V6	10-20% MeOH/CH_2Cl_2	89.9	Red solid
V7	30% MeOH/CH_2Cl_2-100% MeOH	11.7	Red solid

Subfraction V1 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.38, 0.41 and 0.50 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction V2 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.04, 0.19 and 0.26. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 214**.

Table 214 Subfractions obtained from **subfraction V2** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
V21	3.1	Yellow gum
V22	3.9	Yellow gum

Table 214 Continued

Subfraction	Weight (mg)	Physical appearance
V23	5.3	Red gum
V24	3.5	Red gum

Subfraction V21 showed one UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.28. Its ^1H NMR spectrum indicated the presence of **K46**. Further investigation was then not carried out.

Subfraction V22 showed one pale UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.23. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction V23 showed one pale UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.21. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction V24 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction V3 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.04, 0.14 and 0.19. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (6 runs) to afford three bands.

Band 1 (K46) was an orange gum (4.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.34.

$[\alpha]_D^{29}$	-15 (*c* 0.02, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	207 (3.11), 216 (3.13), 249 (2.72), 292 (2.51)
FTIR(neat):υ(cm^{-1})	3310 (O-H stretching), 1681 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	12.65 (*s*, 1H), 7.43 (*s*, 1H), 6.06 (*s*, 1H), 3.95 (*t*, *J* = 3.9 Hz, 1H), 3.91 (*s*, 3H), 3.15 (*d*, *J* = 17.4 Hz, 1H), 3.04 (*d*, *J* = 3.9 Hz, 2H), 2.84 (*d*, *J* = 17.4 Hz, 1H), 1.31 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	190.59, 179.50, 161.11, 159.61, 142.79, 130.55, 120.24, 112.00, 109.42, 71.79, 70.93, 56.60, 40.44, 29.78, 24.73
DEPT 135: CH;	120.24, 109.42, 71.79
CH_2;	40.44, 29.78
CH_3;	56.60, 24.73

Band 2 was a red gum (2.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.24. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was a red gum (2.0 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.22. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction V4 showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07 and 0.16. Its ^{1}H NMR spectrum indicated that the major compound was **K49**. Further investigation was then not carried out.

Subfraction V5 showed three UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.26, 0.33 and 0.47. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 45% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 215**.

Table 215 Subfractions obtained from **subfraction V5** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
V51	1.7	Red gum
V52	8.3	Red gum
V53	4.2	Red gum
V54	15.5	Red gum
V55	12.3	Red gum

Subfraction V51 displayed a long tail under UV-S on reverse phase TLC using 45% methanol in water as a mobile phase. Thus, it was not investigated.

Subfraction V52 showed one UV-active spot on reverse phase TLC using 45% methanol in water as a mobile phase with the R_f value of 0.47. Its ^1H NMR spectrum indicated the presence of **K44**. Further investigation was then not performed.

Subfraction V53 showed one UV-active spot on reverse phase TLC using 45% methanol in water as a mobile phase with the R_f value of 0.33. Its ^1H NMR spectrum indicated the presence of **K43**. Further investigation was then not performed.

Subfraction V54 showed two UV-active spots on reverse phase TLC using 45% methanol in water as a mobile phase with the R_f values of 0.24 and 0.33. It was

further separated by column chromatography over Sephadex LH-20. Elution was performed with 50% methanol in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three fractions as shown in **Table 216**.

Table 216 Subfractions obtained from **subfraction V54** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
V541	4.3	Red gum
V542	8.8	Colorless gum
V543	4.3	Colorless gum

Subfraction V541 showed one pale UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.10. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction V542 (K51) showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.15.

UV λ_{max}(nm)(MeOH)(log ε)	203 (3.56), 260 (3.21)
FTIR(neat):υ(cm^{-1})	3349 (O-H stretching), 1686 (C=O stretching)
^1H NMR(CD_3OD)(δ_{ppm})(300 MHz):	8.01 (*d*, *J* = 8.1 Hz, 1H), 5.90 (*d*, *J* = 4.5 Hz, 1H), 5.70 (*d*, *J* = 8.1 Hz, 1H), 4.18 (*t*, *J* = 5.1 Hz, 1H), 4.14 (*t*, *J* = 5.1 Hz, 1H), 4.00 (*m*, 1H), 3.84 (*dd*, *J* = 12.3, 2.7 Hz, 1H), 3.73 (*dd*, *J* = 12.3, 2.7 Hz, 1H)
^{13}C NMR(CD_3OD)(δ_{ppm})(75 MHz):	164.77, 151.00, 141.32, 101.25, 89.31, 84.96, 74.31, 69.90, 60.87
DEPT 135: CH;	141.32, 101.25, 89.31, 84.96, 74.31, 69.90
CH_2;	60.87

Subfraction V543 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction V55 showed two UV-active spots on reverse phase TLC using 45% methanol in water as a mobile phase with the R_f values of 0.13 and 0.20. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction V6 showed four UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.31, 0.52, 0.47 and 0.64. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 50% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 217**.

Table 217 Subfractions obtained from **subfraction V6** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
V61	50% MeOH/H_2O	40.1	Brown gum
V62	50% MeOH/H_2O	8.5	Yellow gum
V63	50% MeOH/H_2O	3.6	Yellow gum
V64	50% MeOH/H_2O	6.4	Brown gum
V65	50% MeOH/H_2O	2.8	Brown gum
V66	50% MeOH/H_2O	3.0	Brown gum
V67	80% MeOH/H_2O-100% MeOH	20.3	Red solid

Subfraction V61 showed three UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.07 and 0.22. It was further separated by column chromatography over Sephadex LH-20.

Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three fractions as shown in **Table 218**.

Table 218 Subfractions obtained from **subfraction V61** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
V611	3.4	Brown gum
V612	31.0	Brown gum
V613	5.0	Yellow gum

Subfraction V611 showed one pale UV-active spot on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f value of 0.39. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction V612 showed three UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.15 and 0.17. Its ^1H NMR spectrum indicated the presence of sugar as a major compound. Thus, it was not further investigated.

Subfraction V613 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction V62 showed four UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.22, 0.32 and 0.35. Because of low quantity, it was not further investigated.

Subfraction V63 showed three UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.20, 0.28 and 0.32. Because of low quantity, it was not further investigated.

Subfraction V64 showed three UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.24, 0.31 and 0.44. Its ^1H NMR spectrum indicated that the major compound was **K46**. Further investigation was then not performed.

Subfraction V65 showed four UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.22, 0.34, 0.42 and 0.45. Because of the minute quantity, it was not further investigated.

Subfraction V66 showed four UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.26, 0.34, 0.47 and 0.49. Because of the minute quantity, it was not further investigated.

Subfraction V67 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction V7 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Fraction 14CE4 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.04, 0.22, 0.31, 0.47 and 0.95. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 219**.

Table 219 Subfractions obtained from **fraction 14CE4** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
W1	2% MeOH/CH_2Cl_2	7.7	Yellow gum
W2	5% MeOH/CH_2Cl_2	19.4	Orange gum
W3	5% MeOH/CH_2Cl_2	3.0	Red gum
W4	5-10% MeOH/CH_2Cl_2	3.7	Red gum
W5	10% MeOH/CH_2Cl_2	3.6	Red gum
W6	10-20% MeOH/CH_2Cl_2	19.7	Red solid
W7	30% MeOH/CH_2Cl_2-100% MeOH	14.4	Red solid

Subfraction W1 showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.23. Its ^1H NMR spectrum indicated the presence of **K48**. Further investigation was then not performed.

Subfraction W2 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.24, 0.31, 0.44 and 0.79. Its ^1H NMR spectrum indicated that the major compound was **K48**. Further investigation was then not performed.

Subfraction W3 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.19, 0.24, 0.31 and 0.44. Because of the minute quantity, it was not further investigated.

Subfraction W4 showed five UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.31, 0.41, 0.45 and 0.50. Because of low quantity, it was not further investigated.

Subfraction W5 showed three UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.55, 0.31 and 0.45. Because of low quantity, it was not further investigated.

Subfraction W6 showed three UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.31, 0.45 and 0.55. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with the similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three fractions as shown in **Table 220**.

Table 220 Subfractions obtained from **subfraction W6** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
W61	5.6	Brown solid
W62	10.4	Red solid
W63	3.6	Red gum

Subfraction W61 showed four pale UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.19, 0.23 and 0.40. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction W62 showed three pale UV-active spots on normal phase TLC using 5% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.29 and 0.44. Its ^{1}H NMR spectrum indicated the presence of sugar as a major compound. Thus, it was not further investigated.

Subfraction W63 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction W7 displayed a long tail under UV-S on normal phase TLC using 5% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 14CE5 showed five pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.03, 0.14, 0.36, 0.45 and 0.71. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Fraction 14CE6 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

CHAPTER 5.3

RESULTS AND DISCUSSION

One new hydroxynaphthalenone **(K42)** and seven known compounds **(K43-K45** and **K47-50)** were isolated from the broth extract while two known ones **(K46** and **K51)** were obtained from the mycelial extract. The structures were identified by spectroscopic methods.

5.3.1 Compound K43

Compound **K43** was obtained as a colorless gum. The UV spectrum displayed absorption bands at 245, 287 and 359 nm while the IR spectrum showed absorption bands for hydroxyl and conjugated ketone carbonyl groups at 3351 and 1685 cm^{-1}, respectively. The ^{1}H NMR spectrum **(Figure 105) (Table 221)** showed signals for one chelated hydroxy proton (δ 12.72, *s*, 1H), one aromatic proton (δ 6.44, *s*, 1H), four methine protons [δ 4.83 (*d*, J = 10.2 Hz, 1H), 3.49 (*brd*, J = 12.3 Hz, 1H), 2.47 (*td*, J = 12.3 and 3.9 Hz, 1H) and 2.25 (*dm*, J = 12.9 Hz, 1H)], two sets of nonequivalent methylene protons [δ 2.43 (*dd*, J = 12.6 and 5.1 Hz, 1H)/1.34 (*dd*, J = 12.6 and 11.7 Hz, 1H) and 2.32 (*m*, 1H)/1.64 (*q*, J = 12.3 Hz, 1H)], one methoxyl group (δ 3.90, *s*, 3H) and one methyl group (δ 1.28, *s*, 3H). The ^{13}C NMR **(Figure 106) (Table 221)** and DEPT 135 **(Table 221)** spectra displayed seven quaternary (δ 202.51, 158.62, 155.57, 137.78, 127.41, 107.94 and 69.85), five methine (δ 98.99, 73.73, 72.47, 46.07 and 40.92), two methylene (δ 40.75 and 29.41) and two methyl (δ 55.51 and 26.49) carbons. The chelated hydroxy proton, 5-OH (δ 12.72), showed HMBC correlations **(Table 222)** with C-5 (δ 158.62), C-6 (δ 98.99) and C-10a (δ 107.94). The aromatic proton, H-6 (δ 6.44), was correlated with C-5, C-7 (δ 155.75) and C-10a while the methoxy protons, H_3-11 (δ 3.90), gave a HMBC cross peak with C-7. Thus, the aromatic proton and the methoxyl group were attached at C-6

and C-7, respectively. This assignment was supported by NOEDIFF experiment **(Table 222)**. Irradiation of H-6 affected signal intensity of H_3-11. The substituent at C-8 (δ 137.78) of the benzene ring was a hydroxyl group according to its carbon chemical shift. The oxymethine proton, H-9 (δ 4.83), showed a ^{1}H-^{1}H COSY cross peak **(Table 222)** with the methine proton, H-9a (δ 2.25), which was further coupled with the methylene protons, H_{ab}-1 (δ 2.43 and 1.34), and the methine proton, H-4a (δ 2.47). In addition, the remaining methylene protons, H_{ab}-4 (δ 2.32 and 1.64), gave ^{1}H-^{1}H COSY cross peaks with the oxymethine proton, H-3 (δ 3.49), and H-4a. The HMBC correlations of H_3-12 (δ 1.28) with C-1 (δ 40.75), C-2 (δ 69.85) and C-3 (δ 73.73) established the location of H_3-12 at C-2. The substituents at C-2 and C-3 were hydroxyl groups on the basis of the chemical shifts of C-2 and C-3. These results constructed a cyclohexane having two hydroxyl groups at C-2 and C-3, the methyl group at C-2 and the oxymethine unit at C-9a (δ 40.92). Bond formation between C9 (δ 72.47)-C8a (δ 127.41) and C4a (δ 46.07)-C10 (δ 202.51) was established based on the HMBC correlations of H-9/C-8a and C-10a and H_{ab}-4/C-10, respectively, to form a hydroanthraquinone derivative. Irradiation of H-9 in the NOEDIFF experiment **(Table 222)**, affected signal intensity of H-4a, but not H-9a, indicating *trans* ring fusion and *cis*-relationship between H-4a and H-9. Both H-4a and H-9 were located at axial position on the basis of the large coupling constants of 12.3 ($J_{H4a,H9a}$) and 10.2 ($J_{H9,H9a}$) Hz. Signal enhancement of H-4a and H_3-12, upon irradiation of H-3, indicating that H-3 and H_3-12 were located at axial and equatorial position, respectively. The observed optical rotation of **K43**, $[\alpha]_D^{29}$ -8 (*c* 0.16, MeOH), was almost identical to that of (2*S*,3*R*,4a*S*,9*R*,9a*S*)-hydroxydihydrodesoxybostrycin, $[\alpha]_D^{29}$ -5.6 (*c* 0.16, MeOH), indicating that they had the same absolute configuration. Therefore, **K43** was identified as (2*S*,3*R*,4a*S*,9*R*,9a*S*)-hydroxydihydrodesoxybostrycin which was previously isolated from the endophytic fungus PSU-N24 (Sommart, *et al.*, 2008).

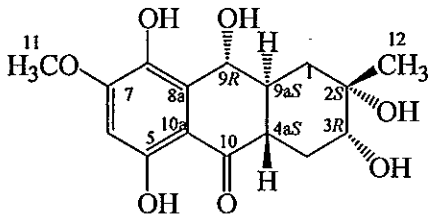


Table 221 The ^{1}H and ^{13}C NMR data of compound **K43** and **(2*S*,3*R*,4a*S*,9*R*,9a*S*)-Hydroxydihydrodesoxybostrycin** in acetone-d_6

Position	K43		(2*S*,3*R*,4a*S*,9*R*,9a*S*)-Hydroxydihydrodesoxybostrycin	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	a: 2.43 (*dd*, 12.6, 5.1) b: 1.34 (*dd*, 12.6, 11.7)	40.75 (CH_2)	a: 2.43 (*m*) b: 1.38 (*dd*, 13.2, 11.7)	40.8 (CH2)
2	-	69.85 (C)	-	69.8 (C)
3	3.49 (*brd*, 12.3)	73.73 (CH)	3.50 (*ddd*, 11.4, 6.3, 4.8)	73.7 (CH)
4	a: 2.32 (*m*) b: 1.64 (*q*, 12.3)	29.41 (CH_2)	a: 2.33 (*m*) b: 1.64 (*q*, 11.4)	29.3 (CH_2)
4a	2.47 (*td*, 12.3, 3.9)	46.07 (CH)	2.48 (*td*, 11.4, 3.9)	46.1 (CH)
5-OH	12.72 (*s*)	158.62 (C)	12.72 (*s*)	158.7 (C)
6	6.44 (*s*)	98.99 (CH)	6.44 (*s*)	99.0 (CH)
7	-	155.75 (C)	-	155.8 (CH)
8	-	137.78 (C)	-	137.8 (C)
8a	-	127.41 (C)	-	127.5 (C)
9	4.83 (*d*, 10.2)	72.47 (CH)	4.84 (*dd*, 9.9, 5.4)	72.5 (CH)
9-OH	-	-	5.54 (*d*, 5.4)	-
9a	2.25 (*dm*, 12.9)	40.92 (CH)	2.25 (*m*)	40.9 (CH)
10	-	202.51 (C)	-	202.5 (C)
10a	-	107.94 (C)	-	108.0 (C)
11	3.90 (*s*)	55.51 (CH_3)	3.90 (*s*)	55.5 (CH_3)
12	1.28 (*s*)	26.49 (CH_3)	1.28 (*s*)	26.5 (CH_3)

Table 222 The HMBC, COSY and NOEDIFF data of compound **K43** in acetone-d_6

Proton	HMBC	COSY	NOEDIFF
H_a-1	C-3, C-4a, C-9, C-9a, C-12	H_b-1, H-9a	H-3, H-9
H_b-1	C-3, C-4a, C-9, C-9a, C-12	H_a-1, H-9a	H-3, H-4a, H-9
H-3	-	H_2-4	H_b-1, H-4a, H_3-12
H_a-4	C-1, C-2, C-3, C-4a, C-9a, C-10	H-3, H_b-4, H-4a	-
H_b-4	C-1, C-2, C-3, C-4a, C-9a, C-10	H-3, H_a-4, H-4a	-
H-4a	C-1, C-9a	H_{ab}-4, H-9a	H_b-1, H-3, H-9
5-OH	C-5, C-6, C-10a	-	-
H-6	C-5, C-7, C-10a	-	H_3-11
H-9	C-1, C-4a, C-8, C-8a, C-10a	H-9a	H_b-1, H-4a
H-9a	C-1, C-2, C-4a	H_{ab}-1, H-4a, H-9	H_b-4
H_3-11	C-7	-	H-6
H_3-12	C-1, C-2, C-3	-	H-3, H-4a

5.3.2 Compound K42

Compound **K42** with the molecular formula $C_{16}H_{20}O_7$ from EIMS (*m/z* 324) **(Figure 102)** was obtained as a colorless gum. The UV and IR spectra were almost identical to those of **K43**. Their ^{1}H NMR **(Figure 103) (Table 223)** and ^{13}C NMR **(Figure 104) (Table 223)** spectra were also similar with the difference in the coupling constant between of H-9 (δ5.00, *t*, J = 3.5 Hz) and H-9a (δ2.16, *tt*, J = 11.5 and 3.5 Hz). A small coupling constant of 3.5 Hz indicated the location of H-9 at equatorial position. This assignment was supported by NOEDIFF results **(Table 224)** since irradiation of H-4a (δ2.82) did not affect signal intensity of H-9. Thus, **K42** was identified as a 9β epimer of **K43**. The absolute configuration at C-2, C-3, C-4a and C-9a in **K42** was proposed to be *S*, *R*, *S* and *S*, respectively, identical to those of **K43**. Thus, C-9 has *S* configuration.

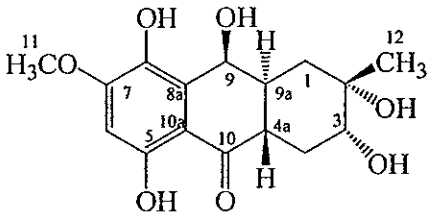


Table 223 The ^{1}H, ^{13}C NMR and HMBC data of compound **K42** in acetone-d_6

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1	a: 1.87 (*t*, 13.5)	40.23 (CH_2)	C-4a, C-9, C-9a
	b: 1.76 (*dd*, 13.5, 3.5)		C-2, C-3, C-4a
2-OH	3.21 (*s*)	70.48 (C)	C-1, C-2, C-3
3	3.47 (*ddd*, 11.0, 6.5, 4.5)	74.08 (CH)	C-4
3-OH	3.81 (*d*, 6.5)	-	C-2, C-3, C-4
4	a: 2.38 (*dt*, 12.5, 4.0)	29.61 (CH_2)	C-2, C-3, C-4a, C-9a
	b: 1.56 (*q*, 12.5)		
4a	2.82 (*td*, 12.0, 3.5)	41.45 (CH)	C-1, C-3, C-4, C-9, C-9a
5-OH	12.76 (*s*)	158.72 (C)	C-5, C-6, C-10a
6	6.44 (*s*)	98.77 (CH)	C-5, C-7, C-8, C-10a
7	-	155.00 (C)	-
8	-	136.00 (C)	-
8a	-	129.00 (C)	-
9	5.00 (*t*, 3.5)	62.42 (CH)	C-4a, C-8, C-8a, C-10a
9-OH	4.15 (*d*, 3.5)	-	C-8a, C-9, C-9a
9a	2.16 (*tt*, 11.5, 3.5)	38.45 (CH)	C-4
10	-	203.00 (C)	-
10a	-	107.80 (C)	-
11	3.93 (*s*)	55.65 (CH_3)	C-7
12	1.29 (*s*)	26.66 (CH_3)	C-1, C-2, C-3

Table 224 The COSY and NOEDIFF data of compound **K42** in acetone-d_6

Proton	COSY	NOEDIFF
H_a-1	H_b-1, H-9a	H_3-12
H_b-1	H_a-1, H-9a	H_3-12
H-3	3-OH, H_2-4	H-4a, H_3-12
3-OH	H-3	-
H_a-4	H-3, H_b-4, H-4a	H_b-4
H_b-4	H-3, H_a-4, H-4a	H_a-4
H-4a	H_{ab}-4, H-9a	H-3
H-6	-	H_3-11, 5-OH
H-9	9-OH, H-9a	H_b-1, 9-OH, H-9a
9-OH	H-9	H-9
H-9a	H_{ab}-1, H-4a, H-9	-
H_3-11	-	H-6
H_3-12	-	H-3

5.3.3 Compound K44

Compound **K44** was obtained as a colorless gum with $[\alpha]_D^{29}$-93 (*c* 0.15, MeOH). The UV and IR spectra were almost identical to those of **K43**. Their ^{1}H NMR spectra were also similar except for the replacement of signals for methylene protons in **K43** with an oxymethine proton (δ 4.06, *dt*, *J* = 9.0 and 3.0 Hz) **(Figure 107) (Table 225)**. The ^{1}H-^{1}H COSY cross peaks **(Table 226)** of this oxymethine proton with H-3 (δ 3.33, *d*, *J* = 9.0 Hz, 1H), 4-OH (δ 4.41, *d*, *J* = 3.0 Hz, 1H) and H-4a (δ 2.45, *t*, *J* = 9.0 Hz, 1H) indicated the location of this proton at C-4 (δ 71.34). A large coupling constant of 9.0 Hz between H-4 with H-3 and H-4a established the orientation of H-4 at axial position. Thus, **K44** was assigned as (2S,3*R*,4*S*,4a*R*,9*R*,9a*S*)-hydroxyhalorosellinia A, $[\alpha]_D^{29}$-95.8 (*c* 0.15, MeOH), which was previously isolated from the endophytic fungus PSU-N24 (Sommart, *et al.*, 2008).

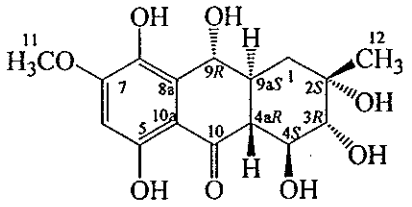


Table 225 The ^{1}H and ^{13}C NMR data of compound **K44** and **(2S,3*R*,4*S*,4a*R*,9*R*,9a*S*)-Hydroxyhalorosellinia A** in acetone-d_6

Position	K44		(2S,3*R*,4*S*,4a*R*,9*R*,9a*S*)-Hydroxyhalorosellinia A	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	a: 2.37 (*brd*, 12.0)	39.47 (CH_2)	a: 2.38 (*dm*, 12.0)	39.5 (CH_2)
	b: 1.45 (*brd*, 12.0)		b: 1.45 (*t*, 12.0)	
2	-	70.25 (C)	-	70.3 (C)
3	3.33 (*d*, 9.0)	78.04 (CH)	3.31 (*dd*, 9.0, 5.0)	78.1 (CH)
3-OH	-	-	3.27 (*d*, 5.0)	-
4	4.06 (*td*, 9.0, 3.0)	71.34 (CH)	4.06 (*td*, 9.0, 3.0)	71.4 (CH)
4-OH	4.41 (*d*, 3.0)	-	4.41 (*d*, 3.0)	-
4a	2.45 (*t*, 9.0)	51.63 (CH)	2.44 (*t*, 9.0)	51.6 (CH)
5-OH	12.25 (*s*)	158.91 (C)	12.28 (*s*)	158.9 (C)
6	6.47 (*s*)	99.08 (CH)	6.48 (*s*)	99.1 (CH)
7	-	156.51 (C)	-	156.5 (C)
8	-	137.92 (C)	-	137.9 (C)
8a	-	127.51 (C)	-	127.5 (C)
9	4.90 (*d*, 9.0)	72.16 (CH)	4.91 (*dd*, 9.0, 6.0)	72.2 (CH)
9-OH	-		5.61 (*d*, 6.0)	
9a	2.37 (*d*, 12.0)	39.41 (CH)	2.38 (*m*)	39.5 (CH)
10	-	204.65 (C)	-	205.1 (C)
10a	-	108.08 (C)	-	108.1(C)
11	3.92 (*s*)	55.65 (CH_3)	3.92 (*s*)	55.7 (CH_3)
12	1.29 (*s*)	26.67 (CH_3)	1.29 (*s*)	26.7 (CH_3)

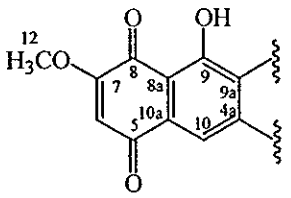
Table 226 The HMBC, COSY and NOE data of compound **K44** in acetone-d_6

Proton	HMBC	COSY	NOEDIFF
H_a-1	C-3, C-4a, C-9a, C-10, C-12	H_b-1, H-9a	H_b-1, H-4a, H_3-12
H_b-1	C-3, C-4a, C-9a, C-10, C-12	H_a-1, H-9a	H_a-1, H-9, H_3-12
H-3	C-4	H-4	H_a-4, H_3-12
H-4	C-3	H-3, H-4a	4-OH
4-OH	-	H-4	H-4
H-4a	C-3, C-4, C-9a	H-4, H-9a	H-3
5-OH	C-5, C-6, C-10a	-	-
H-6	C-5, C-7, C-8, C-10a	-	H_3-11
H-9	C-8, C-8a	H-9a	-
H-9a	-	H_{ab}-1, H-4a, H-9	-
H_3-11	C-7	-	H-6
H_3-12	C-1, C-2, C-3	-	H-3

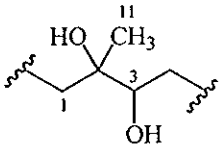
5.3.4 Compound K45

Compound **K45** was obtained as a yellow gum. The UV spectrum displayed maximum absorption bands at 217, 249 and 290 nm. The IR spectrum exhibited absorption bands at 3329 cm^{-1} for a hydroxyl group and 1692 and 1681 cm^{-1} for conjugated ketone carbonyl groups. The ^1H NMR spectrum **(Figure 109)** **(Table 227)** consisted of signals for one chelated hydroxy proton (δ 12.19, *s*, 1H), one aromatic proton (δ 7.40, *s*, 1H), one olefinic proton (δ 6.10, *s*, 1H), two sets of methylene protons [δ 3.10 (*m*, 2H) and 3.03 (*d*, J = 18.5 Hz, 1H)/2.87 (*d*, J = 18.5 Hz, 1H)], one methoxyl group (δ 3.90, *s*, 3H) and one methyl group (δ 1.36, *s*, 3H). The ^{13}C NMR **(Figure 110)** **(Table 227)** and DEPT 135 **(Table 227)** spectra showed nine quaternary (δ 184.65, 184.08, 160.43, 160.25, 143.62, 129.99, 129.50, 112.00 and 70.71), three methine (δ 119.59, 110.34 and 71.95), two methylene (δ 35.83 and 34.34) and two methyl (δ 56.56 and 25.51) carbons. The olefinic proton, H-6 (δ 6.10), showed HMBC cross peaks **(Table 228)** with C-5 (δ 184.08), C-7 (δ 160.25), C-8

(δ 184.65) and C-10a (δ 129.50) while the methoxy protons, H_3-12 (δ 3.90), were correlated with C-7. The location of the methoxyl group at C-7 was supported by signal enhancement of H_3-12 upon irradiation of H-6 in the NOEDIFF experiment **(Table 228)**. These results constructed a *p*-quinone moiety with the methoxyl group at C-7. The chelated hydroxy proton, 9-OH (δ 12.19), showed HMBC cross peaks with C-8a (δ 112.00), C-9 (δ 160.43) and C-9a (δ 129.99). The aromatic proton, H-10 (δ 7.40), gave HMBC cross peaks with C-4a (δ 143.62), C-5, C-8, C-8a and C-9a. Consequently, substructure 1 was established. The methyl protons, H_3-11 (δ 1.36), showed HMBC correlations with C-1 (δ 34.34), C-2 (δ 70.71) and C-3 (δ 71.95) while the methine proton, H-3 (δ 3.90), showed a ^{1}H-^{1}H COSY **(Table 228)** cross peak with H_2-4 (δ3.10). These data constructed substructure 2.



substructure 1



substructure 2

Bond formation between C1-C9a and C4-C4a was established based on HMBC correlations of H_2-1/C-9 and C-9a and H_2-4/C-4a and C-10 (δ 119.59), respectively. Irradiation of H_3-11 in the NOEDIFF experiment, affected signal intensity of H-3, indicating their *cis*-relationship. The observed optical rotation of **K45**, $[\alpha]_D^{25}$ -10 (*c* 0.01, $CHCl_3$), was almost identical to that of (2*S*,3*R*)-nigrosporin A, $[\alpha]_D^{25}$ -10.8 (*c* 0.01, $CHCl_3$), indicating that they had the same configuration at C-2 and C-3. Consequently, **K45** was assigned as (2*S*,3*R*)-nigrosporin A which was previously isolated from *Nigrospora oryzae* (Tanaka, *et al.*, 1997).

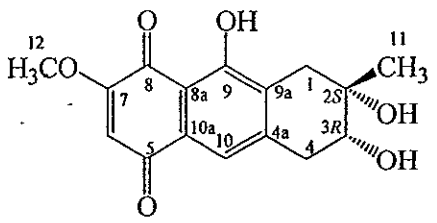


Table 227 The ^{1}H and ^{13}C NMR data of compound **K45** in $CDCl_3$ and **(2*S*,3*R*)-Nigrosporin A** in DMSO-d_6

Position	K45		(2*S*,3*R*)-Nigrosporin A	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	a: 3.03 (*d*, 18.5)	34.34 (CH_2)	a: 2.84 (*d*, 18.0)	35.5 (CH_2)
	b: 2.87 (*d*, 18.5)		b: 2.60 (*d*, 18.0)	
2	-	70.71 (C)	-	69.2 (C)
3	3.90 (*d*, 1.8)	71.95 (CH)	3.63 (*m*)	70.7 (CH)
4	3.10 (*m*)	35.83 (CH_2)	3.97 (*m*)	36.0 (CH_2)
4a	-	143.62 (C)	-	145.5 (C)
5	-	184.08 (C)	-	183.8 (C)
6	6.10 (*s*)	110.34 (CH)	6.30 (*s*)	110.4 (CH)
7	-	160.25 (C)	-	160.2 (C)
8	-	184.65 (C)	-	184.3 (C)
8a	-	112.00 (C)	-	111.3 (C)
9-OH	12.19 (*s*)	160.43 (C)	12.10 (s)	159.2 (C)
9a	-	129.99 (C)	-	130.6 (C)
10	7.40 (*s*)	119.59 (CH)	7.27 (*s*)	118.7 (CH)
10a	-	129.50 (C)	-	128.8 (C)
11	1.36 (*s*)	25.51 (CH_3)	1.21 (*s*)	25.5 (CH_3)
12	3.90 (*s*)	56.56 (CH_3)	3.90 (*s*)	56.7 (CH_3)

Table 228 The HMBC, COSY and NOEDIFF data of compound **K45** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H_a-1	C-2, C-3, C-4a, C-9, C-9a, C-11	H_b-1	-
H_b-1	C-2, C-4a, C-9, C-9a, C-11	H_a-1	-
H-3	-	H_2-4	H_3-11
H_2-4	C-2, C-3, C-4a, C-9a, C-10	H-3	-
H-6	C-5, C-7, C-8, C-10a	-	H_3-12
9-OH	C-8a, C-9, C-9a	-	-

Table 228 Continued

Proton	HMBC	COSY	NOEDIFF
H-10	C-4, C-4a, C-5, C-8, C-8a, C-9a	-	-
H_3-11	C-1, C-2, C-3	-	H-3
H_3-12	C-7	-	H-6

5.3.5 Compound K46

Compound **K46** was obtained as an orange gum. The UV and IR spectra were almost identical to those of **K45**. Their ^{1}H NMR **(Figure 111)** **(Table 229)**, ^{13}C NMR **(Figure 112)** **(Table 229)** and DEPT 135 **(Table 229)** spectra were similar except for the chemical shift of a chelated hydroxy proton. The chelated hydroxy proton, 10-OH (δ 12.65), showed HMBC cross peaks **(Table 230)** with C-4a (δ 130.55), C-10 (δ 159.61) and C-10a (δ 112.00). These established the attachment of the hydroxyl group at C-10 (δ 159.61), not C-9 as found in **K45**. In addition, HMBC cross peaks between the aromatic proton, H-9 (δ 7.43), and C-1 (δ 40.44), C-4a, C-8 (δ 179.50) and C-10a and those of H-6 (δ 6.06) with C-5 (δ 190.59), C-8 and C-10a supported above assignment. Therefore, **K46** was identified as (2*S*,3*R*)-nigrosporin B which was previously isolated from *Nigrospora oryzae* (Tanaka, *et al.*, 1997).

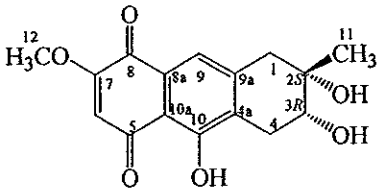


Table 229 The ^{1}H and ^{13}C NMR data of compound **K46** in $CDCl_3$ and **(2*S*,3*R*)-Nigrosporin B** in DMSO-d_6

Position	K46		(2*S*,3*R*)-Nigrosporin B	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	a: 3.15 (*d*, 17.4) b: 2.84 (*d*, 17.4)	40.44 (CH_2)	a: 2.95 (*d*, 17.6) b: 2.77 (*d*, 17.6)	41.2 (CH_2)

Table 229 Continued

Position	K46		(2*S*,3*R*)-Nigrosporin B	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
2	-	70.93 (C)	-	69.1 (C)
3	3.95 (*t*, 3.9)	71.79 (CH)	3.64 (*m*)	70.1 (CH)
4	3.04 (*d*, 3.9)	29.78 (CH_2)	a: 2.87 (*dd*, 18.0, 5.5)	29.3 (CH_2)
			b: 2.73 (*dd*, 18.0, 7.2)	
4a	-	130.55 (C)	-	131.3 (C)
5	-	190.59 (C)	-	190.4 (C)
6	6.06 (*s*)	109.42 (CH)	6.29 (*s*)	108.9 (CH)
7	-	161.11 (C)	-	160.9 (C)
8	-	179.50 (C)	-	178.5 (C)
8a	-	130.55 (C)	-	127.6 (C)
9	7.43 (*s*)	120.24 (CH)	7.26 (*s*)	119.0 (CH)
9a	-	142.79 (C)	-	143.6 (C)
10-OH	12.65 (*s*)	159.61 (C)	12.68 (*s*)	158.0 (C)
10a	-	112.00 (C)	-	110.2 (C)
11	1.31 (*s*)	24.73 (CH_3)	1.15 (*s*)	24.7 (CH_3)
12	3.91 (*s*)	56.60 (CH_3)	3.87 (*s*)	56.4 (CH_3)

Table 230 The HMBC, COSY and NOEDIFF data of compound **K46** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H_a-1	C-2, C-4a, C-9a, C-11	H_b-1	-
H_b-1	C-2, C-4a, C-9a, C-11	H_a-1	-
H-3	C-1, C-4a	H_2-4	H_3-11
H_2-4	C-2, C-4a, C-9a	H-3	-
H-6	C-5, C-7, C-8, C-10a	-	H_3-12
H-9	C-1, C-4a, C-8, C-8a, C-10a	-	-
10-OH	C-4a, C-10, C-10a	-	-
H_3-11	C-1, C-2, C-3	-	H-3
H_3-12	C-7	-	H-6

5.3.6 Compound K47

Compound **K47** was obtained as a red gum. The UV and IR spectra were almost identical to those of **K45**. The ^{1}H NMR spectral data **(Figure 113) (Table 231)** were similar to those of **K45** except for an additional signal of the methyl protons (δ 2.23, *s*, 3H) of an acetoxyl group. In addition, signals for one aromatic proton and two methylene protons were replaced by those of a chelated hydroxy proton (δ 13.51, *s*, 1H) and one oxymethine proton (δ 5.18, *d*, J = 7.2 Hz, 1H), respectively. HMBC correlations **(Table 231)** of the chelated hydroxy proton, 10-OH (δ 13.51), with C-4a (δ 138.43), C-5 (δ 184.77), C-10 (δ 159.55) and C-10a (δ 108.61) indicated the location of the chelated hydroxyl group at C-10. The oxymethine proton (δ 5.18) was assigned as H-4 based on a ^{1}H-^{1}H COSY correlation **(Table 232)** of H-4 with the oxymethine proton, H-3 (δ 5.25, *d*, J = 7.2 Hz, 1H). In addition, H-3 gave a HMBC cross peak with C-13 (δ 170.47) of the acetoxyl group, revealing the attachment of the acetoxyl group at C-3. Irradiation of H-4 did not enhance signal intensity of H-3, indicating their *trans*-relationship. The observed optical rotation of **K47**, $[\alpha]_D^{29}$ -39 (*c* 0.80, acetone), was almost identical to that of (2*S*,3*R*,4*S*)-bostrycin, $[\alpha]_D^{29}$ -30 (*c* 0.80, acetone), indicating that they had the same absolute configuration at all chiral centers. Therefore, **K47** was identified as a monoacetate derivative of (2*S*,3*R*,4*S*)-bostrycin, previously isolated from *Bostrychonema alpestre* (Noda, *et al.*, 1968).

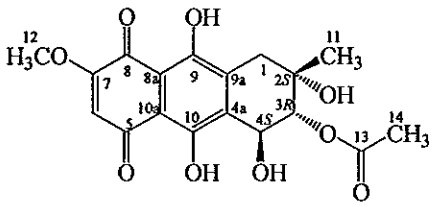


Table 231 The ^{1}H, ^{13}C NMR and HMBC data of compound **K47** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1	a: 3.17 (*d*, 18.6)	36.00 (CH_2)	C-2, C-3, C-4a, C-9, C-9a, C-10, C-11
	b: 2.83 (*d*, 18.6)		
2	-	70.76 (C)	-

Table 231 Continued

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
3	5.25 (*d*, 7.2)	78.06 (CH)	C-1, C-4, C-4a, C-13
4	5.18 (*d*, 7.2)	68.68 (CH)	C-3, C-4a, C-9a, C-10
4a	-	138.43 (C)	-
5	-	184.77 (C)	-
6	6.22 (*s*)	109.67 (CH)	C-5, C-7, C-8, C-10a
7	-	160.96 (C)	-
8	-	178.16 (C)	-
8a	-	110.36 (C)	-
9-OH	12.63 (*s*)	160.80 (C)	C-8, C-8a, C-9, C-9a
9a	-	135.22 (C)	-
10-OH	13.51 (*s*)	159.55 (C)	C-4a, C-5, C-10, C-10a
10a	-	108.61 (C)	-
11	1.40 (*s*)	25.99 (CH_3)	C-1, C-2, C-3
12	3.96 (*s*)	56.88 (CH_3)	C-6, C-7
13	-	170.47 (C)	-
14	2.23 (*s*)	20.99 (CH_3)	C-13

Table 232 The COSY and NOEDIFF data of compound **K47** in $CDCl_3$

Proton	COSY	NOEDIFF
H_a-1	H_b-1	H_a-1, H_3-11
H_b-1	H_a-1	H_b-1, H-3, H_3-11
H-3	H-4	H_b-1, H_3-11
H-4	H-3	10-OH
H-6	-	H_3-12
H_3-11	-	H_{ab}-1, H-3
H_3-12	-	H-6
H_3-14	-	H-3

5.3.7 Compound K48

Compound **K48** was obtained as a red gum. It exhibited UV absorption bands at 218, 253 and 281 nm, revealing the presence of an anthraquinone chromophore (Cai., *et al.*, 2004). Hydroxyl (3338 cm^{-1}) and conjugated ketone carbonyl (1686 and 1681 cm^{-1}) functional groups were found in the IR spectrum. The ^{1}H NMR spectrum **(Figure 115) (Table 233)** displayed signals for two chelated hydroxy protons [δ 13.70 (*s*, 1H) and 13.40 (*s*, 1H)], two *ortho*-coupled aromatic protons [δ 8.17 (*d*, J = 7.8 Hz, 1H) and 7.56 (*d*, J = 7.8 Hz, 1H)], two singlet aromatic protons [δ 8.08 (*s*, 1H) and 6.64 (*s*, 1H)], one methoxyl group (δ 3.90, *s*, 3H) and one methyl group (δ 2.48, *s*, 3H). The ^{13}C NMR **(Figure 116) (Table 233)** and DEPT 135 **(Table 233)** spectra showed fifteen carbon resonances for two anthraquinone carbonyl (δ 188.00 and 184.67), seven quaternary (δ 160.65, 157.50, 150.00, 145.50, 133.50, 131.50 and 111.27), four methine (δ 135.53, 127.31, 127.06 and 106.09) and two methyl (δ 56.65 and 21.94) carbons. One of the chelated hydroxy protons at δ 13.70 was placed at C-8 (δ 160.65) and gave HBMC correlations **(Table 234)** with C-7 (δ 106.09) and C-8 and C-8a (δ 145.50). The methine carbon, C-7, exhibited a HMQC cross peak **(Table 233)** with the singlet aromatic proton at δ 6.64. Therefore, this aromatic proton was attributed to H-7. Irradiation of H-7 in the NOEDIFF experiment **(Table 234)** enhanced signal intensity of the methoxy protons, H_3-11 (δ 3.90), indicating the location of the methoxyl group at C-6 (δ 157.50). A HMBC correlation of H_3-11 with C-6 supported this assignment. The other chelated hydroxy proton (δ 13.40) displayed HMBC cross peaks with C-5 (δ 150.00), C-6 and C-10a (δ 111.27), thus connecting the hydroxyl group at C-5. One of the *ortho*-coupled aromatic protons (δ 8.17) was correlated in the HMBC spectrum with C-8a, C-9 (δ 184.67) and C-9a (δ 133.50) and the other one (δ 7.56) gave the same cross peaks with C-1 (δ 127.06), C-3 (δ 131.50), C-4 (δ 127.31) and C-12 (δ 21.94). Thus, the aromatic protons resonating at δ 8.17 and 7.56 were assigned as H-1 and H-2, respectively. The remaining aromatic proton and the methyl group were then located at C-4 and C-3, respectively. The location of H_3-12 (δ 2.48) was confirmed by its ^{3}J HMBC cross peaks with C-2 (δ 135.53) and C-4. Moreover, this assignment was supported by signal enhancement

of H-2 and H-4 (δ 8.08) upon irradiation of H_3-12 in the NOEDIFF experiment. Therefore, **K48** was identified as austrocortirubin which was previously isolated from the mangrove-derived endophytic fungus *Halorosellinia* sp. (No. 1403) (Xia, *et al.*, 2007).

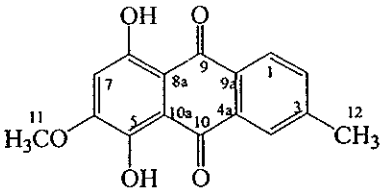


Table 233 The ^{1}H and ^{13}C NMR data of compound **K48** and **Austrocortirubin** in $CDCl_3$

Position	**K48**		**Austrocortirubin**	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	8.17 (*d*, 7.8)	127.06 (CH)	8.23 (*d*, 7.3)	127.1 (CH)
2	7.56 (*d*, 7.8)	135.53 (CH)	7.60 (*d*, 7.3)	139.5 (CH)
3	-	131.50 (C)	-	145.1 (C)
4	8.08 (*s*)	127.31 (CH)	8.15 (*s*)	127.3 (CH)
4a	-	131.50 (C)	-	131.8 (C)
5-OH	13.40 (*s*)	150.00 (C)	13.47 (*s*)	150.2 (C)
6	-	157.50 (C)	-	157.5 (C)
7	6.64 (*s*)	106.09 (CH)	6.71 (*s*)	106.9 (CH)
8-OH	13.70 (*s*)	160.65 (C)	13.59 (*s*)	160.6 (C)
8a	-	145.50 (C)	-	106.2 (C)
9	-	184.67 (C)	-	184.7 (C)
9a	-	133.50 (C)	-	133.2 (C)
10	-	188.00 (C)	-	187.6 (C)
10a	-	111.27 (C)	-	112.7 (C)
11	3.90 (*s*)	56.65 (CH_3)	3.92 (*s*)	56.6 (CH_3)
12	2.48 (*s*)	21.94 (CH_3)	2.20 (*s*)	21.9 (CH_3)

Table 234 The HMBC, COSY and NOEDIFF data of compound **K48** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-1	C-9, C-9a	H-2	H-2
H-2	C-1, C-3, C-4, C-12	H-1	H-1, H_3-12
H-4	C-2, C-4a, C-10, C-12	-	H_3-12
5-OH	C-5, C-6, C-10a	-	-
H-7	C-5, C-6, C-8	-	H-8, H_3-11
8-OH	C-7, C-8, C-8a	-	-
H_3-11	C-6	-	H-7
H_3-12	C-2, C-4	-	H-2, H-4

5.3.8 Compound K49

Compound **K49** was obtained as a colorless gum. The UV spectrum showed characteristic absorption bands of a conjugated chromophore at 211, 221, 240, 247 and 290 nm (Kashiwada, *et al.*, 1984). Its IR spectrum displayed absorption bands at 3310 and 1686 cm^{-1} for hydroxyl and conjugated ketone carbonyl functional groups, respectively. The ^1H NMR spectrum **(Figure 117) (Table 235)** consisted of signals for a 2-hydroxypropyl unit [δ 4.23 (*m*, 1H), 2.65 (*d*, J = 5.7 Hz, 2H) and 1.31 (*d*, J = 6.3 Hz, 3H), two *meta*-coupled aromatic protons [δ 6.60 (*brs*, 1H) and 6.57 (*brs*, 1H)], one olefinic proton (δ 6.04, *s*, 1H) and one methyl group (δ 2.70, *s*, 3H). The ^{13}C NMR **(Figure 118) (Table 235)** and DEPT 135 **(Table 235)** spectra showed one ketone carbonyl (δ 180.34), five quaternary (δ 164.60, 160.81, 159.81, 142.64 and 115.07), four methine (δ 116.89, 111.80, 100.77 and 65.39), one methylene (δ 43.31) and two methyl (δ 23.12 and 22.79) carbons. The methyl protons, H_3-9 (δ 2.70), showed HMBC cross peaks **(Table 236)** with C-4a (δ 115.07), C-5 (δ 142.64) and C-6 (δ 116.89). One of the *meta*-coupled aromatic protons, H-6 (δ 6.57), was correlated with C-6 in the HMQC spectrum **(Table 235)**. In addition, the other *meta*-coupled aromatic proton exhibited HMBC cross peaks with C-4a, C-6, C-7 (δ 159.81) and C-8a (δ 160.81). The aromatic protons at δ 6.60 and 6.57 were then

assigned as H-8 and H-6, respectively. The chemical shift of C-7 established the substituent to be a hydroxyl group. The olefinic proton, H-3 (δ 6.04), showed HMBC cross peaks with C-2 (δ 164.60), C-4 (δ 180.43) and C-4a. An ether linkage between C-2 (δ 164.60) and C-8a was formed on the basis of the chemical shifts of C-2 and C-8a and the UV data. In addition, the methylene protons, H_2-10 (δ 2.65), of the 2-hydroxypropyl unit gave HMBC correlations with C-2 and C-3 (δ 111.80), thus connecting this unit at C-2. The observed optical rotation of **K49**, $[\alpha]_D^{25}$ +37 (*c* 0.89, MeOH), was almost identical to that of 2-(2′*S*-hydroxypropyl)-5-methyl-7-hydroxychromone, $[\alpha]_D^{25}$ +38.4 (*c* 0.89, MeOH), suggesting that C-11 in **K49** also had *s*-configuration. Therefore, **K49** was identified as 2-(2′*S*-hydroxypropyl)-5-methyl-7-hydroxychromone which was previously isolated from rhubarb (*Rhei rhizoma*) (Kashiwada, *et al.*, 1984).

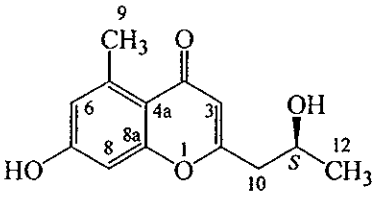


Table 235 The ^{1}H and ^{13}C NMR data of compound **K49** in $CDCl_3$+CD_3OD and **2-(2′*S* Hydroxypropyl)-5-methyl-7-hydroxychromone** in DMSO-d_6

Position	K49		2-(2′*S*-Hydroxypropyl)-5-methyl-7-hydroxychromone	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
2	-	164.60 (C)	-	164.9 (C)
3	6.04 (*s*)	111.80 (CH)	5.97 (*s*)	116.4 (CH)
4	-	180.34 (C)	-	178.9 (C)
4a	-	115.07 (C)	-	114.4 (C)
5	-	142.64 (C)	-	141.5 (C)
6	6.57 (*brs*)	116.89 (CH)	6.61 (*s*)	111.4 (CH)
7	-	159.81 (C)	-	160.6 (C)
8	6.60 (*brs*)	100.77 (CH)	6.61 (*s*)	100.4 (CH)
8a	-	160.81 (C)	-	159.1 (C)

Table 235 Continued

Position	K49		2-(2′*S*-Hydroxypropyl)-5-methyl-7-hydroxychromone	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
9	2.70 (*s*)	22.79 (CH_3)	2.66 (*s*)	22.3 (CH_3)
10	2.65 (*d*, 5.7)	43.31 (CH_2)	2.58 (*d*, 7.0)	42.7 (CH_2)
11	4.23 (*m*)	65.39 (CH)	4.02 (*m*)	64.0 (CH)
12	1.31 (*d*, 6.3)	23.12 (CH_3)	1.15 (*d*, 7.0)	23.2 (CH_3)

Table 236 The HMBC, COSY, and NOEDIFF data of compound **K49** in $CDCl_3$+CD_3OD

Proton	HMBC	COSY	NOEDIFF
H-3	C-2, C-4, C-4a, C-10	-	H_2-10
H-6	C-4a	H-8	H_3-9
H-8	C-4a, C-6, C-7, C-8a	H-6	-
H_3-9	C-4a, C-5, C-6	-	H-6
H_2-10	C-2, C-3, C-11	H-11	H-3
H-11	-	H_2-10, H_3-12	-
H_3-12	C-10, C-11	H-11	-

5.3.9 Compound K50

Compound **K50** was obtained as a colorless gum. Its UV and IR data were similar to those of **K2**. Their ^{1}H NMR spectra were also similar except for the replacement of the (1*Z*,3*E*)-1-carboxyl-2-methyl-1,3-butadienyl signals in **K2** with signals of a *E*-2-oxobutenyl unit [δ 6.76 (*d*, J = 15.5 Hz, 1H), 6.40 (*d*, J = 15.5 Hz, 1H) and 2.24 (*s*, 3H)] **(Figure 119) (Table 237)**. The presence of one ketone carbonyl carbon (δ 196.77) and two methine carbons (δ 144.87 and 130.37) in the ^{13}C NMR **(Figure 120) (Table 237)** and DEPT 135 **(Table 237)** spectra supported above

conclusion. HMBC correlations of H-7 (δ6.76) with C-5 (δ160.09) and C-6 (δ79.32) established the linkage of the oxobutenyl unit at C-6 of the cyclohexenone ring. Irradiation of H-7 of the butenyl unit in the NOEDIFF experiment **(Table 238)** affected signal intensity of H_3-12 (δ0.96), indicating *cis*-relationship of H_3-12 and the butenyl unit. The observed rotation of **K50**, $[\alpha]_D^{29}$+133 (*c* 0.42, MeOH), was similar to that of (6*S*)-(+)-dehydrovomifoliol, $[\alpha]_D^{29}$ +139 (*c* 0.42, MeOH), indicating that they had the same configuration. Therefore, **K50** was (6*S*)-(+)-dehydrovomifoliol which was previously isolated from the leaves of *Cucumis sativus* (Kai, *et al.*, 2007).

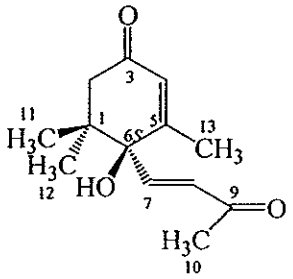


Table 237 The ^{1}H and ^{13}C NMR data of compound **K50** in $CDCl_3$ and **(6*S*)-(+)-Dehydrovomifoliol** in CD_3OD

Position	K50		(6*S*)-(+)-Dehydrovomifoliol	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	-	41.41 (C)	-	42.6 (C)
2	a: 2.44 (*d*, 17.0)	49.58 (CH_2)	a: 2.59 (*d*, 18.3)	50.4 (CH_2)
	b: 2.37 (*d*, 17.0)		b: 2.28 (*d*, 18.3)	
3	-	197.19 (C)	-	199.9 (C)
4	5.89 (*q*, 1.5)	127.85 (CH)	5.93 (*s*)	127.7 (CH)
5	-	160.09 (C)	-	164.2 (C)
6	-	79.32 (C)	-	79.8 (C)
7	6.76 (*d*, 15.5)	144.87 (CH)	6.99 (*d*, 15.7)	148.0 (CH)
8	6.40 (*d*, 15.5)	130.37 (CH)	6.43 (*d*, 15.7)	131.4 (CH)
9	-	196.77 (C)	-	200.1 (C)
10	2.24 (*s*)	28.42 (CH_3)	2.30 (*s*)	27.6 (CH_3)
11	1.04 (*s*)	22.93 (CH_3)	1.06 (*s*)	23.5 (CH_3)
12	0.96 (*s*)	24.34 (CH_3)	1.01 (*s*)	24.7 (CH_3)
13	1.82 (*d*, 1.5)	18.64 (CH_3)	1.89 (*d*, 1.5)	19.1 (CH_3)

Table 238 The HMBC, COSY and NOEDIFF data of compound **K50** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H_a-2	C-1, C-3, C-6, C-11, C-12	H_b-2	-
H_b-2	C-3, C-4, C-6, C-11, C-12	H_a-2	-
H-4	C-2, C-6, C-13	H_3-13	H_3-13
H-7	C-5, C-6, C-8, C-9	H-8	H_3-10, H_3-12
H-8	C-6, C-7, C-9, C-10	H-7	H_3-10
H_3-10	C-8, C-9	-	-
H_3-11	C-1, C-2, C-6, C-12	-	-
H_3-12	C-1, C-2, C-3, C-6, C-11	-	H-7
H_3-13	C-4, C-5, C-6	H-4	H-4

5.3.10 Compound K51

Compound **K51** was obtained as a colorless gum. It exhibited UV spectrum absorption bands at 203 and 260 nm while hydroxyl and carbonyl absorption bands at 3349 and 1686 cm^{-1}, respectively, were observed in the IR spectrum. The ^1H NMR spectrum **(Figure 121) (Table 239)** showed characteristic signals for a cytosine moiety [δ 8.01 (*d*, *J* = 8.1 Hz, 1H) and 5.70 (*d*, *J* = 8.1 Hz, 1H)] and a ribose unit [δ 5.90 (*d*, *J* = 5.1 Hz, 1H), 4.18 (*t*, *J* = 5.1 Hz, 1H), 4.14 (*t*, *J* = 5.1 Hz, 1H), 4.00 (*m*, 1H), 3.84 (*dd*, *J* = 12.3 and 2.7 Hz, 1H) and 3.73 (*dd*, *J* = 12.3 and 2.7 Hz, 1H)]. The ^{13}C NMR **(Figure 122) (Table 239)** and DEPT 135 **(Table 239)** spectra showed nine carbon resonances for two quaternary (δ 164.77 and 151.00), six methine (δ 141.32, 101.25, 89.31, 84.96, 74.31 and 69.90) and one methylene (δ 60.87) carbons. The HMBC correlations **(Table 239)** from H-7 (δ 5.90) of the ribose unit with C-3 (δ 151.00) and C-5 (δ 141.32) of the cytosine moiety formed a bond between C-7 of the ribose unit with N-4 of the cytosine unit. Therefore, **K51** was identified as uridine (Rukachaisirikul, *et al.*, 2008).

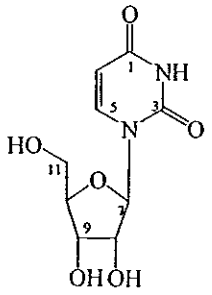


Table 239 The NMR data of compound **K51** in CD_3OD

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC	COSY	NOEDIFF
1	-	164.77 (C)	-		
3	-	151.00 (C)	-		
5	8.01 (*d*, 8.1)	141.32 (CH)	C-1, C-3, C-6, C-7	H-6	H-6
6	5.70 (*d*, 8.1)	101.25 (CH)	C-1, C-5	H-5	H-5
7	5.90 (*d*, 5.1)	89.31 (CH)	C-3, C-5, C-8, C-9	H-8	
8	4.18 (*t*, 5.1)	74.31 (CH)	C-10	H-7, H-9	H-9
9	4.14 (*t*, 5.1)	69.90 (CH)	C-7, C-11	H-8, H-10	H-8
10	4.00 (*m*)	84.96 (CH)	C-9	H-9, H_{ab}-11	
11	a: 3.84 (*dd*, 12.3, 2.7)	60.87 (CH_2)	C-9, C-10	H-10, H_b-11	
	b: 3.73 (*dd*, 12.3, 2.7)			H-10, H_a-11	

PART VI

METABOLITES FROM THE MARINE-DERIVED FUNGUS *CURVULARIA* SP. PSU-F22

CHAPTER 6.1

INTRODUCTION

6.1.1 Introduction

The genus *Curvularia* is a rich source of biologically active secondary metabolites. Fungal metabolites isolated from the genus *Curvularia* are summarized in **Table 240** based on SciFinder Scholar Database. The marine-derived fungus *Curvularia* sp. PSU-F22 was isolated from the sea fan *Annella* sp., collected in Phangnga Province, Thailand. This fungus was deposited as PSU-F22 at the Department of Microbiology, Faculty of Science, Prince of Songkla University.

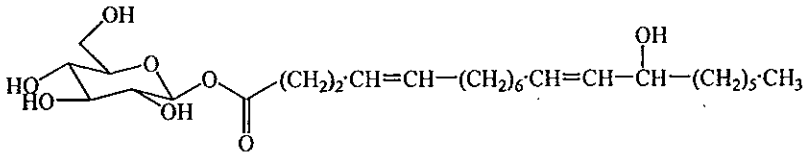
Table 240 Compounds isolated from the *Curvularia* genus

Scientific name	Compound	Activity	Reference
C. andropogonis	1-*O*-*β*-D-(14-hydroxy-4,12-eicosadienoyl)glucoside, **17**	Phytotoxic	Alam, *et al.*, 1997
C. inaequalis	Pyrenocine A, **18** Pyrenocine B, **19**	Phytotoxic	Kim, *et al.*, 2000
C. lunata	Lunatin, **20** Cytoskyrin A, **21** (+)-Abscisic acid, **22**	Antibacterial	Jadulco, *et al.*, 2002
	Methyl 2-acetyl-3,5-dihydroxyphenylacetate, **23** Curvulinic acid, **24** Methyl 2-acetyl-5-hydroxy-3-methoxyphenylacetate, **25** Curvulin, **26** 4-Epiradicinol, **27**	Antibacterial	Varma, *et al.*, 2006

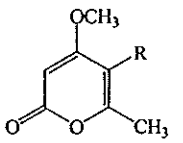
Table 240 Continued

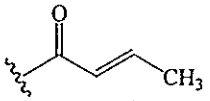
Scientific name	Compound	Activity	Reference
Curvularia sp.	2-Methyl-5-methoxybenzopyran-4-one, **28** 2-(Propan-2′-ol)-5-hydroxy-benzopyran-4-one, **29** (*2R*)-2,3-Dihydro-2-methyl-5-methoxybenzopyran-4-one, **30** (*2R,4R*)-2,3-Dihydro-2-methylbenzopyran-4,5-diol, **31**	Antifungal	Teles, *et al.*, 2005

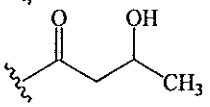
Structures of compounds isolated from the *Curvularia* genus

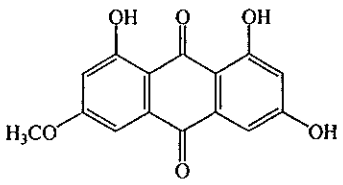


17: 1-*O*-*β*-D-(14-hydroxy-4,12-eicosadienoyl)glucoside

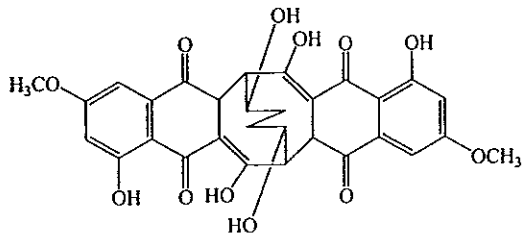


18: R =  : Pyrenocine A

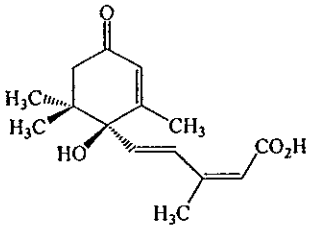
19: R =  : Pyrenocine B



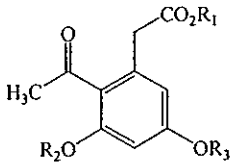
20: Lunatin



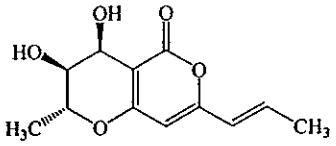
21: Cytoskyrin A



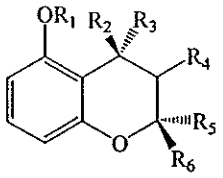
22: (+)-Abscisic acid



23: $R_1 = CH_3$, $R_2 = R_3 = H$: Methyl 2-acetyl-3,5-dihydroxyphenylacetate
24: $R_1 = R_2 = R_3 = H$: Curvulinic acid
25: $R_1 = R_2 = CH_3 = R_3 = H$: Methyl 2-acetyl-5-hydroxy-3-methoxyphenylacetate
26: $R_1 = CH_2CH_3$, $R_2 = R_3 = H$: Curvulin



27: 4-Epiracidinol



28: $R_1 = CH_3$, $R_2+R_3 = O$, R_4+R_5 = double bond, $R_6 = CH_3$
: 2-Methyl-5-methoxybenzopyran-4-one
29: $R_1 = H$, $R_2+R_3 = O$, R_4+R_5 = double bond,
R_6 = (*2R*)-2-hydroxypropyl
: 2-(Propan-2′-ol)-5-hydroxybenzopyran-4-one
30: $R_1 = R_6 = CH_3$, $R_2+R_3 = O$, $R_4 = R_5 = H$
: (*2R*)-2,3-Dihydro-2-methyl-5-methoxybenzopyran-4-one
31: $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = OH$, $R_6 = CH_3$
: (*2R,4R*)-2,3-Dihydro-2-methylbenzopyran-4,5-diol

6.1.2 The objectives

1. To isolate the secondary metabolites from the marine-derived fungus *Curvularia* sp. PSU-F22.

2. To elucidate the structure of the isolated metabolites.

CHAPTER 6.2

EXPERIMENTAL

6.2.1 Fermentation and extraction

The fermentation and the extraction of the culture broth (15 L) and mycelia were performed using the same procedure as those of *Nigrospora* sp. PSU-F5 to afford both crude extracts as a brown gum in 1.30 g and 380 mg from the culture broth and mycelia, respectively. Each extract was subjected to chromatographic separation.

6.2.2 Purification of the broth extract

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six fractions as shown in **Table 241.**

Table 241 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
22BA	35.5	Brown gum
22BB	144.8	Brown gum
22BC	1,065	Brown gum
22BD	60.1	Brown gum
22BE	16.6	Brown gum
22BF	2.4	Brown gum

Fraction 22BA displayed a long tail under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Fraction 22BB showed seven UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.12, 0.21, 0.30, 0.37, 0.47, 0.65 and 0.81. It was further separated by column chromatography over silica gel. Elution was performed initially with 20% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 242.**

Table 242 Subfractions obtained from **fraction 22BB** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
BB1	20% EtOAc/Petrol	6.3	Yellow gum
BB2	30% EtOAc/Petrol	4.0	Yellow gum
BB3	30% EtOAc/Petrol	15.8	Yellow gum
BB4	50-70% EtOAc/Petrol	31.6	Yellow gum
BB5	100% EtOAc	30.1	Yellow gum
BB6	100% EtOAc- 2% MeOH/EtOAc	11.3	Yellow gum
BB7	2-10% MeOH/EtOAc	9.3	Yellow gum
BB8	20% MeOH/EtOAc- 100% MeOH	50.2	Yellow gum

Subfraction BB1 displayed a long tail under UV-S on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction BB2 showed one pale UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.74. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction BB3 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.40, 0.51 and 0.74. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 243.**

Table 243 Subfractions obtained from **subfraction BB3** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
BB31	4.0	Colorless gum
BB32	4.2	Colorless gum
BB33	2.2	Colorless gum
BB34	4.1	Yellow gum

Subfraction BB31 showed none of UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase but showed three gray spots with the R_f values of 0.13, 0.23 and 0.40 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BB32 (K56) showed one gray spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.38 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate.

$[\alpha]_D^{29}$	+40 (*c* 0.25, MeOH)
UVλ_{max}(nm)(MeOH)(log ε)	207 (3.22)
FTIR(neat):υ(cm^{-1})	3416 (O-H stretching), 1698 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	5.88 (*dd*, *J* = 12.6, 2.4 Hz, 1H), 5.87 (*dd*, *J* = 12.6, 1.2 Hz, 1H), 5.69 (*ddd*, *J* = 15.6, 11.1, 3.6 Hz, 1H), 5.54 (*ddd*, *J* = 15.6, 7.8, 1.5 Hz, 1H), 5.32 (*m*, 1H), 4.75 (*brd*, *J* = 6.9 Hz, 1H), 2.39 (*dm*, *J* = 13.5 Hz, 1H), 2.20 (*m*, 1H), 1.68 (*m*, 2H), 1.22 (*d*, *J* = 6.6 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	168.86, 136.47, 135.43, 129.09, 122.58, 72.43, 71.79, 33.88, 30.96, 21.49
DEPT 135: CH;	136.47, 135.43, 129.09, 122.58, 72.43, 71.79
CH_2;	33.88, 30.96
CH_3;	21.49

Subfraction BB33 showed two spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.38 (gray) and 0.45 (pink) after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K56**. Further investigation was then not performed.

Subfraction BB34 displayed a long tail under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction BB4 showed four UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.23, 0.41, 0.46 and 0.53. It was further separated by column chromatography over silica gel. Elution was performed initially with 30% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol.

Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 244**.

Table 244 Subfractions obtained from **subfraction BB4** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
BB41	30% EtOAc/Petrol	3.4	Colorless gum
BB42	50% EtOAc/Petrol	2.4	Colorless gum
BB43	70% EtOAc/Petrol-100% EtOAc	17.6	Colorless gum
BB44	5% MeOH/EtOAc-100% MeOH	4.3	Colorless gum

Subfraction BB41 displayed a long tail under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction BB42 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.30, 0.40 and 0.47. Because of the minute quantity, it was not further investigated.

Subfraction BB43 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.16, 0.21 and 0.37. It was further separated by column chromatography over silica gel. Elution was performed initially with 20% acetone in petroleum ether and gradually enriched with acetone and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 245**.

Table 245 Subfractions obtained from **subfraction BB43** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
BB43A	20% Acetone/Petrol	1.2	Colorless gum
BB43B	30% Acetone/Petrol	3.8	Colorless gum
BB43C	70% Acetone/Petrol-3% MeOH/Acetone	9.5	Colorless gum
BB43D	5% MeOH/Acetone-100% MeOH	4.0	Colorless gum

Subfraction BB43A (K58) showed one UV-active spot on normal phase TLC using 30% acetone in petroleum ether as a mobile phase with the R_f value of 0.42.

$[\alpha]_D^{29}$	-257 (*c* 0.67, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	204 (2.86), 221 (2.41), 262 (2.06)
FTIR(neat):υ(cm^{-1})	1700 and 1686 (C=O stretching), 1619 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	6.75 (*dd*, *J* = 17.0, 6.5 Hz, 1H), 6.63 (*d*, *J* = 12.5 Hz, 1H), 6.20 (*dd*, *J* = 17.0, 1.0 Hz, 1H), 6.14 (*d*, *J* = 12.5 Hz, 1H), 5.58 (*qd*, *J* = 7.0, 2.0 Hz, 1H), 3.82 (*ddd*, *J* = 6.5, 4.5, 1.5 Hz, 1H), 3.25 (*dd*, *J* = 4.5, 2.0 Hz, 1H), 1.52 (*d*, *J* = 7.0 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	192.90, 166.63, 145.70, 134.58, 133.52, 128.71, 66.19, 60.88, 54.76, 16.79
DEPT 135: CH;	145.70, 134.58, 133.52, 128.71, 66.19, 60.88, 54.76
CH_3;	16.79

Subfraction BB43B showed three UV-active spots on normal phase TLC using 30% acetone in petroleum ether as a mobile phase with the R_f values of 0.28, 0.37 and 0.40. Because of low quantity, it was not further investigated.

Subfraction BB43C showed one UV-active spot on normal phase TLC using 30% acetone in petroleum ether as a mobile phase with the R_f value of 0.37 and one gray spot with the R_f value of 0.19 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Further investigation was then not performed.

Subfraction BB43D showed one pale UV-active spot on normal phase TLC using 30% acetone in petroleum ether as a mobile phase with the R_f value of 0.22. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction BB44 showed three pale UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.27, 0.36 and 0.41. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction BB5 showed five UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.23, 0.37, 0.55, 0.62 and 0.67. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 246.**

Table 246 Subfractions obtained from **subfraction BB5** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
BB51	2% MeOH/CH_2Cl_2	4.5	Colorless gum
BB52	2% MeOH/CH_2Cl_2	3.0	Colorless gum
BB53	4% MeOH/CH_2Cl_2	3.7	Colorless gum
BB54	4-8% MeOH/CH_2Cl_2	5.0	Colorless gum
BB55	10% MeOH/CH_2Cl_2	6.7	Colorless gum
BB56	20% MeOH/CH_2Cl_2-100% MeOH	5.3	Yellow gum

Subfraction BB51 showed none of UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase but showed many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction BB52 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.33 and one pink spot with the R_f value of 0.13 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K52**. Further investigation was then not performed.

Subfraction BB53 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.31 and 0.33 and one pink spot with the R_f value of 0.38 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K52**. Further investigation was then not performed.

Subfraction BB54 showed none of UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase but showed many spots after

dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BB55 showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.17 and 0.24 and one yellow spot with the R_f value of 0.12 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BB56 displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction BB6 showed three gray spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.11, 0.18 and 0.30 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K55**. Further investigation was then not performed.

Subfraction BB7 showed none of UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase but showed many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BB8 displayed a long tail under UV-S on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Fraction 22BC showed five UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.12, 0.21, 0.30, 0.37 and 0.81. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar

chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 247**.

Table 247 Subfractions obtained from **fraction 22BC** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
BC1	15.5	Yellow gum
BC2	23.3	Yellow gum
BC3	164.6	Yellow gum
BC4	423.8	Yellow gum
BC5	361.2	Yellow gum
BC6	66.4	Yellow gum
BC7	3.6	Yellow gum

Subfraction BC1 showed none of major spots under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction BC2 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.15, 0.21 and 0.39. It was further separated by column chromatography over silica gel. Elution was performed initially with 4% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 248**.

Table 248 Subfractions obtained from **subfraction BC2** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
BC21	4% MeOH/CH_2Cl_2	5.5	Colorless gum

Table 248 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
BC22	4% MeOH/CH_2Cl_2	3.0	Colorless gum
BC23	4% MeOH/CH_2Cl_2	3.1	Colorless gum
BC24	7-15% MeOH/CH_2Cl_2	4.0	Colorless gum
BC25	15% MeOH/CH_2Cl_2	1.9	Colorless gum
BC26	20% MeOH/CH_2Cl_2-100% MeOH	4.8	Yellow gum

Subfraction BC21 showed none of UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase but showed many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BC22 showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.27 and 0.34 and one purple spot with the R_f value of 0.19 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction BC23 showed one purple spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.10 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated the presence of **K55**. Further investigation was then not performed.

Subfraction BC24 displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction BC25 showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.22. Its ^1H

NMR spectrum indicated the presence of **K53**. Further investigation was then not performed.

Subfraction BC26 displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction BC3 showed four UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.08, 0.13, 0.22 and 0.39 and two yellow spots with the R_f values of 0.36 and 0.56 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was further separated by column chromatography over silica gel. Elution was performed initially with 30% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford twelve subfractions as shown in **Table 249**.

Table 249 Subfractions obtained from **subfraction BC3** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
BC31	30% EtOAc/Petrol	10.5	Colorless gum
BC32	30% EtOAc/Petrol	8.4	Colorless gum
BC33	30% EtOAc/Petrol	3.5	Colorless gum
BC34	30% EtOAc/Petrol	6.1	Colorless gum
BC35	40% EtOAc/Petrol	20.0	Colorless gum
BC36	40% EtOAc/Petrol	12.5	Colorless gum
BC37	50-70% EtOAc/Petrol	9.0	Colorless gum
BC38	70% EtOAc/Petrol	6.3	Colorless gum
BC39	100% EtOAc	16.3	Colorless gum
BC310	5% MeOH/EtOAc	18.0	Colorless gum
BC311	5-20% MeOH/EtOAc	35.7	Colorless gum
BC312	40% MeOH/EtOAc-100% MeOH	2.8	Yellow gum

Subfraction BC31 showed none of UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase but showed many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BC32 showed one purple spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.33 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated the presence of **K56**. Further investigation was then not carried out.

Subfraction BC33 showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.25 and one purple spot with the R_f value of 0.30 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Subfraction BC34 showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.25 and two purple spots with the R_f values of 0.18 and 0.33 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was then purified by precoated TLC with 30% ethyl acetate in petroleum ether as a mobile phase (3 runs) to afford two bands.

Band 1 was a colorless gum (2.0 mg). Its chromatogram showed one purple spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.51 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 (K57) was a colorless gum (4.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.43.

$[\alpha]_D^{29}$	-180 (*c* 0.20, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	207 (3.37), 253 (2.89)
FTIR(neat):υ(cm^{-1})	3422 (O-H stretching), 1700 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	6.62 (*ddd*, *J* = 12.0, 3.0, 1.0 Hz, 1H), 6.12 (*dd*, *J* = 15.0, 1.0 Hz, 1H), 5.85 (*dd*, *J* = 12.0, 3.0 Hz, 1H), 5.74 (*ddd*, *J* = 15.0, 9.0, 3.0 Hz, 1H), 4.98 (*qnd*, *J* = 6.0, 3.0 Hz, 1H), 4.24 (*td*, *J* = 9.0, 3.0 Hz, 1H), 2.09 (*ddd*, *J* = 15.0, 9.0, 6.0 Hz, 1H), 1.84 (*ddd*, *J* = 15.0, 9.0, 3.0 Hz, 1H), 1.75 (*m*, 1H), 1.62 (*m*, 1H), 1.12 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	168.27, 140.17, 139.61, 126.64, 125.62, 73.67, 73.27, 37.37, 30.38, 21.36
DEPT 135: CH;	140.17, 139.61, 126.64, 125.62, 73.67, 73.27
CH_2;	37.37, 30.38
CH_3;	21.36

Subfraction BC35 showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.43 and two yellow spots with the R_f values of 0.20 and 0.30 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction BC36 showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.20 and two yellow spots with the R_f values of 0.30 and 0.35 after dipping the TLC plate in

anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K52**. Further investigation was then not performed.

Subfraction BC37 showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.20 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K52**. Further investigation was then not further performed.

Subfraction BC38 displayed a long tail under UV-S on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction BC39 showed two UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.08 and 0.10. Its ^{1}H NMR spectrum indicated that the major compound was **K55**. Further investigation was then not performed.

Subfraction BC310 showed two UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.09 and 0.11. It was further separated by column chromatography over silica gel. Elution was performed with 4% methanol in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 250**.

Table 250 Subfractions obtained from **subfraction BC310** by column chromatograph over silica gel

Subfraction	Weight (mg)	Physical appearance
BC310A	4.1	Colorless gum
BC310B	4.6	Colorless gum
BC310C	5.6	Colorless gum

Subfraction BC310A showed one UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.34 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Further investigation was then not performed.

Subfraction BC310B showed two purple spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.19 and 0.21 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K55**. Further investigation was then not performed.

Subfraction BC310C showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.15 and one purple spot with the R_f value of 0.17 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was then purified by precoated TLC with 4% methanol in dichloromethane as a mobile phase (7 runs) to afford a colorless gum (2.0 mg). Its chromatogram showed one purple spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.17 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction BC311 showed two UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.25 and 0.40. It was further separated by column chromatography over silica gel. Elution was performed with 3% methanol in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 251**.

Table 251 Subfractions obtained from **subfraction BC311** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
BC311A	4.2	Colorless gum
BC311B	4.4	Colorless gum
BC311C	25.7	Yellow gum

Subfraction BC311A showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.40, 0.63 and 0.83 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BC311B showed three spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.08 (yellow), 0.13 (purple) and 0.20 (yellow) after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BC311C showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.23 and 0.40. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 252.**

Table 252 Subfractions obtained from **subfraction BC311C** by column chromatography over Sephadex LH-20

Subfraction	Elution	Weight (mg)
BC311C1	7.8	Yellow gum

Table 252 Continued

Subfraction	Weight (mg)	Physical appearance
BC311C2	11.4	Yellow gum
BC311C3	6.3	Yellow gum

Subfraction BC311C1 showed one pale UV-active spot on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f value of 0.14. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction BC311C2 showed two UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.17 and 0.23. It was then purified by precoated TLC with 2% methanol in dichloromethane as a mobile phase (8 runs) to afford two bands.

Band 1 was a colorless gum (2.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.11. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 (K53) was a colorless gum (5.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.18.

$[\alpha]_D^{29}$	+112 (*c* 0.30, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	207 (2.90), 257 (2.27)
FTIR(neat):υ(cm^{-1})	3422 (O-H stretching), 1703 (C=O stretching)
^1H NMR(CD_3OD)(δ_{ppm})(300 MHz):	7.28 (*dd*, *J* = 15.3, 11.4 Hz, 1H), 6.36 (*t*, *J* = 11.4 Hz, 1H), 5.94 (*dt*, *J* = 15.3, 6.9 Hz, 1H), 5.62 (*d*, *J* = 11.4 Hz, 1H), 3.75 (*sextet*,

	J = 6.3 Hz, 1H), 2.18 (*brd*, J = 6.9 Hz, 2H), 1.51 (*m*, 2H), 1.48 (*m*, 2H), 1.16 (*d*, J = 6.3 Hz, 3H)
^{13}C NMR(CD_3OD)(δ_{ppm})(75 MHz):	173.00, 140.41, 139.33, 127.78, 121.74, 66.98, 38.30, 32.44, 24.94, 22.05
DEPT 135: CH;	140.41, 139.33, 127.78, 121.74, 66.98
CH_2;	38.30, 32.44, 24.94
CH_3;	22.05
EIMS *m/z* (% relative intensity):	184 (2), 151 (8), 124 (28), 79 (100), 67 (19)

Subfraction BC311C3 displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction BC312 displayed a long tail under UV-S on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction BC4 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.08, 0.15 and 0.32 and three yellow spots with the R_f values of 0.22, 0.36 and 0.56 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was further separated by column chromatography over silica gel. Elution was performed initially with 30% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford twelve subfractions as shown in **Table 253**.

Table 253 Subfractions obtained from **subfraction BC4** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
BC41	30% EtOAc/Petrol	4.1	Yellow gum
BC42	30% EtOAc/Petrol	17.8	Yellow gum

Table 253 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
BC43	30% EtOAc/Petrol	10.6	Yellow gum
BC44	30% EtOAc/Petrol	196.7	Yellow gum
BC45	30% EtOAc/Petrol	41.1	Yellow gum
BC46	50% EtOAc/Petrol	20.1	Yellow gum
BC47	50% EtOAc/Petrol	16.2	Yellow gum
BC48	70% EtOAc/Petrol	7.0	Yellow gum
BC49	70% EtOAc/Petrol	36.7	Yellow gum
BC410	70% EtOAc/Petrol-100% EtOAc	12.5	Yellow gum
BC411	2-10% MeOH/EtOAc	5.8	Yellow gum
BC412	20% MeOH/EtOAc - 100% MeOH	44.5	Yellow gum

Subfraction BC41 showed none of UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase but showed many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BC42 showed one gray spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.53 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated the presence of **K56**. Further investigation was then not carried out.

Subfraction BC43 showed two UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.46 and 0.55. Its ^{1}H NMR spectrum indicated that the major compound was **K57**. Further investigation was then not carried out.

Subfraction BC44 showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.46 and two yellow spots with the R_f values of 0.37 and 0.44 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction BC45 showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.23 and three spots with the R_f values of 0.30 (pink), 0.37 (yellow) and 0.44 (yellow) after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because the ^1H NMR spectrum indicated the presence of a mixture of **K52** and **K56**, it was not investigated.

Subfraction BC46 showed one UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.30 and two yellow spots with the R_f values of 0.47 and 0.53 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated that the major compound was **K52**. Further investigation was then not performed.

Subfraction BC47 showed two UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.23 and 0.30 and one yellow spot with the R_f value of 0.23 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated that the major compound was **K52**. Further investigation was then not performed.

Subfraction BC48 showed one UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.37 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Thus, it was not investigated.

Subfraction BC49 showed two gray spots on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.12 and 0.14 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^1H NMR spectrum indicated that the major compound was **K55**. Further investigation was then not performed.

Subfraction BC410 showed one UV-active spot on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.14 and one gray spot with the R_f value of 0.07 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was further separated by column chromatography over silica gel. Elution was performed initially with 70% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate until pure ethyl acetate. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 254**.

Table 254 Subfractions obtained from **subfraction BC410** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
BC410A	70% EtOAc/Petrol	3.5	Colorless gum
BC410B	70% EtOAc/Petrol	2.7	Colorless gum
BC410C	70% EtOAc/Petrol	2.9	Colorless gum
BC410D	80% EtOAc/Petrol-100% EtOAc	2.7	Colorless gum

Subfraction BC410A showed none of UV-active spots on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase but showed many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of low quantity, it was not further investigated.

Subfraction BC410B showed one purple spot on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.40 after

dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated the presence of **K55**. Further investigation was then not carried out.

Subfraction BC410C showed one UV-active spot on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.36 and one gray spot with the R_f value of 0.19 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction BC411 displayed a long tail under UV-S on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction BC412 displayed a long tail under UV-S on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction BC5 showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.17 and 0.19 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction BC6 showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.35 and two yellow spots with the R_f values of 0.34 and 0.41 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction BC7 showed one pale UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.17 Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Fraction 22BD showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.14, 0.37 and 0.74 and many spots after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 255**.

Table 255 Subfractions obtained from **fraction 22BD** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
BD1	6.4	Yellow gum
BD2	28.5	Yellow gum
BD3	8.1	Yellow gum
BD4	8.0	Yellow gum
BD5	7.5	Yellow gum

Subfraction BD1 showed none of major spots under UV-S on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase. Thus, it was not investigated.

Subfraction BD2 showed two pale UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.20 and 0.25 and many spots after dipping the TLC plate in anisaldehyde reagent and

subsequently heating the plate. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction BD3 showed one pale UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.13. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction BD4 showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.18 and 0.45. It was then purified by precoated TLC with 30% ethyl acetate in petroleum ether as a mobile phase (3 runs) to afford two bands.

Band 1 (K59) was a colorless gum (4.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.45.

$[\alpha]_D^{29}$	+215 (*c* 0.11, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	208 (3.78), 257 (3.50), 273 (3.27)
FTIR(neat):υ(cm^{-1})	1739 and 1720 (C=O stretching), 1655 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	7.03 (*dd*, *J* = 9.9, 6.0 Hz, 1H), 6.23 (*d*, *J* = 9.9 Hz, 1H), 6.10 (*ddd*, *J* = 10.5, 6.6, 4.5 Hz, 1H), 6.05 (*m*, 1H), 5.93 (*m*, 1H), 5.90 (*m*, 1H), 5.08 (*dd*, *J* = 6.0, 2.4 Hz, 1H), 4.49 (*dd*, *J* = 11.1, 2.4 Hz, 1H), 4.32 (*d*, *J* = 4.5 Hz, 1H), 4.28 (*t*, *J* = 6.0 Hz, 1H), 3.06 (*m*, 1H), 3.02 (*m*, 1H), 2.03 (*s*, 3H) 1.14 (*d*, *J* = 6.9 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	170.00, 162.12, 140.19, 137.50, 136.94, 126.30, 125.07, 124.44, 86.43, 77.65, 75.93,

	63.16, 52.62, 50.08, 20.68, 14.25
DEPT 135: CH;	140.19, 137.50, 136.94, 126.30, 125.07, 124.44, 86.43, 77.65, 75.93, 63.16, 52.62, 50.08
CH_3;	20.68, 14.25

Band 2 (K60) was a colorless gum (2.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.18.

$[\alpha]_D^{29}$	+83 (*c* 0.59, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	206 (3.08), 256 (2.56), 265 (2.46)
FTIR(neat):υ(cm^{-1})	3418 (O-H stretching), 1721 (C=O stretching), 1655 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	6.97 (*dd*, *J* = 9.6, 6.3 Hz, 1H), 6.19 (*m*, 1H), 6.05 (*d*, *J* = 6.9 Hz, 1H), 6.02 (*m*, 1H), 5.89 (*m*, 1H), 5.85 (*m*, 1H), 4.36 (*t*, *J* = 5.4 Hz, 1H), 4.28 (*dd*, *J* = 10.5, 2.4 Hz, 1H), 4.25 (*d*, *J* = 4.5 Hz, 1H), 4.00 (*brt*, *J* = 5.4 Hz, 1H), 2.95 (*m*, 1H), 2.91 (*q*, *J* = 6.9 Hz, 1H), 1.06 (*d*, *J* = 6.9 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	163.27, 144.34, 137.39, 137.19, 126.09, 124.79, 122.90, 86.14, 79.49, 76.89, 62.08, 51.73, 14.32
DEPT 135: CH;	144.34, 137.39, 137.19, 126.09, 124.79, 122.90, 86.14, 79.49, 76.89, 62.08, 51.73
CH_3;	14.32

Subfraction BD5 showed three gray spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.23, 0.39 and 0.48 after dipping the TLC plate in anisaldehyde reagent and subsequently

heating the plate. It was then purified by precoated TLC with 30% ethyl acetate in petroleum ether as a mobile phase (8 runs) to afford two bands.

Band 1 was a colorless gum (3.3 mg). Its chromatogram showed two gray spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.49 and 0.58 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

Band 2 (K54) was a colorless gum (5.5 mg). Its chromatogram showed one yellow spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.24 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate

$[\alpha]_D^{29}$	-48 (*c* 0.10, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	208 (3.48)
FTIR(neat):υ(cm^{-1})	3385 (O-H stretching), 1715 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz) :	6.12 (*dd*, *J* = 16.0, 7.5 Hz, 1H), 6.05 (*ddd*, *J* = 16.0, 7.5, 1.5 Hz, 1H), 5.97 (*dd*, *J* = 12.5, 3.5 Hz, 1H), 5.90 (*dd*, *J* = 12.5, 2.0 Hz, 1H), 5.66 (*m*, 1H), 4.90 (*brs*, 1H), 4.71 (*m*, 1H), 3.83 (*m*, 1H), 1.34 (*d*, *J* = 6.5 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz) :	167.77, 136.78, 131.50, 128.02, 122.43, 75.96, 71.24, 68.10, 58.84, 17.21
DEPT 135: CH;	136.78, 131.50, 128.02, 122.34, 75.96, 71.24, 68.10, 58.84
CH_3;	17.21
EIMS *m/z* (% relative intensity):	214 (7), 197 (17), 141 (100), 123 (73), 95 (80)

Fraction 22BE showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.74. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Fraction 22BF showed many spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Because of the minute quantity, it was not further investigated.

6.2.3 Purification of the EtOAc extract from mycelia

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four fractions as shown in **Table 256**.

Table 256 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
22CA	101.9	Brown gum
22CB	203.6	Brown gum
22CC	64.8	Brown gum
22CD	6.4	Brown gum

Fraction 22CA displayed a long tail under UV-S on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 22CB showed three UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.28,

0.63 and 0.91. It was further separated by column chromatography over silica gel. Elution was performed initially with 50% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 257**.

Table 257 Subfractions obtained from **fraction 22CB** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
CB1	50% EtOAc/Petrol	10.0	Yellow gum
CB2	50% EtOAc/Petrol	6.5	Yellow gum
CB3	50% EtOAc/Petrol	14.8	Yellow gum
CB4	70% EtOAc/Petrol	12.7	Colorless gum
CB5	70% EtOAc/Petrol	15.8	Colorless gum
CB6	70% EtOAc/Petrol-100% EtOAc	11.8	Yellow gum
CB7	2-20% MeOH/EtOAc	31.9	Yellow gum
CB8	30% MeOH/EtOAc-100% MeOH	92.9	Yellow gum

Subfraction CB1 displayed a long tail under UV-S on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction CB2 showed three UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.13, 0.21 and 0.45. It was then purified by precoated TLC with 40% ethyl acetate in petroleum ether as a mobile phase (6 runs) to afford three bands.

Band 1 was a colorless gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.44. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a colorless gum (1.0 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.23. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 3 (K52) was a colorless gum (3.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.16.

$[\alpha]_D^{29}$	-147 (*c* 0.38, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	213 (3.62), 226 (3.65), 251 (3.70), 320 (3.81)
FTIR(neat):υ(cm^{-1})	3394 (O-H stretching), 1683 (C=O stretching)
^1H NMR(acetone-d_6)(δ_{ppm})(300 MHz):	6.61 (*dq*, *J* = 15.6, 6.9 Hz, 1H), 6.16 (*dq*, *J* = 15.6, 1.5 Hz, 1H), 5.98 (*s*, 1H), 4.49 (*d*, *J* = 5.4 Hz, 1H), 4.29 (*qn*, *J* = 6.6 Hz, 1H), 3.70 (*dd*, *J* = 6.6, 5.4 Hz, 1H), 1.89 (*dd*, *J* = 6.9, 1.5 Hz, 3H), 1.46 (*d*, *J* = 6.6 Hz, 3H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(75 MHz):	164.00, 163.10, 158.24, 133.86, 123.07, 100.85, 98.98, 76.97, 71.87, 66.40, 17.42, 16.40
DEPT 135: CH;	133.86, 123.07, 98.98, 76.97, 72.87, 66.40
CH_3;	17.42, 16.40
EIMS *m/z* (% relative intensity):	238 (11), 181 (100), 152 (8), 111 (50), 69 (56)

Subfraction CB3 showed three UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.12, 0.21 and 0.35 and two gray spots with the R_f values of 0.45 and 0.52 after dipping the TLC plate in anisaldehyde reagent and subsequently heating the plate. Its ^{1}H NMR spectrum indicated that the major compound was **K52**. Further investigation was then not performed.

Subfraction CB4 showed four UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.26, 0.43, 0.50 and 0.61. Its ^{1}H NMR spectrum indicated that the major compound was **K55**. Further investigation was then not performed.

Subfraction CB5 (K55) showed one UV-active spot on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.33.

$[\alpha]_D^{29}$	+40 (*c* 0.25, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	208 (3.48)
FTIR(neat):υ(cm^{-1})	3224 (O-H stretching), 1714 (C=O stretching), 1638 (C=C stretching)
^{1}H NMR(acetone-d_6)(δ_{ppm})(300 MHz):	5.87 (*d*, *J* = 11.7 Hz, 2H), 5.60 (*dd*, *J* = 15.3, 6.9 Hz, 1H), 5.58 (*dd*, *J* = 15.3, 6.9 Hz, 1H), 5.26 (*dqd*, *J* = 10.5, 6.3, 1.8 Hz, 1H), 4.73 (*brs*, 1H), 4.49 (*brs*, 1H), 4.13 (*m*, 1H), 1.94 (*ddd*, *J* = 14.0, 3.0, 1.8 Hz, 1H), 1.73 (*dt*, *J* = 14.0, 10.5 Hz, 1H), 1.24 (*d*, *J* = 6.3 Hz, 3H)
^{13}C NMR(acetone-d_6)(δ_{ppm})(75 MHz):	167.93, 137.81, 137.26, 129.68, 121.33, 71.55, 71.01, 68.27, 43.16, 20.78
DEPT 135: CH;	137.81, 137.26, 129.68, 121.33, 71.55, 71.01, 68.27
CH_2;	43.16
CH_3;	20.78

Subfraction CB6 showed four UV-active spots on normal phase TLC using 60% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.05, 0.12, 0.33 and 0.45. Its ^1H NMR spectrum indicated that the major compound was **K55**. Further investigation was then not performed.

Subfraction CB7 showed three UV-active spots on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.33, 0.52 and 0.64. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Subfraction CB8 displayed a long tail under UV-S on normal phase TLC using 70% ethyl acetate in petroleum ether as a mobile phase and its ^1H NMR spectrum showed broad signals. Thus, it was not further purified.

Fraction 22CC showed four UV-active spots on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.21, 0.30, 0.43 and 0.91. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not further purified.

Fraction 22CD displayed a long tail under UV-S on normal phase TLC using 50% ethyl acetate in petroleum ether as a mobile phase. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

CHAPTER 6.3

RESULTS AND DISCUSSION

One new carboxylic acid **(K53)** and one new macrolide **(K54)** together with five known ones **(K56-K60)** were isolated from the broth extract. In addition, one new pyrone **(K52)** and one known macrolide **(K55)** were obtained from the mycelial extract. The structures were identified by spectroscopic methods.

6.3.1 Compound K52

Compound **K52** with the molecular formula $C_{12}H_{16}O_6$ from EIMS (*m/z* 256) **(Figure 123)** was obtained as a colorless gum. The UV spectrum with maximum absorption bands at 213, 226, 251 and 320 nm indicated that **K52** had a conjugated pyrone chromophore. The IR spectrum showed absorption bands at 3394 and 1683 cm^{-1} for hydroxyl and conjugated carbonyl groups, respectively. The ^1H NMR spectrum **(Figure 124)** **(Table 258)** displayed characteristic signals for a *trans*-propenyl unit [δ 6.61 (*dq*, J = 15.6 and 6.9 Hz, 1H), 6.16 (*dq*, J = 15.6 and 1.5 Hz, 1H) and 1.89 (*dd*, J = 6.9 and 1.5 Hz, 3H)], a 1,2,3-trihydroxybutyl unit [δ 4.49 (*d*, J = 5.4 Hz, 1H), 4.29 (*qn*, J = 6.6 Hz, 1H), 3.70 (*dd*, J = 6.6 and 5.4 Hz, 1H) and 1.46 (*d*, J = 6.6 Hz, 3H)] and one olefinic proton (δ 5.98, *s*, 1H). The ^{13}C NMR **(Figure 125)** **(Table 258)** and DEPT 135 **(Table 258)** spectra showed four quaternary, six methine and two methyl carbons. The olefinic proton (δ 5.98) was attributed to H-5 on the basis of its HMBC correlations with C-3 (δ 100.85), C-4 (δ 164.00) and C-6 (δ 158.24). These results together with the chemical shift of C-4 established a hydroxyl group at C-4 of the pyrone unit. The *trans*-propenyl fragment was attached at C-6 of the pyrone ring based on HMBC correlations of both H-7 (δ 6.16) and H-8 (δ 6.61) of the *trans*-propenyl unit with C-6. Thus, the remaining unit, the 1,2,3-trihydroxybutyl group, was located at C-3. This assignment was confirmed

by HMBC cross peaks of the hydroxymethine proton, H-10 (δ 4.49), with C-2 (δ 163.10), C-3 and C-4. Therefore, **K52** was identified as a new α-pyrone derivative. Because of minute amount, no attempts were made to assign the absolute configuration.

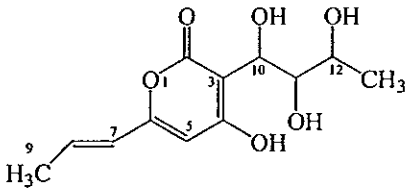


Table 258 The ^{1}H, ^{13}C NMR and HMBC data of compound **K52** in acetone-d_6

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
2	-	163.10 (C)	-
3	-	100.85 (C)	-
4	-	164.00 (C)	-
5	5.98 (*s*)	98.98 (CH)	C-3, C-4, C-6, C-7, C-10
6	-	158.24 (C)	-
7	6.16 (*dq*, 15.6, 1.5)	123.07 (CH)	C-6, C-9
8	6.61 (*dq*, 15.6, 6.9)	133.86 (CH)	C-6, C-9
9	1.89 (*dd*, 6.9, 1.5)	17.42 (CH_3)	C-7, C-8
10	4.49 (*d*, 5.4)	66.40 (CH)	C-2, C-3, C-4, C-11, C-12
11	3.70 (*dd*, 6.6, 5.4)	71.87 (CH)	C-3, C-10, C-12, C-13
12	4.29 (*qn*, 6.6)	76.97 (CH)	C-10, C-11, C-13
13	1.46 (*d*, 6.6)	16.40 (CH_3)	C-11, C-12

Table 259 The COSY and NOEDIFF data of compound **K52** in acetone-d_6

Proton	COSY	NOEDIFF
H-5	-	H-7
H-7	H-8, H_3-9	H-5, H_3-9
H-8	H-7, H_3-9	H_3-9
H_3-9	H-7, H-8	H-7, H-8
H-10	H-11	H-11, H-12

Table 259 Continued

Proton	COSY	NOEDIFF
H-11	H-10, H-12	H-10, H-12, H_3-13
H-12	H-11, H_3-13	H-10, H-11, H_3-13
H_3-13	H-12	H-11, H-12

6.3.2 Compound K53

Compound **K53** with the molecular formula $C_{10}H_{16}O_3$ from EIMS (*m/z* 184) **(Figure 126)** was obtained as a colorless gum. The UV spectrum displayed absorption bands at 207 and 257 nm while hydroxyl (3422 cm^{-1}) and conjugated carboxylic carbonyl (1704 cm^{-1}) absorption bands were found in the IR spectrum. The ^1H NMR spectrum **(Figure 127) (Table 260)** displayed characteristic signals for *trans*-olefinic protons [δ 7.28 (*dd*, J = 15.3 and 11.4 Hz, 1H) and 5.94 (*dt*, J = 15.3 and 6.9 Hz, 1H)], *cis*-olefinic protons [δ 6.36 (*t*, J = 11.4 Hz, 1H) and 5.62 (*d*, J = 11.4 Hz, 1H)] and a 2-hydroxypentyl unit [δ 3.75 (*sextet*, J = 6.3 Hz, 1H), 2.18 (*brd*, J = 6.9 Hz, 2H), 1.51 (*m*, 2H), 1.48 (*m*, 2H) and 1.16 (*d*, J = 6.3 Hz, 3H)]. The ^1H-^1H COSY correlations **(Table 261)** supported the presence of the 2-hydroxypentyl moiety. One of the *trans*-olefinic protons, H-4 (δ 7.28), showed a ^1H-^1H COSY cross peak with the *cis*-olefinic proton, H-3 (δ 6.36), while the other *trans*-olefinic proton, H-5 (δ 5.94) was correlated with H_2-6 (δ 2.18) of the hydroxypentyl unit. In addition, the *cis*-olefinic proton, H-3, showed a HMBC correlation with a carboxylic carbonyl carbon, C-1 (δ 173.00). Irradiation of H-3 in the NOEDIFF experiment **(Table 261)** affected signal intensity of H-2 (δ 5.62) and H-5, indicating *s-trans* configuration for C3-C4 single bond. Thus, **K53** was identified as a new uncyclized metabolite of modiolide macrolides. Hence, the absolute configuration at C-9 was proposed to be *R*, identical to those of **K55** and **K56**.

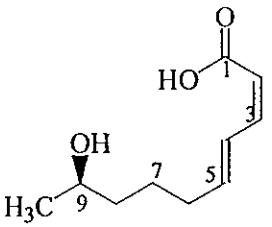


Table 260 The ^{1}H, ^{13}C NMR and HMBC data of compound **K53** in CD_3OD

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1	-	173.00 (C)	-
2	5.62 (*d*, 11.4)	121.74 (CH)	C-4
3	6.36 (*t*, 11.4)	139.33 (CH)	C-1, C-4, C-5
4	7.28 (*dd*, 15.3, 11.4)	127.78 (CH)	C-2, C-3, C-6
5	5.94 (*dt*, 15.3, 6.9)	140.41 (CH)	C-3, C-6, C-7
6	2.18 (*brd*, 6.9)	32.44 (CH_2)	C-4, C-5, C-7, C-8
7	1.51 (*m*)	24.94 (CH_2)	C-8
8	1.48 (*m*)	38.30 (CH_2)	C-6, C-7, C-9, C-10
9	3.75 (*sextet*, 6.3)	66.98 (CH)	C-7, C-8
10	1.16 (*d*, 6.3)	22.05 (CH_3)	C-8, C-9

Table 261 The COSY and NOEDIFF data of compound **K53** in CD_3OD

Proton	COSY	NOEDIFF
H-2	H-3	H-3
H-3	H-2, H-4	H-2, H-5
H-4	H-3, H-5	H_2-6
H-5	H-4, H_2-6	H-3, H_2-6
H_2-6	H-5, H_2-7	H-4, H-5
H_2-7	H_2-6, H_2-8	-
H_2-8	H_2-7, H-9	-
H-9	H_2-8, H_3-10	H_3-10
H_3-10	H-9	H-9

6.3.3 Compound K55

Compound **K55** was obtained as a colorless gum. The UV spectrum displayed an absorption band at 208 nm, while hydroxyl and carbonyl absorption bands at 3224 and 1714 cm^{-1}, respectively, were found in the IR spectrum. The ^1H NMR spectrum **(Figure 132) (Table 262)** consisted of signals for *cis*-olefinic protons (δ 5.87, *d*, *J* = 11.7 Hz, 2H), *trans*-olefinic protons [δ 5.60 (*dd*, *J* = 15.3 and 6.9 Hz, 1H) and 5.58 (*dd*, *J* = 15.3 and 6.9 Hz, 1H)], one hydroxy proton (δ 4.49, *brs*, 1H), three oxymethine protons [δ 5.26 (*dqd*, *J* = 10.5, 6.3 and 1.8 Hz, 1H), 4.73 (*brs*, 1H) and 4.13 (*m*, 1H)], two nonequivalent methylene protons [δ 1.94 (*ddd*, *J* = 14.0, 3.0 and 1.8 Hz, 1H) and 1.73 (*dt*, *J* = 14.0 and 10.5 Hz, 1H)] and one methyl group (δ 1.24, *d*, *J* = 6.3 Hz, 3H). The ^{13}C NMR **(Figure 133) (Table 262)** and DEPT 135 **(Table 262)** spectra displayed one quaternary (δ 167.93), seven methine (δ 137.81, 137.26, 129.68, 121.33, 71.55, 71.01 and 68.27), one methylene (δ 43.16) and one methyl (δ 20.78) carbons. The oxymethine proton, H-4 (δ 4.73), showed ^1H-^1H COSY correlations **(Table 263)** with one of the *cis*-olefinic protons, H-3 (δ 5.87), the hydroxy proton, 4-OH (δ 4.49), and one of the *trans*-olefinic protons, H-5 (δ 5.60). The other *trans*-olefinic proton, H-6 (δ 5.58), was coupled with the oxymethine proton, H-7 (δ 4.13). Furthermore, the methylene protons, H_{ab}-8 (δ 1.94 and 1.73), showed ^1H-^1H COSY cross peaks with H-7 and the oxymethine proton, H-9 (δ 5.26), which was further coupled with the methyl protons, H_3-10 (δ 1.24). The remaining *cis*-olefinic proton, H-2 (δ 5.87), and the oxymethine proton, H-9, showed HMBC correlations **(Table 263)** with the same ester carbonyl carbon, C-1 (δ 167.93), constructing a 10-membered lactone ring. Signal enhancement of H-5 and H-9 upon irradiation of H-7 indicated that they were located at the same side of the molecule. Irradiation of H-5 did not affect signal intensity of H-4, suggesting that H-4 was on the opposite side to H-7 and H-9. The observed optical rotation of **K55**, $[\alpha]_D^{18}$ +40 (*c* 0.25, MeOH), was almost identical to that of (4*R*,7*S*,9*S*)-modiolide A, $[\alpha]_D^{18}$ +42 (*c* 0.25, MeOH). These results indicated that they had the same absolute configuration. Consequently, **K55** was identified as (4*R*,7*S*,9*S*)-modiolide A which was previously isolated from *Paraphaeosphaeria* sp. (N 119) (Tsuda, *et al.*, 2003).

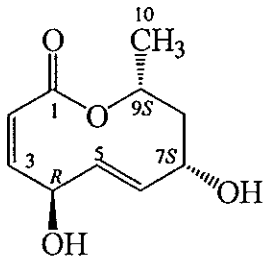


Table 262 The ^{1}H and ^{13}C NMR data of compound **K55** in acetone-d_6 and **(4*R*,7*S*,9*S*)-Modiolide A** in CD_3OD

Position	**K55**		**(4*R*,7*S*,9*S*)-Modiolide A**	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	-	167.93 (C)	-	170.9 (C)
2	5.87 (*d*, 11.7)	121.33 (CH)	5.85 (*dd*, 12.3, 1.5)	123.7 (CH)
3	5.87 (*d*, 11.7)	137.26 (CH)	5.83 (*dd*, 12.3, 3.5)	138.7 (CH)
4	4.73 (*brs*)	71.01 (CH)	4.68 (*brdd*, 7.3, 3.5)	73.0 (CH)
4-OH	4.49 (*brs*)	-	-	-
5	5.60 (*dd*, 15.3, 6.9)	129.68 (CH)	5.61 (*dd*, 15.8, 7.5)	131.8 (CH)
6	5.58 (*dd*, 15.3, 6.9)	137.81 (CH)	5.56 (*dd*, 15.8, 7.5)	139.4 (CH)
7	4.13 (*m*)	71.55 (CH)	4.12 (*ddd*, 11.4, 7.5, 2.5)	73.6 (CH)
8	a: 1.94 (*ddd*, 14.0, 3.0, 1.8)	43.16 (CH_2)	a: 1.87 (*dt*, 14.0, 2.5)	44.7 (CH_2)
	b: 1.73 (*dt*, 14.0, 10.5)		b: 1.71 (*dt*, 14.0, 11.4)	
9	5.26 (*dqd*, 10.5, 6.3, 1.8)	68.27 (CH)	5.25 (*dqd*, 11.4, 6.7, 2.5)	70.9 (CH)
10	1.24 (*d*, 6.3)	20.78 (CH_3)	1.22 (*d*, 6.7)	22.4 (CH_3)

Table 263 The HMBC, COSY and NOEDIFF data of compound **K55** in acetone-d_6

Position	HMBC	COSY	NOEDIFF
H-2	C-1, C-3, C-4	H-3	-
H-3	C-1, C-2, C-4, C-5	H-2	-

Table 263 Continued

Position	HMBC	COSY	NOEDIFF
H-4	C-5, C-6	H-5, 4-OH	H-3, H-6
H-5	C-2, C-4, C-6	H-4, H-6	H-7
H-6	C-4, C-5	H-5, H-7	H-4, H-7
H-7	-	H-6, H_{ab}-8	H-5, H-6, H-9
H_a-8	C-6, C-7, C-9, C-10	H-7, H_b-8, H-9	H-7, H_b-8, H-9
H_b-8	C-6, C-7, C-9, C-10	H-7, H_a-8, H-9	H-7, H_a-8, H-9
H-9	C-1, C-6, C-7, C-8, C-10	H_{ab}-8, H_3-10	H-7, H_{ab}-8
H_3-10	C-9, C-8	H-9	H_{ab}-8, H-9

6.3.4 Compound K56

Compound **K56** was obtained as a colorless gum with $[\alpha]_D^{18}$ +40 (*c* 0.25, MeOH). Its UV and IR spectra were similar to those of **K55**. Their ^{1}H NMR spectra **(Figure 134) (Table 264)** were also similar except for the replacement of signal for one of the oxymethine protons in **K55** with those of methylene protons [δ 2.39 (*dm*, *J* = 13.5 Hz, 1H) and 2.20 (*m*, 1H)]. The ^{1}H-^{1}H COSY cross peaks **(Table 265)** of these methylene protons with one of the *trans*-olefinic protons, H-6 (δ 5.69, *ddd*, *J* = 15.6, 11.1 and 3.6 Hz, 1H), and H_2-8, (δ 1.68, *m*, 2H), established the location of these methylene protons at C-7. Thus, **K56** was assigned as (4*R*,9*R*)-modiolide B, $[\alpha]_D^{18}$ +45 (*c* 0.25, MeOH), which was previously isolated from *Paraphaeosphaeria* sp. (N 119) (Tsuda, *et al.*, 2003).

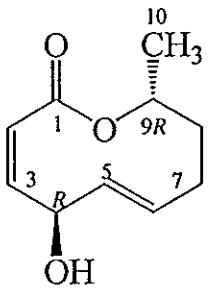


Table 264 The ^{1}H and ^{13}C NMR data of compound **K56** in $CDCl_3$ and **(4*R*,9*R*)-Modiolide B** in CD_3OD

Position	**K56**		**(4*R*,9*R*)-Modiolide B**	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	-	168.86 (C)	-	170.8 (C)
2	5.87 (*dd*, 12.6, 1.2)	122.58 (CH)	5.85 (*dd*, 12.3, 1.4)	123.0 (CH)
3	5.88 (*dd*, 12.6, 2.4)	136.47 (CH)	5.83 (*dd*, 12.3, 2.9)	138.0 (CH)
4	4.75 (*brd*, 6.9)	71.79 (CH)	4.67 (*brdd*, 8.2, 2.9)	72.6 (CH)
5	5.54 (*ddd*, 15.6, 7.8, 1.5)	129.09 (CH)	5.45 (*ddd*, 15.2, 8.2, 1.5)	130.2 (CH)
6	5.69 (*ddd*, 15.6, 11.1, 3.6)	135.43 (CH)	5.69 (*ddd*, 15.2, 11.0, 3.5)	136.2 (CH)
7	a: 2.39 (*dm*, 13.5) b: 2.20 (*m*)	30.96 (CH_2)	a: 2.37 (*dddd*, 13.5, 5.3, 3.5, 2.4) b: 2.15 (*dtd*, 13.5, 11.0, 2.6)	32.0 (CH_2)
8	1.68 (*m*)	33.88 (CH_2)	a: 1.65 (*dtd*, 14.7, 11.0, 2.4) b: 1.71 (*ddt*, 14.7, 5.3, 2.6)	34.9 (CH_2)
9	5.32 (*m*)	72.43 (CH)	5.61 (*dqd*, 11.0, 6.7, 2.6)	73.7 (CH)
10	1.22 (*d*, 6.6)	21.49 (CH_3)	1.22 (*d*, 6.7)	21.7 (CH_3)

Table 265 The HMBC, COSY and NOEDIFF data of compound **K56** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-2	C-1, C-3, C-4, C-5	H-3, H-4	H-3
H-3	C-1, C-2, C-4, C-5	H-2, H-4	H-4
H-4	C-5, C-6	H-2, H-3, H-5	H-3, H-5, H-6

Table 265 Continued

Proton	HMBC	COSY	NOEDIFF
H-5	C-4, C-6, C-7	H-4, H-6, H-7	H-4, H_{ab}-7
H-6	C-3, C-4, C-5, C-7, C-8	H-5, H_{ab}-7	H_{ab}-7, H_2-8, H-9
H_a-7	C-5, C-6, C-8, C-9	H-5, H-6, H_b-7, H_2-8	H_2-8
H_b-7	C-5, C-6, C-8, C-9	H-6, H_a-7, H_2-8	H-5, H-6, H-9
H_2-8	C-6, C-7, C-9, C-10	H_{ab}-7, H-9	H_a-7, H-9, H_3-10
H-9	C-1, C-7, C-8, C-10	H_2-8, H_3-10	H_b-7, H_3-10
H_3-10	C-8, C-9	H-9	H_2-8, H-9

6.3.5 Compound K54

Compound **K54** with the molecular formula $C_{10}H_{14}O_5$ from HREIMS (*m/z* 214) **(Figure 129)** was obtained as a colorless gum. The UV and IR spectra were similar to those of **K56**. Its ^{1}H NMR spectrum **(Figure 130) (Table 266)** was similar to that of **K56** except for the replacement of the resonances for two methylene protons with signals of two hydroxymethine protons [δ 4.71 (*m*, 1H) and 3.83 (*m*, 1H)]. The presence of four oxymethine carbons (δ 75.96, 71.24, 68.10 and 58.84) in the DEPT 135 spectrum **(Table 266)** supported the ^{1}H NMR data. In the ^{1}H-^{1}H COSY spectrum **(Table 267)**, the oxymethine proton resonating at δ 4.71 was correlated with the olefinic proton, H-6 (δ 6.12), and the hydroxymethine proton at δ 3.83 which was further coupled with H-9 (δ 5.66). Thus, the hydroxymethine protons at δ 4.71 and 3.83 were identified as H-7 and H-8, respectively. Irradiation of H-8, in the NOEDIFF experiment **(Table 267)**, affected signal intensity of both H-7 and H_3-10 (δ 1.34), indicating their *cis*-relationship. The absolute configurations of C-4 and C-9 in **K54** were proposed to be both *R* on the basis of known absolute configurations of its cometabolite **K56**. The remaining absolute configurations were then determined to be 7*S* and 8*S*. Consequently, **K54** was identified as a new member of modiolide macrolides.

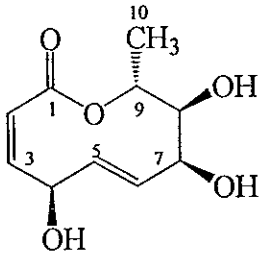


Table 266 The ^{1}H, ^{13}C NMR and HMBC data of compound **K54** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1	-	167.77 (C)	-
2	5.90 (*dd*, 12.5, 2.0)	122.43 (CH)	C-1, C-3, C-4
3	5.97 (*dd*, 12.5, 3.5)	136.78 (CH)	C-1, C-2, C-4, C-5, C-6
4	4.90 (*brs*)	71.24 (CH)	-
5	6.05 (*ddd*, 16.0, 7.5, 1.5)	131.50 (CH)	C-2, C-4, C-6, C-7
6	6.12 (*dd*, 16.0, 7.5)	128.02 (CH)	C-3, C-4, C-5, C-7
7	4.71 (*brs*)	58.84 (CH)	-
8	3.83 (*brs*)	75.96 (CH)	-
9	5.66 (*m*)	68.10 (CH)	-
10	1.34 (*d*, 6.5)	17.21 (CH_3)	C-8, C-9

Table 267 The COSY and NOEDIFF data of compound **K54** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H-3, H-4	-
H-3	H-2, H-4	-
H-4	H-2, H-3, H-5	H-3, H-6
H-5	H-4, H-6	-
H-6	H-5, H-7	-
H-7	H-6, H-8	H-8
H-8	H-7, H-9	H-7, H_3-10
H-9	H-8, H_3-10	H_3-10
H_3-10	H-9	H-8, H-9

6.3.6 Compound K57

Compound **K57** was obtained as a colorless gum. The IR spectrum displayed absorption bands at 3422 and 1700 cm^{-1} for hydroxyl and conjugated carbonyl groups, respectively. Its ^1H NMR spectrum **(Figure 136) (Table 268)** was similar to that of **K56**. Furthermore, they consisted of the same number and types of carbons. However, **K57** exhibited an additional UV absorption band at longer wavelength (λ_{max} 253 nm), indicating that it possessed a longer conjugated chromophore than **K56**. In addition, the difference were found in the ^1H-^1H COSY spectrum **(Table 269)**. The hydroxymethine proton, H-6 (δ 4.24, *td*, J = 9.0 and 3.0 Hz), was correlated with the *trans*-olefinic proton, H-5 (δ 5.74, *ddd*, J = 15.0, 9.0 and 3.0 Hz), and the methylene protons, H_{ab}-7 [δ 2.09 (*ddd*, J = 15.0, 9.0 and 6.0 Hz) and 1.75 (*m*)]. Furthermore, the other *trans*-olefinic proton, H-4 (δ 6.12, *dd*, J = 15.0 and 1.0 Hz), was coupled with one of the *cis*-olefinic protons, H-3 (δ 6.62, *ddd*, J = 12.0, 3.0 and 1.0 Hz, 1H). These data established the location of the hydroxyl group at C-6 (δ 73.76) and the *trans*-double bond at C4-C5, not C-4 and C5-C6, respectively, as found in **K56**. The observed optical rotation of **K57**, $[\alpha]_D^{25}$ -180 (*c* 0.20, $CHCl_3$), was almost identical to that of (6*R*,9*R*)-stagonolide E, $[\alpha]_D^{25}$ -186 (*c* 0.20, $CHCl_3$), indicating that they had the same absolute configuration. Consequently, **K57** was identified as (6*R*,9*R*)-stagonolide E which was previously isolated from *Stagonospora cirsii* (Evidente, *et al.*, 2008).

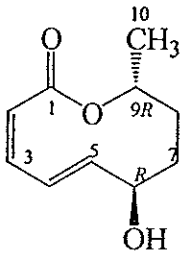


Table 268 The ^1H and ^{13}C NMR data of compound **K57** and **(6*R*,9*R*)-Stagonolide E** in $CDCl_3$

Position	K57		(6*R*,9*R*)-Stagonolide E	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	-	168.27 (C)	-	168.2 (C)

Table 268 Continued

Position	K57		(6*R*,9*R*)-Stagonolide E	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
2	5.85 (*dd*, 12.0, 3.0)	125.62 (CH)	5.84 (*d*, 11.6)	125.6 (CH)
3	6.62 (*ddd*, 12.0, 3.0, 1.0)	139.61 (CH)	6.60 (*brd*, 11.6)	139.6 (CH)
4	6.12 (*dd*, 15.0, 1.0)	126.64 (CH)	6.12 (*brd*, 15.4)	126.6 (CH)
5	5.74 (*ddd*, 15.0, 9.0, 3.0)	140.17 (CH)	5.73 (*dd*, 15.4, 9.6)	140.2 (CH)
6	4.24 (*td*, 9.0, 3.0)	73.67 (CH)	4.24 (*ddd*, 9.6, 9.0, 3.8)	73.7 (CH)
7	a: 2.09 (*ddd*, 15.0, 9.0, 6.0) b: 1.75 (*m*)	37.37 (CH_2)	a: 2.08 (*ddd*, 14.2, 9.0, 3.8) b: 1.73 (*ddd*, 14.2, 9.5, 9.0)	37.4 (CH_2)
8	a: 1.84 (*ddd*, 15.0, 9.0, 3.0) b: 1.62 (*m*)	30.38 (CH_2)	a: 1.85 (*dd*, 15.8, 9.0) b: 1.60 (*m*)	30.4 (CH_2)
9	4.98 (*qnd*, 6.0, 3.0)	73.27 (CH)	4.98 (*dq*, 11.7, 6.5)	73.2 (CH)
10	1.12 (*d*, 6.0)	21.36 (CH_3)	1.21 (*d*, 6.5)	21.4 (CH_3)

Table 269 The HMBC, COSY and NOEDIFF data of compound **K57** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-2	C-1, C-4, C-5	H-3, H-4	H-3
H-3	C-1, C-2, C-5	H-2, H-4, H-5	H-2, H-4
H-4	C-2, C-6	H-2, H-3, H-5	H-3, H-6
H-5	C-3	H-3, H-4, H-6	H-6
H-6	C-3, C-7, C-8	H-5, H_{ab}-7	H-4, H-5, H_b-7
H_a-7	C-5, C-6, C-9	H-6, H_b-7, H_{ab}-8	H-6, H_b-8

Table 269 Continued

Proton	HMBC	COSY	NOEDIFF
H_b-7	C-5, C-6, C-9	H-6, H_a-7, H_{ab}-8	H-6
H_a-8	C-6, C-9	H_{ab}-7, H_b-8, H-9	H-9
H_b-8	C-6, C-9	H_{ab}-7, H_a-8, H-9	-
H-9	C-1, C-7	H_{ab}-8, H_3-10	H_{ab}-8, H_3-10
H_3-10	C-8, C-9	H-9	H-9

6.3.7 Compound K58

Compound **K58** was obtained as a colorless gum. The UV spectrum showed absorption bands at 221 and 262 nm, indicating that **K58** had a conjugated chromophore. The IR spectrum exhibited absorption bands at 1700 and 1619 cm^{-1} for conjugated carbonyl and double bond functional groups, respectively. The ^1H NMR spectrum **(Figure 138) (Table 270)** consisted of signals for *trans*-olefinic protons [δ 6.75 (*dd*, J = 17.5 and 6.5 Hz, 1H) and 6.20 (*dd*, J = 17.0 and 1.0 Hz, 1H), *cis*-olefinic protons [δ 6.63 (*d*, J = 12.5 Hz, 1H) and 6.14 (*d*, J = 12.5 Hz, 1H), three oxymethine protons [δ 5.58 (*qd*, J = 7.0 and 2.0 Hz, 1H), 3.82 (*ddd*, J = 6.5, 4.5 and 1.5 Hz, 1H) and 3.25 (*dd*, J = 4.5 and 2.0 Hz, 1H)] and one methyl group (1.52, *d*, J = 7.0 Hz, 3H). The ^{13}C NMR **(Figure 139) (Table 270)** and DEPT 135 **(Table 270)** spectra showed ten carbon resonances for two quaternary (δ 192.90 and 166.63), seven methine (δ 145.70, 134.58, 133.52, 128.71, 66.19, 60.88 and 54.76) and one methyl (δ 16.79) carbons. In the ^1H-^1H COSY spectrum **(Table 271)**, the *trans*-olefinic proton, H-6 (δ 6.75), was correlated with the oxymethine proton, H-7 (δ 3.82), which was further coupled with the oxymethine proton, H-8 (δ 3.25). In addition, the oxymethine proton, H-9 (δ 5.58), was coupled with H-8 and the methyl protons, H_3-10 (δ 1.52). The *trans*-olefinic proton, H-6, and the *cis*-olefinic proton, H-3 (δ 6.14), showed a HMBC cross peak **(Table 271)** with the same ketone carbonyl carbon, C-4 (δ 192.90). Furthermore, H-9 and the other *cis*-olefinic proton, H-2 (δ 6.63), were correlated with the same ester carbonyl carbon, C-1 (δ 166.63), in the HMBC spectrum, constructing

a 4-oxolactone ring. The chemical shifts of C-7 (δ 54.76) and C-8 (δ 60.88) established an epoxide functionality on these carbons. H-7 and H-9 were enhanced when H-8 was irradiated, in the NOEDIFF experiment **(Table 271)**, indicating their *cis*-relationship. The observed optical rotation of **K58**, $[\alpha]_D^{29}$ -257.4 (*c* 0.67, $CHCl_3$), was almost identical to that of (7*R*,8*S*,9*R*)-pyrenolide A, $[\alpha]_D^{29}$ -262 (*c* 0.67, $CHCl_3$), indicating that all chiral carbons of **K58** possessed the same absolute configuration as those of (7*R*,8*S*,9*R*)-pyrenolide A which was previously isolated from *Pyrenophora teres* IFO 7508 (Nukina, *et al.*, 1980).

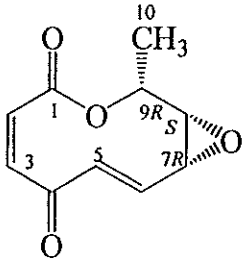


Table 270 The ^1H and ^{13}C NMR data of compound **K58** and **(7*R*,8*S*,9*R*)-Pyrenolide A** in $CDCl_3$

Position	K58		(7*R*,8*S*,9*R*)-Pyrenolide A	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	-	166.63 (C)	-	166.5 (C)
2	6.63 (*d*, 12.5)	134.58 (CH)	6.08 (*d*, 12.0)	134.5 (CH)
3	6.14 (*d*, 12.5)	133.52 (CH)	6.62 (*d*, 12.0)	128.6 (CH)
4		192.90 (C)		192.8 (C)
5	6.20 (*dd*, 17.0, 1.0)	128.71 (CH)	6.12 (*dd*, 17.0, 1.0)	133.4 (CH)
6	6.75 (*dd*, 17.0, 6.5)	145.70 (CH)	6.75 (*dd*, 17.0, 6.0)	145.7 (CH)
7	3.82 (*ddd*, 6.5, 4.5, 1.5)	54.76 (CH)	3.78 (*ddd*, 6.0, 4.5, 1.0)	54.7 (CH)
8	3.25 (*dd*, 4.5, 2.0)	60.88 (CH)	3.20 (*dd*, 4.5, 2.0)	60.8 (CH)
9	5.58 (*qd*, 7.0, 2.0)	66.19 (CH)	5.55 (*qd*, 7.0, 2.0)	66.1 (CH)
10	1.52 (*d*, 7.0)	16.79 (CH_3)	1.50 (*d*, 7.0)	16.8 (CH_3)

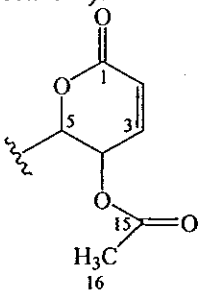
Table 271 The HMBC, COSY and NOEDIFF data of compound **K58** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-2	C-1, C-3, C-4	H-3	H-3
H-3	C-1, C-4, C-5	H-2	H-2
H-5	C-3, C-6, C-7	H-6, H-7	-
H-6	C-4, C-5, C-7	H-5, H-7	-
H-7	C-5, C-6, C-8	H-5, H-6, H-8	H-8
H-8	C-7, C-9	H-7, H-9	H-7, H-9
H-9	C-1, C-8, C-10	H-8, H_3-10	H-8, H_3-10
H_3-10	C-8, C-9	H-9	H-9

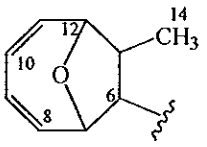
6.3.8 Compound K59

Compound **K59** was obtained as a colorless gum. The UV spectrum showed absorption bands at 208, 257 and 273 nm, indicating that **K59** had a conjugated chromophore. The IR spectrum displayed absorption bands at 1739 and 1720 cm^{-1} for conjugated carbonyl and 1655 cm^{-1} for double bond functional groups. The ^1H NMR spectrum **(Figure 140) (Table 272)** consisted of signals for *cis*-olefinic protons of an α,β-unsaturated carbonyl unit [δ 7.03 (*dd*, J = 9.9 and 6.0 Hz, 1H) and 6.23 (*d*, J = 9.9 Hz, 1H)], four olefinic protons [δ 6.10 (*ddd*, J = 10.5, 6.6 and 4.5 Hz, 1H), 6.05 (*m*, 1H), 5.93 (*m*, 1H) and 5.90 (*m*, 1H)], six methine protons [δ 5.08 (*dd*, J = 6.0 and 2.4 Hz, 1H), 4.49 (*dd*, J = 11.1 and 2.4 Hz, 1H), 4.32 (*d*, J = 4.5 Hz, 1H), 4.28 (*t*, J = 6.0 Hz, 1H), 3.06 (*m*, 1H) and 3.02 (*m*, 1H)], one acetyl group (δ 2.03, *s*, 3H) and one methyl group (δ 1.14, *d*, J = 6.9 Hz, 3H). The ^{13}C NMR **(Figure 141) (Table 272)** and DEPT 135 **(Table 272)** spectra showed two quaternary (δ 170.00 and 162.12), twelve methine (δ 140.19, 137.50, 136.94, 126.30, 125.07, 124.44, 86.43, 77.65, 75.93, 63.16, 52.62 and 50.08) and two methyl (δ 20.68 and 14.25) carbons. The *cis*-olefinic proton, H-3 (δ 7.03), of the α,β-unsaturated carbonyl unit showed ^1H-^1H COSY cross peaks **(Table 273)** with the other *cis*-olefinic proton, H-2 (δ 6.23), and the oxymethine proton, H-4 (δ 5.08), which was further coupled with the

oxymethine proton, H-5 (δ 4.49). In addition, the oxymethine proton, H-4, showed a HMBC correlation **(Table 273)** with the carbonyl carbon, C-15 (δ 170.00), of the acetyl group. These results together the chemical shift of C-1 (δ 162.12) and C-5 (δ 77.65) revealed the presence of a 4,5-dihydropyrone ring having the acetoxyl group at C-4 (substructure 1).



Substructure 1



Substructure 2

In the ^{1}H-^{1}H COSY spectrum, the methine proton, H-6 (δ 3.06), was correlated with the oxymethine proton, H-7 (δ 4.28), and the methine proton, H-13 (δ 3.02), which was coupled with the oxymethine proton, H-12 (δ 4.32), and the methyl protons, H_3-14 (δ 1.14). H-12 was correlated with the olefinic proton, H-11 (δ 6.10). The olefinic proton, H-10 (δ 5.90), showed ^{1}H-^{1}H COSY cross peaks with H-9 (δ 5.93) and H-11. Furthermore, the remaining olefinic proton, H-8 (δ 6.05), showed the same cross peaks with H-7 and H-9. These results established a cyclooctadiene unit having double bonds at C8-C9 and C10-C11 as well as the methyl group at C-13. The HMBC correlations of H_3-14 with C-6 (δ 50.08), C-12 and C-13 (δ 52.62) confirmed the assigned location of this methyl group. In the HMBC spectrum, the oxymethine proton, H-7, showed a cross peak with the C-12 (δ 86.43), thus forming an ether bridge between C-7 (δ 75.93) and C-12. These results established substructure 2. The bond construction between C-5 (δ 77.65) of the dihydropyrone ring with C-6 of substructure 2 was established according to the ^{1}H-^{1}H COSY correlation of H-5 with H-6 as well as the HMBC cross peaks of H-5 with C-6 and C-13. The relative configuration of **K59** was established by the NOEDIFF results. Signals of H-4, H-7 and H_3-14 were enhanced when H-5 was irradiated. Moreover, irradiation of H_3-14 enhanced the signal intensity of H-12. These results indicated that H-4, H-5, H-7, H-12 and H_3-14 were all *cis*, but *trans* to H-6. The observed optical

rotation of **K59**, $[\alpha]_D^{29}$ +215 (*c* 0.11, MeOH), was almost identical to that of mycoepoxydiene, $[\alpha]_D^{29}$+210 (*c* 0.106, MeOH), indicating that they had the same absolute configuration. Therefore, **K59** was identified as mycoepoxydiene which was previously isolated from the endophytic fungus *Phomosis* sp. (Prachya, *et al.*, 2007).

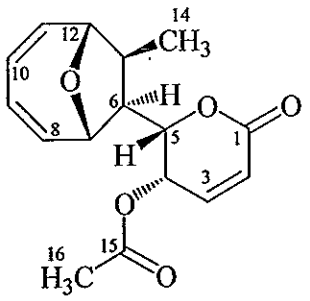


Table 272 The ^1H and ^{13}C NMR data of compound **K59** and **Mycoepoxydiene** in $CDCl_3$

Position	K59		Mycoepoxydiene	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	-	162.12 (C)	-	162.2 (C)
2	6.23 (*d*, 9.9)	125.07 (CH)	6.23 (*d*, 10.0)	125.1 (CH)
3	7.03 (*dd*, 9.9, 6.0)	140.19 (CH)	7.02 (*dd*, 10.0, 6.0)	140.2 (CH)
4	5.08 (*dd*, 6.0, 2.4)	63.16 (CH)	5.05 (*dd*, 6.0, 2.4)	63.3 (CH)
5	4.49 (*dd*, 11.1, 2.4)	77.65 (CH)	4.47 (*dd*, 10.0, 2.4)	77.8 (CH)
6	3.06 (*m*)	50.08 (CH)	3.03 (*m*)	50.2 (CH)
7	4.28 (*t*, 6.0)	75.93 (CH)	4.27 (*dd*, 6.0, 5.6)	76.0 (CH)
8	6.05 (*m*)	136.94 (CH)	6.02 (*brdd*, 11.0, 6.0)	137.0 (CH)
9	5.93 (*m*)	126.30 (CH)	5.88 (*m*)	126.3 (CH)
10	5.90 (*m*)	124.44 (CH)	5.90 (*m*)	124.5 (CH)
11	6.10 (*ddd*, 10.5, 6.6, 4.5)	137.50 (CH)	6.08 (*brdd*, 11.0, 6.0)	137.8 (CH)
12	4.32 (*d*, 4.5)	86.43 (CH)	4.31 (*d*, 4.4)	86.6 (CH)
13	3.02 (*m*)	52.62 (CH)	3.01 (*m*)	52.8 (CH)
14	1.14 (*d*, 6.9)	14.25 (CH_3)	1.12 (*d*, 7.0)	14.5 (CH_3)
15	-	170.00 (C)	-	170.0 (C)
16	2.03 (*s*)	20.68 (CH_3)	2.01 (*s*)	21.0 (CH_3)

Table 273 The HMBC, COSY and NOEDIFF data of compound **K59** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-2	C-1, C-4	H-3	H-3
H-3	C-1, C-2, C-4	H-2, H-4	H-2
H-4	C-2, C-3, C-5, C-15	H-3, H-5	H-3, H-5, H-7
H-5	C-4, C-6, C-13	H-4, H-6	H-4, H-7, H_3-14
H-6	C-4, C-5, C-7, C-8, C-13, C-14	H-5, H-7, H-13	H-7, H-13
H-7	C-5, C-6, C-9, C-10, C-12, C-13	H-6, H-8	H-4, H-5, H-8
H-8	C-7, C-9, C-12	H-7, H-9	H-7
H-9	C-7, C-11	H-8, H-10	H-8, H-10
H-10	C-8, C-12	H-9, H-11	H-9, H-11
H-11	C-8, C-9, C-12	H-10, H-12	H-10, H-12
H-12	C-6, C-7, C-11, C-13, C-14	H-11, H-13	H-11, H_3-14
H-13	C-6, C-7, C-14	H-6, H-12, H_3-14	H-6, H_3-14
H_3-14	C-6, C-12, C-13	H-13	H-5, H-12, H-13
H_3-16	C-15	-	-

6.3.9 Compound K60

Compound **K60** was obtained as a colorless gum with $[\alpha]_D^{20}$ +83.0 (*c* 0.59, MeOH). The UV and IR spectra were almost identical to those of **K59**. Their ^1H NMR data were also similar except for the absence of the acetyl signal in **K60**. The ^{13}C NMR **(Figure 143) (Table 274)** and DEPT 135 **(Table 274)** spectra showed only one ester carbonyl carbon (δ 163.27) and one methyl carbon (δ 14.32), supporting above conclusion. Therefore, **K60** was identified as deacetylmycoepoxydiene, $[\alpha]_D^{20}$ +83 (*c* 0.59, MeOH), which was previously isolated from the endophytic fungus *Phomosis* sp. (Prachya, *et al.*, 2007).

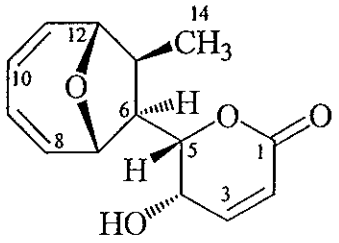


Table 274 The ^{1}H and ^{13}C NMR data of compound **K60** and **Deacetylmycoepoxydiene** in $CDCl_3$

Position	K60		Deacetylmycoepoxydiene	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	-	163.27 (C)	-	163.1 (C)
2	6.05 (*d*, 6.9)	122.90 (CH)	6.14 (*d*, 9.7)	123.1 (CH)
3	6.97 (*dd*, 9.6, 6.3)	144.34 (CH)	7.05 (*dd*, 9.7, 6.0)	144.1 (CH)
4	4.00 (*brt*, 5.4)	62.08 (CH)	4.09 (*dd*, 6.1, 2.3)	62.0 (CH)
5	4.28 (*dd*, 10.5, 2.4)	79.49 (CH)	4.35 (*dd*, 10.6, 2.2)	79.3 (CH)
6	2.95 (*m*)	51.73 (CH)	3.06 (*ddd*, 10.6, 7.0, 5.6)	51.7 (CH)
7	4.36 (*t*, 5.4)	76.89 (CH)	4.44 (*t*, 5.6)	76.8 (CH)
8	6.19 (*m*)	137.39 (CH)	6.24-6.29 (*m*)	137.2 (CH)
9	5.89 (*m*)	126.09 (CH)	5.95-5.99 (*m*)	126.3 (CH)
10	5.85 (*m*)	124.79 (CH)	5.90-5.96 (*m*)	124.8 (CH)
11	6.02 (*m*)	137.19 (CH)	6.10-6.15 (*m*)	137.3 (CH)
12	4.25 (*d*, 4.5)	86.14 (CH)	4.33 (*brd*, 2.5)	86.1 (CH)
13	2.91 (*q*, 6.9)	51.73 (CH)	2.98 (*qnd*, 7.1, 1.3)	51.6 (CH)
14	1.06 (*d*, 6.9)	14.32 (CH_3)	1.14 (*d*, 6.9)	14.2 (CH_3)

Table 275 The HMBC, COSY and NOEDIFF data of compound **K60** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-2	C-4	H-3	H-3
H-3	C-2, C-4, C-5	H-2, H-4	H-2, H-4
H-4	C-2	H-3, H-5	H-3, H-5, H-7

Table 275 Continued

Proton	HMBC	COSY	NOEDIFF
H-5	C-4, C-6, C-7	H-4, H-6	H-4, H_3-14
H-6	C-4, C-5, C-7, C-8	H-5, H-7, H-13	H-6
H-7	C-5, C-6, C-8, C-9, C-12	H-6, H-8	H-4, H-8
H-8	C-6, C-7, C-9, C-10, C-11	H-7, H-9	H-7, H-9
H-9	C-7, C-11, C-12	H-8, H-10	H-8, H-10
H-10	C-7, C-8, C-12	H-9, H-11	
H-11	C-8, C-9, C-10, C-12	H-9, H-10, H-12	H-10, H-12
H-12	C-6, C-9, C-10, C-11, C-14	H-11	H-11, H_3-14
H-13	C-6, C-7, C-11, C-14	H-6, H_3-14	H-6
H_3-14	C-12, C-13	H-13	H-5, H-12

PART VII

METABOLITES FROM THE MARINE-DERIVED FUNGUS *ASPERGILLUS* SP. PSU-F154

CHAPTER 7.1

INTRODUCTION

7.1.1 Introduction

Fungal metabolites of the genus *Aspergillus* reported since the year 2006 are summarized in **Table 276**. The marine-derived fungus *Aspergillus* sp. PSU-F154 was isolated from the sea fan of the genus *Annella*, collected in Phangnga Province, Thailand, in 2005. This fungus was deposited as PSU-F154 at the Department of Microbiology, Faculty of Science, Prince of Songkla University. The ethyl acetate extract from the culture broth of this marine-derived fungus exhibited interesting antioxidation activity with the IC_{50} values of 0.05 ($DPPH^{\cdot}$), 2.56 ($OH^{\cdot}$) and 0.36 ($O_2^{\cdot-}$) mg/mL, antibacterial activity against SA, MRSA and antifungal activity against *Microsporum gypseum* SH-MU-4 (MG) with the MIC values of 128, 128 and 200 μg/mL, respectively. Moreover, the mycelial extract displayed antioxidation activity with the IC_{50} values of 0.13 ($DPPH^{\cdot}$), 3.64 ($OH^{\cdot}$) and 1.81 ($O_2^{\cdot-}$) mg/mL.

Table 276 Compounds isolated from the *Aspergillus* genus

Scientific name	Compound	Activity	Reference
A. carbonarius	8′-*O*-Demethylnigerone, **32** 8′-*O*-Demethylisonigerone, **33** 10,10′-Bifonsecin B, **34** 6′-*O*-Demethylnigerone, **35** Nigerone, **36**	Antimycobacterial	Zhang, *et al.*, 2008

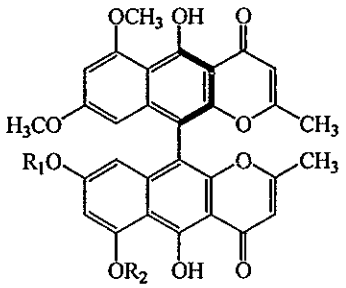
Table 276 Continued

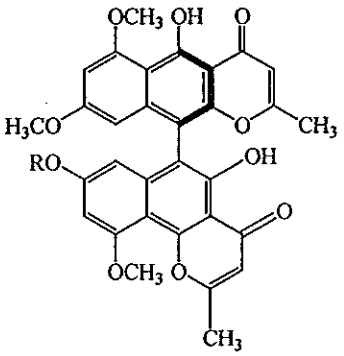
Scientific name	Compound	Activity	Reference
A. carbonarius	Isonigerone, **37** Fonsecin, **38** Rubrofusarin B, **39** TMC256A1, **40** Flavasperone, **41**	Antimycobacterial	Zhang, *et al.*, 2008
A.candidus IF10	Prenylterphenyllin, **42** 4″-Deoxyprenylterphenyllin, **43** 4″-Deoxyisoterprenin, **44** 4″-Deoxyterprenin, **45**	Cytotoxic	Wei, *et al.*, 2007
A. fumigatus	Fumiquinone A, **46** Fumiquinone B, **47** Spinulosin, **48** LL-S490β, **49** Pseurotin A, **50**	Nematicidal	Hayashi, *et al.*, 2007
A. niger	Methyl (*Z*)-4-{[(*Z*)-1-(hydroxymethyl)-2-phenyl-1-ethenyl] amino}-4-oxo-2-butenoate, **51**	Antioxidant	Yuan, *et al.*, 2006
	(*Z*)-5-Ethylidene-8-hydroxy-3,4,5,6,7,8-hexahydro-1*H*-pyrano[3,4-*c*]pyridine-1-one, **52**	Anti-inflammatory	Chang, *et al.*, 2008
A.ostianus	Aspergillide A, **53** Aspergillide B, **54** Aspergillide C, **55**	Cytotoxic	Kito, *et al.*, 2008
A. versicolor	Kipukasin A, **56** Kipukasin B, **57** Kipukasin C, **58**	Antibacterial	Jiao, *et al.*, 2007

Table 276 Continued

A. versicolor	Kipukasin D, **59** Kipukasin E, **60** Kipukasin F, **61** Kipukasin G, **62**	Antibacterial	Jiao, *et al.*, 2007
	Decumbenone A, **63** Decumbenone B, **64** Versiol, **65**	Cytotoxic and antibacterial	Lee, *et al.*, 2007
Aspergillus sp.	Sch725681, **66**	Antifungal	Yang, *et al.*, 2006

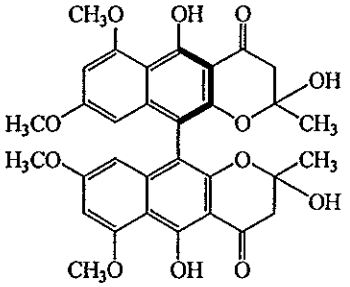
Structures of compounds isolated from the *Aspergillus* genus



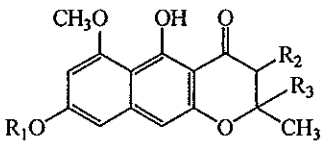


32: $R_1 = H$, $R_2 = CH_3$: 8′-*O*-Demethylnigerone
35: $R_1 = CH_3$, $R_2 = H$: 6′-*O*-Demethylnigerone
36: $R_1 = R_2 = CH_3$: Nigerone

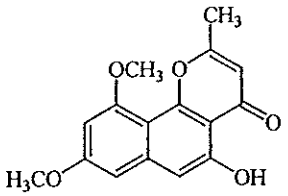
33: R = H : 8′-*O*-Demethylisonigerone
37: R = CH_3 : Isonigerone



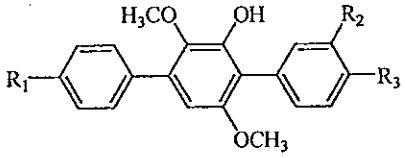
34: 10,10′-Bifonsecin B



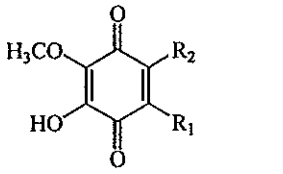
38: $R_1 = R_2 = H$, $R_3 = OH$: Fonsecin
39: $R_1 = CH_3$, $R_2 + R_3$ = double bond : Rubrofusarin B
40: $R_1 = H$, $R_2 + R_3$ = double bond : TMC256A1

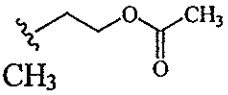


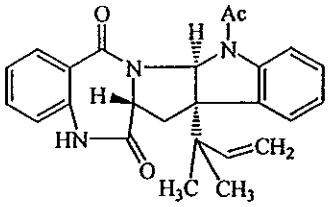
41: Flavasperone



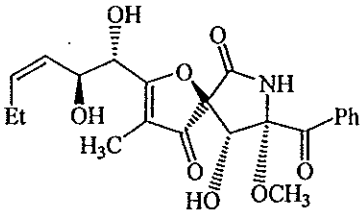
42: $R_1 = R_3 = OH$, R_2 = prenyl : Prenylterphenyllin
43: R_1 = H, R_2 = prenyl , $R_3 = OH$: 4″-Deoxyprenylterphenyllin
44: R_1 = H, R_2 = *O*-prenyl, $R_3 = OH$: 4″-Deoxyisoterprenin
45: R_1 = H, $R_2 = OH$, R_3 = *O*-prenyl : 4″-Deoxyterprenin



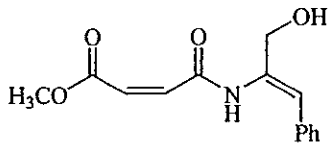
46: $R_1 = CH_3$, R_2 =  : Fumiquinone A
47: $R_1 = OH$, $R_2 = CH_3$: Fumiquinone B
48: $R_1 = CH_3$, $R_2 = OH$: Spinulosin



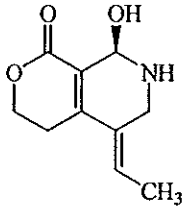
49: LL-S490β



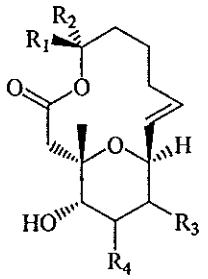
50: Pseurotin A



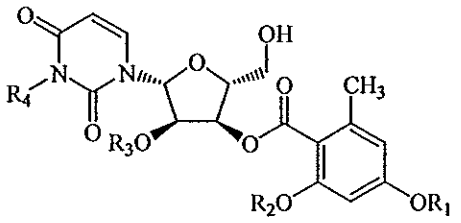
51: Methyl (*Z*)-4-{[(*Z*)-1-(hydroxymethyl)-2-phenyl-1-ethenyl]amino}-4-oxo-2-butenoate



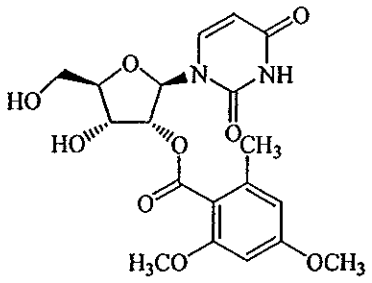
52: (*Z*)-5-Ethylidene-8-hydroxy-3,4,5,6,7,8-hexahydro-1*H*-pyrano[3,4-*c*]pyridine-1-one



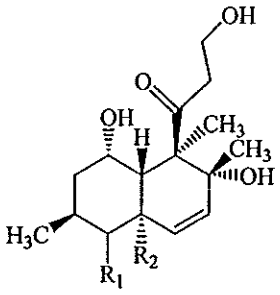
53: $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$: Aspergillide A
54: $R_1 = R_3 = R_4 = H$, $R_2 = CH_3$: Aspergillide B
55: $R_1 = CH_3$, $R_2 = H$, $R_3 + R_4$ = double bond : Aspergillide C



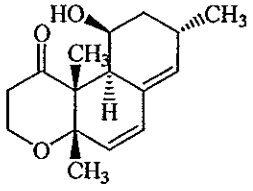
56: $R_1 = R_2 = CH_3$, R_3 = Ac, $R_4 = H$: Kipukasin A
57: $R_1 = R_4 = H$, $R_2 = CH_3$, R_3 = Ac : Kipukasin B
58: $R_1 = CH_3$, $R_2 = R_4 = H$, R_3 = Ac : Kipukasin C
59: $R_1 = R_2 = CH_3$, $R_3 = R_4 = H$: Kipukasin D
61: $R_1 = H$, $R_2 = R_4 = CH_3$, R_3 = Ac : Kipukasin F
62: $R_1 = R_3 = H$, $R_2 = R_4 = CH_3$: Kipukasin G



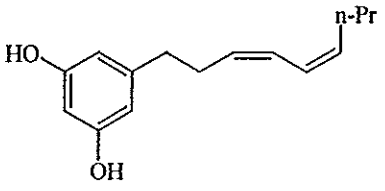
60: Kipukasin E



63: $R_1 + R_2$ = double bond : Decumbenone A
64: $R_1 = R_2 = H$: Decumbenone B



65: Versiol



66: Sch725681

7.1.2 The objectives

1. To isolate the secondary metabolites from the marine-derived fungus *Aspergillus* sp. PSU-F154.
2. To elucidate the structure of the isolated metabolites.

CHAPTER 7.2

EXPERIMENTAL

7.2.1 Fermentation and extraction

The fermentation and the extraction of the culture broth (15 L) and mycelia were performed using the same procedure as those of *Nigrospora* sp. PSU-F18 to afford a brown gum (1.7 g) and a brown gum (900 mg) from the culture broth and mycelia, respectively. Each extract was subjected to chromatographic separation.

7.2.2 Purification of the broth extract

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six fractions as shown in **Table 277.**

Table 277 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
100-1	15.0	Brown gum
100-2	573.6	Brown gum
100-3	630.1	Brown gum
100-4	269.6	Brown gum
100-5	169.5	Brown gum
100-6	17.1	Brown gum

Fraction 100-1 displayed a long tail under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Fraction 100-2 showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.29, 0.39, 0.56 and 0.77. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 278**.

Table 278 Subfractions obtained from **fraction 100-2** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
100-21	9.9	Yellow gum
100-22	501.3	Yellow gum
100-23	57.3	Yellow gum
100-24	5.1	Yellow gum

Subfraction 100-21 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.12 and 0.47. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 100-22 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.14, 0.29 and 0.47. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 70% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 279**.

Table 279 Subfractions obtained from **subfraction 100-22** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
100-221	70% MeOH/H_2O	83.5	Yellow gum
100-222	70% MeOH/H_2O	23.5	Yellow gum
100-223	70% MeOH/H_2O	45.2	Yellow gum
100-224	70% MeOH/H_2O	190.3	Yellow gum
100-225	80% MeOH/H_2O	28.7	Yellow gum
100-226	80% MeOH/H_2O	61.1	Yellow gum
100-227	80% MeOH/H_2O	4.8	Yellow gum
100-228	80% MeOH/H_2O	26.4	Yellow gum
100-229	100% MeOH	30.4	Yellow gum

Subfraction 100-221 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.03, 0.08 and 0.33. Its ^1H NMR spectrum indicated the presence of sugar as a major compound. Thus, it was not further investigated.

Subfraction 100-222 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.13, 0.18, 0.33 and 0.38. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 4% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 280**.

Table 280 Subfractions obtained from **subfraction 100-222** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
222A	4% MeOH/CH_2Cl_2	2.6	Yellow gum

Table 280 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
222B	4% MeOH/CH_2Cl_2	3.0	Yellow gum
222C	6% MeOH/CH_2Cl_2	4.6	Yellow gum
222D	10% MeOH/CH_2Cl_2-100% MeOH	11.8	Yellow gum

Subfraction 222A showed four UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.36, 0.42, 0.50 and 0.84. Because of the minute quantity, it was not further investigated.

Subfraction 222B showed two UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.34 and 0.39. It was then purified by precoated TLC with 40% ethyl acetate in petroleum ether as a mobile phase (5 runs) to afford two bands.

Band 1 was a colorless gum (1.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.45. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 (K65) was a colorless gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.25.

$[\alpha]_D^{29}$	+ 170 (*c* 0.06, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	210 (3.89), 230 (3.71), 280 (3.30), 320 (3.10)
FTIR(neat):υ(cm^{-1})	3400 (O-H stretching), 1725 and 1697 (C=O stretching), 1649 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	11.97 (*s*, 1H), 6.94 (*s*, 1H), 6.76 (*s*, 1H),

	4.76 (*brs*, 2H), 4.09 (*dd*, J = 10.5, 4.0 Hz, 1H), 3.85 (*s*, 3H), 2.96 (*m*, 1H), 2.87 (*m*, 1H), 2.31 (*m*, 1H), 2.21 (*m*, 1H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	180.00, 172.00, 167.22, 160.70, 156.00, 151.00, 117.00, 116.80, 108.91, 104.33, 76.00, 72.69, 64.37, 53.31, 26.03, 24.21
DEPT 135: CH;	108.91, 104.33, 72.69
CH_2;	64.37, 26.03, 24.21
CH_3;	53.31
EIMS *m/z* (% relative intensity):	336 (9), 277 (100), 259 (70), 231 (45), 166 (7)

Subfraction 222C showed two pale UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.13 and 0.34. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 222D showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.13, 0.28 and 0.39. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 100-223 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.15, 0.33 and 0.77. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 281**.

Table 281 Subfractions obtained from **subfraction 100-223** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
223A	2% MeOH/CH_2Cl_2	4.2	Yellow gum
223B	2% MeOH/CH_2Cl_2	3.1	Yellow gum
223C	2% MeOH/CH_2Cl_2	4.7	Yellow gum
223D	2% MeOH/CH_2Cl_2	3.1	Yellow gum
223E	4% MeOH/CH_2Cl_2	4.9	Yellow gum
223F	4-6% MeOH/CH_2Cl_2	5.0	Yellow gum
223G	6-20% MeOH/CH_2Cl_2	3.3	Yellow gum
223H	20% MeOH/CH_2Cl_2-100% MeOH	12.1	Yellow gum

Subfraction 223A showed one pale UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.75. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 223B showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.22 and 0.26. It was then purified by precoated TLC with 40% ethyl acetate in chloroform as a mobile phase (4 runs) to afford two bands.

Band 1 was a colorless gum (1.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in chloroform as a mobile phase with the R_f value of 0.75. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a colorless gum (1.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in chloroform as a

mobile phase with the R_f value of 0.25. Its ^1H NMR spectrum indicated the presence of **K65**. Further investigation was then not performed.

Subfraction 223C showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.59 and 0.71. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (4 runs) to afford **K69** in 2.1 mg as a colorless gum. Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.48.

$[\alpha]_D^{29}$	+ 160 (*c* 0.06, $CHCl_3$)
UV λ_{max}(nm)(MeOH)(log ε)	207 (3.12), 227 (3.35), 282 (3.12)
FTIR(neat):υ(cm^{-1})	3391 (O-H stretching), 1732 and 1686 (C=O stretching)
^1H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	11.90 (*s*, 1H), 6.73 (*s*, 1H), 6.63 (*s*, 1H), 4.11 (*dd*, *J* = 10.0, 3.5 Hz, 1H), 3.87 (*s*, 3H), 2.93 (*ddd*, *J* = 19.0, 6.5, 4.5 Hz, 1H), 2.85 (*ddd*, *J* = 19.0, 9.0, 6.5 Hz, 1H), 2.42 (*s*, 3H), 2.33 (*m*, 1H), 2.21 (*m*, 1H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	182.10, 172.81, 166.81, 160.25, 156.15, 147.83, 116.65, 112.33, 108.10, 107.40, 76.72, 72.75, 53.37, 26.10, 24.23, 22.48
DEPT 135: CH;	112.33, 107.40, 72.75
CH_2;	26.10, 24.23
CH_3;	53.37, 22.48

Subfraction 223D showed two pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.32 and 0.35. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 223E showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.35, 0.40 and 0.45. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction 223F showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.16, 0.20, 0.28 and 0.53. Because of low quantity, it was not further investigated.

Subfraction 223G showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.18, 0.24, and 0.30. Because of the minute quantity, it was not further investigated.

Subfraction 223H showed two UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.10 and 0.28. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Subfraction 100-224 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction 100-225 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.13, 0.21, 0.36 and 0.44. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 282.**

Table 282 Subfractions obtained from **subfraction 100-225** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
225A	2% MeOH/CH_2Cl_2	3.8	Yellow gum
225B	2% MeOH/CH_2Cl_2	6.6	Yellow gum
225C	2% MeOH/CH_2Cl_2	2.2	Yellow gum
225D	4% MeOH/CH_2Cl_2	2.8	Yellow gum
225E	4-8% MeOH/CH_2Cl_2	5.3	Yellow gum
225F	10% MeOH/CH_2Cl_2-100% MeOH	5.4	Yellow gum

Subfraction 225A showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.56, 0.73, 0.78 and 0.93. Because of low quantity, it was not further investigated.

Subfraction 225B showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.43, 0.51 and 0.56. It was then purified by precoated TLC with 20% ethyl acetate in petroleum ether as a mobile phase (3 runs) to afford three bands.

Band 1 was a colorless gum (1.7 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.73. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a colorless gum (3.5 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.37. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was a colorless gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.25. Its ^{1}H NMR spectrum indicated the presence of **K61**. Further investigation was then not performed.

Subfraction 225C showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.46 and 0.53. Its ^{1}H NMR spectrum indicated that the major compound was **K61**. Further investigation was then not performed.

Subfraction 225D showed one pale UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.26. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 225E showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.26, 0.31 and 0.39. Its ^{1}H NMR spectrum indicated that the major compound was **K67**. Further investigation was then not performed.

Subfraction 225F displayed a long tail under UV-S on normal phase TLC using 3% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction 100-226 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.13, 0.28 and 0.41. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 283**.

Table 283 Subfractions obtained from **subfraction 100-226** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
226A	2% MeOH/CH_2Cl_2	4.5	Colorless gum
226B	2% MeOH/CH_2Cl_2	8.4	Colorless gum
226C	2% MeOH/CH_2Cl_2	5.9	Colorless gum
226D	4% MeOH/CH_2Cl_2	5.9	Colorless gum
226E	4-8% MeOH/CH_2Cl_2	28.2	Yellow gum
226F	10% MeOH/CH_2Cl_2-100% MeOH	4.3	Yellow gum

Subfraction 226A showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.56, 0.73, 0.78 and 0.93. Because of low quantity, it was not further investigated.

Subfraction 226B (K61) showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.29.

UV λ_{max}(nm)(MeOH)(log ε)	207 (3.21), 228 (3.10), 270 (3.21)
FTIR(neat):υ(cm^{-1})	3313 (O-H stretching), 1635 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	7.07 (*d*, *J* = 7.8 Hz, 1H), 6.92 (*d*, *J* = 1.5 Hz, 1H), 6.87 (*dd*, *J* = 7.8, 1.5 Hz, 1H), 5.69 (*brs*, 1H), 5.55 (*tq*, *J* = 7.2, 1.2 Hz, 1H), 4.62 (*s*, 2H), 2.32 (*q*, *J* = 7.2 Hz, 2H), 1.98 (*d*, *J* = 1.2 Hz, 3H), 1.60 (*m*, 1H), 1.34 (*m*, 2H), 0.93 (*d*, *J* = 6.6 Hz, 6H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	152.08, 141.05, 132.05, 131.57, 130.47, 128.48, 118.64, 113.78, 65.06, 38.60, 27.81, 26.37, 22.51, 17.87
DEPT 135: CH;	132.05, 128.84, 118.64, 113.78, 27.81
CH_2;	65.06, 38.60, 26.37

CH_3;	22.51, 17.87
EIMS *m/z* (% relative intensity):	234 (37), 175 (97), 147 (100), 133 (31), 91 (43)

Subfraction 226C showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.27, 0.31, and 0.42. Its ^{1}H NMR spectrum indicated that the major compound was **K63**. Further investigation was then not performed.

Subfraction 226D showed four UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.10, 0.15, 0.35 and 0.38. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 226E showed three UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.15, 0.17 and 0.19. It was further separated by flash column chromatography over silica gel. Elution was performed with 4% methanol in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 284.**

Table 284 Subfractions obtained from **subfraction 226E** by flash column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
226E1	4.0	Colorless gum
226E2	9.7	Colorless gum
226E3	12.3	Colorless gum

Subfraction 226E1 (K62) showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.75.

UV λ_{max}(nm)(MeOH)(log ε)	219 (4.20), 250 (4.01), 266 (3.81), 295 (3.65), 303 (3.10)
FTIR(neat):υ(cm^{-1})	3312 (O-H stretching), 1721 (C=O stretching), 1623 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	8.04 (*s*, 1H), 7.89 (*d*, *J* = 8.1 Hz, 1H), 7.37 (*d*, *J* = 8.1 Hz, 1H), 2.67 (*t*, *J* = 7.5 Hz, 2H), 2.10 (*s*, 3H), 1.55 (*m*, 2H), 1.49 (*m*, 1H), 0.86 (*d*, *J* = 6.0 Hz, 6H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	172.00, 158.84, 153.22, 135.64, 124.16, 118.22, 112.73, 109.96, 36.88, 27.67, 24.48, 22.34, 7.81
DEPT 135: CH;	124.16, 118.22, 112.73, 27.67
CH_2;	36.88, 24.48
CH_3;	22.34, 7.81
EIMS *m/z* (% relative intensity):	246 (31), 189 (100), 175 (13), 115 (11)

Subfraction 226E2 showed three pale UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.15, 0.24 and 0.35. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 226E3 showed two UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.25 and 0.35. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Subfraction 226F displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction 100-227 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.18

and 0.33. It was then purified by precoated TLC with 40% ethyl acetate in petroleum ether as a mobile phase (5 runs) to afford two bands.

Band 1 (K63) was a colorless gum (2.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.74.

$[\alpha]_D^{29}$	+ 2 (*c* 1.9, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	230 (3.73), 283 (3.15)
FTIR(neat):υ(cm^{-1})	3400 (OH stretching), 1720 (C=O stretching), 1659 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	8.96 (*s*, 1H), 7.49 (*s*, 1H), 7.49 (*d*, *J* = 8.5 Hz, 1H), 7.03 (*d*, *J* = 8.5 Hz, 1H), 3.16 (*s*, 3H), 1.77 (*m*, 1H), 1.75 (*m*, 1H), 1.55 (*s*, 3H), 1.42 (*m*, 1H), 1.27 (*m*, 1H), 1.05 (*m*, 3H), 0.75 (*d*, *J* = 6.5 Hz, 6H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	169.38, 156.05, 133.78, 129.89, 127.46, 121.18, 118.61, 83.03, 50.57, 39.82, 39.04, 27.77, 22.54, 22.45, 22.18, 21.55
DEPT 135: CH;	127.46, 121.18, 118.61, 27.77
CH_2;	39.82, 39.04, 21.55
CH_3;	50.57, 22.18, 22.54, 22.45
EIMS *m/z* (% relative intensity):	280 (3), 246 (30), 178 (100), 165 (38), 83 (20)

Band 2 (K67) was a colorless gum (1.4 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.45.

$[\alpha]_D^{29}$	+ 2 (*c* 2.0 MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	232 (3.87), 281 (3.13)
FTIR(neat):υ(cm^{-1})	3349 (O-H stretching), 1720 (C=O stretching),

	1657 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	7.57 (*brs*, 1H), 7.55 (*dd*, *J* = 8.1, 1.2 Hz, 1H), 7.08 (*d*, *J* = 8.1 Hz, 1H), 1.94 (*dt*, *J* = 14.7, 8.1 Hz, 1H), 1.82 (*dt*, *J* = 14.7, 8.1 Hz, 1H), 1.68 (*s*, 3H), 1.50 (*m*, 1H), 1.31 (*m*, 2H), 1.17 (*m*, 2H), 0.84 (*d*, *J* = 6.6 Hz, 3H), 0.82 (*d*, *J* = 6.6 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	171.50, 156.71, 135.10, 129.72, 126.37, 121.10, 119.36, 79.17, 42.88, 38.96, 29.10, 27.76, 22.52, 22.49, 21.61,
DEPT 135: CH;	126.37, 121.10, 119.36, 27.76
CH_2;	42.88, 38.96, 21.61
CH_3;	22.52, 22.49, 29.10

Subfraction 100-228 showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.08, 0.15, 0.24, 0.62, 0.61 and 0.88. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction 100-229 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction 100-23 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.17 and 0.46. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 285**.

Table 285 Subfractions obtained from **subfraction 100-23** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
100-231	2% MeOH/CH_2Cl_2	3.0	Yellow gum
100-232	2% MeOH/CH_2Cl_2	1.2	Yellow gum
100-233	2% MeOH/CH_2Cl_2	2.4	Yellow gum
100-234	3% MeOH/CH_2Cl_2	5.0	Yellow gum
100-235	3% MeOH/CH_2Cl_2	2.3	Yellow gum
100-236	5% MeOH/CH_2Cl_2	0.9	Yellow gum
100-237	5% MeOH/CH_2Cl_2	18.5	Yellow gum
100-238	5-10% MeOH/CH_2Cl_2	4.8	Yellow gum
100-239	20% MeOH/CH_2Cl_2-100% MeOH	15.2	Yellow solid

Subfraction 100-231 showed none of major spots under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 100-232 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.53. Its ^1H NMR spectrum indicated that the major compound was **K71**. Further investigation was then not performed.

Subfraction 100-233 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.43 and 0.53. It was then purified by precoated TLC with 10% ethyl acetate in dichloromethane as a mobile phase (3 runs) to afford two bands.

Band 1 was a colorless gum (1.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% ethyl acetate in dichloromethane as

a mobile phase with the R_f value of 0.50. Its ^1H NMR spectrum indicated that the major compound was **K71**. Further investigation was then not performed.

Band 2 (K64) was a colorless gum (1.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% ethyl acetate in dichloromethane as a mobile phase with the R_f value of 0.40.

$[\alpha]_D^{29}$	-1.4 (*c* 0.4, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	220 (4.13), 263 (3.81), 292 (3.79), 304 (3.15)
FTIR(neat):υ(cm^{-1})	3380 (O-H stretching), 1745 and 1681 (C=O stretching), 1653 (C=C stretching)
^1H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	12.30 (*s*, 1H), 6.71 (*brs*, 1H), 6.63 (*brs*, 1H), 6.61 (*dd*, *J* = 10.0, 5.0 Hz, 1H), 6.45 (*d*, *J* = 10.0 Hz, 1H), 4.87 (*m*, 1H), 4.29 (*d*, *J* = 3.5 Hz, 1H), 3.73 (*s*, 3H), 2.41 (*brs*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	180.97, 170.86, 160.33, 158.50, 155.61, 147.23, 137.83, 122.96, 112.38, 110.13, 108.85, 107.47, 65.21, 52.78, 44.87, 22.37
DEPT 135: CH;	137.83, 122.96, 112.38, 107.47, 65.21, 44.87
CH_3;	52.78, 22.37
EIMS *m/z* (% relative intensity):	284 (10), 252 (22), 242 (100), 197 (5)

Subfraction 100-234 showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.20, 0.23, 0.28, 0.33, 0.35 and 0.43. Because of low quantity, it was not further investigated.

Subfraction 100-235 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values 0.15 and 0.20. Its ^1H NMR spectrum indicated that the major compound was **K68**. Further investigation was then not performed.

Subfraction 100-236 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.13, 0.22 and 0.28. Because of the minute quantity, it was not further investigated.

Subfraction 100-237 showed four UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.13, 0.19, 0.38 and 0.50. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 286.**

Table 286 Subfractions obtained from **subfraction 100-237** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
237A	4.7	Yellow gum
237B	3.9	Yellow gum
237C	4.5	Yellow gum
237D	5.3	Yellow gum

Subfraction 237A showed four UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.24, 0.31, 0.34 and 0.46. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 237B showed two UV-active spots on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f values of 0.22 and 0.24. Its ^1H NMR spectrum indicated that the major compounds were **K68** and **K71**. Further investigation was then not performed.

Subfraction 237C showed one UV-active spot on reverse phase TLC using 50% methanol in water as a mobile phase with the R_f value of 0.24. Its ^1H NMR

spectrum indicated that the major compound was **K71**. Further investigation was then not performed.

Subfraction 237D displayed a long tail under UV-S on reverse phase TLC using 50% methanol in water as a mobile phase. Its ^{1}H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction 100-238 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.13, 0.24, 0.31, 0.41 and 0.48. Because of low quantity, it was not further investigated.

Subfraction 100-239 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 100-24 showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.17 and 0.40. It was then purified by precoated TLC with 5% ethyl acetate in petroleum ether as a mobile phase (4 runs) to afford **K71** in 2.1 mg as a colorless gum. Its chromatogram showed one UV-active spot on normal phase TLC using 5% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.65.

UV λ_{max}(nm)(MeOH)(log ε)	233 (3.26), 257 (3.11), 291 (2.89), 301 (2.54)
FTIR(neat):υ(cm^{-1})	3400 (O-H stretching), 1736 and 1697 (C=O stretching), 1651 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	12.21 (*s*, 1H), 7.76 (*dd*, *J* = 8.5, 7.0 Hz, 1H), 7.56 (*dd*, *J* = 8.5, 1.0 Hz, 1H), 7.33 (*dd*, *J* = 7.0, 1.0 Hz, 1H), 6.99 (*s*, 1H), 6.77 (*s*, 1H), 4.78 (*s*, 2H), 4.04 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	180.62, 169.00, 162.00, 156.50, 151.80, 135.00, 133.63, 122.67, 119.82, 115.25, 108.24, 108.21, 104.22, 64.44, 53.20

DEPT 135: CH; 135.00, 122.67, 119.82, 108.21, 104.22

CH_2; 64.44

CH_3; 53.20

Fraction 100-3 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.17, 0.29 and 0.44. It was then separated by column chromatography over reverse phase silica gel. Elution was performed initially with 60% methanol in water followed by increasing amount of methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 287.**

Table 287 Subfractions obtained from **fraction 100-3** by column chromatography over reverse phase silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
100-31	60% MeOH/H_2O	69.0	Yellow gum
100-32	60% MeOH/H_2O	260.3	Yellow gum
100-33	60% MeOH/H_2O	154.1	Yellow gum
100-34	60% MeOH/H_2O	26.3	Yellow gum
100-35	60% MeOH/H_2O	10.4	Yellow gum
100-36	70% MeOH/H_2O	35.6	Yellow gum
100-37	70% MeOH/H_2O	14.6	Yellow gum
100-38	70-80% MeOH/H_2O	24.2	Yellow gum
100-39	100% MeOH	21.4	Yellow gum

Subfraction 100-31 showed five UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.12, 0.17, 0.18 and 0.23. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 4% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol.

Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 288.**

Table 288 Subfractions obtained from **subfraction 100-31** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
31A	4% MeOH/CH_2Cl_2	2.8	Colorless gum
31B	4% MeOH/CH_2Cl_2	2.9	Colorless gum
31C	4-6% MeOH/CH_2Cl_2	4.4	Colorless gum
31D	6% MeOH/CH_2Cl_2	5.4	Colorless gum
31E	10-20% MeOH/CH_2Cl_2	6.8	Colorless gum
31F	40% MeOH/CH_2Cl_2-100% MeOH	41.2	Colorless gum

Subfraction 31A showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.54, 0.57 and 0.63. Because of the minute quantity, it was not further investigated.

Subfraction 31B showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.50 and 0.61. It was then purified by precoated TLC with 1% methanol in dichloromethane as a mobile phase (8 runs) to afford **K75** in 2.0 mg as a colorless gum. Its chromatogram showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.76.

UV λ_{max}(nm)(MeOH)(log ε)	207 (3.15), 217 (3.07), 274 (2.89)
FTIR(neat):υ(cm^{-1})	3349 (O-H stretching), 1639 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	6.42 (*d*, *J* = 2.0 Hz, 1H), 6.40 (*d*, *J* = 2.0 Hz, 1H), 6.30 (*t*, *J* = 2.0 Hz, 1H), 2.27 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	158.10, 156.46, 140.99, 112.23, 111.15, 103.47, 21.47

DEPT 135:	CH;	112.23, 111.15, 103.47
	CH_3;	21.47

Subfraction 31C showed four UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.44, 0.48, 0.53 and 0.63. Because of low quantity, it was not further investigated.

Subfraction 31D showed two UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.22 and 0.42. It was then purified by precoated TLC with 4% methanol in dichloromethane as a mobile phase (5 runs) to afford a colorless gum in 3.5 mg. Its chromatogram showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.26. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 31E showed six UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.19, 0.24, 0.29, 0.33 and 0.39. Because of low quantity, it was not further investigated.

Subfraction 31F displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 100-32 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.24, 0.33 and 0.42. Its ^{1}H NMR spectrum indicated that the major compound was **K68**. Further investigation was then not performed.

Subfraction 100-33 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.33. Its ^{1}H NMR spectrum displayed high field signals. Thus, it was not investigated.

Subfraction 100-34 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12, 0.17, 0.21 and 0.26. It was further separated by flash column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 289**.

Table 289 Subfractions obtained from **subfraction 100-34** by flash column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
34A	2% MeOH/CH_2Cl_2	1.9	Yellow gum
34B	2% MeOH/CH_2Cl_2	1.2	Yellow gum
34C	4-6% MeOH/CH_2Cl_2	5.1	Yellow gum
34D	6% MeOH/CH_2Cl_2	6.2	Colorless gum
34E	10-20% MeOH/CH_2Cl_2	8.1	Yellow gum
34F	40% MeOH/CH_2Cl_2-100% MeOH	3.0	Yellow gum

Subfraction 34A showed two UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.50 and 0.59. Its ^1H NMR spectrum indicated that the major compound was **K71**. Further investigation was then not performed.

Subfraction 34B displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 34C showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.21 and 0.27. Its ^1H NMR spectrum indicated that the major compound was **K71**. Further investigation was then not performed.

Subfraction 34D (K74) showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.27.

$[\alpha]_D^{29}$	-206 (*c* 1.0, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	213 (3.51), 261 (3.43), 310 (2.83)
FTIR(neat):υ(cm^{-1})	3321 (O-H stretching), 1690 (C=O stretching)
^{1}H NMR(DMSO-d_6)(δ_{ppm}) (300 MHz):	10.90 (*d*, *J* = 1.5 Hz, 1H), 7.93 (*d*, *J* = 2.1 Hz, 1H), 7.71 (*d*, *J* = 2.4 Hz, 1H), 7.49 (*d*, *J* = 7.8 Hz, 1H), 7.32 (*d*, *J* = 7.8 Hz, 1H), 7.16 (*m*, 3H), 7.08 (*t*, *J* = 7.8 Hz, 1H), 7.00 (*t*, *J* = 7.8 Hz, 1H), 6.96 (*d*, *J* = 1.5 Hz, 1H), 6.69 (*dd*, *J* = 7.5, 1.8 Hz, 2H), 3.98 (*m*, 1H), 3.85 (*m*, 1H), 2.81 (*dd*, *J* = 14.9, 4.5 Hz, 1H), 2.53 (*dd*, *J* = 14.9, 5.7 Hz, 1H), 2.45 (*dd*, *J* = 13.2, 4.8 Hz, 1H), 1.81 (*dd*, *J* = 13.2, 7.2 Hz, 1H)
^{13}C NMR(DMSO-d_6)(δ_{ppm}) (75 MHz):	167.33, 166.70, 136.91, 136.48, 130.14, 127.96, 126.86, 126.81, 124.90, 121.39, 119.22, 118.92, 111.80, 109.20, 56.07, 55.71, 40.25, 30.13
DEPT 135: CH;	130.14, 126.86, 126.81, 124.90, 121.39, 119.22, 118.92, 111.80, 56.07, 55.71
CH_2;	40.25, 30.13

Subfraction 34E showed three pale UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.23, 0.35 and 0.41. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 34F displayed a long tail under UV-S on normal phase TLC using 4% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 100-35 showed none of major spots under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 100-36 showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.21, 0.31, 0.36, 0.47 and 0.54. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 290.**

Table 290 Subfractions obtained from **subfraction 100-36** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
36A	2% MeOH/CH_2Cl_2	2.3	Yellow gum
36B	2% MeOH/CH_2Cl_2	1.2	Yellow gum
36C	2% MeOH/CH_2Cl_2	8.4	Yellow gum
36D	2-4% MeOH/CH_2Cl_2	6.3	Yellow gum
36E	4-6% MeOH/CH_2Cl_2	3.2	Yellow gum
36F	10% MeOH/CH_2Cl_2-100% MeOH	13.3	Yellow gum

Subfraction 36A (K70) showed one UV-active spot on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f value of 0.45.

UV λ_{max}(nm)(MeOH)(log ε)	233 (3.22), 257 (3.10), 291 (2.84), 310 (2.65), 361 (2.45)
FTIR(neat):υ(cm^{-1})	3351 (O-H stretching), 1731 and 1695 (C=O stretching), 1657 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	12.09 (*s*, 1H), 7.68 (*dd*, *J* = 8.7, 7.5 Hz, 1H),

	7.46 (*dd*, *J* = 8.7, 0.9 Hz, 1H), 7.24 (*dd*, *J* = 7.5, 0.9 Hz, 1H), 6.70 (*brs*, 1H), 6.58 (*brs*, 1H), 3.96 (*s*, 3H), 2.37 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(75 MHz):	180.00, 169.50, 162.00, 157.00, 156.00, 149.50, 134.74, 133.80, 122.51, 119.41, 117.00, 111.74, 107.38, 106.96, 53.09, 22.67
DEPT 135: CH;	134.74, 122.51, 119.41, 111.74, 107.38
CH_3;	53.09, 22.67

Subfraction 36B showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.54. Its ^{1}H NMR spectrum indicated that the major compound was **K71**. Further investigation was then not performed.

Subfraction 36C showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.19, 0.34, 0.41 and 0.54. Its ^{1}H NMR spectrum indicated that the major compound was **K71**. Further investigation was then not performed.

Subfraction 36D showed four pale UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.17, 0.37, 0.41 and 0.54. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 36E showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.03, 0.11, 0.31, 0.33, 0.42 and 0.45. Because of low quantity, it was not further investigated.

Subfraction 36F displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 100-37 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.17 and 0.71. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Subfraction 100-38 showed two pale UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.12 and 0.38. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 100-39 showed none of major spots under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Fraction 100-4 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.10, 0.17, 0.27 and 0.56. It was further separated by column chromatography over silica gel. Elution was performed initially with 1% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 291.**

Table 291 Subfractions obtained from **fraction 100-4** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
100-41	1% $MeOH/CH_2Cl_2$	6.6	Brown gum
100-42	2% $MeOH/CH_2Cl_2$	8.2	Yellow gum
100-43	2% $MeOH/CH_2Cl_2$	6.8	Yellow gum
100-44	2-3% $MeOH/CH_2Cl_2$	7.3	Yellow gum
100-45	5-7% $MeOH/CH_2Cl_2$	130.1	Yellow gum
100-46	7-10% $MeOH/CH_2Cl_2$	10.3	Yellow gum
100-47	10% $MeOH/CH_2Cl_2$	19.7	Yellow gum

Table 291 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
100-48	20-40% MeOH/CH_2Cl_2	31.3	Yellow gum
100-49	50% MeOH/CH_2Cl_2-100% MeOH	20.0	Yellow gum

Subfraction 100-41 showed three pale UV-active spots on normal phase TLC using 100% dichloromethane as a mobile phase with the R_f values of 0.34, 0.61 and 0.85. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 100-42 showed four UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.36, 0.52, 0.59 and 0.67. It was then purified by precoated TLC with 30% ethyl acetate in petroleum ether as a mobile phase (4 runs) to afford four bands.

Band 1 was a colorless gum (3.0 mg). Its chromatogram showed one pale UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.71. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Band 2 was a yellow gum (2.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.45. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 (K72) was a yellow gum (0.9 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.33.

UV λ_{max}(nm)(MeOH)(log ε)	231 (4.12), 255 (3.83), 289 (3.63), 330 (2.31)
FTIR(neat):υ(cm^{-1})	3351 (O-H stretching), 1739 and 1697 (C=O stretching), 1640 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	12.23 (*s*, 1H), 7.51 (*d*, *J* = 9.0 Hz, 1H), 7.40 (*d*, *J* = 9.0 Hz, 1H), 6.77 (*s*, 1H), 6.66 (*s*, 1H), 4.03 (*s*, 3H), 2.49 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	180.42, 169.10, 161.18, 155.65, 152.00, 150.50, 148.83, 125.41, 122.29, 118.64, 113.00, 111.41, 107.26, 53.07, 22.61
DEPT 135: CH;	125.41, 122.29, 111.41, 107.26
CH_3;	53.07, 22.61

Band 4 was a yellow gum (4.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.31. Its ^{1}H NMR spectrum indicated the presence of **K73**. Further investigation was then not performed.

Subfraction 100-43 showed none of major spots under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 100-44 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.10, 0.11 and 0.19. It was then purified by precoated TLC with 40% ethyl acetate in petroleum ether as a mobile phase (8 runs) to afford three bands.

Band 1 was a colorless gum (1.9 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.89. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a colorless gum (2.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.42. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 (K68) was a colorless gum (2.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.33.

$[\alpha]_D^{29}$	-1 (*c* 0.4, MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	213 (4.21), 261 (4.03), 330 (3.72)
FTIR(neat):υ(cm^{-1})	3391 (O-H stretching), 1734 and 1698 (C=O stretching), 1653 (C=C stretching)
^{1}H NMR($CDCl_3$)(δ_{ppm})(300 MHz):	12.24 (*s*, 1H), 6.80 (*brs*, 1H), 6.66 (*brs*, 1H), 6.56 (*dd*, *J* = 9.9, 5.1 Hz, 1H), 6.37 (*d*, *J* = 9.9 Hz, 1H), 4.78 (*m*, 1H), 4.66 (*s*, 2H), 4.23 (*d*, *J* = 3.6 Hz, 1H), 3.62 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	181.08, 170.79, 160.68, 158.76, 155.84, 149.76, 138.25, 122.83, 110.32, 109.80, 108.95, 104.49, 65.01, 64.40, 52.81, 44.76
DEPT 135: CH;	138.25, 122.83, 108.95, 104.49, 65.01, 44.76
CH_2;	64.40
CH_3;	52.81

Subfraction 100-45 showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.24. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified

Subfraction 100-46 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.41, 0.39

and 0.46. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 100-47 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.19 and 0.53. It was then separated by column chromatography over reverse phase silica gel. Elution was performed with 50% methanol in water. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 292.**

Table 292 Subfractions obtained from **subfraction 100-47** by column chromatography over reverse phase silica gel

Subfraction	Weight (mg)	Physical appearance
100-471	8.3	Yellow gum
100-472	7.1	Yellow gum
100-473	4.1	Yellow gum

Subfraction 100-471 showed one pale UV-active spot on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f value of 0.18. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 100-472 showed two UV-active spots on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f values of 0.05 and 0.18. It was further separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 293.**

Table 293 Subfractions obtained from **subfraction 100-472** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
100-472A	2.4	Yellow gum
100-472B	3.5	Yellow gum
100-472C	1.9	Yellow gum

Subfraction 100-472A showed two pale UV-active spots on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f values of 0.12 and 0.15. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 100-472B showed one UV-active spot on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f value of 0.35. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 100-472C showed none of major spots under UV-S on normal phase TLC using 6% methanol in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 100-473 showed two pale UV-active spots on normal phase TLC using 6% methanol in dichloromethane as a mobile phase with the R_f value of 0.19 and 0.23. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 100-48 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Subfraction 100-49 (K73) showed one UV-active spot on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f value of 0.34.

UV λ_{max}(nm)(MeOH)(log ε)	207 (3.21), 228 (4.31), 263 (3.71), 292 (3.15)
FTIR(neat):υ(cm^{-1})	3321 (OH stretching), 1729 and 1683 (C=O stretching)
^{1}H NMR(DMSO-d_6)(δ_{ppm}) (300 MHz):	12.19 (*s*, 1H), 10.50 (*brs*, 1H), 7.62 (*d*, *J* = 9.0 Hz, 1H), 7.47 (*d*, *J* = 9.0 Hz, 1H), 6.97 (*s*, 1H), 6.73 (*s*, 1H), 5.54 (*brs*, 1H), 4.58 (*s*, 2H), 3.85 (*s* ,3H)
^{13}C NMR(DMSO-d_6)(δ_{ppm}) (75 MHz):	180.78, 167.26, 160.91, 155.97, 154.52, 151.51, 149.36, 125.79, 120.59, 117.66, 117.51, 107.60, 107.05, 104.39, 62.80, 52.71
DEPT 135: CH;	125.79, 120.59, 107.60, 104.39
CH_2;	62.80
CH_3;	52.71

Fraction 100-5 showed five UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.19, 0.24, 0.56 and 0.61. It was further separated by column chromatography over silica gel. Elution was performed initially with 40% ethyl acetate in petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 294.**

Table 294 Subfractions obtained from **fraction 100-5** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
100-51	40% EtOAc/Petrol	4.3	Yellow gum
100-52	40% EtOAc/Petrol	9.4	Yellow gum
100-53	40% EtOAc/Petrol	8.3	Yellow gum
100-54	40-70% EtOAc/Petrol	14.2	Yellow gum
100-55	70% EtOAc/Petrol - 100% EtOAc	20.7	Yellow gum

Table 294 Continued

Subfraction	Elution	Weight (mg)	Physical appearance
100-56	100% EtOAc - 2% MeOH/EtOAc	11.0	Brown solid
100-57	2-10% MeOH/EtOAc	18.2	Brown solid
100-58	20% MeOH/EtOAc - 100% MeOH	53.1	Brown solid

Subfraction 100-51 showed two pale UV-active spots on normal phase TLC using 20% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.33 and 0.89. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 100-52 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.26, 0.51 and 0.77. It was then purified by precoated TLC with 30% ethyl acetate in petroleum ether as a mobile phase (3 runs) to afford two bands.

Band 1 was a colorless gum (3.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.77. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a red gum (4.0 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f value of 0.53. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 100-53 showed three UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.28,

0.51 and 0.65. It was then purified by precoated TLC with 10% ethyl acetate in dichloromethane as a mobile phase (3 runs) to afford two bands.

Band 1 was a colorless gum (1.1 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% ethyl acetate in dichloromethane as a mobile phase with the R_f value of 0.78. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was a yellow gum (2.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 10% ethyl acetate in dichloromethane as a mobile phase with the R_f value of 0.68. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 100-54 showed two UV-active spots on normal phase TLC using 30% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.28 and 0.41. It was further separated by column chromatography over silica gel. Elution was performed initially with 10% ethyl acetate in dichloromethane and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 295.**

Table 295 Subfractions obtained from **subfraction 100-54** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
100-541	10-20% EtOAc/CH_2Cl_2	3.7	Yellow gum
100-542	20-40% EtOAc/CH_2Cl_2	5.0	Colorless gum
100-543	60% EtOAc/CH_2Cl_2-100% MeOH	4.3	Yellow solid

Subfraction 100-541 showed two pale UV-active spots on normal phase TLC using 10% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.76

and 0.80. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 100-542 showed one UV-active spot on normal phase TLC using 10% ethyl acetate in dichloromethane as a mobile phase with the R_f value of 0.22. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 100-543 displayed a long tail under UV-S on normal phase TLC using 10% ethyl acetate in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 100-55 showed three UV-actives spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.17, 0.33 and 0.35. It was further separated by flash column chromatography over silica gel. Elution was performed with 10% ethyl acetate in dichloromethane. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 296.**

Table 296 Subfractions obtained from **subfraction 100-55** by flash column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
100-551	3.1	Yellow gum
100-552	6.9	Yellow gum
100-553	5.8	Yellow gum
100-554	3.0	Yellow gum

Subfraction 100-551 showed three UV-active spots on normal phase TLC using 10% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.65, 0.78 and 0.97. Because of the minute quantity, it was not further investigated.

Subfraction 100-552 showed two UV-active spots on normal phase TLC using 10% ethyl acetate in dichloromethane as a mobile phase with the R_f values of 0.19 and 0.34. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 100-553 showed one UV-active spot on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase with the R_f value of 0.34. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

Subfraction 100-554 displayed a long tail under UV-S on normal phase TLC using 20% ethyl acetate in dichloromethane as a mobile phase. Thus, it was not investigated.

Subfraction 100-56 showed four UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.08, 0.16, 0.20 and 0.77. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 100-57 showed three UV-active spots on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase with the R_f values of 0.08, 0.13 and 0.25. Because its ^1H NMR spectrum showed broad signals, it was not further purified.

Subfraction 100-58 displayed a long tail under UV-S on normal phase TLC using 40% ethyl acetate in petroleum ether as a mobile phase. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

Fraction 100-6 displayed a long tail under UV-S on normal phase TLC using 1% methanol in dichloromethane as a mobile phase. Its ^1H NMR spectrum displayed signals in the high field region. Thus, it was not investigated.

7.2.3 Purification of the EtOAc extract from mycelia

The crude EtOAc extract was separated by column chromatography over Sephadex LH-20. Elution was performed with 100% methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford five fractions as shown in **Table297.**

Table 297 Fractions obtained from the crude EtOAc extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
100-CE1	360.4	Brown gum
100-CE2	179.4	Brown gum
100-CE3	186.2	Brown solid
100-CE4	114.9	Brown solid
100-CE5	57.4	Brown solid

Fraction 100-CE1 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 100-CE2 showed six UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.05, 0.14, 0.16, 0.29, 0.45 and 0.64. It was further separated by column chromatography over silica gel. Elution was performed initially with 2% methanol in dichloromethane followed by increasing the polarity with methanol and finally with pure methanol. Fractions with similar chromatogram were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 298.**

Table 298 Subfractions obtained from **fraction 100-CE2** by column chromatography over silica gel

Subfraction	Elution	Weight (mg)	Physical appearance
100-CE21	2% MeOH/CH_2Cl_2	4.2	Yellow gum
100-CE22	2% MeOH/CH_2Cl_2	11.2	Yellow gum
100-CE23	2% MeOH/CH_2Cl_2	9.1	Yellow gum
100-CE24	4% MeOH/CH_2Cl_2	11.8	Yellow gum
100-CE25	4-6% MeOH/CH_2Cl_2	7.9	Yellow gum
100-CE26	6-10% MeOH/CH_2Cl_2	48.6	Brown gum
100-CE27	10% MeOH/CH_2Cl_2	6.8	Brown solid
100-CE28	10% MeOH/CH_2Cl_2-100% MeOH	58.8	Brown solid

Subfraction 100-CE21 showed three UV-active spots on normal phase TLC using 1% methanol in dichloromethane as a mobile phase with the R_f values of 0.44, 0.56 and 0.85. Its ^{1}H NMR spectrum indicated that the major compound was **K71**. Further investigation was then not performed.

Subfraction 100-CE22 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.32, 0.37, 0.61 and 0.71. Its ^{1}H NMR spectrum indicated that the major compound was **K70**. Further investigation was then not performed.

Subfraction 100-CE23 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.37, 0.45 and 0.51. It was then purified by precoated TLC with 2% methanol in dichloromethane as a mobile phase (3 runs) to afford two bands.

Band 1 was a colorless gum (3.2 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a

mobile phase with the R_f value of 0.53. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 (K66) was a colorless gum (2.3 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f value of 0.36.

$[\alpha]_D^{29}$	- 186 (*c* 1.0 MeOH)
UV λ_{max}(nm)(MeOH)(log ε)	210 (3.11), 217 (3.82), 263 (3.51), 283 (3.01)
FTIR(neat):υ(cm^{-1})	3321 (O-H stretching), 1685 (C=O stretching)
^1H NMR($CDCl_3$)(δ_{ppm})(500 MHz):	7.33 (*dd*, *J* = 7.5, 1.0 Hz, 2H), 7.28 (*d*, *J* = 7.5 Hz, 1H), 7.27 (*d*, *J* = 7.0 Hz, 1H), 7.17 (*t*, *J* = 7.5 Hz, 2H), 7.16 (*td*, *J* = 7.0, 1.0 Hz, 1H), 6.82 (*td*, *J* = 7.0, 1.0 Hz, 1H), 6.67 (*d*, *J* = 7.0 Hz, 1H), 5.49 (*s*, 1H), 5.19 (*s*, 1H), 4.17 (*dm*, *J* = 10.5 Hz, 1H), 3.88 (*ddd*, *J* = 11.0, 6.0, 1.0 Hz, 1H), 3.57 (*dd*, *J* = 14.5, 3.5 Hz, 1H), 2.73 (*dd*, J = 14.5, 11.5 Hz, 1H), 2.69 (*t*, *J* = 11.0 Hz, 1H), 2.58 (*dd*, *J* = 11.0, 6.0 Hz, 1H)
^{13}C NMR($CDCl_3$)(δ_{ppm})(125 MHz):	168.43, 165.73, 150.01, 135.51, 130.05, 129.46, 128.97, 127.76, 126.45, 125.45, 119.22, 110.34, 58.74, 56.19, 36.65, 35.33
DEPT 135: CH;	130.05, 129.46, 128.97, 127.76, 125.45, 119.22, 110.34, 58.74, 56.19
CH_2;	36.65, 35.35

Subfraction 100-CE24 showed two UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.26 and 0.33. Its ^1H NMR spectrum indicated that the major compound was **K68**. Further investigation was then not performed.

Subfraction 100-CE25 showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.26, 0.33 and 0.39. Its ^1H NMR spectrum indicated that the major compound was **K68**. Further investigation was then not performed.

Subfraction 100-CE26 showed three UV-active spots on normal phase TLC using 3% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.17 and 0.24. Its ^1H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Subfraction 100-CE27 showed two UV-active spots on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f values of 0.11 and 0.14. It was then purified by precoated TLC with 4% methanol in dichloromethane as a mobile phase (5 runs) to afford two bands.

Band 1 was a colorless gum (1.6 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.10. Its ^1H NMR spectrum indicated the presence of **K41** (see chapter 4). Further investigation was then not performed.

Band 2 was a colorless gum (1.9 mg). Its chromatogram showed one UV-active spot on normal phase TLC using 4% methanol in dichloromethane as a mobile phase with the R_f value of 0.15. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction 100-CE28 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 100-CE3 showed three UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.21, 0.42 and 0.64. Its ^{1}H NMR spectrum displayed signals of long chain hydrocarbons. Thus, it was not purified.

Fraction 100-CE4 showed four UV-active spots on normal phase TLC using 2% methanol in dichloromethane as a mobile phase with the R_f values of 0.07, 0.19, 0.32 and 0.45. Because its ^{1}H NMR spectrum showed broad signals, it was not further purified.

Fraction 100-CE5 displayed a long tail under UV-S on normal phase TLC using 2% methanol in dichloromethane as a mobile phase. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further investigated.

CHAPTER 7.3

RESULTS AND DISCUSSION

Five new metabolites **(K61-K65)** along with nine known compounds **(K67-75)** were isolated from the broth extract while one new metabolite **(K66)** was isolated from the mycelial extract. The structures were identified by spectroscopic methods.

7.3.1 Compound K61

Compound **K61** was obtained as a colorless gum with the molecular formula $C_{15}H_{22}O_2$ from EIMS (*m/z* 234) **(Figure 144)**. The UV spectrum displayed absorption bands at λ_{max} 207, 228 and 270 nm, indicating that **K61** had an aromatic chromophore. The IR spectrum exhibited absorption bands at 3313 and 1635 cm^{-1} for hydroxyl and double bond functional groups, respectively. The ^1H NMR spectrum **(Figure 145) (Table 299)** showed characteristic signals for three aromatic protons of a 1,2,4-trisubstituted benzene [δ 7.07 (*d*, J = 7.8 Hz, 1H), 6.92 (*d*, J = 1.5 Hz, 1H) and 6.87 (*dd*, J = 7.8 and 1.5 Hz, 1H)], an isopentyl moiety [δ 2.32 (*q*, J = 7.2 Hz, 2H), 1.60 (*m*, 1H), 1.34 (*m*, 2H) and 0.93 (*d*, J = 6.6 Hz, 6H), one hydroxy proton (δ 5.69, *brs*, 1H), one olefinic proton of a trisubstitued double bond (δ 5.55, *tq*, J = 7.2 and 1.2 Hz, 1H), one hydroxymethyl group (δ 4.62, *s*, 2H) and one methyl group (δ 1.98, *d*, J = 1.2 Hz, 3H). The ^{13}C NMR **(Figure 146) (Table 299)** and DEPT 135 **(Table 299)** spectra showed fourteen carbon resonances for fifteen carbons: four quaternary (δ 152.08, 141.05, 131.57 and 130.47), five methine (δ 132.05, 128.48, 118.64, 113.78 and 27.81), three methylene (δ 65.06, 38.60 and 26.37) and two methyl (δ 22.51 and 17.87) carbons. The substituent at C-1 of the 1,2,4-trisubstituted benzene ring was identified as a hydroxyl group according to the chemical shift of C-1 (δ 152.08). Three aromatic protons resonating at δ 7.07, 6.92 and 6.87 were assigned

as H-5, H-2 and H-4, respectively, on the basis of their coupling constants, multiplicity and HMBC correlations. The hydroxymethylene protons, H_2-15 (δ 4.62), showed HMBC correlations **(Table 299)** with C-2 (δ 113.78), C-3 (141.05) and C-4 (118.64), suggesting that the hydroxymethyl unit was located at C-3 of the benzene ring. Signal enhancement of H-2 (δ 6.92) and H-4 (δ 6.87) after irradiation of H_2-15 in the NOEDIFF experiment **(Table 300)** supported the assigned location. In the ^{1}H-^{1}H COSY spectrum **(Table 300)**, the methylene protons, H_2-9 (δ 2.32), of the isopentyl unit showed a cross peak with the olefinic proton, H-8 (δ 5.55), which was further coupled with the methyl protons, H_3-14 (δ 1.98), to form a 1,5-dimethyl-1-hexenyl fragment. The attachment of the 1,5-dimethyl-1-hexenyl unit at C-6 (δ 130.47) of the benzene ring was supported by HMBC correlations of H-8 and H_3-14 with C-6. The configuration of the double bond in the 1,5-dimethyl-1-hexenyl unit was assigned as *E* on the basis of signal enhancement of H_2-9 upon irradiation of H_3-14 in the NOEDIFF experiment. Therefore, **K61** was assigned as a new phenol derivative.

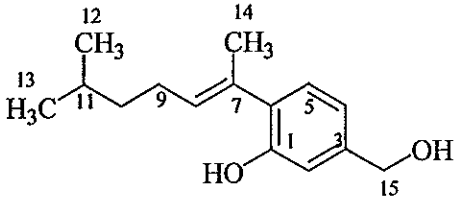


Table 299 The ^{1}H, ^{13}C NMR and HMBC data of compound **K61** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1-OH	5.69 (*brs*)	152.08 (C)	C-1, C-2, C-6
2	6.92 (*d*, 1.5)	113.78 (CH)	C-1, C-4, C-6, C-15
3	-	141.05 (C)	-
4	6.87 (*dd*, 7.8, 1.5)	118.64 (CH)	C-2, C-6, C-15
5	7.07 (*d*, 7.8)	128.48 (CH)	C-1, C-3, C-7
6	-	130.47 (C)	-
7	-	131.57 (C)	-
8	5.55 (*tq*, 7.2, 1.2)	132.05 (CH)	C-6, C-9, C-14
9	2.32 (*q*, 7.2)	26.37 (CH_2)	C-7, C-10, C-11

Table 299 Continued

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
10	1.34 (*m*)	38.60 (CH_2)	C-8, C-9, C-11, C-12, C-13
11	1.60 (*m*)	27.81 (CH)	C-9, C-12, C-13
12	0.93 (*d*, 6.6)	22.51 (CH_3)	C-10, C-11, C-13
13	0.93 (*d*, 6.6)	22.51 (CH_3)	C-10, C-11, C-12
14	1.98 (*d*, 1.2)	17.87 (CH_3)	C-6, C-7, C-8
15	4.62 (*s*)	65.06 (CH_2)	C-2, C-3, C-4

Table 300 The COSY and NOEDIFF data of compound **K61** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H-4	H_2-15
H-4	H-2, H-5	H-5, H_2-15
H-5	H-4	H-4, H_3-14
H-8	H_2-9, H_3-14	H_2-9, H_2-10
H_2-9	H-8, H_2-10, H_3-14	H-8, H-11, H_3-12, H_3-14
H_2-10	H_2-9, H-11	H-8, H_3-12, H_3-13
H-11	H_2-10, H_3-12, H_3-13	-
H_3-12	H-11	H_2-9, H_2-10, H-11
H_3-13	H-11	H_2-9, H_2-10, H-11
H_3-14	H-8, H_2-9	H-5, H_2-9, H_2-10, H-11
H_2-15	-	H-2, H-4

7.3.2 Compound K62

Compound **K62** was obtained as a colorless gum with the molecular formula $C_{15}H_{18}O_3$ on the basis of EIMS (*m/z* 246) **(Figure 147)**. The UV spectrum with the maximum absorption bands at 219, 250, 266, 295 and 303 nm, suggested that **K62** had a benzofuran chromophore (Trofast, *et al.*, 1978). The IR spectrum was similar to that of **K61**. The ^{1}H NMR spectrum **(Figure 148) (Table 301)** was almost

identical to that of **K61** except for the absence of the hydroxy, olefinic and hydroxymethyl protons in **K62**. The major differences were the downfield shift of H-4 (δ7.37), H-5 (δ7.07) and H-7 (δ8.04) in **K62** when they were compared with H-5, H-4 and H-2 in **K61**, respectively. These results together with ^3J HMBC correlations **(Table 301)** of H-5 and H-7 with the same carboxylic carbonyl carbon, C-14 (δ 172.00), indicated the replacement of the hydroxymethyl group in **K61** with a carboxyl group in **K62**. Furthermore, the methyl protons, H_3-13 (δ2.10), gave HMBC cross peaks with C-2 (δ 158.84), C-3 (δ 109.96) and C-3a (δ 135.64). The chemical shifts of C-2 and C-7a (δ 153.22) together with the molecular formula and the UV data confirmed that **K62** had the benzofuran skeleton. Consequently, **K62** was assigned as a new benzofuran derivative.

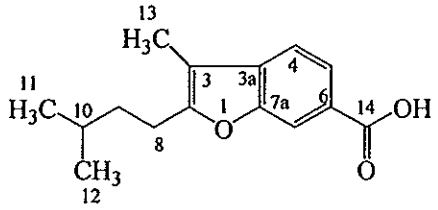


Table 301 The ^{1}H, ^{13}C NMR and HMBC data of compound **K62** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
2	-	158.84 (C)	-
3	-	109.96 (C)	-
3a	-	135.64 (C)	-
4	7.37 (*d*, 8.1)	118.22 (CH)	C-3, C-3a, C-5, C-6, C-7a
5	7.89 (*d*, 8.1)	124.16 (CH)	C-3a, C-6, C-7, C-14
6	-	135.64 (C)	-
7	8.04 (*s*)	112.73 (CH)	C-3a, C-5, C-6, C-7a, C-14
7a	-	153.22 (C)	-
8	2.67 (*t*, 7.5)	24.48 (CH_2)	C-2, C-3, C-9, C-10
9	1.55 (*m*)	36.88 (CH_2)	C-2, C-8, C-10, C-11, C-12
10	1.49 (*m*)	27.67 (CH)	C-9, C-11, C-12
11	0.86 (*d*, 6.0)	22.34 (CH_3)	C-9, C-10, C-12

Table 301 Continued

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
12	0.86 (*d*, 6.0)	22.34 (CH_3)	C-9, C-10, C-11
13	2.10 (*s*)	7.84 (CH_3)	C-2, C-3, C-3a
14	-	172.00 (C)	-

Table 302 The COSY and NOEDIFF data of compound **K62** in $CDCl_3$

Proton	COSY	NOEDIFF
H-4	H-5	H-5, H_3-13
H-5	H-4, H-7	H-4
H-7	H-5	-
H_2-8	H_2-9	H_2-9, H_3-11, H_3-12, H_3-13
H_2-9	H_2-8, H-10	H_2-8, H_3-11, H_3-12
H-10	H_2-9, H_3-11, H_3-12	H_2-8, H_3-11, H_3-12
H_3-11	H-10	H_2-9, H-10
H_3-12	H-10	H_2-9, H-10
H_3-13	-	H-4, H_2-8

7.3.3 Compound K67

Compound **K67** was obtained as a colorless gum. The UV spectrum showed absorption bands at 232 and 281 nm, indicating that **K67** had a benzene chromophore. Hydroxyl (3349 cm^{-1}) and carbonyl (1720 cm^{-1}) groups were found in the IR spectrum. The ^1H NMR spectrum **(Figure 161) (Table 303)** exhibited characteristic signals for three aromatic protons of a 1,2,4-trisubstituted benzene [δ 7.57 (*brs*, 1H), 7.55 (*dd*, J = 8.1 and 1.2 Hz, 1H) and 7.08 (*d*, J = 8.1 Hz, 1H)], an isohexyl unit [δ 1.94 (*dt*, J = 14.7 and 8.1 Hz, 1H), 1.82 (*dt*, J = 14.7 and 8.1 Hz, 1H), 1.50 (*m*, 2H), 1.31 (*m*, 1H), 1.17 (*m*, 2H), 0.84 (*d*, J = 6.6 Hz, 3H) and 0.82 (*d*, J = 6.6 Hz, 3H)] and one singlet methyl protons (δ 1.68, *s*, 3H). The ^{13}C NMR **(Figure 162) (Table 303)** and DEPT 135 **(Table 303)** spectra displayed five quaternary (δ 171.50,

156.17, 135.10, 129.72 and 79.17), three methine (δ 126.37, 121.10 and 119.36), three methylene (δ 42.88, 38.96 and 21.61) and three methyl (δ 29.10, 22.52 and 22.49) carbons. The substituent at C-1 (δ 156.17) of the benzene ring was identified as a hydroxyl group on the basis of its chemical shift. The aromatic protons at δ 7.57, 7.55 and 7.08 were assigned to be H-2, H-4 and H-5, respectively, according to their coupling constants and multiplicity. H-4 (δ 7.55) showed a HMBC correlation **(Table 304)** with a carboxylic carbonyl carbon, C-14 (δ 171.50), indicating that the carboxyl group was located at C-3 (δ 129.72). The methyl protons, H_3-15 (δ 1.68), were correlated with C-7 (δ 79.17) and C-8 (δ 42.88) of the isohexyl unit. These results together with the chemical shift of C-7 constructed a 2-hydroxy-6-methyl-heptyl fragment. The linkage between C-6 (δ 135.10) of the benzene ring and C-7 of the heptyl unit was established according to the HMBC cross peaks of H_{ab}-8 (δ 1.94 and 1.82) and H_3-15 with C-6. The observed optical rotation of **K67**, $[\alpha]^{23}$ +2 (*c* 2.0, MeOH), was almost identical to that of (+)-(7*S*)-sydonic acid, $[\alpha]^{20}$ +2.73 (*c* 2.3, MeOH), indicating that they had the same absolute configuration at C-7. Therefore, **K67** was determined as (+)-(7*S*)-sydonic acid which was previously isolated from *Glonium* sp. (Kudo, *et al.*, 2009).

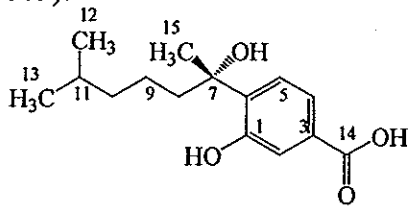


Table 303 The ^1H and ^{13}C NMR data of compound **K67** in $CDCl_3$ and **(+)-(7*S*)-Sydonic acid** in CD_3OD

Position	K67		(+)-(7*S*)- Sydonic acid	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})[a]	δ_C (C-type)[b]
1	-	156.17 (C)	-	156.91 (C)
2	7.57 (*brs*)	119.36 (CH)	7.36 (*d*, 1.6)	118.60 (CH)
3	-	129.72 (C)	-	131.58 (C)
4	7.55 (*dd*, 8.1, 1.2)	121.10 (CH)	7.43 (*dd*, 7.9, 1.6)	121.50 (CH)
5	7.08 (*d*, 8.1)	126.37 (CH)	7.25 (*d*, 7.9)	127.77 (CH)
6	-	135.10 (C)	-	138.00 (C)

Table 303 Continued

Position	K67		(+)-(7*S*)- Sydonic acid	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})[a]	δ_C (C-type)[b]
7	-	79.17 (C)	-	77.88 (C)
8	a: 1.94 (*dt*, 14.7, 8.1)	42.88 (CH_2)	a: 1.94 (*ddd*, 13.7,	43.57 (CH_2)
	b: 1.82 (*dt*, 14.7, 8.1)		12.2, 4.5)	
			b: 1.77 (*ddd*,	
			13.7, 11.6, 4.8)	
9	1.31 (*m*)	21.61 (CH_2)	a: 1.33 (*m*)	22.86 (CH_2)
			b: 1.18 (*m*)	
10	1.17 (*m*)	38.96 (CH_2)	1.13 (*m*)	40.43 (CH_2)
11	1.50 (*m*)	27.76 (CH)	1.47 (*m*)	28.82 (CH)
12	0.84 (*d*, 6.6)	22.52 (CH_3)	0.81 (*d*, 6.5)	22.90 (CH_3)
13	0.82 (*d*, 6.6)	22.49 (CH_3)	0.81 (*d*, 6.5)	22.98 (CH_3)
14	-	171.50 (C)	-	169.86 (C)
15	1.68 (*s*)	29.10 (CH_3)	1.59 (*s*)	28.99 (CH_3)

[a] ^{1}H NMR in CD_3OD (400 MHz), [b] ^{13}C NMR in CD_3OD (125 MHz)

Table 304 The HMBC, COSY and NOEDIFF data of compound **K67** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
H-2	C-4	H-4	-
H-4	C-2, C-5, C-6, C-14	H-2, H-5	H-5
H-5	C-1, C-3, C-14	H-4	H-4, H_3-15
H_a-8	C-6, C-7, C-9, C-10, C-15	H_b-8, H_2-9	-
H_b-8	C-6, C-7, C-9, C-10, C-15	H_a-8, H_2-9	-
H_2-9	C-7, C-8, C-10, C-11	H_{ab}-8, H_2-10	-
H_2-10	C-8, C-11, C-12, C-13	H_2-9, H-11	-
H-11	C-9, C-10, C-12, C-13	H_2-10, H_3-12, H_3-13	-
H_3-12	C-10, C-11, C-13	H-11	-
H_3-13	C-10, C-11, C-12	H-11	-
H_3-15	C-6, C-7, C-8	-	H-5

7.3.4 Compound K63

Compound **K63** was obtained as a colorless gum whose molecular formula was assigned as $C_{16}H_{24}O_4$ by EIMS (*m/z* 280) **(Figure 150)**. The UV and IR spectra were almost identical to those of **K67**. Its ^{1}H NMR spectrum **(Figure 151)** **(Table 305)** was similar to that of **K67** except for the presence of an additional signal for a methoxyl group (δ 3.16, *s*, 3H). The DEPT 135 spectrum **(Table 305)** showed the presence of a methoxy carbon (δ 50.57), supporting above conclusion. The methoxy protons, H_3-16, showed a HMBC correlation **(Table 305)** with C-7 (δ 83.03), indicating that the hydroxyl group in **K67** was replaced with the methoxyl group in **K63**. The observed optical rotation of **K63**, $[\alpha]^{23}$ +2 (*c* 1.9, MeOH), was almost identical to that of (+)-(7*S*)-sydonic acid, $[\alpha]^{20}$ +2.73 (*c* 2.3, MeOH), indicating that they had the same absolute configuration at C-7. Therefore, **K63** was identified as a new methyl ether of (+)-(7*S*)-sydonic acid.

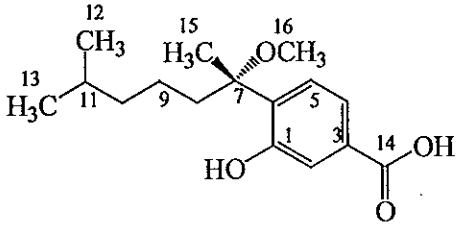


Table 305 The ^{1}H, ^{13}C NMR and HMBC data of compound **K63** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1-OH	8.96 (*s*)	156.05 (C)	-
2	7.49 (*s*)	118.61 (CH)	C-1, C-3
3	-	129.89 (C)	-
4	7.49 (*d*, 8.5)	121.18 (CH)	C-6, C-7, C-14
5	7.03 (*d*, 8.5)	127.46 (CH)	C-1, C-3, C-7, C-14
6	-	133.78 (C)	-
7	-	83.03 (C)	-
8	a: 1.77 (*m*)	39.82 (CH_2)	C-7, C-9, C-10
	b: 1.75 (*m*)		C-7, C-9, C-10

Table 305 Continued

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
9	a: 1.27 (*m*)	21.55 (CH_2)	C-10
	b: 1.05 (*m*)		C-10
10	1.05 (*m*)	39.04 (CH_2)	C-8, C-9, C-11
11	1.42 (*m*)	27.77 (CH)	C-10, C-12, C-13
12	0.75 (*d*, 6.5)	22.54 (CH_3)	C-10, C-11, C-13
13	0.75 (*d*, 6.5)	22.45 (CH_3)	C-10, C-11 ,C-12
14	-	169.38 (C)	-
15	1.55 (*s*)	22.18 (CH_3)	C-6, C-7, C-8
16	3.16 (*s*)	50.57 (CH_3)	C-7

Table 306 The COSY and NOEDIFF data of compound **K63** in $CDCl_3$

Proton	COSY	NOEDIFF
H-4	H-5	H-5
H-5	H-4	H-4, H_3-15
H_a-8	H_b-8, H_{ab}-9	-
H_b-8	H_a-8, H_{ab}-9	-
H_a-9	H_{ab}-8, H_b-9	-
H_b-9	H_{ab}-8, H_a-9	-
H_2-10	H_{ab}-9, H-11	-
H-11	H_2-10, H_3-12, H_3-13	-
H_3-12	H-11	-
H_3-13	H-11	-
H_3-15	-	H-5, H_3-16
H_3-16	-	H_3-15

7.3.5 Compound K68

Compound **K68** was obtained as a colorless gum. The UV spectrum displayed absorption bands at 213, 261 and 330 nm while absorption bands at 3391, 1734 and 1698 cm^{-1} in the IR spectrum indicated the presence of hydroxyl, conjugated ester carbonyl and conjugated ketone carbonyl groups, respectively. The ^1H NMR spectrum **(Figure 163) (Table 307)** consisted of signals for one chelated hydroxy proton (δ 12.24, *s*, 1H), two *meta*-coupled aromatic protons [δ 6.80 (*brs*, 1H) and 6.66 (*brs*, 1H)], two *cis*-olefinic protons [δ 6.56 (*dd*, J = 9.9 and 5.1 Hz, 1H) and 6.37 (*d*, J = 9.9 Hz, 1H)], two methine protons [δ 4.78 (*m*, 1H) and 4.23 (*d*, J = 2.3 Hz, 1H)], one hydroxymethyl group (δ 4.66, *s*, 2H) and one methoxyl group (δ 3.62, *s*, 3H). The ^{13}C NMR **(Figure 164) (Table 307)** and DEPT 135 **(Table 307)** spectra showed two carbonyl (δ 181.08 and 170.79), six quaternary (δ 160.68, 158.76, 155.84, 149.76, 110.32 and 109.80), six methine (δ 138.25, 122.83, 108.95, 104.49, 65.01 and 44.76), one oxymethylene (δ 64.40) and one methoxy (δ 52.81) carbons. The chelated hydroxy proton, 1-OH (δ 12.14), was placed at C-1 (δ 160.68), a *peri*-position of the carbonyl group, and gave HMBC correlations **(Table 308)** with C-1, C-2 (δ 108.95) and C-9a (δ 109.80). The *meta*-coupled aromatic proton resonating at 6.66 was assigned as H-2 on the basis of its HMQC correlation with C-2 **(Table 307)**. Thus, the remaining *meta*-coupled aromatic proton at δ 6.80 was attributed to H-4. The hydroxymethyl protons, H_2-11 (δ 4.66), showed HMBC cross peaks with C-2, C-3 (δ 149.76) and C-4 (δ 104.49), indicating the location of the hydroxymethyl group at C-3. Irradiation of H_2-11 in the NOEDIFF experiment **(Table 308)** enhanced signal intensity of H-2 and H-4, supporting the above conclusion. One of the *cis*-olefinic protons, H-5 (δ 6.37), showed HMBC cross peaks with C-6 (δ 138.25), C-7 (δ 65.01) and C-8a (δ 110.32). The other one, H-6 (δ 6.56), gave a ^1H-^1H COSY cross peak **(Table 308)** with the oxymethine proton, H-7 (δ 4.78), which was further coupled with the methine proton, H-8 (δ 4.23). A dihydroxanthone skeleton was established on the basis of a HMBC correlation of H-8 with C-8a as well as the chemical shift of the ketone carbonyl carbon, C-9 (δ 181.08). The methoxy protons, H_3-13 (δ 3.62), and H-8 showed a HMBC correlation with the same ester carbonyl carbon, C-12

(δ 170.79), revealing that the methoxycarbonyl group was connected to C-8. Irradiation of H-7, did not affect signal intensity of H-8, suggesting that they were *trans*-oriented. The observed optical rotation of **K68**, $[\alpha]^{25}$ -1 (*c* 0.4, MeOH), was almost identical to that of (7*R*,8*R*)-AGI-B4, $[\alpha]^{25}$ -1.6 (*c* 0.4, MeOH), indicating that they had the same absolute configuration. Therefore, **K68** was assigned as (7*R*,8*R*)-AGI-B4 which was previously isolated from *Aspergillus* sp. Y80118 (Kim, *et al.*, 2002).

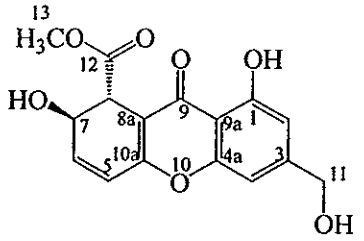


Table 307 The ^{1}H and ^{13}C NMR data of compound **K68** in $CDCl_3$ and **(7*R*,8*R*)-AGI- B4** in acetone-d_6

Position	K68		(7*R*,8*R*)-AGI-B4	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1-OH	12.24 (*s*)	160.68 (C)	-	161.5 (C)
2	6.66 (*brs*)	108.95 (CH)	6.78 (*brs*)	109.7 (CH)
3	-	149.76 (C)	-	152.5 (C)
4	6.80 (*brs*)	104.49 (CH)	7.01 (*brs*)	105.7 (CH)
4a	-	155.84 (C)	-	157.1 (C)
5	6.37 (*d*, 9.9)	122.83 (CH)	6.52 (*d*, 9.9)	123.2 (CH)
6	6.56 (*dd*, 9.9, 5.1)	138.25 (CH)	6.60 (*dd*, 9.9, 4.8)	140.6 (CH)
7	4.78 (*m*)	65.01 (CH)	4.71 (*dd*, 4.8, 3.6)	65.8 (CH)
8	4.23 (*d*, 3.6)	44.76 (CH)	4.15 (*d*, 3.6)	46.2 (CH)
8a	-	110.32 (C)	-	111.6 (C)
9	-	181.08 (C)	-	182.5 (C)
9a	-	109.80 (C)	-	110.4 (C)
10a	-	158.76 (C)	-	160.8 (C)

Table 307 Continued

Position	K68		(7*R*,8*R*)-AGI-B4	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_H (*mult.*, J_{Hz})
11	4.66 (*s*)	64.40 (CH_2)	4.67 (*s*)	64.3 (CH_2)
12	-	170.79 (C)	-	172.8 (C)
13	3.62 (*s*)	52.81 (CH_3)	3.70 (*s*)	53.2 (CH_3)

Table 308 The HMBC, COSY and NOEDIFF data of compound **K68** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
1-OH	C-1, C-2, C-9a	-	-
H-2	C-1, C-4, C-11	H-4, H_3-11	H_2-11
H-4	C-2, C-4a, C-11	H-2, H_3-11	H_2-11
H-5	C-6, C-7, C-8a	H-6	H-5
H-6	C-7, C-8, C-10a	H-5, H-7	H-6
H-7	-	H-6, H-8	-
H-8	C-6, C-7, C-8a, C-12	H-7	-
H_2-11	C-2, C-3, C-4	H-2, H-4	H-2, H-4
H_3-13	C-12	-	-

7.3.6 Compound K64

Compound **K64** with the molecular formula $C_{16}H_{14}O_4$ from EIMS (*m/z* 302) **(Figure 153)** was obtained as a colorless gum. The UV and IR spectra were almost identical to those of **K68**. The ^1H NMR **(Figure 154) (Table 309)** spectrum was similar to that of **K68** except for the presence of an aromatic methyl protons at δ 2.41 instead of the hydroxymethyl group in **K68**. The appearance of the aromatic methyl group was supported by signal of a methyl carbon at δ 22.37 in the ^{13}C NMR spectrum **(Table 309)**. The location of the methyl group, H_3-11 (δ 2.41), was

confirmed by the HMBC correlations **(Table 309)** of H_3-11 with C-2 (δ 112.38), C-3 (δ 147.23) and C-4 (δ 107.47) as well as signal enhancement of H-2 (δ 6.63) and H-4 (δ 6.71) upon irradiation of H_3-11 in the NOEDIFF experiment **(Table 310)**. Consequently, **K64** was characterized as a new dihydroxanthone derivative.

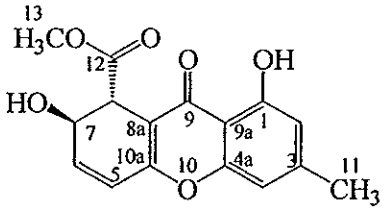


Table 309 The ^1H, ^{13}C NMR and HMBC data of compound **K64** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1-OH	12.30 (*s*)	160.33 (C)	C-1, C-2, C-9a
2	6.63 (*brs*)	112.38 (CH)	C-1, C-4, C-9a, C-11
3	-	147.23 (C)	-
4	6.71 (*brs*)	107.47 (CH)	C-2, C-4a, C-9, C-9a, C-11
4a	-	155.61 (C)	-
5	6.45 (*d*, 10.0)	122.96 (CH)	C-6, C-7, C-8a, C-9, C-10a
6	6.61 (*dd*, 10.0, 5.0)	137.83 (CH)	C-5, C-7, C-8, C-10a
7	4.87 (*m*)	65.21 (CH)	-
8	4.29 (*d*, 3.5)	44.87 (CH)	C-6, C-7, C-8a, C-9, C-10a, C-12
8a	-	110.13 (C)	-
9	-	180.97 (C)	-
9a	-	108.85 (C)	-
10a	-	158.50 (C)	-
11	2.41 (*brs*)	22.37 (CH_3)	C-2, C-3, C-4
12	-	170.86 (C)	-
13	3.73 (*s*)	52.78 (CH_3)	C-12

Table 310 The COSY and NOEDIFF data of compound **K64** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H-4, H_3-11	H_3-11
H-4	H-2, H_3-11	H_3-11
H-5	H-6	H-6
H-6	H-5, H-7	H-5
H-7	H-6, H-8	-
H-8	H-7	-
H_3-11	H-2, H-4	-

7.3.7 Compound K70

Compound **K70** was obtained as a yellow gum. The UV and IR data revealed the presence of a xanthone skeleton. The ^{1}H NMR spectrum **(Figure 167)** **(Table 311)** was similar to that of **K64** except for the replacement of signals for the *cis*-olefinic, hydroxymethine and methine protons in ring A of **K64** with signals of three aromatic protons of a 1,2,3-trisubstituted benzene [δ 7.68 (*dd*, J = 8.7 and 7.5 Hz, 1H), 7.46 (*dd*, J = 8.7 and 0.9 Hz, 1H) and 7.24 (*dd*, J = 7.5 and 0.9 Hz, 1H). These protons were attributed to H-6, H-5 and H-7 on the basis of their coupling constants, multiplicity and the following ^3J HMBC correlations **(Table 312)**; H-5 (δ 7.46)/C-7 (δ 122.51) and C-8a (δ 117.00), H-6 (δ 7.68)/C-8 (δ 133.80) and C-10a (δ 156.00) and H-7 (δ 7.24)/C-5 (δ 119.41), C-8a and C-12 (δ 169.50). Thus, **K70** was determined to be methyl 1-hydroxy-3-methyl-9-oxo-*9H*-xanthene-8-carboxylate which was previously isolated from *Penicillium* sp. (ZZF 32#) (Shao, *et al.*, 2008).

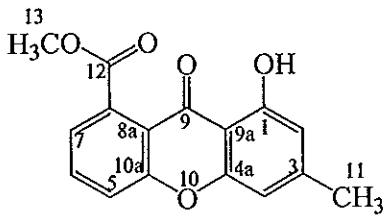


Table 311 The ^{1}H and ^{13}C NMR data of compound **K70** and **Methyl 1-hydroxy-3-methyl-9-oxo-*9H*-xanthene-8-carboxylate** in $CDCl_3$

Position	K70		Methyl 1-hydroxy-3-methyl-9-oxo-*9H*-xanthene-8-carboxylate	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1-OH	12.09 (*s*)	162.00 (C)	12.14 (*s*)	161.5 (C)
2	6.58 (*brs*)	111.74 (CH)	6.63 (*d*, 1.2)	111.7 (CH)
3	-	149.50 (C)	-	149.4 (C)
4	6.70 (*brs*)	107.38 (CH)	6.75 (*d*, 1.2)	107.4 (CH)
4a	-	157.00 (C)	-	155.7 (C)
5	7.46 (*dd*, 8.7, 0.9)	119.41 (CH)	7.53 (*dd*, 8.4, 1.2)	119.4 (CH)
6	7.68 (*dd*, 8.7, 7.5)	134.74 (CH)	7.74 (*dd*, 8.4, 7.2)	134.8 (CH)
7	7.24 (*dd*, 7.5, 0.9)	122.51 (CH)	7.31 (*dd*, 7.2, 1.2)	122.5 (CH)
8	-	133.80 (C)	-	133.6 (C)
8a	-	117.00 (C)	-	117.6 (C)
9	-	180.00 (C)	-	180.4 (C)
9a	-	106.96 (C)	-	107.0 (C)
10a		156.00 (C)	-	156.0 (C)
11	2.37 (*s*)	22.67 (CH_3)	2.50 (*s*)	22.6 (CH_3)
12	-	169.50 (C)	-	169.7 (C)
13	3.96 (*s*)	53.09 (CH_3)	4.01 (*s*)	53.1 (CH_3)

Table 312 The HMBC and COSY data of compound **K70** in $CDCl_3$

Proton	HMBC	COSY
1-OH	C-1, C-2, C-9a	-
H-2	C-4, C-11	H-4, H_3-11
H-4	C-2, C-4a, C-9a, C-11	H-2, H_3-11
H-5	C-7, C-8a	H-6, H-7
H-6	C-8, C-10a	H-5, H-7
H-7	C-5, C-8a, C-12	H-5, H-6

Table 312 Continued

Proton	HMBC	COSY
H_3-11	C-2, C-3, C-4	H-2, H-4
H_3-13	C-12	-

7.3.8 Compound K71

Compound **K71** was obtained as a yellow gum. The UV and IR data were identical to those of **K70**. Its ^{1}H NMR spectrum **(Figure 169) (Table 313)** revealed the replacement of signal for the aromatic methyl protons in **K70** with a singlet resonance of hydroxymethylene protons (δ 4.78, *s*, 2H) in **K71**. The hydroxymethyl protons, H_2-11 (δ4.78), showed HMBC correlations **(Table 314)** with C-2 (δ 108.21), C-3 (δ 151.80) and C-4 (δ 104.22). These results together with signal enhancement of both H-2 (δ 6.77) and H-4 (δ 6.99) after irradiation of H_2-11 in the NOEDIFF experiment **(Table 314)** confirmed the location of the hydroxymethyl group at C-3. Therefore, **K71** was assigned as sydowinin A which was previously isolated from *Aspergillus sydowi* (Hamasaki, *et al.*, 1975).

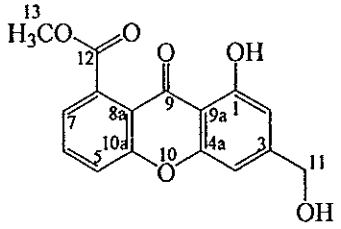


Table 313 The ^{1}H and ^{13}C NMR data of compound **K71** in $CDCl_3$ and **Sydowinin A** in DMSO-d_6

Position	K71		Sydowinin A
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})
1-OH	12.21 (*s*)	162.00 (C)	11.92 (*s*)
2	6.77 (*s*)	108.21 (CH)	6.72 (*d*, 1.5)
3	-	151.80 (C)	-

Table 313 Continued

Position	K71		Sydowinin A
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})
4	6.99 (*s*)	104.22 (CH)	6.93 (*d*, 1.5)
4a	-	156.50 (C)	-
5	7.56 (*dd*, 8.5, 1.0)	119.82 (CH)	7.60 (*dd*, 9.0, 2.0)
6	7.76 (*dd*, 8.5, 7.0)	135.00 (CH)	7.85 (*dd*, 9.0, 6.0)
7	7.33 (*dd*, 7.0, 1.0)	122.67 (CH)	7.46 (*dd*, 6.0, 2.0)
8	-	133.63 (C)	-
8a	-	115.25 (C)	-
9	-	180.62 (C)	-
9a	-	108.24 (C)	-
10a	-	156.50 (C)	-
11	4.78 (*s*)	64.44 (CH_2)	4.56 (*d*, 6.0)
11-OH	-	-	5.43 (*t*, 6.0)
12	-	169.00 (C)	-
13	4.04 (*s*)	53.20 (CH_3)	3.88 (*s*)

Table 314 The HMBC and COSY data of compound **K71** in $CDCl_3$

Proton	HMBC	COSY
1-OH	C-1, C-2, C-3, C-9a	-
H-2	C-1, C-5, C-11	H-4, H_2-11
H-4	C-2, C-4a, C-8a, C-11	H-2, H_2-11
H-5	C-7, C-8a, C-10a	H-6, H-7
H-6	C-8, C-10a	H-5, H-7
H-7	C-5, C-8a, C-12	H-6, H-8
H_2-11	C-2, C-3, C-4	H-2, H-4
H_3-13	C-12	-

7.3.9 Compound K72

Compound **K72** was obtained as a yellow gum. The UV and IR data were similar to those of **K70**. The ^{1}H NMR spectrum **(Figure 171) (Table 315)** was also similar to that of **K70** except for the fact that signals for aromatic protons in ring A of **K70** were replaced by signals of two *ortho*-coupled aromatic protons [δ 7.51 (*d*, J = 9.0 Hz, 1H) and 7.40 (*d*, J = 9.0 Hz, 1H)]. The aromatic protons at δ 7.51 and 7.40 were assigned as H-5 and H-6, respectively, according to the following HMBC correlations **(Table 316)**: H-5/C-7 (δ 152.00), C-8a (δ 118.64) and C-10a (δ 150.50) and H-6/C-7, C-8 (δ 113.00), C-10a and C-12 (δ 169.10). The substituent at C-7 was identified to be a hydroxyl group on the basis of its chemical shift (δ 152.00). Consequently, **K72** was characterized as pinselin which was previously isolated from *Talaromyces bacillosporus* (Yamazaki, *et al.*, 1980).

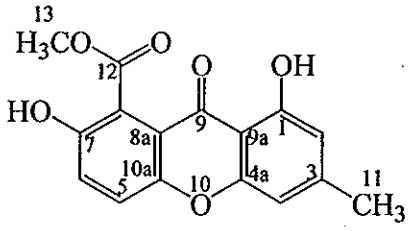


Table 315 The ^{1}H and ^{13}C NMR data of compound **K72** in $CDCl_3$ and **Pinselin**

Position	K72		Pinselin	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})[a]	δ_C (C-type)[b]
1-OH	12.23 (*s*)	161.18 (C)	12.12 (*s*)	161.7 (C)
2	6.66 (*s*)	111.41 (CH)	6.52 (*s*)	111.2 (CH)
3	-	148.83 (C)	-	149.1 (C)
4	6.77 (*s*)	107.26 (CH)	6.71 (*s*)	107.5 (CH)
4a	-	155.65 (C)	-	156.1 (C)
5	7.51 (*d*, 9.0)	122.29 (CH)	7.45 (*d*, 9.0)	120.1 (CH)
6	7.40 (*d*, 9.0)	125.41 (CH)	7.45 (*d*, 9.0)	125.5 (CH)
7-OH	-	152.00 (C)	9.07 (*brs*)	152.2 (C)
8	-	113.08 (C)	-	119.1 (C)

Table 315 Continued

Position	K72		Pinselin	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})[a]	δ_C (C-type)[b]
8a	-	118.64 (C)	-	118.6 (C)
9	-	180.42 (C)	-	181.2 (C)
9a	-	107.26 (C)	-	107.0 (C)
10a	-	150.50 (C)	-	167.3 (C)
11	2.49 (*s*)	22.61 (CH_3)	2.38 (*s*)	22.2 (CH_3)
12		169.10 (C)		168.1 (C)
13	4.03 (*s*)	53.07 (CH_3)	3.90 (s)	52.5 (CH_3)

[a] in acetone-d_6, [b] in pyridine-d_5

Table 316 The HMBC, COSY and NOEDIFF data of compound **K72** in $CDCl_3$

Proton	HMBC	COSY	NOEDIFF
1-OH	C-1, C-2, C-9a	-	-
H-2	C-1, C-4, C-11	H-4, H_3-11	H_3-11
H-4	C-2, C-4a, C-11	H-2, H_3-11	H_3-11
H-5	C-7, C-8a, C-10a	H-6	H-6
H-6	C-7, C-8, C-10a, C-12	H-5	H-5
H_3-11	C-2, C-3, C-4	H-2, H-4	H-2, H-4
H_3-13	C-12	-	-

7.3.10 Compound K73

Compound **K73** was obtained as a yellow gum. The UV and IR data were similar to those of **K72**. Its ^{1}H NMR spectrum **(Figure 173) (Table 317)** revealed the replacement of signal for the aromatic methyl protons in **K72** with a singlet resonance of hydroxymethylene protons (δ 4.58, *s*, 2H) in **K73**. The hydroxymethyl group, H_2-11 (δ 4.58), showed HMBC correlations **(Table 318)** with C-2 (δ 107.60), C-3 (δ 154.52) and C-4 (δ 104.39). These results together with signal

enhancement of both H-2 (δ 6.73) and H-4 (δ 6.97) after irradiation of H_2-11 in the NOEDIFF experiment **(Table 318)** confirmed the location of the hydroxymethyl group at C-3. Therefore, **K73** was assigned as sydowinin B which was previously isolated from *Aspergillus sydowi* (Hamasaki, *et al.*, 1975).

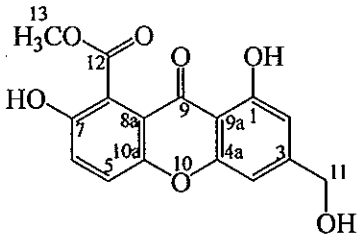


Table 317 The ^1H and ^{13}C NMR data of compound **K73** and **Sydowinin B** in DMSO-d_6

Position	**K73**		**Sydowinin B**
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})
1-OH	12.19 (*s*)	160.91 (C)	12.20 (*s*)
2	6.73 (*s*)	107.60 (CH)	6.47 (*d*, 1.0)
3	-	154.52 (C)	-
4	6.97 (*s*)	104.39 (CH)	6.98 (*d*, 1.0)
4a	-	155.97 (C)	-
5	7.62 (*d*, 9.0)	120.59 (CH)	7.62 (*d*, 8.0)
6	7.47 (*d*, 9.0)	125.79 (CH)	7.46 (*d*, 8.0)
7-OH	10.50 (*brs*)	149.36 (C)	10.40 (*brs*)
8	-	117.66 (C)	-
8a	-	117.51 (C)	-
9	-	180.78 (C)	-
9a	-	107.05 (C)	-
10a	-	151.51 (C)	-
11	4.58 (*s*)	62.80 (CH_2)	4.60 (*brs*)
11-OH	5.54 (*brs*)	-	5.50 (*brd*)
12	-	167.26 (C)	-
13	3.85 (*s*)	52.71 (CH_3)	3.86 (*s*)

Table 318 The HMBC, COSY and NOEDIFF data of compound **K73** in DMSO-d_6

Proton	HMBC	COSY	NOEDIFF
1-OH	C-1, C-2, C-3, C-9, C-9a	-	-
H-2	C-1, C-4, C-9, C-9a, C-11	H-4, H_2-11	H_2-11, 1-OH
H-4	C-2, C-4a, C-9, C-9a, C-11	H-2, H_2-11	H_2-11
H-5	C-6, C-8a, C-9, C-10a	H-6	H-7
H-6	C-7, C-8, C-8a, C-12	H-5	H-5
H_2-11	C-2, C-3, C-4	H-2, H-4, 11-OH	H-2, H-4
H_3-13	C-6, C-12	-	-

7.3.11 Compound K69

Compound **K69** was obtained as a colorless gum. The UV spectrum displayed absorption bands at 207, 227 and 282 nm, indicating that **K69** had a benzene chromophore. The absorption bands at 3391 (hydroxyl), 1732 (ester carbonyl) and 1686 (conjugated ketone carbonyl) cm^{-1} were observed in the IR spectrum. The ^1H NMR spectrum **(Figure 165) (Table 319)** was similar to that of **K64** except for the presence of two sets of nonequivalent methylene protons [δ 2.93 (*ddd*, J = 19.0, 6.5 and 4.5 Hz, 1H)/2.85 (*ddd*, J = 19.0, 9.0 and 6.5 Hz, 1H) and 2.33 (*m*, 1H)/2.21 (*m*, 1H)] instead of the *cis*-olefinic protons in **K64**. In the ^1H-^1H COSY spectrum **(Table 320)**, the methylene protons resonating at δ 2.33 and 2.21 were correlated with the oxymethine proton, H-7 (δ 4.11), and the other methylene protons, (δ 2.93 and 2.85). The latter methylene protons were correlated with C-8a (δ 116.65) and C-10a (δ 166.81) in the HMBC spectrum **(Table 319)**. Thus, the methylene protons at δ 2.93/2.85 and 2.33/2.21 were identified as H_{ab}-5 and H_{ab}-6, respectively. In addition, the methine proton at C-8 in **K64** was replaced by a hydroxyl group in **K69** according to the chemical shift of C-8 (δ 76.72). The observed optical rotation of **K69**, $[\alpha]^{25}$ + 160 (*c* 0.06, $CHCl_3$), was similar to that of (7*R*,8*S*)-β-diversonolic ester, $[\alpha]^{25}$ + 181.7 (*c* 0.06, $CHCl_3$), indicating that they had the same absolute configuration

at C-7 and C-8. Therefore, **K69** was identified as (7*R*,8*S*)-*β*-diversonolic ester which was previously isolated from *Penicillium diversum* (Holker, *et al.*, 1983).

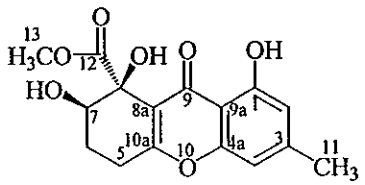


Table 319 The ^{1}H, ^{13}C NMR and HMBC data of compound **K69** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1-OH	11.90 (*s*)	160.25 (C)	C-1, C-2, C-9a
2	6.63 (*s*)	112.33 (CH)	C-1, C-4, C-9a, C-11
3	-	147.83 (C)	-
4	6.73 (*s*)	107.40 (CH)	C-2, C-4a, C-9, C-9a, C-11
4a	-	156.15 (C)	-
5	a: 2.93 (*ddd*, 19.0, 6.5, 4.5)	26.10 (CH_2)	C-6, C-7, C-8a, C-10a
	b: 2.85 (*ddd*, 19.0, 9.0, 6.5)		C-6, C-7, C-8a, C-10a
6	a: 2.33 (*m*)	24.23 (CH_2)	C-5, C-7, C-8, C-10a
	b: 2.21 (*m*)		C-5, C-7, C-8, C-10a
7	4.11 (*dd*, 10.0, 3.5)	72.75 (CH)	C-5, C-8, C-12
8	-	76.72 (C)	-
8a	-	116.65 (C)	-
9	-	182.10 (C)	-
9a	-	108.18 (C)	
10a	-	166.81 (C)	-
11	2.42 (*s*)	22.48 (CH_3)	C-2, C-3, C-4
12	-	172.81 (C)	-
13	3.87 (*s*)	53.37 (CH_3)	C-12

Table 320 The COSY and NOEDIFF data of compound **K69** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H-4, H_3-11	H_3-11
H-4	H-2, H_3-11	H_3-11
H_a-5	H_b-5, H_{ab}-6	-
H_b-5	H_a-5, H_{ab}-6	-
H_a-6	H_{ab}-5, H_b-6, H-7	-
H_b-6	H_{ab}-5, H_a-6, H-7	-
H-7	H_{ab}-6	-
H_3-11	H-2, H-4	H-2, H-4

7.3.12 Compound K65

Compound **K65** was obtained as a colorless gum whose molecular formula $C_{16}H_{16}O_8$ (*m/z* 336) was deduced by EIMS **(Figure 156)**. The UV and IR spectra were almost identical to those of **K69**. Its ^{1}H NMR spectrum **(Figure 157)** **(Table 321)** was similar to that of **K69** except that the aromatic methyl signal in **K69** was replaced by a hydroxymethyl signal (δ 4.76, *s*, 2H). The ^3J HMBC correlations **(Table 321)** of the hydroxymethyl protons, H_2-11 (δ 4.76), with C-2 (δ 108.91), C-3 (δ 151.00) and C-4 (δ 104.33) as well as signal enhancement of both H-2 (δ 6.76) and H-4 (δ 6.94) upon irradiation of H_2-11 in the NOEDIFF experiment **(Table 322)**, supported the location of the hydroxymethyl group at C-3. Accordingly, **K65** determined as a new tetrahydroxanthone derivative.

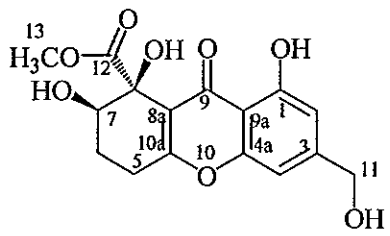


Table 321 The ^{1}H, ^{13}C NMR and HMBC data of compound **K65** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC
1-OH	11.97 (*s*)	160.70 (C)	C-1, C-2, C-9a
2	6.76 (*s*)	108.91 (CH)	C-1, C-4, C-11
3	-	151.00 (C)	-
4	6.94 (*s*)	104.33 (CH)	C-2, C-4a, C-11
4a	-	156.00 (C)	-
5	a: 2.96 (*m*)	26.03 (CH_2)	C-6, C-7, C-8a, C-10a
	b: 2.87 (*m*)		C-6, C-7, C-8a, C-10a
6	a: 2.31 (*m*)	24.21 (CH_2)	C-5, C-8
	b: 2.21 (*m*)		C-5, C-8
7	4.09 (*dd*, 10.5, 4.0)	72.69 (CH)	C-8, C-12
8	-	76.00 (C)	-
8a	-	117.00 (C)	-
9	-	180.00 (C)	-
9a	-	116.80 (C)	-
10a	-	167.22 (C)	-
11	4.76 (*brs*)	64.37 (CH_2)	C-2, C-3, C-4
12	-	172.00 (C)	-
13	3.85 (*s*)	53.31 (CH_3)	C-12

Table 322 The COSY and NOEDIFF data of compound **K65** in $CDCl_3$

Proton	COSY	NOEDIFF
H-2	H_2-11	H-2
H-4	H_2-11	H-4
H_a-5	H_b-5, H_{ab}-6	-
H_b-5	H_a-5, H_{ab}-6	-
H_a-6	H_{ab}-5, H_b-6, H-7	-
H_b-6	H_{ab}-5, H_a-6, H-7	-
H-7	H_{ab}-6	-
H_2-11	H-2, H-4	H-2, H-4

7.3.13 Compound K74

Compound **K74** was obtained as a colorless gum. The UV spectrum displayed absorption bands at 213, 261 and 310 nm, indicating that **K74** had an indole chromophore (Carić, *et al.*, 2004). The absorption bands at 3221 (amino), 1690 (amide carbonyl) and 1653 (double bond) cm^{-1} were found in the IR spectrum. The ^{1}H NMR spectrum **(Figure 175) (Table 323)** displayed signals for three amino protons [δ 10.90 (*d*, J = 1.5 Hz, 1H), 7.93 (*d*, J = 2.1 Hz, 1H) and 7.71 (*d*, J = 2.4 Hz, 1H)], five aromatic protons of a monosubstituted benzene [δ 7.16 (*m*, 3H) and 6.69 (*dd*, J = 7.5 and 1.8 Hz, 2H)] and four aromatic protons of a 1,2-disubstituted benzene [δ 7.49 (*d*, J = 7.8 Hz, 1H), 7.32 (*d*, J = 7.8 Hz, 1H), 7.08 (*t*, J = 7.8 Hz, 1H) and 7.00 (*t*, J = 7.8 Hz, 1H)], one olefinic proton (δ 6.96, *d*, J = 1.5 Hz, 1H), two aminomethine protons [δ 3.98 (*m*, 1H) and 3.85 (*m*, 1H)] and two sets of nonequivalent methylene protons [δ 2.81 (*dd*, J = 14.9 and 4.5 Hz, 1H)/2.53 (*dd*, J = 14.9 and 5.7 Hz, 1H) and 2.45 (*dd*, J = 13.2 and 4.8 Hz, 1H)/1.81 (*dd*, J = 13.2 and 7.2 Hz, 1H). The ^{13}C NMR **(Figure 176) (Table 323)** and DEPT 135 **(Table 323)** spectra showed eighteen signals for twenty carbons, which are six quaternary (δ 167.33, 166.70, 136.48, 136.91, 127.96 and 109.20), ten methine (δ 130.14, 128.81, 126.86, 124.90, 121.39, 119.22, 118.92, 111.80, 56.07 and 55.71) and two methylene (δ 40.25 and 30.13) carbons. The aromatic protons resonating at δ 7.49, 7.32, 7.08 and 7.00 were assigned to be H-5, H-8, H-7 and H-6, respectively, on the basis of the coupling constant and multiplicity. The olefinic proton at δ 6.96 was identified as H-2 according to its coupling with 1-NH. HMBC correlations **(Table 324)** of both H-2 and H-5 with C-3 (δ 109.20) and C-9 (δ 136.48) established a 3-substituted indole moiety. The aminomethine proton, H-11 (δ 3.98), showed ^{1}H-^{1}H COSY correlations **(Table 324)** with the amino proton, 12-NH (δ 7.93), and the methylene protons, H_{ab}-10 (δ 2.81 and 2.53) while the remaining aminomethine proton, H-14 (δ 3.85), gave the same correlations with the amino, 15-NH (δ 7.71), and the methylene protons, H_{ab}-17 (δ 2.45 and 1.81). In addition, the aminomethine protons, H-11 and H-14, showed a HMBC correlation with C-13 (δ 166.70) and C-16 (δ 167.33), respectively. These results constructed a 2,5-diketopiperazine having the methylene groups attached at

C-11 (δ 55.71) and C-14 (δ 56.07). The 3-substituted indole unit and the monosubstituted benzene ring were attached at C-10 (δ 30.13) and C-17 (δ 40.25), respectively, according to the following HMBC correlations: H_{ab}-10/C-2 (δ 124.90), C-3 and C-4 (δ 127.96) and H_{ab}-17/C-18 (δ 136.91), C-19 (δ 130.14) and C-23 (δ 130.14). The observed optical rotation of **K74**, $[\alpha]^{20}$ -206 (*c* 1.0, MeOH), was similar to that of (11*S*,14*S*)-cyclo-(*L*-try-*L*-phe), $[\alpha]^{20}$ -254.9 (*c* 1.0, MeOH), indicating that they had the same absolute configuration. Therefore, **K74** was assigned as (11*S*,14*S*)-cyclo-(*L*-try-*L*-phe) which was previously isolated from *Penicillium* sp. (Kimura, *et al.*, 1996).

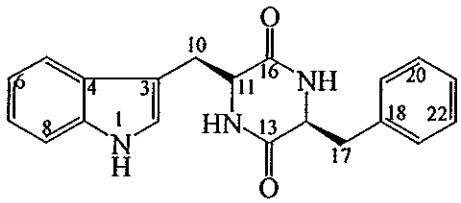


Table 323 The ^{1}H and ^{13}C NMR data of compound **K74** and **(11*S*,14*S*)-*Cyclo*-(L-*Trp*-L-*Phe*)** in DMSO-d_6

Position	K74		(11*S*,14*S*)-*Cyclo*-(L-*Trp*-L-*Phe*)	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1-NH	10.90 (*d*, 1.5)	-	10.89 (*s*)	-
2	6.96 (*d*, 1.5)	124.90 (CH)	6.96 (*d*, 2.0)	120.89 (CH)
3	-	109.20 (C)	-	108.85 (C)
4	-	127.96 (C)	-	127.54 (C)
5	7.49 (*d*, 7.8)	119.22 (CH)	7.48 (*dd*, 7.2, 1.0)	118.42 (CH)
6	7.00 (*t*, 7.8)	118.92 (CH)	6.98 (*ddd*, 7.5, 7.2, 1.5)	118.76 (CH)
7	7.08 (*t*, 7.8)	121.39 (CH)	7.08 (*ddd*, 7.7, 7.5, 1.5)	124.41 (CH)
8	7.32 (*d*, 7.8)	111.80 (CH)	7.32 (*dd*, 7.7, 7.5)	111.33 (CH)
9	-	136.48 (C)	-	136.07 (C)
10	a: 2.81 (*dd*, 14.9, 4.5)	30.13 (CH_2)	a: 2.81 (*dd*, 14.5, 4.5)	29.69 (CH_2)
	b: 2.53 (*dd*, 14.9, 5.7)		b: 2.52 (*dd*, 14.5, 5.7)	
11	3.98 (*m*)	55.71 (CH)	3.98 (*m*)	55.29 (CH)
12-NH	7.93 (*d*, 2.1)	-	7.91 (*d*, 2.0)	-

Table 323 Continued

Position	K74		(11*S*,14*S*)-*Cyclo*-(L-*Trp*-L-*Phe*)	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
13	-	166.70 (C)	-	166.22 (C)
14	3.85 (*m*)	56.07 (CH)	3.89 (*m*)	55.64 (CH)
15-NH	7.71 (*d*, 2.4)	-	7.71 (*d*, 2.0)	-
16	-	167.33 (C)	-	166.88 (C)
17	a: 2.45 (*dd*, 13.2, 4.8)	40.25 (CH_2)	a: 2.45 (*dd*, 13.5, 4.7)	39.89 (CH_2)
	b: 1.81 (*dd*, 13.2, 7.2)		b: 1.85 (*dd*, 13.5, 7.0)	
18	-	136.91 (C)	-	136.56 (C)
19	6.69 (*dd*, 7.5, 1.8)	130.14 (CH)	7.16 (*m*)	128.03 (CH)
20	7.16 (*m*)	126.86 (CH)	6.71 (*m*)	129.70 (CH)
21	7.16 (*m*)	128.81 (CH)	7.17 (*m*)	126.36 (CH)
22	7.16 (*m*)	126.86 (CH)	6.71 (*m*)	129.70 (CH)
23	6.69 (*dd*, 7.5, 1.8)	130.14 (CH)	7.16 (*m*)	128.03 (CH)

Table 324 The HMBC, COSY and NOEDIFF data of compound **K74** in DMSO-d_6

Proton	HMBC	COSY	NOEDIFF
1-NH	-	H-2	H-2, H-8
H-2	C-3, C-9	1-NH	1-NH, H_{ab}-10, H-11
H-5	C-3, C-4, C-7, C-9	H-6	H-6, H_{ab}-10, 12-NH
H-6	C-7, C-8	H-5, H-7	H-5, H-7
H-7	C-5, C-9	H-6, H-8	H-6, H-8
H-8	C-4, C-6	H-7	1-NH, H-7
H_a-10	C-2, C-3, C-4, C-11, C-16	H-2, H_b-10, H-11	H-2, H-5, H_b-10, H-11
H_b-10	C-2, C-3, C-4, C-11, C-16	H_a-10, H-11	H-2, H-5, H_a-10, H-11, 12-NH
H-11	C-3, C-10, C-13, C-16	H_{ab}-10, 12-NH	H-2, H_{ab}-10, 12-NH
12-NH	C-14, C-16	H-11	H-5, H_b-10, H-11

Table 324 Continued

Proton	HMBC	COSY	NOEDIFF
H-14	C-13, C-16, C-17, C-18	15-NH, H_{ab}-17	15-NH, H_{ab}-17, H-19, H-23
15-NH	C-11, C-13	H-14	H-14, H_{ab}-17, H-19, H-23
H_a-17	C-13, C-14, C-18, C-19, C-23	H-14, H_b-17, H-19	H-14, H_b-17, H-19, H-23
H_b-17	C-13, C-14, C-18, C-19, C-23	H-14, H_a-17, H-19	H-14, H_a-17, H-19, H-23
H-19	C-17, C-20, C-21, C-23	H_{ab}-17, H-20, H-21	H-14, 15-NH, H_{ab}-17, H-20
H-20	C-18, C-19, C-21, C-22	H-19, H-21	H-19, H-21
H-21	C-19, C-20, C-22, C-23	H-20, H-22	H-20, H-22
H-22	C-18, C-20, C-21, C-23	H-21, H-23	H-21, H-23
H-23	C-17, C-19, C-21, C-22	H_{ab}-17, H-21, H-22	H-14, 15-NH, H_{ab}-17, H-22

7.3.14 Compound K66

Compound **K66** was obtained as a colorless gum. The UV and IR data were almost identical to those of **K74**. The ^{1}H NMR spectrum **(Figure 159) (Table 325)** was similar to that of **K74** except for the replacement of the 3-substituted indole signals in **K74** with signals of a 2-substituted phenol [δ 7.27 (*d*, J = 7.0 Hz, 1H), 7.16 (*td*, J = 7.0 and 1.0 Hz, 1H), 6.82 (*td*, J = 7.0 and 1.0 Hz, 1H) and 6.67 (*d*, J = 7.0 Hz, 1H)] in **K66**. The protons resonating at δ 7.27, 7.16, 6.82 and 6.67 were assigned as H-5, H-3, H-4 and H-2, respectively, according to their coupling constants and multiplicity. This unit was confirmed by the following ^3J HMBC correlations: H-5 (δ 7.27)/C-1 (δ 150.01) and C-3 (δ 130.05), H-4 (δ 6.82)/C-2 (δ 110.34) and C-6 (δ 126.45), H-3 (δ 7.16)/C-1 and C-5 (δ 125.45) and H-2 (δ 6.67)/C-4 (δ 119.22) and

C-6. The attachment of the 2-substituted phenol at C-7 (δ 35.33) was confirmed by the HMBC correlations of H_{ab}-7 (δ 2.69 and 2.58) with C-6 of the 2-substituted phenol unit. The observed optical rotation of **K66**, $[\alpha]^{20}$ -186 (*c* 1.0, MeOH), was similar to that of **K74**, $[\alpha]^{20}$ -206 (*c* 1.0, MeOH), indicating that they had the same configuration at C-6 and C-11. Thus, **K66** was identified as a new 2,5-diketopiperazine.

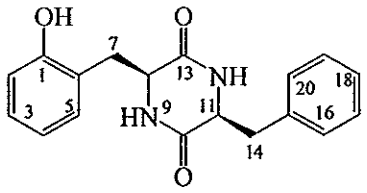


Table 325 The NMR data of compound **K66** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC	COSY
1	-	150.01 (C)	-	-
2	6.67 (*d*, 7.0)	110.34 (CH)	C-4, C-6	H-3
3	7.16 (*td*, 7.0, 1.0)	130.05 (CH)	C-1, C-5	H-2, H-4
4	6.82 (*td*, 7.0, 1.0)	119.22 (CH)	C-2, C-6	H-2, H-3, H-5
5	7.27 (*d*, 7.0)	125.45 (CH)	C-1, C-3, C-8	H-3, H-4
6	-	126.45 (C)	-	-
7	a : 2.69 (*t*, 11.0)	35.33 (CH_2)	C-6, C-8, C-13	H_b-7, H-8
	b : 2.58 (*dd*, 11.0, 6.0)		C-6, C-8	H_a-7, H-8
8	3.88 (*ddd*, 11.0, 6.0, 1.0)	58.74 (CH)	C-7, C-13	H_{ab}-7
9-NH	5.19 (*s*)	-	C-8	-
10	-	165.73 (C)	-	-
11	4.17 (*dm*, 10.5)	56.19 (CH)	C-10, C-15	H_{ab}-14
12-NH	5.49 (*s*)	-	C-8, C-10, C-11, C-13	-
13	-	168.43 (C)	-	-

Table 325 Continued

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC	COSY
14	a: 3.57 (*dd*, 14.5, 3.5)	36.65 (CH_2)	C-10, C-11, C-15, C-16	H-11, H_b-14
	b: 2.73 (*dd*, 14.5, 11.5)		C-10, C-11, C-15, C-16	H-11, H_a-14
15	-	135.51 (C)	-	-
16	7.33 (*dd*, 7.5, 1.0)	129.46 (CH)	C-15	H-17, H-18
17	7.17 (*t*, 7.5)	128.97 (CH)	C-14, C-18	H-16, H-18
18	7.28 (*d*, 7.5)	127.76 (CH)	C-17, C-19	H-17, H-19
19	7.17 (*t*, 7.5)	128.97 (CH)	C-14, C-18	H-18, H-20
20	7.33 (*dd*, 7.5, 1.0)	129.46 (CH)	C-15	H-18, H-19

7.3.15 Compound K75

Compound **K75** was obtained as a colorless gum. The UV spectrum displayed an aromatic chromophore at 207, 217 and 274 nm, while hydroxyl (3349 cm^{-1}) and double bond (1639 cm^{-1}) functional groups were found in the IR spectrum. The ^1H NMR spectrum **(Figure 177) (Table 326)** showed characteristic signals for three aromatic protons of a 1,3,5-trisubstituted benzene [δ 6.42 (*d*, J = 2.0 Hz, 1H), 6.40 (*d*, J = 2.0 Hz, 1H) and 6.30 (*t*, J = 2.0 Hz, 1H)] and an aromatic methyl group (δ 2.27, *s*, 3H). The ^{13}C NMR **(Figure 178) (Table 326)** and DEPT 135 **(Table 326)** spectra exhibited three quaternary (δ 158.10, 156.46 and 140.99), three aromatic methine (δ 112.23, 111.15 and 103.47) and one methyl (δ 21.47) carbons. The methyl protons, H_3-7 (δ 2.27), showed HMBC correlations **(Table 327)** with C-1 (δ 140.99), C-2 (δ 111.15) and C-6 (δ 112.23), indicating that the methyl group was attached at C-1. The substituents at C-3 (δ 156.46) and C-5 (δ 158.10) were assigned as hydroxyl groups according to the chemical shifts of these carbons. Therefore, **K75** was

identified as orcinal which was previously isolated from *Lilium maximowiczii* (Monde, *et al.*, 1998).

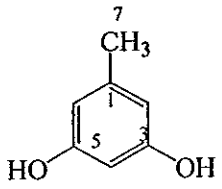


Table 326 The ^{1}H and ^{13}C NMR data of compound **K75** and **Orcinol** in $CDCl_3$

Position	K75		Orcinol	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz}) [a]	δ_C (C-type) [b]
1		140.99 (C)	-	141.0 (C)
2	6.40 (*d*, 2.0)	111.15 (CH)	6.24 (*d*, 2.0)	108.7 (CH)
3	-	156.46 (C)	-	156.6 (C)
4	6.30 (*t*, 2.0)	103.47 (CH)	6.17 (*t*, 2.0)	99.9 (CH)
5	-	158.10 (C)	-	156.6 (C)
6	6.42 (*d*, 2.0)	112.23 (CH)	6.24 (*d*, 2.0)	108.7 (CH)
7	2.27 (*s*)	21.47 (CH_3)	2.23 (*s*)	21.4 (CH_3)

[a] ^{1}H NMR 90 MHz, [b] ^{13}C NMR 22.5 MHz

Table 327 The HMBC and COSY data of compound **K75** in $CDCl_3$

Proton	HMBC	COSY
H-2	C-1, C-3, C-4, C-6, C-7	H-4, H-6, H_3-7
H-4	C-2, C-3, C-5, C-6	H-2, H-6
H-6	C-2, C-4, C-5	H-2, H-4, H_3-7
H_3-7	C-1, C-2, C-6	H-2, H-6

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APPENDIX

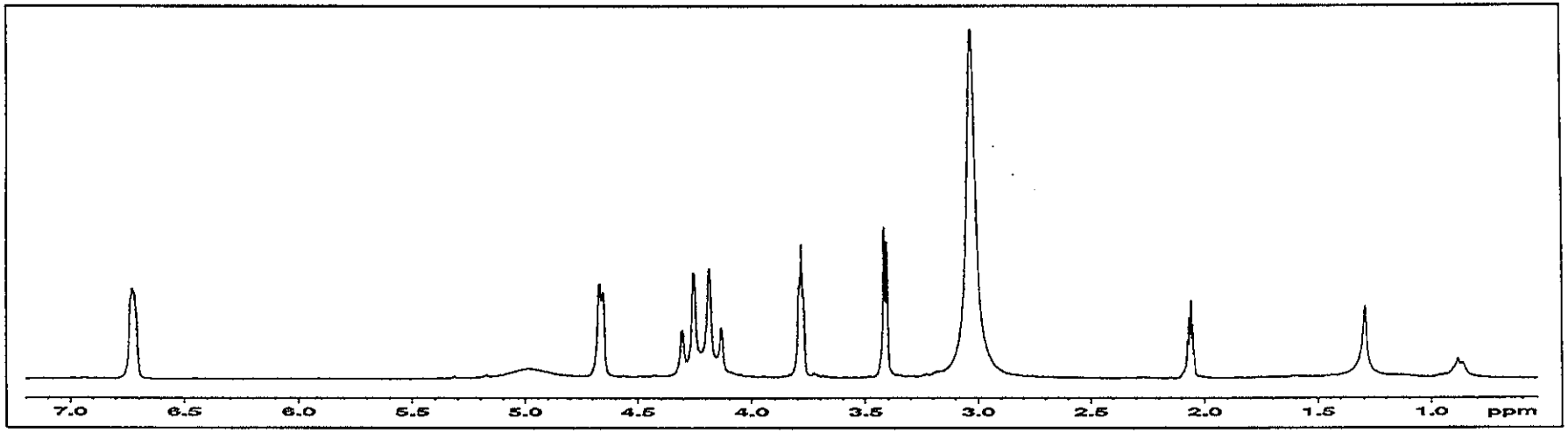

Figure 1 The 300 MHz ^{1}H NMR spectrum of compound **K1** in acetone-d_6

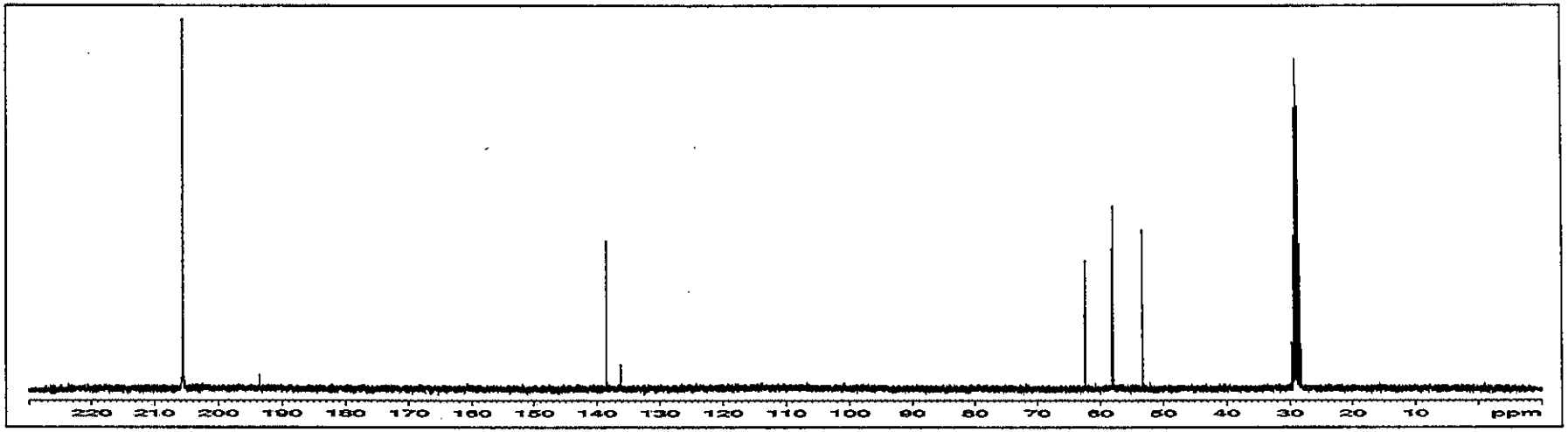

Figure 2 The 75 MHz ^{13}C NMR spectrum of compound **K1** in acetone-d_6

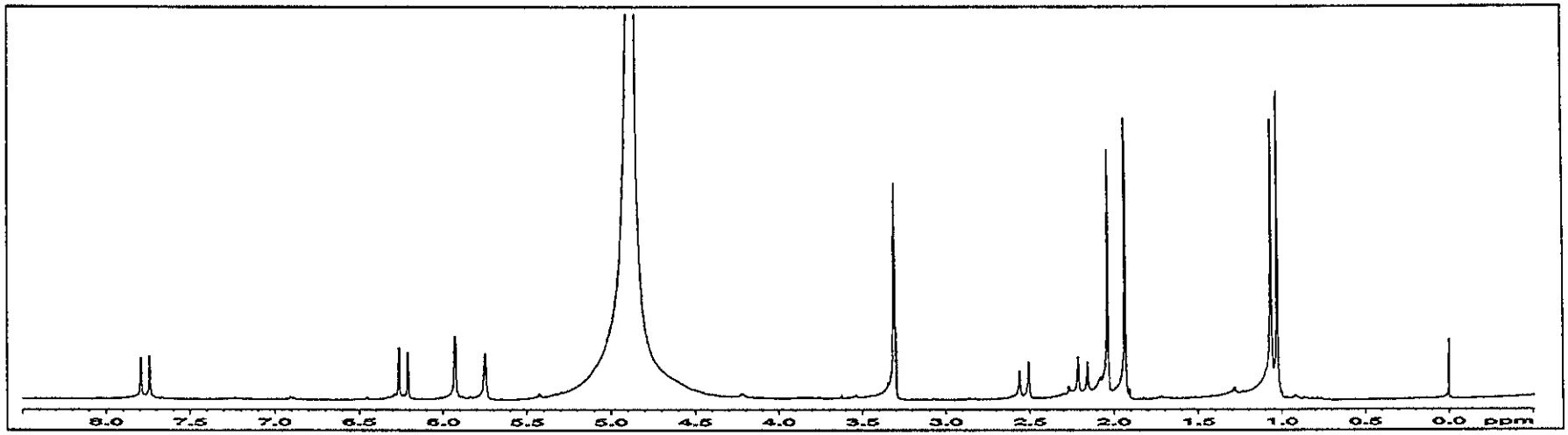

Figure 3 The 300 MHz ^{1}H NMR spectrum of compound **K2** in CD_3OD

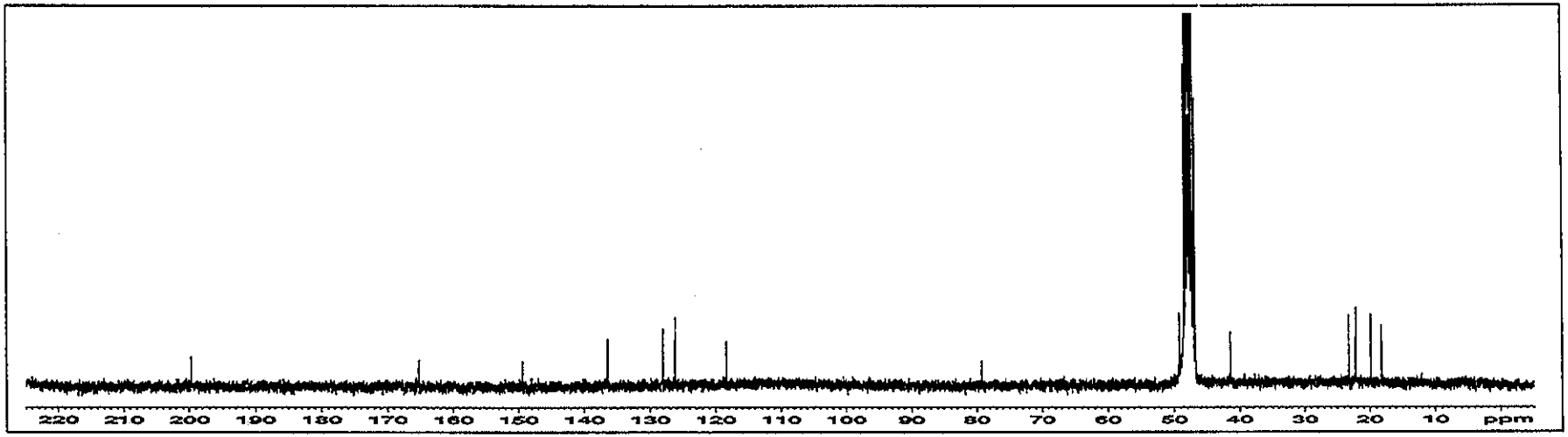

Figure 4 The 75 MHz ^{13}C NMR spectrum of compound **K2** in CD_3OD

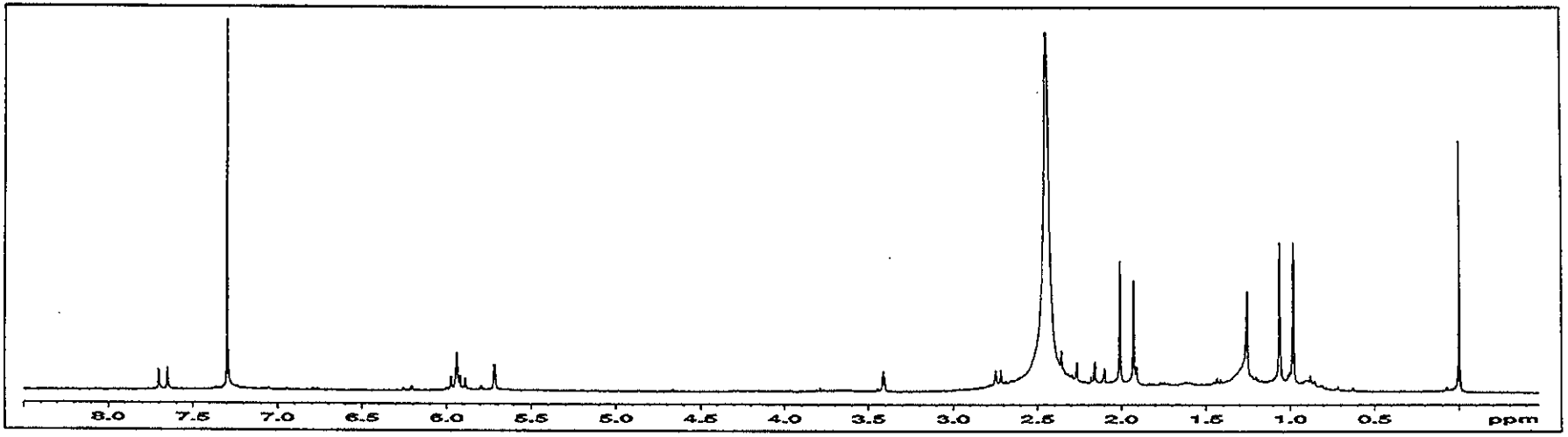

Figure 5 The 300 MHz ^{1}H NMR spectrum of compound **K3** in $CDCl_3$+CD_3OD

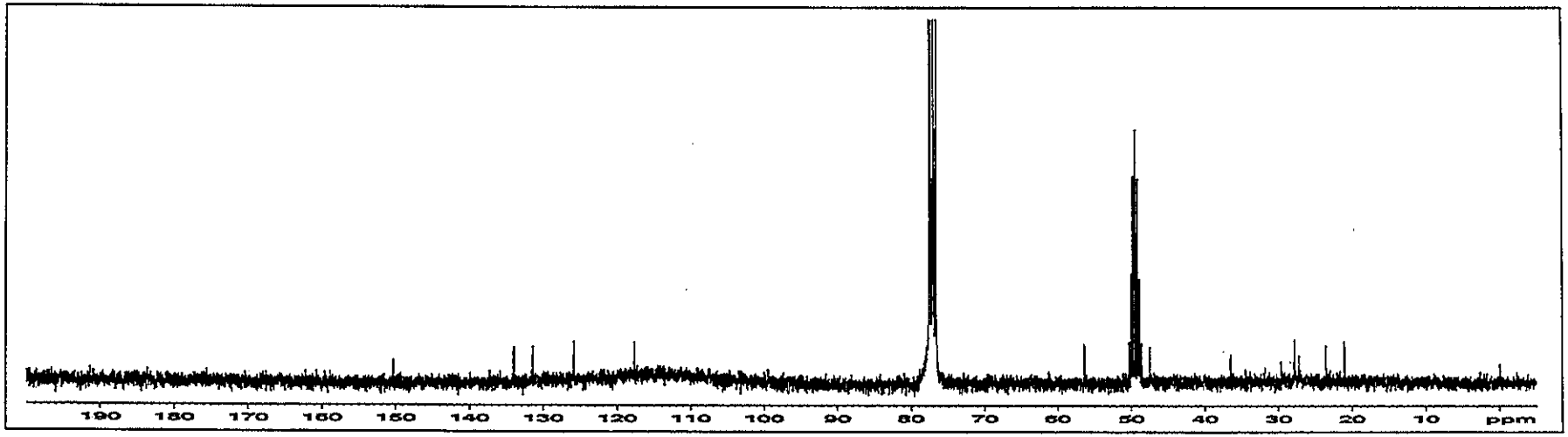

Figure 6 The 75 MHz ^{13}C NMR spectrum of compound **K3** in $CDCl_3$+CD_3OD

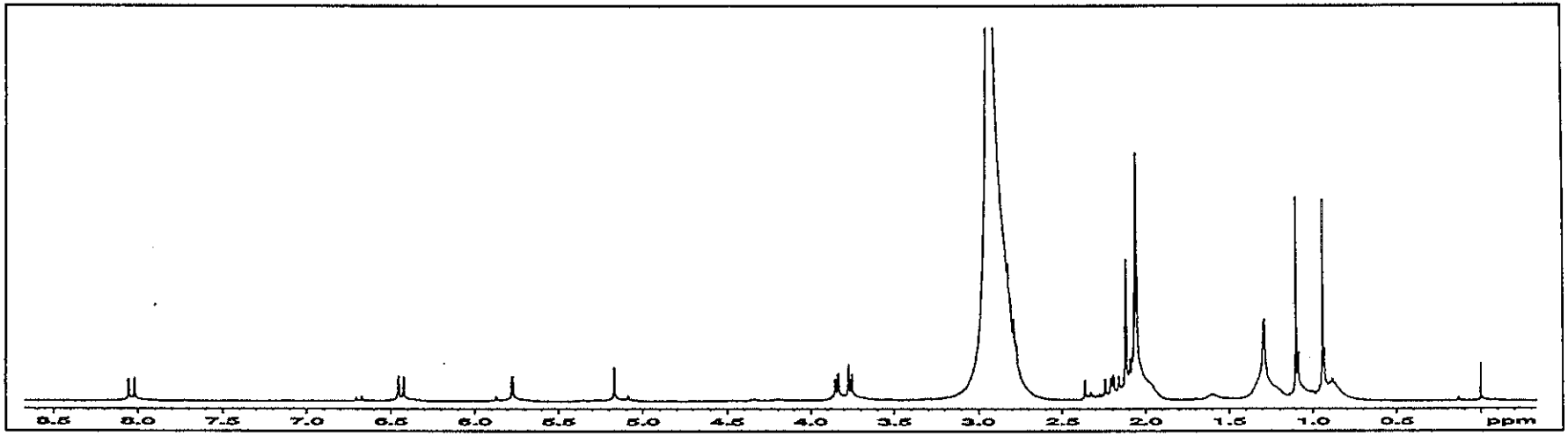

Figure 7 The 500 MHz ^{1}H NMR spectrum of compound **K4** in acetone-d_6

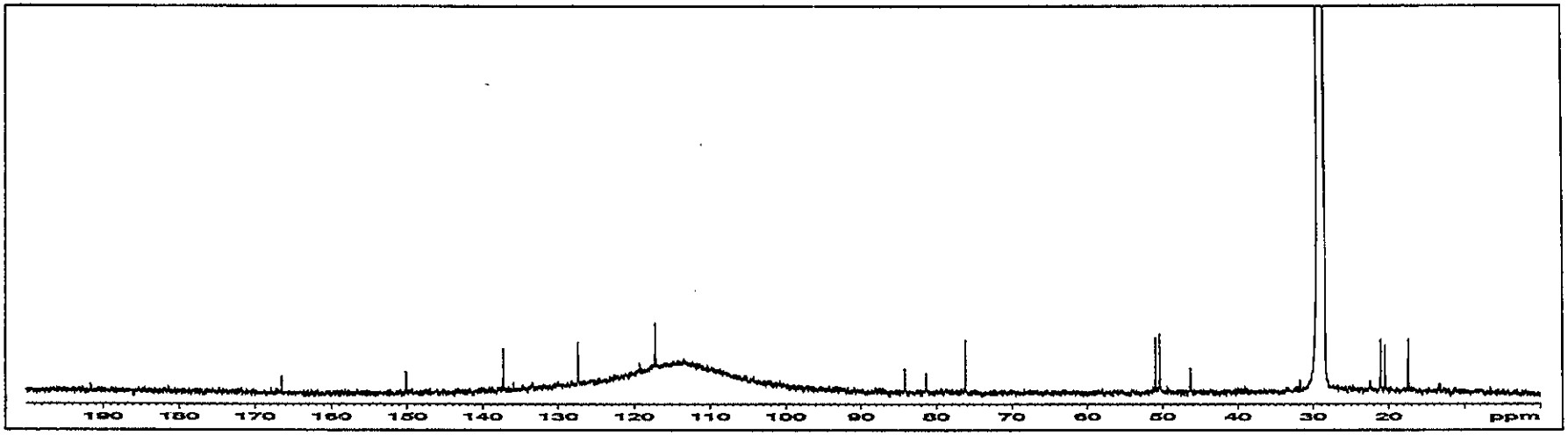

Figure 8 The 125 MHz ^{13}C NMR spectrum of compound **K4** in acetone-d_6

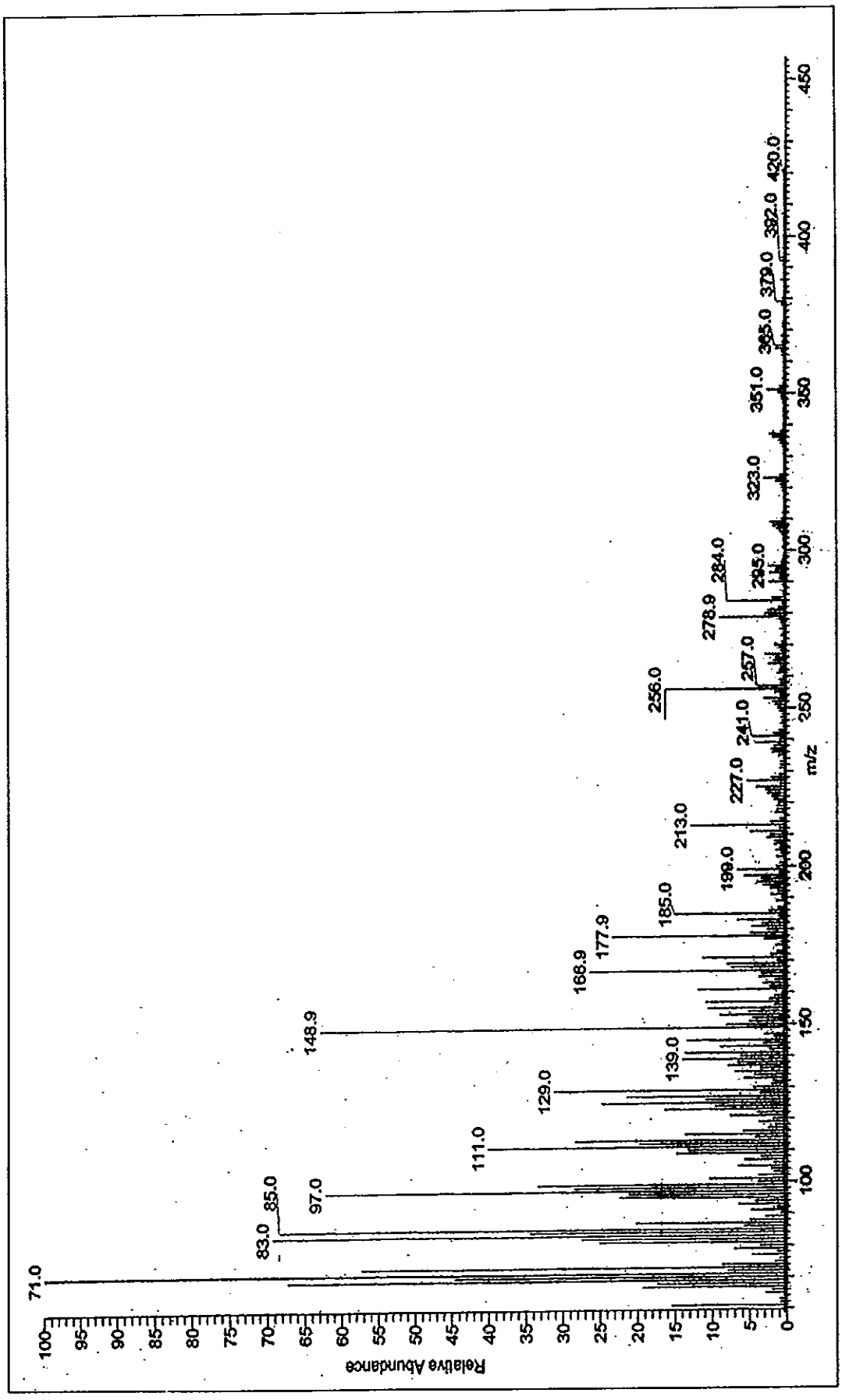

Figure 9 The mass spectrum of compound K5

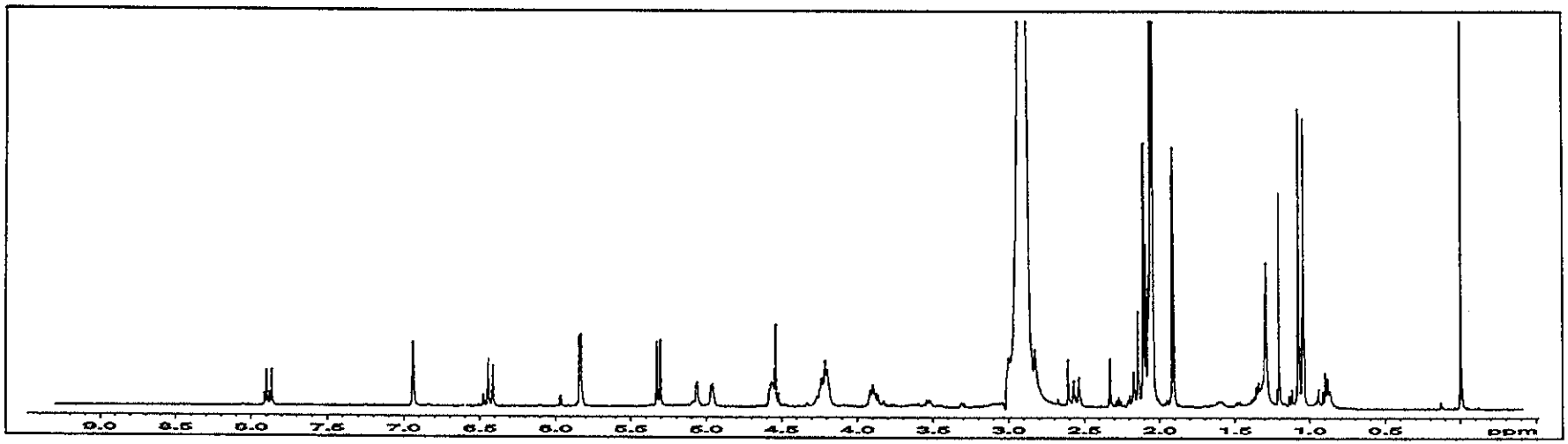

Figure 10 The 500 MHz ^{1}H NMR spectrum of compound **K5** in acetone-d_6

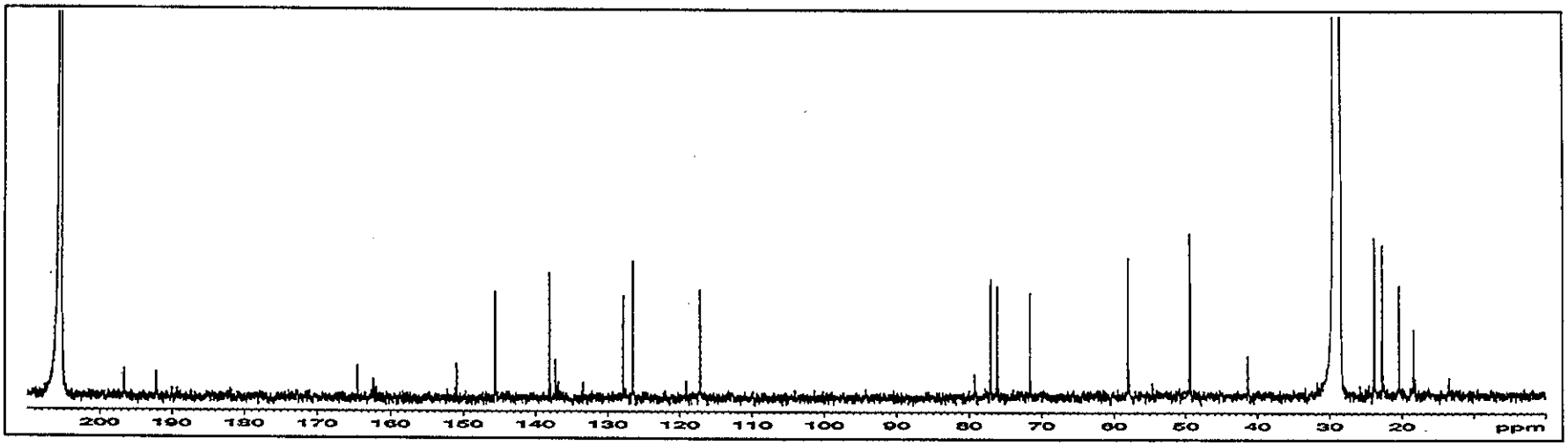

Figure 11 The 125 MHz ^{13}C NMR spectrum of compound **K5** in acetone-d_6

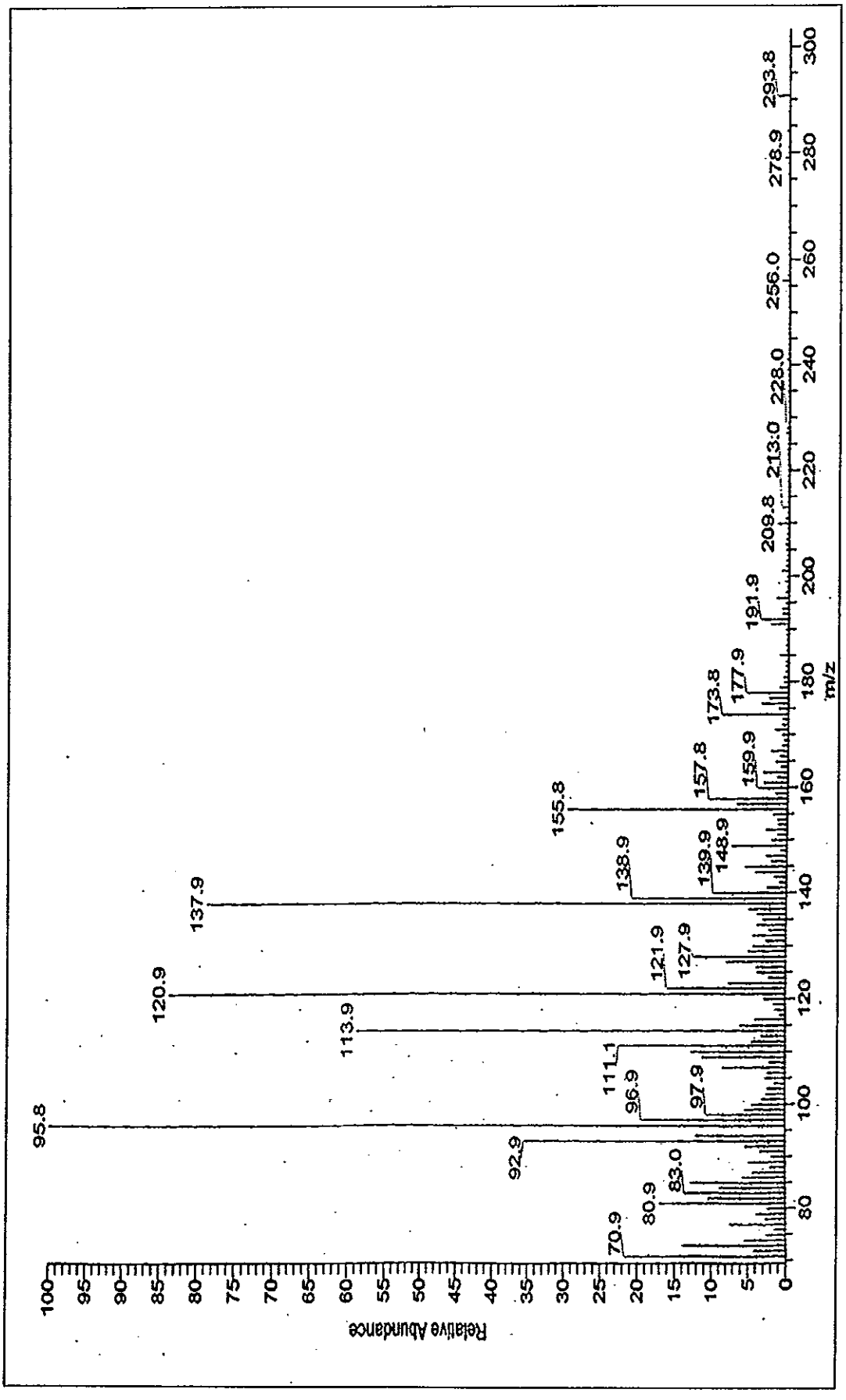

Figure 12 The mass spectrum of compound K6

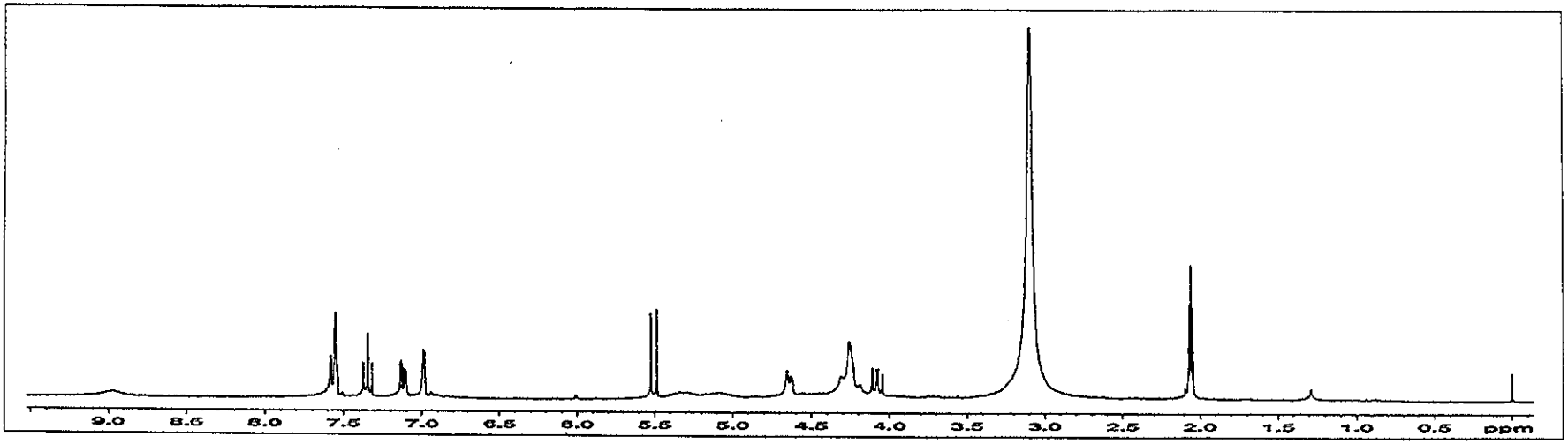

Figure 13 The 300 MHz ^{1}H NMR spectrum of compound **K6** in acetone-d_6

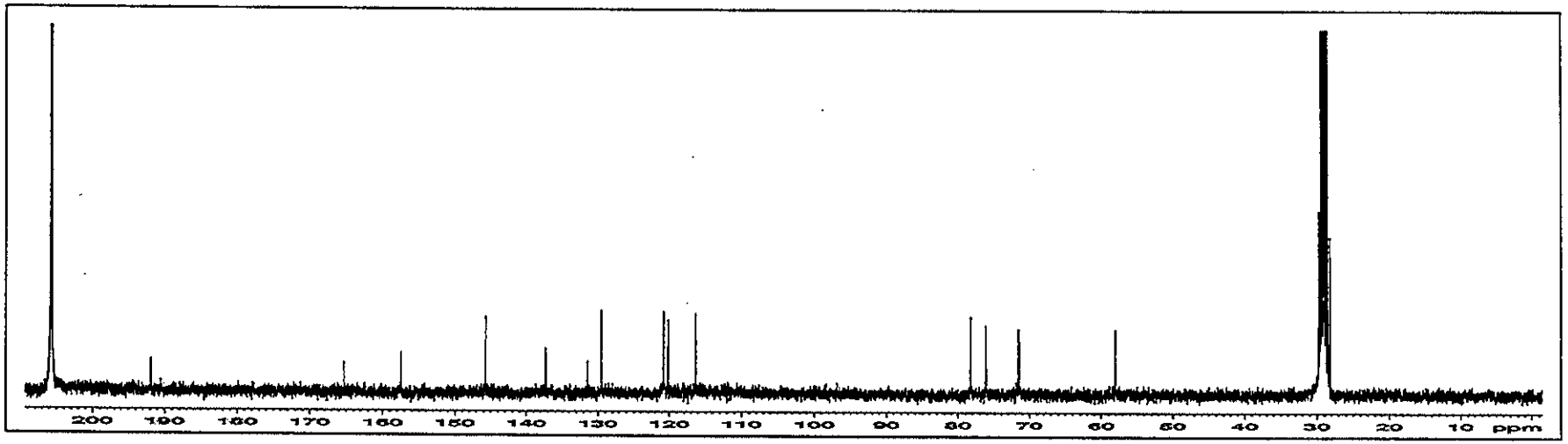

Figure 14 The 75 MHz ^{13}C NMR spectrum of compound **K6** in acetone-d_6

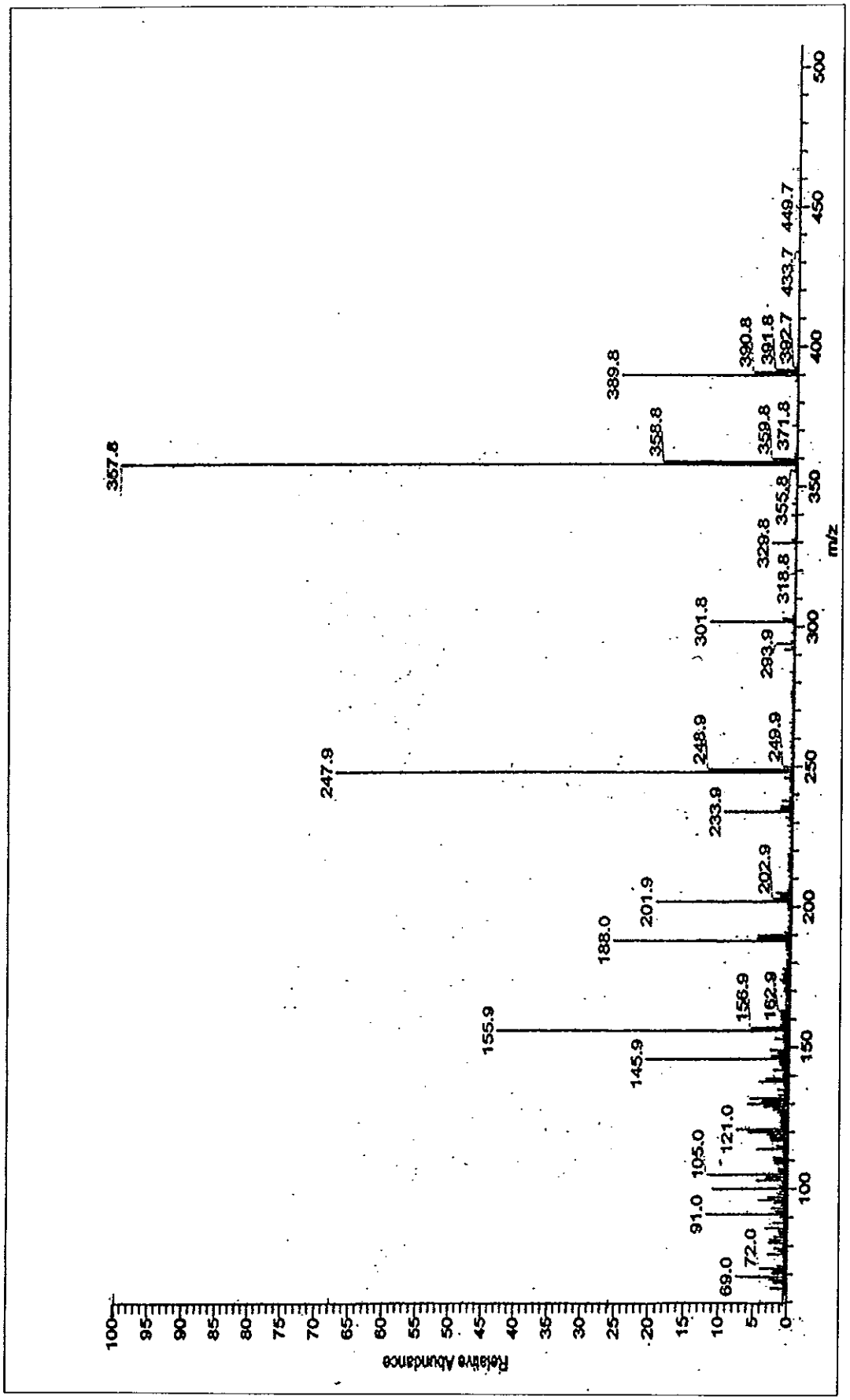

Figure 15 The mass spectrum of compound K7

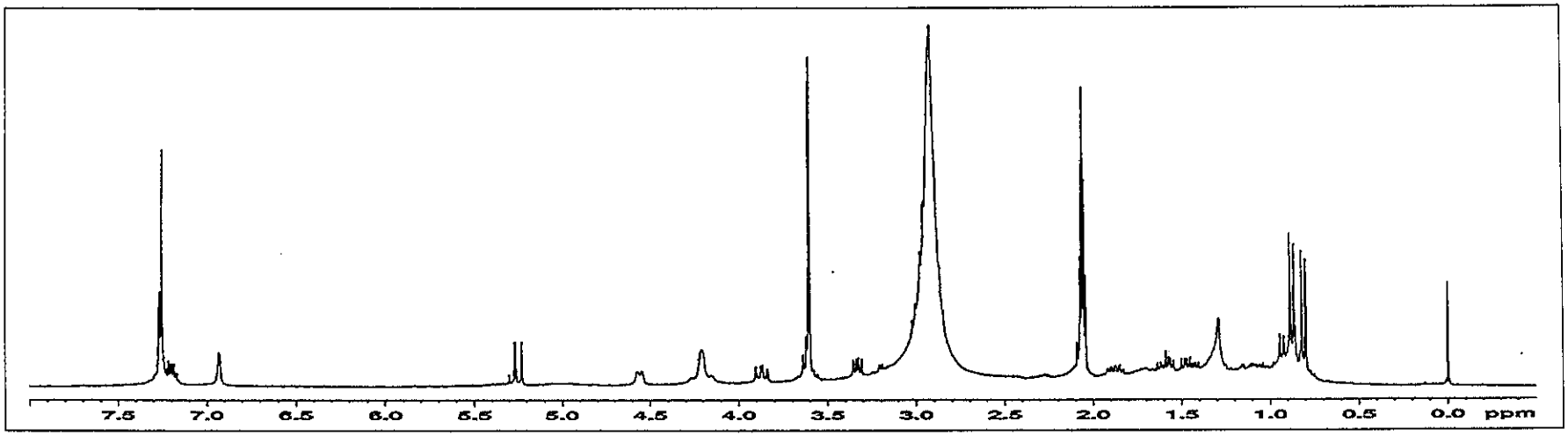

Figure 16 The 500 MHz ^{1}H NMR spectrum of compound **K7** in acetone-d_6

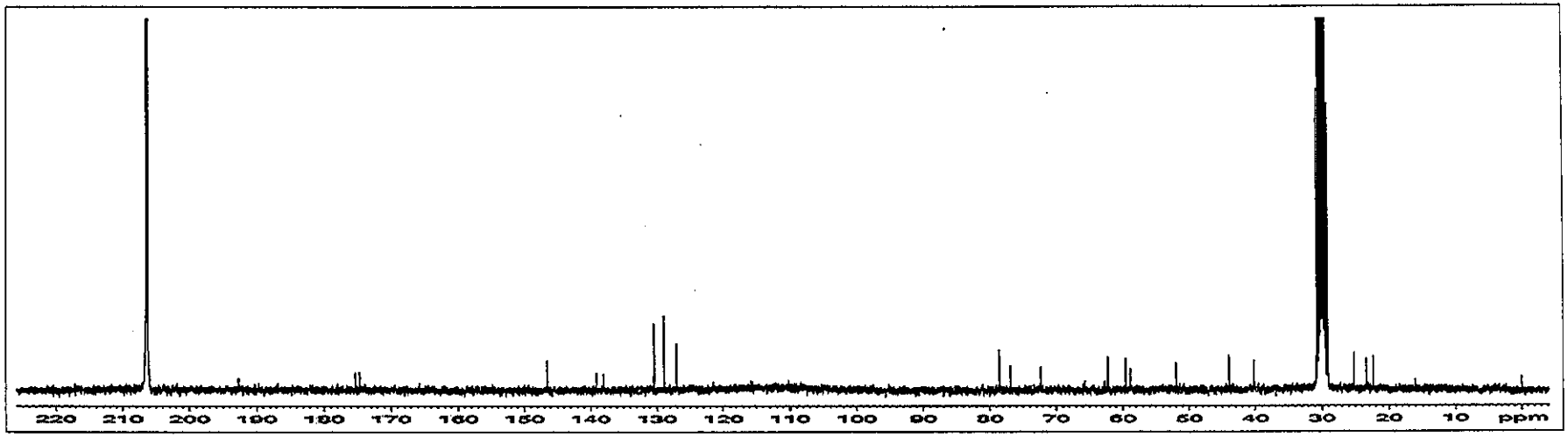

Figure 17 The 125 MHz ^{13}C NMR spectrum of compound **K7** in acetone-d_6

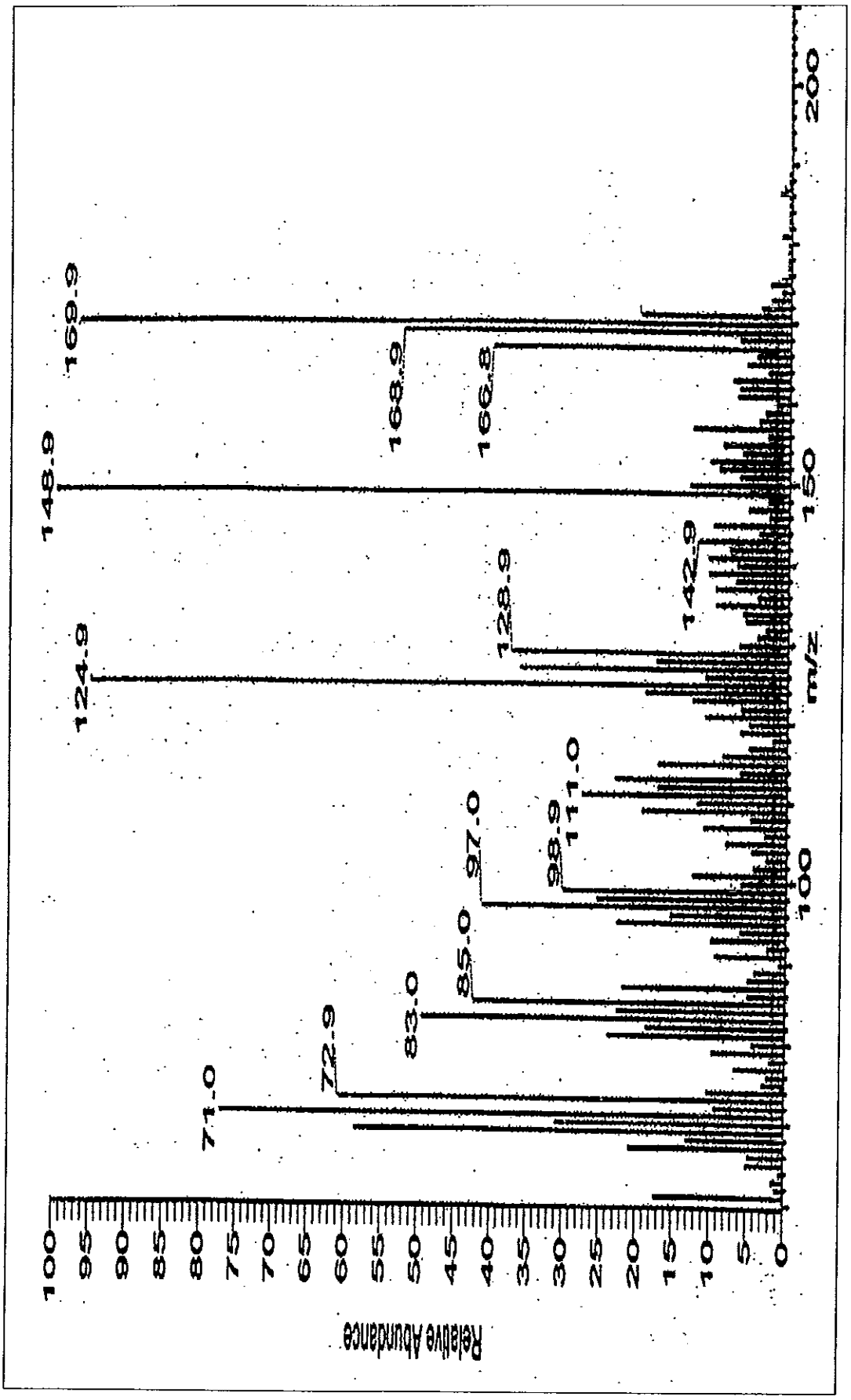

Figure 18 The mass spectrum of compound K8

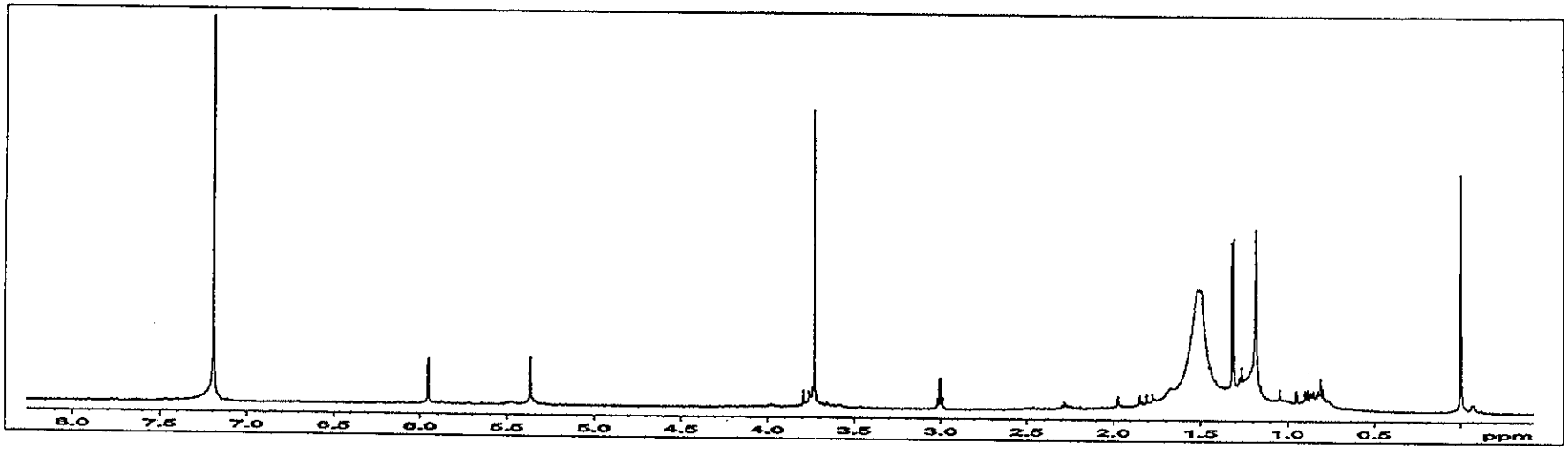

Figure 19 The 500 MHz ^{1}H NMR spectrum of compound **K8** in $CDCl_3$

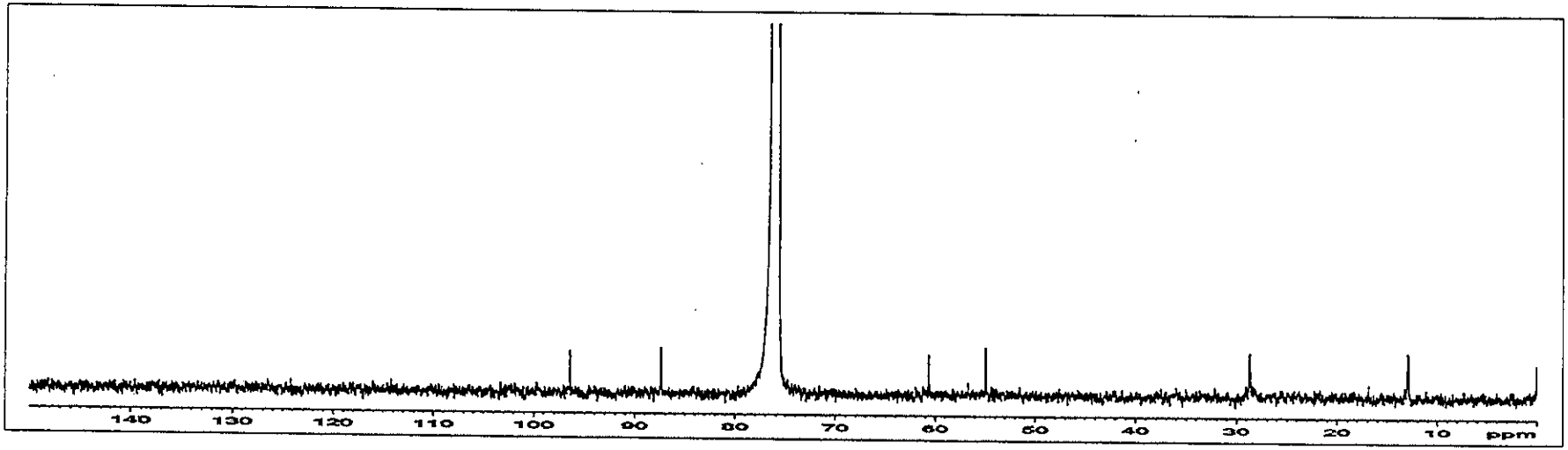

Figure 20 The 125 MHz ^{13}C NMR spectrum of compound **K8** in $CDCl_3$

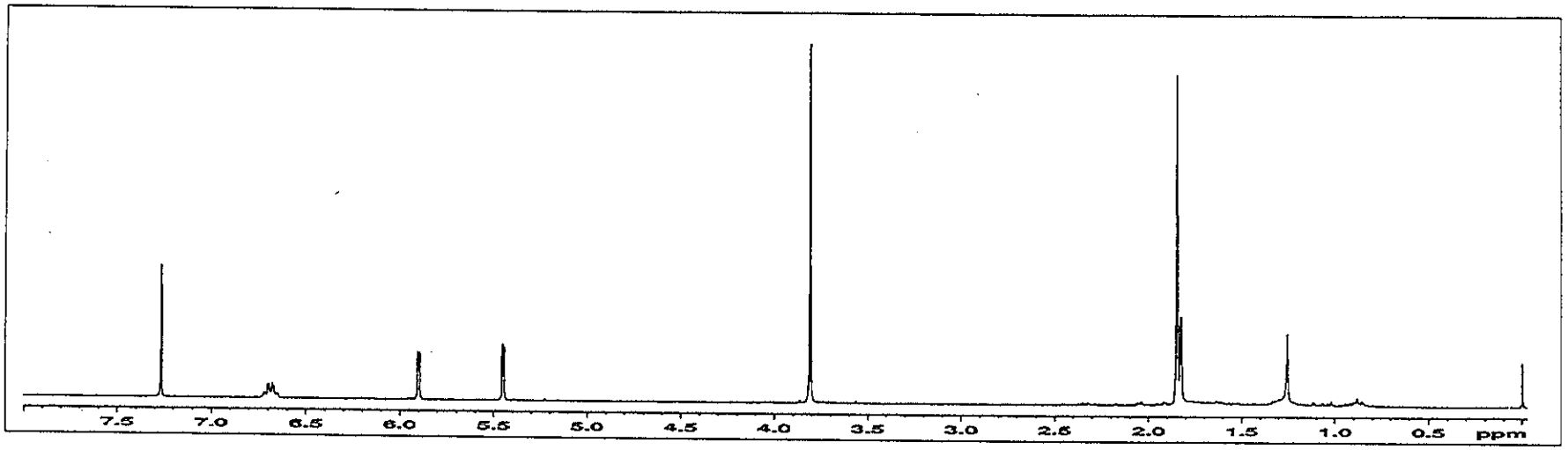

Figure 21 The 300 MHz ^{1}H NMR spectrum of compound **K9** in $CDCl_3$

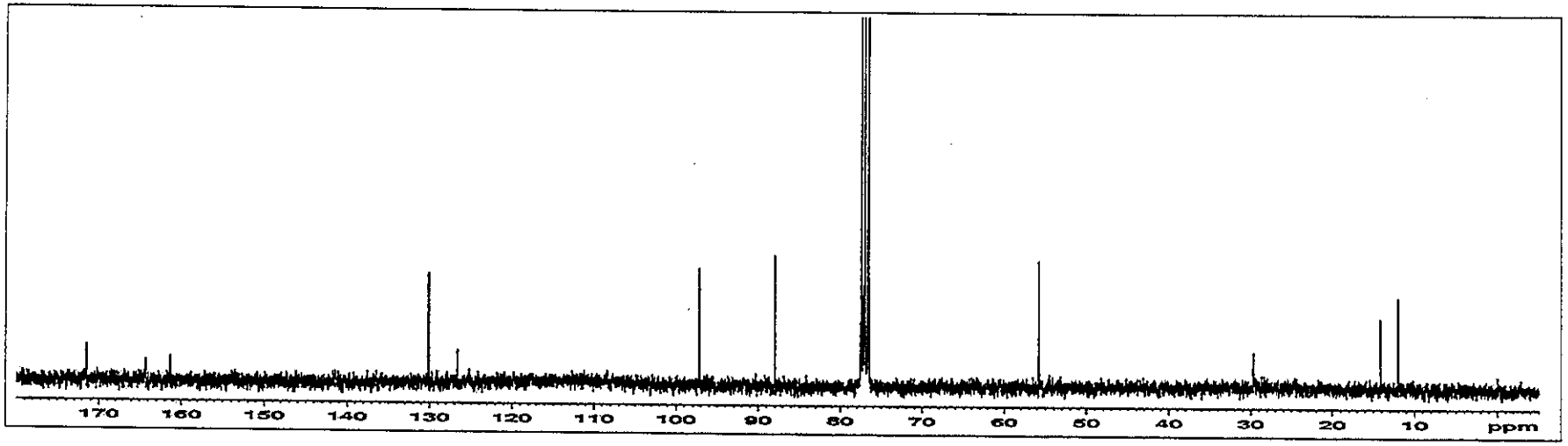

Figure 22 The 75 MHz ^{13}C NMR spectrum of compound **K9** in $CDCl_3$

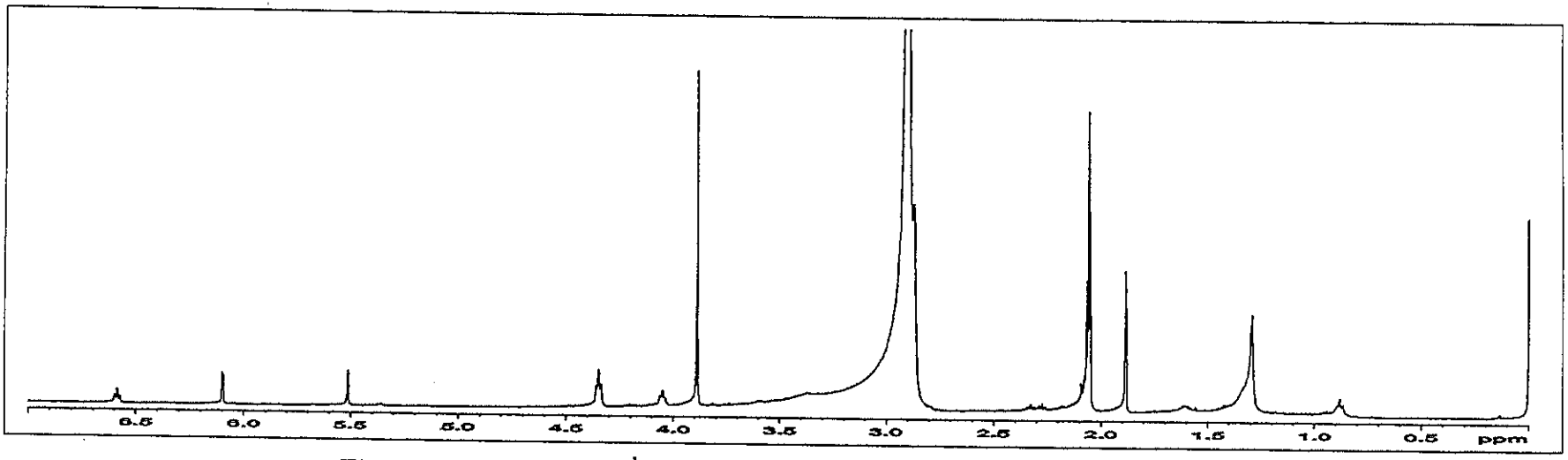

Figure 23 The 500 MHz ^{1}H NMR spectrum of compound **K10** in acetone-d_6

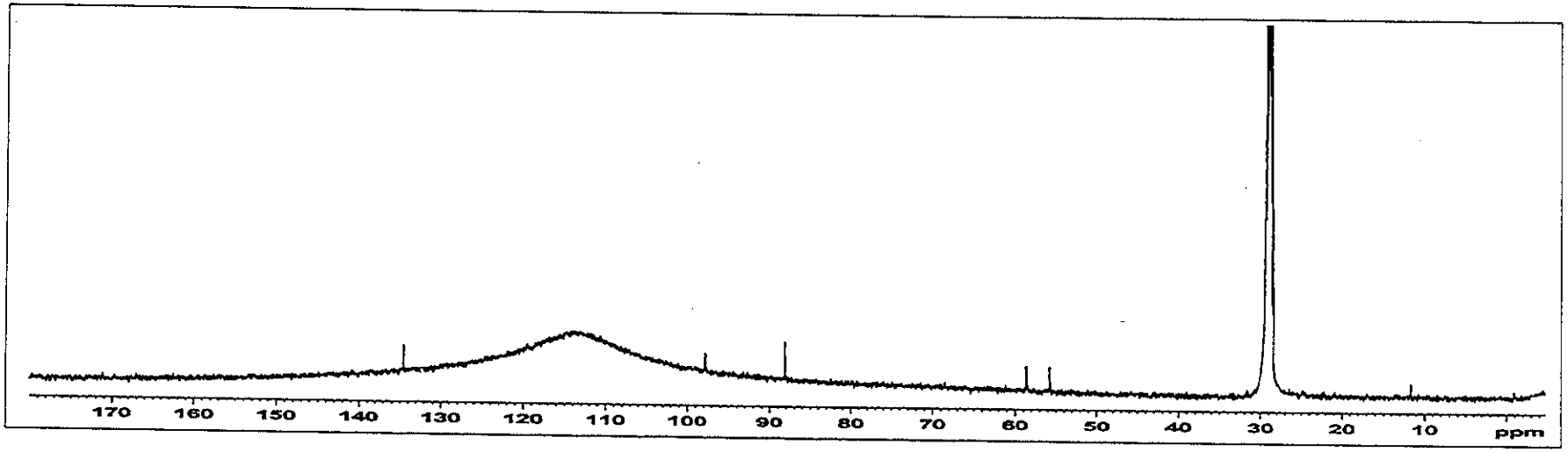

Figure 24 The 125 MHz ^{13}C NMR spectrum of compound **K10** in acetone-d_6

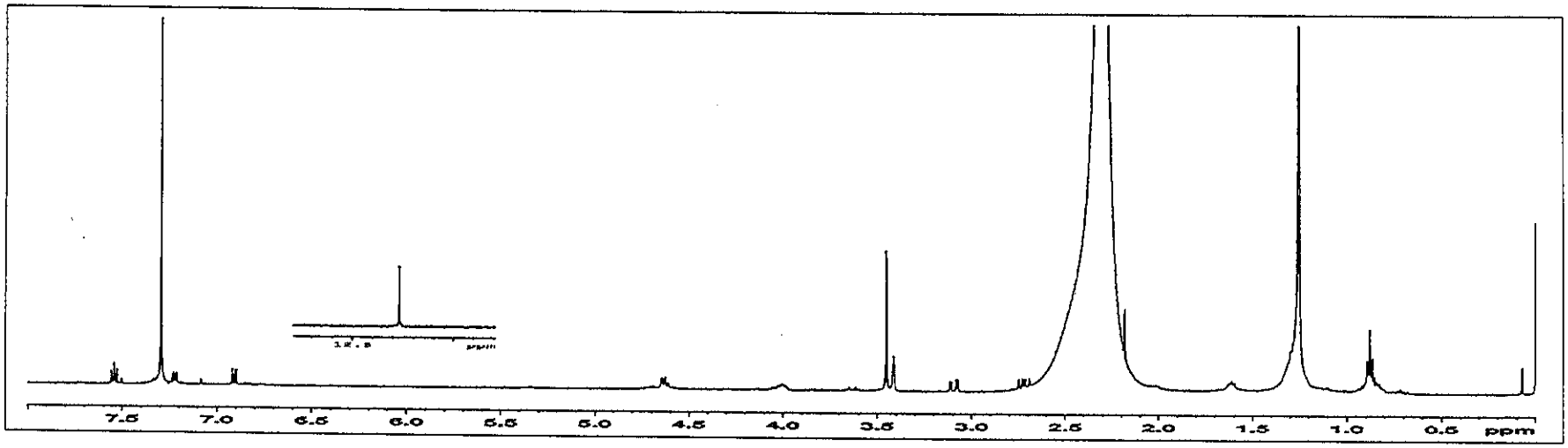

Figure 25 The 500 MHz ^{1}H NMR spectrum of compound **K11** in $CDCl_3$+CD_3OD

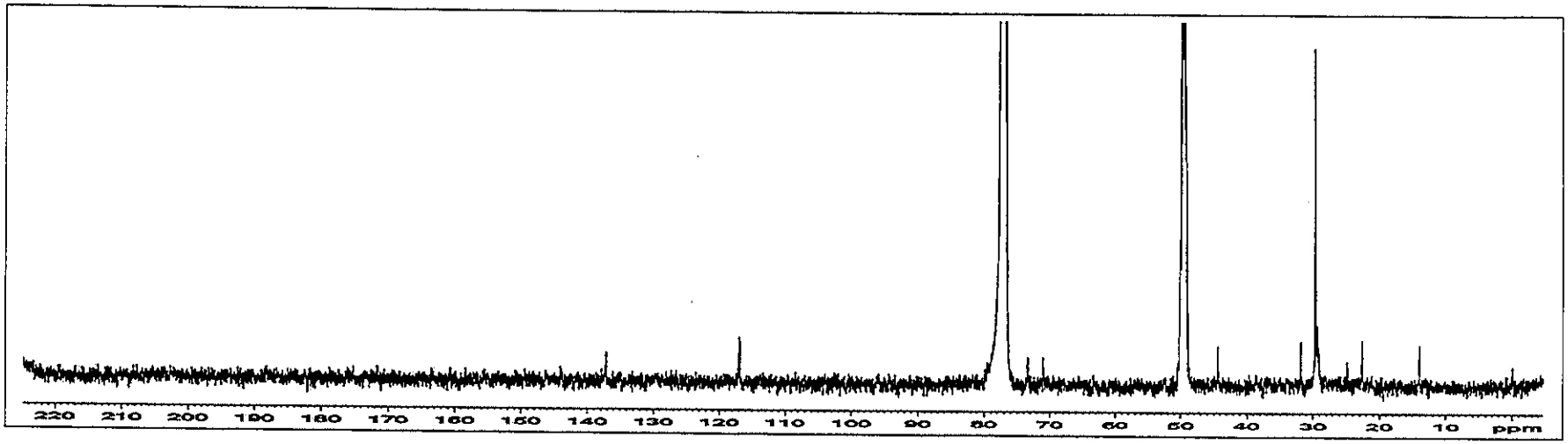

Figure 26 The 125 MHz ^{13}C NMR spectrum of compound **K11** in $CDCl_3$+CD_3OD

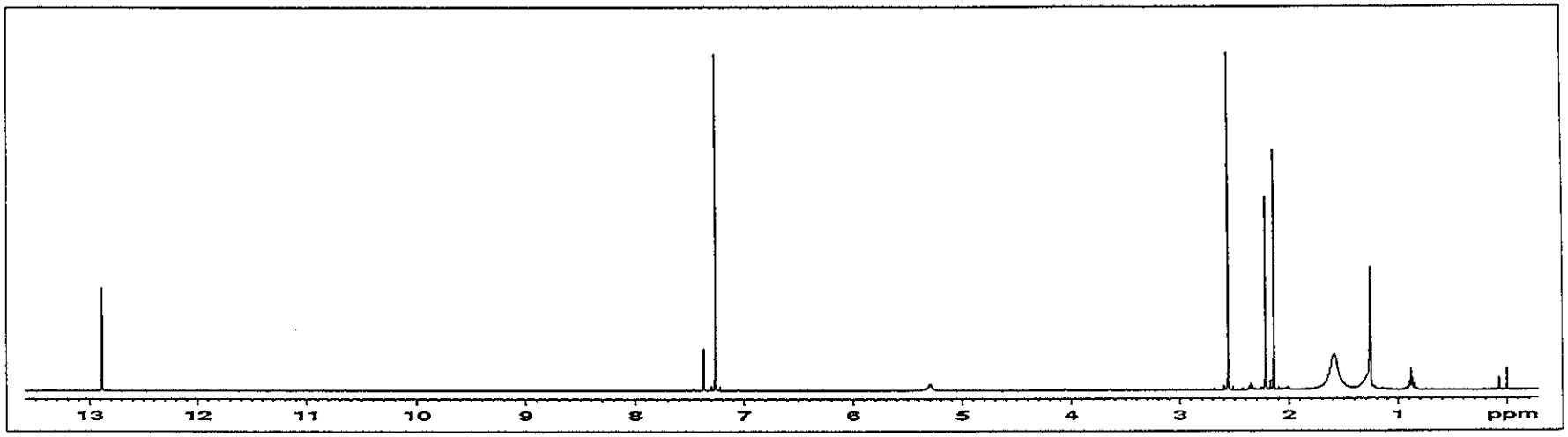

Figure 27 The 300 MHz ^{1}H NMR spectrum of compound **K12** in $CDCl_3$

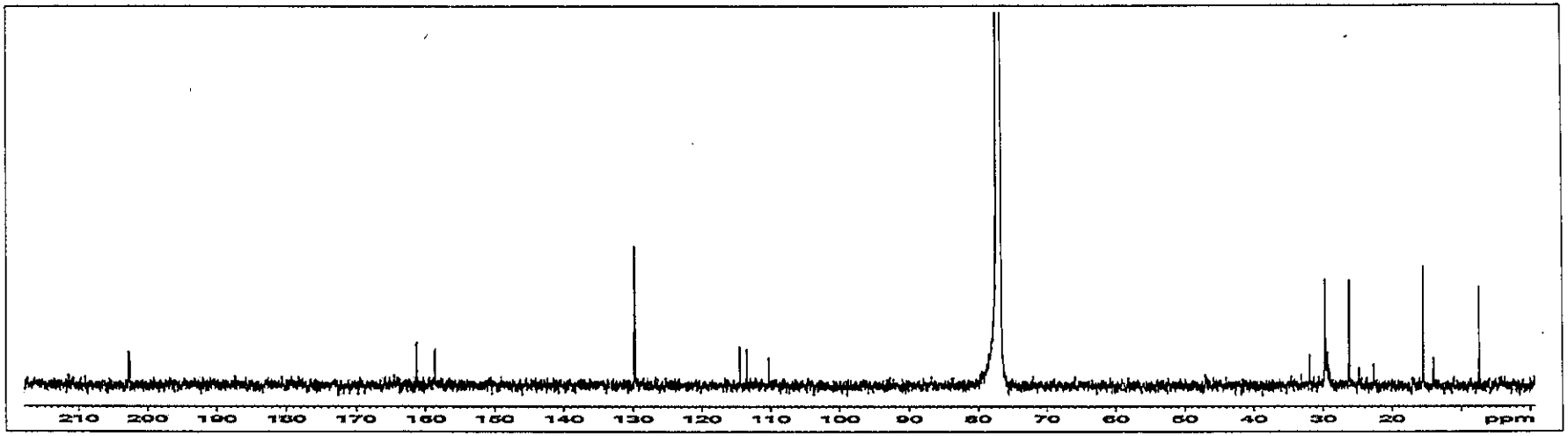

Figure 28 The 75 MHz ^{13}C NMR spectrum of compound **K12** in $CDCl_3$

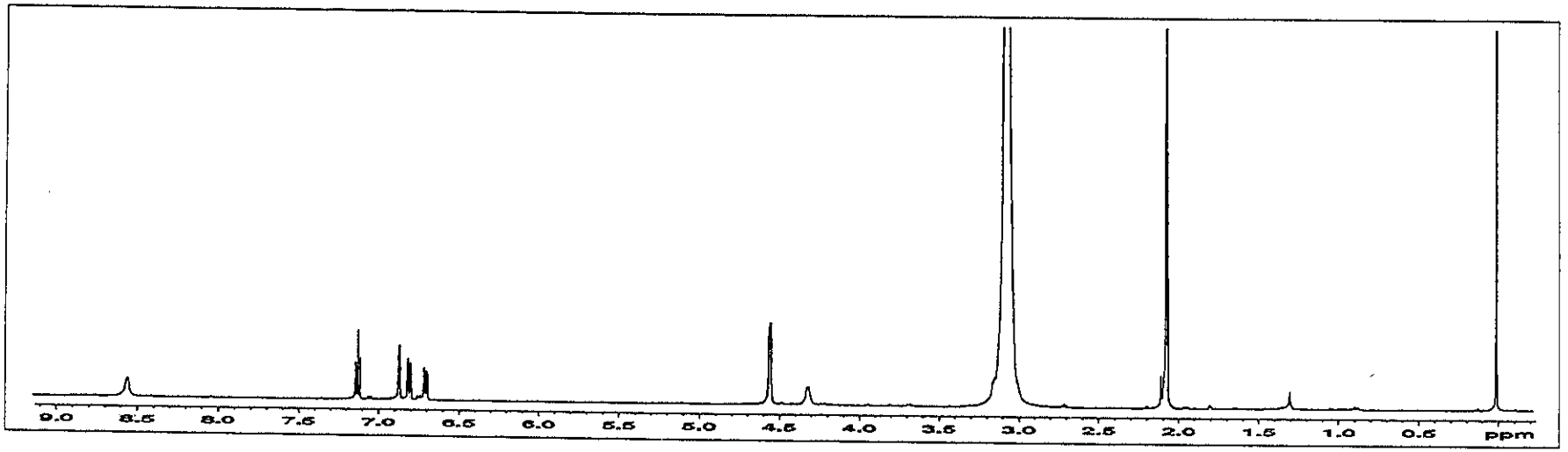

Figure 29 The 300 MHz ^{1}H NMR spectrum of compound **K13** in acetone-d_6

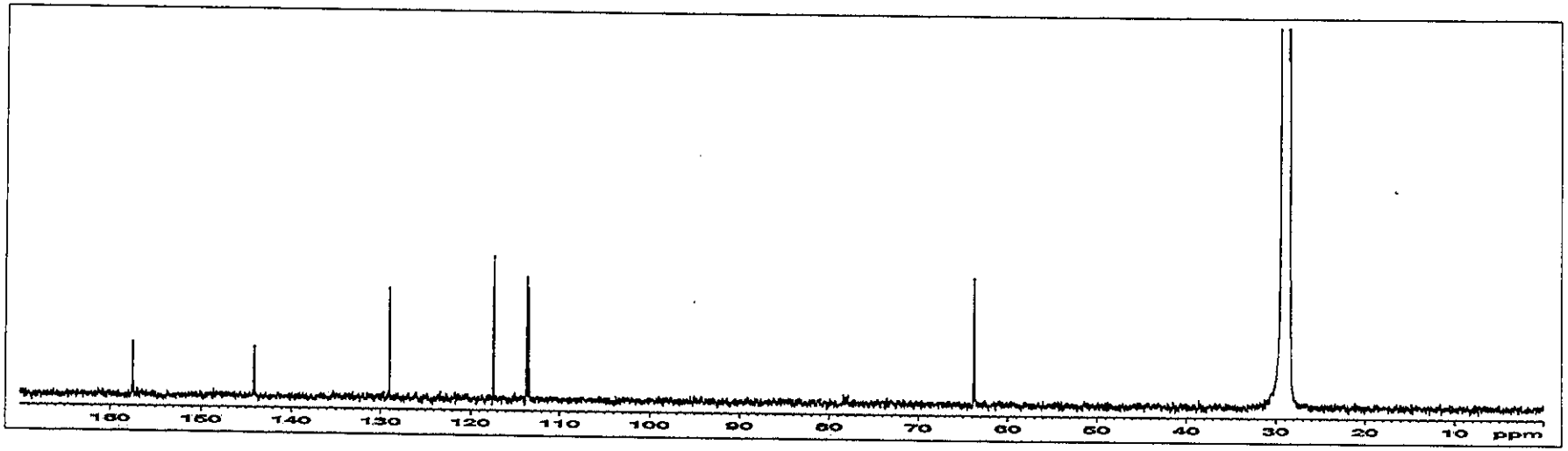

Figure 30 The 75 MHz ^{13}C NMR spectrum of compound **K13** in acetone-d_6

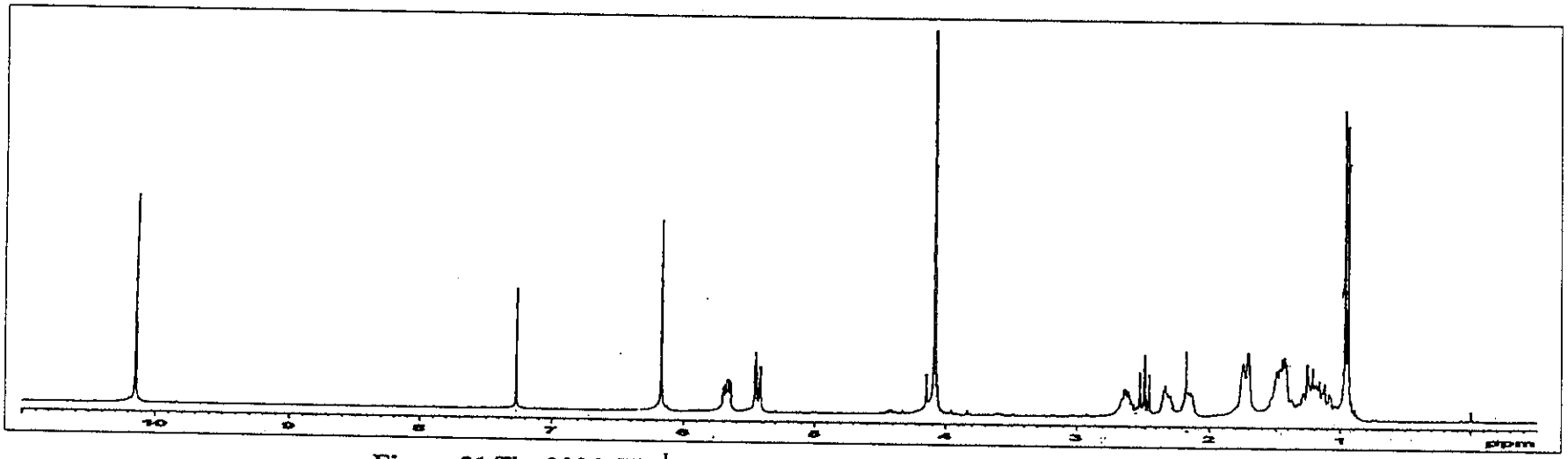

Figure 31 The 300 MHz ^{1}H NMR spectrum of compound **K14** in $CDCl_3$

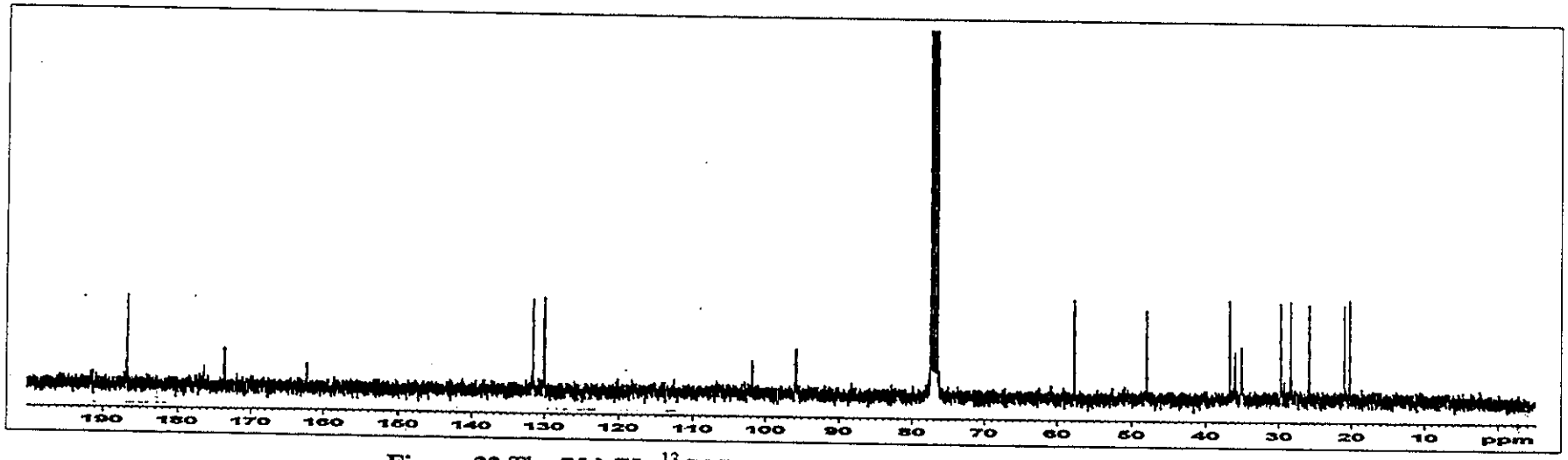

Figure 32 The 75 MHz ^{13}C NMR spectrum of compound **K14** in $CDCl_3$

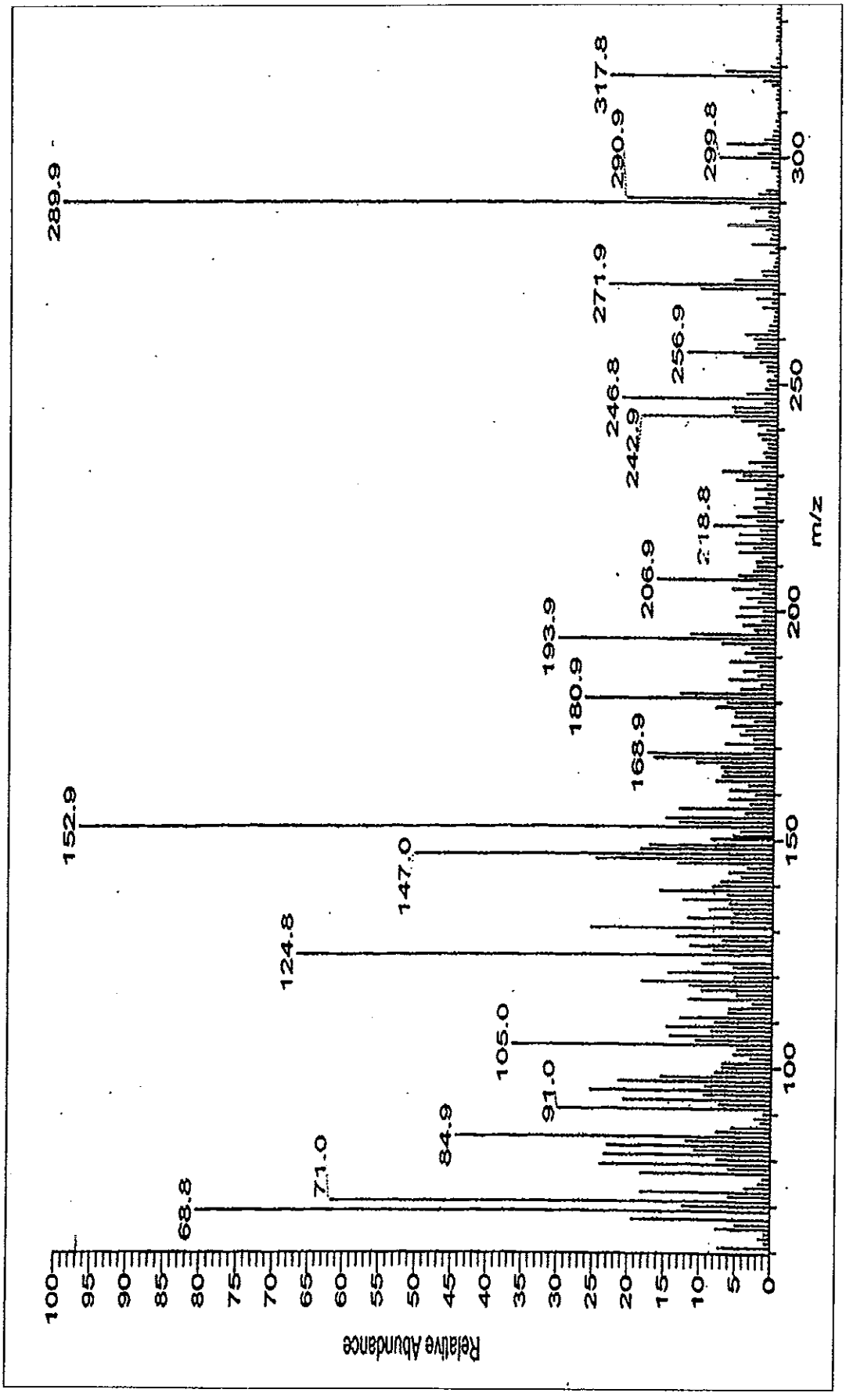

Figure 33 The mass spectrum of compound K15

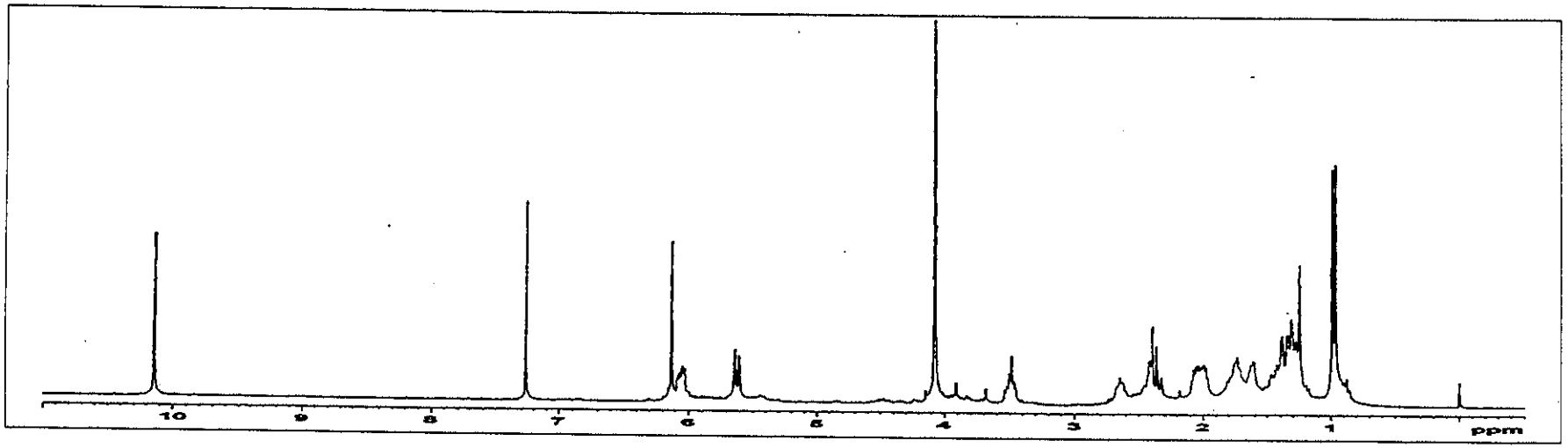

Figure 34 The 300 MHz ^{1}H NMR spectrum of compound **K15** in $CDCl_3$

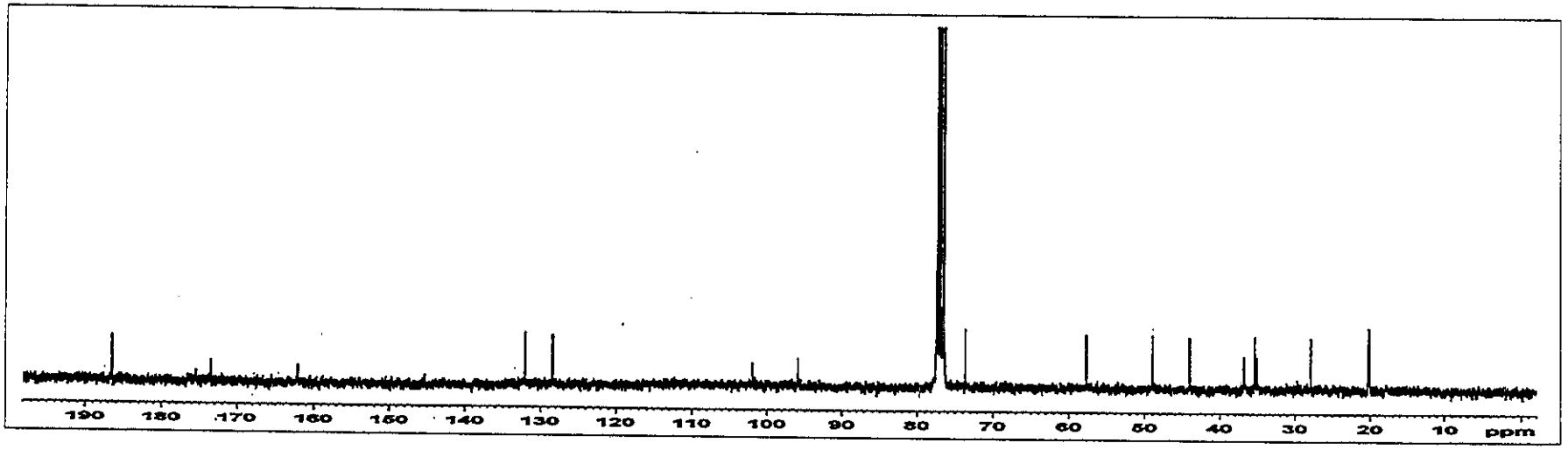

Figure 35 The 75 MHz ^{13}C NMR spectrum of compound **K15** in $CDCl_3$

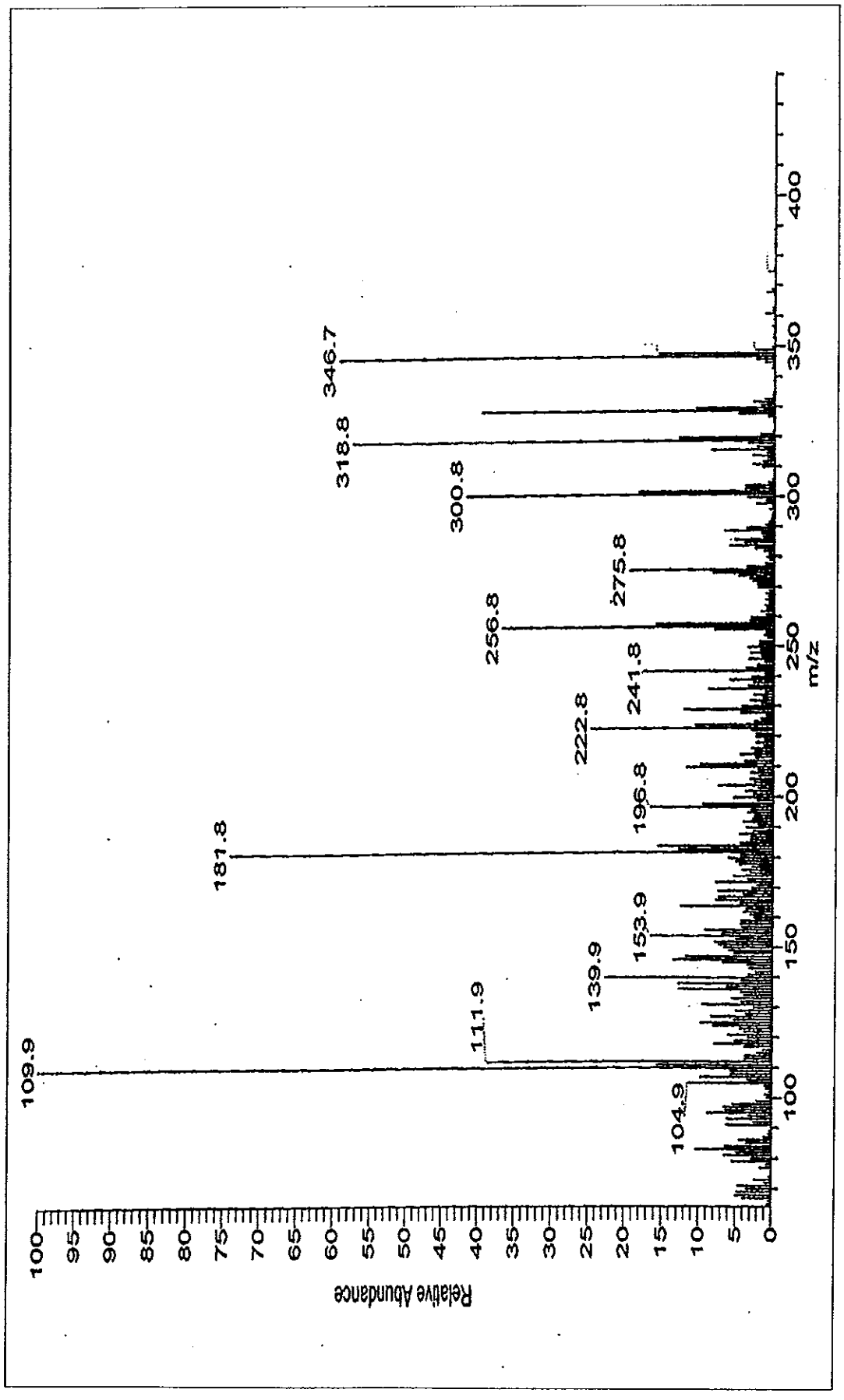

Figure 36 The mass spectrum of compound K16

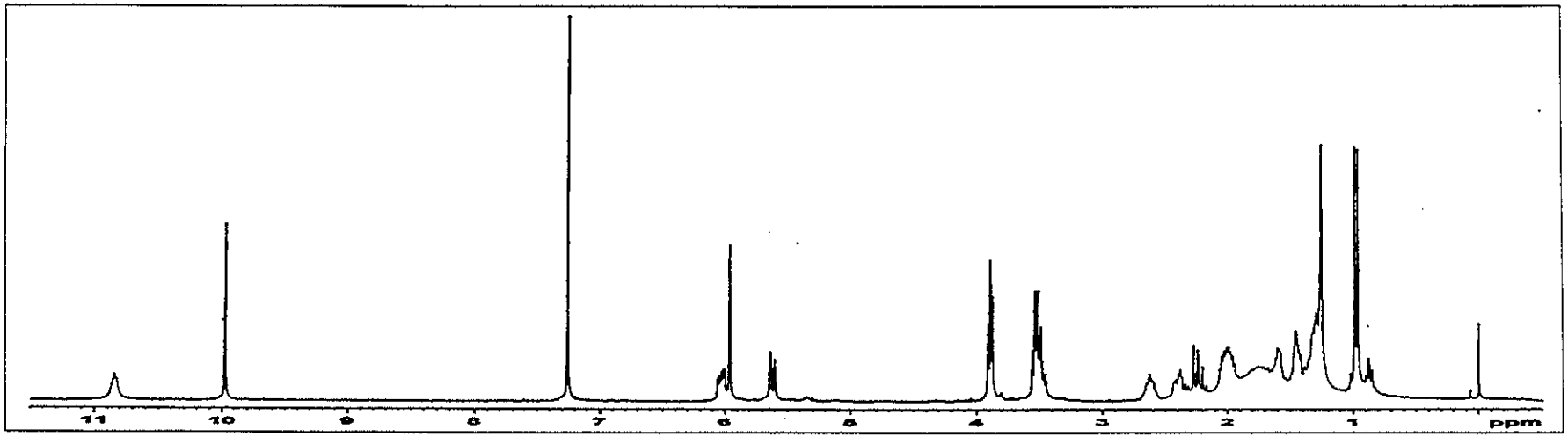

Figure 37 The 300 MHz ^{1}H NMR spectrum of compound **K16** in $CDCl_3$

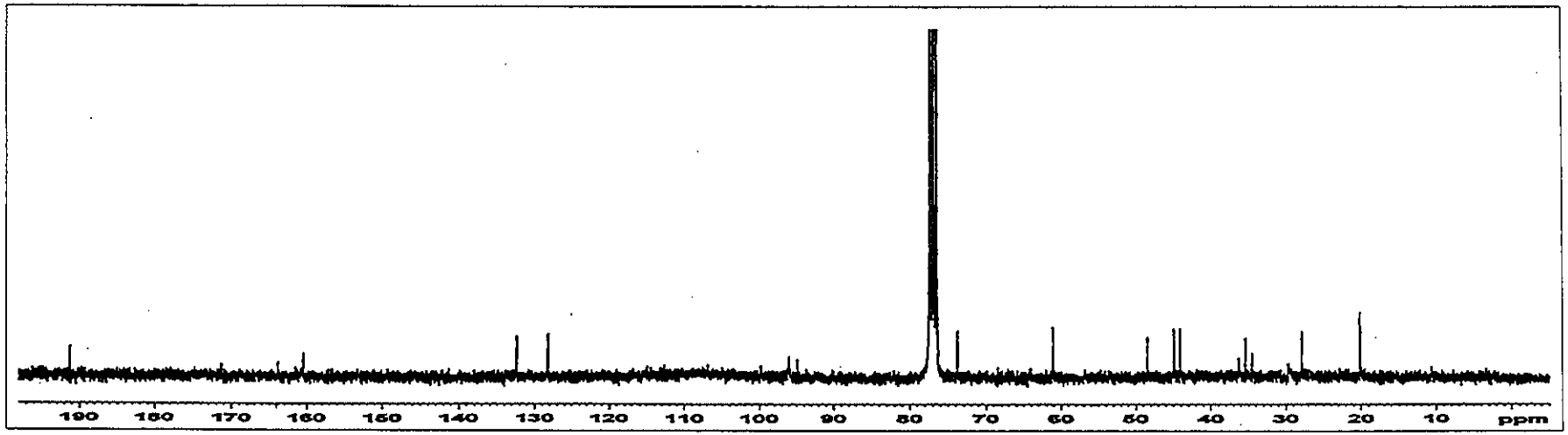

Figure 38 The 75 MHz ^{13}C NMR spectrum of compound **K16** in $CDCl_3$

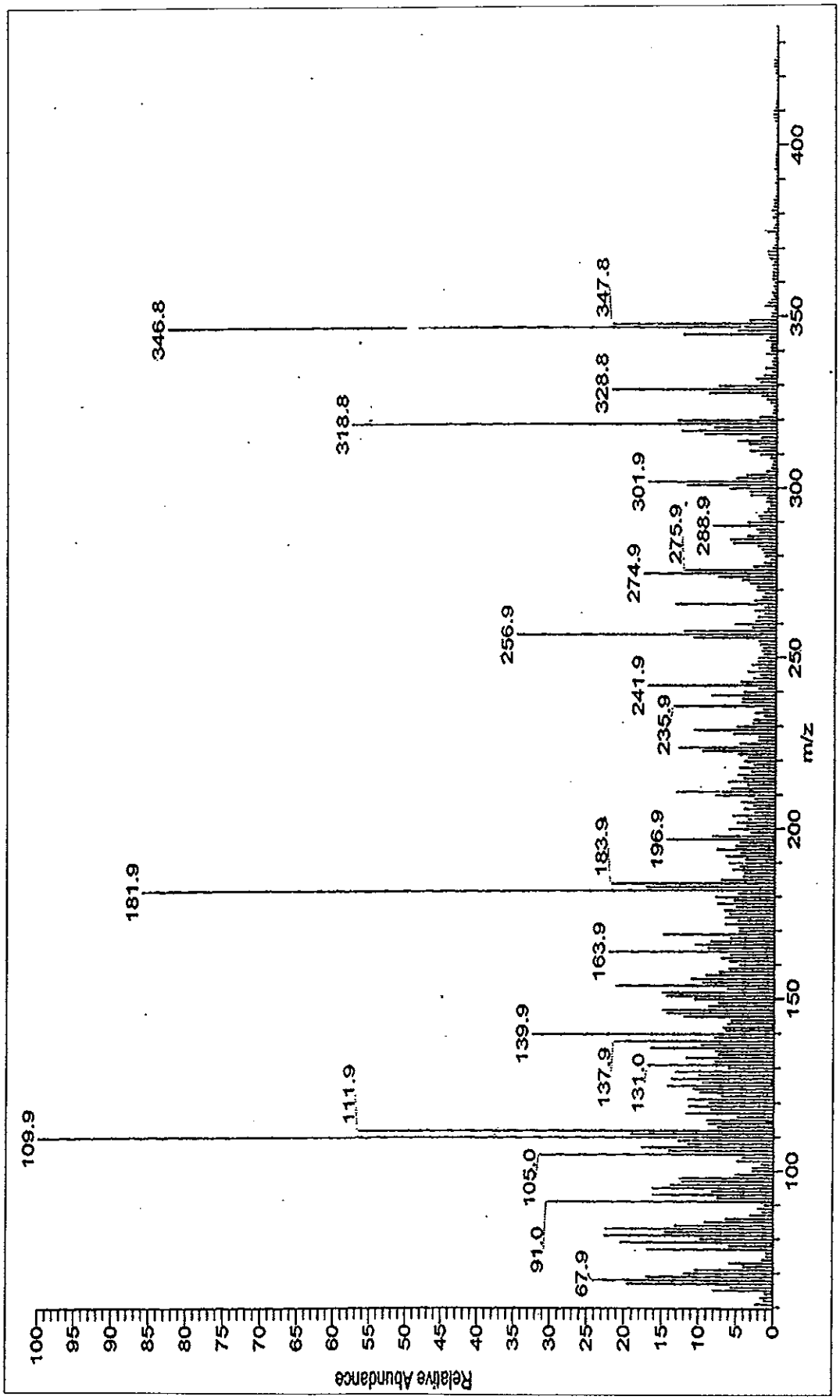

Figure 39 The mass spectrum of compound **K17**

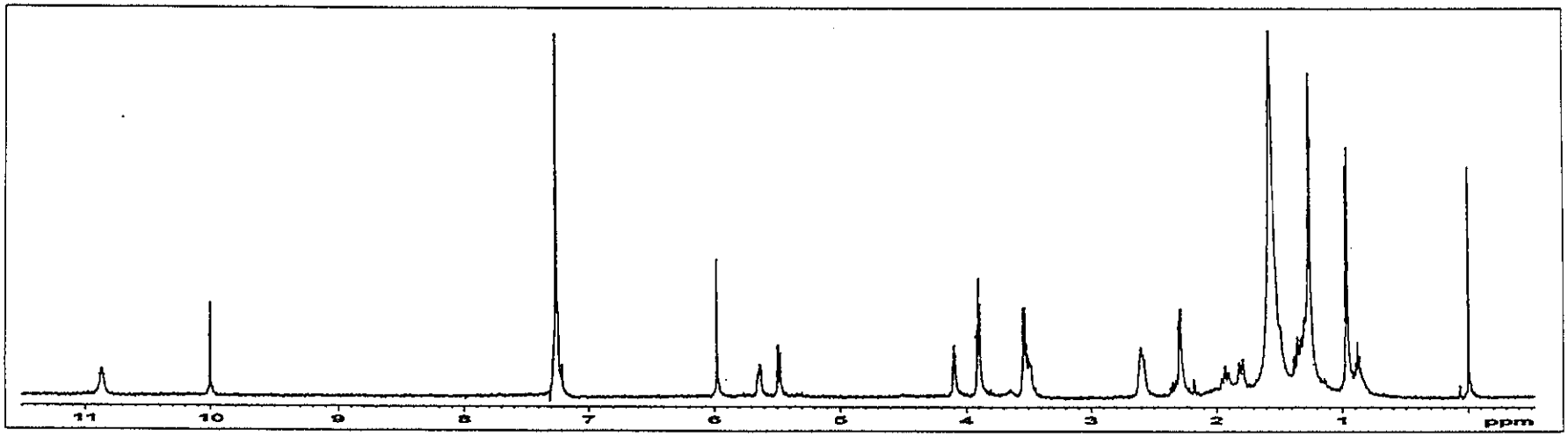

Figure 40 The 300 MHz ^{1}H NMR spectrum of compound **K17** in $CDCl_3$

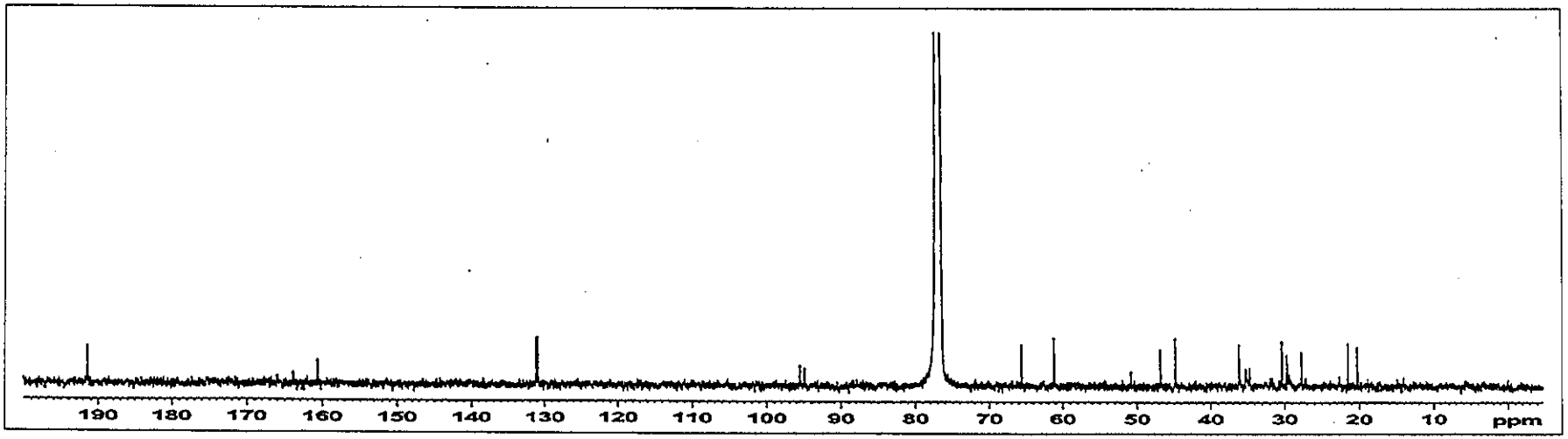

Figure 41 The 75 MHz ^{13}C NMR spectrum of compound **K17** in $CDCl_3$

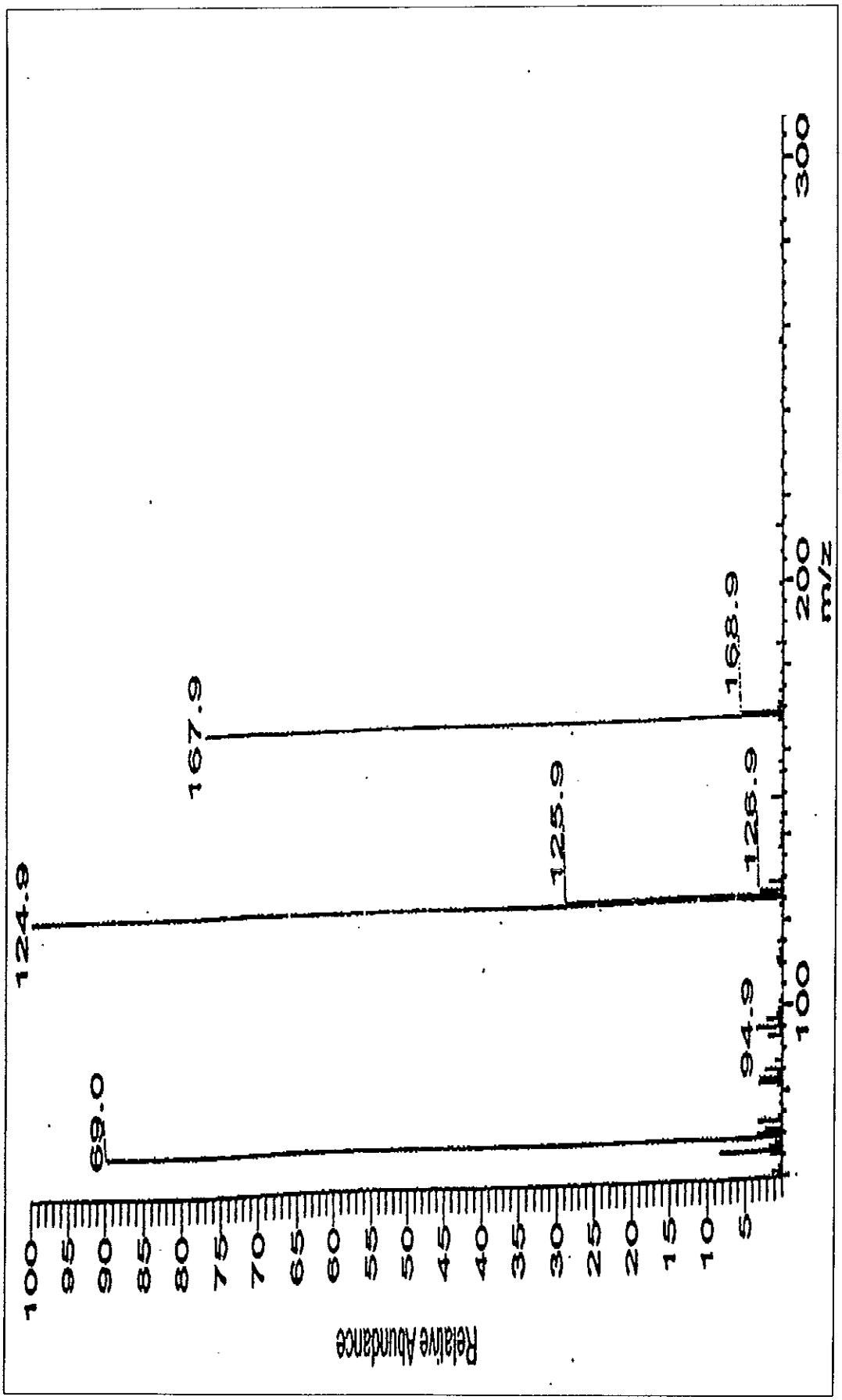

Figure 42 The mass spectrum of compound K18

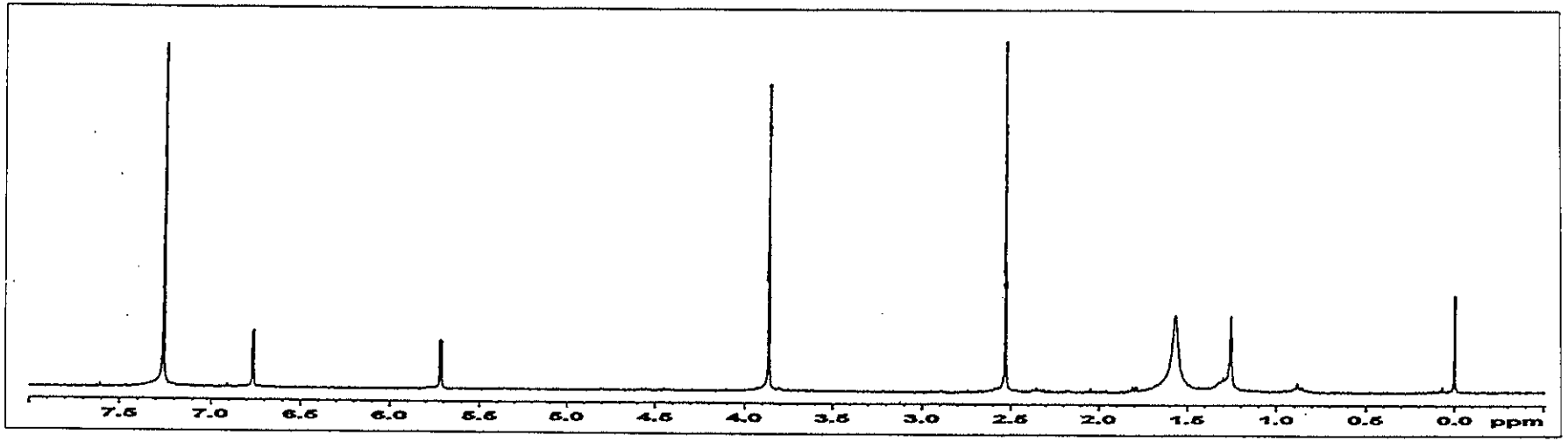

Figure 43 The 300 MHz ^{1}H NMR spectrum of compound **K18** in $CDCl_3$

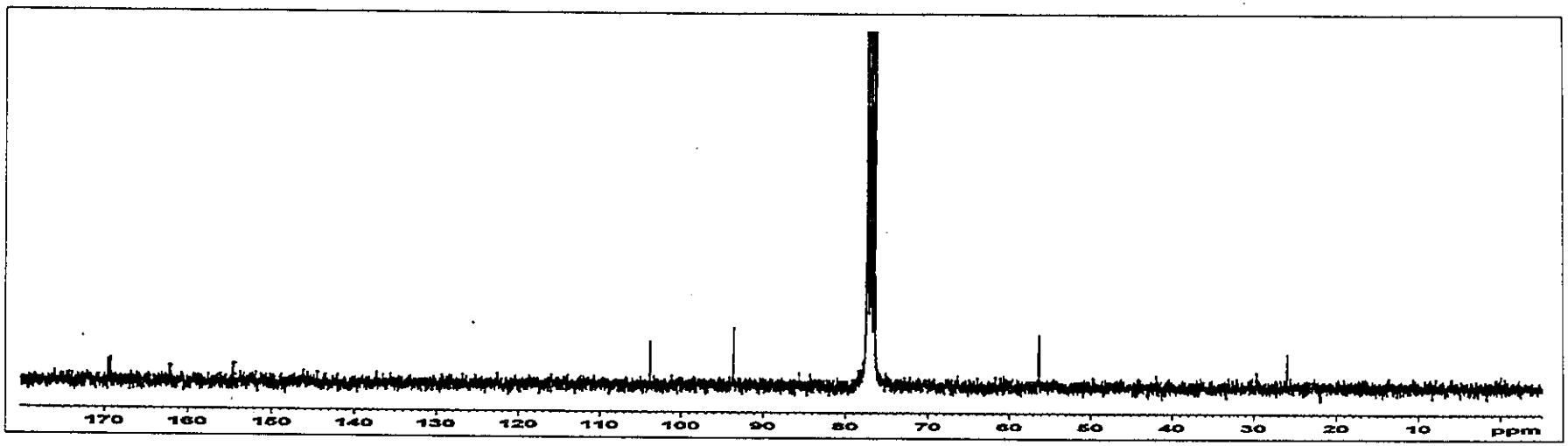

Figure 44 The 75 MHz ^{13}C NMR spectrum of compound **K18** in $CDCl_3$

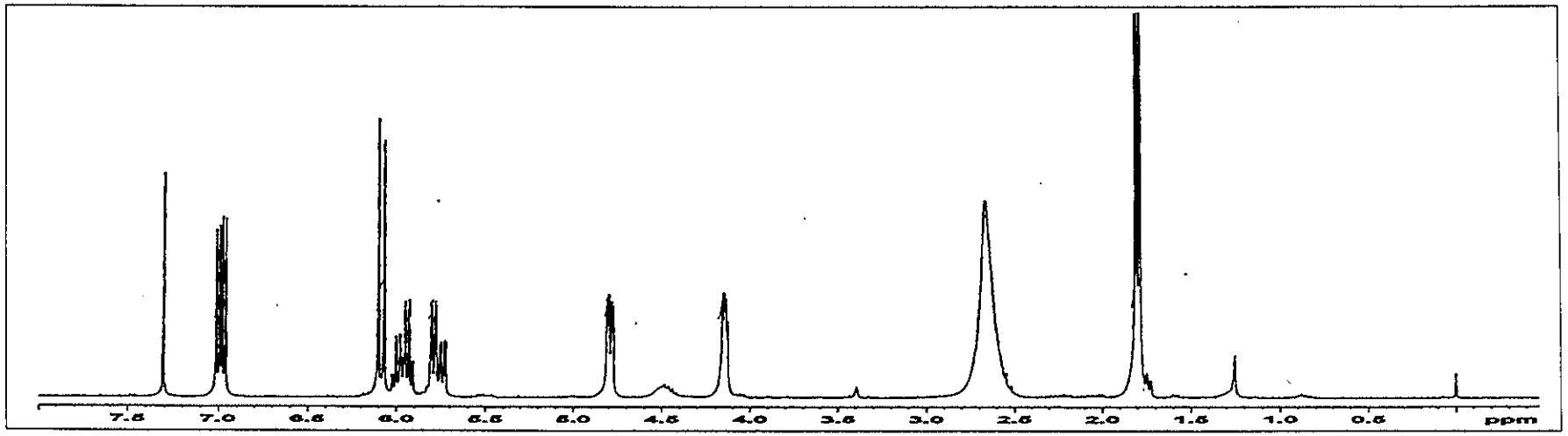

Figure 45 The 300 MHz ^{1}H NMR spectrum of compound **K19** in $CDCl_3$+CD_3OD

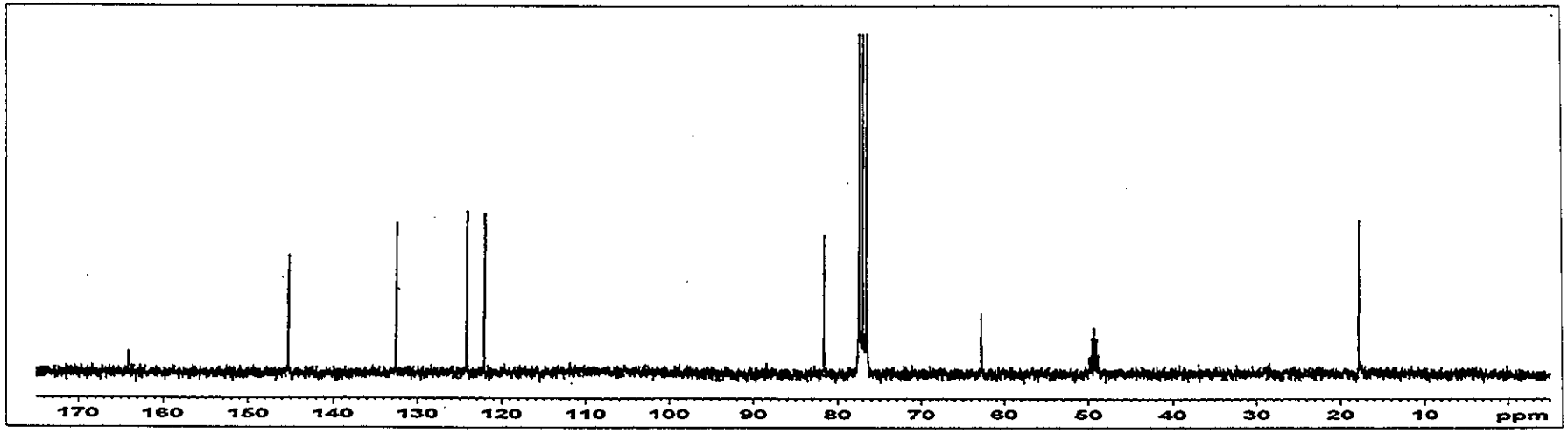

Figure 46 The 75 MHz ^{13}C NMR spectrum of compound **K19** in $CDCl_3$+CD_3OD

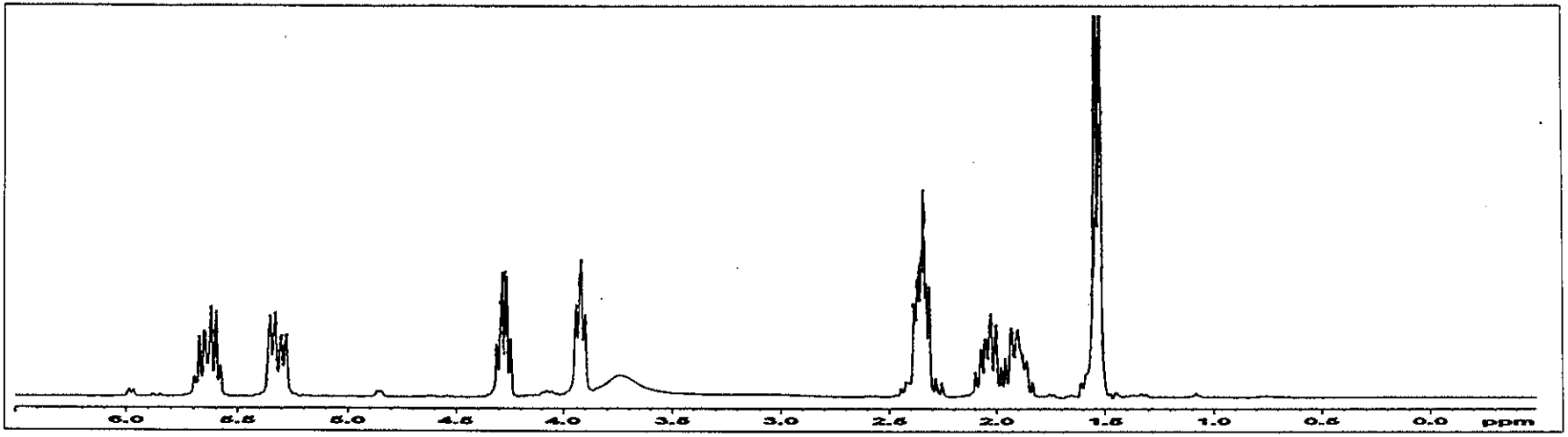

Figure 47 The 300 MHz ^{1}H NMR spectrum of compound **K20** in $CDCl_3$

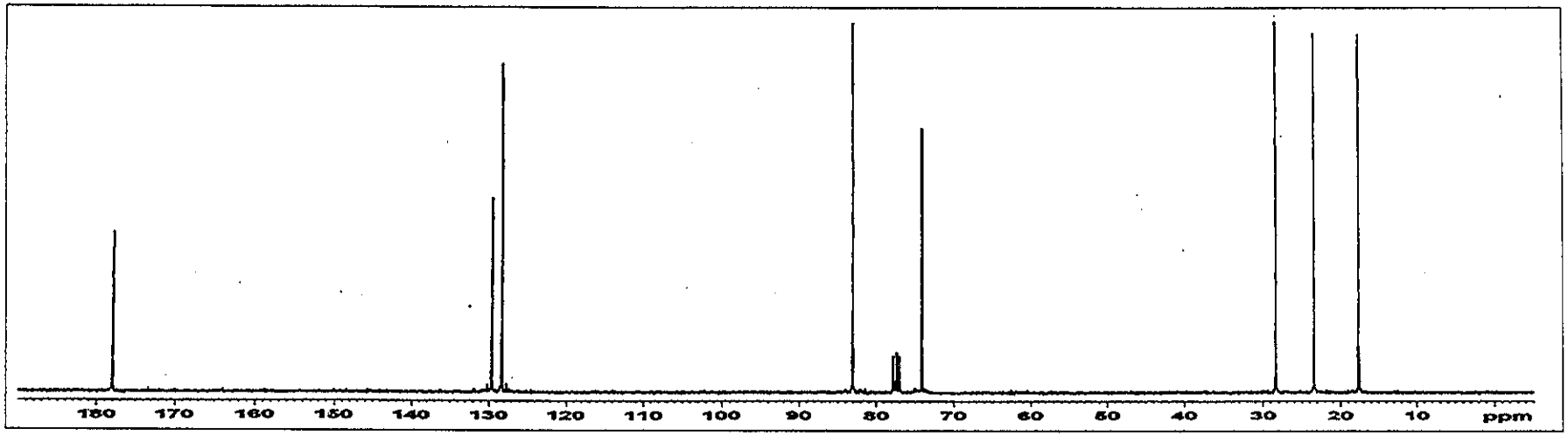

Figure 48 The 75 MHz ^{13}C NMR spectrum of compound **K20** in $CDCl_3$

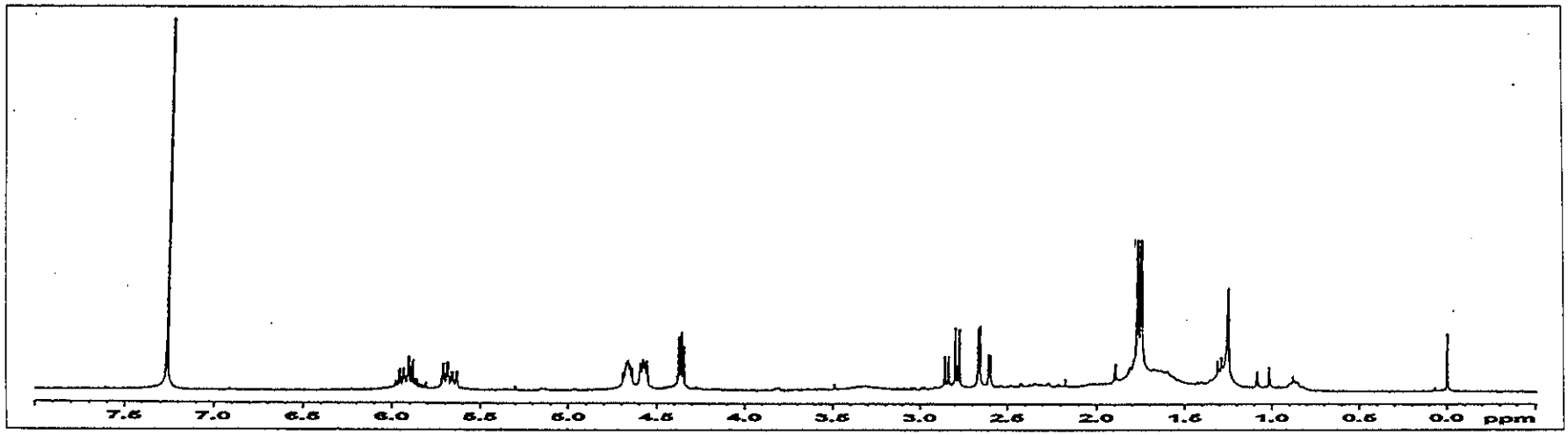

Figure 49 The 300 MHz ^{1}H NMR spectrum of compound **K21** in $CDCl_3$

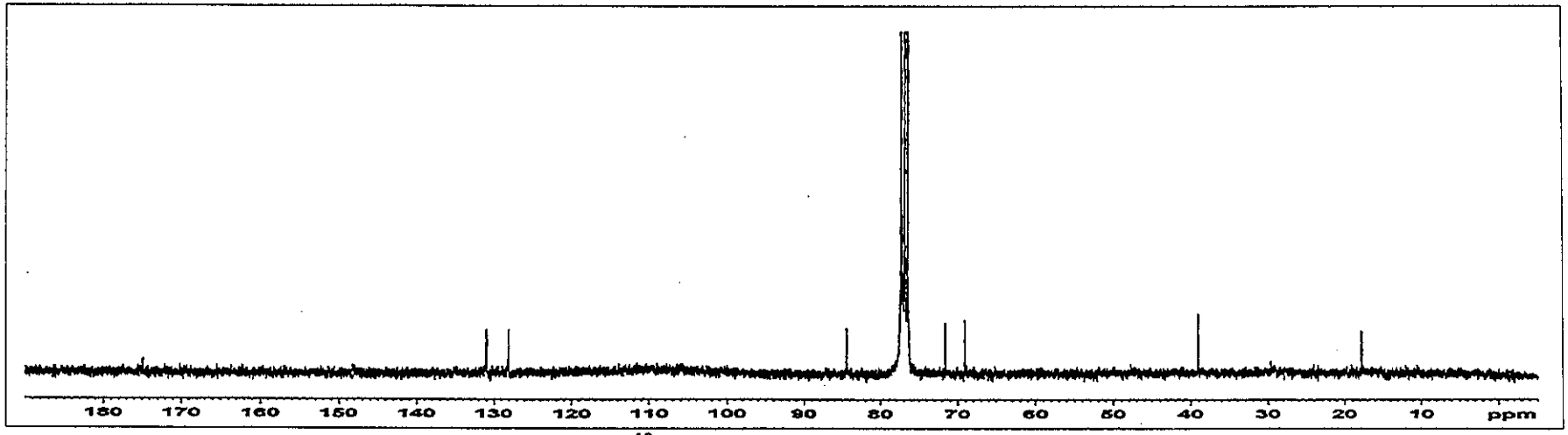

Figure 50 The 75 MHz ^{13}C NMR spectrum of compound **K21** in $CDCl_3$

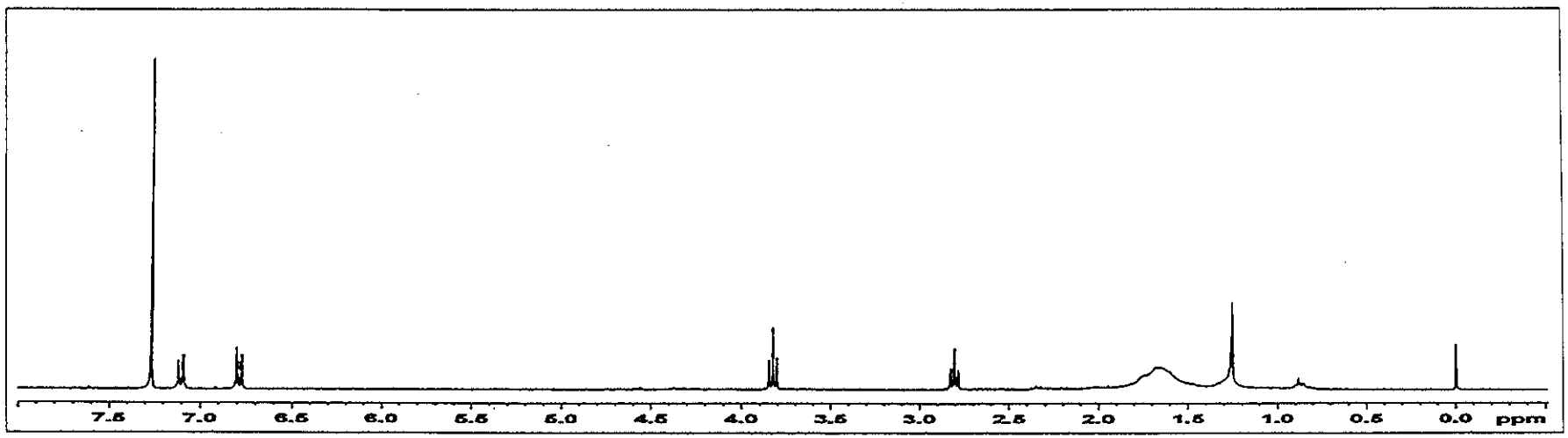

Figure 51 The 300 MHz ^{1}H NMR spectrum of compound **K22** in $CDCl_3$

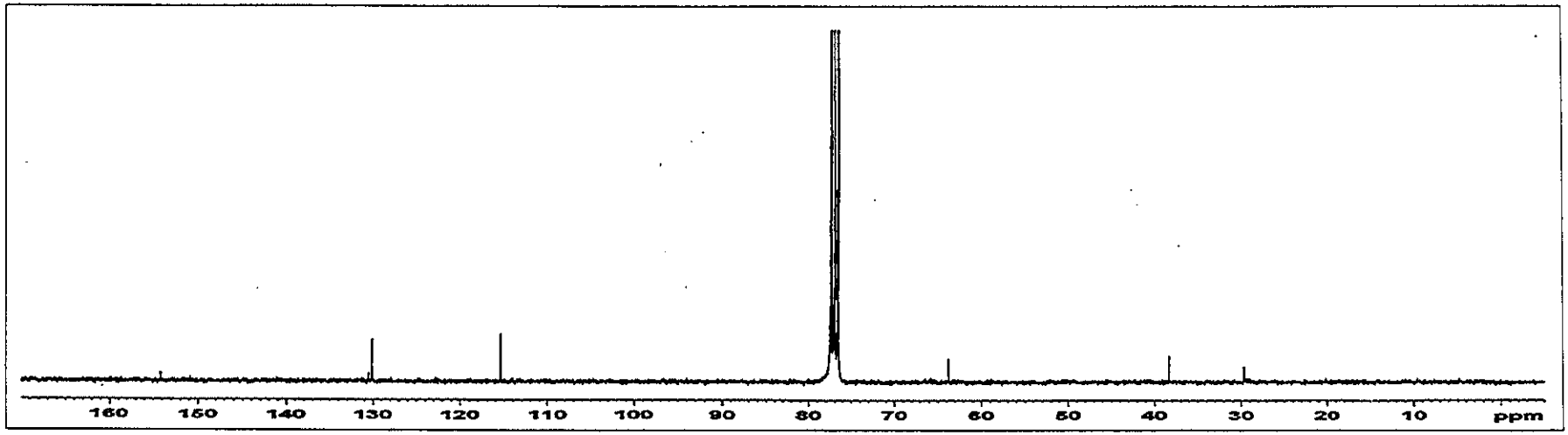

Figure 52 The 75 MHz ^{13}C NMR spectrum of compound **K22** in $CDCl_3$

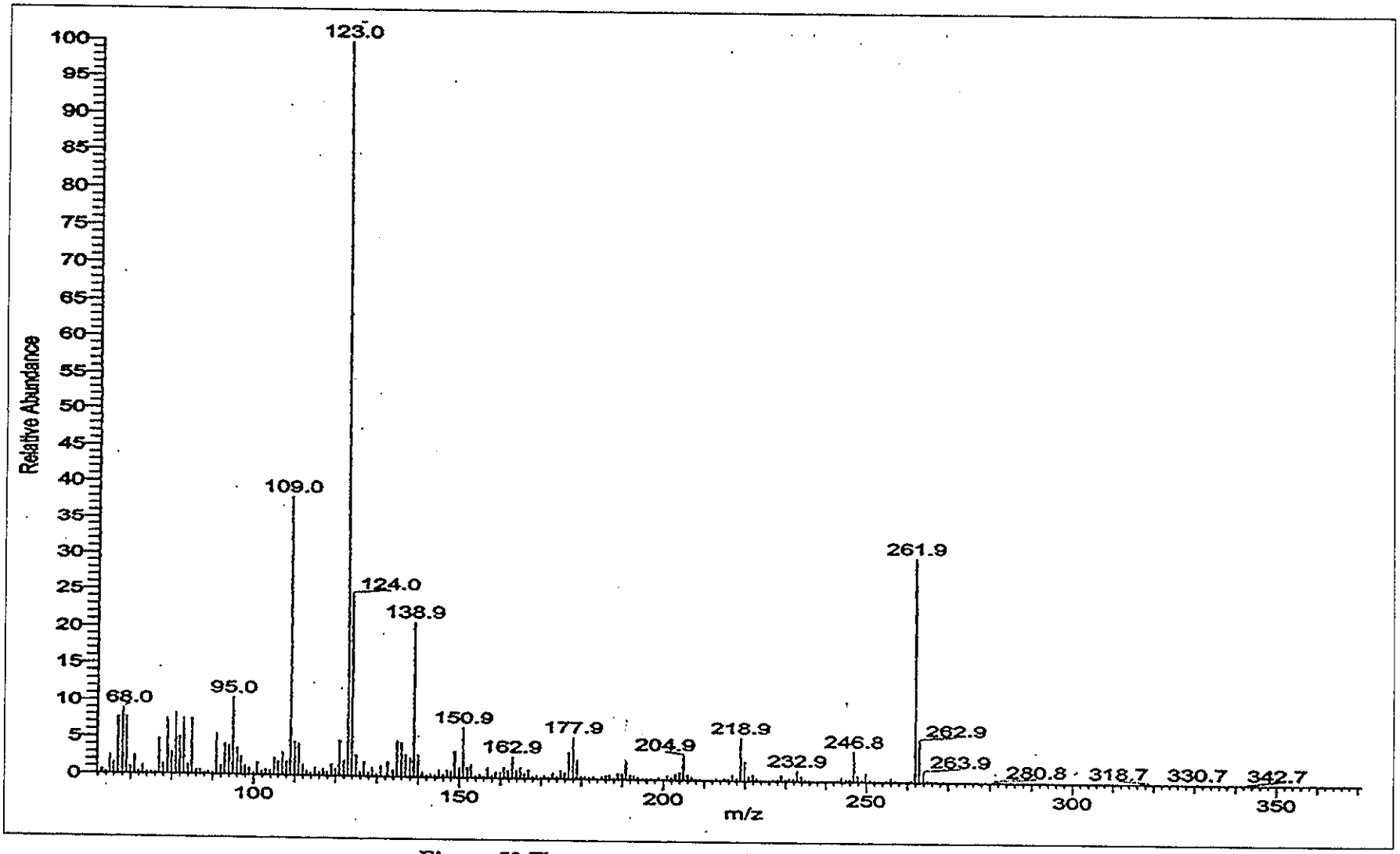

Figure 53 The mass spectrum of compound **K23**

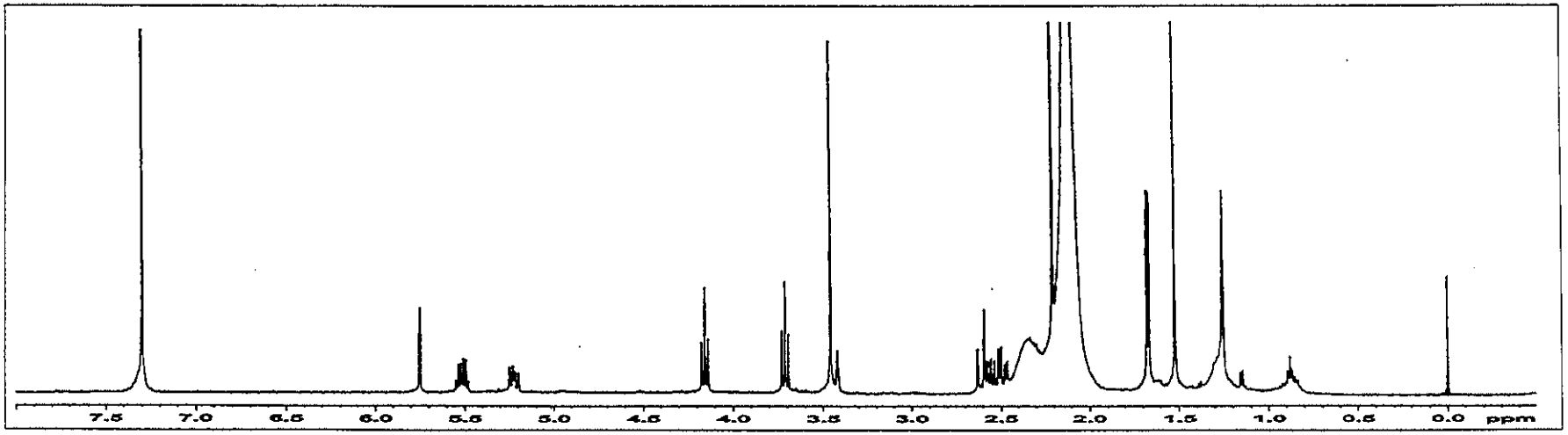

Figure 54 The 300 MHz ^{1}H NMR spectrum of compound **K23** in $CDCl_3$+CD_3OD

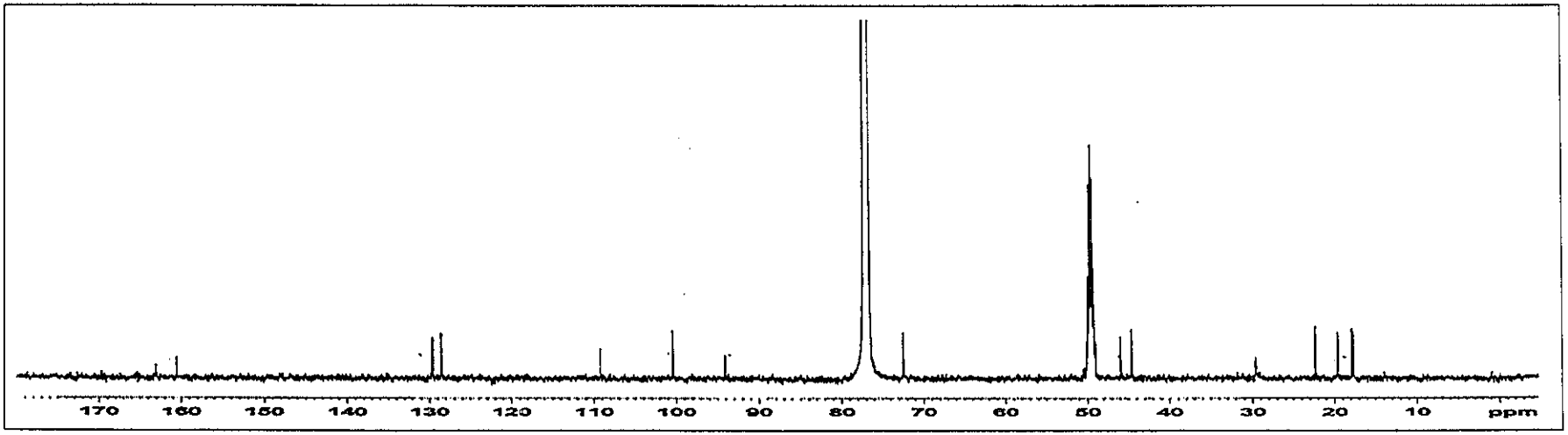

Figure 55 The 75 MHz ^{13}C NMR spectrum of compound **K23** in $CDCl_3$+CD_3OD

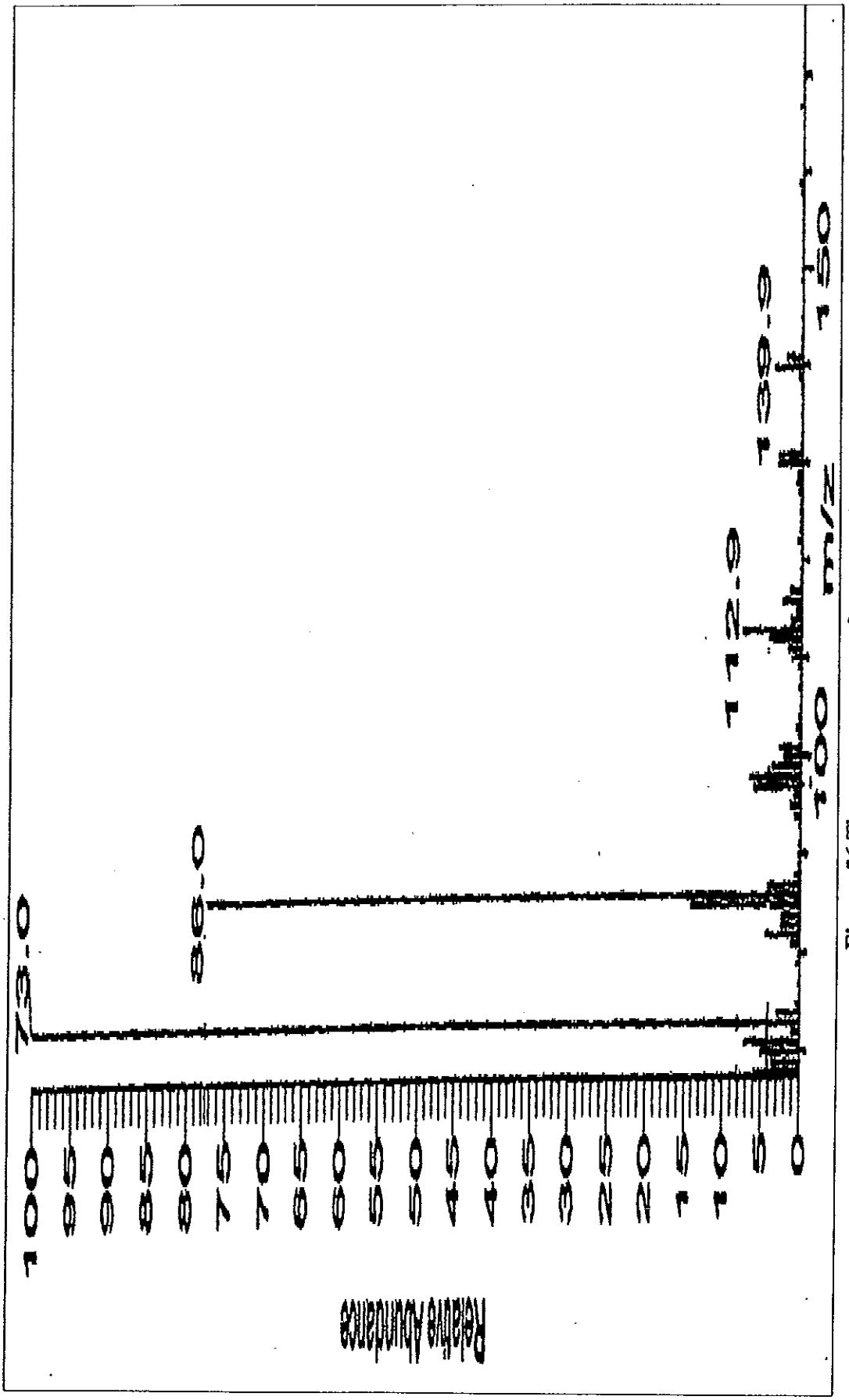

Figure 56 The mass spectrum of compound K24

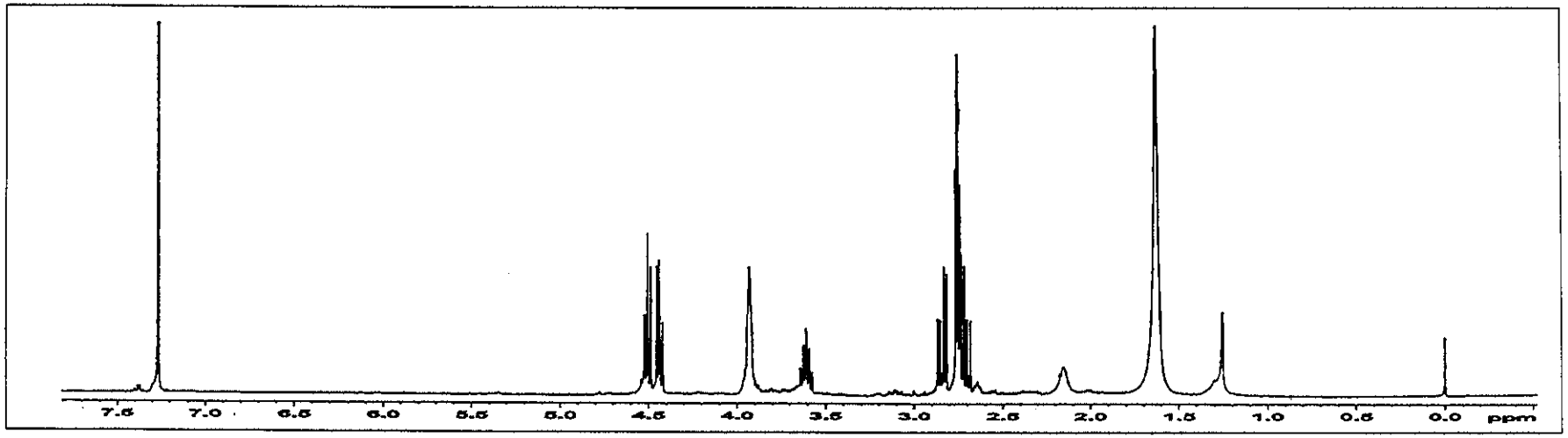

Figure 57 The 500 MHz ^{1}H NMR spectrum of compound **K24** in $CDCl_3$

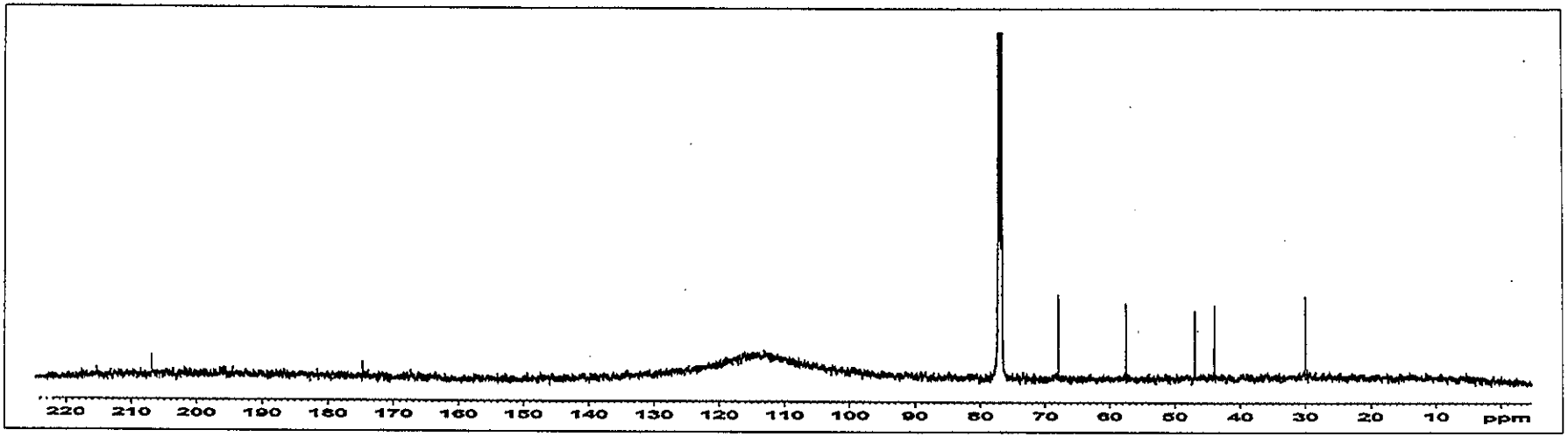

Figure 58 The 125 MHz ^{13}C NMR spectrum of compound **K24** in $CDCl_3$

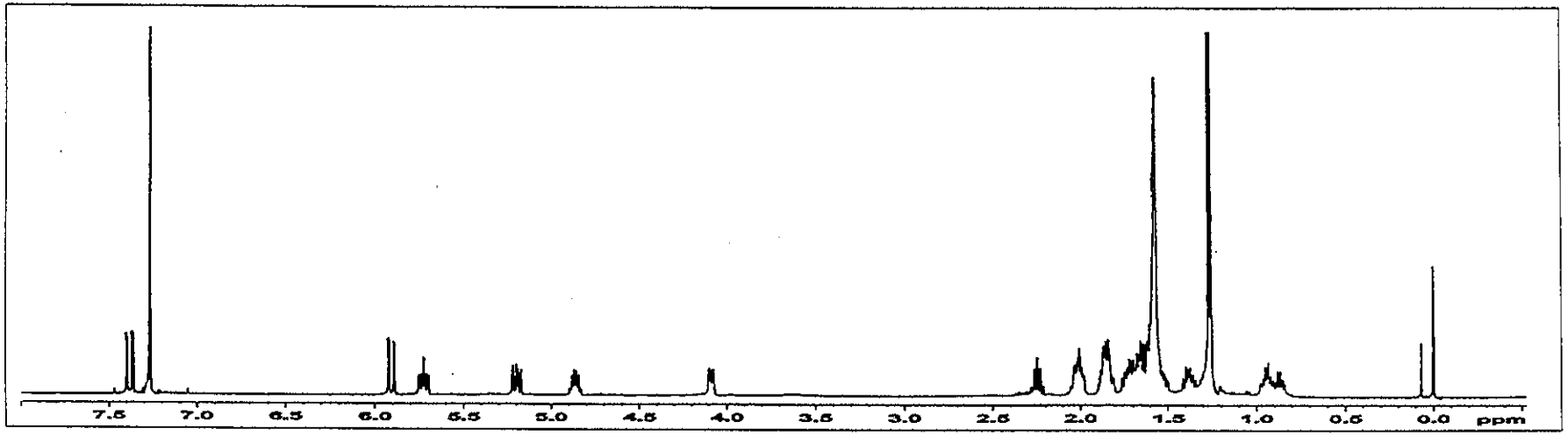

Figure 59 The 300 MHz ^{1}H NMR spectrum of compound **K25** in $CDCl_3$

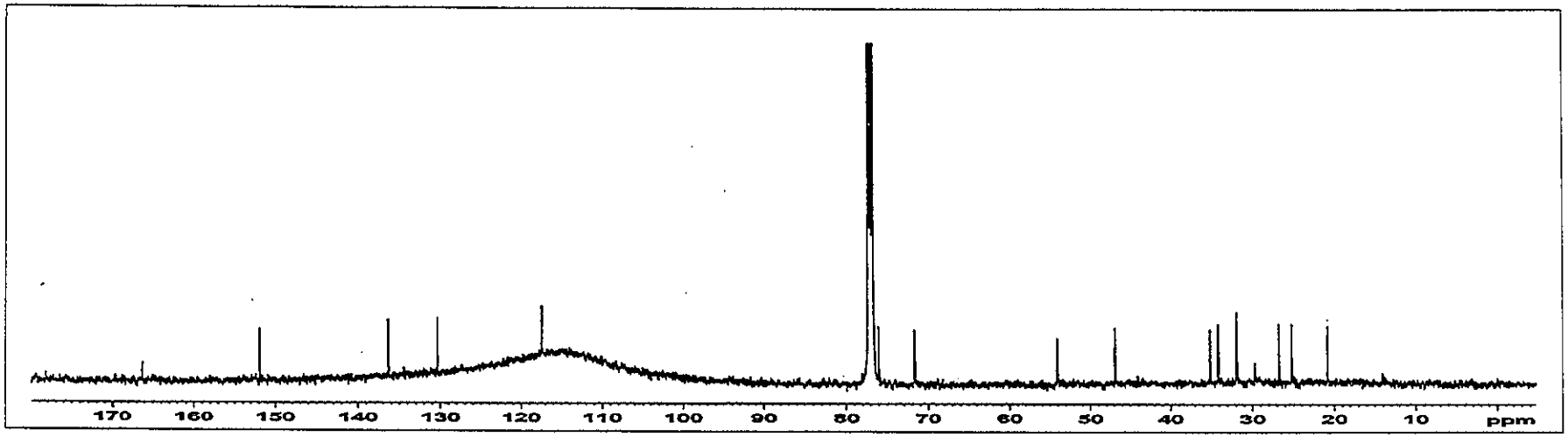

Figure 60 The 75 MHz ^{13}C NMR spectrum of compound **K25** in $CDCl_3$

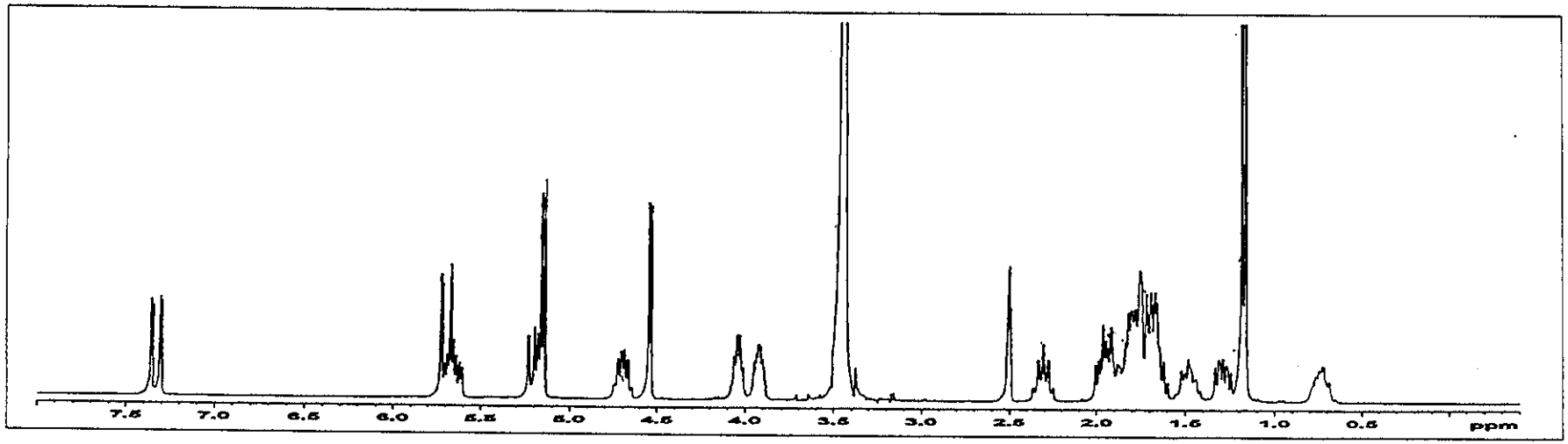

Figure 61 The 300 MHz ^{1}H NMR spectrum of compound **K26** in DMSO-d_6

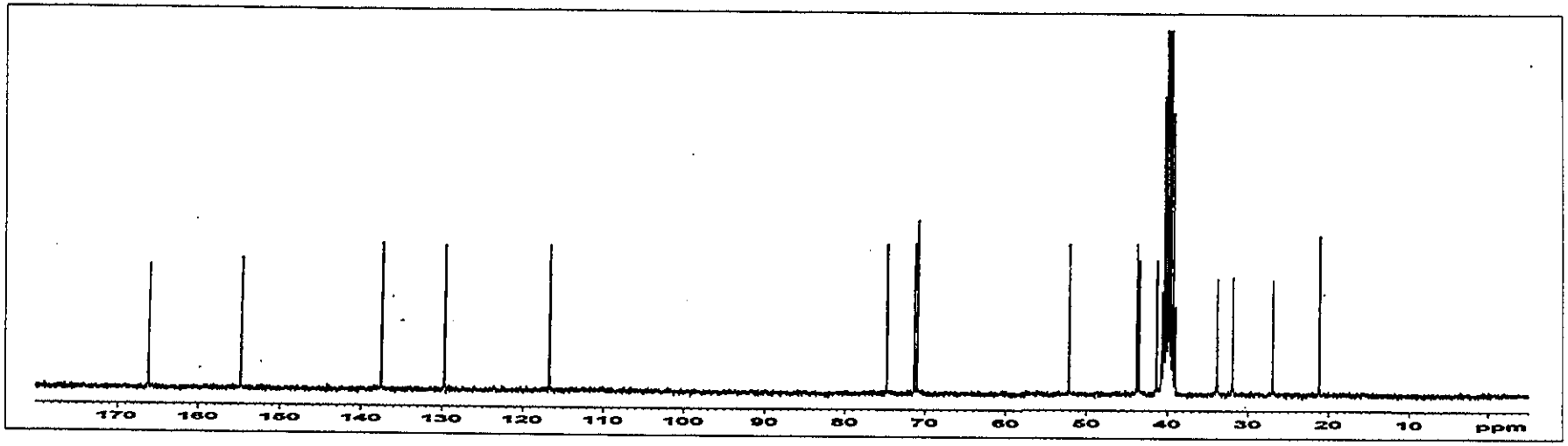

Figure 62 The 75 MHz ^{13}C NMR spectrum of compound **K26** in DMSO-d_6

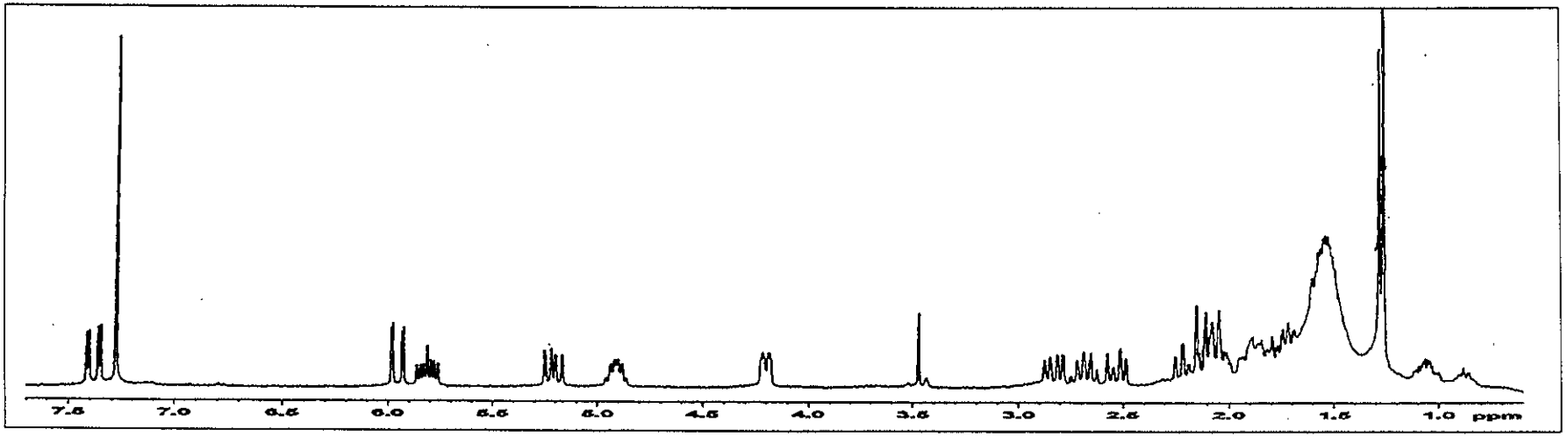

Figure 63 The 300 MHz ^{1}H NMR spectrum of compound **K27** in $CDCl_3$+CD_3OD

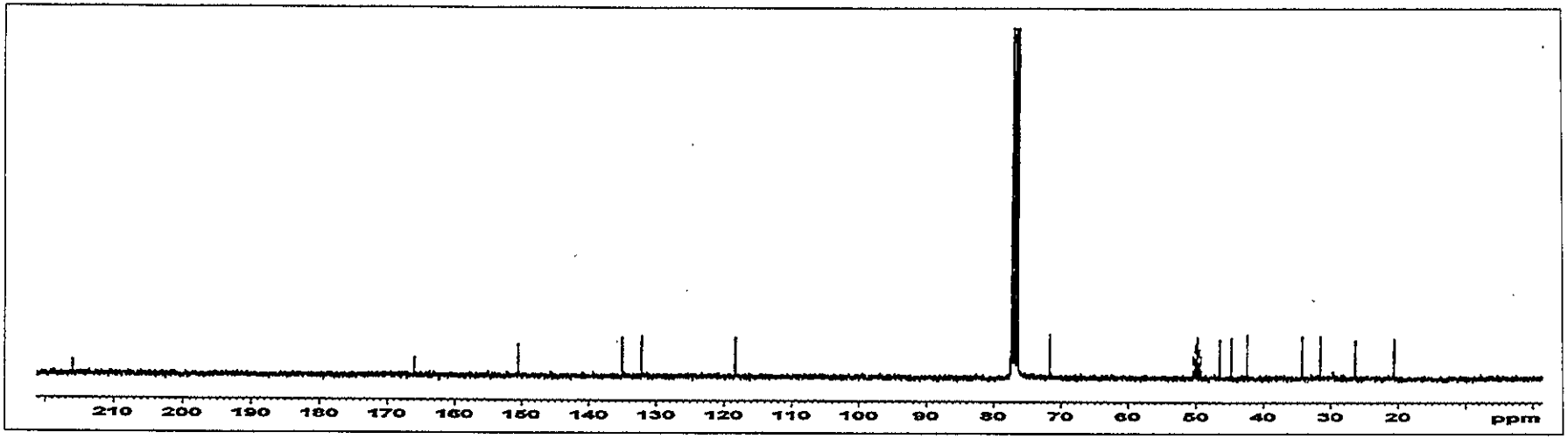

Figure 64 The 75 MHz ^{13}C NMR spectrum of compound **K27** in $CDCl_3$+CD_3OD

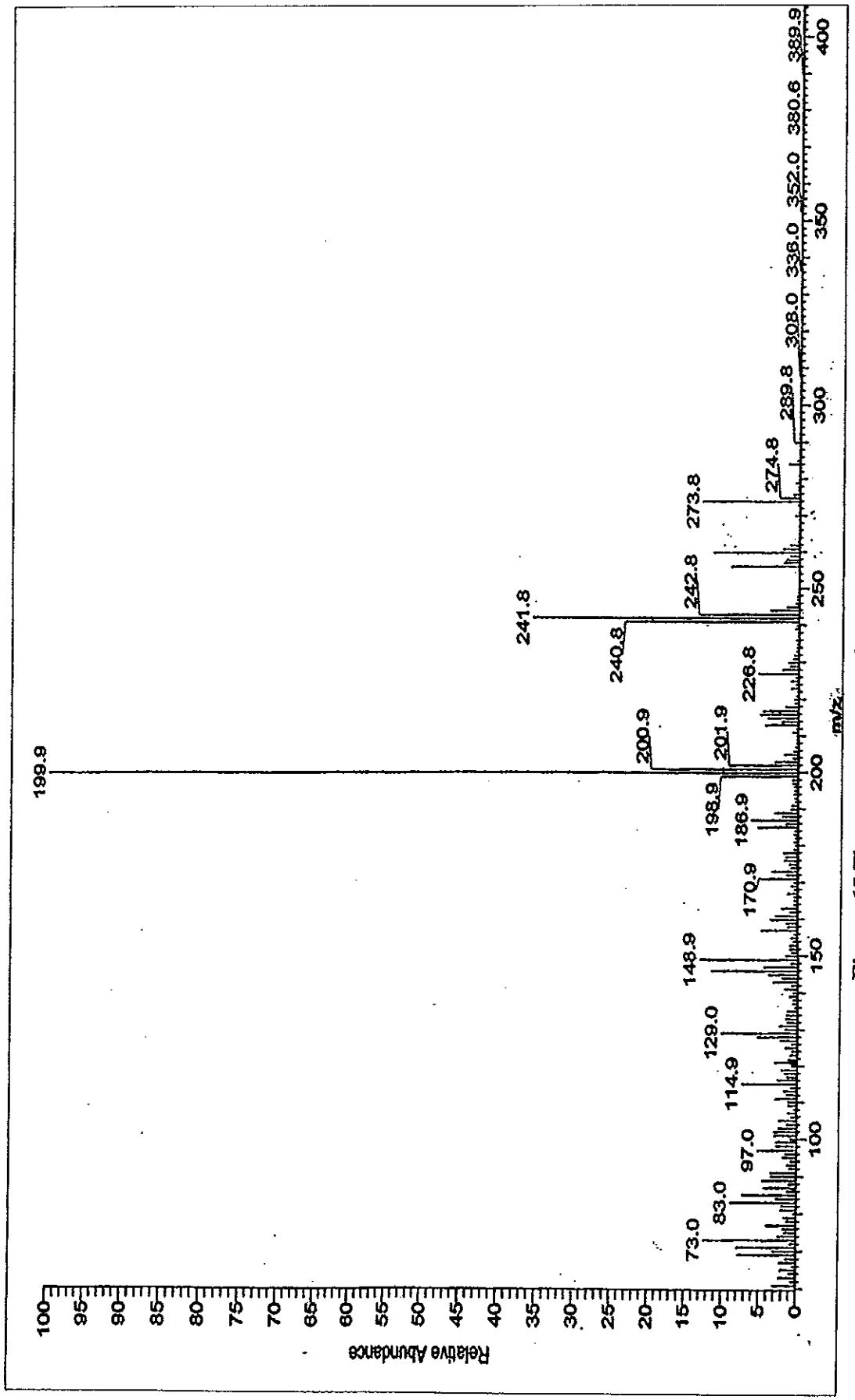

Figure 65 The mass spectrum of compound K28

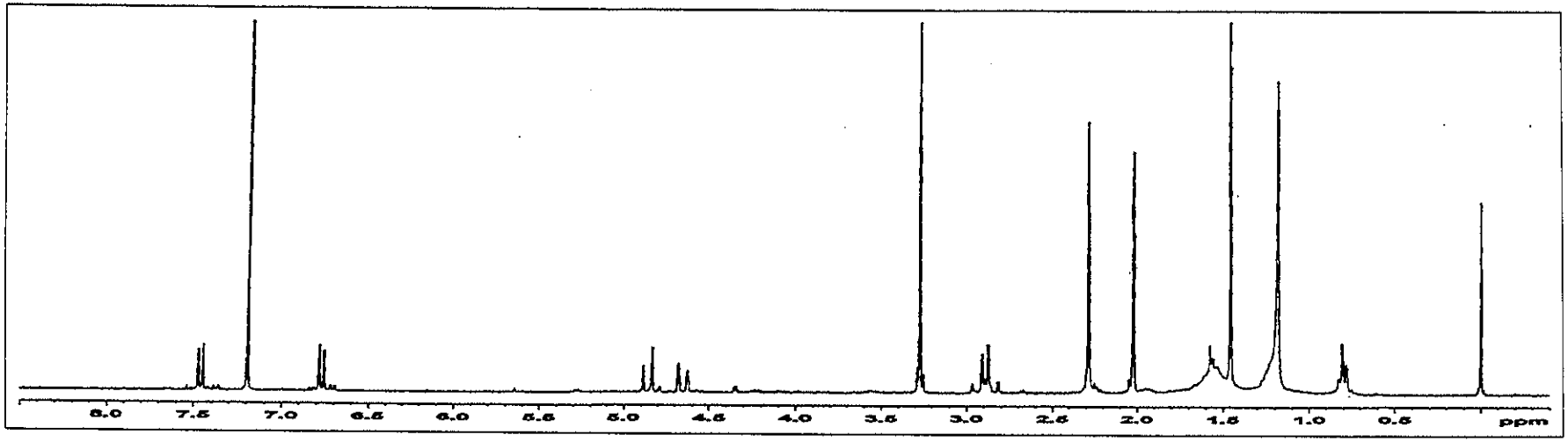

Figure 66 The 300 MHz ^{1}H NMR spectrum of compound **K28** in $CDCl_3$

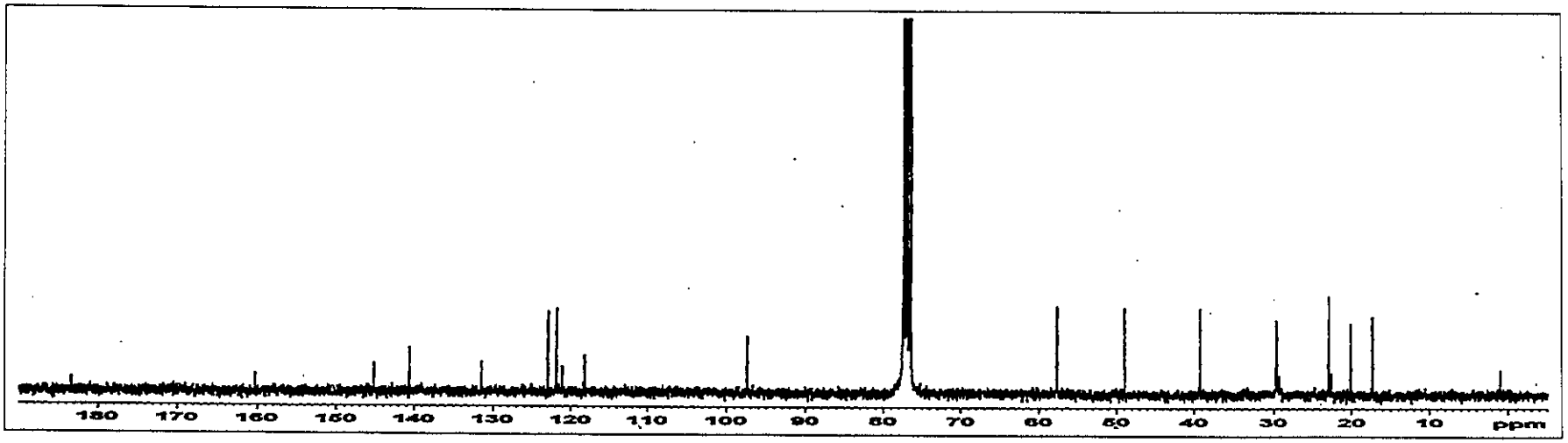

Figure 67 The 75 MHz ^{13}C NMR spectrum of compound **K28** in $CDCl_3$

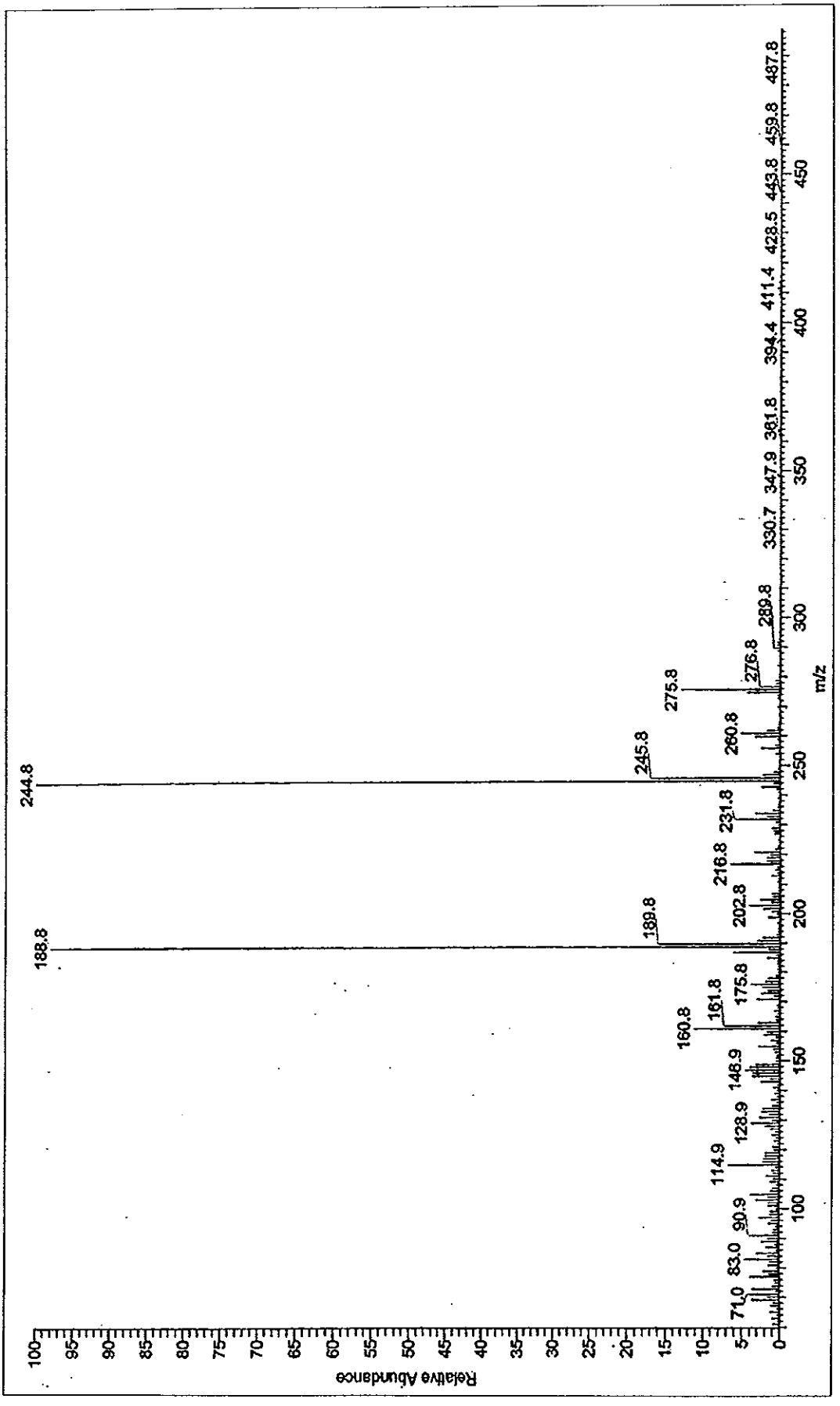

Figure 68 The mass spectrum of compound K29

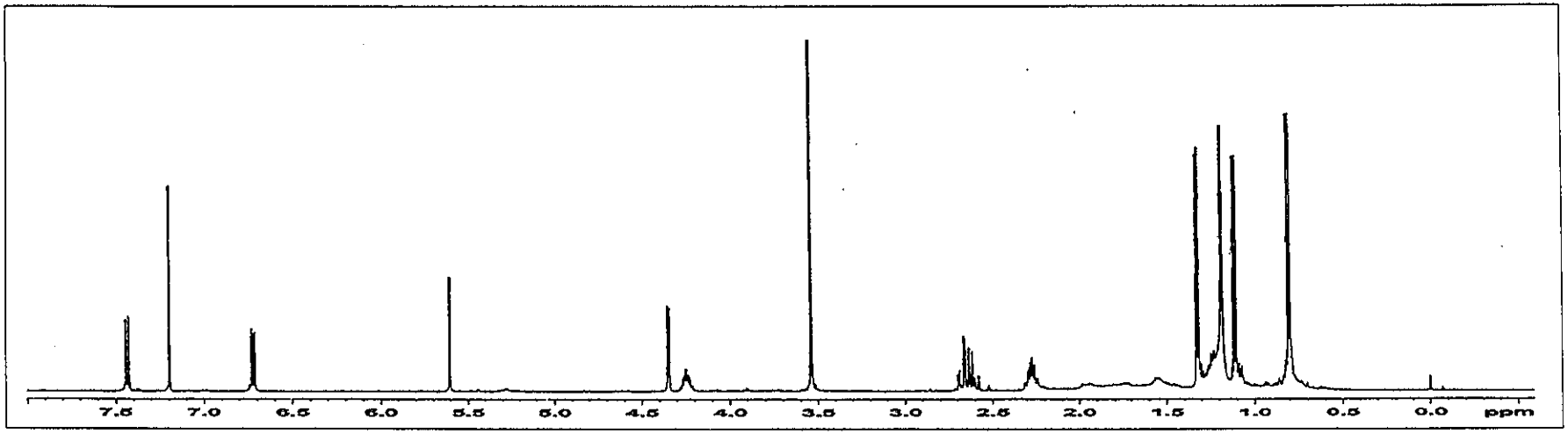

Figure 69 The 500 MHz ^{1}H NMR spectrum of compound **K29** in $CDCl_3$

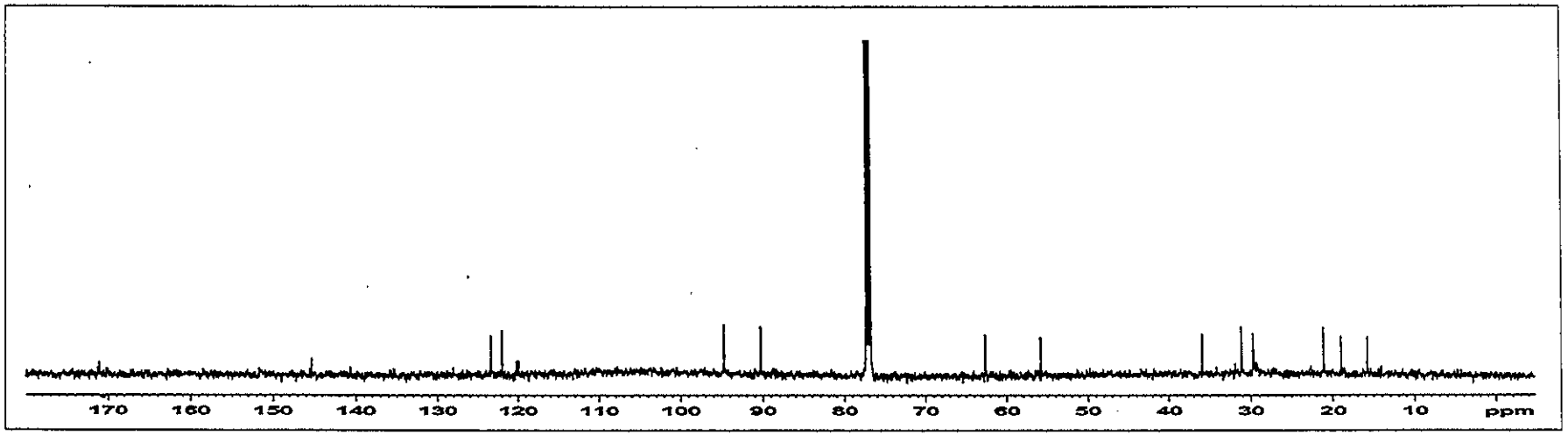

Figure 70 The 125 MHz ^{13}C NMR spectrum of compound **K29** in $CDCl_3$

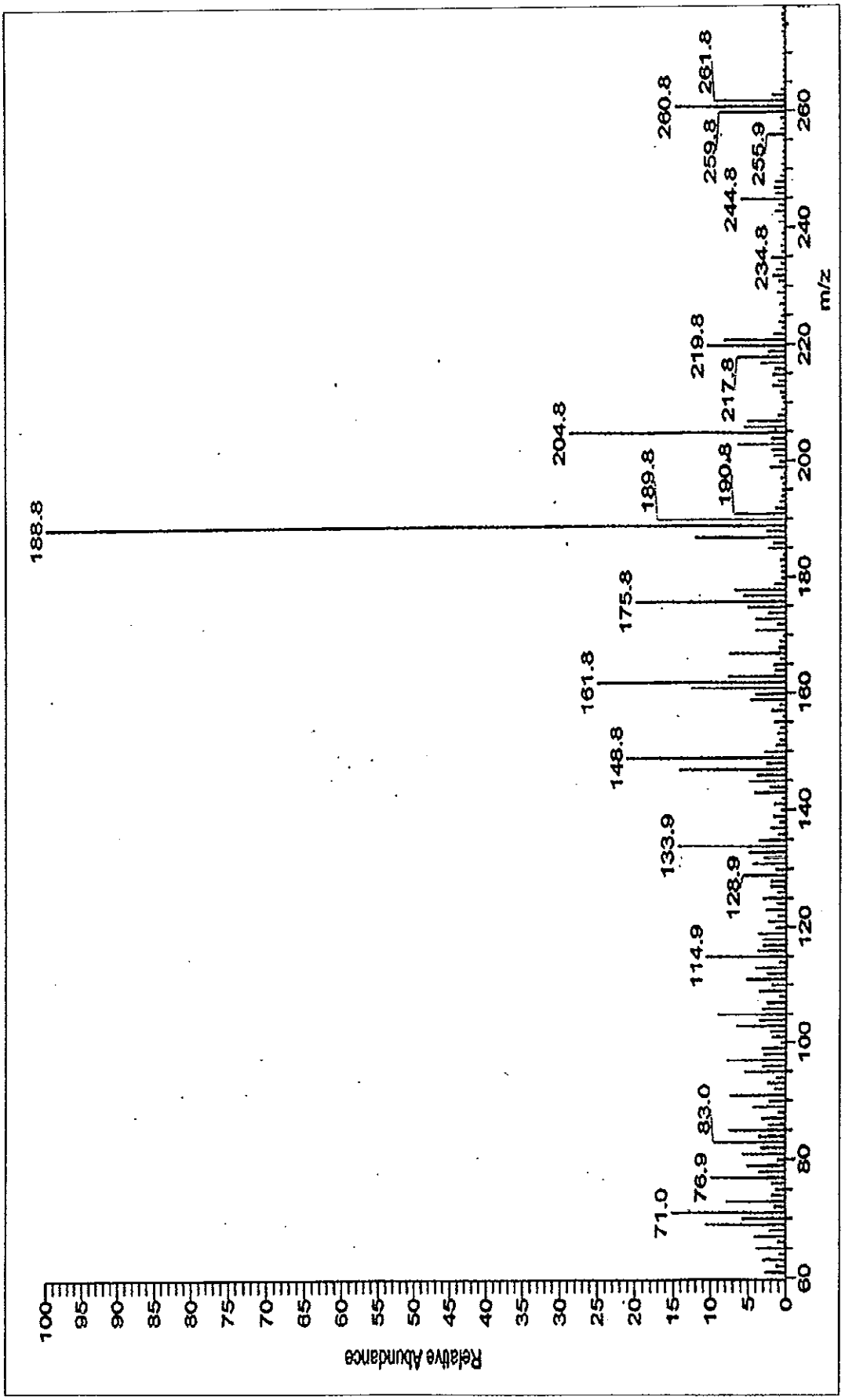

Figure 71 The mass spectrum of compound K30

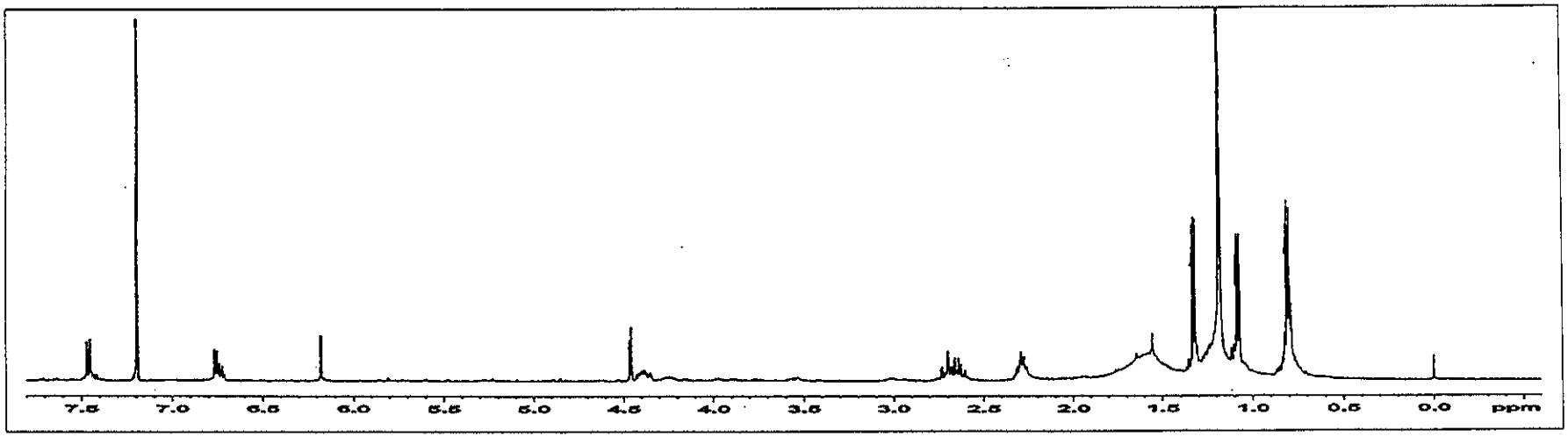

Figure 72 The 500 MHz ^{1}H NMR spectrum of compound **K30** in $CDCl_3$

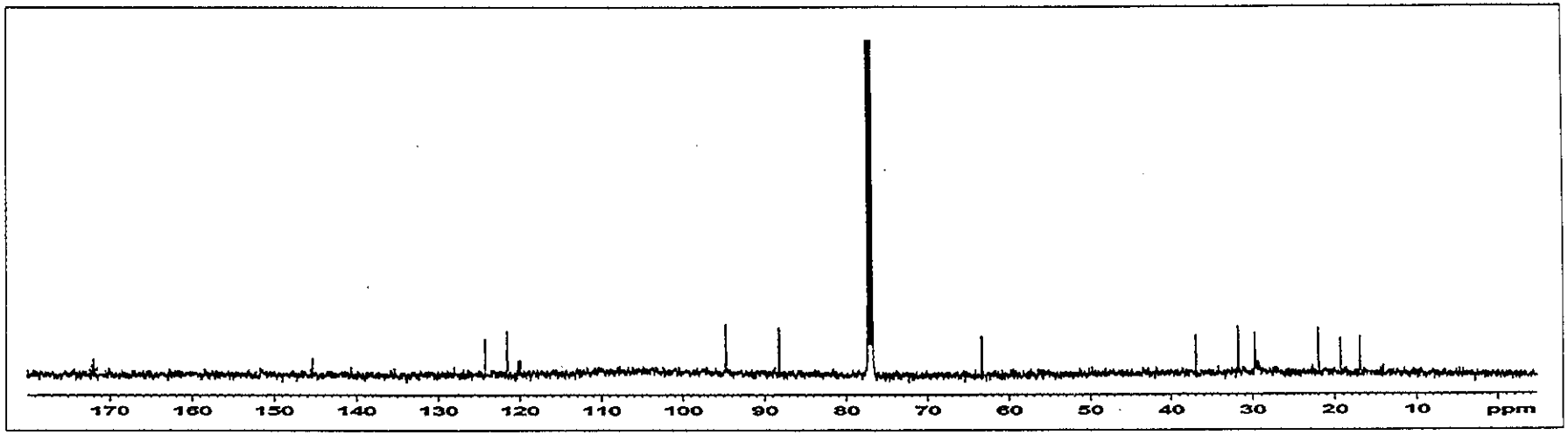

Figure 73 The 125 MHz ^{13}C NMR spectrum of compound **K30** in $CDCl_3$

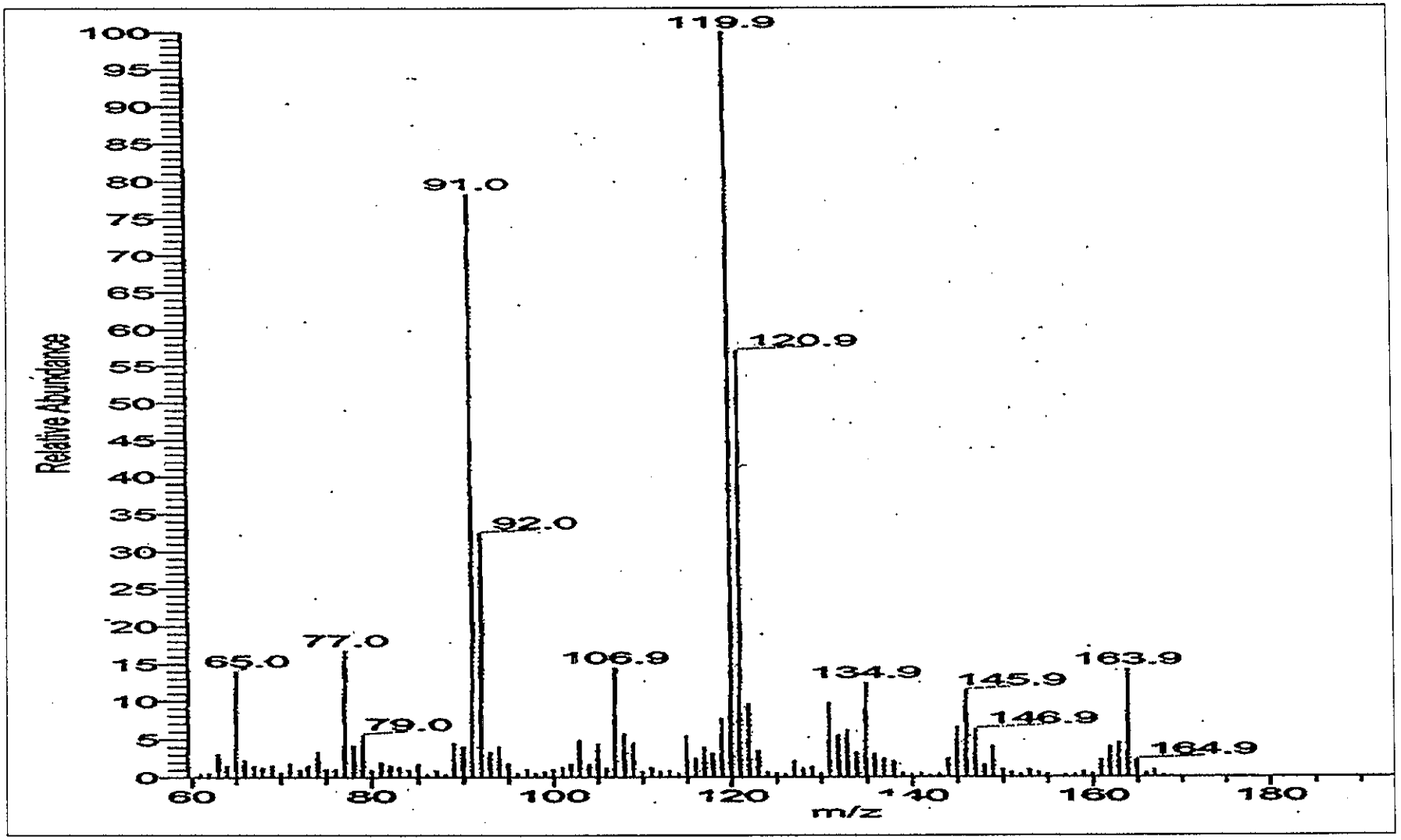

Figure 74 The mass spectrum of compound K31

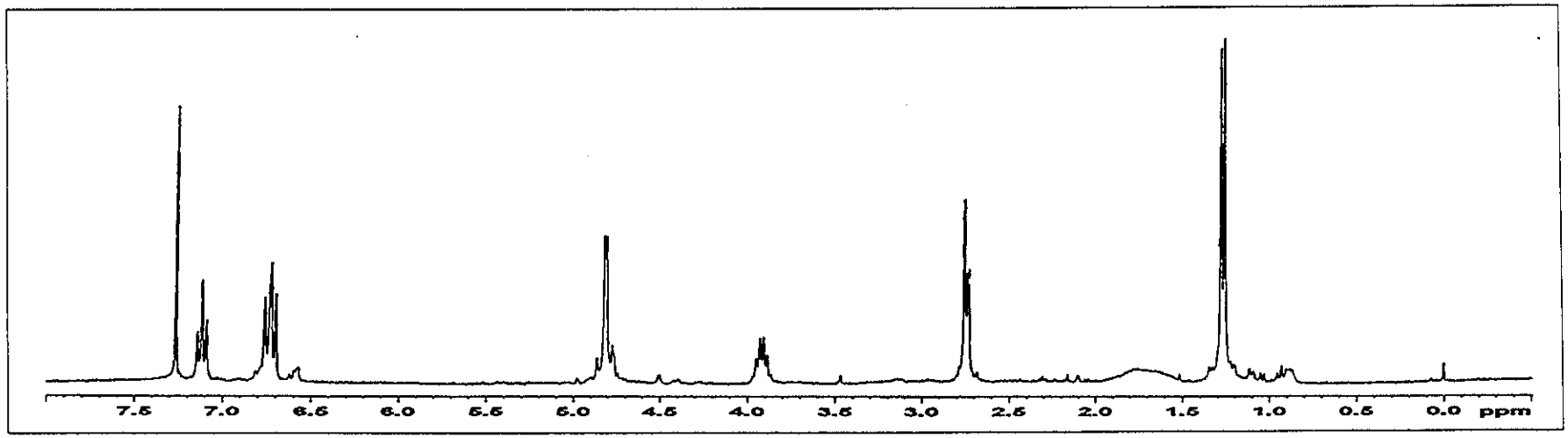

Figure 75 The 300 MHz ^{1}H NMR spectrum of compound **K31** in $CDCl_3$

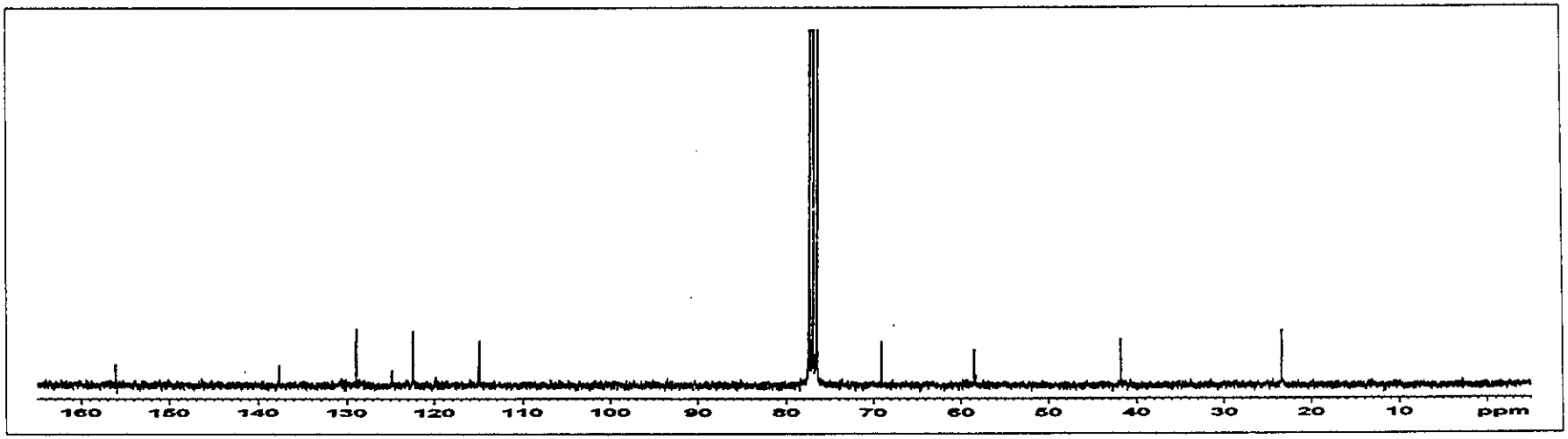

Figure 76 The 75 MHz ^{13}C NMR spectrum of compound **K31** in $CDCl_3$

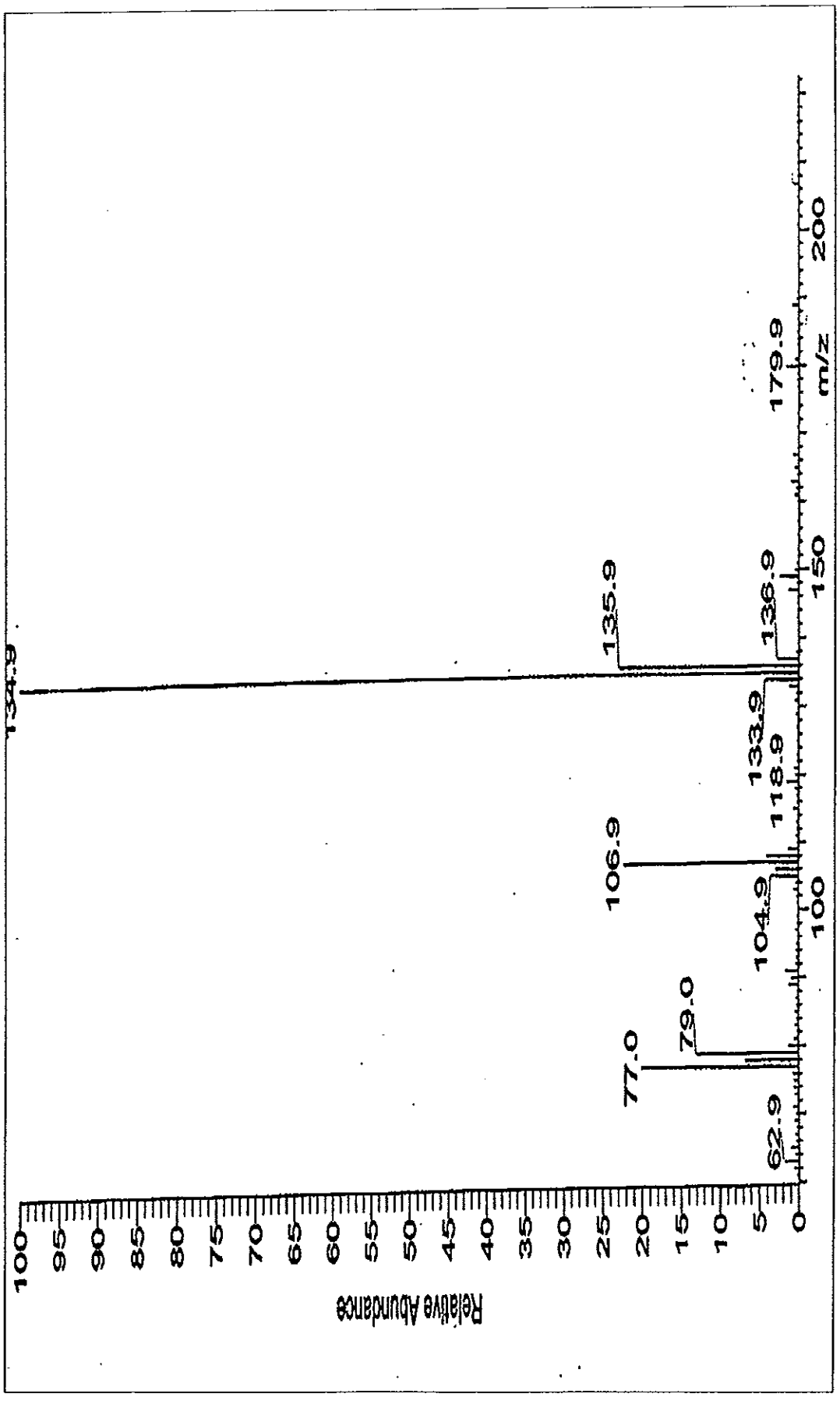

Figure 77 The mass spectrum of compound K32

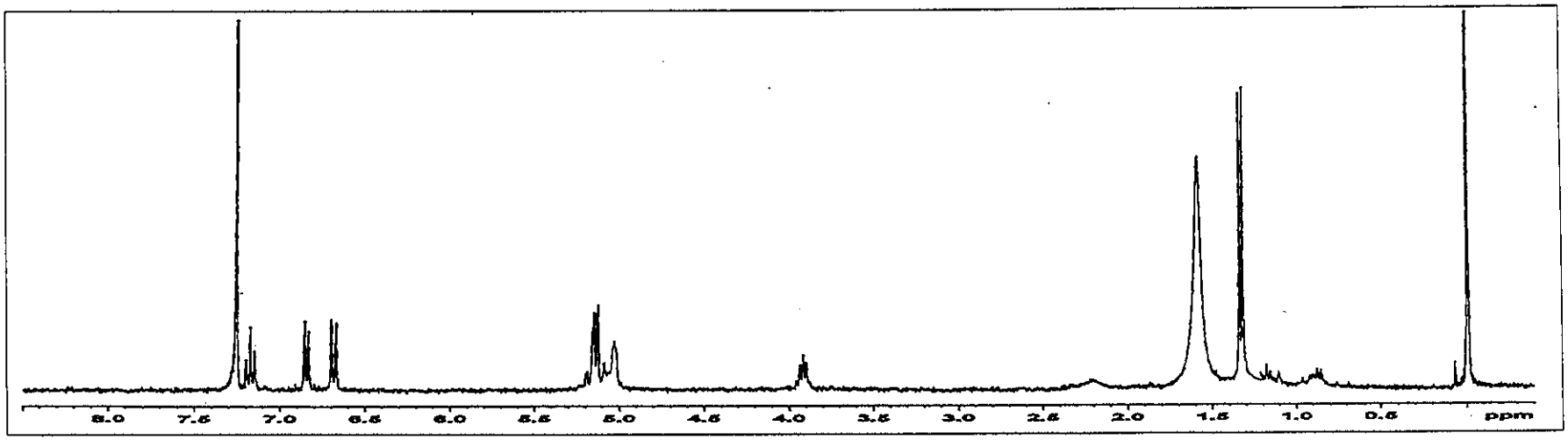

Figure 78 The 300 MHz ^{1}H NMR spectrum of compound **K32** in $CDCl_3$

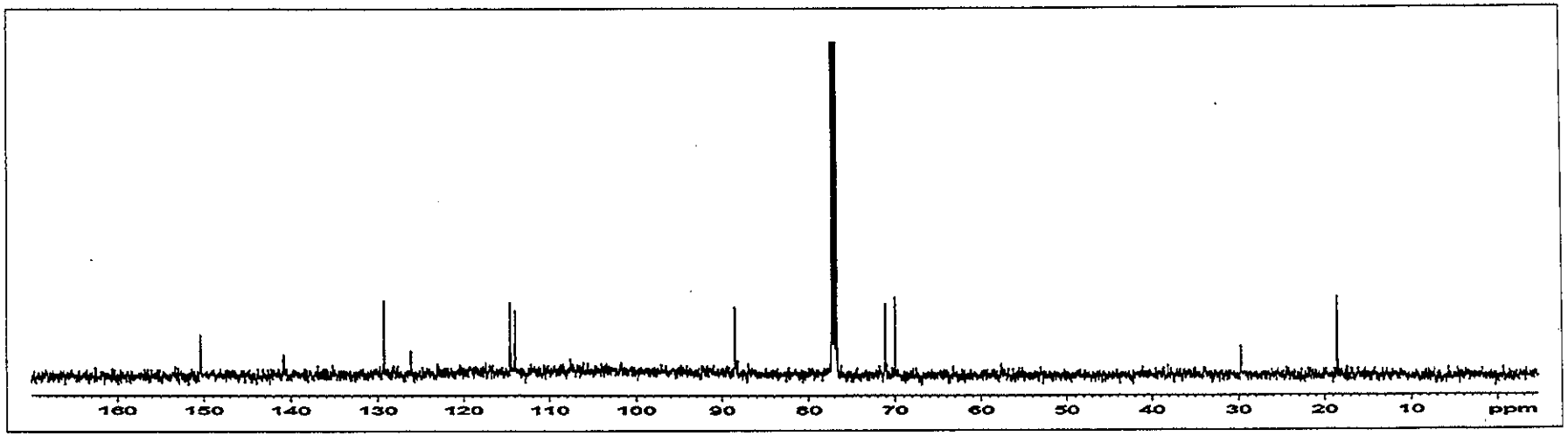

Figure 79 The 75 MHz ^{13}C NMR spectrum of compound **K32** in $CDCl_3$

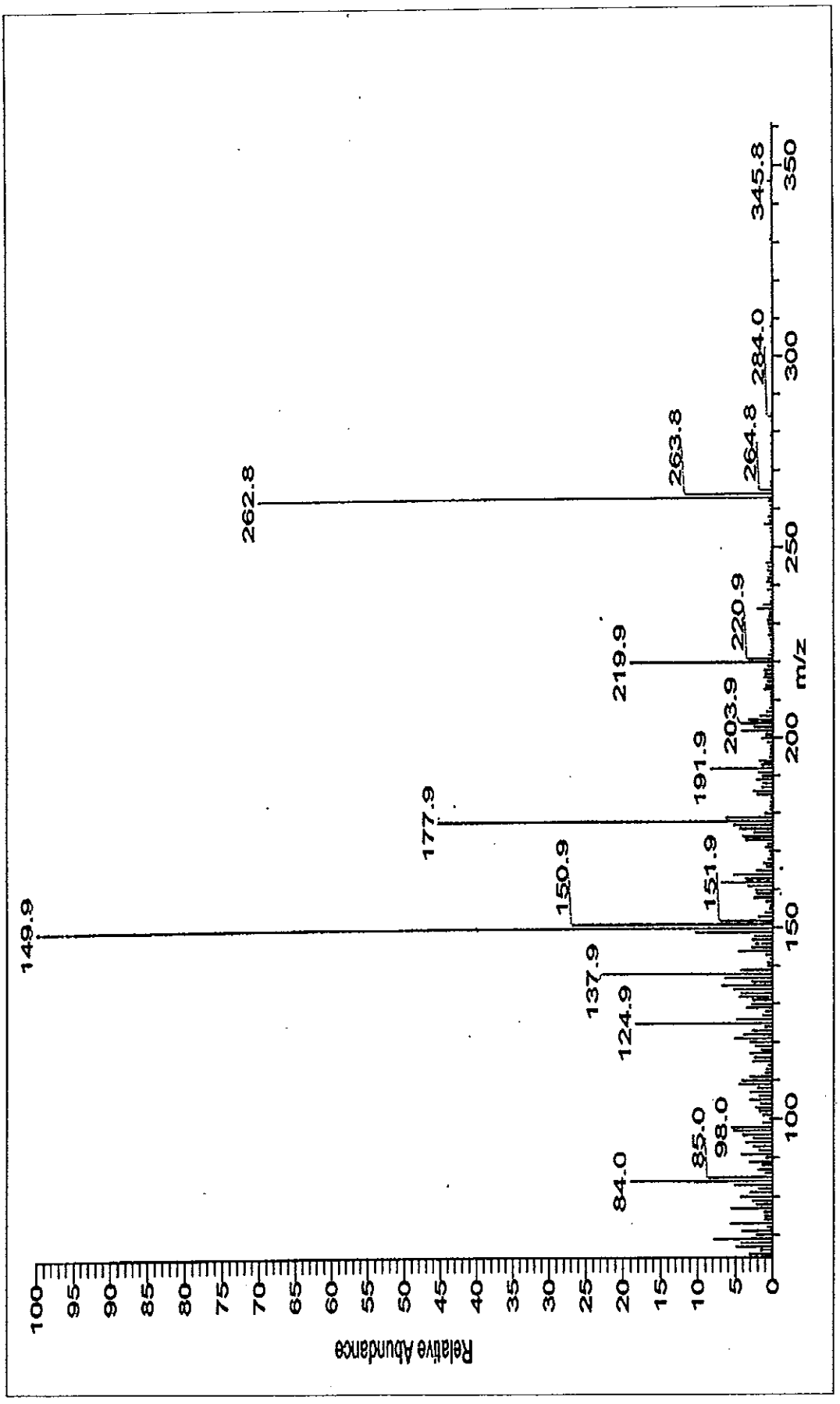

Figure 80 The mass spectrum of compound K33

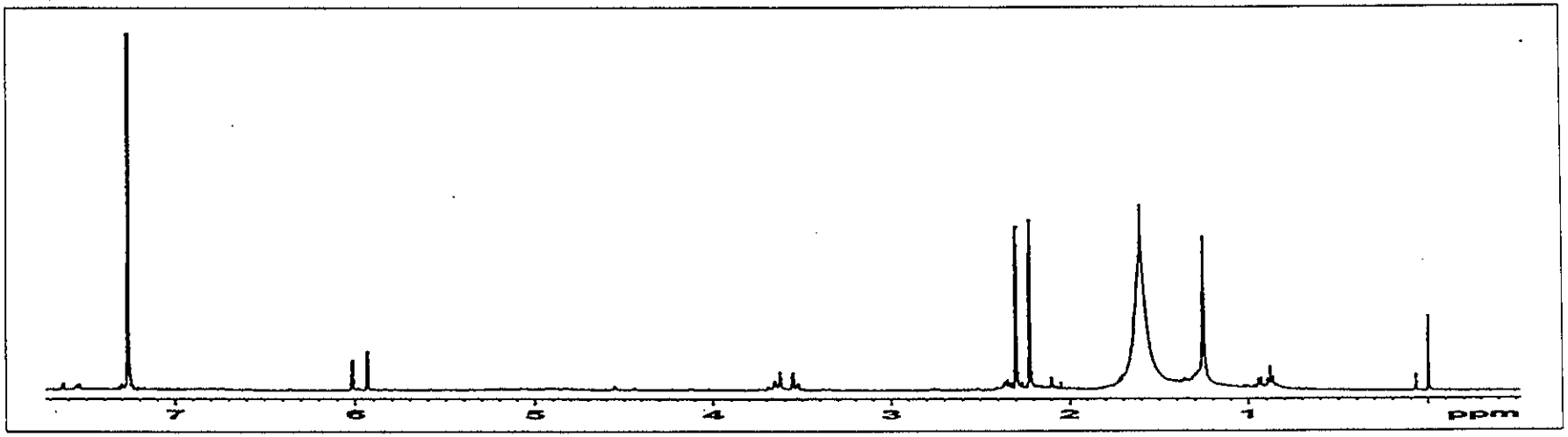

Figure 81 The 300 MHz ^{1}H NMR spectrum of compound **K33** in $CDCl_3$

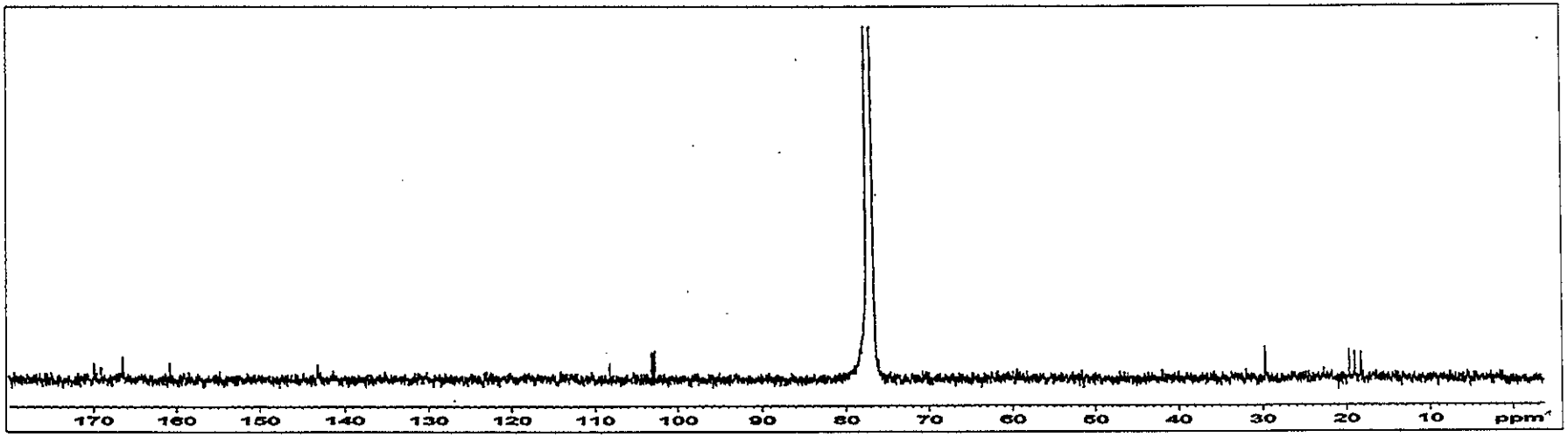

Figure 82 The 75 MHz ^{13}C NMR spectrum of compound **K33** in $CDCl_3$

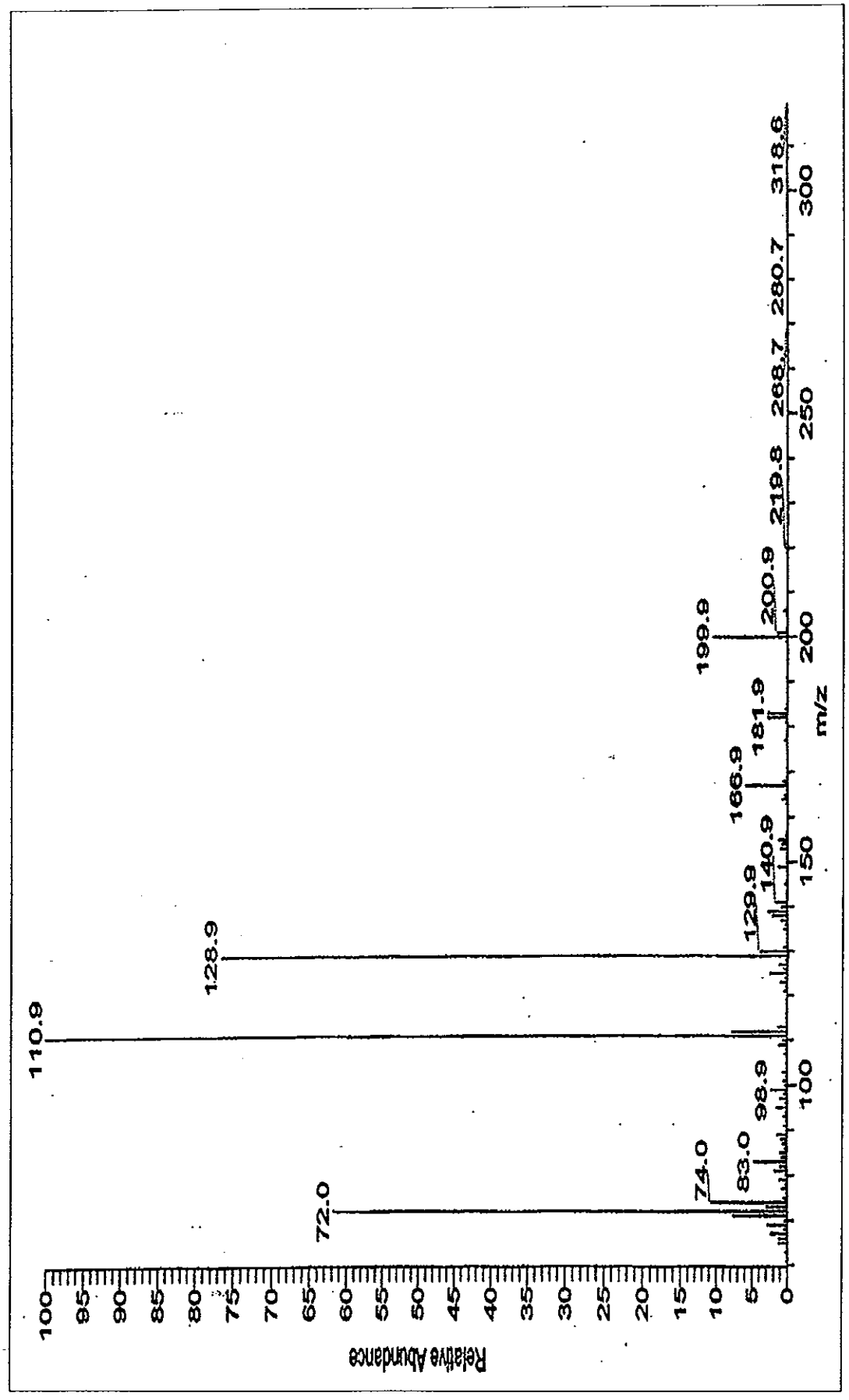

Figure 83 The mass spectrum of compound K34

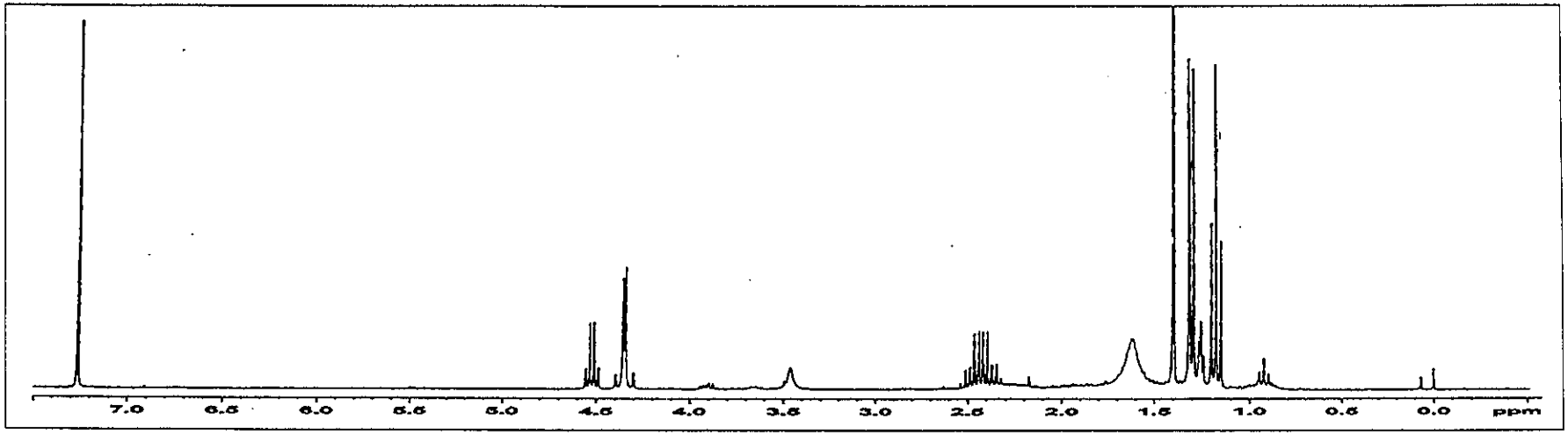

Figure 84 The 300 MHz ^{1}H NMR spectrum of compound **K34** in $CDCl_3$

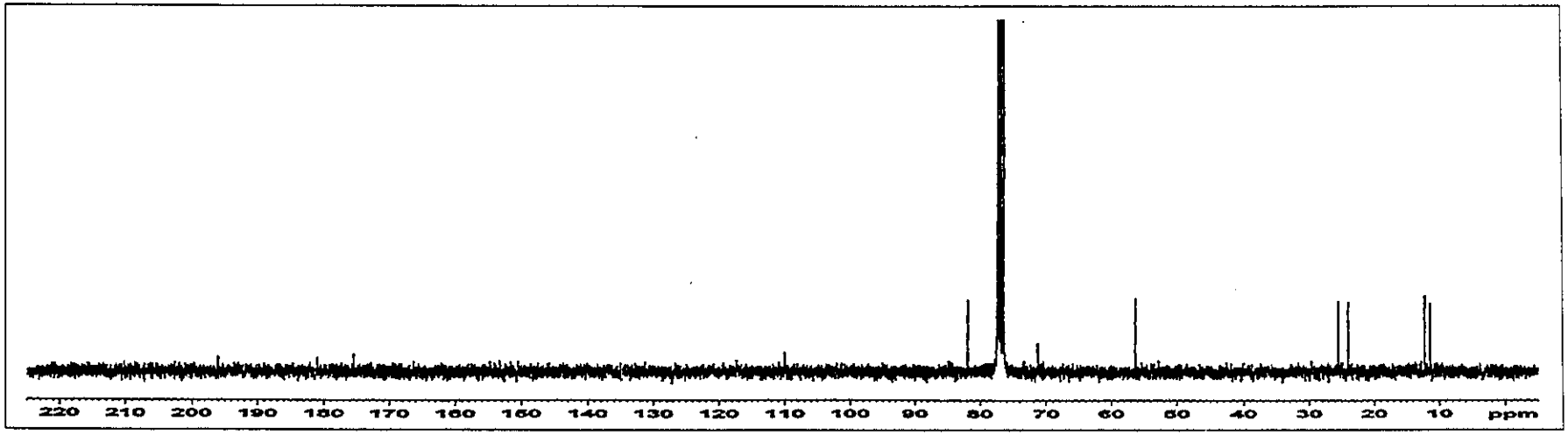

Figure 85 The 75 MHz ^{13}C NMR spectrum of compound **K34** in $CDCl_3$

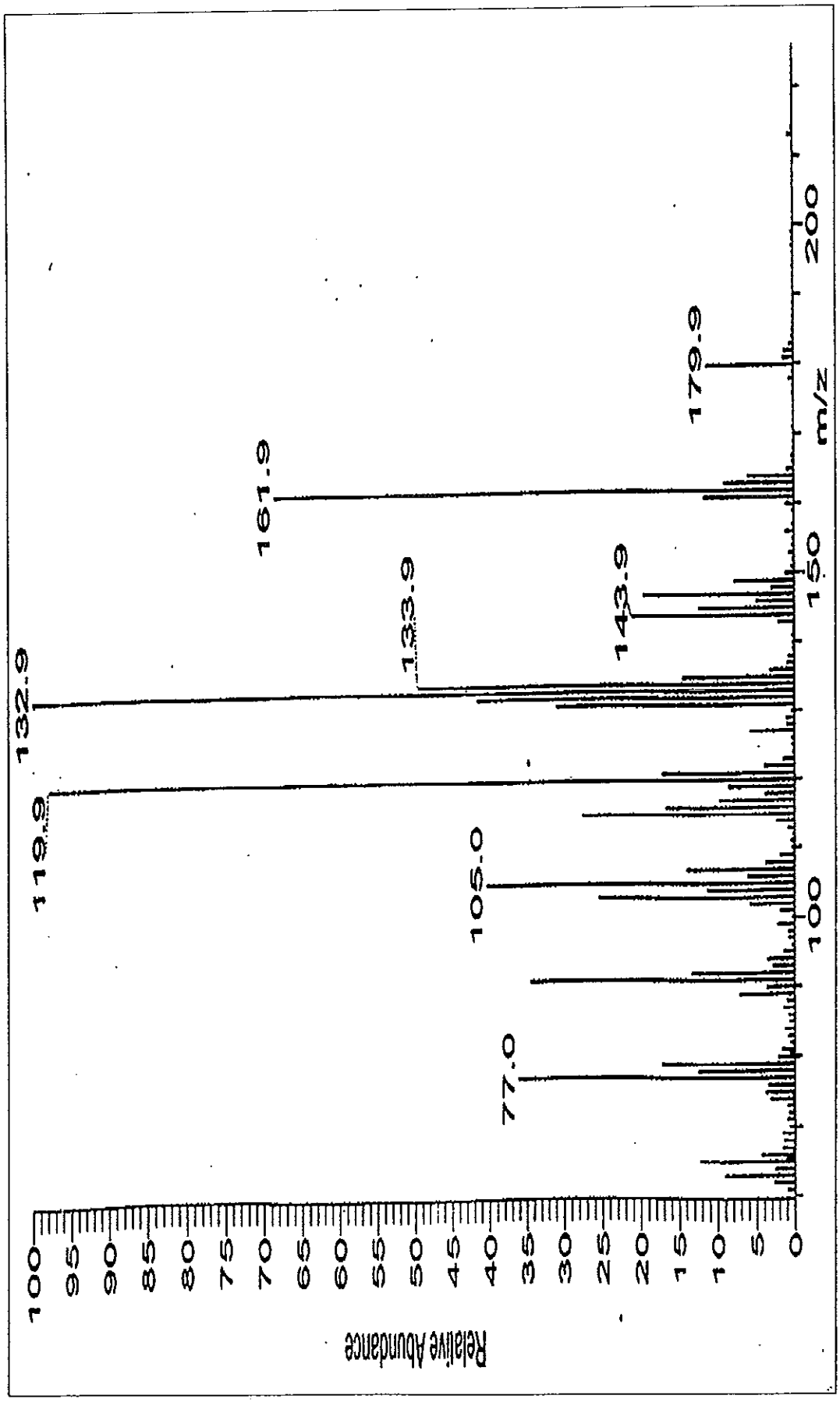

Figure 86 The mass spectrum of compound K35

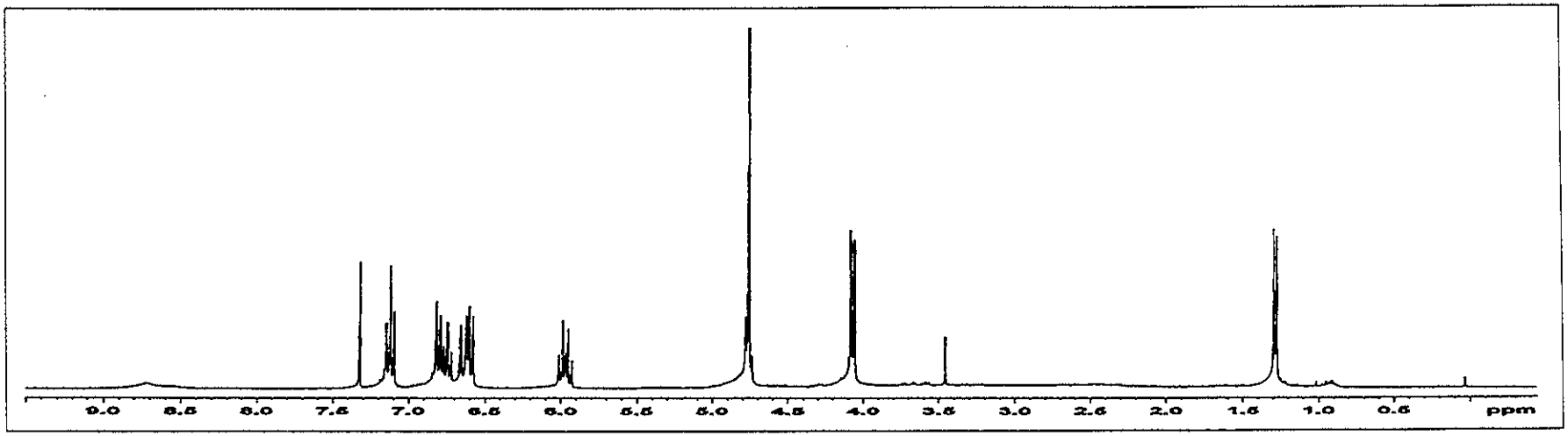

Figure 87 The 300 MHz ^{1}H NMR spectrum of compound **K35** in $CDCl_3$+CD_3OD

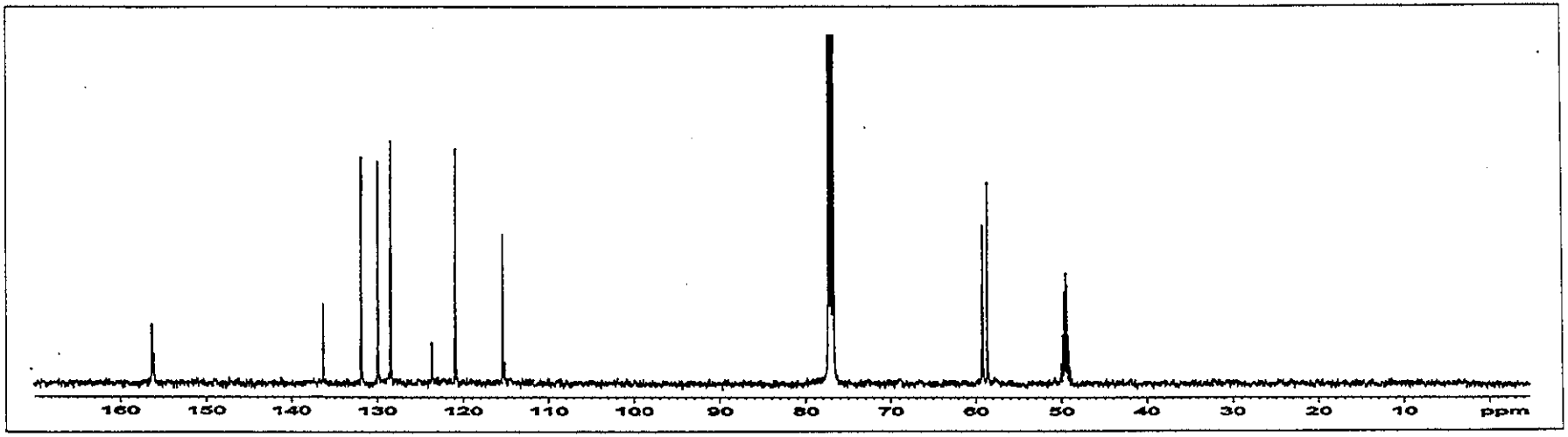

Figure 88 The 75 MHz ^{13}C NMR spectrum of compound **K35** in $CDCl_3$+CD_3OD

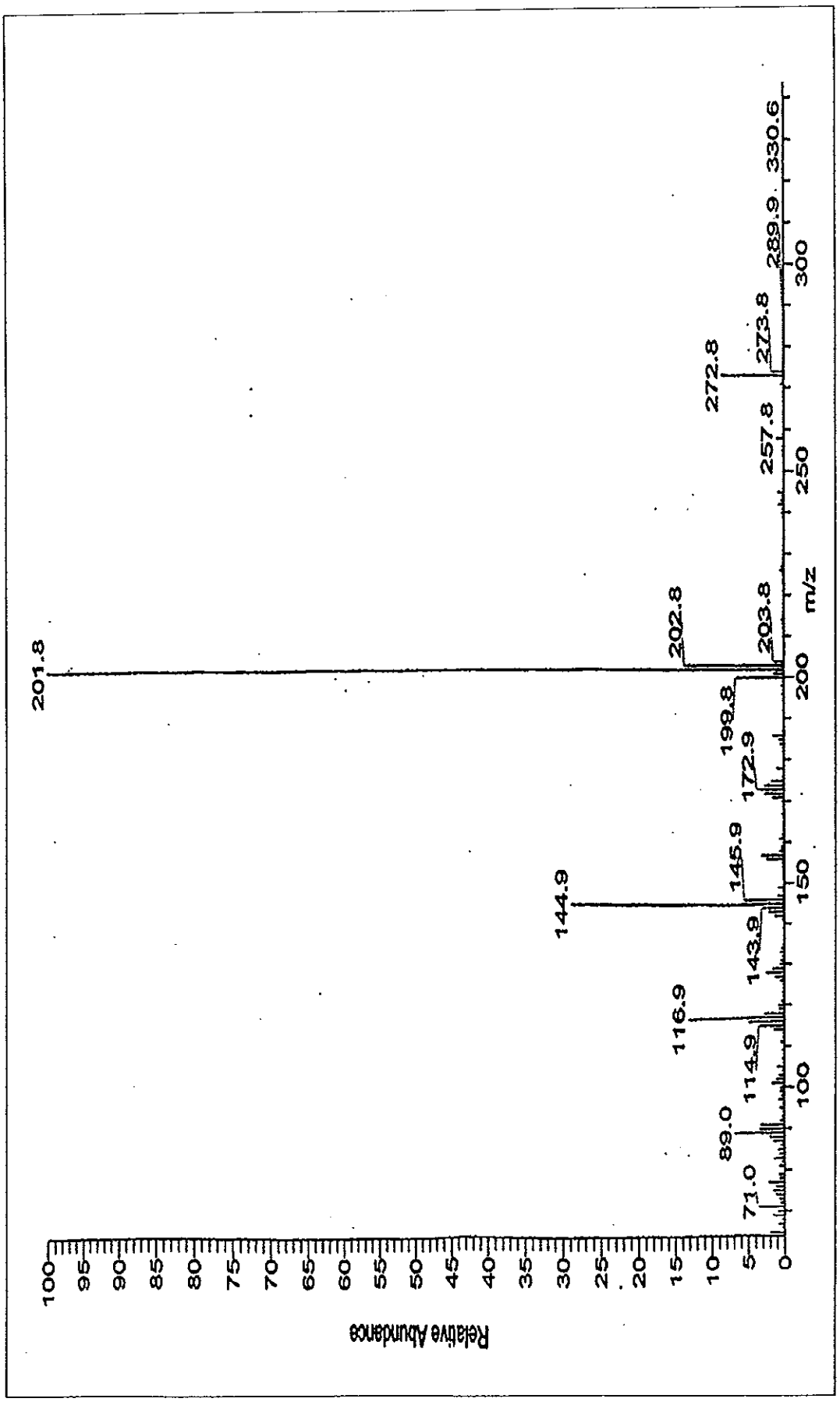

Figure 89 The mass spectrum of compound K36

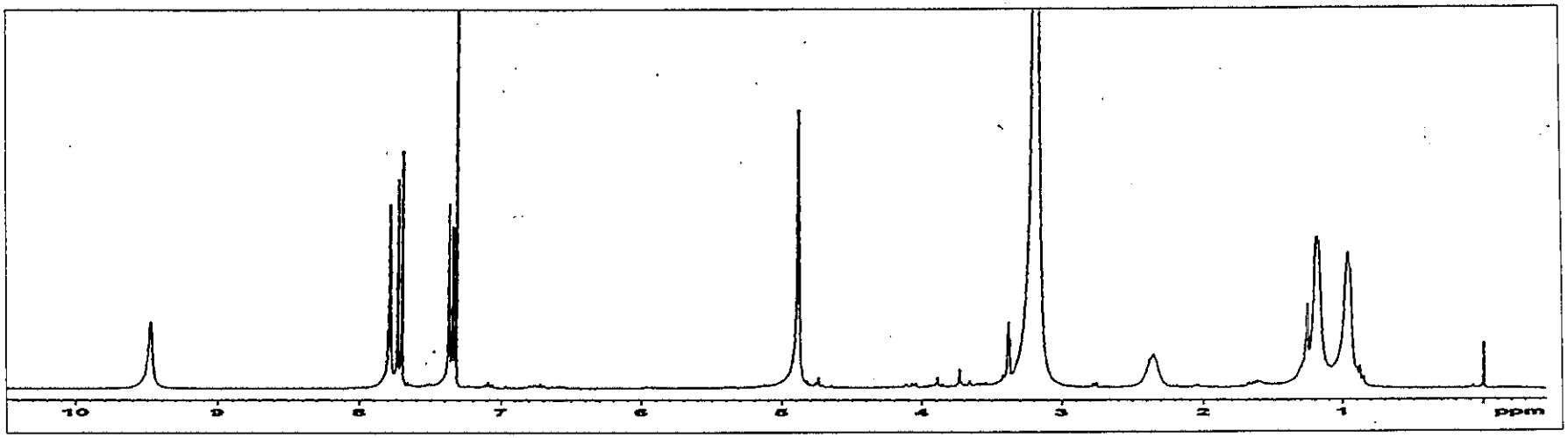

Figure 90 The 300 MHz ^{1}H NMR spectrum of compound **K36** in $CDCl_3$+CD_3OD

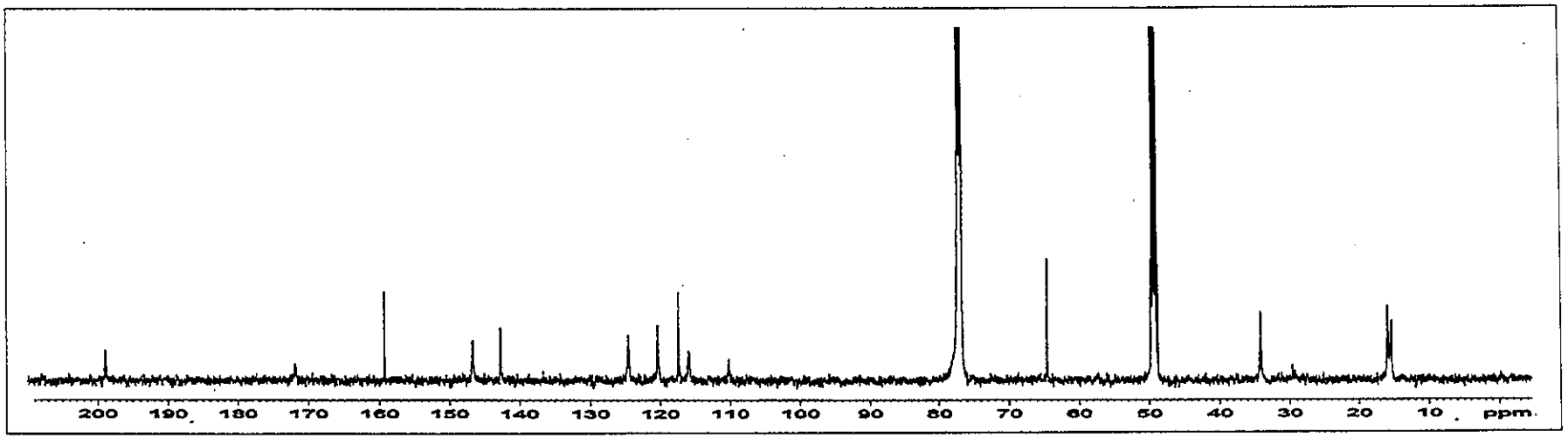

Figure 91 The 75 MHz ^{13}C NMR spectrum of compound **K36** in $CDCl_3$+CD_3OD

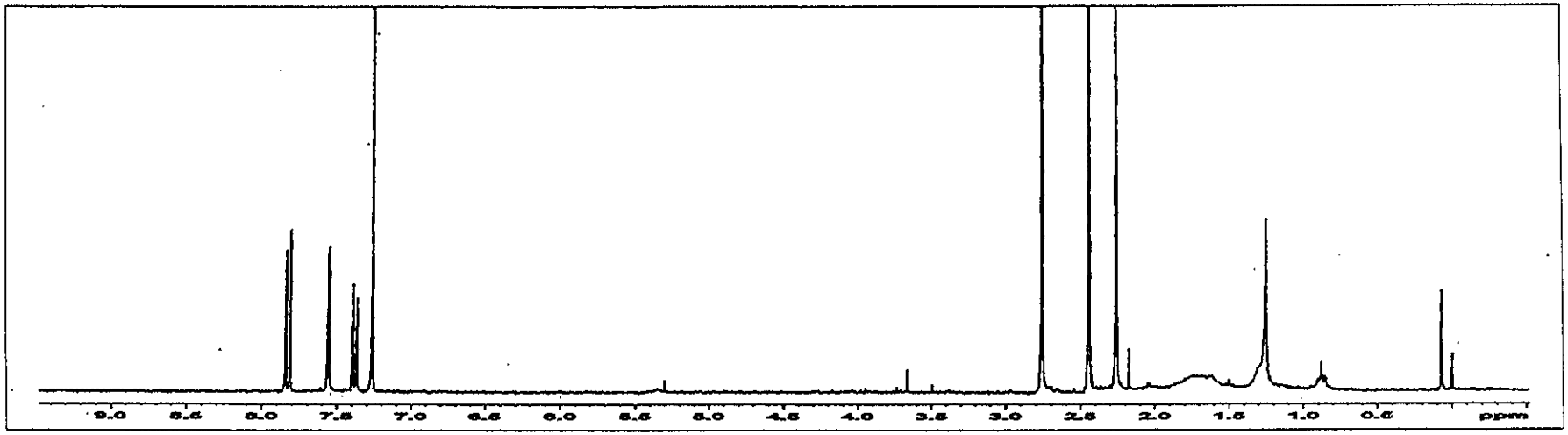

Figure 92 The 300 MHz ^{1}H NMR spectrum of compound **K37** in $CDCl_3$

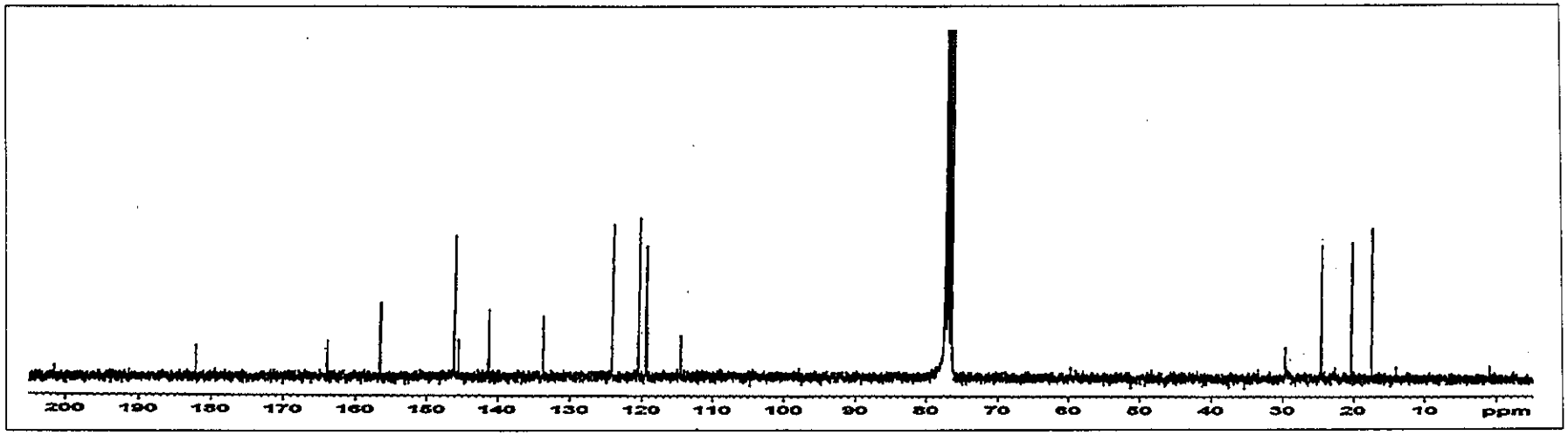

Figure 93 The 75 MHz ^{13}C NMR spectrum of compound **K37** in $CDCl_3$

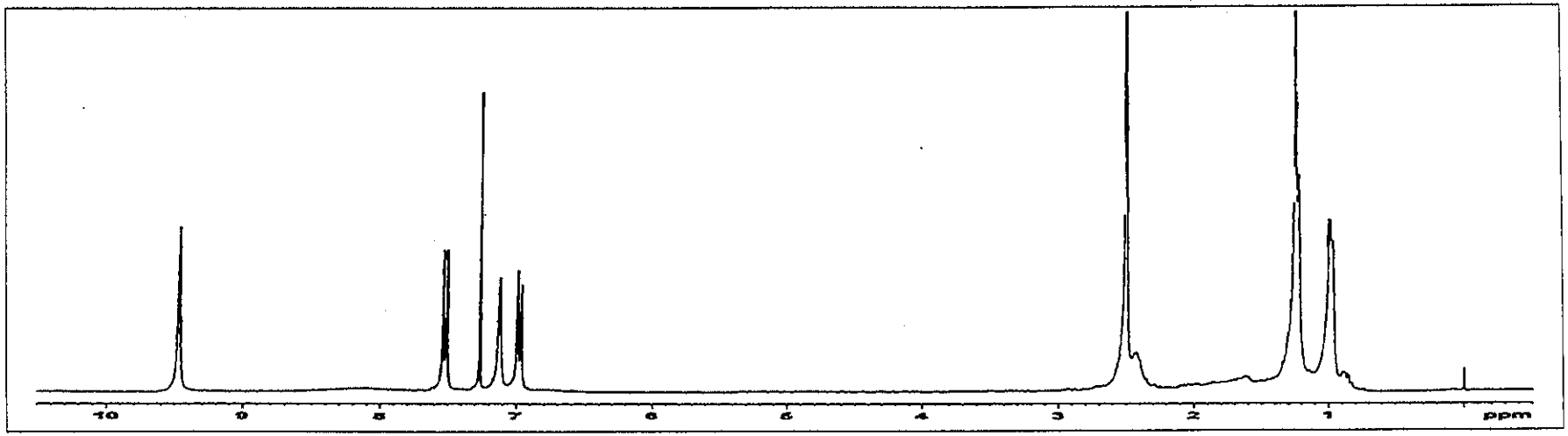

Figure 94 The 300 MHz ^{1}H NMR spectrum of compound **K38** in $CDCl_3$

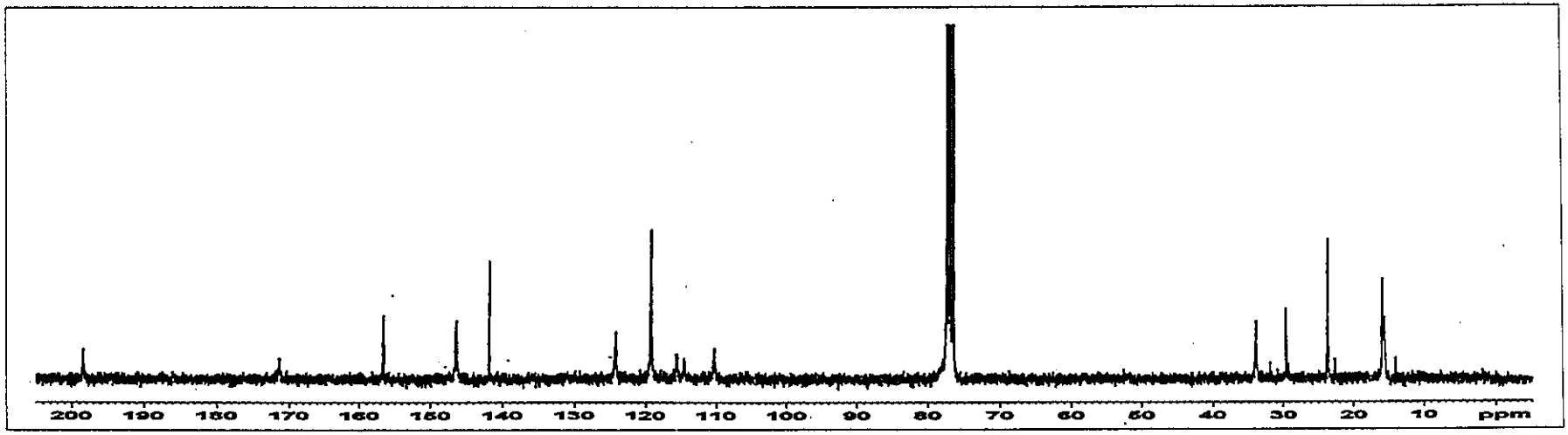

Figure 95 The 75 MHz ^{13}C NMR spectrum of compound **K38** in $CDCl_3$

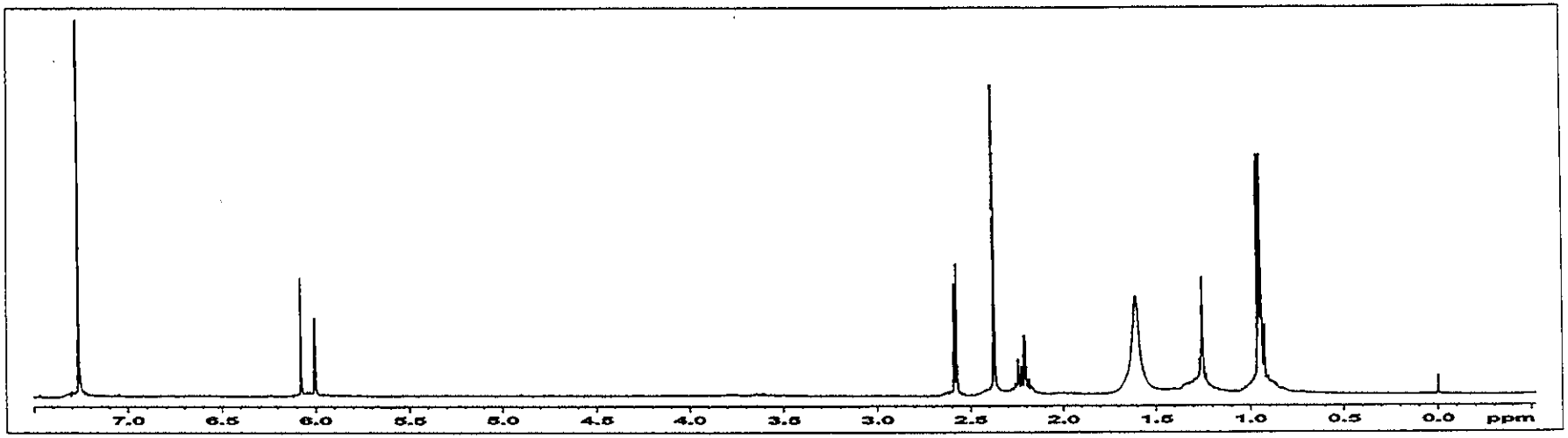

Figure 96 The 300 MHz ^{1}H NMR spectrum of compound **K39** in $CDCl_3$

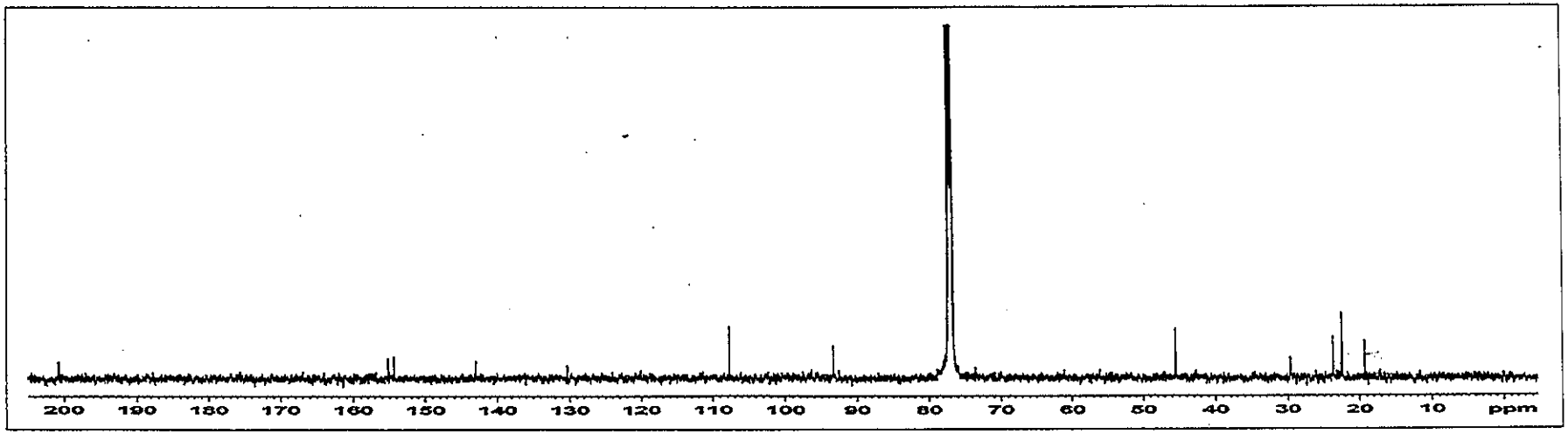

Figure 97 The 75 MHz ^{13}C NMR spectrum of compound **K39** in $CDCl_3$

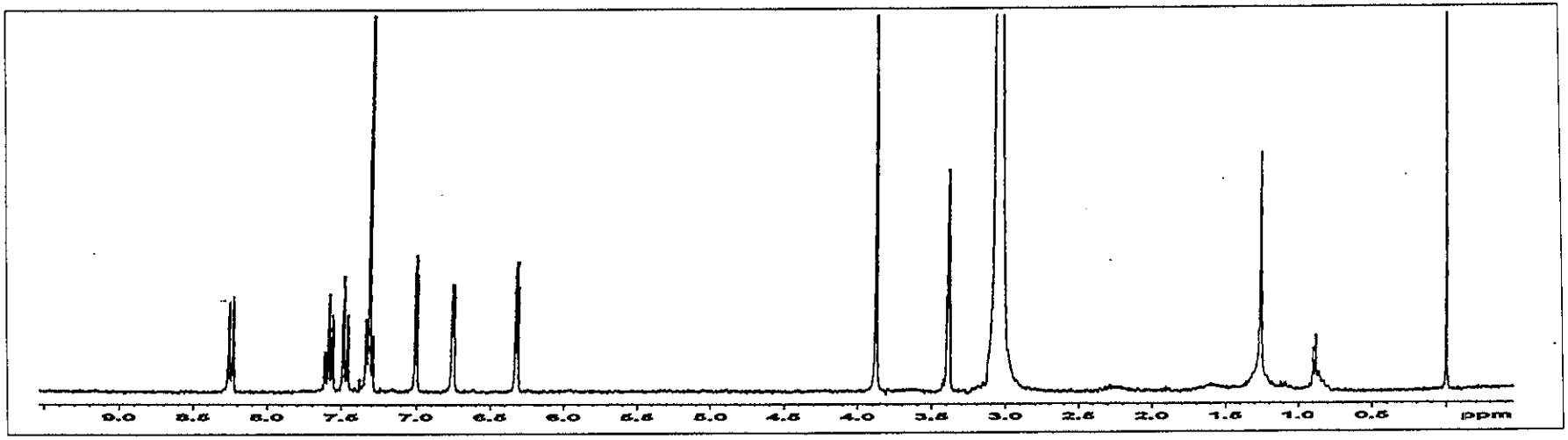

Figure 98 The 300 MHz ^{1}H NMR spectrum of compound **K40** in $CDCl_3$+CD_3OD

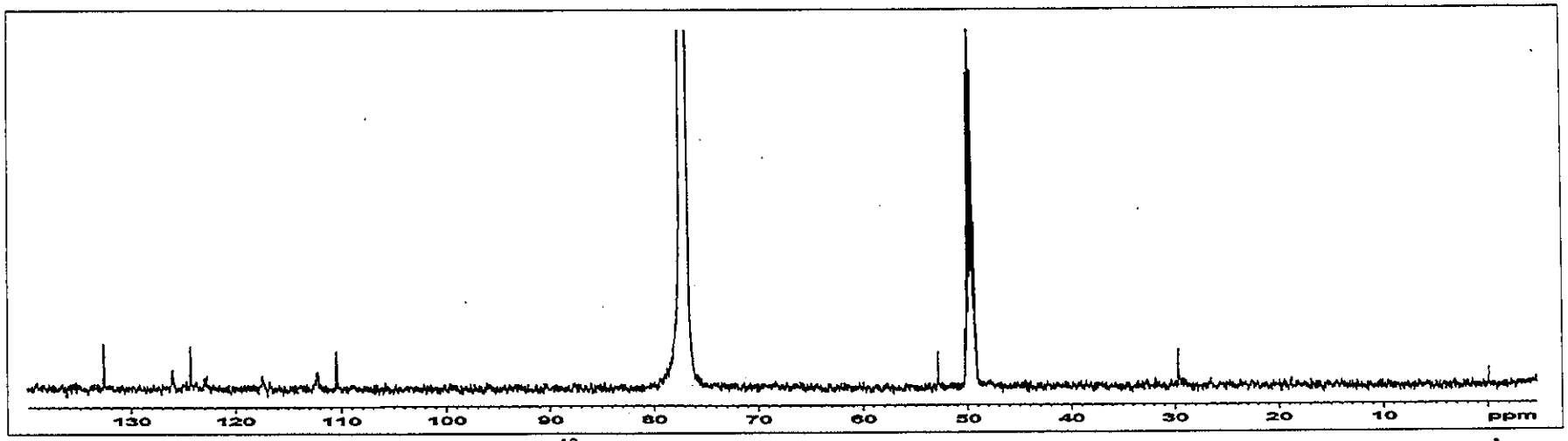

Figure 99 The 75 MHz ^{13}C NMR spectrum of compound **K40** in $CDCl_3$+CD_3OD

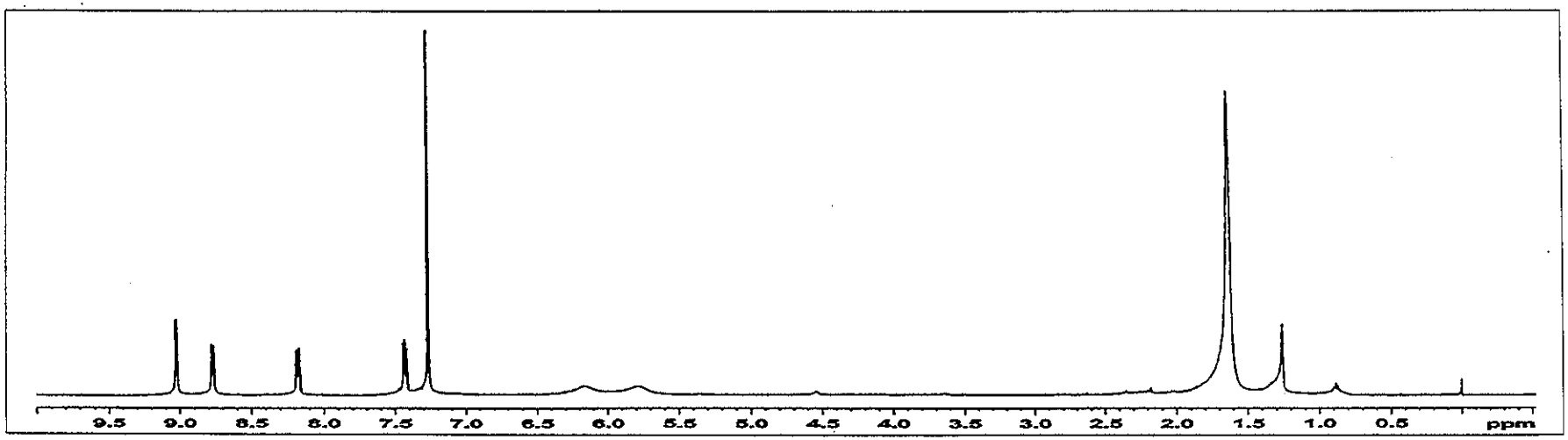

Figure 100 The 300 MHz ^{1}H NMR spectrum of compound **K41** in $CDCl_3$

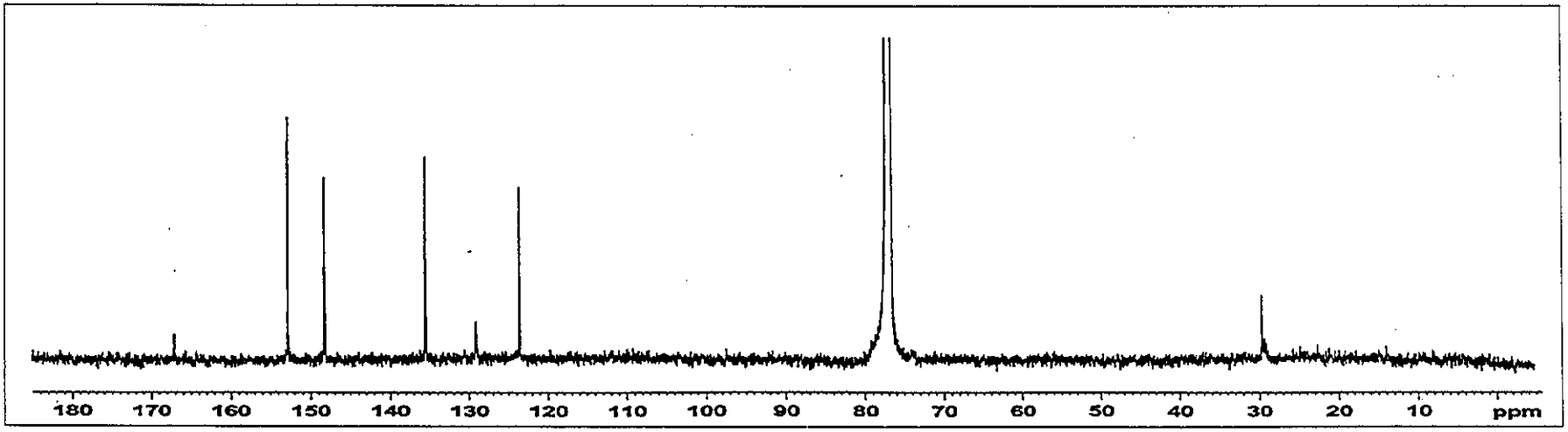

Figure 101 The 75 MHz ^{13}C NMR spectrum of compound **K41** in $CDCl_3$

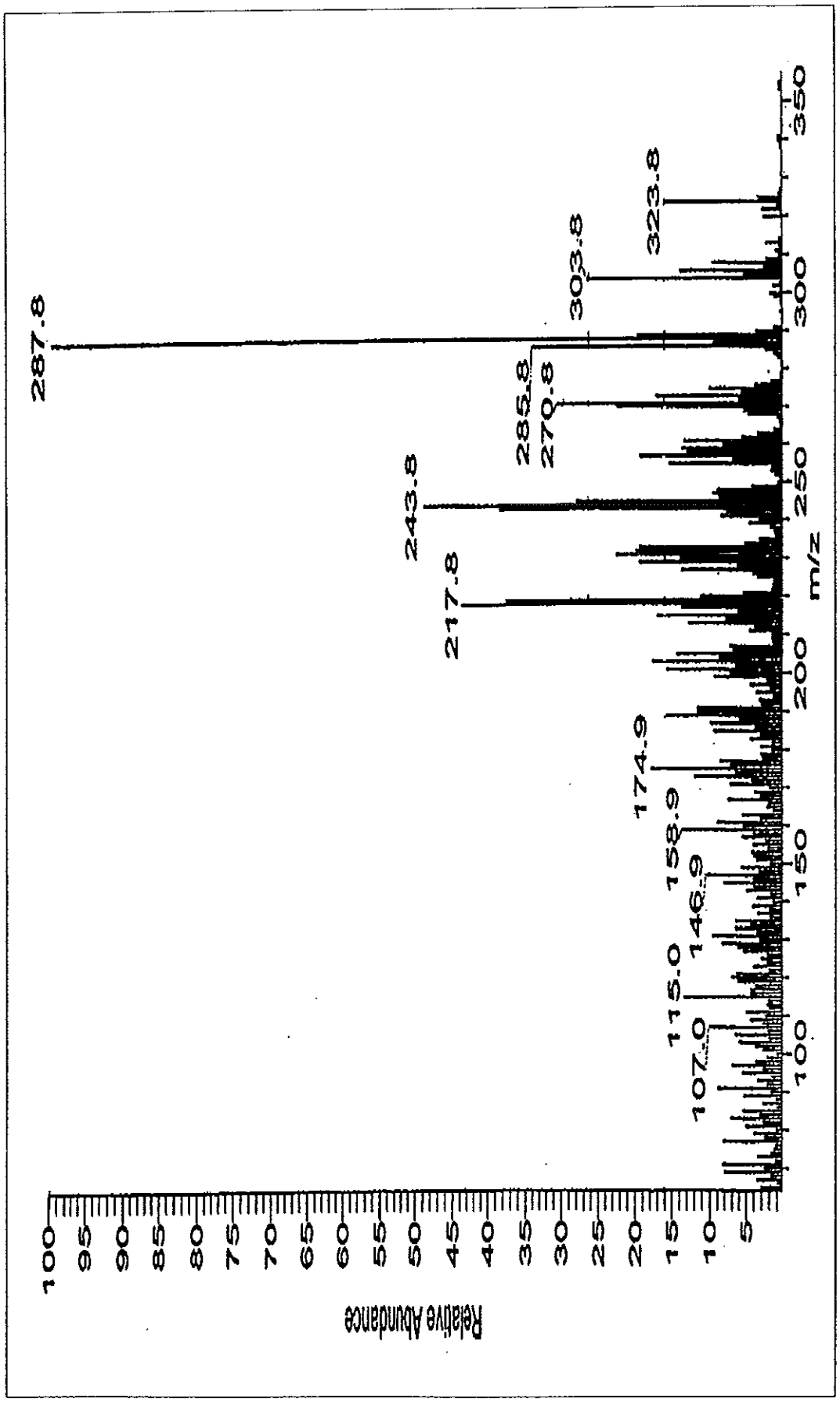

Figure 102 The mass spectrum of compound K42

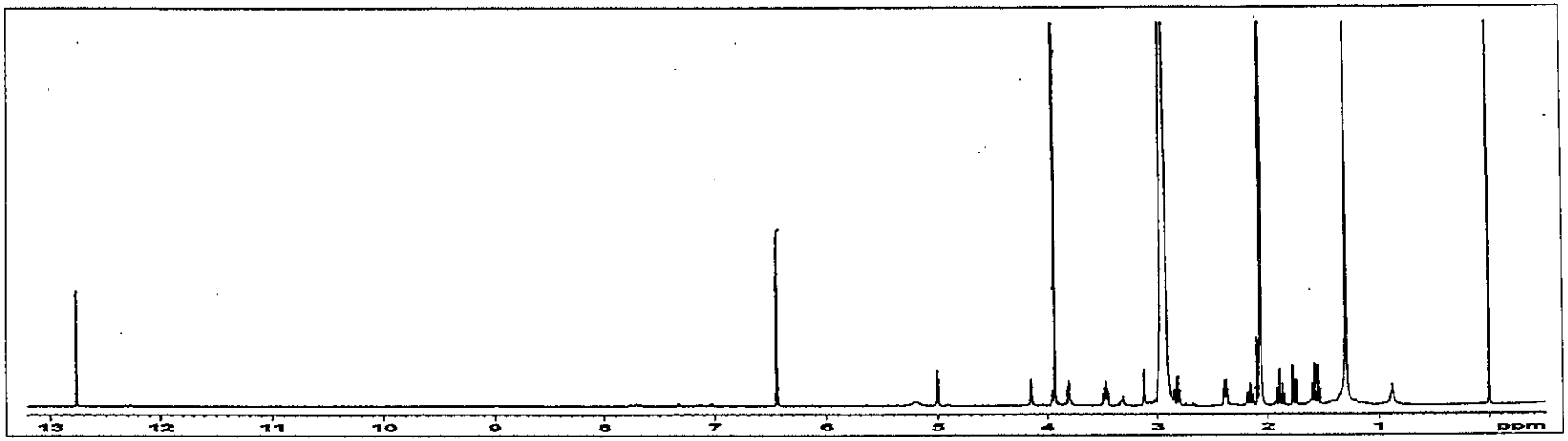

Figure 103 The 300 MHz ^{1}H NMR spectrum of compound **K42** in acetone-d_6

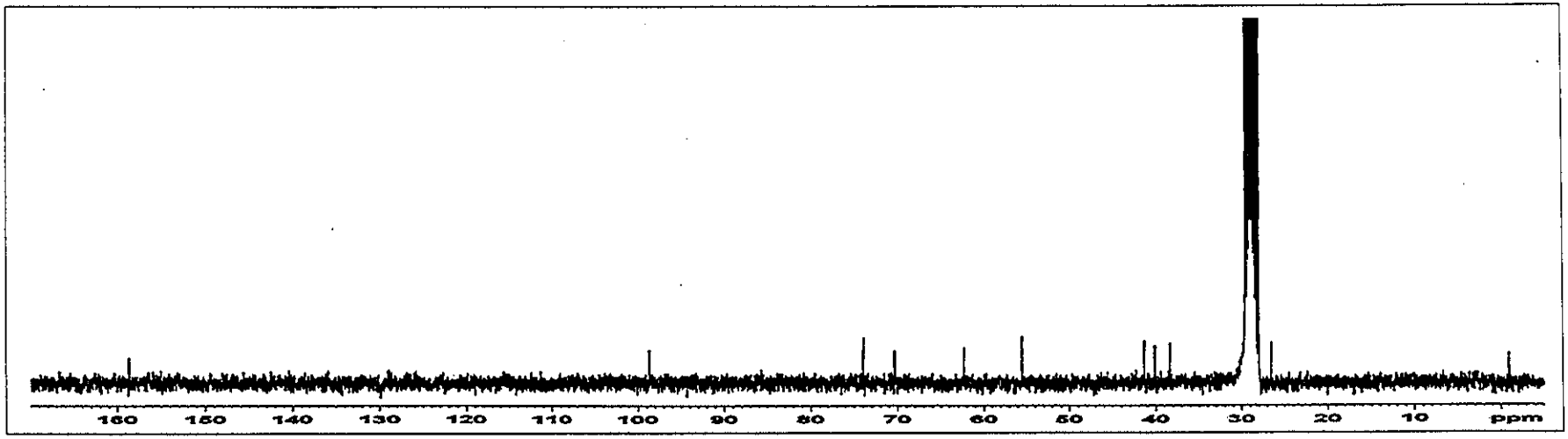

Figure 104 The 75 MHz ^{13}C NMR spectrum of compound **K42** in acetone-d_6

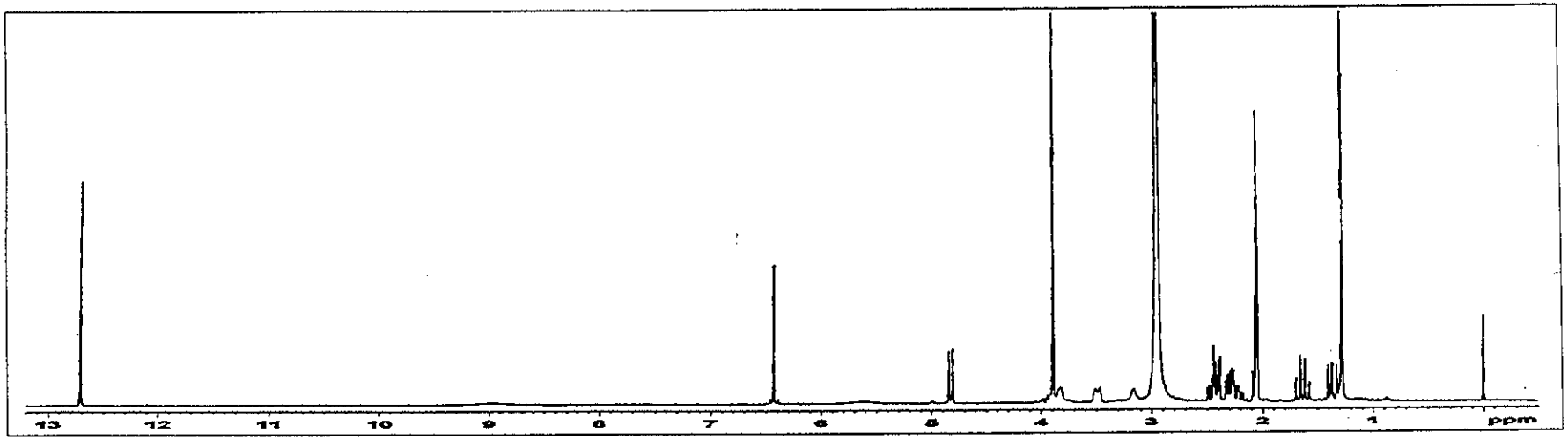

Figure 105 The 300 MHz ^{1}H NMR spectrum of compound **K43** in acetone-d_6

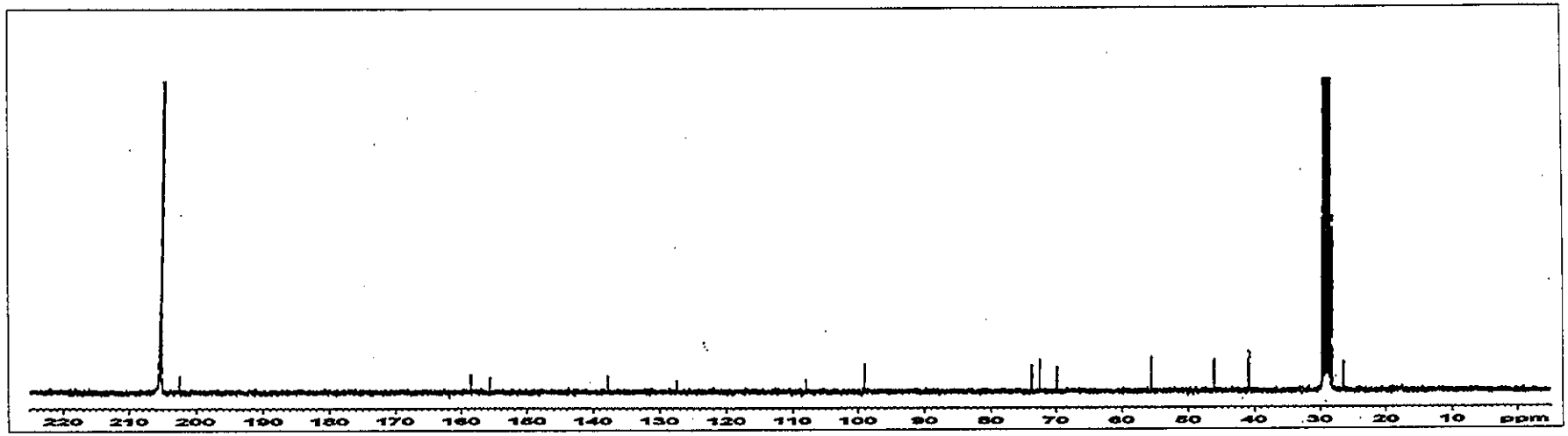

Figure 106 The 75 MHz ^{13}C NMR spectrum of compound **K43** in acetone-d_6

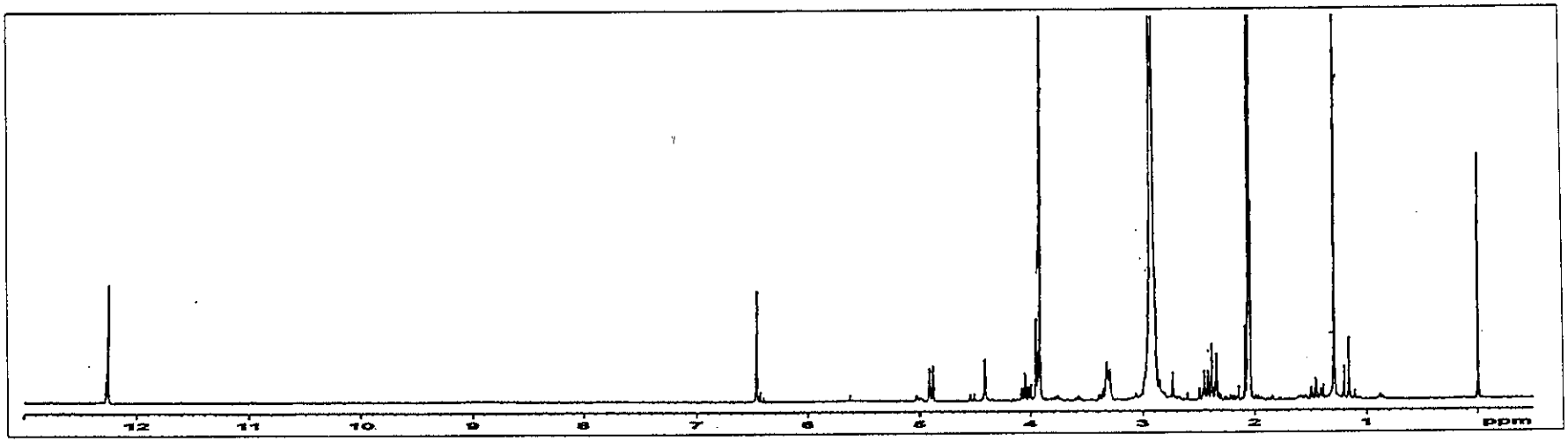

Figure 107 The 300 MHz ^{1}H NMR spectrum of compound **K44** in acetone-d_6

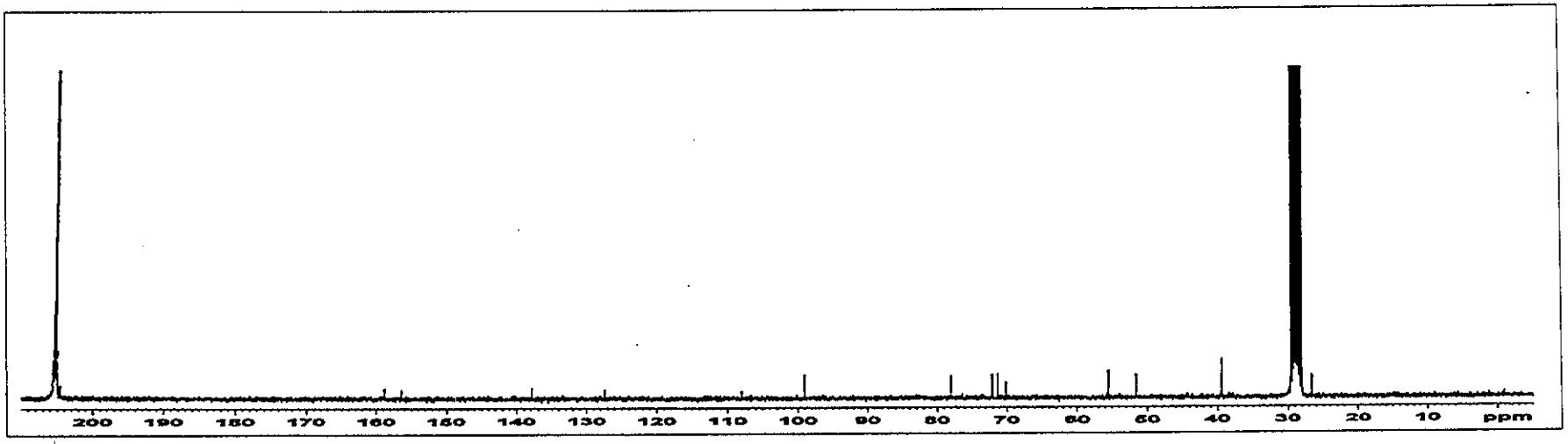

Figure 108 The 75 MHz ^{13}C NMR spectrum of compound **K44** in acetone-d_6

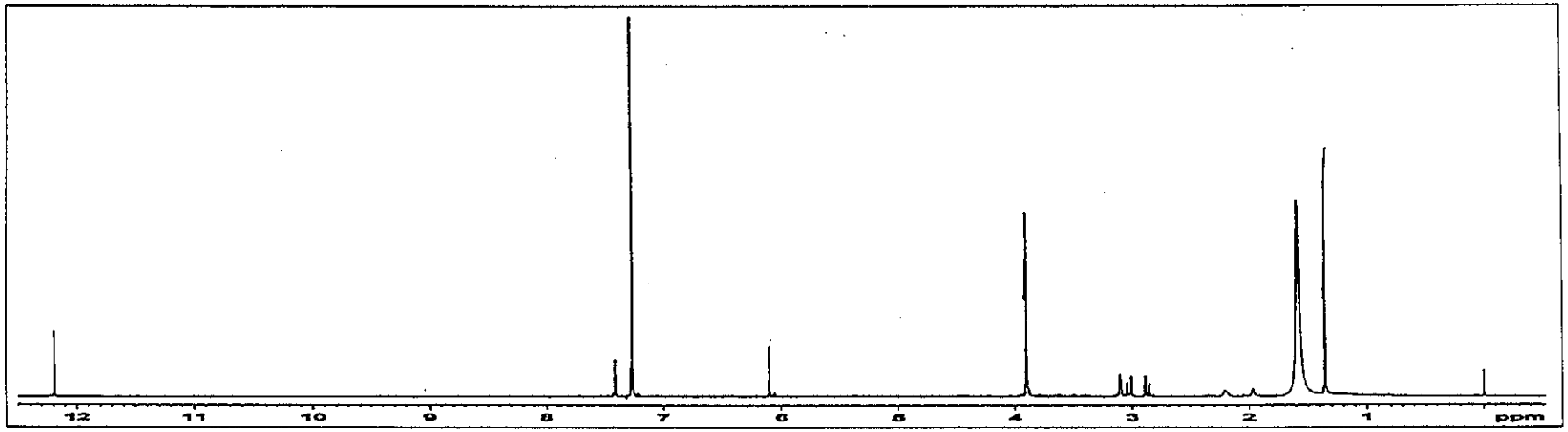

Figure 109 The 300 MHz ^{1}H NMR spectrum of compound **K45** in $CDCl_3$

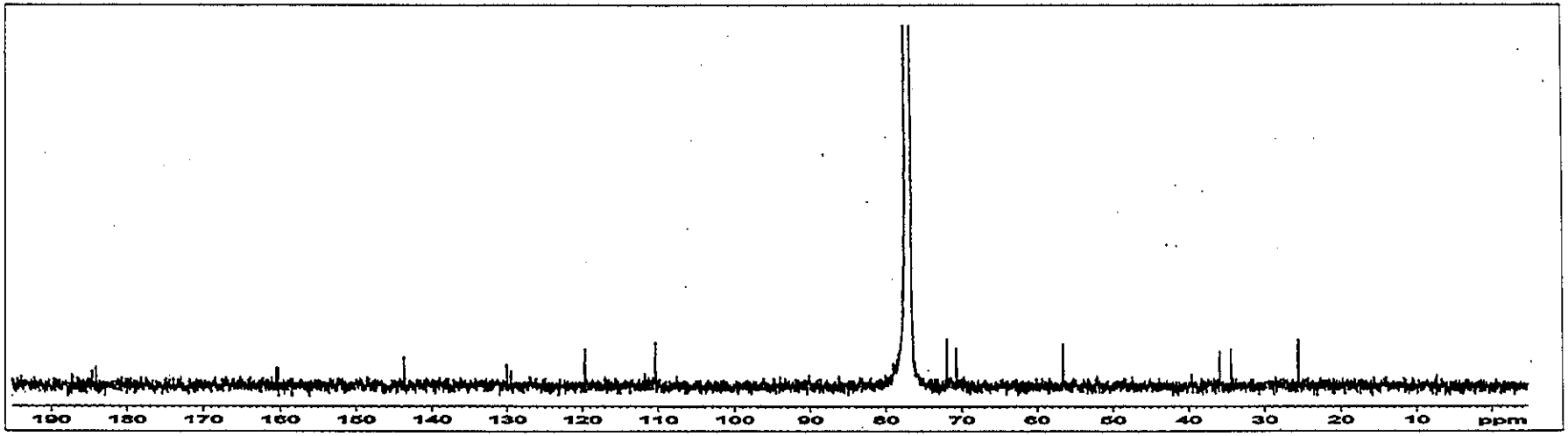

Figure 110 The 75 MHz ^{13}C NMR spectrum of compound **K45** in $CDCl_3$

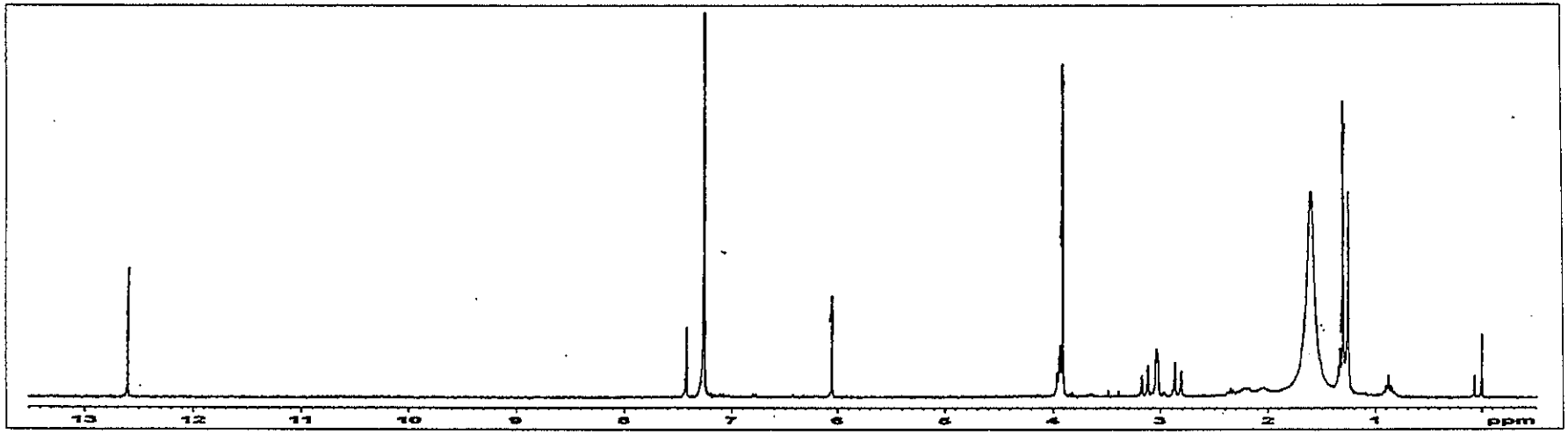

Figure 111 The 300 MHz ^{1}H NMR spectrum of compound **K46** in $CDCl_3$

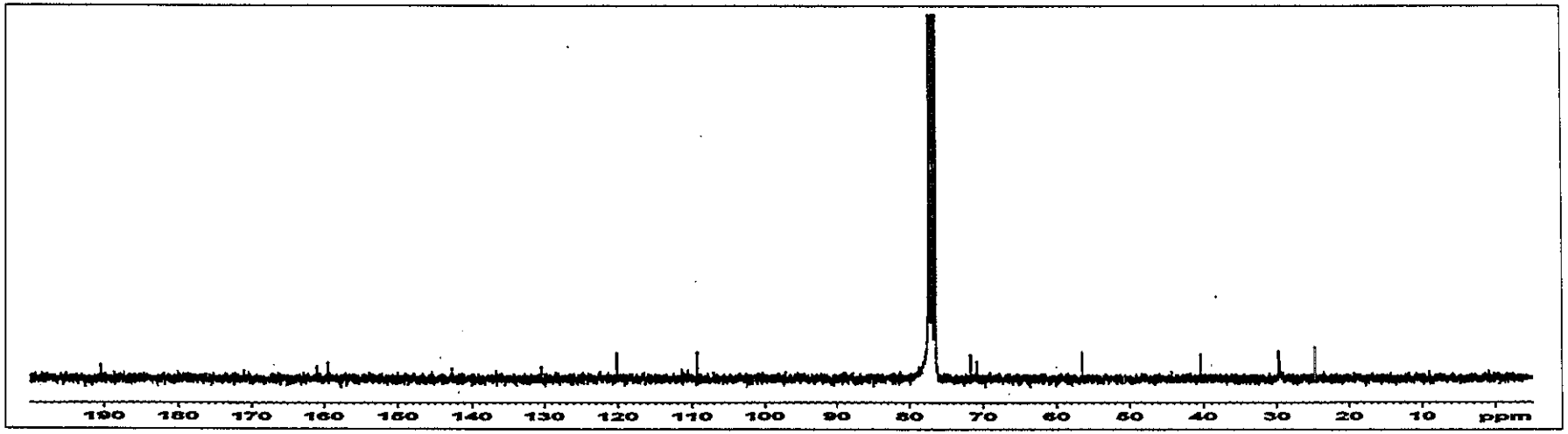

Figure 112 The 75 MHz ^{13}C NMR spectrum of compound **K46** in $CDCl_3$

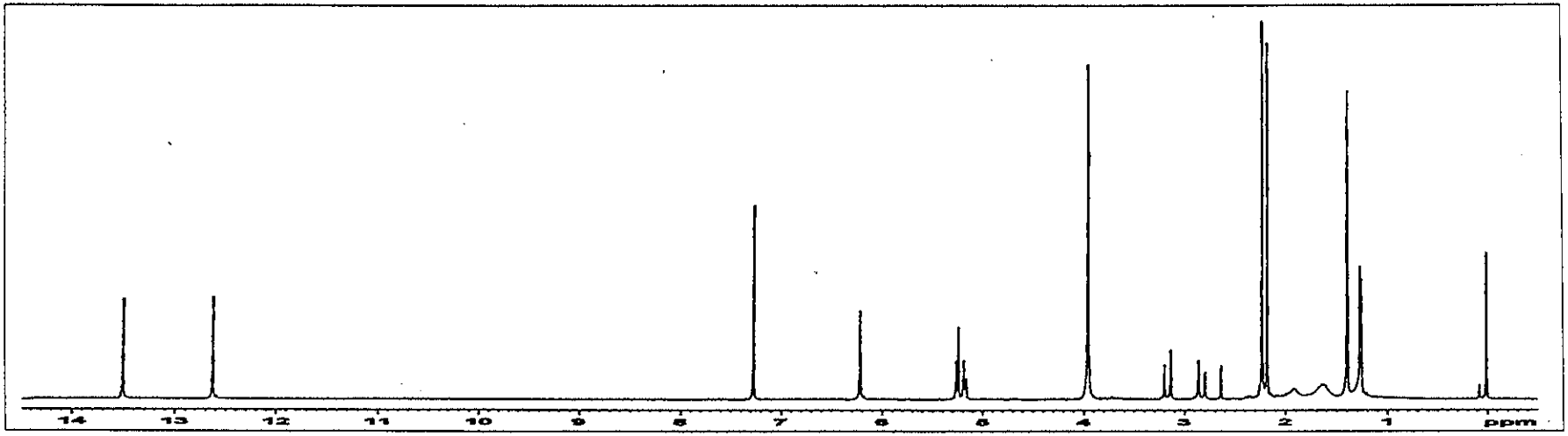

Figure 113 The 300 MHz ^{1}H NMR spectrum of compound **K47** in $CDCl_3$

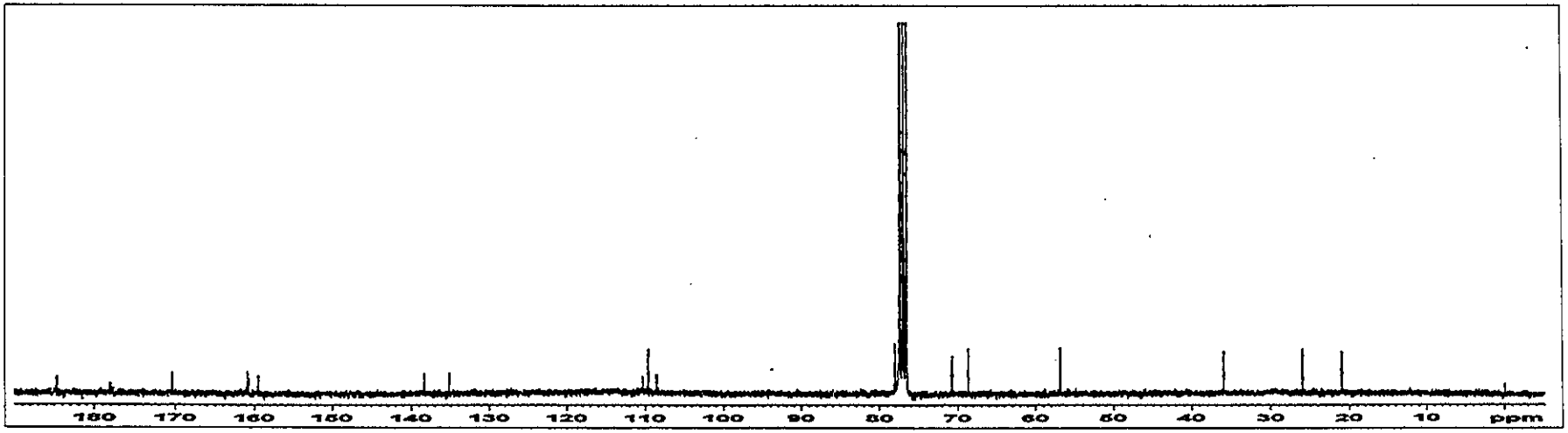

Figure 114 The 75 MHz ^{13}C NMR spectrum of compound **K47** in $CDCl_3$

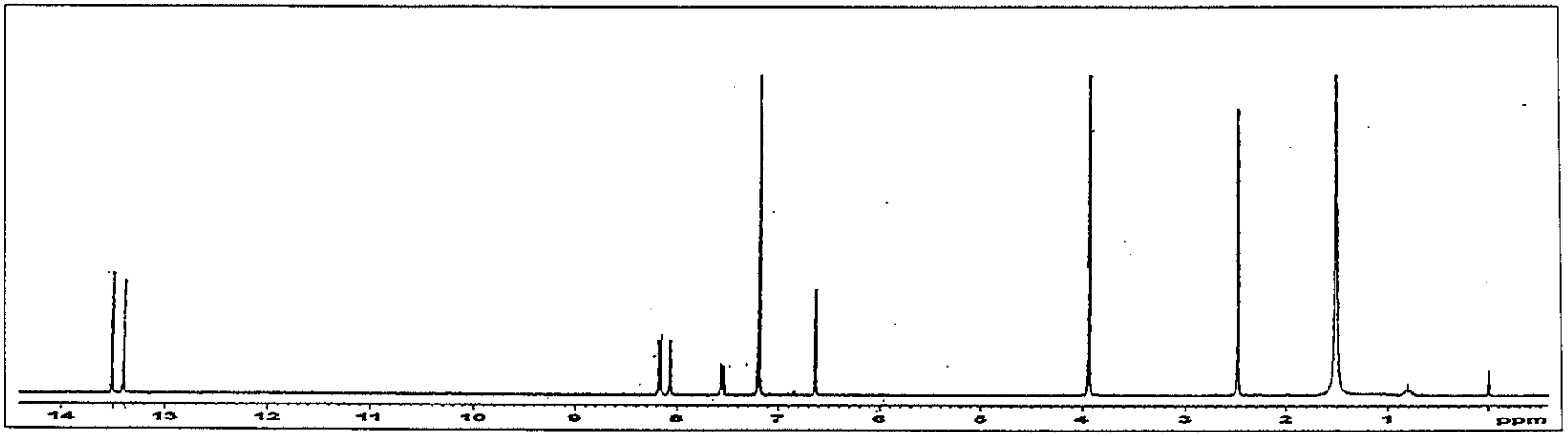

Figure 115 The 300 MHz ^{1}H NMR spectrum of compound **K48** in $CDCl_3$

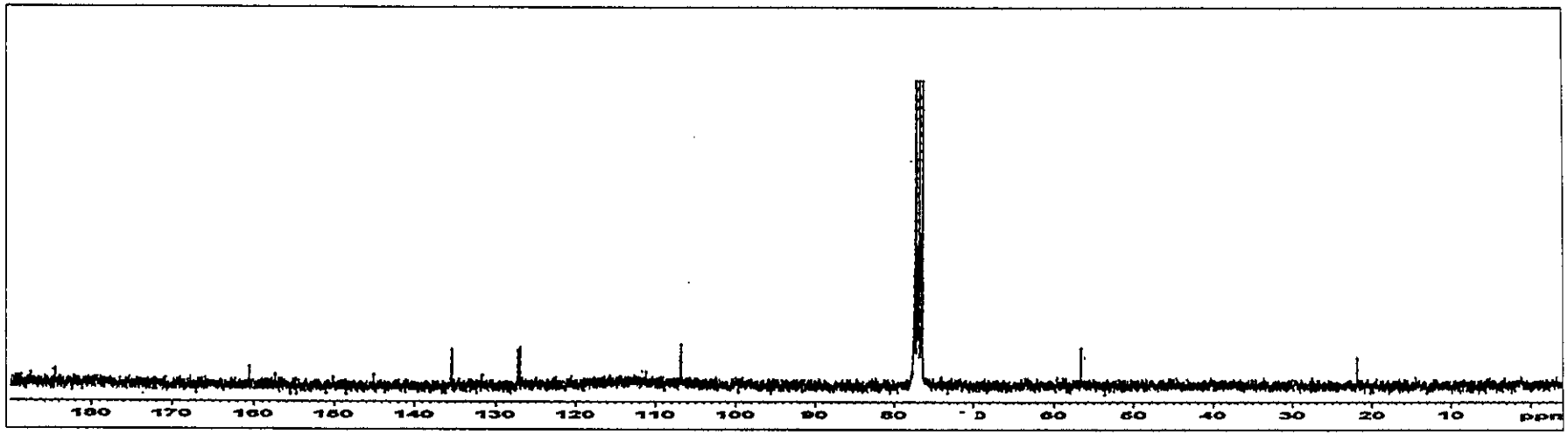

Figure 116 The 75 MHz ^{13}C NMR spectrum of compound **K48** in $CDCl_3$

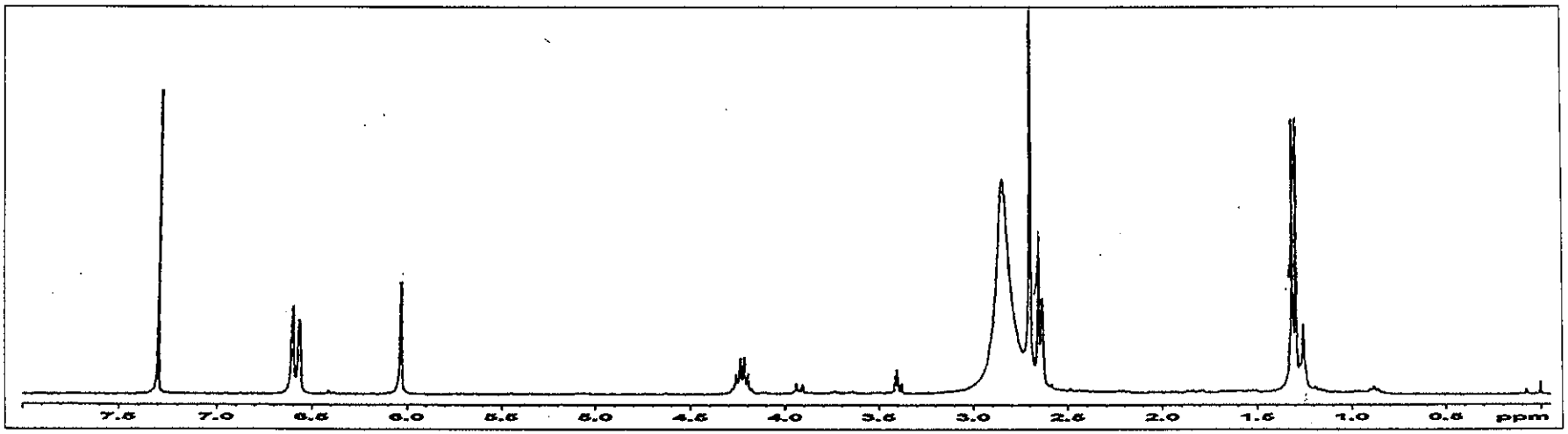

Figure 117 The 300 MHz ^{1}H NMR spectrum of compound **K49** in $CDCl_3$+CD_3OD

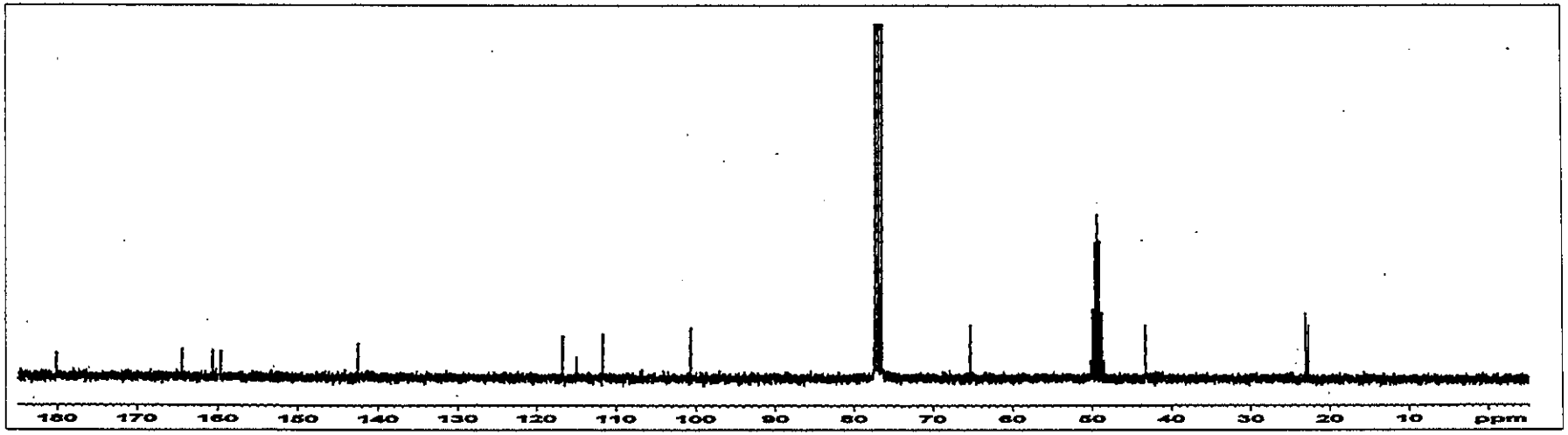

Figure 118 The 75 MHz ^{13}C NMR spectrum of compound **K49** in $CDCl_3$+CD_3OD

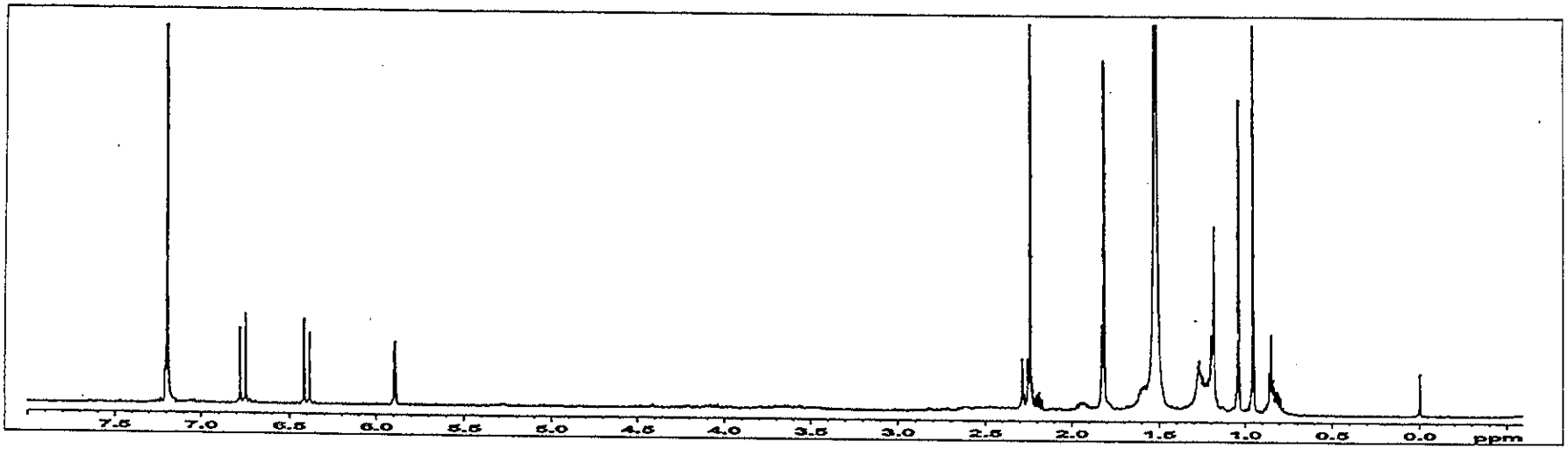

Figure 119 The 300 MHz ^{1}H NMR spectrum of compound **K50** in $CDCl_3$

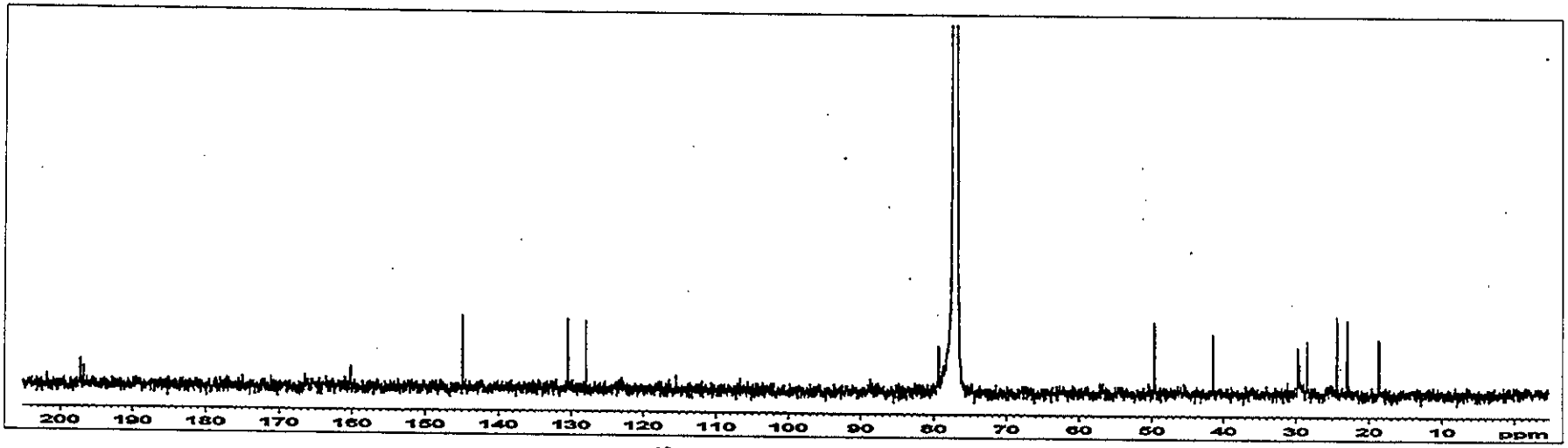

Figure 120 The 75 MHz ^{13}C NMR spectrum of compound **K50** in $CDCl_3$

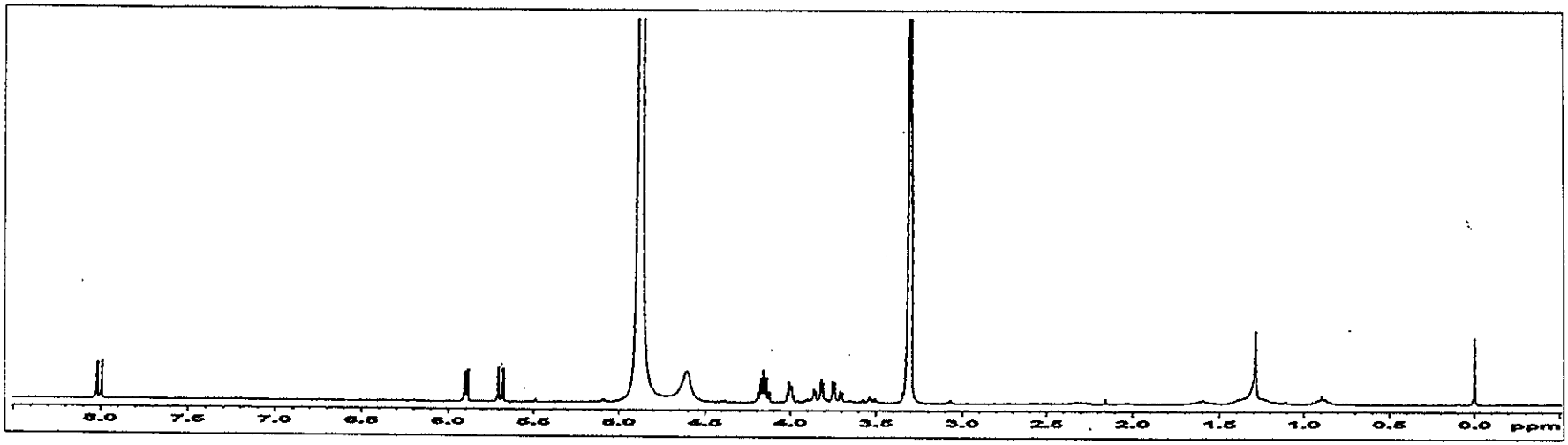

Figure 121 The 300 MHz ^{1}H NMR spectrum of compound **K51** in CD_3OD

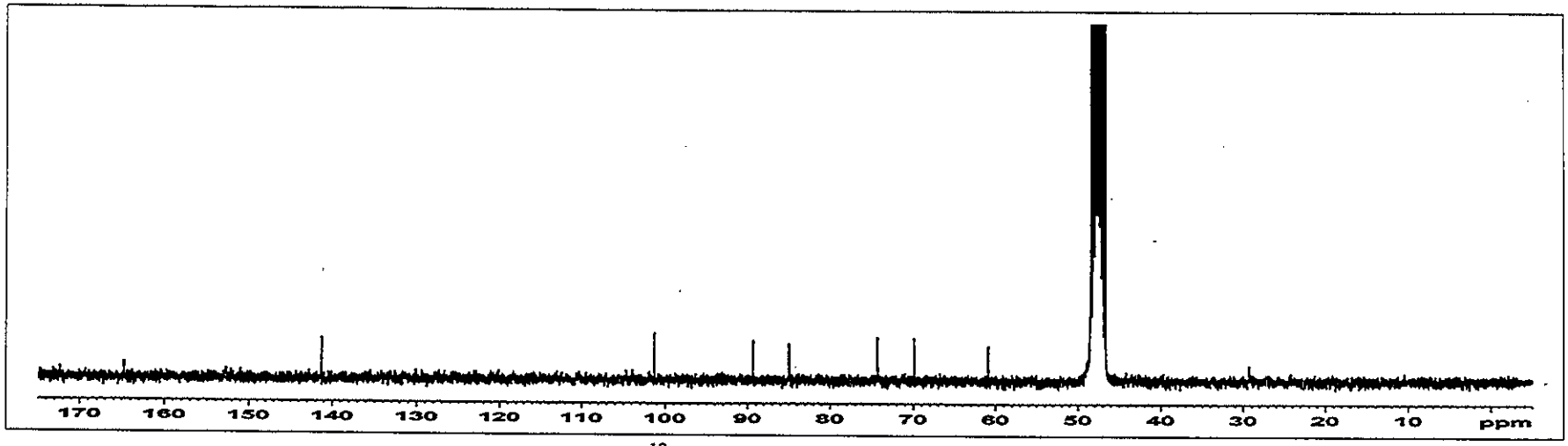

Figure 122 The 75 MHz ^{13}C NMR spectrum of compound **K51** in CD_3OD

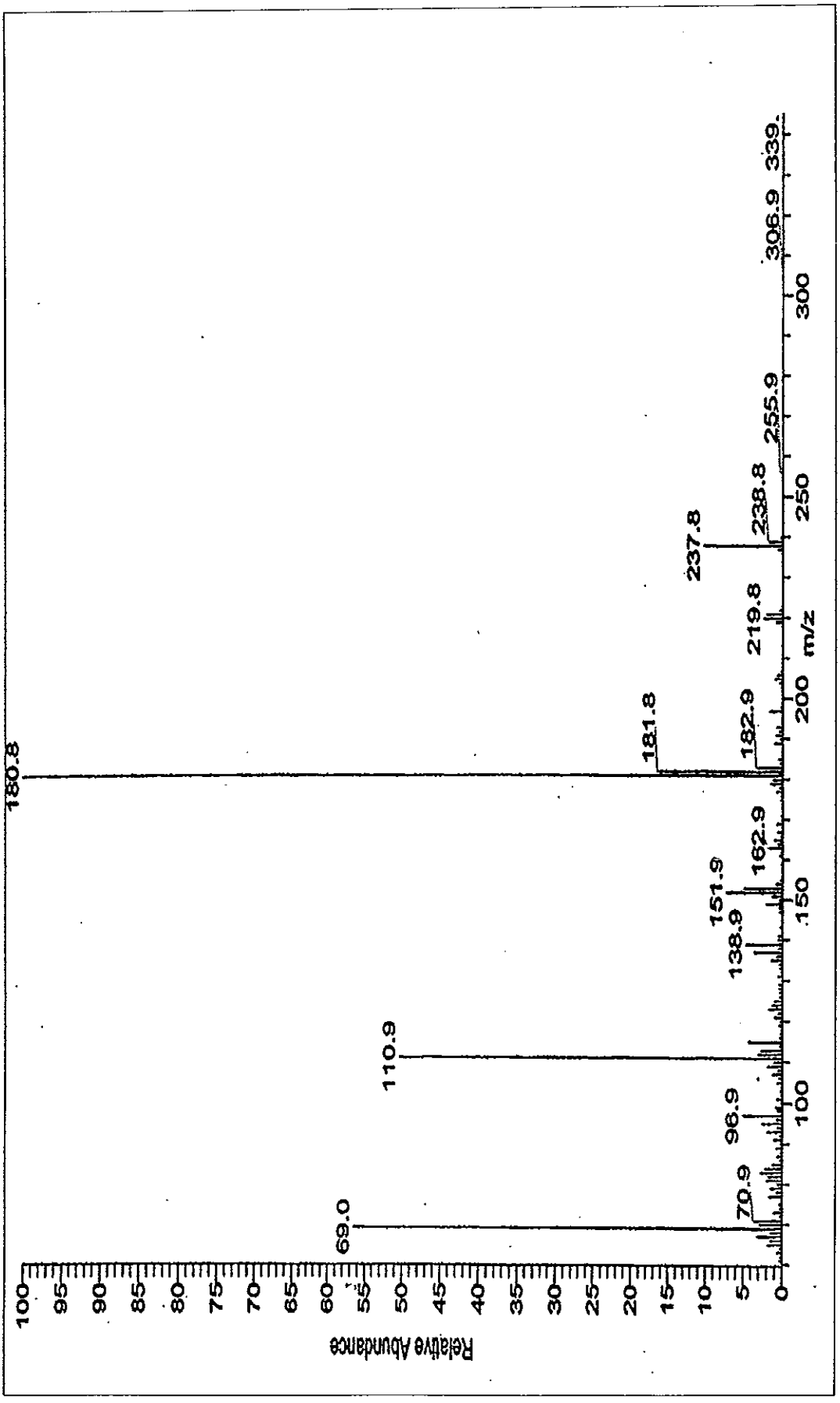

Figure 123 The mass spectrum of compound K52

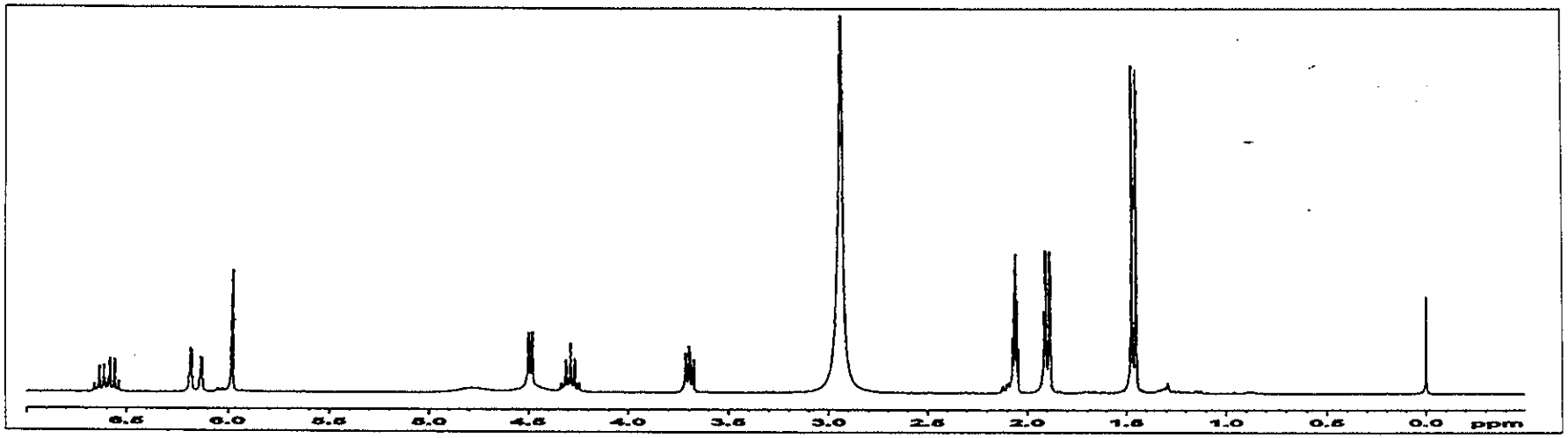

Figure 124 The 300 MHz ^{1}H NMR spectrum of compound **K52** in acetone-d_6

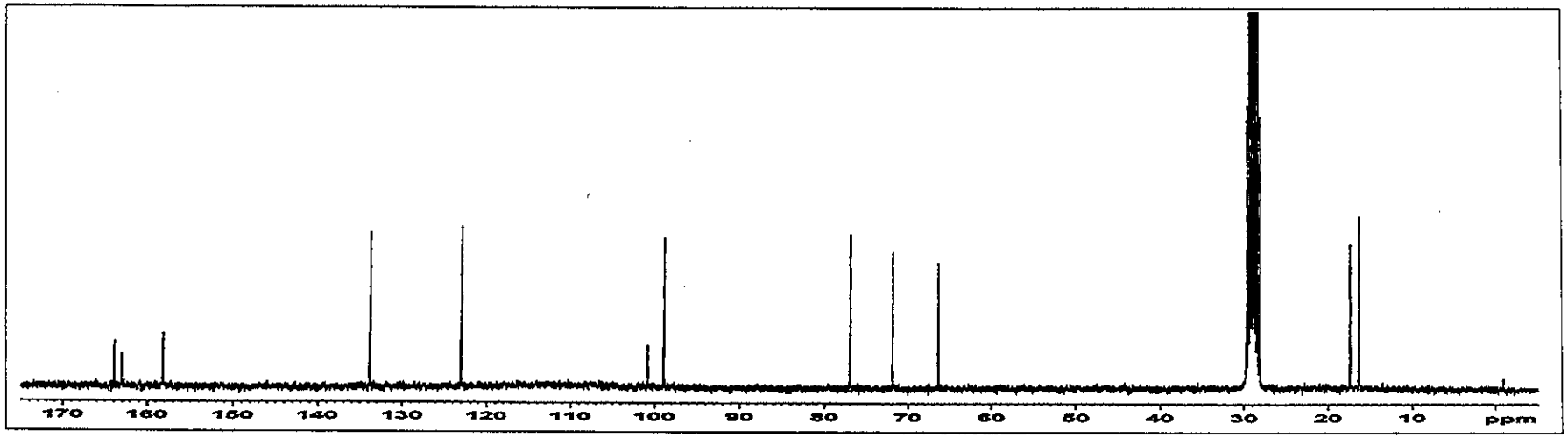

Figure 125 The 75 MHz ^{13}C NMR spectrum of compound **K52** in acetone-d_6

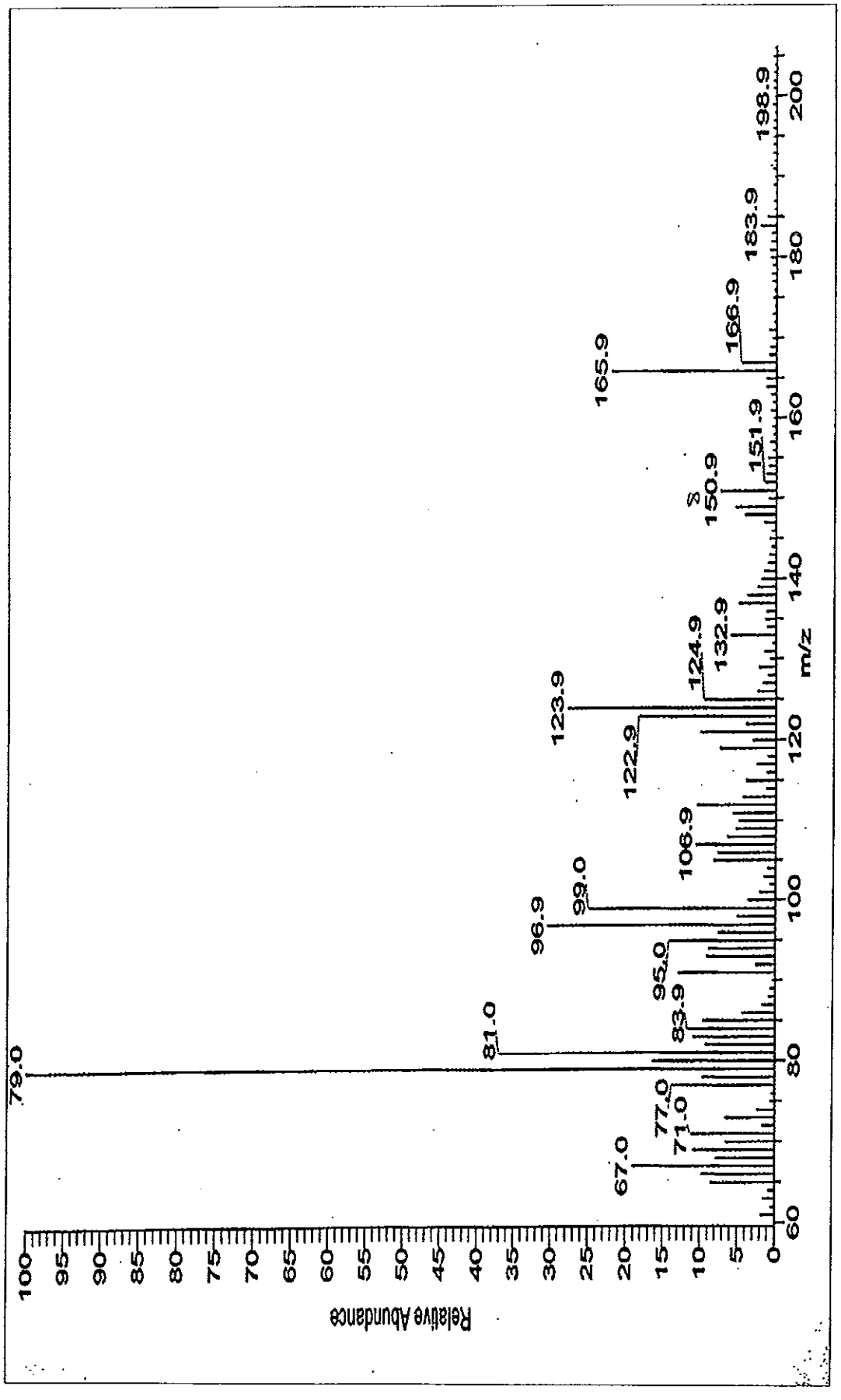

Figure 126 The mass spectrum of compound K53

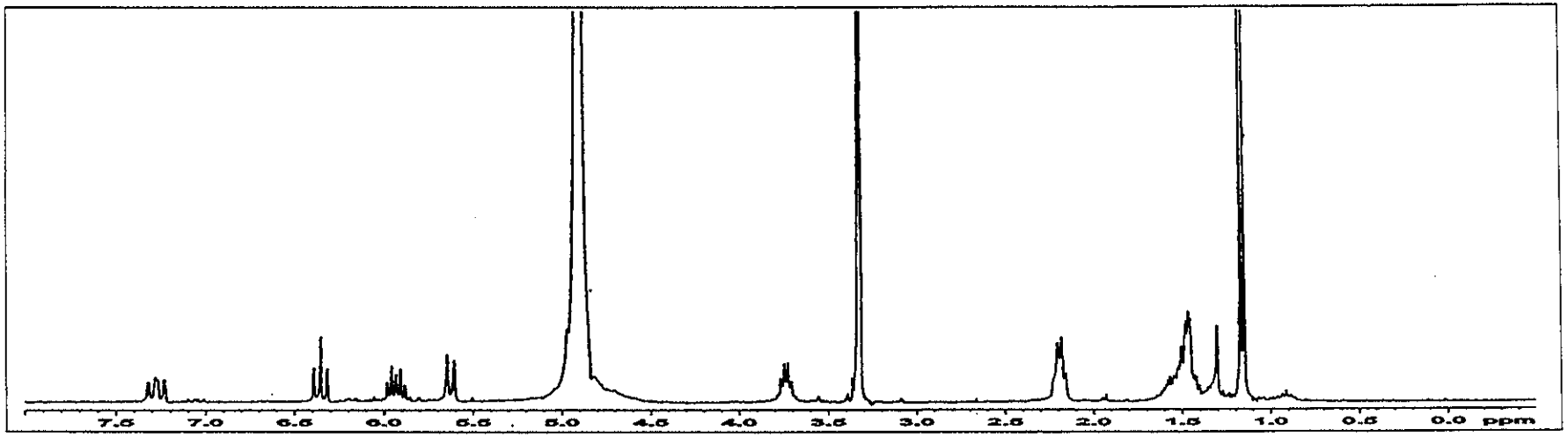

Figure 127 The 300 MHz ^{1}H NMR spectrum of compound **K53** in CD_3OD

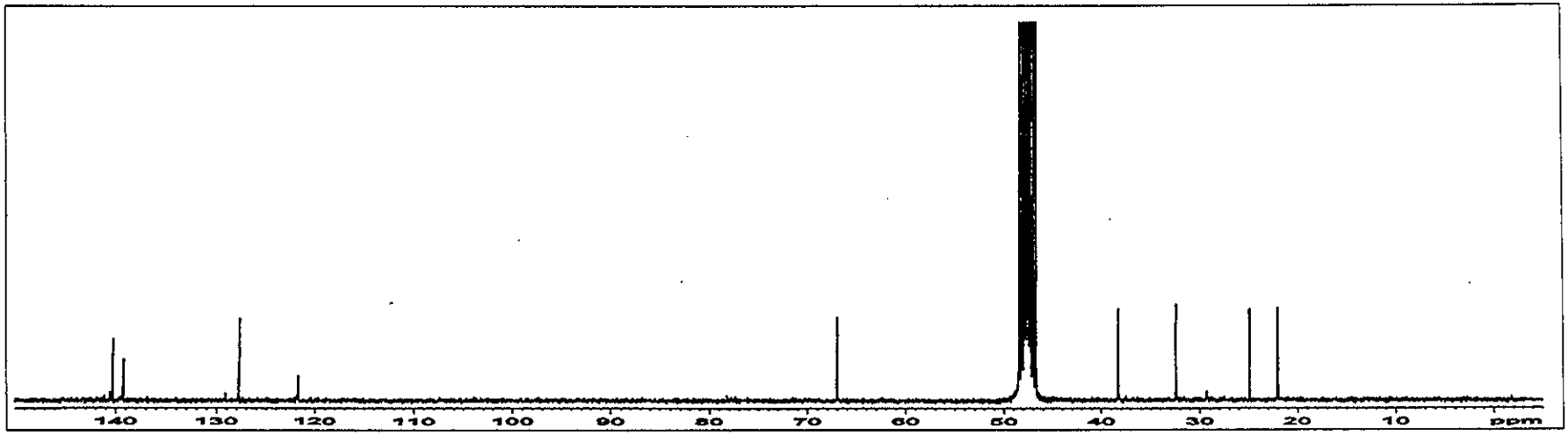

Figure 128 The 75 MHz ^{13}C NMR spectrum of compound **K53** in CD_3OD

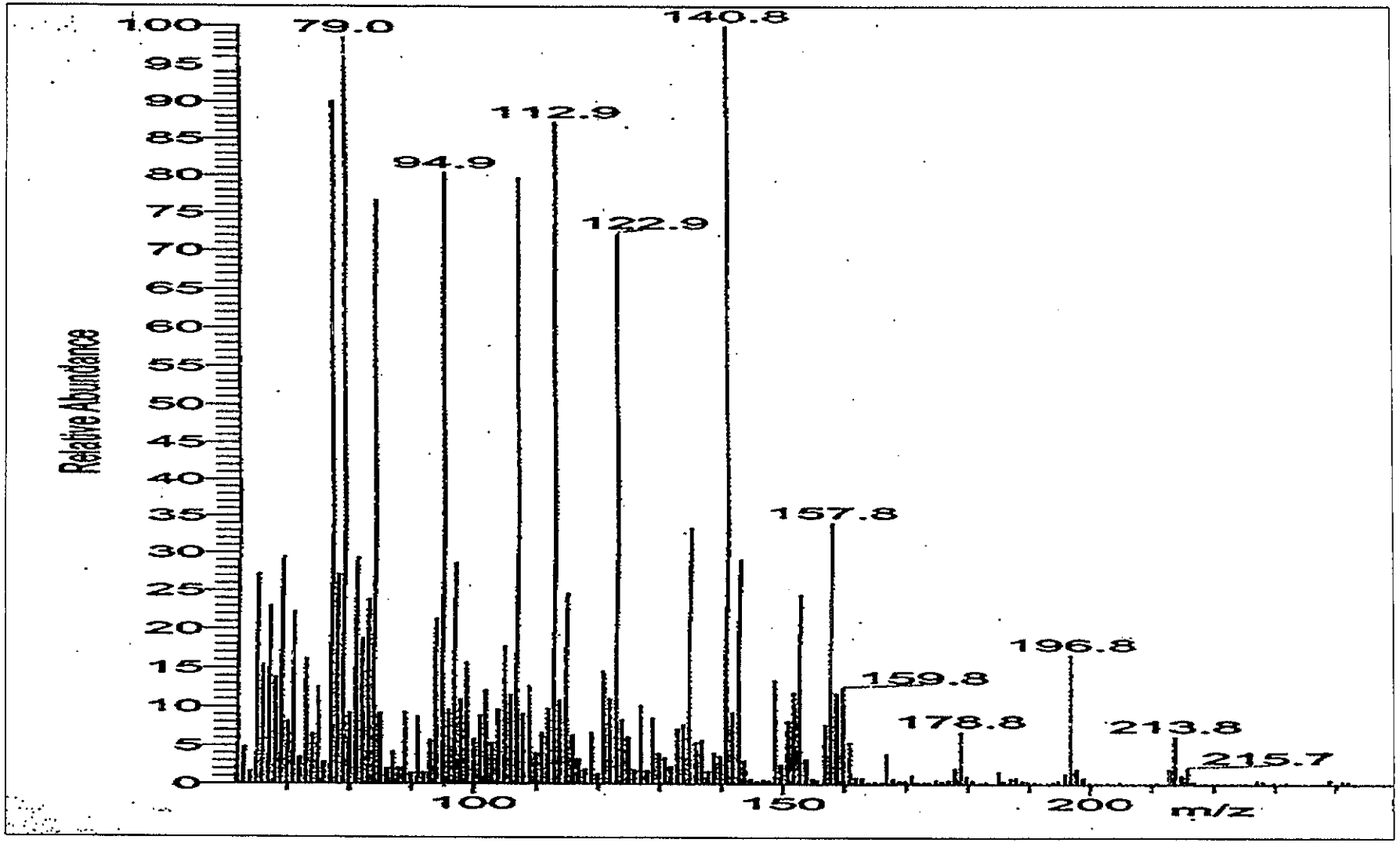

Figure 129 The mass spectrum of compound K54

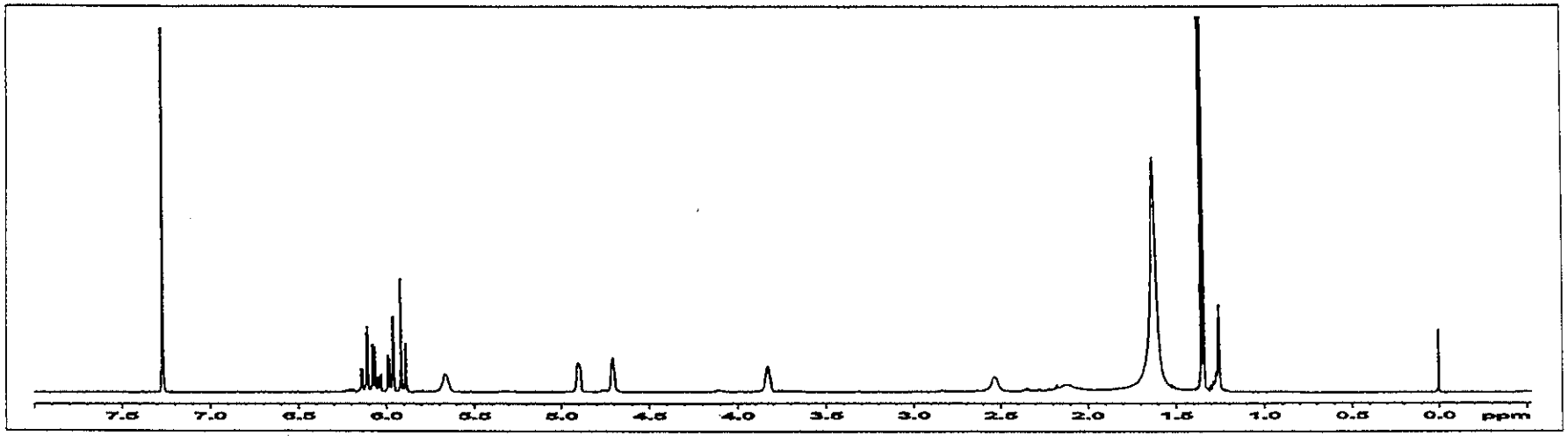

Figure 130 The 500 MHz ^{1}H NMR spectrum of compound **K54** in $CDCl_3$

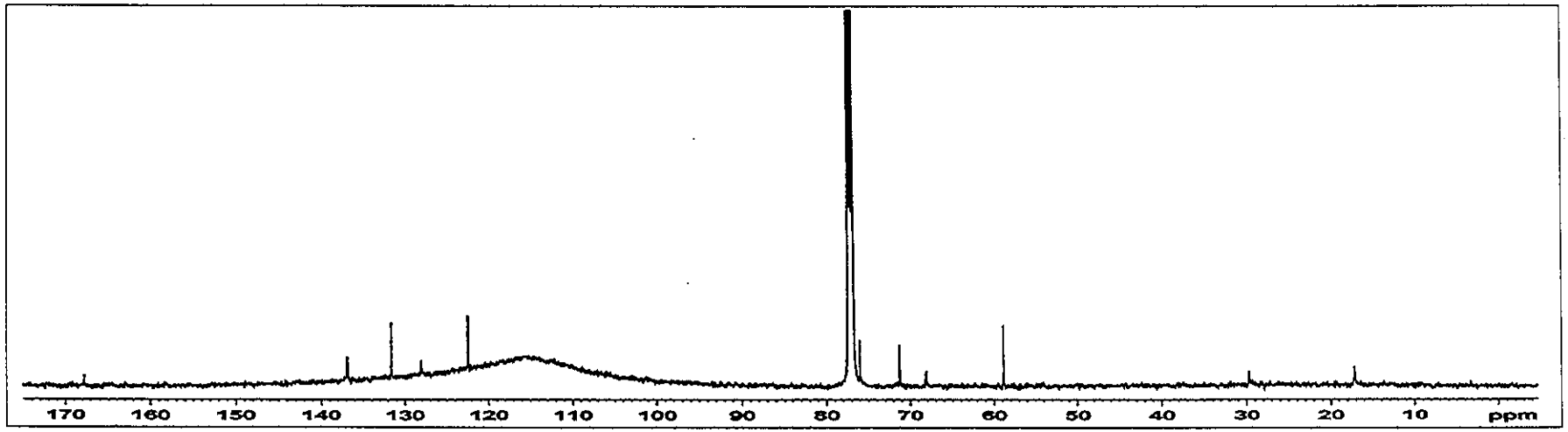

Figure 131 The 125 MHz ^{13}C NMR spectrum of compound **K54** in $CDCl_3$

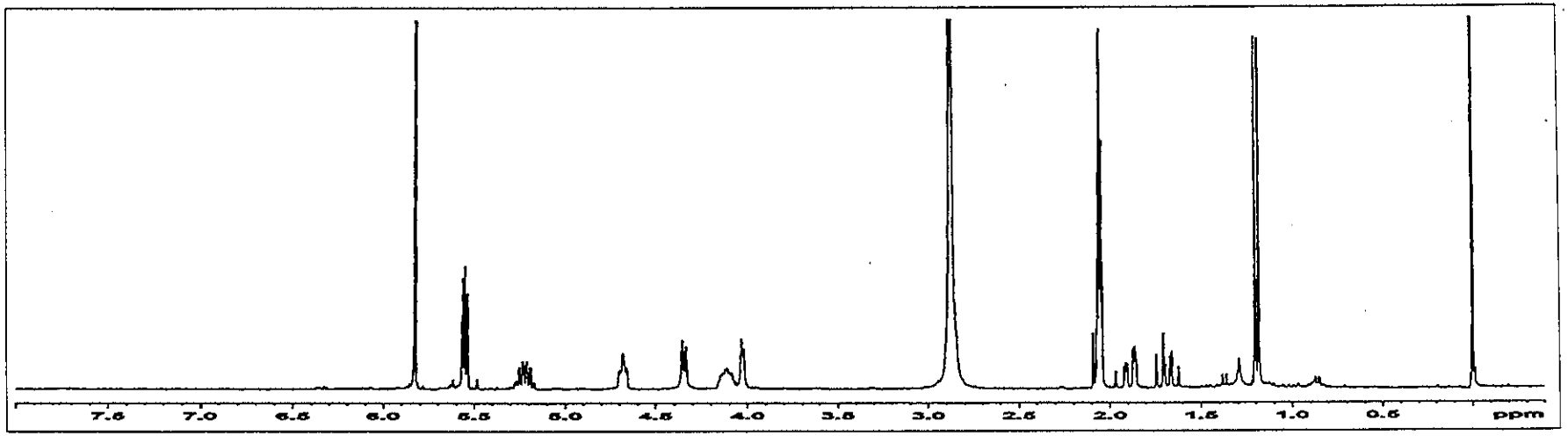

Figure 132 The 300 MHz ^{1}H NMR spectrum of compound **K55** in acetone-d_6

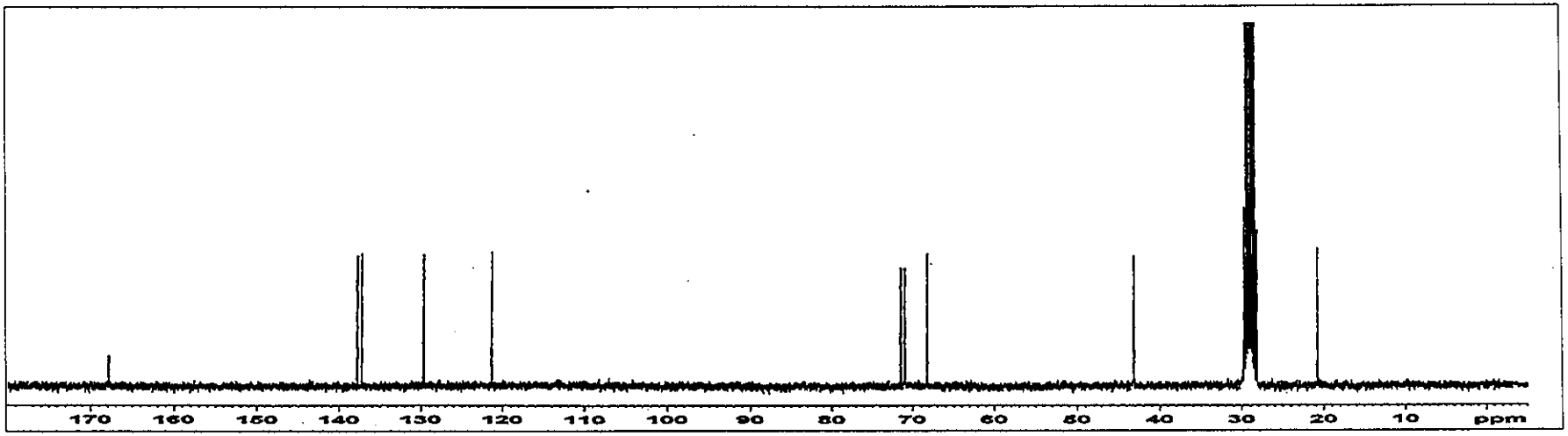

Figure 133 The 75 MHz ^{13}C NMR spectrum of compound **K55** in acetone-d_6

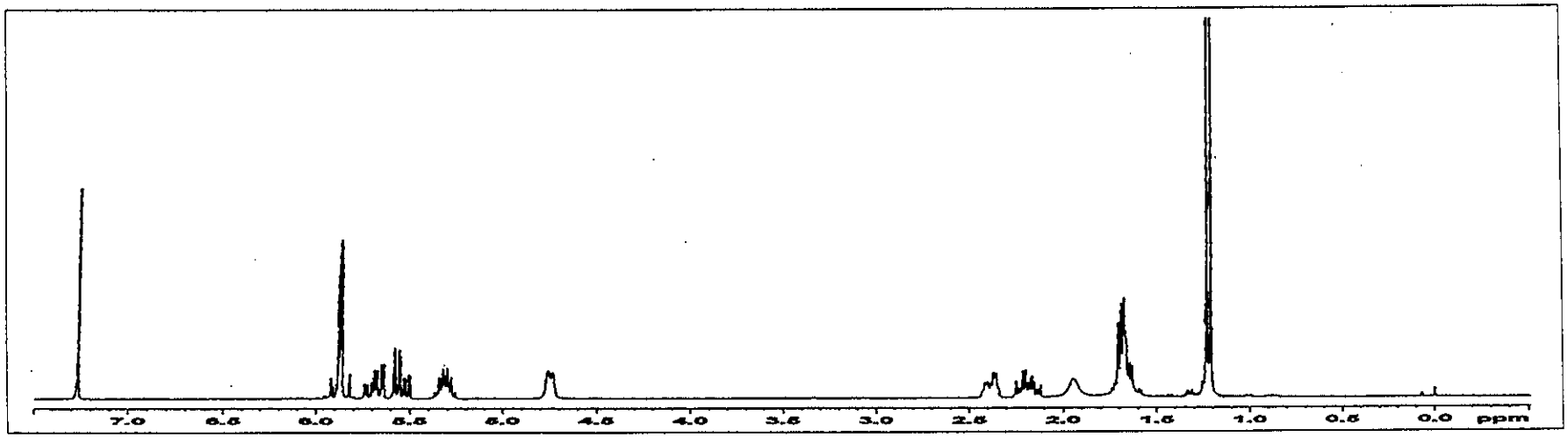

Figure 134 The 300 MHz ^{1}H NMR spectrum of compound **K56** in $CDCl_3$

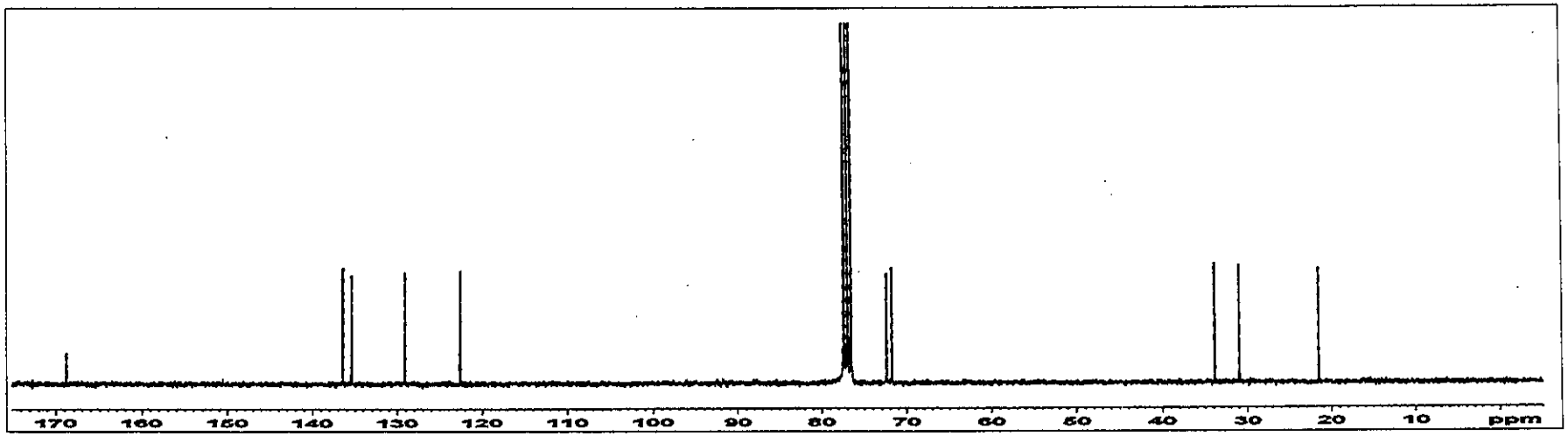

Figure 135 The 75 MHz ^{13}C NMR spectrum of compound **K56** in $CDCl_3$

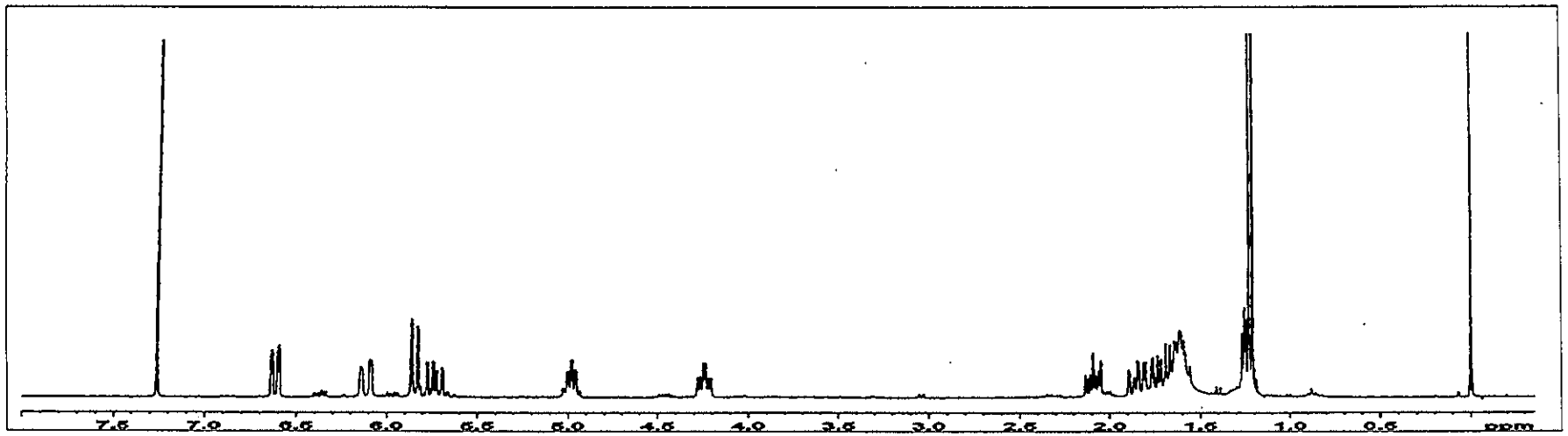

Figure 136 The 300 MHz ^{1}H NMR spectrum of compound **K57** in $CDCl_3$

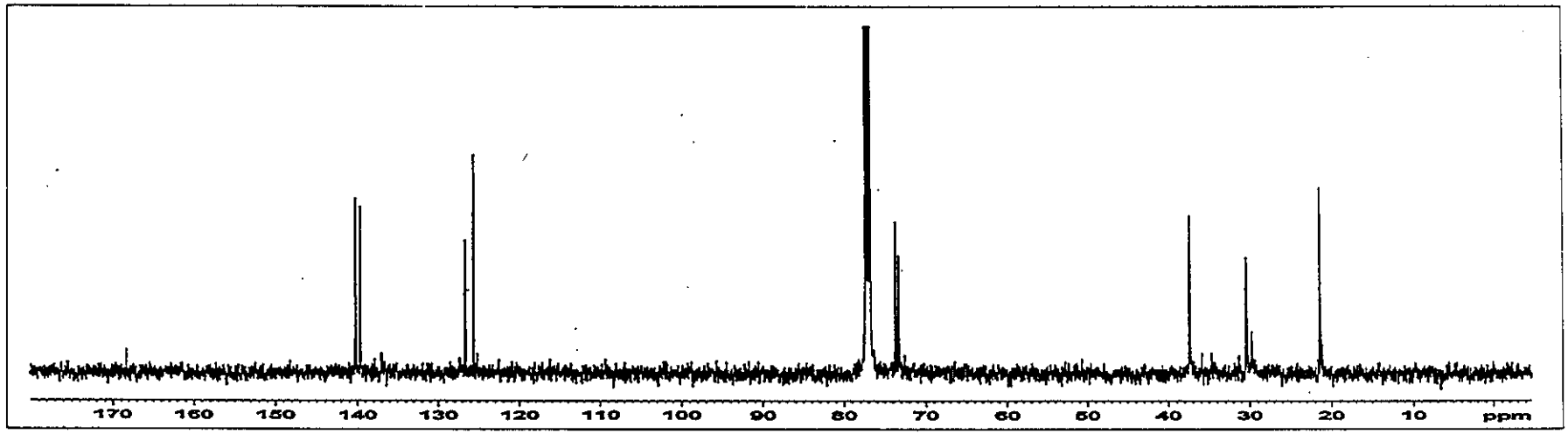

Figure 137 The 75 MHz ^{13}C NMR spectrum of compound **K57** in $CDCl_3$

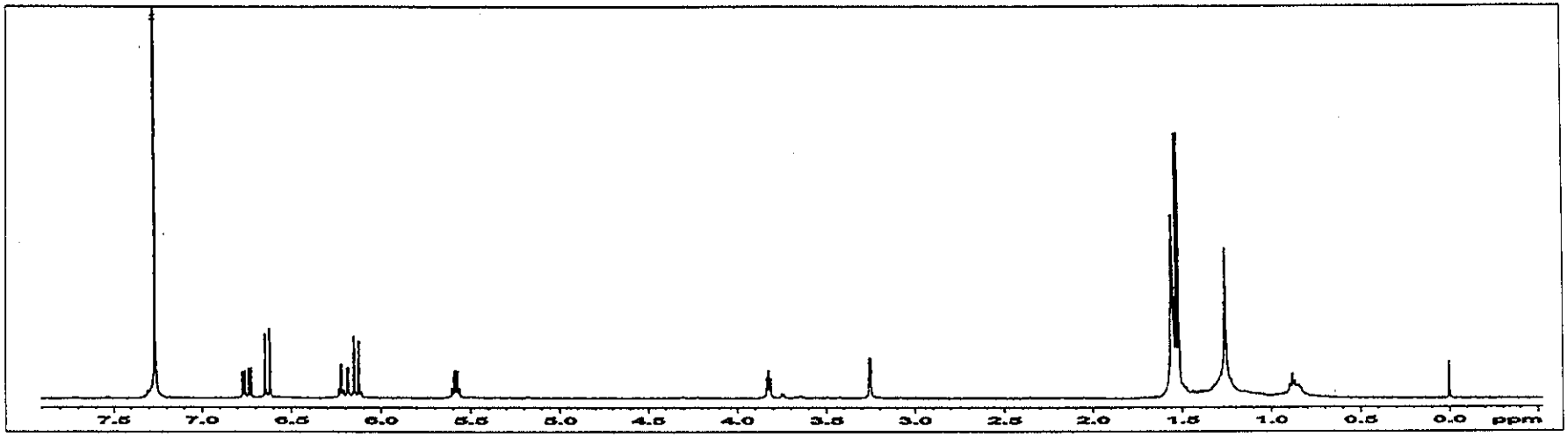

Figure 138 The 300 MHz ^{1}H NMR spectrum of compound **K58** in $CDCl_3$

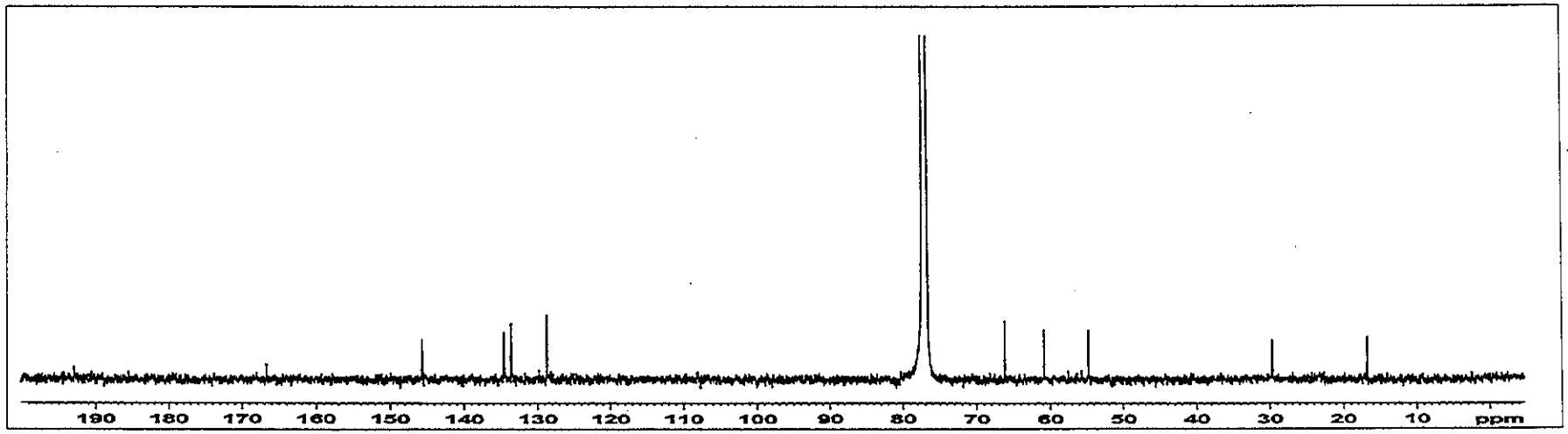

Figure 139 The 75 MHz ^{13}C NMR spectrum of compound **K58** in $CDCl_3$

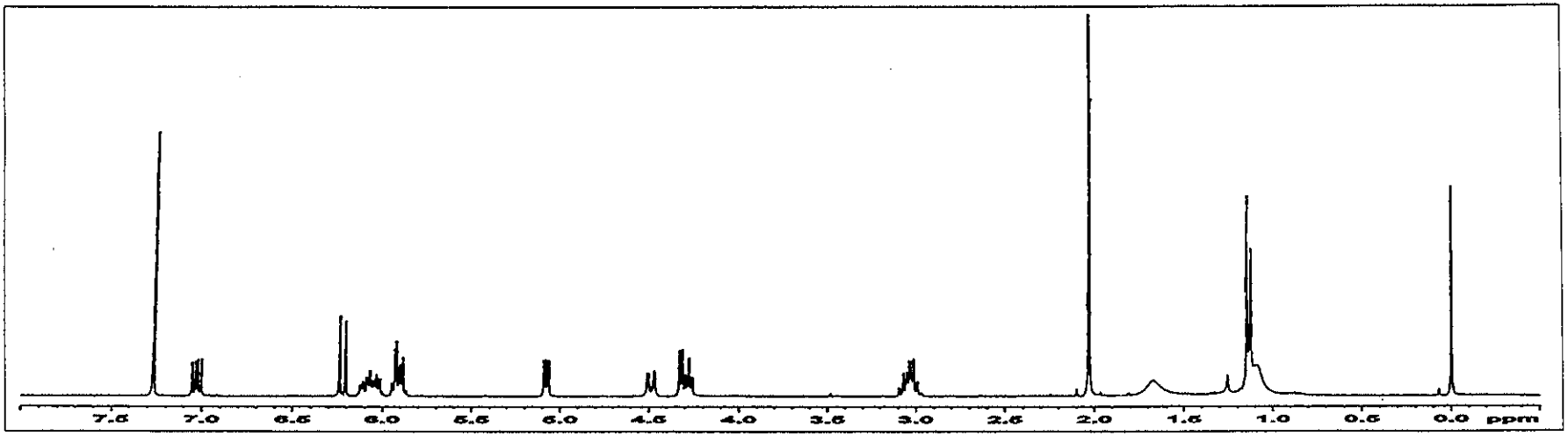

Figure 140 The 300 MHz ^{1}H NMR spectrum of compound **K59** in $CDCl_3$

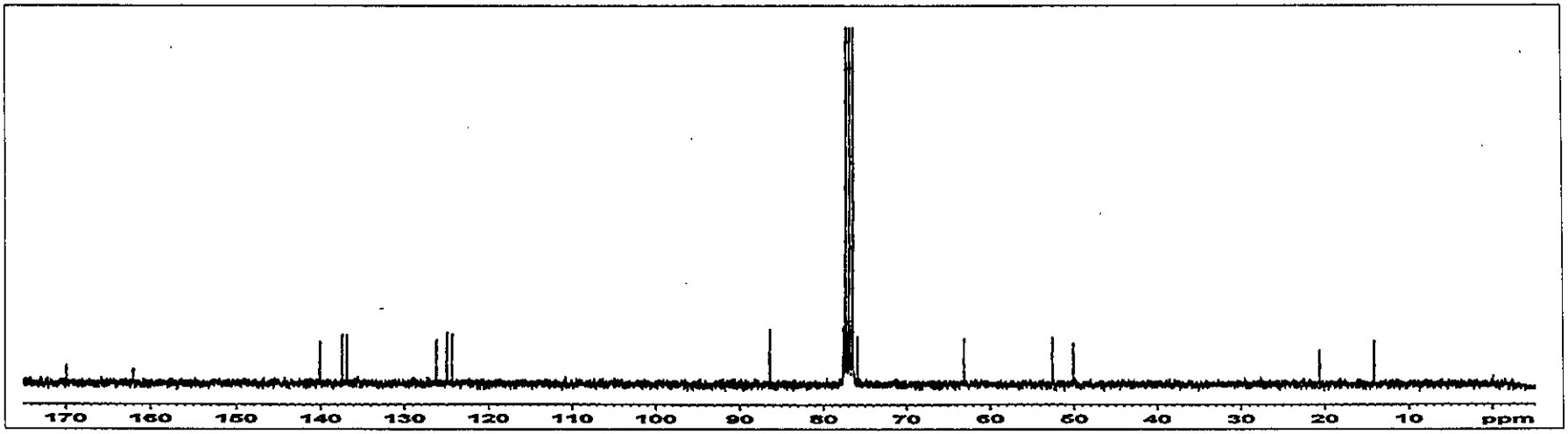

Figure 141 The 75 MHz ^{13}C NMR spectrum of compound **K59** in $CDCl_3$

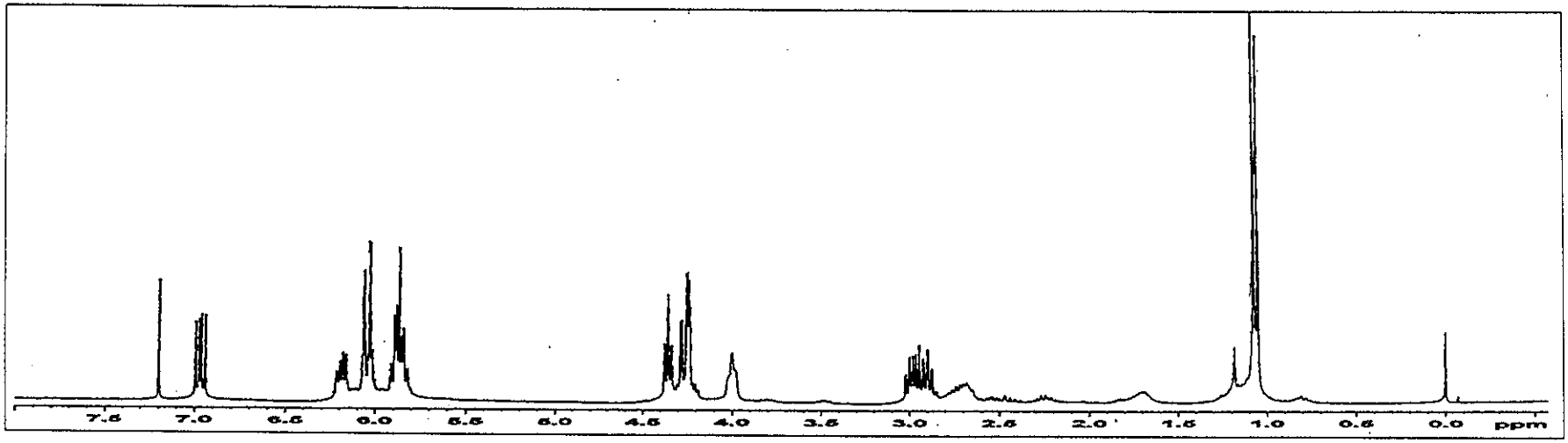

Figure 142 The 300 MHz ^{1}H NMR spectrum of compound **K60** in $CDCl_3$

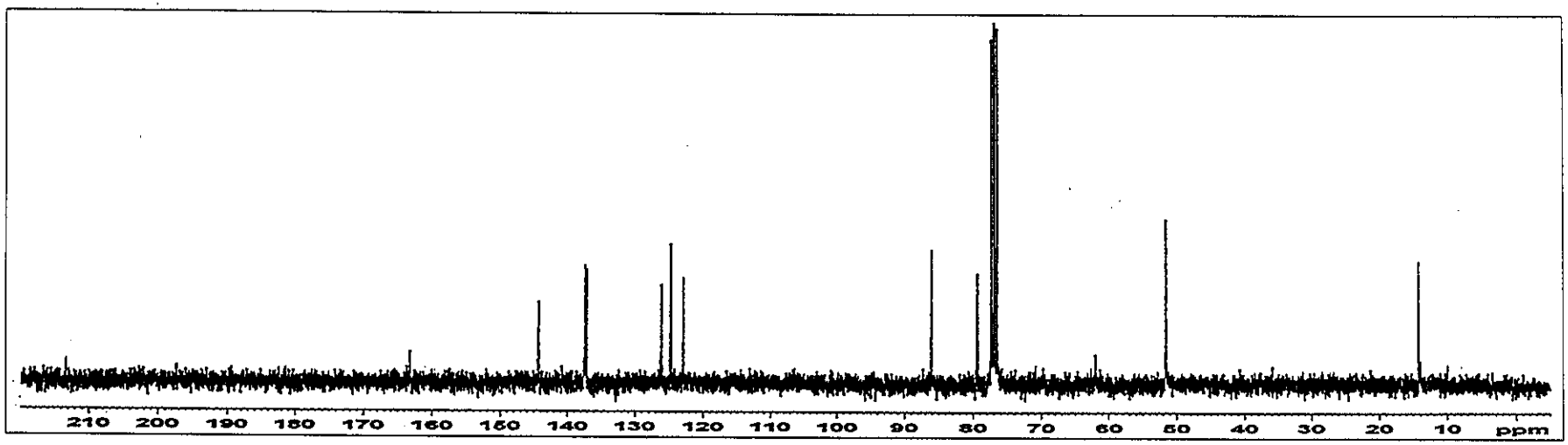

Figure 143 The 75 MHz ^{13}C NMR spectrum of compound **K60** in $CDCl_3$

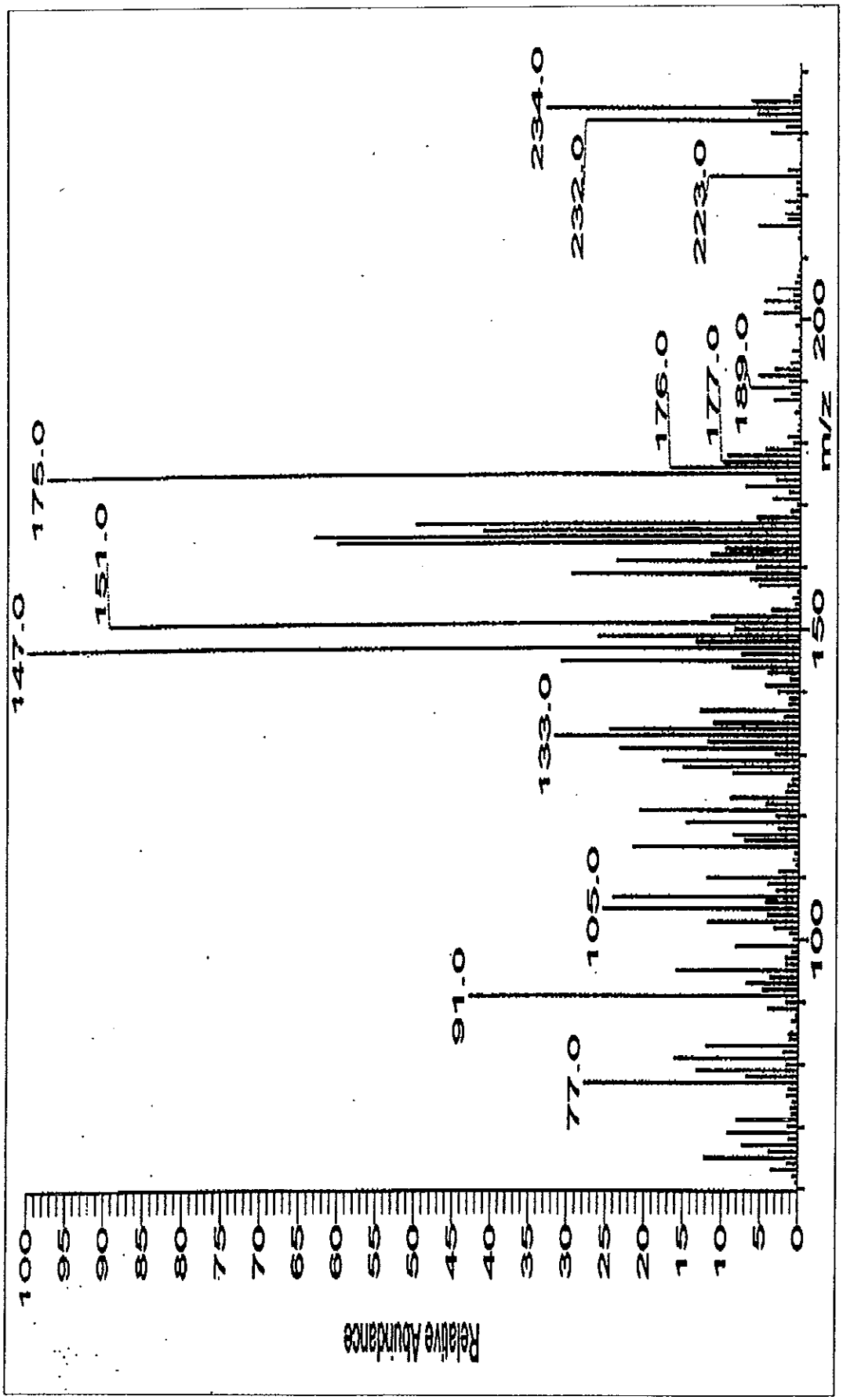

Figure 144 The mass spectrum of compound K61

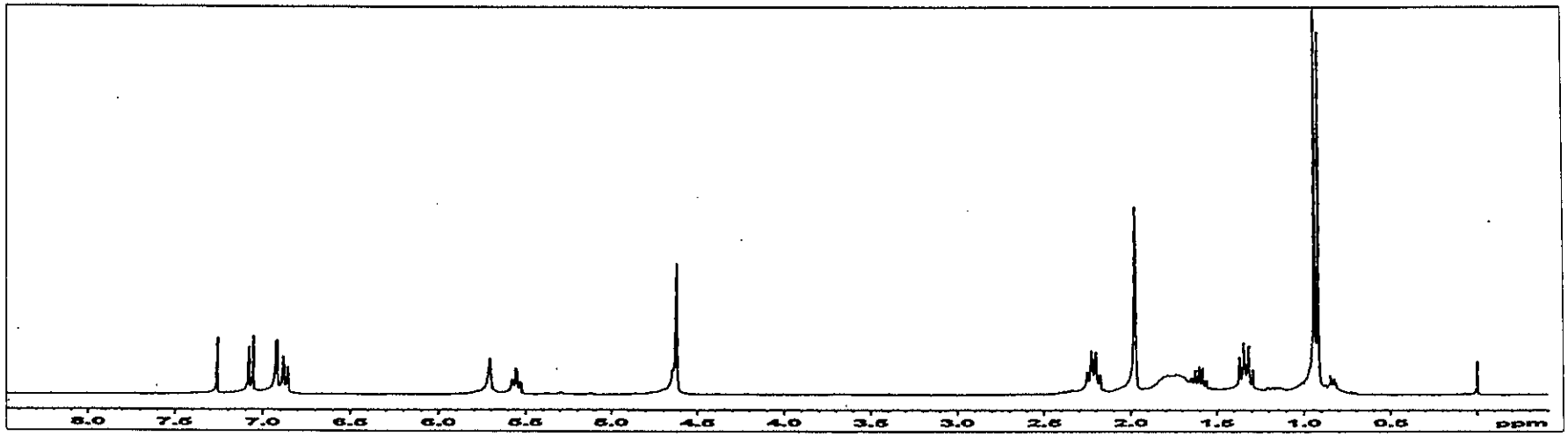

Figure 145 The 300 MHz ^{1}H NMR spectrum of compound **K61** in $CDCl_3$

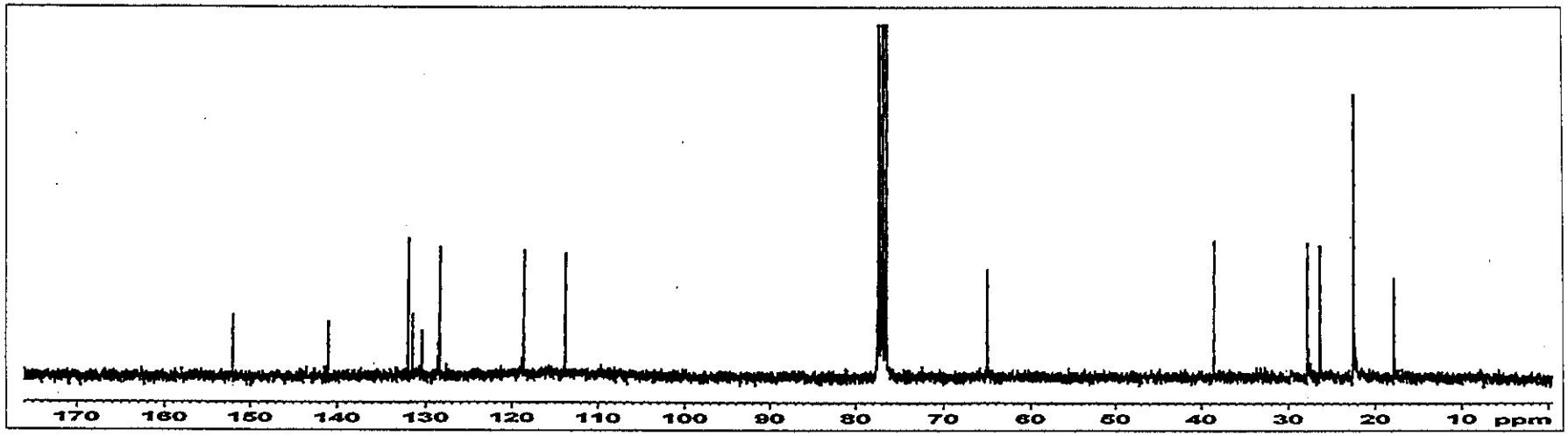

Figure 146 The 75 MHz ^{13}C NMR spectrum of compound **K61** in $CDCl_3$

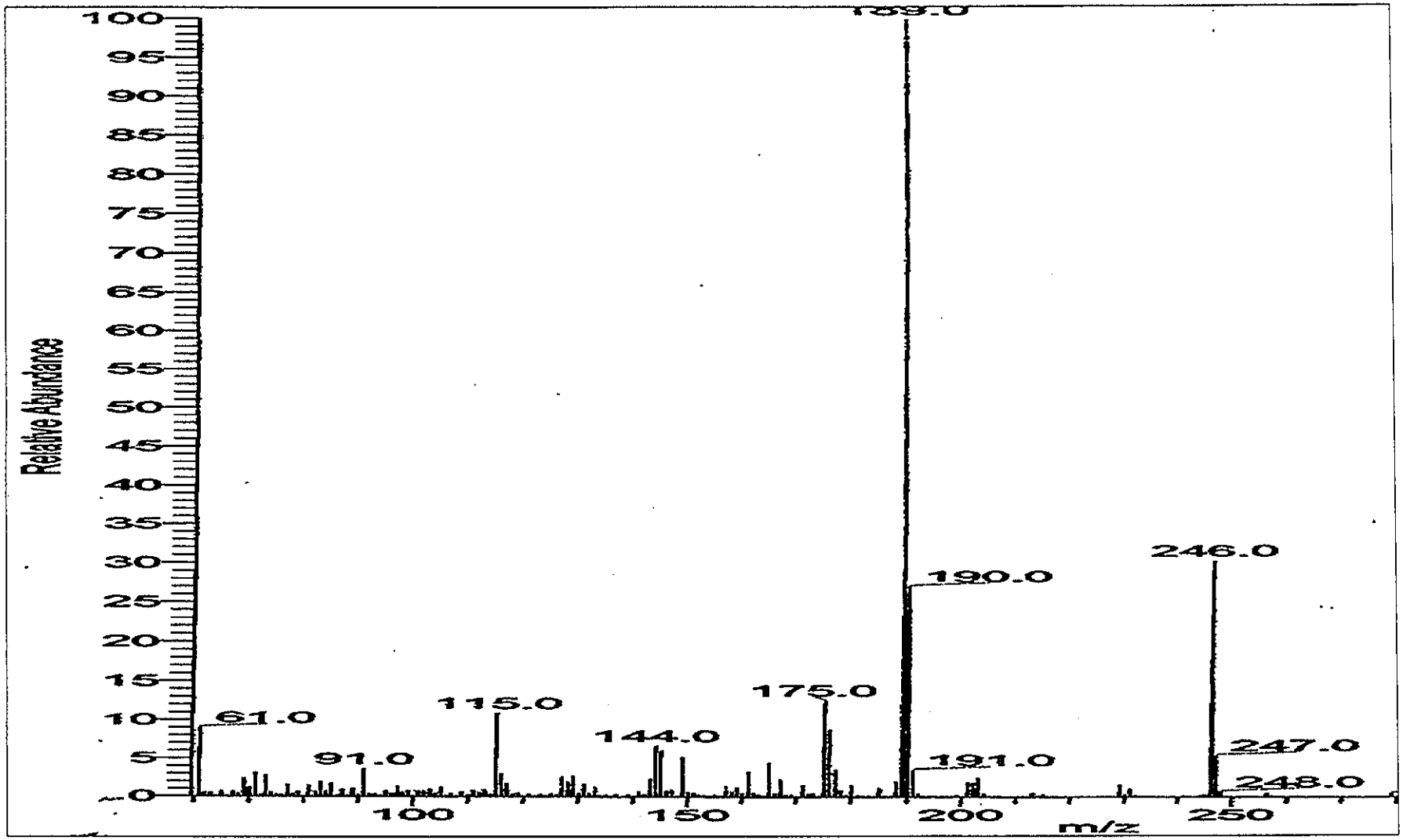

Figure 147 The mass spectrum of compound K62

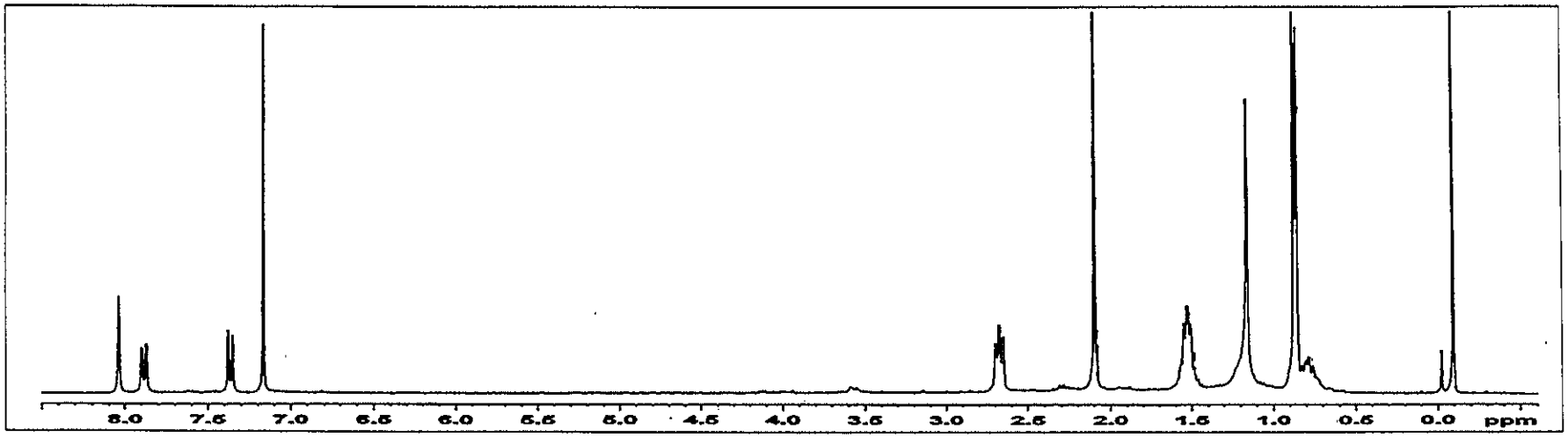

Figure 148 The 300 MHz ^{1}H NMR spectrum of compound **K62** in $CDCl_3$

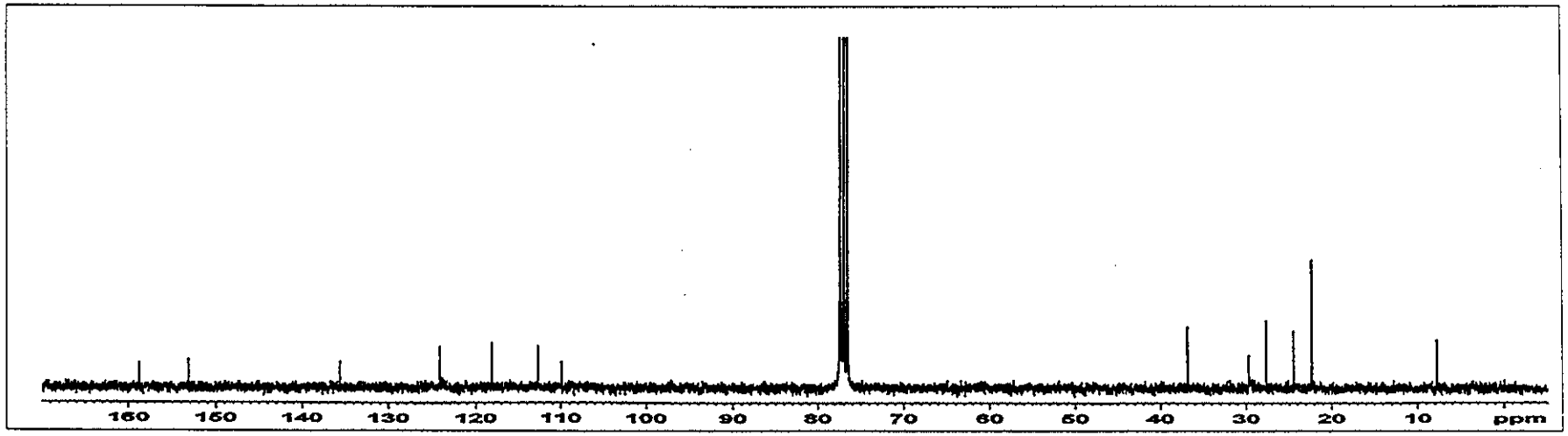

Figure 149 The 75 MHz ^{13}C NMR spectrum of compound **K62** in $CDCl_3$

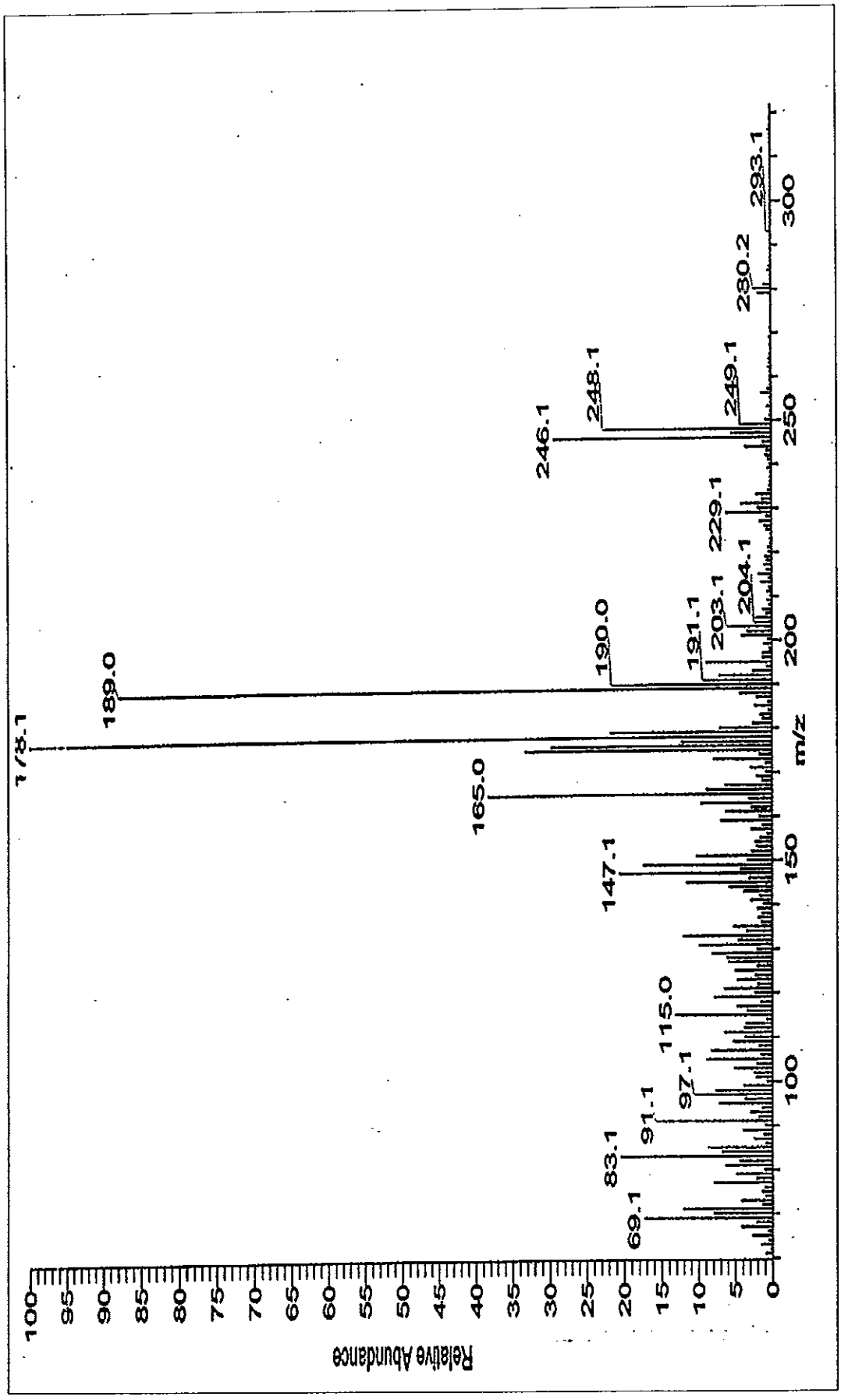

Figure 150 The mass spectrum of compound K63

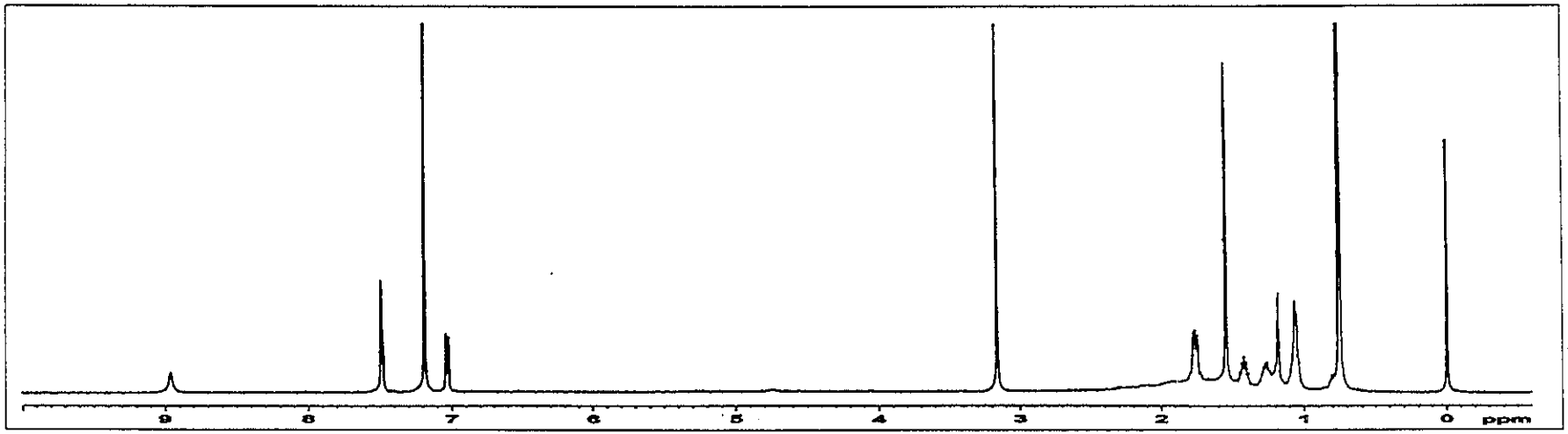

Figure 151 The 500 MHz ^{1}H NMR spectrum of compound **K63** in $CDCl_3$

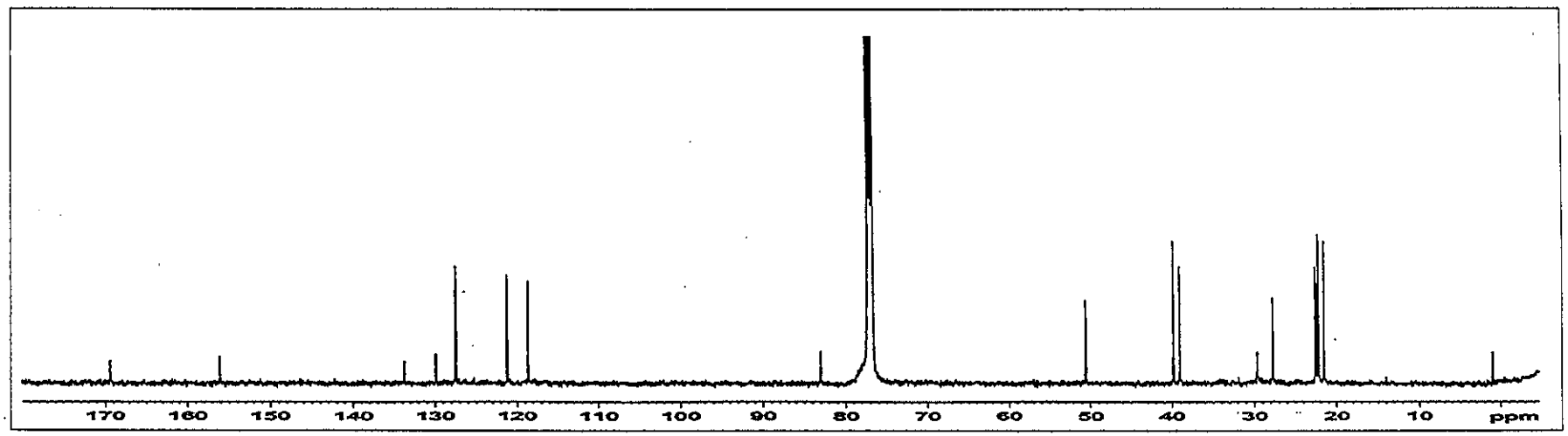

Figure 152 The 125 MHz ^{13}C NMR spectrum of compound **K63** in $CDCl_3$

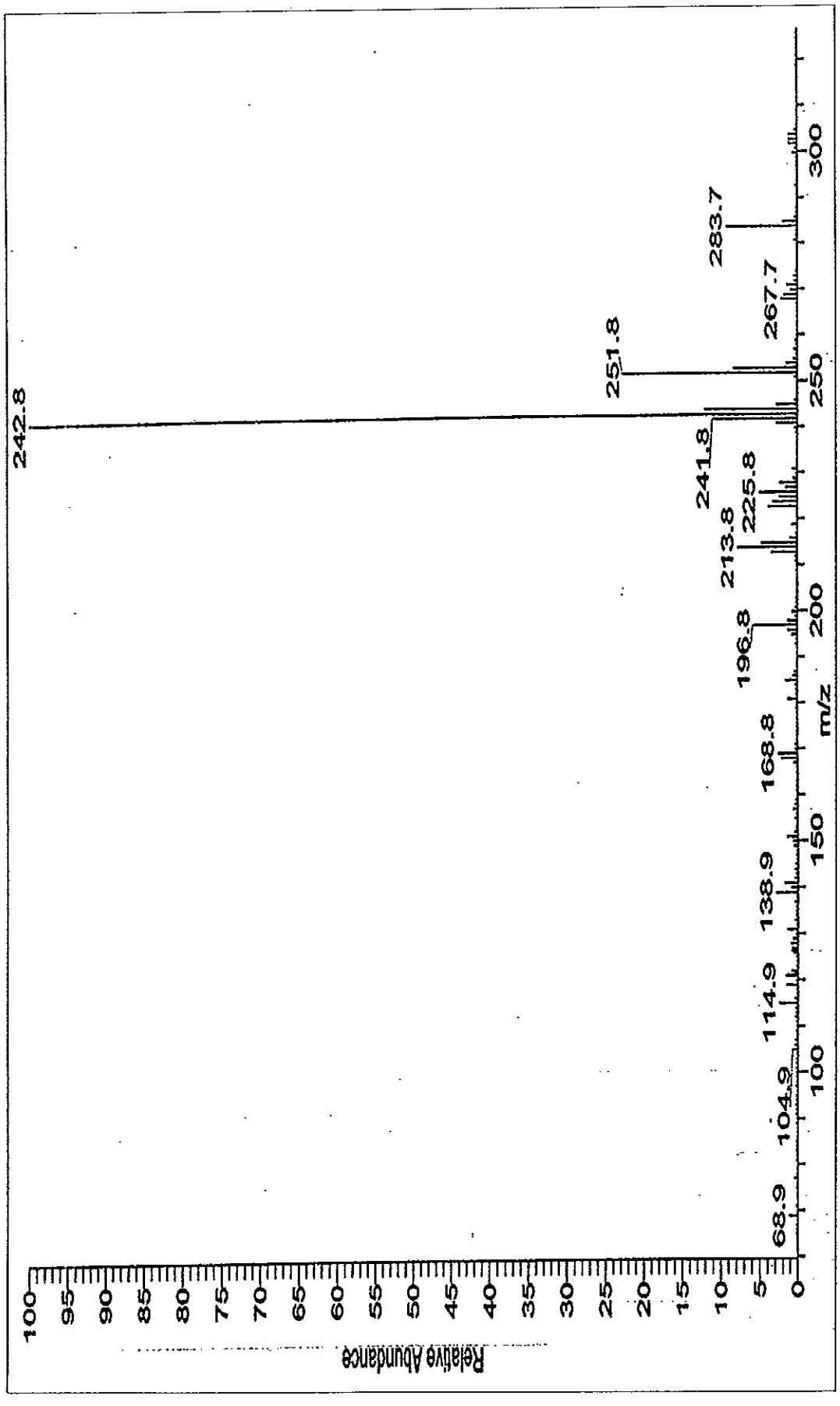

Figure 153 The mass spectrum of compound K64

Figure 154 The 500 MHz ^{1}H NMR spectrum of compound **K64** in $CDCl_3$

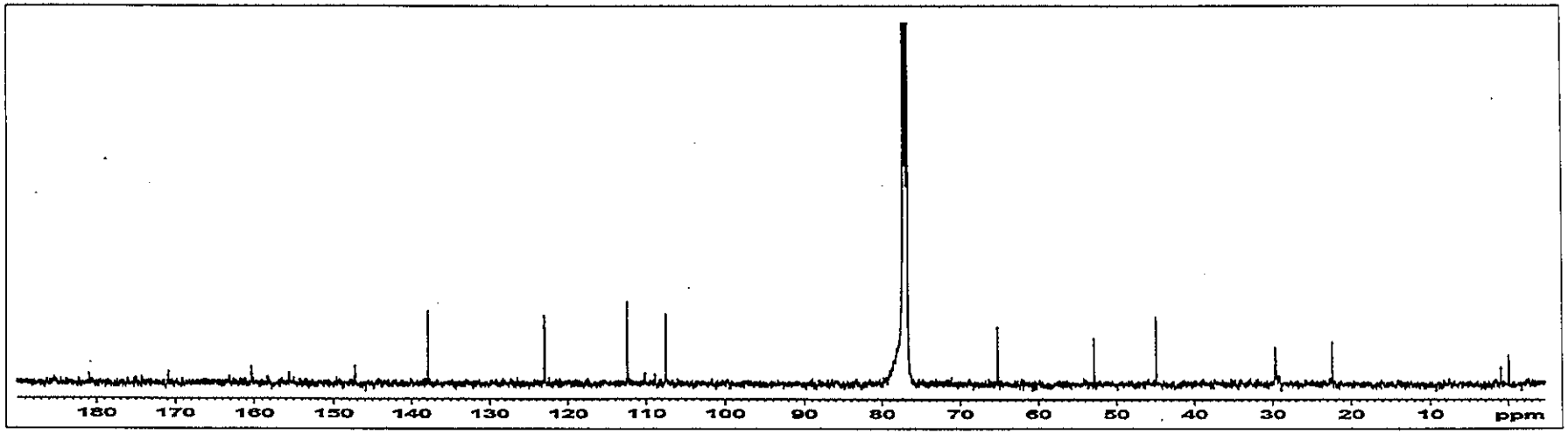

Figure 155 The 125 MHz ^{13}C NMR spectrum of compound **K64** in $CDCl_3$

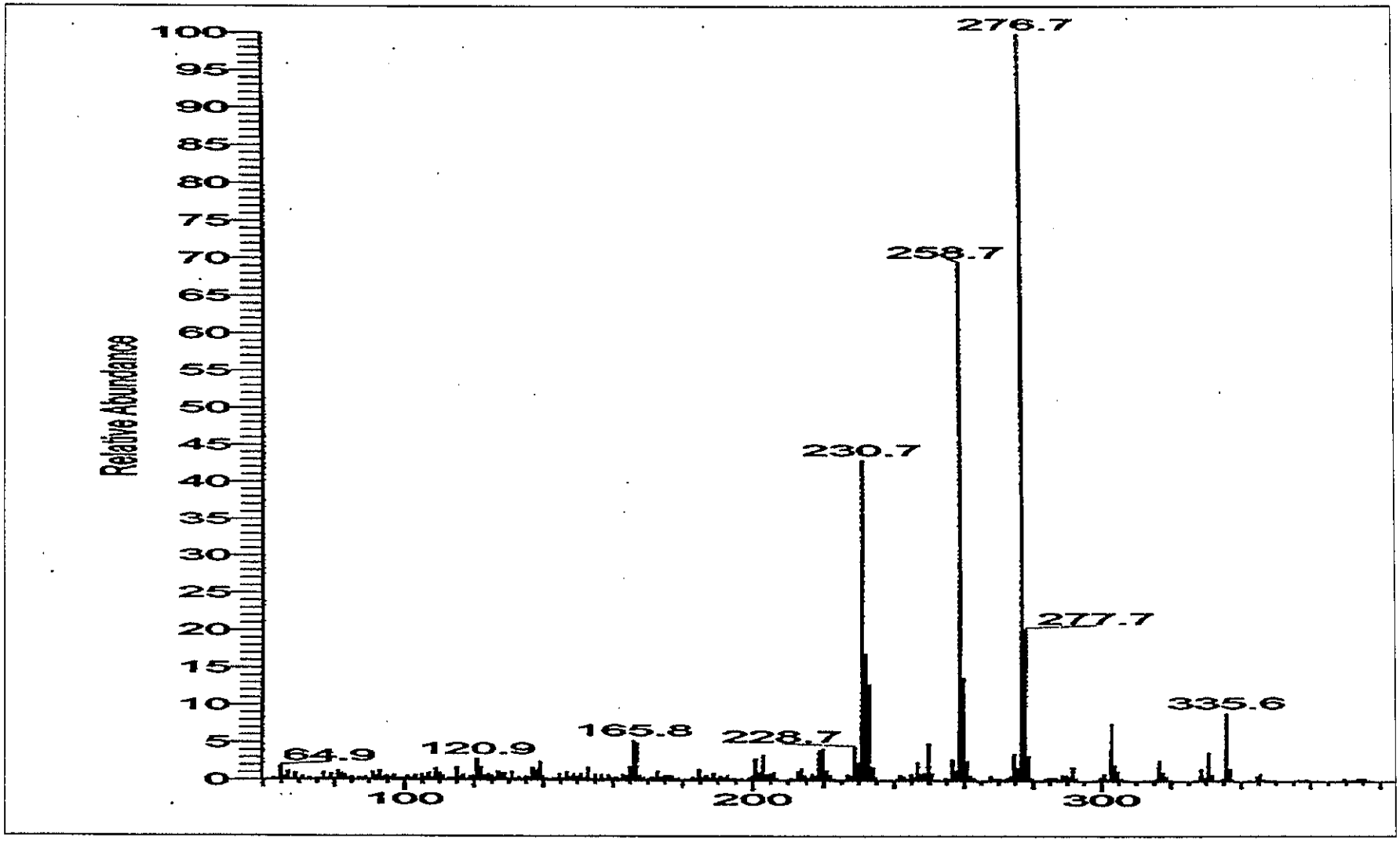

Figure 156 The mass spectrum of compound K65

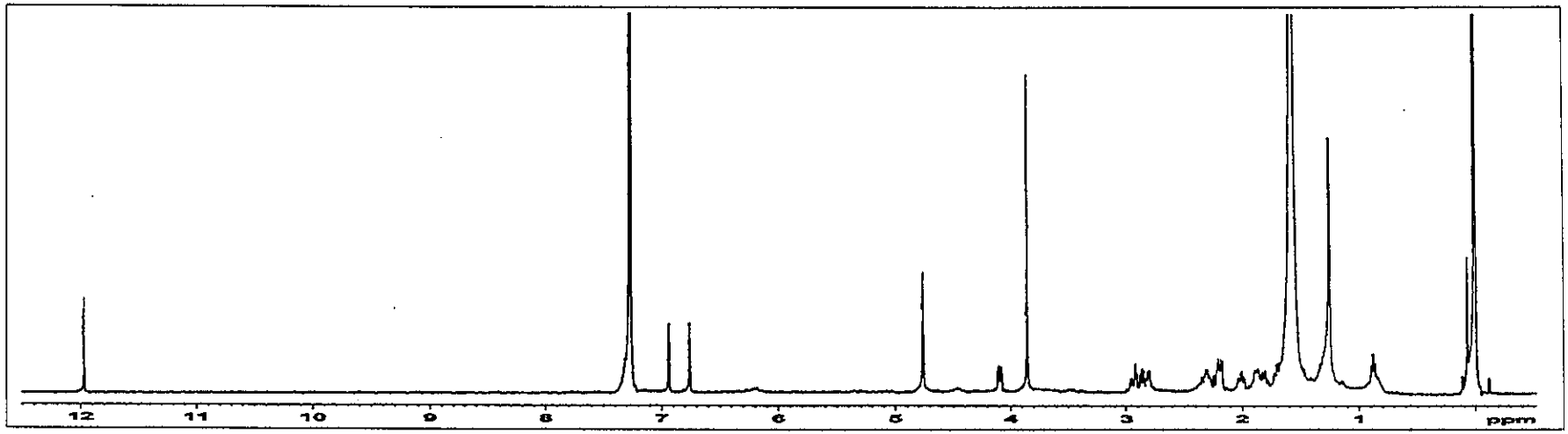

Figure 157 The 500 MHz ^{1}H NMR spectrum of compound **K65** in $CDCl_3$

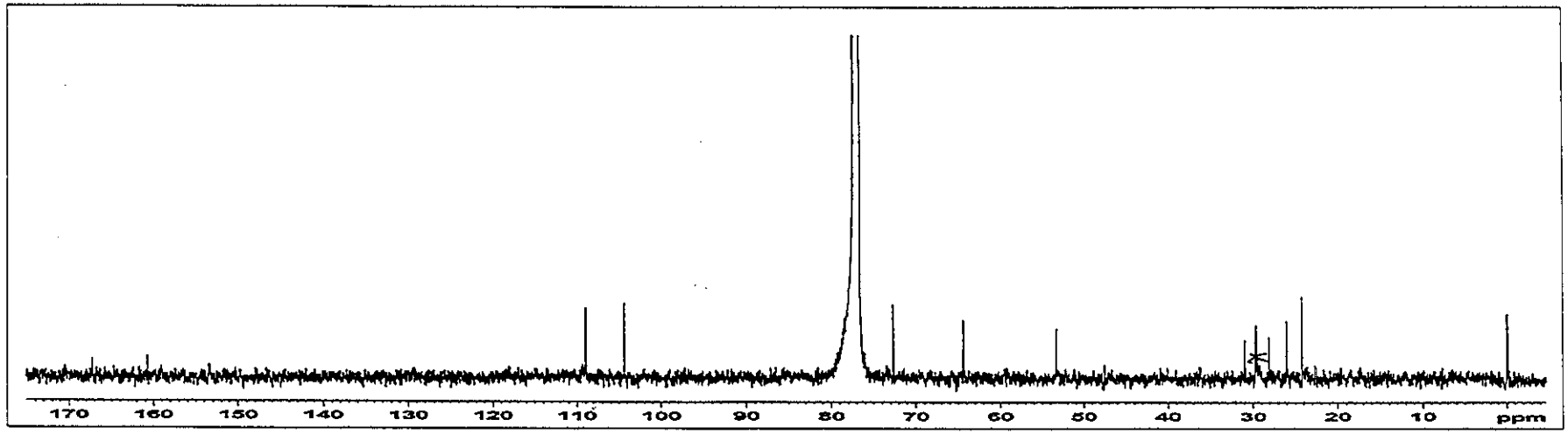

Figure 158 The 125 MHz ^{13}C NMR spectrum of compound **K65** in $CDCl_3$

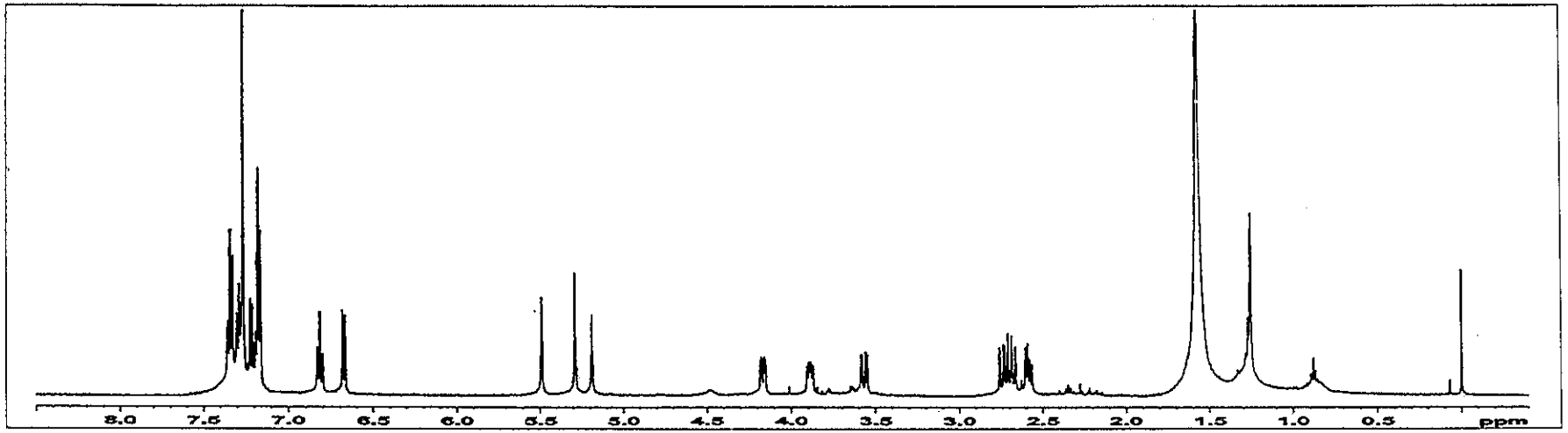

Figure 159 The 500 MHz ^{1}H NMR spectrum of compound **K66** in $CDCl_3$

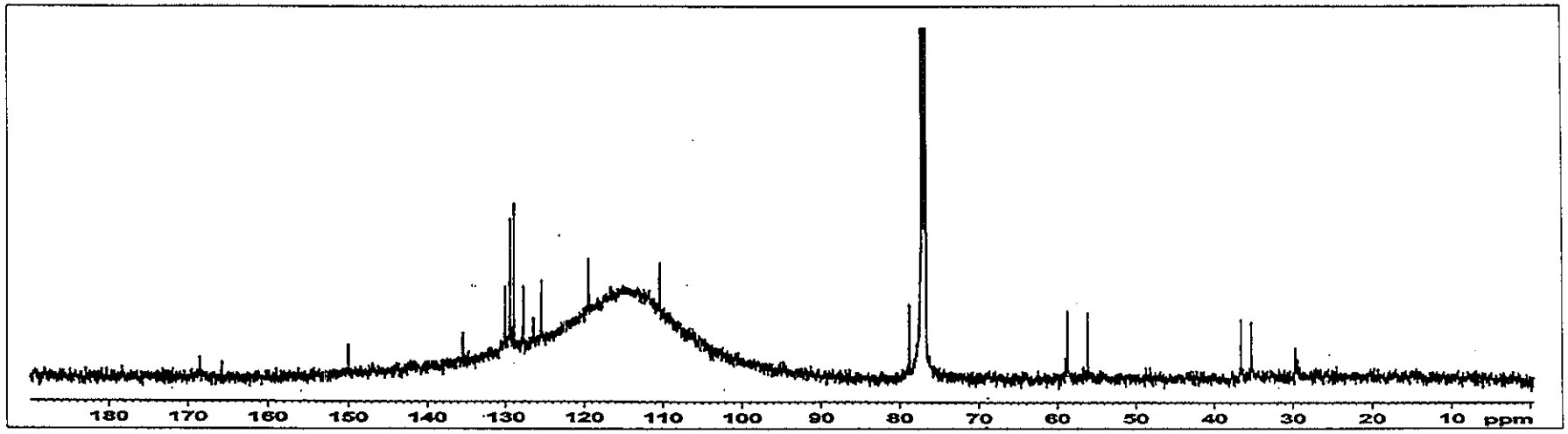

Figure 160 The 125 MHz ^{13}C NMR spectrum of compound **K66** in $CDCl_3$

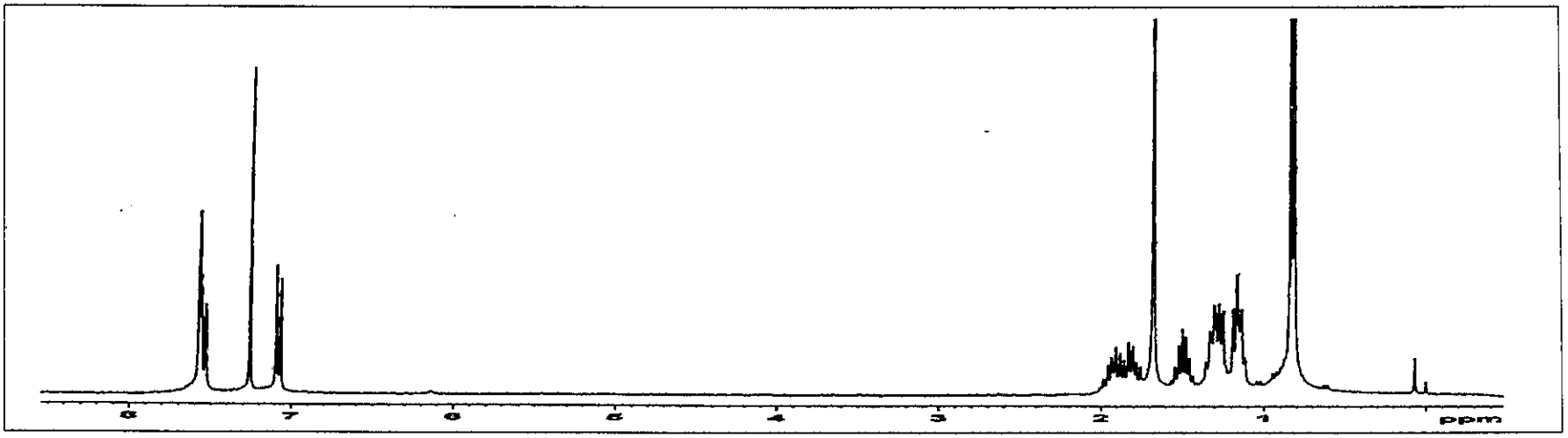

Figure 161 The 300 MHz ^{1}H NMR spectrum of compound **K67** in $CDCl_3$

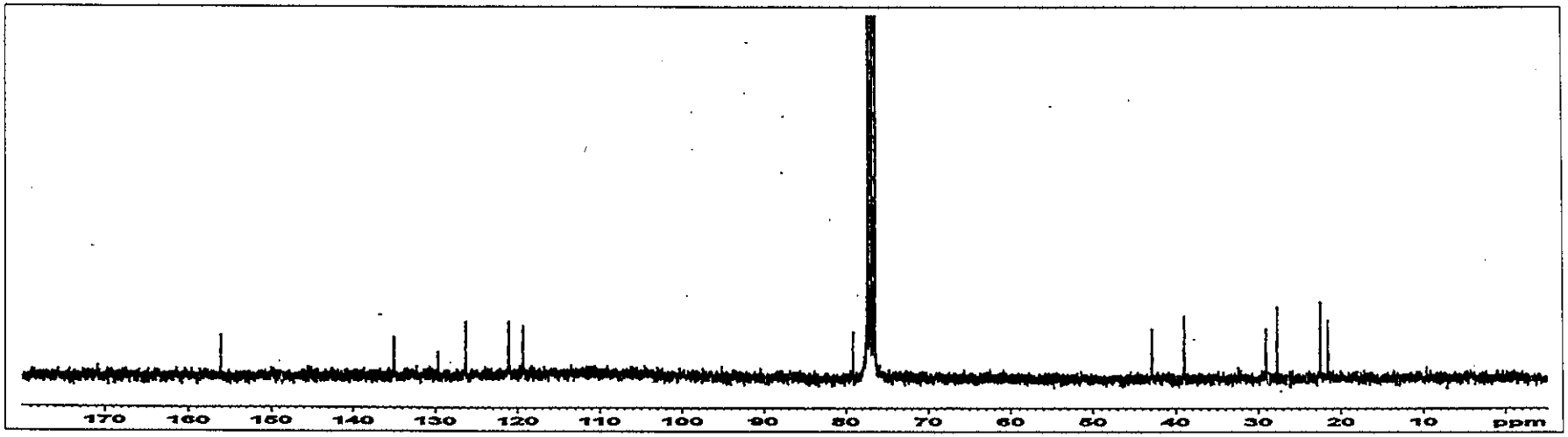

Figure 162 The 75 MHz ^{13}C NMR spectrum of compound **K67** in $CDCl_3$

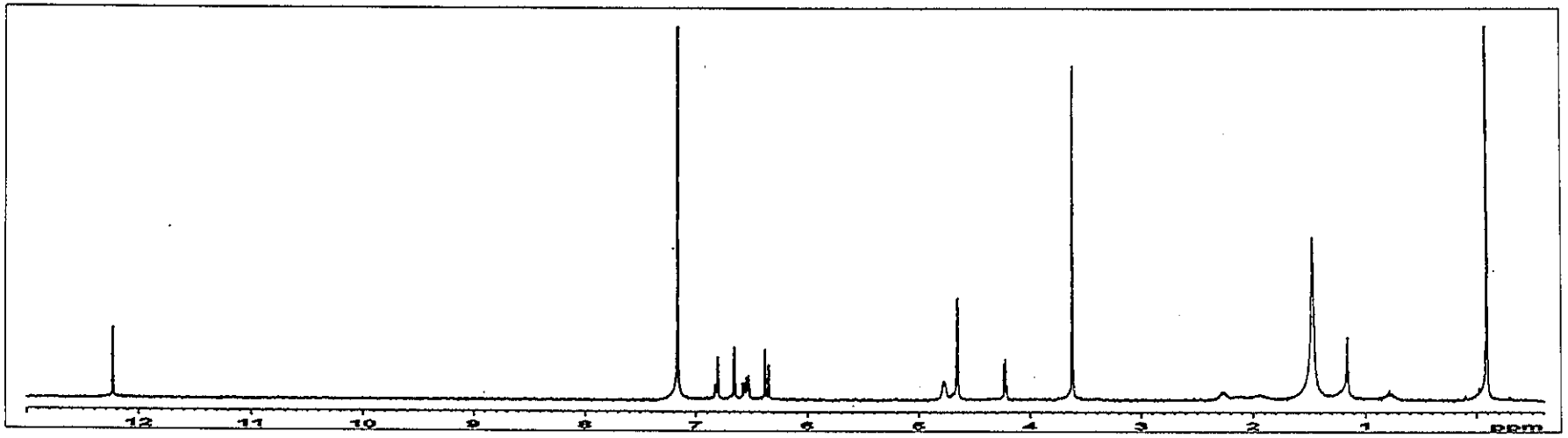

Figure 163 The 300 MHz ^{1}H NMR spectrum of compound **K68** in $CDCl_3$

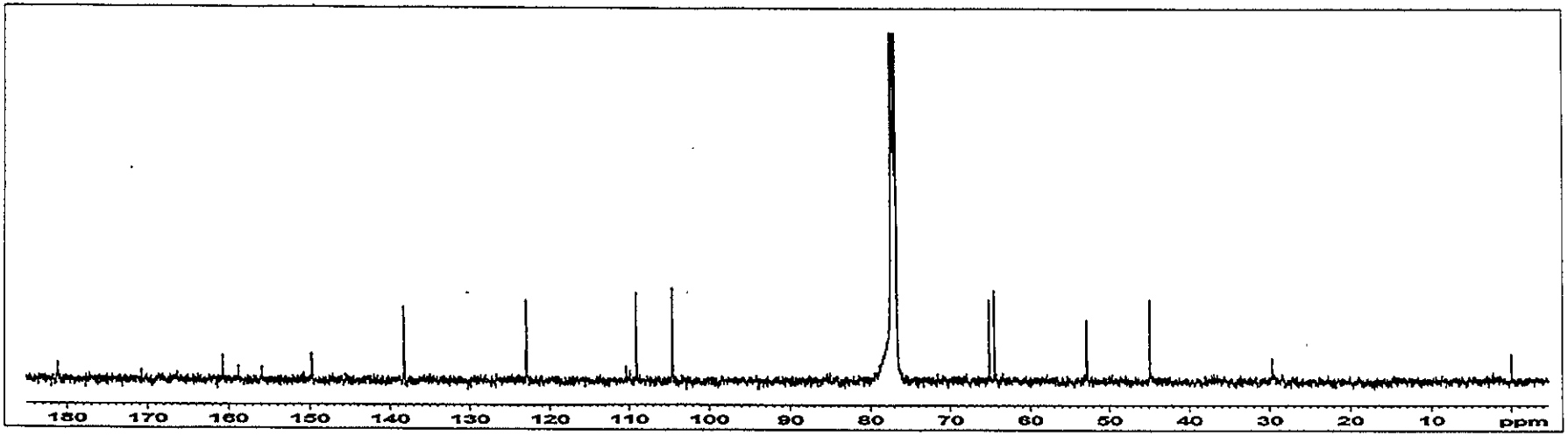

Figure 164 The 125 MHz ^{13}C NMR spectrum of compound **K68** in $CDCl_3$

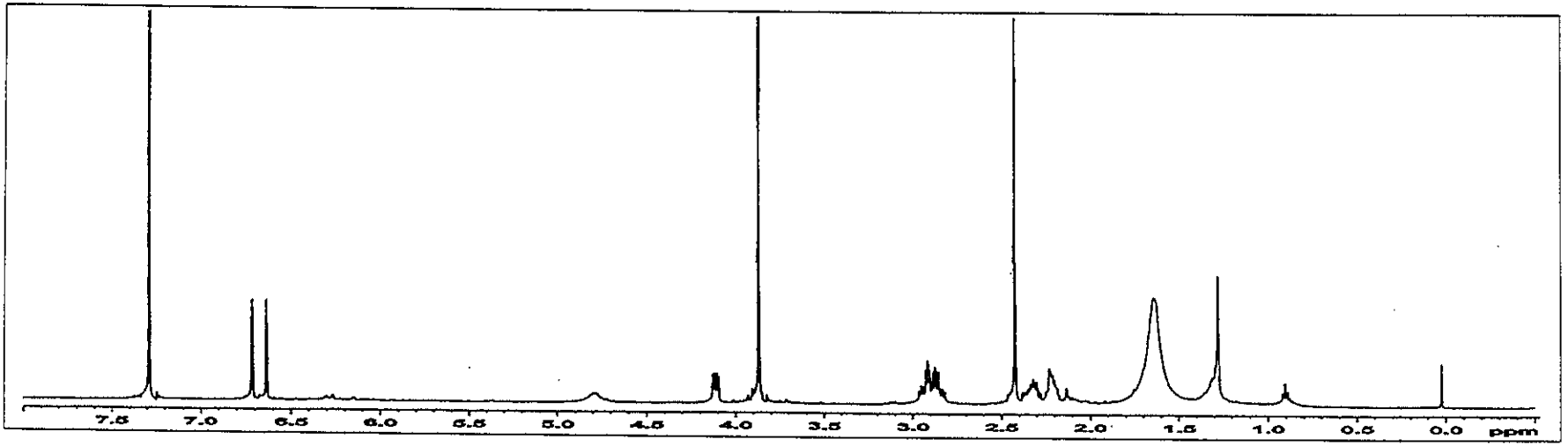

Figure 165 The 500 MHz ^{1}H NMR spectrum of compound **K69** in $CDCl_3$

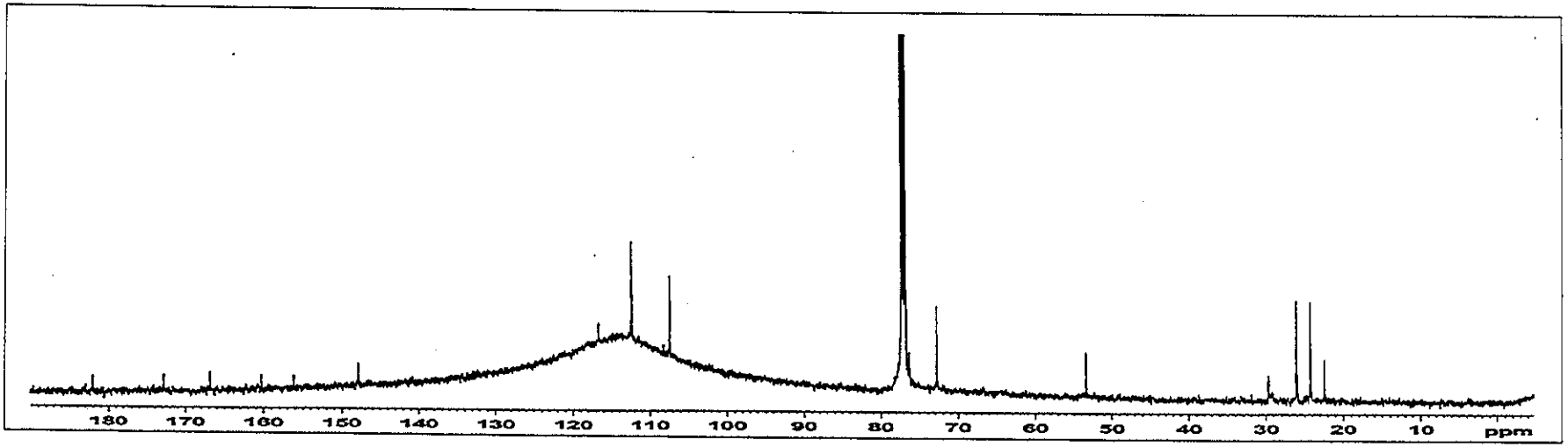

Figure 166 The 125 MHz ^{13}C NMR spectrum of compound **K69** in $CDCl_3$

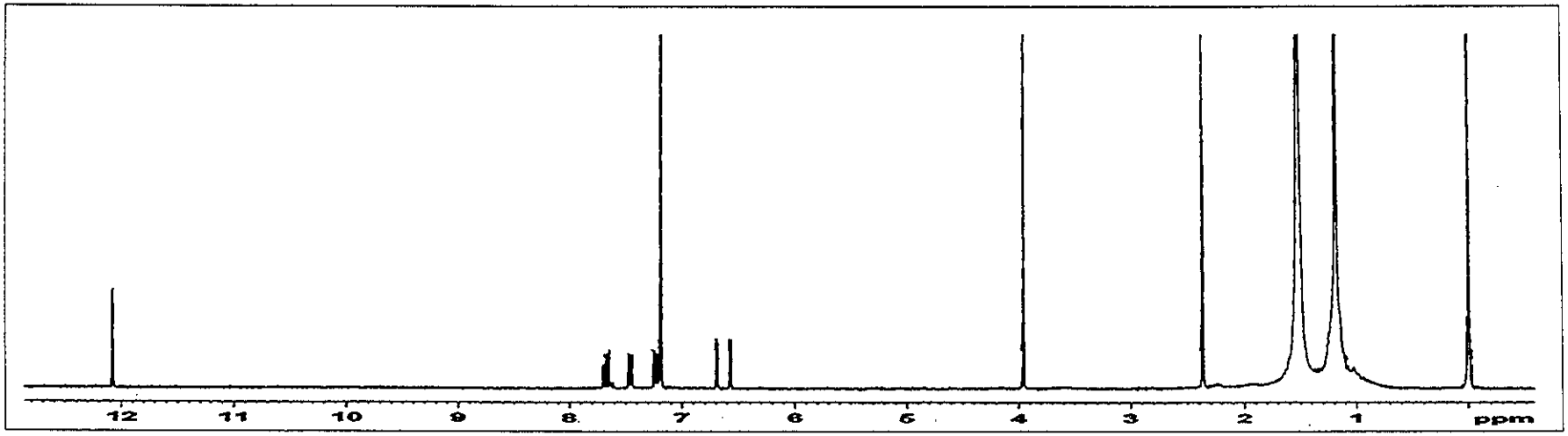

Figure 167 The 300 MHz ^{1}H NMR spectrum of compound **K70** in $CDCl_3$

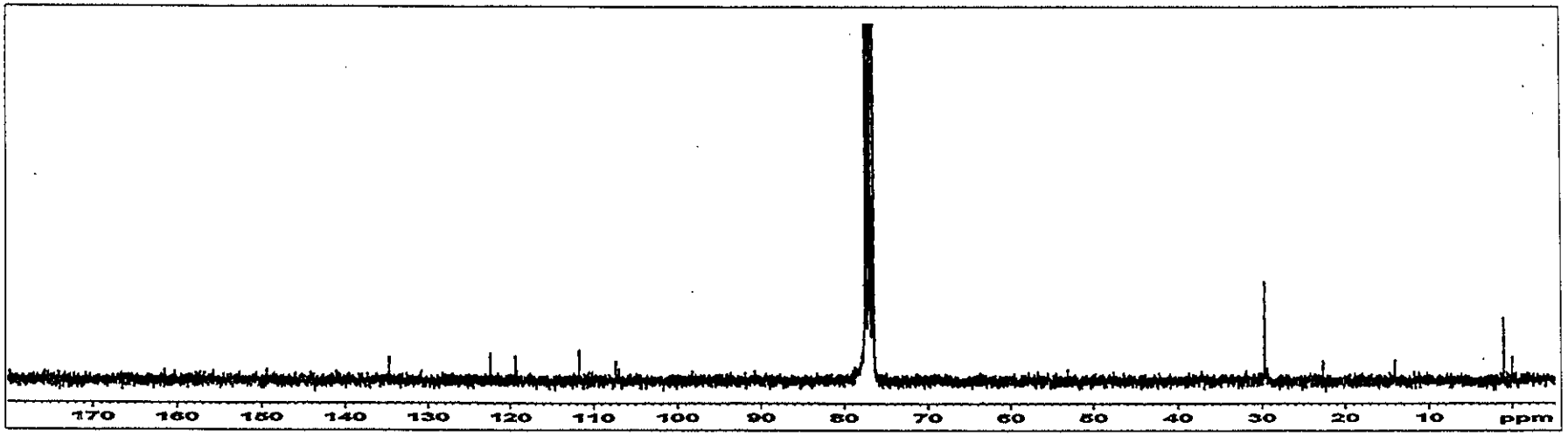

Figure 168 The 75 MHz ^{13}C NMR spectrum of compound **K70** in $CDCl_3$

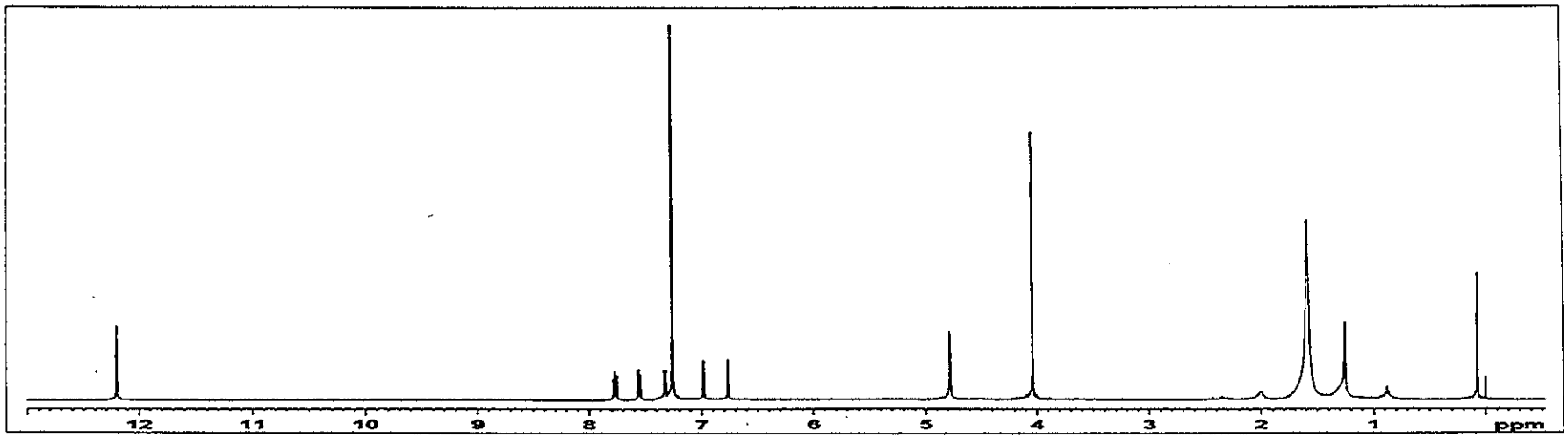

Figure 169 The 500 MHz ^{1}H NMR spectrum of compound **K71** in $CDCl_3$

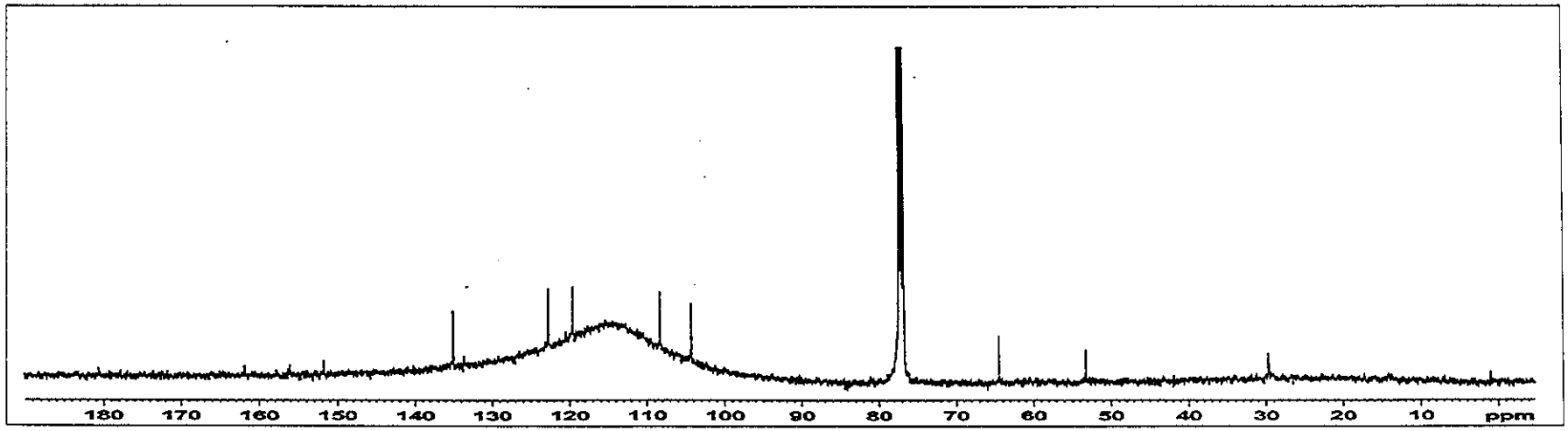

Figure 170 The 125 MHz ^{13}C NMR spectrum of compound **K71** in $CDCl_3$

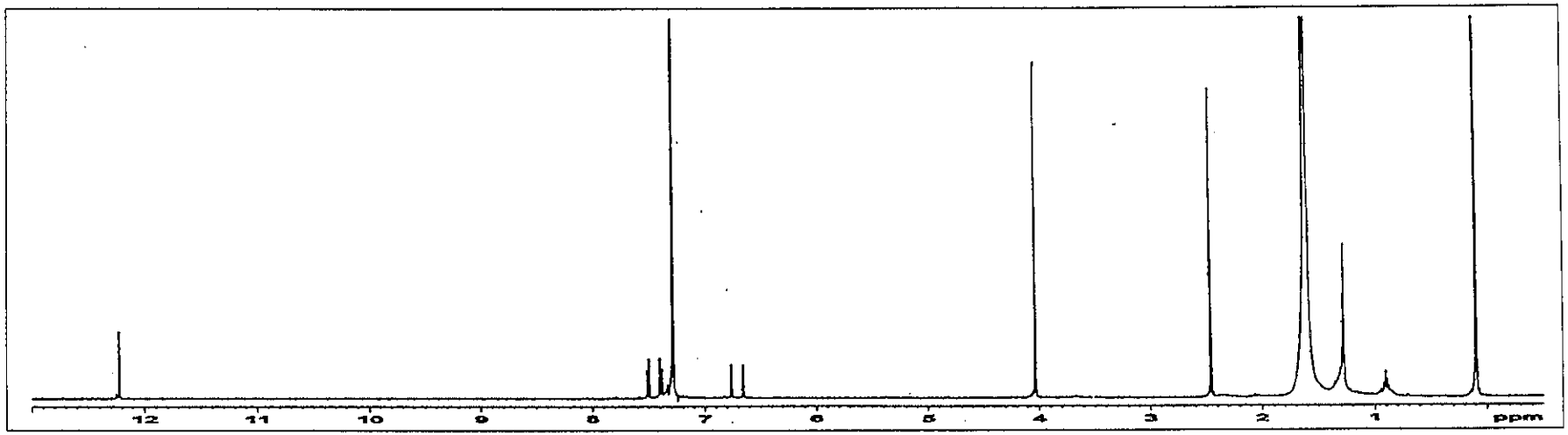

Figure 171 The 500 MHz ^{1}H NMR spectrum of compound **K72** in $CDCl_3$

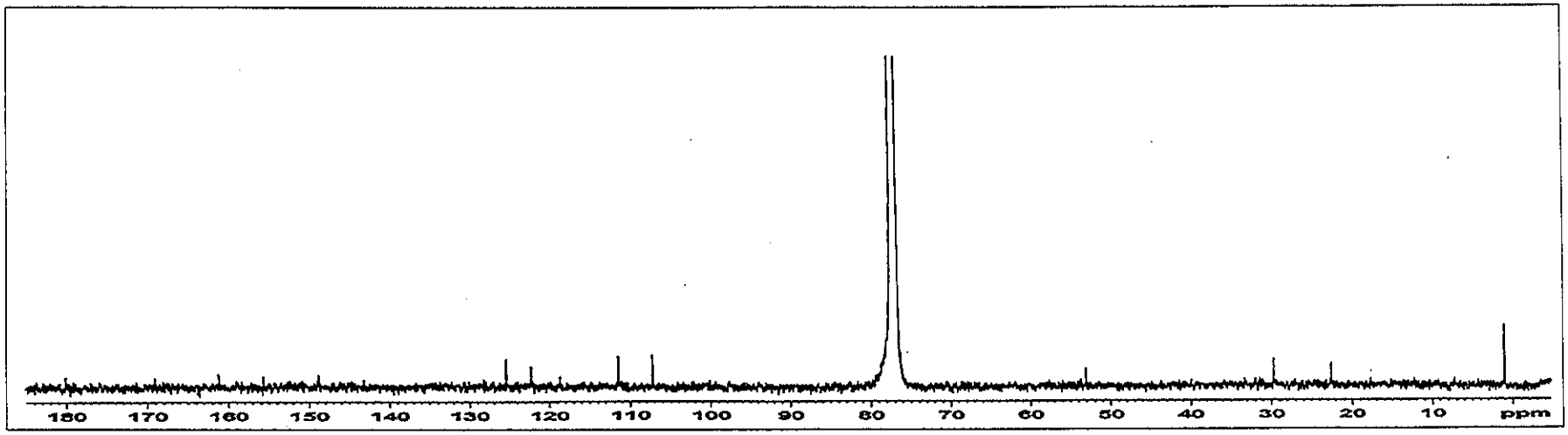

Figure 172 The 125 MHz ^{13}C NMR spectrum of compound **K72** in $CDCl_3$

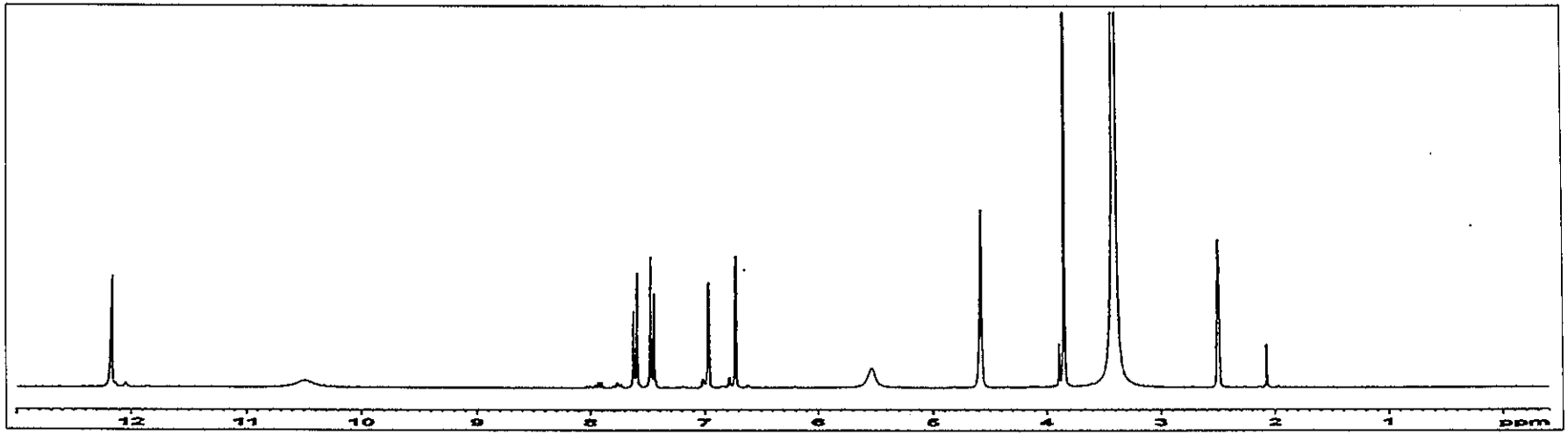

Figure 173 The 300 MHz ^{1}H NMR spectrum of compound **K73** in DMSO-d_6

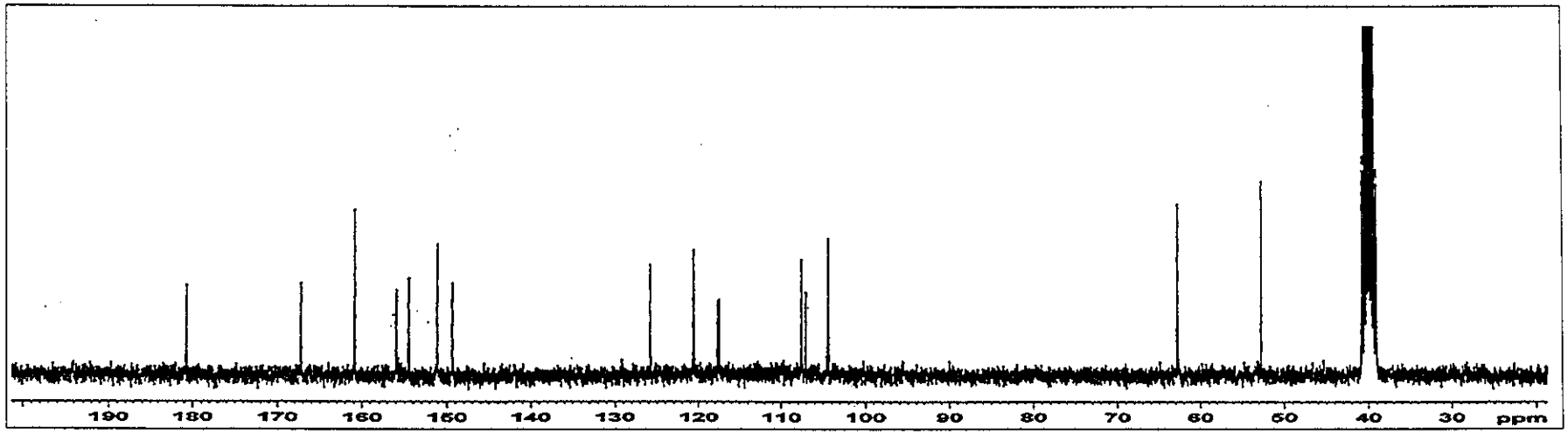

Figure 174 The 75 MHz ^{13}C NMR spectrum of compound **K73** in DMSO-d_6

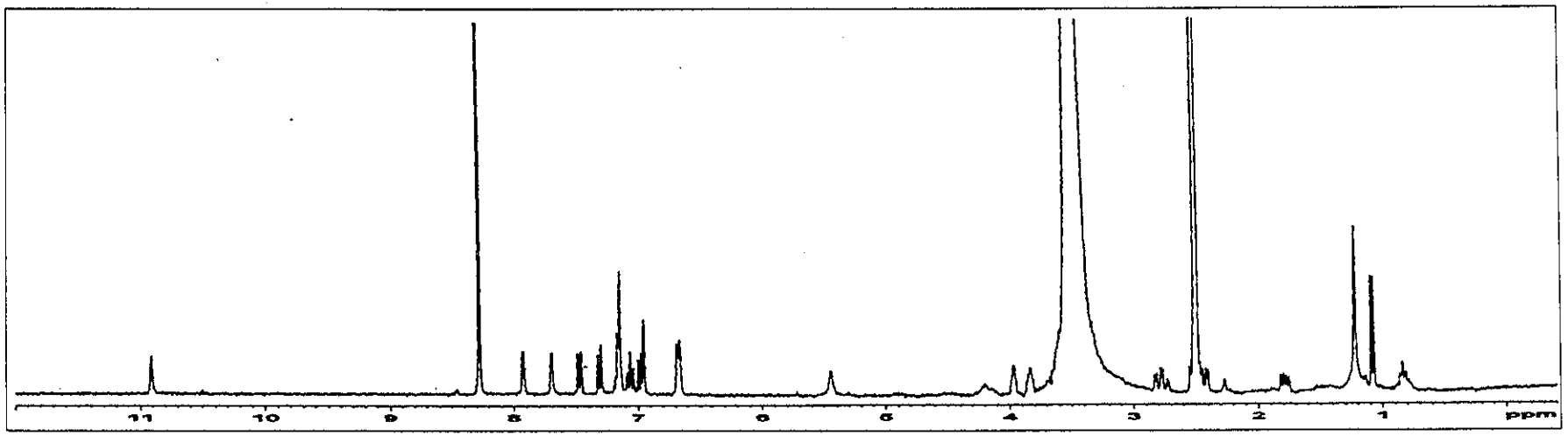

Figure 175 The 300 MHz ^{1}H NMR spectrum of compound **K74** in DMSO-d_6

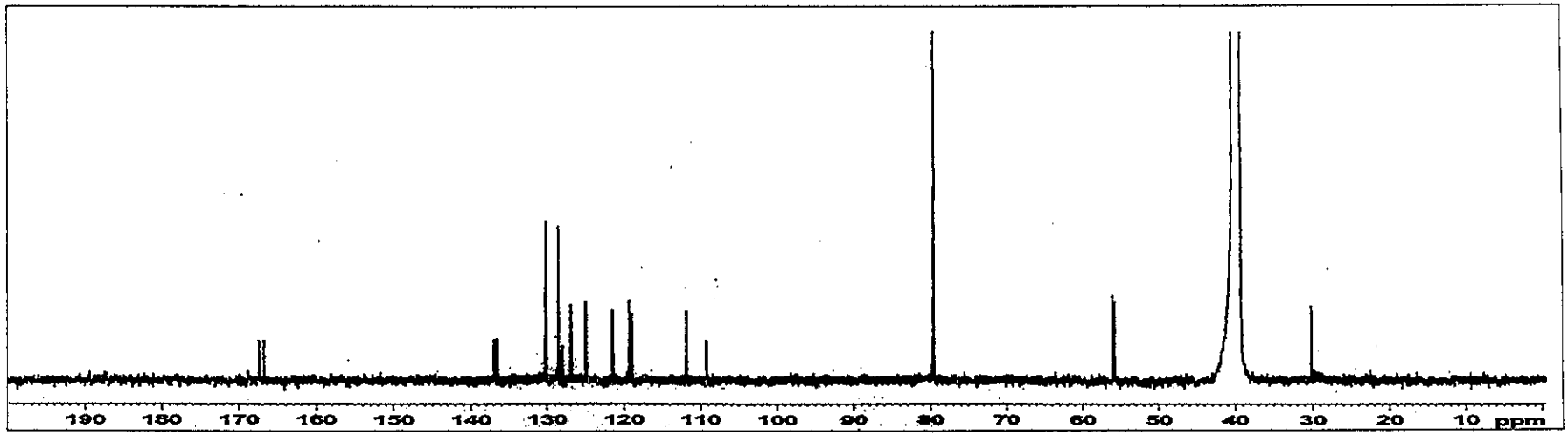

Figure 176 The 75 MHz ^{13}C NMR spectrum of compound **K74** in DMSO-d_6

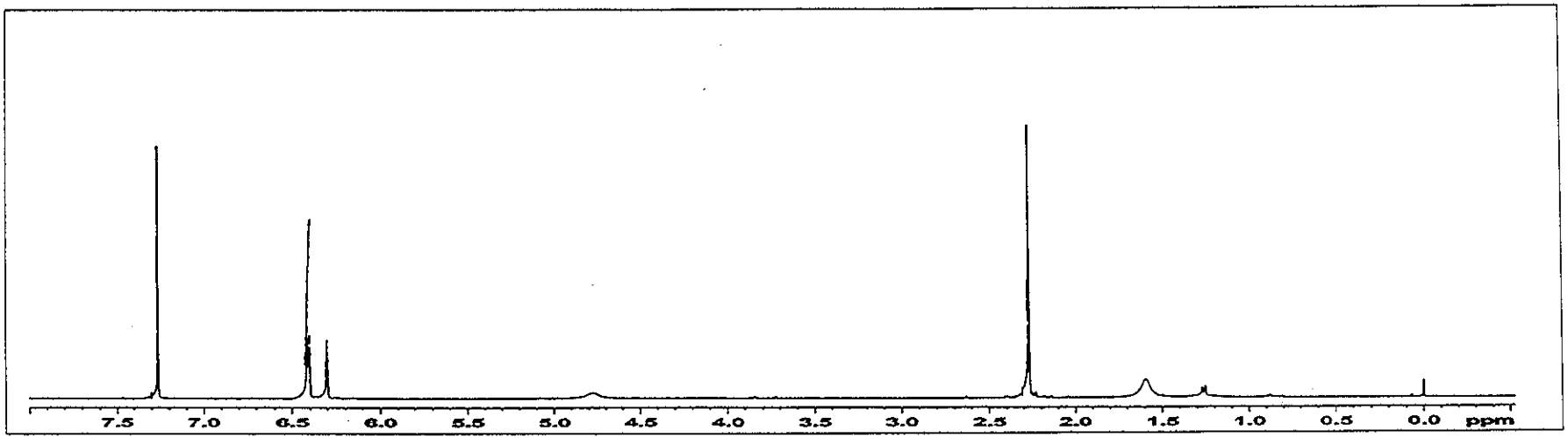

Figure 177 The 500 MHz ^{1}H NMR spectrum of compound **K75** in $CDCl_3$

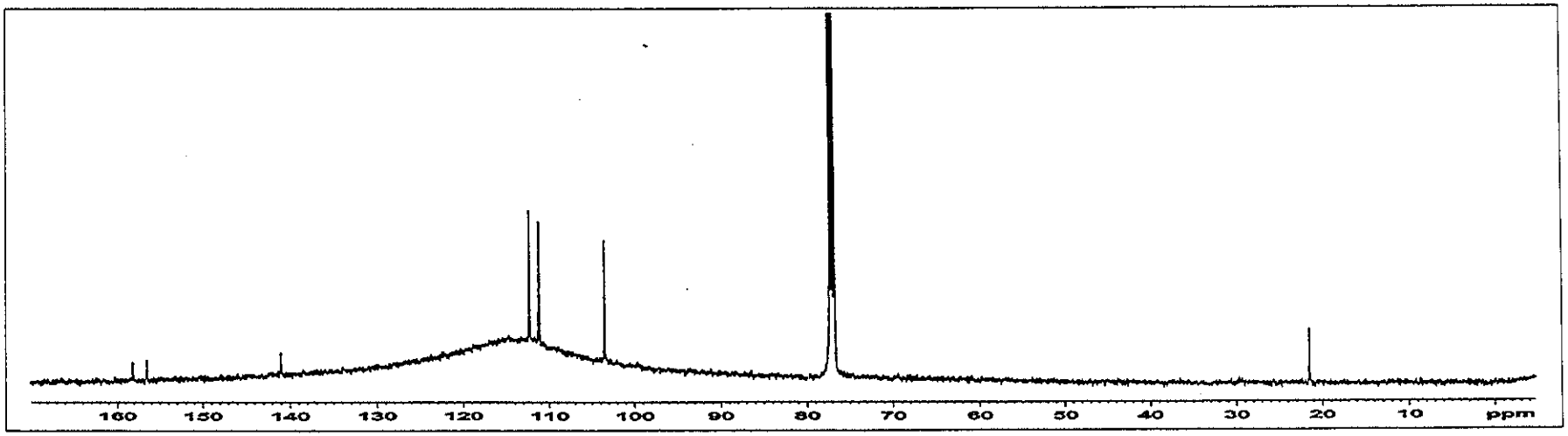

Figure 178 The 125 MHz ^{13}C NMR spectrum of compound **K75** in $CDCl_3$

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Educational Attainment

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Scholarship Awards during Enrolment

1. The Center for Innovation in Chemistry, Commission on Higher Education, Ministry of Education.
2. The Royal Golden Jubilee Ph.D. Program of the Thailand Research Fund (Grant No. PHD/0109/255).

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1. Rukachaisirikul, V., Trisuwan, K., Sukpondma, Y. and Phongpaichit, S. 2008. A new benzoquinone derivative from the leaves of *Garcinia parvifolia*. Arch. Pharm. Res. 31, 17-20.
2. Trisuwan, K., Rukachaisirikul, V., Sukpondma, Y., Preedanon, S., Phongpaichit, S., Rungjindamai, N. and Sakayaroj, J. 2008. Epoxydons and a pyrone from the marine-derived fungus *Nigrospora* sp. PSU-F5. J. Nat. Prod. 71, 1323-1326.
3. Trisuwan, K., Rukachaisirikul, V., Sukpondma, Y., Preedanon, S., Phongpaichit, S. and Sakayaroj, J. 2009. Pyrone derivatives from the marine-derived fungus *Nigrospora* sp. PSU-F18. Phytochemistry 70, 554-557.

4. Trisuwan, K., Rukachaisirikul, V., Sukpondma, Y., Phongpaichit, S., Preedanon, S. and Sakayaroj, J. 2009. Lactone derivatives from the marine-derived fungus *Penicillium* sp. PSU-F44. Chem. Pharm. Bull. 57, 1100-1102.
5. Trisuwan, K., Rukachaisirikul, V., Sukpondma, Y., Phongpaichit, S., Preedanon, S. and Sakayaroj, J. 2010. Furo[3,2-h]isochroman, furo[3,2-h]isoquinoline, isochroman, phenol, pyranone and pyrone derivatives from the sea fan-derived fungus *Penicillium* sp. PSU-F40. Tetrahedron, in press.

Proceedings

1. Trisuwan, K., Rukachaisirikul, V., Phongpaichit, S. and Preedanon, S. 2006. Chemical constituents from the unidentified fungus PSU-SF5. Proceeding of the 32nd Congress on Science and Technology of Thailand. Queen Sirikit National Convention Center, October 10-12, 2006. pp. 178.
2. Trisuwan, K., Rukachaisirikul, V., Phongpaichit, S., Preedanon, S. and Sakayaroj, J. 2007. Chemical constituents from the Sordariomycetes fungus PSU-SF5. Proceeding of the international Congress for Innovation in Chemistry (PERCH-CIC Congress V). Jomtien Palm Beach Resort Pattaya, Chonburi, May 6-9, 2007. pp. 80.
3. Trisuwan, K., Rukachaisirikul, V., Phongpaichit, S. and Preedanon, S. 2007. Chemical constituents from the unidentified marine-derived fungus PSU-F18. Proceeding of the 33rd Congress on Science and Technology of Thailand. Walailak University, October 18-20, 2007. pp. 171.
4. Trisuwan, K., Rukachaisirikul, V., Phongpaichit, S. and Preedanon, S. 2008. Fungal metabolites from the marine-derived fungus *Nigrospora* sp. PSU-F18. Proceeding of the 6th Regional IMT-GT Uninet Conference 2008. Penang, Malaysia, August 28-30, 2008. pp. 256.

5. Trisuwan, K., Rukachaisirikul, V., Phongpaichit, S. and Preedanon, S. 2008. Metabolites from the marine-derived fungus *Penicillium* sp. PSU-F44. Proceeding of the 34th Congress on Science and Technology of Thailand. Queen Sirikit National Convention Center, October 31- November 2, 2008. pp. 152.
6. Trisuwan, K., Rukachaisirikul, V., Phongpaichit, S., Preedanon, S. and Sakayaroj, J. 2009. Metabolites from the marine-derived fungus *Penicillium* sp. PSU-F40. Proceeding of the international Congress for Innovation in Chemistry (PERCH-CIC Congress VI). Jomtien Palm Beach Resort Pattaya, Chonburi, May 3-6, 2009. pp. 243.
7. Trisuwan, K., Rukachaisirikul, V., Phongpaichit, S., Preedanon, S. and Sakayaroj, J. 2010. Fungal metabolites from the marine-derived fungi *Nigrospora* sp. PSU-F5 and PSU-F18. Proceeding of the RGJ-Ph.D Congress XI. Jomtien Palm Beach Resort Pattaya, Chonburi, April 1-3, 2010. pp. 149.