Metabolites from the Marine-derived Fungi *Pseudopestalotiopsis* sp. PSU-AMF45 and *Trichoderma longibrachiatum* PSU-AMF274

Haryadi Nugraha Putra

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry (International Program)

Prince of Songkla University

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Author	Mr. Haryadi Nugraha Putra
Major Program	Chemistry (International Program)

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(Prof. Dr. Vatcharin Rukachaisirikul)

……..……..Committee
(Assoc. Prof. Dr. Kanda Panthong)

……..……..Committee
(Asst. Prof. Dr. Yaowapa Sukpondma)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Chemistry (International Program)

…..…….…….
(Prof. Dr. Damrongsak Faroongsarng)
Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

…….Signature
(Prof. Dr. Vatcharin Rukachaisirikul)
Major Advisor

…….Signature
(Mr. Haryadi Nugraha Putra)
Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

……...Signature
(Mr. Haryadi Nugraha Putra)
Candidate

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Author Mr. Haryadi Nugraha Putra

Major Program Chemistry

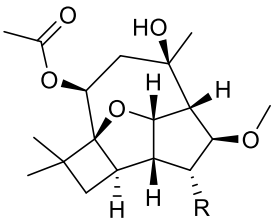
Academic Year 2018

ABSTRACT

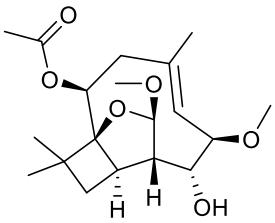
This research focused on the chemical investigation of the crude extracts from two marine-derived fungi, *Pseudopestalotiopsis* sp. PSU-AMF45 and *Trichoderma longibrachiatum* PSU-AMF274. Purification of these extracts was performed by using various chromatographic techniques, resulting in the isolation of nineteen compounds with diverse structures. Their structures were assigned based on the spectroscopic data analysis, such as 1D and 2D NMR spectra, and further supported by HRESIMS (only new compounds) as well as comparison of NMR data with those previously reported. The determination of the relative configuration of the isolated compound was carried out on the basis of the NOEDIFF results and/or coupling constants, while that of the absolute configuration was established using X-ray data with a graphite-monochromatic CuK_α radiation (λ = 1.54178 Å) at 100(2) K, the modified Mosher's method, electronic circular dichroism (ECD) calculation, or comparison of the specific rotations with those of structurally-related known compounds.

- Sixteen compounds including three new caryophyllene sesquiterpenoids (**H2**, **H3** and **H10**), one new chromone (**H4**), one new 10-membered macrolide (**H12**) and one synthetic chromone (**H6**) together with ten known compounds including three caryophyllene sesquiterpenoids (**H1**, **H7** and **H11**), one 10-membered macrolide (**H8**), two xanthones (**H5** and **H13**), two benzophenones (**H9** and **H14**) and two diphenyl ethers (**H15** and **H16**) were isolated from the broth extract of the fungus *Pseudopestalotiopsis* sp. PSU-AMF45. In addition, one diphenyl ether (**H17**) was obtained from the mycelial hexane extract.

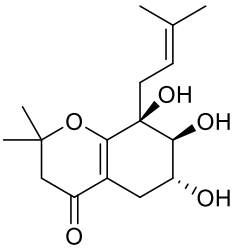
- Two known compounds, vertinolide (**H18**) and sorbicillin (**H19**), were isolated from the broth extract of the fungus *T. longibrachiatum* PSU-AMF274.



H1: R = OMe
H3: R = OH



H2



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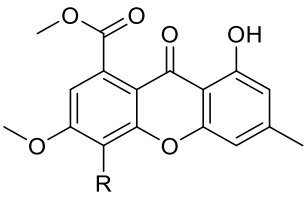
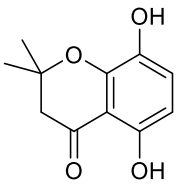


H5: R = H
H13: R = Cl



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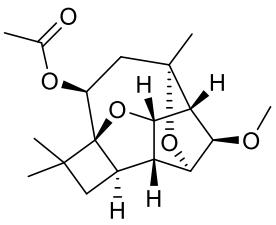

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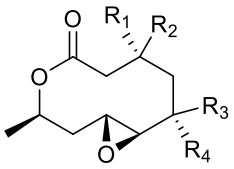
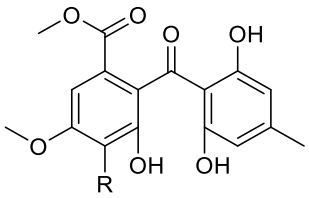
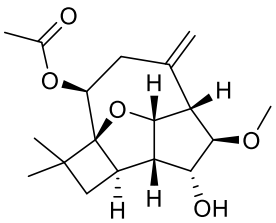


H8: $R_1 + R_2 = =O$, $R_3 = H$, $R_4 = OH$
H12: $R_1 = OH$, $R_2 = H$, $R_3 + R_4 = =O$



H9: R = H
H14: R = Cl



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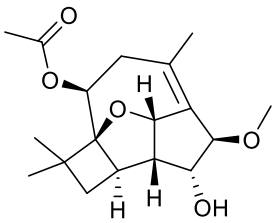

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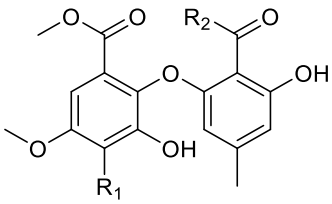
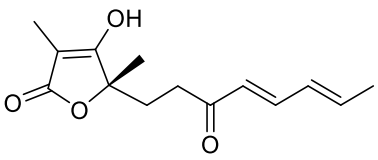
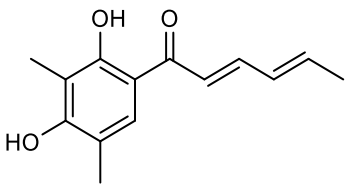


H15: $R_1 = H$, $R_2 = OH$
H16: $R_1 = Cl$, $R_2 = OH$
H17: $R_1 = Cl$, $R_2 = OMe$



H18



H19

ACKNOWLEDGEMENT

First of all, I would like to express my sincere gratitude to my supervisor, Professor Dr. Vatcharin Rukachaisirikul, for the valuable instruction, suggestion, comment and expert guidance during the learning process of this master thesis as well as for the correction of my thesis.

Secondly, I would also like to express my thankfulness to Dr. Pattama Pittayakhajonwut, Assoc. Prof. Dr. Kanda Panthong and Asst. Prof. Dr. Yaowapa Sukpondma for the valuable comments.

Thirdly, I would like to acknowledge to Miss Supattra Kaewtaro for recording 300 and 500 MHz NMR spectra, to the staff of the Department of Chemistry for their support and guidance, to Assist. Prof. Dr. Saowanit Saithong, the Department of Chemistry, for recording X-ray data, to Prof. Dr. Souwalak Phongpaichit, the Department of Microbiology, for the isolation and fermentation of the fungi as well as antimicrobial assay, to Assoc. Prof. Siriporn Jungsuttiwong, Department of Chemistry, Faculty of Science, Ubon Ratchathani University for the calculation of circular dichroism (CD) data and to Dr. Jariya Sakayaroj, School of Science, Walailak University, for the fungi identification.

In addition, my sincere thanks go to the Higher Education Research Promotion and the Thailand's Education Hub for Southern Region of ASEAN Countries Project Office of the Higher Education Commission, to The Royal Property Bureau Foundation for The National Science and Technology Development Agency (NSTDA) Chair Professor Grant to Prof. Dr. Vatcharin Rukachaisirikul, and to the Center of Excellence for Innovation in Chemistry (PERCH-CIC) and Prince of Songkla University for partial support.

The last but not least, I express my profound appreciation to my family who always gives encouragement support. I also thank all of my friends for their help and friendship. This accomplishment would not be possible without them.

Haryadi Nugraha Putra

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Marine-derived fungi have been well-known as a potential source of a lot of bioactive secondary metabolites. Therefore, we have conducted our research on two marine-derived fungi, *Pseudopestalotiopsis* sp. PSU-AMF45 and *Trichoderma longibrachiatum* PSU-AMF274. Fungi PSU-AMF45 and 274 were isolated from ascidian and bryozoan, respectively, which were collected from the Phuket Coastal Fisheries Research and Development Center, Phuket Province, Thailand. This is the first research focusing on the structural investigation of secondary metabolites from the genus *Pseudopestalotiopsis*. The crude extracts from the fungus PSU-AMF45 displayed antifungal activity whereas the PSU-AMF274 crude extracts showed cytotoxic, antibacterial and antifungal activities. Nineteen secondary metabolites including five new ones were isolated from these fungi and the antimicrobial activity of some selected compounds was evaluated.

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LIST OF FIGURES

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LIST OF ABBREVIATIONS AND SYMBOLS

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
q	=	*quartet*
qn	=	*quintet*
sext	=	*sextet*
m	=	*multiplet*
brs	=	*broad singlet*
brd	=	*broad doublet*
dd	=	*doublet of doublets*
dt	=	*doublet of triplets*
dq	=	*doublet of quartets*
dqn	=	*doublet of quintets*
td	=	*triplet of doublets*
tt	=	*triplet of triplets*
ddd	=	*doublet of doublet of doublets*
ddt	=	*doublet of doublet of triplets*
dtd	=	*doublet of triplets of doublets*
dqd	=	*doublet of quartet of doublets*
dddd	=	*doublet of doublet of doublets of doublets*
δ	=	chemical shift relative to TMS
J	=	coupling constant
°C	=	degree Celsius
m/*z*	=	mass-to-charge ratio
R_f	=	retention factor
g	=	gram
mg	=	milligram
μg	=	microgram

LIST OF ABBREVIATIONS AND SYMBOLS (**Continued**)

mL	=	milliliter
μL	=	microliter
L	=	liter
cm^{-1}	=	reciprocal centimeter (wavenumber)
nm	=	nanometer
ppm	=	part per million
λ_{max}	=	maximum wavelength
ν	=	absorption frequency
ε	=	molar extinction coefficient
Hz	=	hertz
MHz	=	megahertz
[α]	=	specific rotation
c	=	concentration
TLC	=	thin-layer chromatography
UV-S	=	Ultraviolet-short wavelength
CD	=	Circular Dichroism
ECD	=	Electronic Circular Dichroism
FT-IR	=	Fourier Transform Infrared
MS	=	Mass Spectroscopy
HRESIMS	=	High Resolution Electrospray Ionization Mass Spectroscopy
NMR	=	Nuclear Magnetic Resonance
1D NMR	=	One Dimensional Nuclear Magnetic Resonance
2D NMR	=	Two Dimensional Nuclear Magnetic Resonance
HMQC	=	Heteronuclear Multiple Quantum Coherence
HMBC	=	Heteronuclear Multiple Bond Correlation
DEPT	=	Distortionless Enhancement by Polarization Transfer
NOEDIFF	=	Nuclear Overhauser Effect Difference

LIST OF ABBREVIATIONS AND SYMBOLS (**Continued**)

COSY	=	Correlation Spectroscopy
TMS	=	tetramethylsilane
acetone-d_6	=	hexadeuteroacetone
$CDCl_3$	=	deuterochloroform
CD_3OD	=	tetradeuteromethanol
$CHCl_3$	=	chloroform
CH_2Cl_2	=	dichloromethane
EtOAc	=	ethyl acetate
MeOH	=	methanol
$NaHCO_3$	=	sodium hydrogen carbonate
K_2CO_3	=	potassium carbonate
MeI	=	iodomethane
HPLC	=	High Performance Liquid Chromatography

CHAPTER 1

METABOLITES FROM THE MARINE-DERIVED FUNGUS *PSEUDOPESTALOTIOPSIS* SP. PSU-AMF45

CHAPTER 1.1

INTRODUCTION

1.1.1 Introduction

The genus *Pseudopestalotiopsis* was segregated from the genus *Pestalotiopsis* that is of interest due to its ability to produce bioactive secondary metabolites (Debbab et al., 2013). Xu et al. (2010) reported that the isolated compounds obtained from *Pestalotiopsis theae* collected from branches of *Camellia sinensis* (family *Theaceae*) showed HIV-1 replication inhibitory (in C8166 cells) and antifungal (against *Aspergillus fumigatus* ATCC10894) activities. In addition, cytotoxic chromone derivatives against the murine cancer were obtained from the endophytic fungus *Pestalotiopsis* sp. isolated from the Chinese mangrove plant *Rhizophora mucronata* (Xu et al., 2009). *Pseudopestalotiopsis* sp. PSU-AMF45 was isolated from an ascidian which was collected from the Phuket Coastal Fisheries Research and Development Center, Phuket Province, Thailand. This fungus was cultured at Department of Microbiology, Faculty of Science, Prince of Songkla University. According to SciFinder Database, secondary metabolites isolated from the genus *Pseudopestalotiopsis* have never been reported. Therefore, those from the genus *Pestalotiopsis* were summarized in **Table 1**. The broth ethyl acetate and mycelial hexane extracts from *Pseudopestalotiopsis* sp. PSU-AMF45 showed antifungal activity against *Cryptococcus neoformans* with the MIC values of 32 and 16 μg/mL, respectively. Furthermore, the ^{1}H-NMR spectra of the crude extracts displayed signals of aromatic and olefinic protons. Accordingly, secondary metabolites of this fungus are of interest.

Table 1 Compounds isolated from the genus *Pestalotiopsis*

Scientific name	Compound	Activity	References
Pestalotiosis disseminata	6-Hydroxypunctaporonin A, **1** 6-Hydroxypunctaporonin B, **2** 6-Hydroxypunctaporonin E, **3**	- Antibacterial Antibacterial	Deyrup et al., 2006
Pestalotiopsis sp.	Cytosporin D, **4** Pestaloquinol A, **5** Pestaloquinol B, **6**	- Cytotoxic Cytotoxic	Ding et al., 2011
Pestalotiopsis sp.	Pestalotiolide A, **7** 7-Hydroxy-5-methoxy-4,6-dimethyl-7-*O*-*β*-D-glucopyranosylphthalide, **8** 7-Hydroxy-5-methoxy-4,6-dimethyl-7-*O*-*α*-L-rhamnosyl-phthalide, **9** 7-Hydroxy-5-methoxy-4,6-dimethylphthalide, **10** 5'-*O*-Acetyluridine, **11**	Antiviral Antiviral Antiviral Antiviral -	Jia et al., 2015
Pestalotiopsis sp.	Pestaloporinate A, **12** Pestaloporinate B, **13** Pestaloporinate C, **14** Pestaloporinate D, **15** Pestaloporinate E, **16** Pestaloporinate F, **17** Pestaloporinate G, **18** 14-Acetylhumulane, **19**	- Nitric oxide inhibitory - - - - - -	Liu et al., 2016c

Table 1 (continued)

Scientific name	Compound	Activity	References
Pestalotiopsis sp.	Pestalaphenone A, **20** Pestalachloride G, **21** Pestalone, **22** Ambuic acid, **23** 15-Carbonylambuic acid, **24** Pestalotic acid I, **25** 18-Acetylambuic acid, **26** 10-Hydroxyambuic acid, **27**	Cytotoxic Antibacterial Antibacterial, antifungal - - - - -	Song et al., 2017a
Pestalotiopsis sp.	Pestalactone A, **28** Pestalactone B, **29** Pestalactone C, **30** Pestapyrone D, **31** Pestapyrone E, **32** 7-Hydroxy-5-methoxy-4,6-dimethylphthalide, **10** 5,7-Dimethoxy-4,6-dimethyl-phthalide, **33**	- - Antifungal - - - -	Song et al., 2017b
Pestalotiopsis sp.	Pestalotiopsone A, **34** Pestalotiopsone B, **35** Pestalotiopsone C, **36** Pestalotiopsone D, **37** Pestalotiopsone E, **38** Pestalotiopsone F, **39** 7-Hydroxy-2-(hydroxypropyl)-5-methylchromone, **40**	- - - - - Cytotoxic -	Xu et al., 2009

Table 1 (continued)

Scientific name	Compound	Activity	References
Pestalotiopsis sp. cr013	Pestalpolyol A, **41** Pestalpolyol B, **42** Pestalpolyol C, **43** Pestalpolyol D, **44**	Cytotoxic Cytotoxic - Cytotoxic	Li et al., 2015
Pestalotiopsis sp. EJC07	(4*S*)-4,8-Dihydroxy-1-tetralone, **45** Uracil, **46** Uridin, **47** *p*-Hydroxybenzoic acid, **48** Ergosterol, **49** Ergosterol peroxide, **50** Cerevisterol, **51** Ducitol, **52**	- - - - - - - -	De souza et al., 2016
Pestalotiopsis sp. FT172	Pestalotiotone A, **53** Pestalotiotone B, **54**	- -	Li et al., 2018
Pestalotiopsis sp. HHL101	Pestalotiopisorin B, **55** (*R*)-(-)- Mellein methyl ether, **56** Pestalotiopyrone G, **57** (*R*)-Mevalonolactone, **58** Pestalotiollide A, **59** Pestalotiollide B, **60** Pestalotiopsol A, **61**	Antibacterial - - Calcineurin inhibitory - - -	Xu et al., 2019
Pestalotiopsis sp. PG52	Pestalpolyol E, **62** Pestalpolyol F, **63** Pestalpolyol G, **64** Pestalpolyol H, **65**	- Cytotoxic Cytotoxic Cytotoxic	Xie et al., 2015

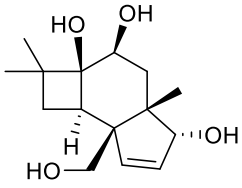
Table 1 (continued)

Scientific name	Compound	Activity	References
Pestalotiopsis sp. PSU-ES194	7-Hydroxydehydroaustin, **66**	-	Arunpanichlert et al., 2015
	11β-Acetoxyisoaustinone, **67**	-	
	Pestalotiorin, **68**	-	
	Pestalotionol, **69**	-	
	Dehydroaustinol, **70**	-	
	Dehydroaustin, **71**	-	
	Acetoxydehydroaustin**, 72**	-	
	Austin, **73**	-	
	Aspergillumarin A, **74**	Antibacterial	
	Aspergillumarin B, **75**	Antifungal, antibacterial	
	Penicillide, **76**	-	
	Purpactin A, **77**	-	
Pestalotiopsis sp. PSU-MA69	Pestalotether A, **78**	Antifungal	Klaiklay et al., 2012
	Pestalotether B, **79**	Antifungal	
	Pestalotether C, **80**	-	
	Pestalotether D, **81**	-	
	Pestalochromone A, **82**	-	
	Pestalochromone B, **83**	-	
	Pestalochromone C, **84**	-	
	Pestaloxanthone, **85**	-	
	Pestalolide, **86**	Antifungal	
	Pesteic acid, **87**	Antifungal	
	Isosulochrin dehydrate, **88**	-	
	Chloroisosulochrin dehydrate, **89**	Antifungal	
	Chloroisosulochrin, **90**	Antifungal	
	Isosulochrin, **91**	-	
	Asperpentyn, **92**	-	

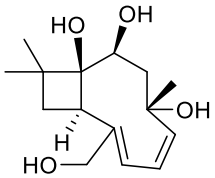
Table 1 (continued)

Scientific name	Compound	Activity	References
	Siccayne, **93**	-	
	(*S*)-Penipratynolene, **94**	Antifungal	
	Seiridin, **95**	-	
	2,2-Dimethyl-2*H*-1-chromone-6-carboxylic acid, **96**	-	
Pestalotiopsis sp. (ZJ-2009-7-6)	Pestarhamnose A, **97**	Cytotoxic, antimicrobial	Xing et al., 2015
	Pestarhamnose B, **98**	Cytotoxic, antimicrobial	
	Pestarhamnose C, **99**	Cytotoxic, antimacrobial	
	(±)-Pestalachloride C, **100**	-	
	(±)-Pestalachloride D, **101**	-	

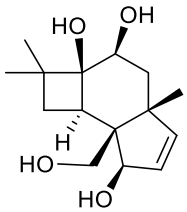
Structures of the metabolites from the genus *Pestalotiopsis*



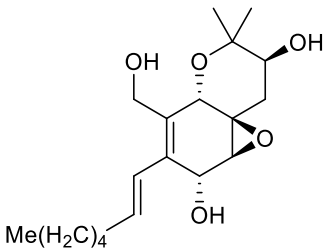
1: 6-Hydroxypunctaporonin A



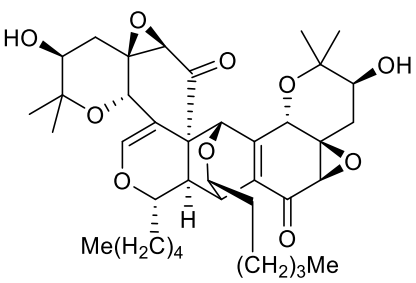
2: 6-Hydroxypunctaporonin B



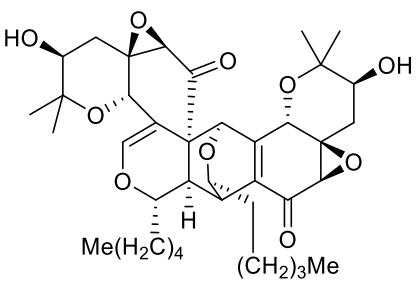
3: 6-Hydroxypunctaporonin E



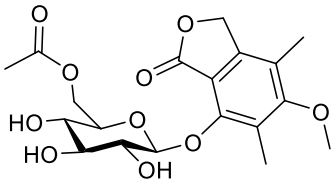
4: Cytosporin D



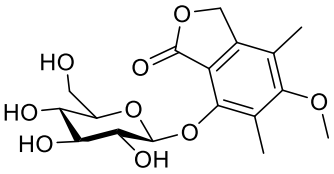
5: Pestaloquinol A



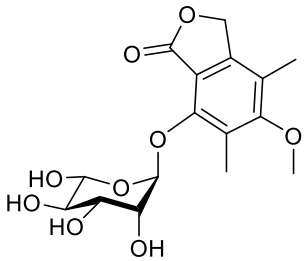
6: Pestaloquinol B



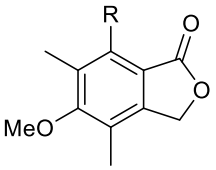
7: Pestalotiolide A



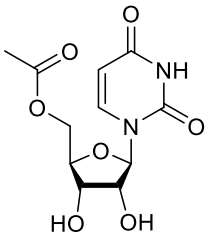
8: 7-Hydroxy-5-methoxy-4,6-dimethyl-7-*O*-*β*-D-glucopyranosylphthalide



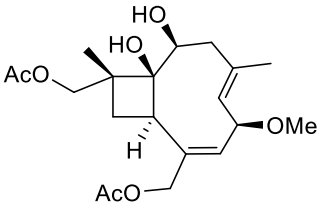
9: 7-Hydroxy-5-methoxy-4,6-dimethyl-7-*O*-*α*-L-rhamnosylphthalide



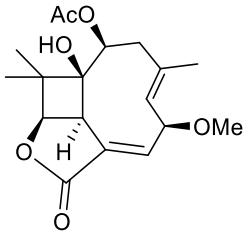
10: R = OH : 7-Hydroxy-5-methoxy-4,6-dimethylphthalide
33: R = OMe : 5,7-Dimethoxy-4,6-dimethylphthalide



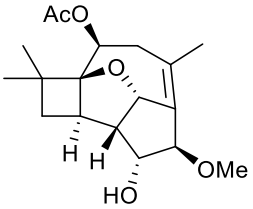
11: 5'-*O*-Acetyluridine



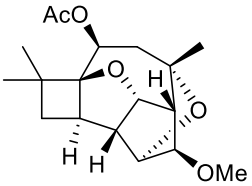
12: Pestaloporinate A



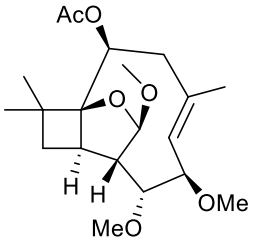
13: Pestaloporinate B



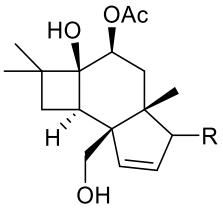
14: Pestaloporinate C



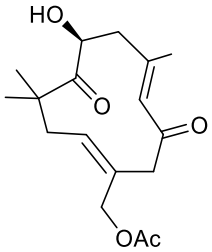
15: Pestaloporinate D



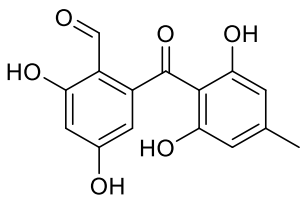
16: Pestaloporinate E



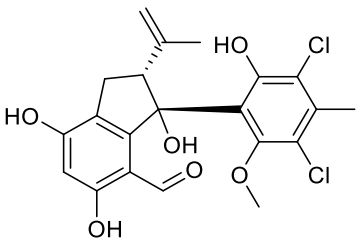
17: R = *α*-OH : Pestaloporinate F
18: R = *β*-OMe : Pestaloporinate G



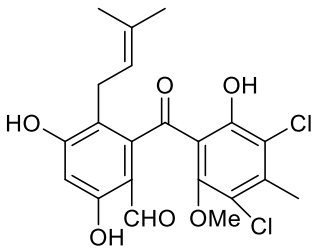
19: 14-Acetylhumulane



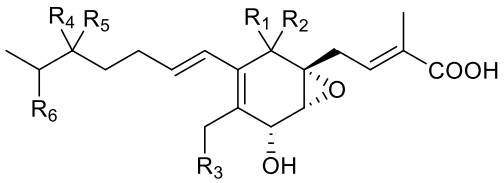
20: Pestalaphenone A



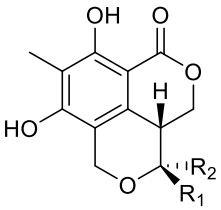
21: Pestalachloride G



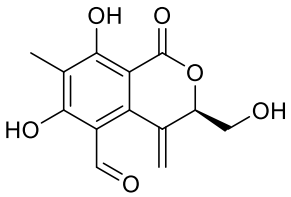
22: Pestalone



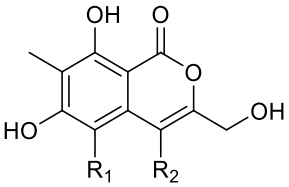
23: $R_1 + R_2 = =O$, $R_3 = OH$, $R_4 = R_5 = R_6 = H$: Ambuic acid
24: $R_1 + R_2 = =O$, $R_3 = OH$, $R_4 + R_5 = =O$, $R_6 = H$: 15-Carbonylambuic acid
25: $R_1 + R_2 = =O$, $R_3 = OH$, $R_4 = R_5 = H$, $R_6 = OH$: Pestalotic acid I
26: $R_1 + R_2 = =O$, $R_3 = OAc$, $R_4 = R_5 = R_6 = H$: 18-Acetylambuic acid
27: $R_1 = \alpha$-OH, $R_2 = R_4 = R_5 = R_6 = H$, $R_3 = OH$: 10-Hydroxyambuic acid



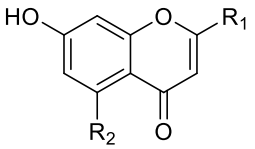
28: $R_1 =$ Me, $R_2 =$ OMe : Pestalactone A
29: $R_1 = CH_2OH$, $R_2 = OH$: Pestalactone B

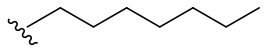
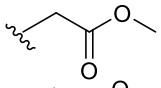


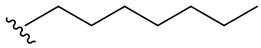
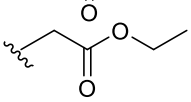
30: Pestalactone C

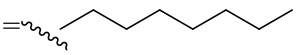
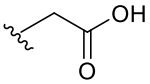


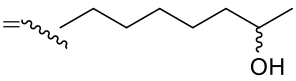
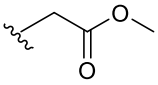
31: $R_1 =$ H, $R_2 =$ Me : Pestapyrone D
32: $R_1 =$ Me, $R_2 =$ H : Pestapyrone E

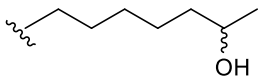
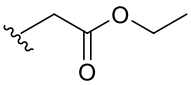


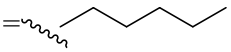
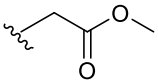
34: R_1 =  R_2 =  : Pestalotiopsone A

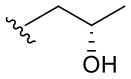
35: R_1 =  R_2 =  : Pestalotiopsone B

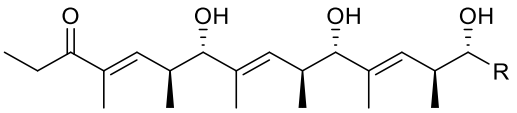
36: R_1 =  R_2 =  : Pestalotiopsone C

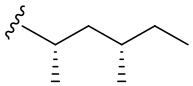
37: R_1 =  R_2 =  : Pestalotiopsone D

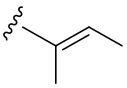
38: R_1 =  R_2 =  : Pestalotiopsone E

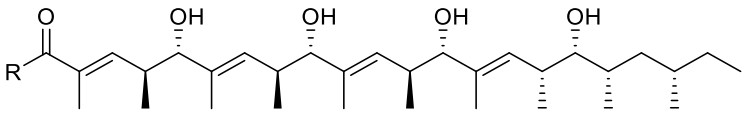
39: R_1 =  R_2 =  : Pestalotiopsone F

40: R_1 =  R_2 = Me : 7-Hydroxy-2-(hydroxypropyl)-5-methylchromone



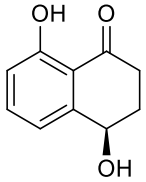
41: R =  : Pestalpolyol A

42: R =  : Pestalpolyol B

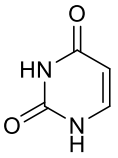


43: R = Me : Pestalpolyol C

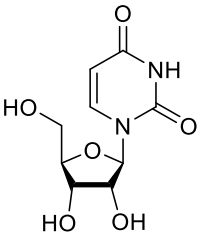
44: R = Et : Pestalpolyol D



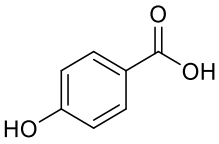
45: (4*S*)-4,8-Dihydroxy-1-tetralone



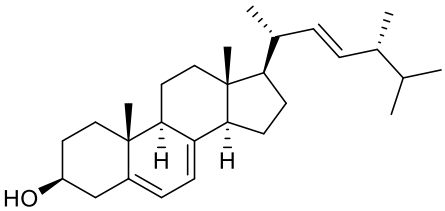
46: Uracil



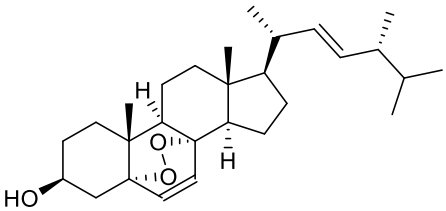
47: Uridin



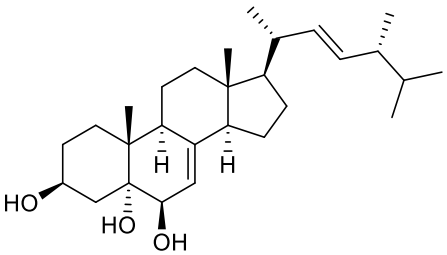
48: *p*-Hydroxybenzoic acid



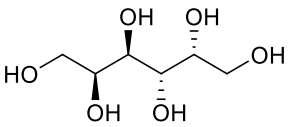
49: Ergosterol



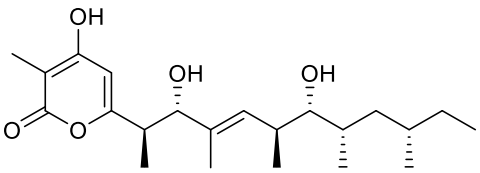
50: Ergosterol peroxide



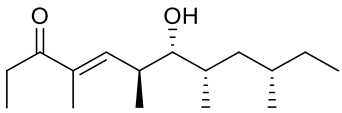
51: Cerevisterol



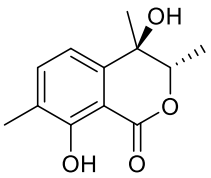
52: Ducitol



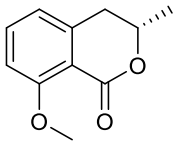
53: Pestalotiotone A



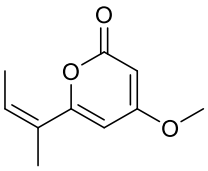
54: Pestalotiotone B



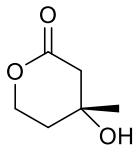
55: Pestalotiopisorin B



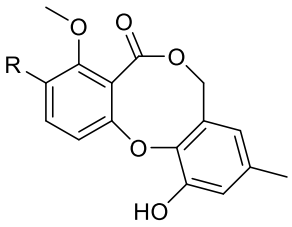
56: (*R*)-(-)-Mellein methyl ether

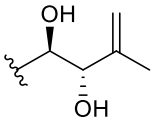


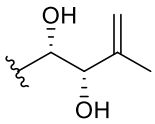
57: Pestalotiopyrone G

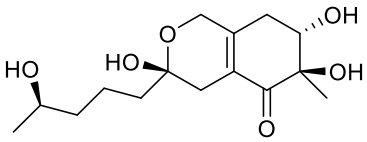


58: (*R*)-Mevalonolactone

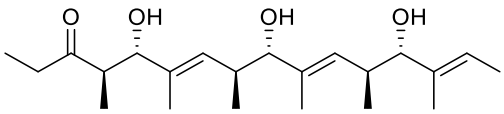


59: R =  : Pestalotiollide A

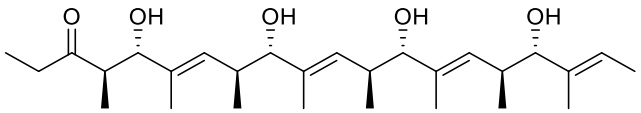
60: R =  : Pestalotiollide B



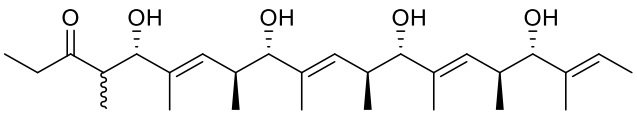
61: Pestalotiopsol A



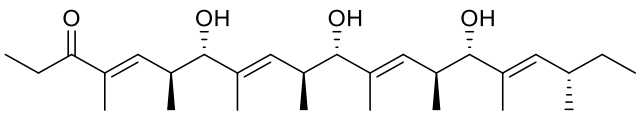
62: Pestalpolyol E



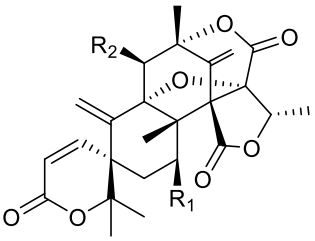
63: Pestalpolyol F



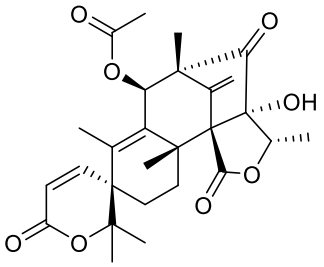
64: Pestalpolyol G



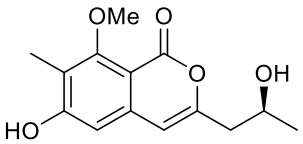
65: Pestalpolyol H



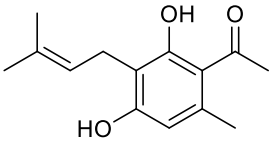
66: R_1 = OH, R_2 = OAc : 7-Hydroxydehydroaustin
69: R_1 = H, R_2 = OH : Pestalotionol
70: R_1 = H, R_2 = OAc : Dehydroaustinol
71: R_1 = R_2 = OAc : Dehydroaustin



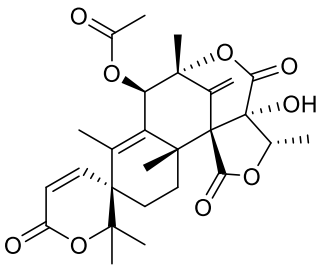
67: 11β-Acetoxyisoaustinone



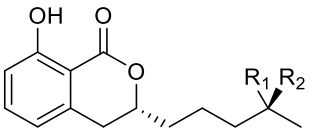
68: Pestalotiorin



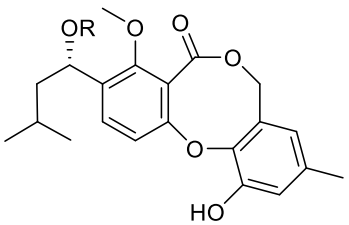
72: Acetoxydehydroaustin



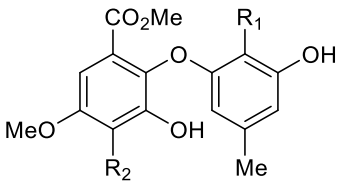
73: Austin



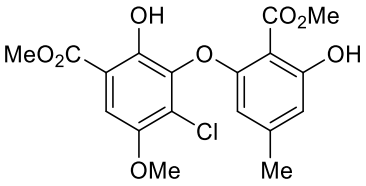
74: R_1 + R_2 = =O : Aspergillumarin A
75: R_1 = H, R_2 = OH : Aspergillumarin B



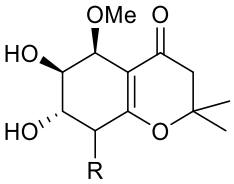
76: R = H : Penicillide
77: R = COMe : Purpactin A



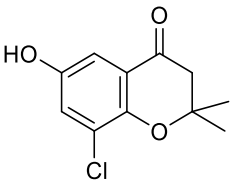
78: R_1 = CO_2Me, R_2 = Cl : Pestalotether A
79: R_1 = H, R_2 = Cl : Pestalotether B
81: R_1 = CO_2Me, R_2 = H : Pestalotether D
87: R_1 = CO_2H, R_2 = Cl : Pesteic acid



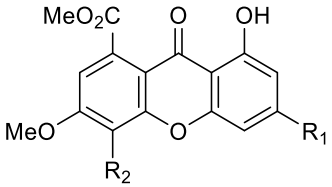
80: Pestalotether C



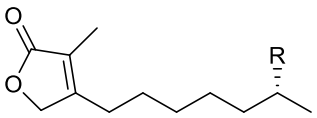
82: R = β-Cl : Pestalochromone A
83: R = α-Cl : Pestalochromone B



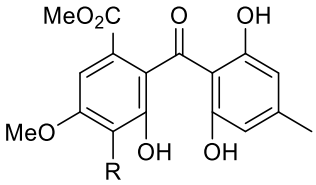
84: Pestalochromone C



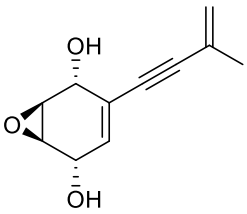
85: R_1 = CO_2H, R_2 = Cl : Pestaloxanthone
88: R_1 = Me, R_2 = H : Isosulochrin dehydrate
89: R_1 = Me, R_2 = Cl : Chloroisosulochrin dehydrate



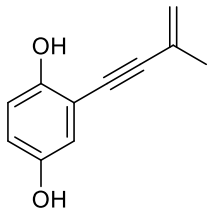
86: R = H : Pestalolide
95: R = OH : Seiridin



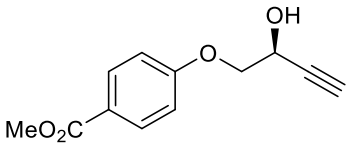
90: R = Cl : Chloroisosulochrin
91: R = H : Isosulochrin



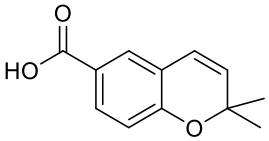
92: Asperpentyn



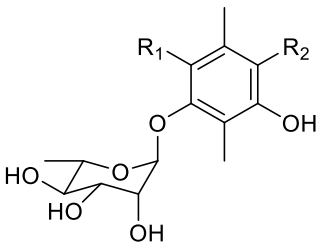
93: Siccayne



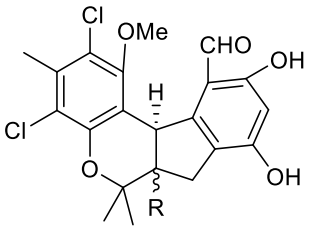
94: (*S*)-Penipratynolene



96: 2,2-Dimethyl-2*H*-1-chromone-6-carboxylic acid



97: R_1 = Me, R_2 = H : Pestarhamnose A
98: R_1 = H, R_2 = Me : Pestarhamnose B
99: R_1 = R_2 = H : Pestarhamnose C



100: R = β-H : (±)-Pestalachloride C
101: R = α-H : (±)-Pestalachloride D

1.1.2 The objectives

The objectives are to isolate secondary metabolites from the marine-derived fungus *Pseudopestalotiopsis* sp. PSU-AMF45 and to identify the structures of the isolated compounds.

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 (IR) spectra were obtained using a Perkin-Elmer spectrum BX FT-IR spectrometer. Ultraviolet (UV) spectra were measured with a Shimadzu UV-2600 UV-Vis spectrophotometer in MeOH. The measurement of specific rotations was performed with a JASCO P-2000 polarimeter. Circular dichroism (CD) spectra were recorded on a JASCO J-815 CD spectrometer. ESI-TOF mass spectra were measured on a TOF/Q-TOF Mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a 300 or 500 MHz Bruker FTNMR Ultra Shield^{TM} spectrometer using tetramethylsilane (TMS) as an internal standard. X-ray crystallographic data were measured on D8 VENTURE Bruker AXS diffractometer equipped with a PHOTON II using a graphite-monochromatic CuK_{α} radiation (λ = 1.54178 Å) at 100(2) K. Thin-layer chromatography (TLC) and preparative TLC were carried out on silica gel 60 GF_{254} (Merck) whereas column chromatography (CC) was conducted on Sephadex LH-20, silica gel (Merck) type 100 (70-230 mesh ASTM) and type 60 (230-400 mesh ASTM), or reverse phase C_{18} silica gel. The organic solvents were distilled at their boiling points prior to use.

1.2.2 Fermentation and extraction of the fungus PSU-AMF45

The flask culture of the fungus *Pseudopestalotiopsis* sp. PSU-AMF45 (15 L) was filtered to separate into the filtrate and wet mycelial cake. The filtrate was divided into 54 fractions and each fraction (500 mL) was extracted three times with EtOAc (3 x 200 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to afford the crude extract as a dark brown gum (BE, 4.3 g). The wet mycelial cake was extracted with MeOH. The MeOH

layer was concentrated under reduced pressure and H_2O (150 mL) was added. The mixture was washed three times with hexane (450 mL). The hexane layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to obtain the crude extract as a dark brown gum (CH, 522.3 mg). The aqueous layer was extracted using the same procedure as BE to give the crude extract as a dark brown gum (CE, 606.1 mg).

1.2.3 Purification of the broth extract of the fungus PSU-AMF45

The broth extract of the fungus *Pseudopestalotiopsis* sp. PSU-AMF45 (4.3 g) was separated by column chromatography over Sephadex LH-20 using 100% methanol as an eluent. All of the obtained fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give six fractions as shown in **Table 2**.

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

Fraction	Weight (mg)	Physical appearance
HA1	63.7	Brown gum
HA2	552.0	Dark brown gum
HA3	1093.2	Dark brown gum
HA4	2013.7	Dark brown gum
HA5	347.5	Dark brown gum
HA6	2.9	Brown gum

Fraction HA1 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one major spot near the baseline under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Fraction HA2 Chromatogram characteristics on normal phase TLC with 25% acetone-chloroform as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over Sephadex LH-20 using 100% methanol as an eluent. Subfractions

containing similar components were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 3**.

Table 3 Subfractions obtained from **fraction HA2** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HA21	130.1	Brown gum
HA22	351.8	Brown gum
HA23	45.4	Brown gum

Subfraction HA21 Chromatogram characteristics on normal phase TLC with 25% acetone-chloroform as a mobile phase displayed one major spot near the baseline under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further purified.

Subfraction HA22 Chromatogram characteristics on normal phase TLC with 25% acetone-chloroform as a mobile phase demonstrated a long tail under UV-S. The ^{1}H NMR spectrum showed signals of aromatic and olefinic protons. It was further purified by column chromatography over Sephadex LH-20 using 100% methanol as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 4**.

Table 4 Subfractions obtained from **subfraction HA22** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HA221	46.8	Brown gum
HA222	253.3	Brown gum
HA223	27.5	Brown gum

Subfraction HA221 Chromatogram characteristics on normal phase TLC with 25% acetone-chloroform as a mobile phase displayed one major spot near the baseline under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not further purified.

Subfraction HA222 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase exhibited a long tail under UV-S. The ^{1}H NMR spectrum showed signals of aromatic and olefinic protons. It was dissolved in chloroform to give a chloroform soluble part (**HA2221**) and a chloroform insoluble one (**HA2222**) as shown in **Table 5**.

Table 5 Subfractions obtained from **subfraction HA222** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HA2221	243.4	Brown gum
HA2222	5.8	Brown gum

Subfraction HA2221 Chromatogram characteristics on normal phase TLC with 50% acetone-dichloromethane as a mobile phase exhibited a long tail under UV-S. The ^{1}H NMR spectrum showed signals of aromatic and olefinic protons. It was further purified by column chromatography over silica gel using 50% acetone-dichloromethane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give seven subfractions as shown in **Table 6**.

Table 6 Subfractions obtained from **subfraction HA2221** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA22211	34.5	Yellow gum
HA22212	39.7	Yellow gum
HA22213	16.5	Yellow gum
HA22214	18.3	Yellow gum
HA22215	11.4	Yellow gum
HA22216	14.4	Yellow gum
HA22217	93.5	Brown gum

Subfraction HA22211 Chromatogram characteristics on normal phase TLC with 50% acetone-dichloromethane as a mobile phase showed one major spot with the R_f value of 0.86 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of **H2** as a major compound. Thus, no further purification was conducted.

Subfraction HA22212 Chromatogram characteristics on normal phase TLC with 50% acetone-dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed the presence of **H2** as a major compound. Therefore, it was not further investigated.

Subfraction HA22213 Chromatogram characteristics on normal phase TLC with 50% acetone-dichloromethane as a mobile phase showed a long tail and one major spot with the R_f value of 0.86 under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic signals. Therefore, it was not further purified.

Subfraction HA22214 Chromatogram characteristics on normal phase TLC with 50% acetone-dichloromethane as a mobile phase demonstrated a long tail and two major spots with the R_f values of 0.51 and 0.86 under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Thus, no further purification was conducted.

Subfraction HA22215 Chromatogram characteristics on normal phase TLC with 50% acetone-dichloromethane as a mobile phase demonstrated a long tail under UV-S. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, no attempts were made to purify this subfraction.

Subfraction HA22216 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid whereas four major spots appeared after being visualized by ceric ammonium molybdate with the R_f values of 0.13, 0.30, 0.48 and 0.53. It was further purified by column chromatography over silica gel using 10% methanol-dichloromethane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford ten subfractions as shown in **Table 7**.

Table 7 Subfractions obtained from **subfraction HA22216** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA22216A	1.0	Yellow gum
HA22216B	2.2	Yellow gum

Table 7 (continued)

Subfraction	Weight (mg)	Physical appearance
HA22216C	1.2	Yellow gum
HA22216D	0.9	Yellow gum
HA22216E	1.0	Yellow gum
HA22216F	0.8	Yellow gum
HA22216G	2.0	Yellow gum
HA22216H	3.1	Yellow gum
HA22216I	1.3	Yellow gum
HA22216J	0.7	Yellow gum

Subfraction HA22216A Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Thus, no further purification was conducted.

Subfraction HA22216B Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Thus, no further purification was performed.

Subfraction HA22216C Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase demonstrated two major spots with the R_f values of 0.35 and 0.40 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity and signals in high field region in the ^{1}H NMR spectrum, no further purification was carried out.

Subfraction HA22216D Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase demonstrated four major spots with the R_f values of 0.35, 0.40, 0.52 and 0.55 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity and signals in high field region in the ^{1}H NMR spectrum, no further purification was carried out.

Subfraction HA22216E Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase demonstrated two major spots with the R_f values of 0.35 and 0.55 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity and signals in high field region in the ^{1}H NMR spectrum, no further purification was carried out.

Subfraction HA22216F Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase displayed one major spot with the R_f value of 0.35 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity and signals in high field region in the ^{1}H NMR spectrum, no further purification was carried out.

Subfraction HA22216G Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase demonstrated a tailing spot and one major spot with the R_f value of 0.35 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because the ^{1}H NMR spectrum exhibited signals in high field region, no further investigation was performed.

Subfraction HA22216H Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase showed two major spots with the R_f values of 0.20 and 0.35 under UV-S and a tailing spot after being visualized by anisaldehyde sulfuric acid. Because the ^{1}H NMR spectrum showed signals in high field region, no further investigation was performed.

Subfraction HA22216I Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase demonstrated one major spot with the R_f value of 0.20 under UV-S. The ^{1}H NMR spectrum showed signals in high field region and the quantity was low. Thus, no further purification was conducted.

Subfraction HA22216J Chromatogram characteristics on normal phase TLC with 0.8% methanol-dichloromethane as a mobile phase displayed one major spot near the baseline under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA22217 Chromatogram characteristics on normal phase TLC with 50% acetone-dichloromethane as a mobile phase displayed one major spot near the baseline under UV-S. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, no attempts were made to purify this subfraction.

Subfraction HA2222 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed one major spot near the baseline under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Therefore, it was not further purified.

Subfraction HA223 Chromatogram characteristics on normal phase TLC with 25% acetone-chloroform as a mobile phase demonstrated one major spot near the baseline under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Thus, no further purification was conducted.

Subfraction HA23 Chromatogram characteristics on normal phase TLC with 25% acetone-chloroform as a mobile phase displayed one major spot near the baseline under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, no further investigation was performed.

Fraction HA3 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and seven major spots with the R_f values of 0.10, 0.23, 0.33, 0.38, 0.60, 0.70 and 0.90 after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel. Elution was initially performed with 2% methanol/dichloromethane, and then gradually enriched with methanol until pure methanol. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 8**.

Table 8 Subfractions obtained from **fraction HA3** by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
HA31	2% methanol/dichloromethane	2.4	Yellow gum

Table 8 (continued)

Subfraction	Eluent	Weight (mg)	Physical appearance
HA32	2% methanol/dichloromethane	53.8	Yellow gum
HA33	5% methanol/dichloromethane	26.7	Yellow gum
HA34	5% methanol/dichloromethane	54.0	Yellow gum
HA35	10% methanol/dichloromethane	124.7	Yellow gum
HA36	10% methanol/dichloromethane	138.6	Brown gum
HA37	20% methanol/dichloromethane	281.8	Brown gum
HA38	40-100% methanol/dichloromethane	403.3	Dark brown gum

Subfraction HA31 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed one major spot with the R_f value of 0.90 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Therefore, no further investigation was carried out.

Subfraction HA32 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (2 runs) as a mobile phase displayed eight major spots with the R_f values of 0.35, 0.40, 0.50, 0.57, 0.65, 0.73, 0.85 and 0.90 after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel. Elution was performed with 25% acetone-n-hexane. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give six subfractions as shown in **Table 9**.

Table 9 Subfractions obtained from **subfraction HA32** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA321	19.5	Yellow gum
HA322	10.4	Yellow gum
HA323	7.3	Yellow gum
HA324	2.9	Yellow gum
HA325	1.6	Yellow gum

Table 9 (continued)

Subfraction	Weight (mg)	Physical appearance
HA326	1.5	Yellow gum

Subfraction HA321 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (2 runs) as a mobile phase demonstrated two major spots with the R_f values of 0.75 and 0.90 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed the presence of **H7** as a major compound. Therefore, it was not investigated.

Subfraction HA322 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (2 runs) as a mobile phase demonstrated two major spots with the R_f values of 0.55 and 0.75 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed the presence of **H7** as a major component. Therefore, it was not investigated.

Subfraction HA323 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (2 runs) as a mobile phase displayed a long tail and one major spot with the R_f value of 0.40 under UV-S and three major spots with the R_f values of 0.45, 0.55 and 0.75 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of **H7** as a major compound. Therefore, it was not investigated.

Subfraction HA324 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (2 runs) as a mobile phase showed one major spot with the R_f value of 0.58 under UV-S. The ^{1}H NMR spectrum revealed the presence of **H6** as a major component. Therefore, it was not investigated.

Subfraction HA325 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (2 runs) as a mobile phase showed two major spots with the R_f values of 0.35 and 0.40 under UV-S. The ^{1}H NMR spectrum showed signals in high field region. Thus, it was not investigated.

Subfraction HA326 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (2 runs) as a mobile phase showed one major spot near the baseline

under UV-S. The ^{1}H NMR spectrum showed signals in high field region. Thus, no attempts were made to purify this subfraction.

Subfraction HA33 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (2 runs) as a mobile phase showed a long tail under UV-S and two major spots with the R_f values of 0.60 and 0.75 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of **H7** as a major compound. Therefore, it was not investigated.

Subfraction HA34 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed five major spots with the R_f values of 0.45, 0.53, 0.73, 0.80 and 0.85 after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel with 25% acetone-n-hexane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 10**.

Table 10 Subfractions obtained from **subfraction HA34** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA341	4.6	Yellow gum
HA342	5.8	Colorless gum
HA343	4.2	Colorless gum
HA344	4.2	Colorless gum
HA345	14.3	Yellow gum
HA346	6.7	Yellow gum
HA347	4.2	Yellow gum
HA348	4.2	Yellow gum

Subfraction HA341 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed no spots under UV-S. Because the ^{1}H NMR spectrum showed the absence of aromatic and olefinic proton signals, further investigation was not performed.

Subfraction HA342 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one major spot with the R_f value of 0.55 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of **H7** as a major compound. Therefore, it was not investigated.

Subfraction HA343 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one major spot with the R_f value of 0.45 under UV-S and one major spot with the R_f value of 0.55 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of **H7** as a major component. Therefore, it was not investigated.

Subfraction HA344 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate as a mobile phase demonstrated seven major spots with the R_f values of 0.18, 0.30, 0.40, 0.43, 0.53, 0.65 and 0.73 after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel with 18:1:1 dichloromethane:chloroform:ethyl acetate as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 11**.

Table 11 Subfractions obtained from **subfraction HA344** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA3441	0.4	Colorless gum
HA3442	0.2	Colorless gum
HA3443	0.1	Colorless gum
HA3444	0.2	Colorless gum
HA3445	0.1	Colorless gum
HA3446	0.1	Colorless gum
HA3447	0.2	Colorless gum
HA3448	3.9	Colorless gum

Subfraction HA3441 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate as a mobile phase showed no spots under

UV-S. The ^{1}H NMR spectrum showed the absence of aromatic and olefinic proton signals. Thus, no attempts were made to purify this subfraction.

Subfraction HA3442 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate as a mobile phase showed no spots under UV-S. Because of minute quantity, no attempts were made to purify this subfraction.

Subfraction HA3443 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate as a mobile phase displayed one major spot with the R_f value of 0.65 after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, no attempts were made to purify this subfraction.

Subfraction HA3444 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate as a mobile phase displayed one major spot with the R_f value of 0.53 after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not further investigated.

Subfraction HA3445 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate as a mobile phase displayed one major spot with the R_f value of 0.43 after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not further investigated.

Subfraction HA3446 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate as a mobile phase demonstrated one major spot with the R_f value of 0.40 after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not further investigated.

Subfraction HA3447 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate as a mobile phase showed one major spot with the R_f value of 0.30 after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not further investigated.

Subfraction HA3448 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase displayed two major spots with the R_f values of 0.50 and 0.65 after being visualized by anisaldehyde

sulfuric acid. It was further purified by column chromatography over silica gel using 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 12**.

Table 12 Subfractions obtained from **subfraction HA3448** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA34481	0.5	Colorless gum
HA34482	0.3	Colorless gum
HA34483	2.2	Colorless solid
HA34484	0.2	Yellow gum

Subfraction HA34481 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed no spots under UV-S. Because of minute quantity, it was not further investigated.

Subfraction HA34482 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed two major spots with the R_f values of 0.50 and 0.60 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of **H1** as a major compound. Therefore, it was not investigated.

Subfraction HA34483 (H1) Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol displayed one spot with the R_f value of 0.50 after being visualized by anisaldehyde sulfuric acid.

Melting point (°C)	:	209-210
$[\alpha]_D^{24}$	:	+24.8 (c 0.09, MeOH)
FT-IR (neat) ν_{max} cm^{-1}	:	3406 (O-H), 1721 (O-C=O)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	:	5.28 (*dd*, *J* = 2.5 and 3.5 Hz, 1H), 5.03 (*dd*, *J* = 7.0 and 10.0 Hz, 1H), 4.08 (*brs*, 1H), 3.68 (*t*, *J* = 7.0 Hz, 1H), 3.37 (*s*, 3H), 3.29 (*s*, 3H), 3.25 (*t*, *J* = 7.0 Hz, 1H), 2.93 (*dt*, *J*

= 4.0 and 7.0 Hz, 1H), 2.59 (*ddd*, *J* = 4.0, 6.0 and 9.5 Hz, 1H), 2.29 (*dd*, *J* = 7.0 and 10.0 Hz, 1H), 2.07 (*s*, 3H), 2.03 (*dd*, *J* = 3.5 and 12.5 Hz, 1H), 1.99 (*dd*, *J* = 9.5 and 12.0 Hz, 1H), 1.97 (*dd*, *J* = 2.5 and 12.5 Hz, 1H), 1.42 (*dd*, *J* = 6.0 and 12.0 Hz, 1H), 1.15 (*s*, 3H), 1.07 (*s*, 3H), 1.03 (*s*, 3H)

^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		:	170.2, 93.9, 87.4, 85.8, 84.4, 74.5, 74.0, 57.5, 57.3, 56.8, 56.1, 40.1, 37.8, 37.4, 34.2, 29.3, 26.0, 24.3, 21.5
DEPT (135^o) ($CDCl_3$)	CH	:	87.4, 85.8, 84.4, 74.0, 56.8, 56.1, 34.2
	CH_2	:	40.1, 37.8
	CH_3	:	57.5, 57.3, 29.3, 26.0, 24.3, 21.5

Subfraction HA34484 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed no spots under UV-S. Because of minute quantity, it was not further investigated.

Subfraction HA345 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate (2 runs) as a mobile phase demonstrated a long tail and five major spots with the R_f values of 0.28, 0.35, 0.40, 0.50 and 0.58 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel using 18:1:1 dichloromethane:chloroform:ethyl acetate as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 13**.

Table 13 Subfractions obtained from **subfraction HA345** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA3451	2.0	Colorless gum
HA3452	0.8	Colorless gum
HA3453	0.6	Colorless gum

Table 13 (continued)

Subfraction	Weight (mg)	Physical appearance
HA3454	3.2	Colorless gum
HA3455	2.3	Colorless gum
HA3456	4.7	Yellow gum

Subfraction HA3451 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate (2 runs) as a mobile phase showed one major spot with the R_f value of 0.58 under UV-S. The ^{1}H NMR spectrum exhibited the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA3452 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate (2 runs) as a mobile phase showed two major spots with the R_f values of 0.50 and 0.58 under UV-S. Because of minute quantity, it was not further investigated.

Subfraction HA3453 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate (2 runs) as a mobile phase showed two major spots with the R_f values of 0.40 and 0.50 under UV-S. Because of minute quantity, no attempts were made to investigate this subfraction.

Subfraction HA3454 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate (2 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.40 under UV-S. Because of low quantity, no attempts were made to investigate this subfraction.

Subfraction HA3455 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate (2 runs) as a mobile phase showed one major spot with the R_f value of 0.35 under UV-S. Because of low quantity, no further investigation was carried out.

Subfraction HA3456 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:chloroform:ethyl acetate (2 runs) as a mobile phase displayed a long tail under UV-S. Because the ^{1}H NMR spectrum revealed the presence of **H1** as a major compound, no further investigation was conducted.

Subfraction HA346 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed two major spots with the R_f values of 0.28 and 0.42 after being visualized by anisaldehyde sulfuric acid. Because the ^1H NMR spectrum indicated the presence of **H2** as a major compound, no further investigation was conducted.

Subfraction HA347 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase demonstrated two major spots with the R_f values of 0.20 and 0.28 after being visualized by anisaldehyde sulfuric acid. Because of low quantity and signals in high field region in the ^1H NMR spectrum, it was not further purified.

Subfraction HA348 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase showed a long tail under UV-S. The ^1H NMR spectrum exhibited signals in high field region. Thus, it was not further investigated.

Subfraction HA35 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (4 runs) as a mobile phase displayed a long tail and three major spots with the R_f values of 0.70, 0.75 and 0.85 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel using 25% acetone-n-hexane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 14**.

Table 14 Subfractions obtained from **subfraction HA35** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA351	5.2	Colorless gum
HA352	3.1	Colorless gum
HA353	3.1	Colorless gum
HA354	6.7	Colorless gum
HA355	39.7	Colorless gum
HA356	33.4	Colorless gum

Table 14 (continued)

Subfraction	Weight (mg)	Physical appearance
HA357	19.3	Colorless gum
HA358	8.8	Yellow gum

Subfraction HA351 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (4 runs) as a mobile phase showed no spots under UV-S. The ^{1}H NMR spectrum exhibited the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA352 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase demonstrated two major spots with the R_f values of 0.40 and 0.50 after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel using 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 15**.

Table 15 Subfractions obtained from **subfraction HA352** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA3521	0.2	Colorless gum
HA3522	1.5	Colorless gum
HA3523	0.4	Colorless gum
HA3524	0.4	Colorless gum
HA3525	0.5	Colorless gum

Subfraction HA3521 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed a long tail after being visualized by anisaldehyde sulfuric acid. Due to minute quantity, it was not investigated.

Subfraction HA3522 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed a long

tail and one major spot with the R_f value of 0.50 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of **H1** as a major component. Therefore, it was not investigated.

Subfraction HA3523 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed a long tail and two major spots with the R_f values of 0.40 and 0.50 after being visualized by anisaldehyde sulfuric acid. Due to minute quantity, it was not investigated.

Subfraction HA3524 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed a long tail and one major spot with the R_f value of 0.40 after being visualized by anisaldehyde sulfuric acid. Due to minute quantity, it was not investigated.

Subfraction HA3525 Chromatogram characteristics on normal phase TLC with 42.5:6.5:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed no spots under UV-S. Due to minute quantity, it was not investigated.

Subfraction HA353 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (3 runs) as a mobile phase showed one major spot with the R_f value of 0.65 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed the presence of **H11** as a major compound. Therefore, it was not further investigated.

Subfraction HA354 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed one major spot with the R_f value of 0.42 after being visualized by anisaldehyde sulfuric acid. Because the ^{1}H NMR spectrum indicated the presence of **H2** as a major compound, no further investigation was conducted.

Subfraction HA355 (H2) Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed one spot with the R_f value of 0.42 after being visualized by anisaldehyde sulfuric acid.

$[\alpha]_D^{24}$		:	+141.4 (c 0.09, MeOH)
FT-IR (neat) ν_{max} cm^{-1}		:	3387 (O-H), 1736 (O-C=O), 1673 (C=C)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		:	5.31 (*brd*, *J* = 2.4 Hz, 1H), 5.24 (*dd*, *J* = 5.4 and 10.8 Hz, 1H), 5.09 (*d*, *J* = 11.7 Hz, 1H), 3.98 (*dd*, *J* = 1.5 and 6.3 Hz, 1H), 3.85 (*dd*, *J* = 6.3 and 11.7 Hz, 1H), 3.52 (*s*, 3H), 3.31 (*s*, 3H), 2.58 (*m*, 1H), 2.54 (*dd*, *J* = 5.4 and 13.2 Hz, 1H), 2.49 (*dd*, *J* = 10.8 and 13.2 Hz, 1H), 2.44 (*m*, 1H), 2.07 (*s*, 3H), 1.96 (*dd*, *J* = 9.6 and 12.3 Hz, 1H), 1.93 (*s*, 3H), 1.60 (*dd*, *J* = 6.3 and 12.3 Hz, 1H), 1.09 (*s*, 3H), 1.02 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		:	170.6, 138.0, 123.9, 116.3, 98.2, 83.2, 77.9, 73.7, 64.1, 56.2 (2x), 42.3, 41.2, 40.0, 38.0, 27.4, 23.9, 21.6, 17.8
DEPT (135^o) ($CDCl_3$)	CH	:	123.9, 116.3, 83.2, 77.9, 73.7, 64.1, 38.0
	CH_2	:	42.3, 41.2
	CH_3	:	56.2 (2x), 27.4, 23.9, 21.6, 17.8
HRESIMS *m*/*z*		:	377.1935, $C_{19}H_{30}O_6Na$, $[M+Na]^+$

Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of compound **H2**

Compound **H2** (1.4 mg, 0.004 mmol) was dissolved with CH_2Cl_2 (200 µL). Pyridine (100 µL) followed by (+)-(*R*)-MTPACl (10 µL, 0.05 mmol) were added to the solution. After stirring for 12 h at room temperature, water was added. The reaction mixture was then extracted three times with EtOAc (3 x 15 mL). The organic layer was washed gradually using 10% HCl (2 x 10 mL) then $NaHCO_3$ (2 x 10 mL) followed by water (2 x 10 mL). After solvent removal, the product was purified by preparative TLC with 25% acetone-n-hexane to obtain the (*S*)-MTPA ester (3.3 mg). The second reaction of **H2** (2.8 mg, 0.008 mmol) with (-)-(*S*)-MTPACl (15 µL, 0.08 mmol) was conducted using the same procedure as the first reaction. The (*R*)-MTPA ester (4.0 mg) was obtained after purification using preparative TLC with the same mobile phase.

Subfraction HA356 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed one major spot with the R_f value of 0.42 after being visualized by anisaldehyde sulfuric acid. Because the ^{1}H NMR spectrum indicated the presence of **H2** as a major compound, no further investigation was conducted.

Subfraction HA357 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed one major spot with the R_f value of 0.33 under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not further purified.

Subfraction HA358 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed one major spot with the R_f value of 0.33 under UV-S. The ^{1}H NMR spectrum exhibited signals of **H8**. Therefore, it was not further purified.

Subfraction HA36 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (7 runs) as a mobile phase demonstrated a long tail and five major spots with the R_f values of 0.13, 0.15, 0.22, 0.28 and 0.38 under UV-S and five major spots with the R_f values of 0.34, 0.40, 0.47, 0.51 and 0.58 after being visualized by anisaldehyde sulfuric acid. It was further purified by flash column chromatography over silica gel using 25% acetone-n-hexane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 16**.

Table 16 Subfractions obtained from **subfraction HA36** by flash column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA361	2.0	Yellow gum
HA362	5.6	Yellow gum
HA363	28.5	Yellow gum
HA364	10.9	Colorless gum
HA365	25.6	Yellow gum

Table 16 (continued)

Subfraction	Weight (mg)	Physical appearance
HA366	11.7	Yellow gum
HA367	30.5	Yellow gum

Subfraction HA361 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed one major spot with the R_f value of 0.33 under UV-S. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, it was not further investigated.

Subfraction HA362 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed five major spots with the R_f values of 0.36, 0.40, 0.47, 0.51 and 0.58 after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not further investigated.

Subfraction HA363 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Because the ^{1}H NMR spectrum indicated the presence of **H2** as a major compound, no further investigation was conducted.

Subfraction HA364 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform as a mobile phase demonstrated four major spots with the R_f values of 0.33, 0.48, 0.53 and 0.93 under UV-S and a long tail after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel using 25% ethyl acetate-chloroform as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 17**.

Table 17 Subfractions obtained from **subfraction HA364** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA3641	1.4	Colorless gum
HA3642	0.2	Colorless gum
HA3643	1.8	Colorless gum

Table 17 (continued)

Subfraction	Weight (mg)	Physical appearance
HA3644	0.8	Colorless gum
HA3645	2.0	Colorless gum
HA3646	3.6	Colorless crystals
HA3647	1.0	Yellow gum

Subfraction HA3641 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform as a mobile phase displayed one major spot with the R_f value of 0.93 under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Therefore, no further investigation was carried out.

Subfraction HA3642 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform as a mobile phase displayed one major spot under UV-S with the R_f value of 0.53. Due to minute quantity, it was not further purified.

Subfraction HA3643 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform as a mobile phase displayed two major spots under UV-S with the R_f values of 0.48 and 0.33. Due to minute quantity, it was not further purified.

Subfraction HA3644 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform as a mobile phase displayed one major spot under UV-S with the R_f value of 0.33. Due to minute quantity, it was not further purified.

Subfraction HA3645 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed one major spot with the R_f value of 0.33 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because the ^{1}H NMR spectrum indicated the presence of **H3** as a major compound, no further investigation was conducted.

Subfraction HA3646 (H3) Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed one spot with the R_f value of 0.33 under UV-S and after being visualized by anisaldehyde sulfuric acid.

Melting point (°C)		:	172-173
$[\alpha]_D^{24}$		:	+46.7 (c 0.09, MeOH)
FT-IR (neat) ν_{max} cm^{-1}		:	3394 (O-H), 1735 (O-C=O)
^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)		:	5.29 (*dd*, *J* = 2.4 and 4.5 Hz, 1H), 5.00 (*dd*, *J* = 7.0 and 9.6 Hz, 1H), 4.18 (*t*, *J* = 7.0 Hz, 1H), 3.44 (*s*, 3H), 3.27 (*t*, *J* = 7.0 Hz, 1H), 2.78 (*dt*, *J* = 3.6 and 7.0 Hz, 1H), 2.72 (*ddd*, *J* = 3.6, 6.0 and 9.5 Hz, 1H), 2.30 (*dd*, *J* = 7.0 and 9.6 Hz, 1H), 2.11 (*dd*, *J* = 4.5 and 14.7 Hz, 1H), 2.07 (*s*, 3H), 2.01 (*dd*, *J* = 9.5 and 12.0 Hz, 1H), 1.98 (*dd*, *J* = 2.4 and 14.7 Hz, 1H), 1.42 (*dd*, *J* = 6.0 and 12.0 Hz, 1H), 1.16 (*s*, 3H), 1.06 (*s*, 3H), 1.03 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		:	170.3, 94.0, 88.7, 84.1, 76.9, 74.5, 74.0, 59.3, 57.7, 56.6, 40.4, 38.1, 37.3, 34.6, 29.4, 26.0, 24.3, 21.5
DEPT (135°) ($CDCl_3$)	CH	:	88.7, 84.1, 76.9, 74.0, 59.3, 56.6, 34.6
	CH_2	:	40.4, 38.1
	CH_3	:	57.7, 29.4, 26.0, 24.3, 21.5
HRESIMS *m/z*		:	363.1778, $C_{18}H_{28}O_6Na$, $[M+Na]^+$

Subfraction HA3647 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed a tailing spot under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to low quantity, it was not further investigated.

Subfraction HA365 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed two major spots with the R_f values of 0.15 and 0.33 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because the ^1H NMR spectrum indicated the presence of **H3** as a major compound, no further investigation was conducted.

Subfraction HA366 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed one major spot with the R_f value of 0.13 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because the ^1H NMR spectrum indicated the absence of major components. Due to low quantity, it was not further purified.

Subfraction HA367 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Because the ^1H NMR spectrum showed signals in high field region. Thus, it was not further purified.

Subfraction HA37 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase exhibited a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. It was subjected to column chromatography over silica gel using a gradient solvent system, starting from 25% acetone-n-hexane until pure methanol. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford eight subfractions as shown in **Table 18**.

Table 18 Subfractions obtained from **subfraction HA37** by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
HA371	25% acetone-n-hexane	11.0	Yellow gum
HA372	25% acetone-n-hexane	13.1	Yellow gum
HA373	50% acetone-n-hexane	15.2	Yellow gum
HA374	50% acetone-n-hexane	73.3	Yellow gum
HA375	50% acetone-n-hexane	32.1	Yellow gum
HA376	50% acetone-n-hexane	24.6	Yellow gum
HA377	50-75% acetone-n-hexane	40.7	Yellow gum
HA378	100% acetone and 100% methanol	70.9	Yellow gum

Subfraction HA371 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed no spots under UV-S. Because of low quantity, it was not further investigated.

Subfraction HA372 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase displayed two major spots with the R_f values of 0.28 and 0.33 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of **H3** as a major component. Thus, it was not further investigated.

Subfraction HA373 Chromatogram characteristics on normal phase TLC with 50% acetone-n-hexane as a mobile phase showed a long tail under UV-S. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, no attempts were made to purify this subfraction.

Subfraction HA374 Chromatogram characteristics on normal phase TLC with 50% acetone-n-hexane as a mobile phase showed a long tail under UV-S. The ^{1}H NMR spectrum indicated the presence of aromatic and olefinic protons. Further purification was performed by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane. Subfractions displaying similar components were combined and evaporated to dryness under reduced pressure to yield three subfractions as shown in **Table 19**.

Table 19 Subfractions obtained from **subfraction HA374** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HA3741	12.6	Yellow gum
HA3742	46.1	Yellow gum
HA3743	1.3	Yellow gum

Subfraction HA3741 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed a long tail after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HA3742 Chromatogram characteristics on normal phase TLC with 5:3:1:1 n-hexane:chloroform:ethyl acetate:acetone as a mobile phase showed a long tail under UV-S and six major spots with the R_f values of 0.28, 0.48, 0.55, 0.65, 0.73 and 0.83 after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over silica gel using 5:3:1:1 n-hexane:chloroform:ethyl acetate:acetone as an eluent. Subfractions displaying similar components were combined and evaporated to dryness under reduced pressure to yield four subfractions as shown in **Table 20**.

Table 20 Subfractions obtained from **subfraction HA3742** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA37421	3.2	Yellow gum
HA37422	2.2	Yellow gum
HA37423	2.9	Yellow gum
HA37424	37.7	Yellow gum

Subfraction HA37421 Chromatogram characteristics on normal phase TLC with 5:3:1:1 n-hexane:chloroform:ethyl acetate:acetone as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not further purified.

Subfraction HA37422 Chromatogram characteristics on normal phase TLC with 5:3:1:1 n-hexane:chloroform:ethyl acetate:acetone as a mobile phase showed one major spot with the R_f value of 0.48 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not further purified.

Subfraction HA37423 Chromatogram characteristics on normal phase TLC with 5:3:1:1 n-hexane:chloroform:ethyl acetate:acetone as a mobile phase showed one major spot with the R_f value of 0.35 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not further purified.

Subfraction HA37424 Chromatogram characteristics on normal phase TLC with 5:2:2:1 n-hexane:chloroform:ethyl acetate:acetone as a mobile phase showed three

major spots with the R_f values of 0.15, 0.28 and 0.53 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was further investigated by column chromatography over silica gel with 5:2:2:1 n-hexane:chloroform:ethyl acetate:acetone as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 21**.

Table 21 Subfractions obtained from **subfraction HA37424** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA37424A	2.4	Yellow gum
HA37424B	3.0	Yellow gum
HA37424C	3.1	Yellow gum
HA37424D	3.0	Yellow gum
HA37424E	16.0	Yellow gum
HA37424F	10.2	Yellow gum

Subfraction HA37424A Chromatogram characteristics on normal phase TLC with 5:2:2:1 n-hexane:chloroform:ethyl acetate:acetone as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not further purified.

Subfraction HA37424B Chromatogram characteristics on normal phase TLC with 5:2:2:1 n-hexane:chloroform:ethyl acetate:acetone as a mobile phase showed one major spot with the R_f value of 0.60 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not further investigated.

Subfraction HA37424C Chromatogram characteristics on normal phase TLC with 5:2:2:1 n-hexane:chloroform:ethyl acetate:acetone as a mobile phase showed three major spots with the R_f values of 0.48, 0.55 and 0.80 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity and the absence of aromatic and olefinic proton signals in the ^{1}H NMR spectrum, it was not further purified.

Subfraction HA37424D Chromatogram characteristics on normal phase TLC with 5:2:2:1 n-hexane:chloroform:ethyl acetate:acetone as a mobile phase showed four major spots with the R_f values of 0.33, 0.43, 0.50 and 0.80 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity and the presence of many components without major components in the ^{1}H NMR spectrum, it was not further investigated.

Subfraction HA37424E Chromatogram characteristics on normal phase TLC with 50% chloroform-ethyl acetate as a mobile phase showed four major spots with the R_f values of 0.43, 0.60, 0.70 and 0.75 under UV-S and after being visualized by anisaldehyde sulfuric acid. Purification was performed by column chromatography over silica gel. Elution was conducted with 50% chloroform-ethyl acetate. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give seven subfractions as shown in **Table 22**.

Table 22 Subfractions obtained from **subfraction HA37424E** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA37424E1	1.0	Colorless gum
HA37424E2	0.9	Colorless gum
HA37424E3	1.1	Colorless gum
HA37424E4	6.2	Colorless gum
HA37424E5	0.2	Colorless gum
HA37424E6	4.1	Colorless gum
HA37424E7	2.3	Colorless gum

Subfraction HA37424E1 Chromatogram characteristics on normal phase TLC with 50% chloroform-ethyl acetate as a mobile phase showed no spots under UV-S. Therefore, no further investigation was carried out.

Subfraction HA37424E2 Chromatogram characteristics on normal phase TLC with 50% chloroform-ethyl acetate as a mobile phase showed one major spot with the R_f

value of 0.55 under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Because of minute quantity, no further investigation was carried out.

Subfraction HA37424E3 Chromatogram characteristics on normal phase TLC with 50% chloroform-ethyl acetate as a mobile phase showed three major spots with the R_f values of 0.28, 0.40 and 0.50 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Because of minute quantity, no further investigation was performed.

Subfraction HA37424E4 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol (2 runs) as a mobile phase displayed one major spot under UV-S with the R_f value of 0.48. It was dissolved with n-hexane to give a n-hexane soluble part (**HA37424E4A**) and a n-hexane insoluble one (**HA37424E4B**) as shown in **Table 23**.

Table 23 Subfractions obtained from **subfraction HA37424E4** by dissolving with n-hexane

Subfraction	Weight (mg)	Physical appearance
HA37424E4A	0.2	Colorless gum
HA37424E4B	6.0	Colorless gum

Subfraction HA37424E4A Chromatogram characteristics on normal phase TLC 18:1:1 dichloromethane:ethyl acetate:methanol (2 runs) as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not further purified.

Subfraction HA37424E4B (H4) Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol (2 runs) as a mobile phase showed one spot under UV-S with the R_f value of 0.48.

$[\alpha]_D^{24}$	:	-121.3 (c 0.05, MeOH)
UV: λ_{max} (nm) (MeOH) (log ε)	:	277 (3.87)
FT-IR (neat) ν_{max} cm^{-1}	:	3420 (O-H), 1653 (C=O)

^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		:	4.94 (*septt*, *J* = 1.2 and 8.4 Hz, 1H), 3.77 (*ddd*, *J* = 5.7, 9.9 and 10.2 Hz, 1H), 3.42 (*d*, *J* = 9.9 Hz, 1H), 2.93 (*dd*, *J* = 5.7 and 15.9 Hz, 1H), 2.65 (*dd*, *J* = 8.4 and 14.4 Hz, 1H), 2.57 (*d*, *J* = 16.5 Hz, 1H), 2.53 (*dd*, *J* = 8.4 and 14.4 Hz, 1H), 2.47 (*d*, *J* = 16.5 Hz, 1H), 1.92 (*dd*, *J* = 10.2 and 15.9 Hz, 1H), 1.71 (*brs*, 3H), 1.69 (*brs*, 3H), 1.46 (*s*, 3H), 1.41 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		:	192.3, 164.8, 137.0, 117.4, 108.2, 80.6, 74.8, 74.5, 67.8, 47.4, 34.5, 27.4, 26.5, 26.1, 24.8, 18.1
DEPT (135^{o}) ($CDCl_3$)	CH	:	117.4, 74.5, 67.8
	CH_2	:	47.4, 34.5, 26.5
	CH_3	:	27.4, 26.1, 24.8, 18.1
HRESIMS *m*/*z*		:	319.1530, $C_{16}H_{24}O_5Na$, $[M+Na]^+$
CD $\Delta\varepsilon$ (λ nm)		:	+18.0 (187), -7.2 (274), -3.0 (324) (c 0.0009 M, MeOH)

Subfraction HA37424E5 Chromatogram characteristics on normal phase TLC with 50% chloroform-ethyl acetate as a mobile phase demonstrated one major spot with the R_f value of 0.23 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed the presence of many components without major components. Because of minute quantity, no further investigation was carried out.

Subfraction HA37424E6 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase displayed two major spots with the R_f values of 0.60 and 0.70. Purification was conducted by preparative TLC using 18:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase to afford three subfractions as shown in **Table 24**.

Table 24 Subfractions obtained from **subfraction HA37424E6** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA37424E6A	0.4	Colorless gum
HA37424E6B	2.2	Colorless gum
HA37424E6C	0.3	Colorless gum

Subfraction HA37424E6A Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase demonstrated one major spot with the R_f value of 0.47 after being visualized by anisaldehyde sulfuric acid. Because of minute quantity. Thus, no further investigation was carried out.

Subfraction HA37424E6B Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase demonstrated one major spot with the R_f value of 0.43 after being visualized by anisaldehyde sulfuric acid. It was dissolved with n-hexane to give a n-hexane soluble part (**HA37424E6B1**) and a n-hexane insoluble one (**HA37424E6B2**) as shown in **Table 25**.

Table 25 Subfractions obtained from **subfraction HA37424E6B** by dissolving with n-hexane

Subfraction	Weight (mg)	Physical appearance
HA37424E6B1	0.8	Colorless gum
HA37424E6B2	1.3	Colorless gum

Subfraction HA37424E6B1 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase displayed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed signals in high field region. Thus, no further investigation was carried out.

Subfraction HA37424E6B2 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase displayed one major spot with the R_f value of 0.43 after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, no further investigation was carried out.

Subfraction HA37424E6C Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase displayed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, further investigation was not conducted.

Subfraction HA37424E7 Chromatogram characteristics on normal phase TLC with 50% chloroform-ethyl acetate as a mobile phase demonstrated one major spot with the R_f value of 0.23 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed the presence of many components without major components. Because of low quantity, no further investigation was carried out.

Subfraction HA37424F Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail and one major spot with the R_f value of 0.80 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed signals in high field region. Thus, it was not purified.

Subfraction HA3743 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not further purified.

Subfraction HA375 Chromatogram characteristics on normal phase TLC with 50% acetone-n-hexane as a mobile phase showed a long tail under UV-S. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, no attempts were made to purify this subfraction.

Subfraction HA376 Chromatogram characteristics on normal phase TLC with 17:2:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase showed a long tail and one major spot with the R_f value of 0.38 under UV-S. It was further purified by column chromatography over silica gel. The eluent was 17:2:1 dichloromethane:ethyl acetate:methanol. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 26**.

Table 26 Subfractions obtained from **subfraction HA376** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA3761	3.0	Yellow gum
HA3762	5.0	Yellow gum
HA3763	3.4	Yellow gum
HA3764	4.1	Yellow gum
HA3765	2.4	Yellow gum
HA3766	4.7	Yellow gum

Subfraction HA3761 Chromatogram characteristics on normal phase TLC with 17:2:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase demonstrated a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not investigated.

Subfraction HA3762 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (4 runs) as a mobile phase exhibited two major spots under UV-S with the R_f values of 0.75 and 0.83 as well as a long tail after being visualized by anisaldehyde sulfuric acid. It was subjected to preparative TLC using 5% methanol-dichloromethane (4 runs) as a mobile phase to afford two subfractions as shown in **Table 27**.

Table 27 Subfractions obtained from **subfraction HA3762** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA3762A	1.1	Colorless gum
HA3762B	0.9	Colorless gum

Subfraction HA3762A Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (4 runs) as a mobile phase exhibited four major spots under UV-S with the R_f values of 0.45, 0.50, 0.68 and 0.80. Because of the minute quantity, it was not investigated.

Subfraction HA3762B Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (4 runs) as a mobile phase exhibited four major spots under UV-S with the R_f values of 0.30, 0.38, 0.45 and 0.60. Because of the minute quantity, it was not investigated.

Subfraction HA3763 Chromatogram characteristics on normal phase TLC with 17:2:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase demonstrated a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not futher purified.

Subfraction HA3764 Chromatogram characteristics on normal phase TLC with 17:2:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase demonstrated a long tail and one major spot with the R_f value of 0.38 under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Because of low quantity, it was not investigated.

Subfraction HA3765 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.73 under UV-S and one major spot with the R_f value of 0.63 after being visualized by anisaldehyde sulfuric acid. Purification was performed by preparative TLC using 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase to afford two subfractions as shown in **Table 28**.

Table 28 Subfractions obtained from **subfraction HA3765** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA3765A	0.3	Colorless gum
HA3765B	2.0	Colorless gum

Subfraction HA3765A Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase exhibited one major spot under UV-S with the R_f value of 0.73. Because of minute quantity, it was not investigated.

Subfraction HA3765B Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase exhibited one major

spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.63. It was dissolved with n-hexane to give a n-hexane soluble part (**HA3765B1**) and a n-hexane insoluble one (**HA3765B2**) as shown in **Table 29**.

Table 29 Subfractions obtained from **subfraction HA3765B** by dissolving with n-hexane

Subfraction	Weight (mg)	Physical appearance
HA3765B1	0.7	Colorless gum
HA3765B2	1.2	Colorless gum

Subfraction HA3765B1 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, no further investigation was carried out.

Subfraction HA3765B2 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase showed one major spot with the R_f value of 0.63 after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, no further investigation was carried out.

Subfraction HA3766 Chromatogram characteristics on normal phase TLC with 17:2:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase demonstrated a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not investigated.

Subfraction HA377 Chromatogram characteristics on normal phase TLC with 50% acetone-n-hexane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Because the ^{1}H NMR spectrum indicated the presence of **H14** as a major compound, no further investigation was conducted.

Subfraction HA378 Chromatogram characteristics on normal phase TLC with 50% acetone-n-hexane as a mobile phase showed a long tail under UV-S. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, no further purification was performed.

Subfraction HA38 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase exhibited a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to yield a chloroform soluble part (**HA38A**) and a chloroform insoluble one (**HA38B**) as shown in **Table 30**.

Table 30 Subfractions obtained from **subfraction HA38** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HA38A	159.8	Brown gum
HA38B	143.9	Brown gum

Subfraction HA38A Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase demonstrated a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not investigated.

Subfraction HA38B Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed signals in high field region. Thus, it was not purified.

Fraction HA4 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S and ten major spots with the R_f values of 0.13, 0.20, 0.25, 0.33, 0.50, 0.55, 0.68, 0.75, 0.80 and 0.87 after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel using 2% methanol-dichloromethane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 31**.

Table 31 Subfractions obtained from **fraction HA4** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA41	20.6	Yellow gum
HA42	298.3	Brown gum
HA43	61.6	Brown gum
HA44	1437.3	Dark brown gum

Subfraction HA41 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated two major spots under UV-S with the R_f values of 0.73 and 0.75. It was purified by preparative TLC using 100% dichloromethane as a mobile phase to afford five subfractions as shown in **Table 32**.

Table 32 Subfractions obtained from **subfraction HA41** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA411	2.3	Yellow gum
HA412	3.8	Pale yellow solid
HA413	4.9	Yellow gum
HA414	5.2	Yellow gum
HA415	4.3	Yellow gum

Subfraction HA411 Chromatogram characteristics on normal phase TLC 100% dichloromethane as a mobile phase exhibited no spots under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic protons. Thus, no attempts were made to purify this subfraction.

Subfraction HA412 (H5) Chromatogram characteristics on normal phase TLC 100% dichloromethane as a mobile phase exhibited one spot under UV-S with the R_f value of 0.45.

Melting point (°C) : 188-190

UV: λ_{max} (nm) (MeOH) (log ε) : 234 (3.63), 303 (3.39), 352 (2.99)

FT-IR (neat) ν_{max} cm^{-1}		:	3429 (O-H), 1733 (O-C=O), 1650 (C=O)
^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)		:	12.28 (*s*, 1H), 6.90 (*d*, *J* = 2.7 Hz, 1H), 6.87 (*d*, *J* = 2.7 Hz, 1H), 6.70 (*brs*, 1H), 6.61 (*brs*, 1H), 4.02 (*s*, 3H), 3.94 (*s*, 3H), 2.42 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		:	179.7, 169.3, 164.7, 161.6, 158.1, 155.8, 148.6, 135.2, 112.1, 111.8, 111.4, 107.2, 106.7, 101.5, 56.2, 53.1, 22.5
DEPT (135°) ($CDCl_3$)	CH	:	112.1, 111.8, 107.2, 101.5
	CH_3	:	56.2, 53.1, 22.5

Subfraction HA413 Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane as a mobile phase exhibited one major spot under UV-S with the R_f value of 0.58. It was dissolved with n-hexane to yield a n-hexane soluble part (**HA413A**) and a n-hexane insoluble one (**HA413B**) as shown in **Table 33**.

Table 33 Subfractions obtained from **subfraction HA413** by dissolving with n-hexane

Subfraction	Weight (mg)	Physical appearance
HA413A	2.0	Yellow gum
HA413B	2.7	Yellow gum

Subfraction HA413A Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane as a mobile phase exhibited one major spot under UV-S with the R_f value of 0.58. The ^1H NMR spectrum displayed the presence of **H6** as a major compound. Because of the minute quantity, it was not further purified.

Subfraction HA413B (H6) Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane as a mobile phase exhibited one spot under UV-S with the R_f value of 0.58.

UV (MeOH) λ_{max} nm (log ε)	:	238 (3.79), 275 (3.91), 386 (3.46)
FT-IR (neat) ν_{max} cm^{-1}	:	3400 (O-H), 1637 (C=O)

^{1}H NMR (acetone-d_6) (δ ppm) (500 MHz)		:	11.03 (s, 1H), 7.50 (s, 1H), 7.05 (*d*, *J* = 8.5 Hz, 1H), 6.31 (*d*, *J* = 8.5 Hz, 1H), 2.86 (*s*, 2H), 1.48 (*s*, 6H)
^{13}C NMR (acetone-d_6) (δ ppm) (125 MHz)		:	199.5, 155.0, 147.4, 139.1, 125.7, 108.3, 107.9, 80.5, 48.8, 26.6 (2x)
DEPT (135^{o}) (acetone-d_6)	CH	:	125.7, 107.9
	CH_2	:	48.8
	CH_3	:	26.6 (2x)

Subfraction HA414 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed two major spots under UV-S with the R_f values of 0.63 and 0.88. Further purification using preparative TLC was performed with 100% dichloromethane as a mobile phase to afford three subfractions as shown in **Table 34**.

Table 34 Subfractions obtained from **subfraction HA414** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA414A	2.1	White solid
HA414B	2.0	White solid
HA414C	1.1	Brown solid

Subfraction HA414A Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed one major spot with the R_f value of 0.88 under UV-S. The ^{1}H NMR spectrum indicated the absence of major components. Due to low quantity, it was not further purified.

Subfraction HA414B Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed one major spot under UV-S with the R_f value of 0.13. The ^{1}H NMR spectrum revealed the presence of **H16** as a major compound. Thus, no further investigation was not conducted.

Subfraction HA414C Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated a long tail near the baseline under

UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA415 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed a long tail and no major spots under UV-S. Because of low quantity, it was not investigated.

Subfraction HA42 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed five major spots under UV-S with the R_f values of 0.35, 0.50, 0.63, 0.73 and 0.75. It was purified over silica gel using 2% methanol-dichloromethane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford ten subfractions as shown in **Table 35**.

Table 35 Subfractions obtained from **subfraction HA42** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA421	2.0	Yellow gum
HA422	4.1	Yellow gum
HA423	16.0	Yellow gum
HA424	18.2	Yellow gum
HA425	31.3	Brown gum
HA426	55.0	Brown gum
HA427	98.8	Brown gum
HA428	15.0	Brown gum
HA429	10.8	Brown gum
HA4210	15.4	Dark brown gum

Subfraction HA421 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed one major spot with the R_f value of 0.80 under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Therefore, no further investigation was carried out.

Subfraction HA422 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed four major spots under UV-S with the R_f values of 0.48, 0.58, 0.78 and 0.80. The ^{1}H NMR spectrum indicated the presence of a mixture of **H5** and **H13**. Therefore, no further investigation was carried out.

Subfraction HA423 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated three major spots under UV-S with the R_f values of 0.30, 0.58 and 0.63. It was further purified by preparative TLC with 100% dichloromethane as a mobile phase to afford three subfractions as shown in **Table 36**.

Table 36 Subfractions obtained from **subfraction HA423** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4231	0.5	Yellow gum
HA4232	1.9	Pale yellow solid
HA4233	2.5	Pale yellow solid

Subfraction HA4231 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed one major spot under UV-S with the R_f value of 0.45. The ^{1}H NMR spectrum indicated that it contained **H5** as a major compound.

Subfraction HA4232 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed one major spot under UV-S with the R_f value of 0.40. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to minute quantity, it was not further purified.

Subfraction HA4233 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed one major spot under UV-S with the R_f value of 0.28. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to low quantity, it was not further purified.

Subfraction HA424 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated two major spots under UV-S with the R_f values of 0.50 and 0.65. It was further purified by column chromatography over silica gel. Elution was conducted with 100% dichloromethane. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford two subfractions as shown in **Table 37**.

Table 37 Subfractions obtained from **subfraction HA424** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA4241	1.4	Yellow gum
HA4242	16.5	Yellow gum

Subfraction HA4241 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HA4242 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase demonstrated two major spots under UV-S with the R_f values of 0.15 and 0.23. It was further purified by column chromatography over silica gel. Elution was conducted with 1% methanol-dichloromethane. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give six subfractions as shown in **Table 38**.

Table 38 Subfractions obtained from **subfraction HA4242** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA4242A	2.2	White solid
HA4242B	3.0	Yellow gum
HA4242C	6.6	White solid
HA4242D	2.1	White solid
HA4242E	0.2	Yellow gum

Table 38 (continued)

Subfraction	Weight (mg)	Physical appearance
HA4242F	2.3	Yellow gum

Subfraction HA4242A Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase displayed no spots under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HA4242B Characteristics on normal phase TLC with 30% acetone-n-hexane (2 runs) as a mobile phase exhibited two major spots after being visualized by ceric ammonium molybdate with the R_f values of 0.73 and 0.78. It was subjected to preparative TLC using 30% acetone-n-hexane as a mobile phase to afford two subfractions as shown in **Table 39**.

Table 39 Subfractions obtained from **subfraction HA4242B** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4242B1	0.6	Colorless gum
HA4242B2	2.2	Colorless gum

Subfraction HA4242B1 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (2 runs) as a mobile phase displayed one major spot under UV-S with the R_f value of 0.78. Because of minute quantity, no further investigation was carried out.

Subfraction HA4242B2 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (2 runs) as a mobile phase displayed one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.75. The ^{1}H NMR spectrum revealed that it contained **H7** as a major compound.

Subfraction HA4242C Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (2 runs) as a mobile phase showed one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.73. Further purification using preparative TLC was performed with 30% acetone-n-hexane (2 runs) as a mobile phase to afford one **subfraction HA4242CA (H7)** which was a colorless solid (4.5 mg).

The chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (2 runs) as a mobile phase displayed one spot after being visualized by ceric ammonium molybdate with the R_f value of 0.75.

Melting point (°C)		:	103-105
$[\alpha]_D^{24}$		:	+123.3 (c 0.06, MeOH)
FT-IR (neat) ν_{max} cm^{-1}		:	1736 (O-C=O)
^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)		:	5.24 (*dd*, 4.5 and 7.8 Hz, 1H), 5.21 (*dd*, 7.2 and 8.4 Hz, 1H), 4.27 (*t*, *J* = 2.7 Hz, 1H), 3.99 (*t*, *J* = 2.7 Hz, 1H), 3.30 (*s*, 3H), 2.74 (*t*, *J* = 8.7 Hz, 1H), 2.59 (*dd*, *J* = 2.7 and 8.1 Hz, 1H), 2.30 (*dd*, *J* = 7.2 and 14.1 Hz, 1H), 2.29 (*m*, 1H), 2.03 (*s*, 3H), 1.87 (*dd*, *J* = 8.4 and 14.1 Hz, 1H), 1.74 (*dd*, *J* = 8.7 and 10.8 Hz, 1H), 1.62 (*dd*, *J* = 9.0 and 10.8 Hz, 1H), 1.21 (*s*, 3H), 1.13 (*s*, 3H), 1.09 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		:	170.3, 94.5, 88.8, 87.9, 79.7, 76.9, 75.9, 57.4, 51.7, 49.9, 41.3, 38.6, 38.5, 37.4, 31.5, 25.1, 23.0, 21.3
DEPT (135°) ($CDCl_3$)	CH	:	88.8, 87.9, 76.9, 75.9, 51.7, 49.9, 38.6
	CH_2	:	41.3, 37.4
	CH_3	:	57.4, 31.5, 25.1, 23.0, 21.3

Subfraction HA4242D Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase showed one major spot under UV-S with the R_f value of 0.45. The ^1H NMR spectrum indicated the absence of major compounds. Due to low quantity, it was not further purified.

Subfraction HA4242E Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase showed four major spots under UV-S with the R_f values of 0.13, 0.45, 0.63 and 0.70. The ^1H NMR spectrum indicated the absence of major components. Due to minute quantity, it was not further purified.

Subfraction HA4242F Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase demonstrated no spots under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA425 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated two major spots under UV-S with the R_f values of 0.40 and 0.65. It was further purified by column chromatography over silica gel. Elution was conducted with 100% dichloromethane. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to yield three subfractions as shown in **Table 40**.

Table 40 Subfractions obtained from **subfraction HA425** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA4251	5.2	Brown gum
HA4252	1.0	Yellow gum
HA4253	25.0	Brown gum

Subfraction HA4251 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HA4252 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated one major spot under UV-S with the R_f value of 0.28. The ^{1}H NMR spectrum indicated the absence of major components. Due to minute quantity, it was not further purified.

Subfraction HA4253 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase demonstrated six major spots under UV-S with the R_f values of 0.30, 0.40, 0.45, 0.53, 0.68 and 0.95 as well as one additional spot with the R_f value of 0.43 after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel. Elution was conducted with 1% methanol-dichloromethane. Subfractions containing similar components were

combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 41**.

Table 41 Subfractions obtained from **subfraction HA4253** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA4253A	1.8	Yellow gum
HA4253B	1.5	Yellow gum
HA4253C	17.7	Yellow gum
HA4253D	1.3	Yellow gum
HA4253E	2.6	Dark yellow gum

Subfraction HA4253A Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase showed two major spots under UV-S with the R_f values of 0.68 and 0.95. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to minute quantity, it was not further purified.

Subfraction HA4253B Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase showed two major spots under UV-S with the R_f values of 0.45 and 0.53. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to minute quantity, it was not further purified.

Subfraction HA4253C Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:n-hexane:chloroform (5 runs) as a mobile phase demonstrated three major spots after being visualized by ceric ammonium molybdate with the R_f values of 0.50, 0.65 and 0.73. It was further purified by preparative TLC using 18:1:1 dichloromethane:n-hexane:chloroform as a mobile phase to afford three subfractions as shown in **Table 42**.

Table 42 Subfractions obtained from **subfraction HA4253C** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4253C1	4.0	Yellow gum
HA4253C2	4.9	Colorless gum

Table 42 (continued)

Subfraction	Weight (mg)	Physical appearance
HA4253C3	4.2	Yellow gum

Subfraction HA4253C1 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:n-hexane:chloroform (5 runs) as a mobile phase demonstrated one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.73. The ^{1}H NMR spectrum indicated the absence of major components. Due to low quantity, it was not further purified.

Subfraction HA4253C2 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:n-hexane:chloroform (5 runs) as a mobile phase showed one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.65. The ^{1}H NMR spectrum revealed the presence of **H7** as a major compound. Thus, it was not further purified.

Subfraction HA4253C3 Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:n-hexane:chloroform (5 runs) as a mobile phase demonstrated one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.50. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to low quantity, it was not further purified.

Subfraction HA4253D Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase showed two major spots under UV-S with the R_f values of 0.30 and 0.40. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to minute quantity, it was not further purified.

Subfraction HA4253E Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase demonstrated a long tail near the baseline under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA426 Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane (2 runs) as a mobile phase demonstrated two major spots under UV-S with the R_f values of 0.28 and 0.50. It was further purified by column chromatography

over silica gel. Elution was conducted with 20% acetone-n-hexane. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give three subfractions as shown in **Table 43**.

Table 43 Subfractions obtained from **subfraction HA426** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA4261	39.4	Yellow gum
HA4262	10.3	Yellow gum
HA4263	5.2	Brown solid

Subfraction HA4261 Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane (2 runs) as a mobile phase showed one major spot under UV-S with the R_f value of 0.13. The ^{1}H NMR spectrum revealed the presence of **H14** as a major compound. Thus, it was not further investigated.

Subfraction HA4262 Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane (2 runs) as a mobile phase demonstrated three major spots under UV-S with the R_f values of 0.25, 0.33 and 0.40. The ^{1}H NMR spectrum indicated the absence of major components. Thus, it was not further purified.

Subfraction HA4263 Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane (2 runs) as a mobile phase demonstrated a long tail near the baseline under UV-S. The ^{1}H NMR spectrum indicated the absence of major components. Thus, it was not further purified.

Subfraction HA427 Chromatogram characteristics on reverse phase TLC with 50% methanol-water (2 runs) as a mobile phase demonstrated two major spots under UV-S with the R_f values of 0.30 and 0.35 as well as one additional spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.85. It was further purified by column chromatography over reverse phase C_{18} silica gel. Elution was conducted with 50% methanol-water. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford seven subfractions as shown in **Table 44**.

Table 44 Subfractions obtained from **subfraction HA427** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HA4271	9.9	Colorless gum
HA4272	17.6	Yellow gum
HA4273	5.6	Yellow gum
HA4274	21.8	Dark yellow gum
HA4275	4.3	Yellow gum
HA4276	7.1	Yellow gum
HA4277	31.0	Brown gum

Subfraction HA4271 (H8) Chromatogram characteristics on reverse phase TLC with 50% methanol-water (2 runs) as a mobile phase demonstrated one spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.85.

$[\alpha]_D^{24}$: -40.9 (c 1.00, MeOH)

FT-IR (neat) ν_{max} cm^{-1} : 3443 (O-H), 1746 (O-C=O), 1713 (C=O)

^{1}H NMR (acetone-d_6) (δ ppm) (300 MHz) : 5.11 (*dqd*, *J* = 1.2, 6.6 and 11.4 Hz, 1H), 4.31 (*brs*, 1H), 3.74 (*m*, 1H), 3.50 (*d*, *J* = 14.4 Hz, 1H), 3.43 (*d*, *J* = 14.4 Hz, 1H), 3.09 (*ddd*, *J* = 4.2, 4.2 and 11.4 Hz, 1H), 2.96 (*dd*, *J* = 5.7 and 13.5 Hz, 1H), 2.88 (*dd*, *J* = 4.2 and 9.0 Hz, 1H), 2.66 (*dd*, *J* = 3.6 and 13.5 Hz, 1H), 2.35 (*ddd*, *J* = 1.2, 4.2 and 14.4 Hz, 1H), 1.47 (*dt*, *J* = 11.4 and 14.4 Hz, 1H), 1.28 (*d*, *J* = 6.6 Hz, 3H)

^{13}C NMR (acetone-d_6) (δ ppm) (75 MHz) : 201.2, 166.4, 69.6, 68.2, 61.4, 56.1, 52.4, 49.9, 37.4, 20.8

DEPT (135°) (acetone-d_6)	CH	:	69.6, 68.2, 61.4, 56.1
	CH_2	:	52.4, 49.9, 37.4
	CH_3	:	20.8

Subfraction HA4272 Chromatogram characteristics on reverse phase TLC with 50% methanol-water (2 runs) as a mobile phase demonstrated two major spots under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.38 and 0.85. It was further purified by column chromatography over reverse phase C_{18} silica gel. Elution was conducted with 50% methanol-water. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 45**.

Table 45 Subfractions obtained from **subfraction HA4272** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HA4272A	1.6	Dark yellow gum
HA4272B	2.0	Yellow gum
HA4272C	7.0	Yellow gum
HA4272D	2.6	Pale yellow gum
HA4272E	1.4	Yellow gum
HA4272F	2.0	Yellow gum

Subfraction HA4272A Chromatogram characteristics on reverse phase TLC with 50% methanol-water (2 runs) as a mobile phase showed no spots under UV-S. Because of low quantity, no further investigation was carried out.

Subfraction HA4272B Chromatogram characteristics on reverse phase TLC with 50% methanol-water (2 runs) as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.85. The ^1H NMR spectrum revealed the presence of **H8** as a major compound. Thus, it was not further purified.

Subfraction HA4272C Chromatogram characteristics on reverse phase TLC with 50% methanol-water (2 runs) as a mobile phase demonstrated a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no further investigation was carried out.

Subfraction HA4272D (H9) Chromatogram characteristics on reverse phase TLC with 20% methanol-water (2 runs) as a mobile phase demonstrated one spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.38.

UV (MeOH) λ_{max} nm (log ε)		:	209 (3.89), 282 (3.41)
FT-IR (neat) ν_{max} cm^{-1}		:	3367 (O-H), 1718 (O-C=O), 1637 (C=O)
^{1}H NMR (acetone-d_6) (δ ppm) (500 MHz)		:	6.98 (*d, J* = 2.5 Hz, 1H), 6.68 (*d, J* = 2.5 Hz, 1H), 6.19 (*s*, 2H), 3.81 (*s*, 3H), 3.66 (*s*, 3H), 2.19 (*s*, 3H)
^{13}C NMR (acetone-d_6) (δ ppm) (125 MHz)		:	200.7, 167.0, 162.8 (2x), 161.1, 156.1, 148.1, 130.7, 127.2, 110.8, 108.8 (2x), 106.7, 106.5, 55.9, 52.2, 21.9
DEPT (135^o) (acetone-d_6)	CH	:	108.8 (2x), 106.7, 106.5
	CH_3	:	55.9, 52.2, 21.9

Subfraction HA4272E Chromatogram characteristics on reverse phase TLC with 20% methanol-water (2 runs) as a mobile phase showed two major spots under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.32 and 0.38. The ^{1}H NMR spectrum revealed the presence of **H9** as a major compound. Thus, it was not further purified.

Subfraction HA4272F Chromatogram characteristics on reverse phase TLC with 20% methanol-water (2 runs) as a mobile phase demonstrated a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no further investigation was carried out.

Subfraction HA4273 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase showed one major spot under UV-S with the R_f value of 0.53. Further purification using preparative TLC was performed with 2% methanol-dichloromethane (3 runs) as a mobile phase to afford one subfraction (**HA4273A**) which was a yellow gum (5.3 mg). Its chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase displayed one major spot under UV-S with the R_f value of 0.53. The ^{1}H NMR spectrum revealed the presence of **H9** as a major compound. Thus, it was not further purified.

Subfraction HA4274 Characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase exhibited two major spots under UV-S with the R_f values of 0.35 and 0.53. It was subjected to preparative TLC using 2% methanol-dichloromethane (4 runs) as a mobile phase to afford three subfractions as shown in **Table 46**.

Table 46 Subfractions obtained from **subfraction HA4274** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4274A	1.9	White solid
HA4274B	7.2	White solid
HA4274C	6.4	Yellow gum

Subfraction HA4274A Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.93. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to minute quantity, it was not further purified.

Subfraction HA4274B Characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase exhibited one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.35. The ^{1}H NMR spectrum revealed the presence of **H14** as a major compound. Thus, it was not further purified.

Subfraction HA4274C Characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase exhibited one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.53. The ^{1}H NMR spectrum revealed the presence of **H9** as a major compound. Thus, it was not further purified.

Subfraction HA4275 Characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase exhibited one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.43. It was subjected to preparative TLC using 2% methanol-dichloromethane (4 runs) as a mobile phase to afford two subfractions as shown in **Table 47**.

Table 47 Subfractions obtained from **subfraction HA4275** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4275A	2.4	White gum
HA4275B	1.5	Colorless gum

Subfraction HA4275A Characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase exhibited one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.25. The ^{1}H NMR spectrum revealed the presence of **H14** as a major compound. Thus, it was not further purified.

Subfraction HA4275B (H10) Characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase exhibited one spot after being visualized by ceric ammonium molybdate with the R_f value of 0.43.

$[\alpha]_D^{24}$: +18.5 (c 0.09, MeOH)

FT-IR (neat) ν_{max} cm^{-1} : 3431 (O-H), 1726 (O-C=O), 1647 (C=C)

^{1}H NMR (acetone-d_6) (δ ppm) (500 MHz) : 5.14 (*t*, *J* = 3.5 Hz, 1H), 4.93 (*dd*, *J* = 7.5 and 9.5 Hz, 1H), 4.83 (*d*, *J* = 2.5 Hz, 1H), 4.72 (*brs*, 1H), 4.37 (*d J* = 4.0 Hz, 1H), 4.08 (*m*, 1H), 3.58 (*t*, *J* =

			5.0 Hz, 1H), 3.28 (*s*, 3H), 2.95 (*dd*, *J* = 5.0 and 9.5 Hz, 1H), 2.90 (*ddd*, *J* = 3.0, 6.0 and 9.5 Hz, 1H), 2.80 (*dd*, *J* = 3.0 and 7.5 Hz, 1H), 2.75 (*ddd*, *J* = 1.0, 3.5 and 15.0 Hz, 1H), 2.39 (*dd*, *J* = 3.5 and 15.0 Hz, 1H), 1.99 (*s*, 3H), 1.92 (*dd*, *J* = 9.5 and 11.5 Hz, 1H), 1.39 (*dd*, *J* = 6.0 and 11.5 Hz, 1H), 1.03 (*s*, 3H), 0.98 (*s*, 3H)
^{13}C NMR (acetone-d_6) (δ ppm) (125 MHz)		:	170.6, 144.9, 117.0, 94.5, 92.7, 86.4, 76.5, 72.8, 59.5, 58.1, 57.5, 41.3, 37.4, 35.6, 35.5, 26.4, 24.3, 21.3
DEPT (135^o) (acetone-d_6)	CH	:	92.7, 86.4, 76.5, 72.8, 59.5, 58.1, 35.6
	CH_2	:	117.0, 41.3, 35.5
	CH_3	:	57.5, 26.4, 24.3, 21.3
HRESIMS *m*/*z*		:	345.1686, $C_{18}H_{26}O_5Na$, $[M+Na]^+$

Subfraction HA4276 Characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase exhibited one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.43. It was subjected to preparative TLC using 2% methanol-dichloromethane (3 runs) as a mobile phase to afford two subfractions as shown in **Table 48**.

Table 48 Subfractions obtained from **subfraction HA4276** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4276A	3.5	Pale yellow gum
HA4276B	3.1	Colorless gum

Subfraction HA4276A Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase showed one major spot under

UV-S with the R_f value of 0.58 The ^1H NMR spectrum indicated the absence of major compounds. Due to low quantity, it was not further purified.

Subfraction HA4276B Characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase exhibited one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.43. The ^1H NMR spectrum revealed the presence of **H10** as a major compound. Thus, it was not further purified.

Subfraction HA4277 Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane (2 runs) as a mobile phase showed a long tail and two major spots after being visualized by ceric ammonium molybdate with the R_f values of 0.33 and 0.43. Further purification was performed by column chromatography over Sephadex LH-20 using an isocratic system of 50% dichloromethane:methanol. Subfractions displaying similar components were combined and evaporated to dryness under reduced pressure to yield four subfractions as shown in **Table 49**.

Table 49 Subfractions obtained from **subfraction HA4277** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HA4277A	4.7	Brown gum
HA4277B	10.5	Yellow gum
HA4277C	12.1	Yellow gum
HA4277D	2.9	Yellow gum

Subfraction HA4277A Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane (2 runs) as a mobile phase displayed no spots under UV-S. The ^1H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HA4277B Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (3 runs) as a mobile phase exhibited two major spots after being visualized by ceric ammonium molybdate with the R_f values of 0.50 and 0.70. It was

subjected to preparative TLC using 25% acetone-n-hexane (3 runs) as a mobile phase to afford two subfractions as shown in **Table 50**.

Table 50 Subfractions obtained from **subfraction HA4277B** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4277B1	2.5	Colorless gum
HA4277B2	2.4	Colorless gum

Subfraction HA4277B1 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (3 runs) as a mobile phase showed one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.70. The ^{1}H NMR spectrum indicated the absence of major components. Due to low quantity, it was not further purified.

Subfraction HA4277B2 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase showed one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.42. The ^{1}H NMR spectrum revealed the presence of **H2** as a major compound. Thus, no further investigation was conducted.

Subfraction HA4277C Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (3 runs) as a mobile phase showed two major spots after being visualized by ceric ammonium molybdate with the R_f values of 0.55 and 0.63. It was purified by column chromatography over silica gel using 25% acetone-n-hexane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford two subfractions as shown in **Table 51**.

Table 51 Subfractions obtained from **subfraction HA4277C** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA4277C1	5.0	Pale yellow gum
HA4277C2	6.5	Yellow gum

Subfraction HA4277C1 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (3 runs) as a mobile phase exhibited one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.65. It was subjected to preparative TLC using 25% acetone-n-hexane (3 runs) as a mobile phase to afford two subfractions as shown in **Table 52**.

Table 52 Subfractions obtained from **subfraction HA4277C1** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4277C1A	3.0	Colorless solid
HA4277C1B	1.8	Colorless gum

Subfraction HA4277C1A (H11) Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (3 runs) as a mobile phase exhibited one spot after being visualized by ceric ammonium molybdate with the R_f value of 0.65.

Melting point (°C)		:	130-133
$[\alpha]_D^{24}$		:	+90.3 (c 0.14, MeOH)
FT-IR (neat) ν_{max} cm^{-1}		:	3436 (O-H), 1735 (O-C=O), 1675 (C=C)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		:	5.29 (*brd*, *J* = 6.3 Hz, 1H), 5.22 (*dd*, *J* = 5.1 and 10.5 Hz, 1H), 4.15 (*d*, *J* = 7.8 Hz, 1H), 3.86 (*brs*, 1H), 3.21 (*dd*, *J* = 10.5 and 13.2 Hz, 1H), 3.21 (*s*, 3H), 2.98 (*ddd*, *J* = 3.0, 6.9 and 9.6 Hz, 1H), 2.60 (*dt*, *J* = 3.0 and 7.8 Hz, 1H), 2.05 (*s*, 3H), 1.97 (*dd*, *J* = 5.1 and 13.2 Hz, 1H), 1.96 (*dd*, *J* = 9.6 and 11.4 Hz, 1H), 1.81 (*d*, *J* = 1.5 Hz, 3H), 1.51 (*dd*, *J* = 6.9 and 11.4 Hz, 1H), 1.11 (*s*, 6H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		:	170.2, 133.7, 129.6, 92.7, 89.5, 85.3, 78.4, 73.8, 55.2, 54.6, 42.3, 39.4, 38.0, 34.4, 26.5, 23.5, 22.0, 21.6
DEPT (135°) ($CDCl_3$)	CH	:	89.5, 85.3, 78.4, 73.8, 54.6, 39.4
	CH_2	:	42.3, 34.4

CH_3 : 55.2, 26.5, 23.5, 22.0, 21.6

Subfraction HA4277C1B Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane as a mobile phase showed one major spot after being visualized by ceric ammonium molybdate with the R_f value of 0.42. The ^{1}H NMR spectrum indicated the presence of **H2** as a major compound. Thus, it was not investigated.

Subfraction HA4277C2 Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (3 runs) as a mobile phase demonstrated a long tail after being visualized by ceric ammonium molybdate. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not purified.

Subfraction HA4277D Chromatogram characteristics on normal phase TLC with 25% acetone-n-hexane (3 runs) as a mobile phase displayed many spots under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HA428 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase demonstrated two major spots under UV-S with the R_f values of 0.60 and 0.68. It was further purified by column chromatography over silica gel. Elution was conducted with 25% ethyl acetate-chloroform. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to yield three subfractions as shown in **Table 53**.

Table 53 Subfractions obtained from **subfraction HA428** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA4281	5.2	Yellow gum
HA4282	8.2	Yellow gum
HA4283	1.4	Yellow gum

Subfraction HA4281 Characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase displayed three major spots with the R_f values of

0.48, 0.58 and 0.90 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was subjected to preparative TLC using 25% ethyl acetate-chloroform (4 runs) as a mobile phase to afford three subfractions as shown in **Table 54**.

Table 54 Subfractions obtained from **subfraction HA4281** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4281A	1.1	Colorless gum
HA4281B	2.1	Yellow gum
HA4281C	1.8	Yellow gum

Subfraction HA4281A Characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase displayed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.65. The ^{1}H NMR spectrum indicated the presence of **H14** as a major component. Thus, it was not further investigated.

Subfraction HA4281B Characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase displayed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.58. The ^{1}H NMR spectrum indicated the presence of **H9** as a major component. Thus, it was not further investigated.

Subfraction HA4281C Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase showed one major spot under UV-S with the R_f value of 0.48. Because of minute quantity, no further investigation was carried out.

Subfraction HA4282 Characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase displayed four major spots under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.33, 0.48, 0.55 and 0.65. It was subjected to preparative TLC using 25% ethyl acetate-chloroform (4 runs) as a mobile phase to afford three subfractions as shown in **Table 55**.

Table 55 Subfractions obtained from **subfraction HA4282** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4282A	1.4	Colorless gum
HA4282B	2.0	Colorless gum
HA4282C	4.7	Colorless gum

Subfraction HA4282A Characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase displayed one major spot with the R_f value of 0.65 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of **H14** as a major component. Thus, it was not further investigated.

Subfraction HA4282B Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.55. Because of minute quantity, no further investigation was carried out.

Subfraction HA4282C Characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase displayed one major spot with the R_f value of 0.33 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was subjected to preparative TLC using 25% ethyl acetate-chloroform (4 runs) as a mobile phase to afford two subfractions as shown in **Table 56**.

Table 56 Subfractions obtained from **subfraction HA4282C** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4282C1	1.6	Colorless gum
HA4282C2	1.1	Colorless gum

Subfractions HA4282C1 and **HA4282C2** Chromatogram characteristics on normal phase TLC of both subfractions with 25% ethyl acetate-chloroform (2 runs) as a mobile phase showed one major spot with the R_f value of 0.35 after being visualized by anisaldehyde sulfuric acid. Their ^{1}H NMR spectrum indicated the presence of **H8** as a major component. Thus, they were not further investigated.

Subfraction HA4283 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, no further investigation was carried out.

Subfraction HA429 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase demonstrated five major spots under UV-S with the R_f values of 0.35, 0.48, 0.58 0.73 and 0.85. It was further purified by column chromatography over silica gel. Elution was conducted with 25% ethyl acetate-chloroform. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 57**.

Table 57 Subfractions obtained from **subfraction HA429** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA4291	2.9	Yellow gum
HA4292	4.1	Yellow gum
HA4293	3.4	Yellow gum
HA4294	4.3	Yellow gum

Subfraction HA4291 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase displayed two major spots under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.78 and 0.85. Because of low quantity, no further investigation was carried out.

Subfraction HA4292 Chromatogram characteristics on normal phase TLC with 10:9:1 n-hexane:chloroform:methanol (4 runs) as a mobile phase displayed four major spots after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.25, 0.33, 0.38 and 0.85. It was subjected to preparative TLC using 10:9:1 n-hexane:chloroform:methanol (5 runs) as a mobile phase to afford three subfractions as shown in **Table 58**.

Table 58 Subfractions obtained from **subfraction HA4292** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA4292A	0.6	Colorless gum
HA4292B	1.8	Colorless gum
HA4292C	1.3	Yellow gum

Subfraction HA4292A Chromatogram characteristics on normal phase TLC with 10:9:1 n-hexane:chloroform:methanol (4 runs) as a mobile phase demonstrated one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.85. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA4292B Chromatogram characteristics on normal phase TLC with 10:9:1 n-hexane:chloroform:methanol (4 runs) as a mobile phase showed two major spots after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.33 and 0.38. Because of minute quantity, no further investigation was carried out.

Subfraction HA4292C Chromatogram characteristics on normal phase TLC with 10:9:1 n-hexane:chloroform:methanol (4 runs) as a mobile phase showed one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.45. The ^{1}H NMR spectrum indicated the presence of **H12** as a major component. Thus, it was not further investigated.

Subfraction HA4293 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated many spots after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Thus, no further investigation was carried out.

Subfraction HA4294 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated no spots after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, no further investigation was carried out.

Subfraction HA4210 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail near the baseline under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic protons. Thus, it was not further purified.

Subfraction HA43 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed three major spots with the R_f values of 0.23, 0.28 and 0.35 under UV-S. It was purified by column chromatography over silica gel using 2% methanol-dichloromethane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford six subfractions as shown in **Table 59**.

Table 59 Subfractions obtained from **subfraction HA43** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA431	2.4	Yellow solid
HA432	5.4	Yellow gum
HA433	5.0	Yellow gum
HA434	18.2	Yellow gum
HA435	19.1	Yellow gum
HA436	11.3	Dark yellow gum

Subfraction HA431 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase showed one major spot with the R_f value of 0.83 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of **H5** as a major component. Thus, it was not further investigated.

Subfraction HA432 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase showed three major spots with the R_f values of 0.30, 0.35 and 0.83 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of a mixture of **H5**, **H9** and **H14**. Thus, it was not further investigated.

Subfraction HA433 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase showed one major spot with the R_f value of 0.35 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of **H9** as a major component. Thus, it was not further investigated.

Subfraction HA434 Chromatogram characteristics on normal phase TLC with 38:1:1 dichloromethane:chloroform:methanol (3 runs) as a mobile phase demonstrated one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.55. It was further purified by column chromatography over silica gel. Elution was conducted with 38:1:1 dichloromethane:chloroform:methanol. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 60**.

Table 60 Subfractions obtained from **subfraction HA434** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA434A	1.4	Yellow gum
HA434B	1.6	Yellow gum
HA434C	5.8	Yellow gum
HA434D	8.0	Yellow gum
HA434E	1.2	Yellow gum

Subfraction HA434A Chromatogram characteristics on normal phase TLC with 38:1:1 dichloromethane:chloroform:methanol (3 runs) as a mobile phase displayed a long tail after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major signals. Thus, it was not investigated.

Subfraction HA434B Chromatogram characteristics on normal phase TLC with 38:1:1 dichloromethane:chloroform:methanol (3 runs) as a mobile phase demonstrated a long tail after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of a mixture of **H2** and **H11**. Thus, it was not further investigated.

Subfraction HA434C Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (3 runs) as a mobile phase demonstrated one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.38. It was further purified by preparative TLC with 25% ethyl acetate-chloroform as a mobile phase to afford two subfractions as shown in **Table 61**.

Table 61 Subfractions obtained from **subfraction HA434C** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA434C1	2.5	Yellow gum
HA434C2	3.1	Yellow gum

Subfraction HA434C1 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (3 runs) as a mobile phase demonstrated no spots after being visualized by anisaldehyde sulfuric acid. Because of low amount, no further investigation was carried out.

Subfraction HA434C2 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.35 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of **H8** as a major component. Thus, it was not further investigated.

Subfraction HA434D Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (3 runs) as a mobile phase demonstrated one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.38. It was further purified by preparative TLC with 25% ethyl acetate-chloroform as a mobile phase to afford three subfractions as shown in **Table 62**.

Table 62 Subfractions obtained from **subfraction HA434D** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA434D1	3.7	Yellow gum
HA434D2	2.5	Brown gum
HA434D3	2.6	Yellow gum

Subfraction HA434D1 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (3 runs) as a mobile phase demonstrated no spots after being visualized by anisaldehyde sulfuric acid. Because of low amount, no further investigation was carried out.

Subfraction HA434D2 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (3 runs) as a mobile phase demonstrated one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.75. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, no attempts were made to purify this subfraction.

Subfraction HA434D3 Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-chloroform (2 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.35 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of **H8** as a major component. Thus, it was not further investigated.

Subfraction HA434E Chromatogram characteristics on normal phase TLC with 38:1:1 dichloromethane:chloroform:methanol (3 runs) as a mobile phase displayed a long tail near the baseline after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major signals. Thus, it was not investigated.

Subfraction HA435 Chromatogram characteristics on normal phase TLC with 38:1:1 dichloromethane:chloroform:methanol (3 runs) as a mobile phase demonstrated a long tail and four major spots after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.45, 0.50, 0.58 and 0.68. It was further investigated by column chromatography over silica gel. Elution was conducted with 38:1:1 dichloromethane:chloroform:methanol. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 63**.

Table 63 Subfractions obtained from **subfraction HA435** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA435A	5.4	Yellow gum
HA435B	9.4	Yellow gum
HA435C	4.2	Yellow gum

Subfraction HA435A Chromatogram characteristics on normal phase TLC with 38:1:1 dichloromethane:chloroform:methanol (3 runs) as a mobile phase exhibited a long tail after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic protons. Thus, no attempts were made to purify this subfraction.

Subfraction HA435B Chromatogram characteristics on normal phase TLC with 10:9:1 n-hexane:chloroform:methanol (4 runs) as a mobile phase showed four major spots after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.45, 0.50, 0.58 and 0.68. Further purification using preparative TLC was performed with 10:9:1 n-hexane:chloroform:methanol (5 runs) as a mobile phase to afford four subfractions as shown in **Table 64**.

Table 64 Subfractions obtained from **subfraction HA435B** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA435B1	1.4	Yellow gum
HA435B2	1.8	Yellow gum
HA435B3	1.5	Yellow gum
HA435B4	3.4	Colorless gum

Subfraction HA435B1 Chromatogram characteristics on normal phase TLC with 10:9:1 n-hexane:chloroform:methanol (4 runs) as a mobile phase showed one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.68. Because of minute quantity, no further investigation was carried out.

Subfraction HA435B2 Chromatogram characteristics on normal phase TLC with 10:9:1 n-hexane:chloroform:methanol (4 runs) as a mobile phase showed one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.58. Because of minute quantity, no further investigation was carried out.

Subfraction HA435B3 Chromatogram characteristics on normal phase TLC with 10:9:1 n-hexane:chloroform:methanol (4 runs) as a mobile phase showed one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.50. Because of minute quantity, no further investigation was performed.

Subfraction HA435B4 (H12) Chromatogram characteristics on normal phase TLC with 10:9:1 n-hexane:chloroform:methanol (4 runs) as a mobile phase showed one spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.45.

$[\alpha]_D^{24}$		:	-6.9 (c 0.60, MeOH)
FT-IR (neat) ν_{max} cm^{-1}		:	3445 (O-H), 1730 (O-C=O), 1716 (C=O)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		:	5.12 (*dqd*, *J* = 1.5, 6.3 and 12.6 Hz, 1H), 4.51 (*dddd*, *J* = 1.2, 4.2, 9.0 and 10.2 Hz, 1H), 3.74 (*d*, *J* = 4.8 Hz, 1H), 3.33 (*ddd*, *J* = 3.9, 4.8 and 9.9 Hz, 1H), 3.16 (*dd*, *J* = 9.0 and 14.4 Hz, 1H), 2.93 (*ddd*, *J* = 0.9, 4.2 and 15.3 Hz, 1H), 2.77 (*d*, *J* = 14.4 Hz, 1H), 2.57 (*dd*, *J* = 10.2 and 15.3 Hz, 1H), 2.23 (*ddd*, *J* = 1.5, 3.9 and 14.7 Hz, 1H), 1.66 (*ddd*, *J* = 9.9, 12.6 and 14.7 Hz, 1H), 1.25 (*d*, *J* = 6.3 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		:	201.6, 168.9, 67.2, 66.1, 57.2, 56.5, 50.6, 44.2, 33.6, 20.6
DEPT (135°) ($CDCl_3$)	CH	:	67.2, 66.1, 57.2, 56.5
	CH_2	:	50.6, 44.2, 33.6
	CH_3	:	20.6

HRESIMS *m/z* : 237.0727, $C_{10}H_{14}O_5Na$, $[M+Na]^+$

Subfraction HA435C Chromatogram characteristics on normal phase TLC with 38:1:1 dichloromethane:chloroform:methanol (3 runs) as a mobile phase exhibited four major spots after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.45, 0.50, 0.58 and 0.68. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, no attempts were made to purify this subfraction.

Subfraction HA436 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase displayed a long tail near the baseline under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA44 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over Sephadex LH-20 using an isocratic system of 100% methanol. Subfractions displaying similar components were combined and evaporated to dryness under reduced pressure to yield four subfractions as shown in **Table 65**.

Table 65 Subfractions obtained from **subfraction HA44** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HA441	442.6	Brown gum
HA442	623.1	Brown gum
HA443	122.8	Brown gum
HA444	111.0	Brown gum

Subfraction HA441 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to give a chloroform soluble part (**HA4411**) and a chloroform insoluble one (**HA4412**) as shown in **Table 66**.

Table 66 Subfractions obtained from **subfraction HA441** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HA4411	89.8	Brown gum
HA4412	318.4	Brown gum

Subfraction HA4411 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HA4412 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HA442 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to give a chloroform soluble part (**HA4421**) and a chloroform insoluble one (**HA4422**) as shown in **Table 67**.

Table 67 Subfractions obtained from **subfraction HA442** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HA4421	191.6	Brown gum
HA4422	428.4	Brown gum

Subfraction HA4421 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, no attempts were made to purify this subfraction.

Subfraction HA4422 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S. The ^{1}H NMR spectrum indicated the presence of many components without major components. Therefore, it was not further purified.

Subfraction HA443 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.40. It was dissolved with chloroform to give a chloroform soluble part (**HA4431**) and a chloroform insoluble one (**HA4432**) as shown in **Table 68**.

Table 68 Subfractions obtained from **subfraction HA443** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HA4431	2.1	Brown gum
HA4432	74.5	Brown gum

Subfraction HA4431 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to low quantity, it was not further purified.

Subfraction HA4432 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed many spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of a mixture of **H15** and **H16**. Therefore, it was not further purified.

Subfraction HA444 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase demonstrated one major spot under UV-S with the R_f value of 0.43. It was further purified by column chromatography over silica gel. Elution was conducted with 8:1:1 dichloromethane:ethyl acetate:methanol. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 69**.

Table 69 Subfractions obtained from **subfraction HA444** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA4441	8.7	Brown gum
HA4442	4.3	Brown gum
HA4443	54.5	Brown gum
HA4444	11.7	Brown gum
HA4445	28.9	Dark brown gum

Subfraction HA4441 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase demonstrated one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.95. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, it was not further investigated.

Subfraction HA4442 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase demonstrated two major spots under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.75 and 0.95. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not further purified.

Subfraction HA4443 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase showed one major spot with the R_f value of 0.38 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with methanol to give a methanol soluble part (**HA44431**) and a methanol insoluble one (**HA44432**) as shown in **Table 70**.

Table 70 Subfractions obtained from **subfraction HA4443** by dissolving with methanol

Subfraction	Weight (mg)	Physical appearance
HA44431	39.7	Brown gum
HA44432	3.5	Brown gum

Subfraction HA44431 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl actetate:methanol (3 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.38 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel. Elution was conducted with 8:1:1 dichloromethane:ethyl actetate:methanol. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 71**.

Table 71 Subfractions obtained from **subfraction HA44431** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA44431A	6.0	Brown gum
HA44431B	9.0	Brown gum
HA44431C	22.2	Brown gum

Subfraction HA44431A Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl actetate:methanol as a mobile phase showed one major spot with the R_f value of 0.83 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, it was not purified.

Subfraction HA44431B Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl actetate:methanol as a mobile phase showed one major spot with the R_f value of 0.25 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of **H16** as a major compound. Thus, it was not purified.

Subfraction HA414431C Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl actetate:methanol as a mobile phase showed two major spots with the R_f values of 0.20 and 0.25 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of a mixture of **H15** and **H16**. Thus, it was not investigated.

Subfraction HA44432 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl actetate:methanol (3 runs) as a mobile phase showed one major spot with the R_f value of 0.38 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA4444 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.38. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA4445 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol (3 runs) as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with methanol to give a methanol soluble part (**HA44451**) and a methanol insoluble one (**HA44452**) as shown in **Table 72**.

Table 72 Subfractions obtained from **subfraction HA4445** by dissolving with methanol

Subfraction	Weight (mg)	Physical appearance
HA44451	20.1	Brown gum
HA44452	0.7	Brown gum

Subfraction HA44451 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl actetate:methanol as a mobile phase showed two major spots with the R_f values of 0.20 and 0.25 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of a mixture of **H15** and **H16**. Thus, it was not investigated.

Subfraction HA44452 Chromatogram characteristics on normal phase TLC with 7% methanol-dichloromethane as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Fraction HA5 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed many spots and a long tail under UV-S. Further purification was performed by column chromatography over silica gel. Elution was initially conducted with 2% methanol-dichloromethane, and then gradually enriched with methanol until pure methanol. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give eight subfractions as shown in **Table 73**.

Table 73 Subfractions obtained from **fraction HA5** by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
HA51	2% methanol-dichloromethane	0.5	Yellow gum
HA52	2% methanol-dichloromethane	5.3	Pale yellow gum
HA53	2% methanol-dichloromethane	2.3	Brown gum
HA54	2% methanol-dichloromethane	35.6	Brown gum
HA55	4-6% methanol-dichloromethane	8.7	Brown gum
HA56	10% methanol-dichloromethane	12.6	Brown gum
HA57	10-50% methanol-dichloromethane	130.6	Dark brown gum
HA58	100% methanol	134.7	Brown gum

Subfraction HA51 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed no spots under UV-S. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HA52 Chromatogram characteristics on normal phase TLC with 10% hexane-dichloromethane as a mobile phase exhibited two major spots under UV-S with the R_f values of 0.50 and 0.55. It was subjected to preparative TLC using 2% methanol-dichloromethane (2 runs) as a mobile phase to afford three subfractions as shown in **Table 74**.

Table 74 Subfractions obtained from **subfraction HA52** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA521	0.8	Pale yellow gum
HA522	0.6	Pale yellow solid
HA523	3.0	Pale yellow gum

Subfraction HA521 Chromatogram characteristics on normal phase TLC with 10% n-hexane-dichloromethane as a mobile phase demonstrated no spots under UV-S. Because of minute quantity, no further investigation was carried out.

Subfraction HA522 (H13) Chromatogram characteristics on normal phase TLC with 10% n-hexane-dichloromethane as a mobile phase displayed one spot with the R_f value of 0.55 under UV-S and after being visualized by anisaldehyde sulfuric acid.

Melting point (°C)		:	198-200
UV (MeOH) λ_{max} nm (log ε)		:	242 (4.43), 308 (4.12), 358 (3.59)
FT-IR (neat) ν_{max} cm^{-1}		:	3410 (O-H), 1734 (O-C=O), 1653 (C=O)
^1H NMR ($CDCl_3$) (δ ppm) (500 MHz)		:	12.08 (*s*, 1H), 6.95 (*s*, 1H), 6.86 (*s*, 1H), 6.65 (*s*, 1H), 4.06 (*s*, 3H), 4.03 (*s*, 3H), 2.44 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		:	179.6, 169.0, 161.5, 160.0, 155.6, 153.1, 149.4, 132.9, 112.5, 112.3, 111.3, 107.7, 107.0, 106.3, 57.1, 53.3, 22.6
DEPT (135°) ($CDCl_3$)	CH	:	112.3, 107.7, 107.0
	CH_3	:	57.1, 53.3, 22.6

Subfraction HA523 Chromatogram characteristics on normal phase TLC with 10% n-hexane-dichloromethane as a mobile phase displayed one major spot with the R_f value of 0.50 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^1H NMR spectrum revealed that it contained **H5** as a major compound.

Subfraction HA53 Chromatogram characteristics on normal phase TLC with 10% n-hexane-dichloromethane as a mobile phase displayed many spots under UV-S and after

being visualized by anisaldehyde sulfuric acid. Due to low quantity, it was not investigated.

Subfraction HA54 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed two major spots with the R_f values of 0.25 and 0.75 under UV-S and after being visualized by anisaldehyde sulfuric acid. Purification by column chromatography over silica gel was performed using 2% methanol-dichloromethane. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give six subfractions as shown in **Table 75**.

Table 75 Subfractions obtained from **subfraction HA54** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HA541	2.2	brown gum
HA542	0.4	brown gum
HA543	2.5	brown gum
HA544	4.1	Pale yellow gum
HA545	20.2	brown gum
HA546	5.5	brown gum

Subfraction HA541 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase displayed one major spot under UV-S with the R_f value of 0.83. The ^{1}H NMR spectrum revealed the presence of **H5** as a major compound. Thus, it was not further purified.

Subfraction HA542 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed no major spots under UV-S. The ^{1}H NMR spectrum showed signals in high field region. Thus, it was not further purified.

Subfraction HA543 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed one major spot under UV-S

with the R_f value of 0.25. The ^1H NMR spectrum revealed the presence of **H14** as a major compound. Thus, it was not further investigated.

Subfraction HA544 (H14) Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed one spot under UV-S with the R_f value of 0.25.

UV (MeOH) λ_{max} nm (log ε)		:	213 (4.53), 273 (4.16)
FT-IR (neat) ν_{max} cm^{-1}		:	3367 (O-H), 1718 (O-C=O), 1633 (C=O)
^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)		:	8.88 (*brs*, 1H), 7.12 (*s*, 1H), 6.40 (*brs*, 1H), 6.23 (*s*, 2H), 3.97 (*s*, 3H), 3.71 (*s*, 3H), 2.25 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		:	196.8, 166.3, 160.3 (2x), 155.9, 149.9, 148.7, 127.9, 123.9, 113.7, 109.5, 109.4 (2x), 105.1, 56.7, 52.7, 22.0
DEPT (135°) ($CDCl_3$)	CH	:	109.4 (2x), 105.1
	CH_3	:	56.7, 52.7, 22.0

Subfraction HA545 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed one major spot under UV-S with the R_f value of 0.25. The ^1H NMR spectrum revealed the presence of **H14** as a major compound. Therfore, it was not further purified.

Subfraction HA546 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed no major spots under UV-S. Because of low quantity, no further investigation was carried out.

Subfraction HA55 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase displayed two major spots under UV-S with the R_f values of 0.25 and 0.75. It was subjected to preparative TLC using 2% methanol-dichloromethane (3 runs) as a mobile phase to afford five subfractions as shown in **Table 76**.

Table 76 Subfractions obtained from **subfraction HA55** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HA551	1.1	Brown gum
HA552	0.4	brown gum
HA553	0.3	brown gum
HA554	1.0	brown gum
HA555	1.4	brown gum

Subfraction HA551 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase displayed one major spot under UV-S with the R_f value of 0.83. The ^{1}H NMR spectrum revealed the presence of **H5** as a major compound. Thus, it was not further investigated.

Subfraction HA552 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Because of minute amount, no further investigation was carried out.

Subfraction HA553 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.30. The ^{1}H NMR spectrum indicated the absence of major compounds. Because of minute amount, no further investigation was conducted.

Subfraction HA554 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated two major spots under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.30 and 0.25. Because of minute amount, no further investigation was performed.

Subfraction HA555 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase displayed two major spots under

UV-S with the R_f values of 0.35 and 0.83. The ^{1}H NMR spectrum revealed the presence of a mixture of **H5** and **H9**. Thus, it was not further investigated.

Subfraction HA56 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and many spots after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, it was not purified.

Subfraction HA57 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase showed two major spots with the R_f values of 0.57 and 0.64 and long tail near the baseline after being visualized by anisaldehyde sulfuric acid. It was subjected to column chromatography over reverse phase C_{18} silica gel using 50% methanol-water. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give six subfractions as shown in **Table 77**.

Table 77 Subfractions obtained from **subfraction HA57** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HA571	1.2	Brown gum
HA572	30.9	Brown gum
HA573	61.6	Colorless solid
HA574	3.7	Brown gum
HA575	3.5	Brown gum
HA576	28.3	Brown gum

Subfraction HA571 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Because of minute amount, no further investigation was carried out.

Subfraction HA572 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated two major spots under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.57 and 0.64. It was subjected to column chromatography over reverse phase C_{18} silica gel using 50% methanol-water. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 78**.

Table 78 Subfractions obtained from **subfraction HA572** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HA5721	1.3	Brown solid
HA5722	13.1	Colorless gum
HA5723	0.1	Dark yellow gum
HA5724	10.9	Brown gum
HA5725	4.5	Dark yellow gum

Subfraction HA5721 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase displayed no spots under UV-S. Due to minute quantity, it was not investigated.

Subfraction HA5722 (H15) Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated one spot under UV-S with the R_f value of 0.64.

UV (MeOH) λ_{max} nm (log ε)	:	212 (4.68), 247 (4.10), 306 (3.87)
FT-IR (neat) ν_{max} cm^{-1}	:	3418 (O-H), 1714 (O-C=O), 1629 (COOH)
^{1}H NMR (acetone-d_6) (δ ppm) (300 MHz)	:	6.79 (*d, J* = 3.0 Hz, 1H), 6.62 (*d, J* = 3.0 Hz, 1H), 6.32 (*s*, 1H), 6.00 (*s*, 1H), 3.77 (*s*, 6H), 2.08 (*s*, 3H)
^{13}C NMR (acetone-d_6) (δ ppm) (75 MHz)	:	175.3, 167.1, 162.4, 159.1, 157.9, 154.0, 142.5, 138.8, 127.4, 112.1,

			109.7, 109.0, 107.8, 105.3, 55.9, 52.5, 21.7
DEPT (135°) (acetone-d_6)	CH	:	112.1, 109.0, 107.8, 105.3
	CH_3	:	55.9, 52.5, 21.7

Subfraction HA5723 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated two major spots under UV-S with the R_f values of 0.57 and 0.64. The ^{1}H NMR spectrum revealed the presence of a mixture of **H15** and **H16**. Thus, it was not further purified.

Subfraction HA5724 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated one major spot under UV-S with the R_f value of 0.57. The ^{1}H NMR spectrum revealed the presence of **H16** as a major compound. Thus, it was not further purified.

Subfraction HA5725 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase showed no spots under UV-S. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, no attempts were made to purify this subfraction.

Subfraction HA573 (H16) Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated one spot under UV-S with the R_f value of 0.57.

Melting point (°C)	:	197-200
UV (MeOH) λ_{max} nm (log ε)	:	213 (4.61), 251 (4.04), 306 (3.75)
FT-IR (neat) ν_{max} cm^{-1}	:	3391 (O-H), 1713 (O-C=O), 1633 (COOH)
^{1}H NMR (CD_3OD) (δ ppm) (300 MHz)	:	7.01 (*s*, 1H), 6.39 (*s*, 1H), 5.91 (*s*, 1H), 3.90 (*s*, 3H), 3.76 (*s*, 3H), 2.11 (*s*, 3H)
^{13}C NMR (CD_3OD) (δ ppm) (75 MHz)	:	176.5, 167.4, 162.2, 159.3, 154.4, 150.7, 144.3, 139.7, 124.4, 116.4, 112.6, 109.0 (2x), 103.4, 56.9, 53.0, 21.8

DEPT (135°) (CD_3OD)	CH	:	112.6, 109.0, 103.4
	CH_3	:	56.9, 53.0, 21.8

Subfraction HA574 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated a long tail and one major spot under UV-S with the R_f value of 0.29. The ^1H NMR spectrum indicated the absence of major compounds. Because of low amount, no further investigation was carried out.

Subfraction HA575 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated a long tail near the baseline and one major spot under UV-S with the R_f value of 0.14. The ^1H NMR spectrum indicated the absence of major compounds. Because of low amount, no further investigation was conducted.

Subfraction HA576 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated a long tail near the baseline under UV-S. The ^1H NMR spectrum displayed signals in high field region. Therefore, no further investigation was carried out.

Subfraction HA58 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase demonstrated one major spot near the baseline after being visualized by anisaldehyde sulfuric acid. The ^1H NMR spectrum displayed signals in high field region. Thus, it was not investigated.

Fraction HA6 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one major spot near the baseline under UV-S. The ^1H NMR spectrum displayed no major signals. Because of low quantity, no further purification was conducted.

1.2.4 Purification of the mycelial extracts of the fungus PSU-AMF45

1.2.4.1 The mycelial ethyl acetate extract (CE)

The extract (606.1 mg) was purified by column chromatography over silica gel. Elution was initially conducted with 2% methanol-dichloromethane and then increased the amount of methanol until 100% methanol. All fractions were examined

by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give twelve fractions as shown in **Table 79**.

Table 79 Fractions obtained from **the mycelial ethyl acetate extract** by column chromatography over silica gel

Fraction	Eluent	Weight (mg)	Physical appearance
HB1	2% methanol-dichloromethane	15.4	Yellow gum
HB2	2% methanol-dichloromethane	4.6	Yellow gum
HB3	2% methanol-dichloromethane	1.7	Yellow gum
HB4	2% methanol-dichloromethane	2.0	Yellow gum
HB5	2% methanol-dichloromethane	6.5	Brown gum
HB6	2% methanol-dichloromethane	8.4	Brown gum
HB7	2% methanol-dichloromethane	1.2	Brown gum
HB8	2-10% methanol-dichloromethane	47.2	Brown gum
HB9	20% methanol-dichloromethane	28.0	Brown gum
HB10	40% methanol-dichloromethane	76.6	Brown gum
HB11	60% methanol-dichloromethane	98.1	Brown gum
HB12	80% methanol-dichloromethane and 100% methanol	114.9	Dark brown gum

Fraction HB1 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.90. The ^1H NMR spectrum indicated the presence of long chain hydrocarbons. Thus, it was not further investigated.

Fraction HB2 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-n-hexane (2 runs) as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.43. The ^1H NMR spectrum showed signals of aromatic and olefinic protons. It was subjected to

preparative TLC using 20% ethyl acetate-n-hexane (4 runs) as a mobile phase to afford three subfractions as shown in **Table 80**.

Table 80 Subfractions obtained from **fraction HB2** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HB2A	0.6	Colorless gum
HB2B	1.3	Colorless gum
HB2C	2.2	Colorless gum

Subfraction HB2A Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-n-hexane (2 runs) as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HB2B Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-n-hexane (2 runs) as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.53. The ^{1}H NMR spectrum revealed the presence of **H5** as a major compound. Thus, it was not further purified.

Subfraction HB2C Chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane as a mobile phase exhibited one major spot under UV-S with the R_f value of 0.38. Further purification using preparative TLC was performed with 20% acetone-n-hexane as a mobile phase to afford one subfraction which was a colorless gum (2.0 mg). The chromatogram characteristics on normal phase TLC with 20% acetone-n-hexane as a mobile phase displayed one major spot under UV-S with the R_f value of 0.38. The ^{1}H NMR spectrum indicated the presence of **H17** as a major compound. Therefore, no further investigation was performed.

Fraction HB3 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to minute quantity, it was not further purified.

Fraction HB4 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of many components without major components. Thus, no attempts were made to purify this subfraction.

Fraction HB5 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-n-hexane (4 runs) as a mobile phase showed a long tail and one major spot with the R_f value of 0.73 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was subjected to preparative TLC using 20% ethyl acetate-n-hexane (4 runs) as a mobile phase to afford one subfration which was a yellow gum (4.1 mg). The ^{1}H NMR spectrum indicated the absence of major components. Thus, no attempts were made to purify this subfraction

Fraction HB6 Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-dichloromethane as a mobile phase displayed a long tail and one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.28. It was further purified by column chromatography over silica gel. Elution was conducted with 5% acetone-dichloromethane. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give four subfractions as shown in **Table 81**.

Table 81 Subfractions obtained from **fraction HB6** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HB6A	2.7	Yellow gum
HB6B	1.2	Yellow gum
HB6C	2.5	Yellow gum
HB6D	1.8	Yellow gum

Subfraction HB6A Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-dichloromethane as a mobile phase displayed a long tail after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not further investigated.

Subfraction HB6B Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-dichloromethane as a mobile phase demonstrated a long tail and one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.28. Purification was performed using preparative TLC with 5% ethyl acetate-dichloromethane (3 runs) as a mobile phase to give one subfraction which was a yellow gum (2.5 mg). The ^{1}H NMR spectrum indicated the absence of major components. Due to low quantity, it was not further purified.

Subfraction HB6C Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-dichloromethane as a mobile phase displayed a long tail and two major spots after being visualized by anisaldehyde sulfuric acid with the R_f values of 0.20 and 0.28. Because of low quantity, it was not further investigated.

Subfraction HB6D Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-dichloromethane as a mobile phase demonstrated no spots after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of aromatic and olefinic protons. Thus, it was not further investigated.

Fraction HB7 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.25. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to minute quantity, it was not further purified.

Fraction HB8 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to the presence of signals for long chain hydrocarbons in the ^{1}H NMR spectrum, no further purification was performed.

Fraction HB9 Chromatogram characteristics on normal phase TLC 10% methanol-dichloromethane (2 runs) as a mobile phase exhibited a long tail and one major spot with the R_f value of 0.30 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of **H16** as a major component. Therefore, it was not further investigated

Fraction HB10 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase exhibited a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed signals of aromatic and olefinic protons. It was dissolved in chloroform to obtain two subfractions, a chloroform soluble part (**HB10A**) and a chloroform insoluble one (**HB10B**) as shown in **Table 82**.

Table 82 Subfractions obtained from **fraction HB10** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HB10A	46.1	Brown gum
HB10B	28.4	Brown gum

Subfraction HB10A Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase demonstrated a long tail and one major spot with the R_f value of 0.25 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not further investigated.

Subfraction HB10B Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase demonstrated a long tail and one major spot with the R_f value of 0.25 after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to give three subfractions as shown in **Table 83**.

Table 83 Subfractions obtained from **subfraction HB10B** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HB10B1	5.8	Brown gum
HB10B2	9.3	Brown gum
HB10B3	12.7	Brown gum

Subfraction HB10B1 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not purified.

Subfraction HB10B2 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase showed a long tail and three major spots with the R_f values of 0.40, 0.50 and 0.60 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not purified.

Subfraction HB10B3 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed the presence of **H16** as a major component. Thus, it was not purified.

Fraction HB11 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase exhibited a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed aromatic and olefinic proton signals. It was dissolved in chloroform to obtain two subfractions, a chloroform soluble part (**HB11A**) and a chloroform insoluble one (**HB11B**) as shown in **Table 84**.

Table 84 Subfractions obtained from **fraction HB11** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HB11A	3.8	Brown gum
HB11B	81.0	Brown gum

Subfraction HB11A Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-dichloromethane as a mobile phase displayed a long tail under UV-S and one major spot with the R_f value of 0.50 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed signals in high field region. No further purification was carried out.

Subfraction HB11B Chromatogram characteristics on reverse phase TLC with 50% methanol-water (2 runs) as a mobile phase demonstrated three major spots with the R_f values of 0.50, 0.60 and 0.87 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was subjected to column chromatography over reverse phase C_{18} silica gel with 50% methanol-water as an eluent. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 85**.

Table 85 Subfractions obtained from **subfraction HB11B** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HB11B1	1.2	Brown gum
HB11B2	10.1	Brown gum
HB11B3	5.5	Brown gum
HB11B4	12.3	Brown gum
HB11B5	35.1	Brown gum

Subfraction HB11B1 Chromatogram characteristics on reverse phase TLC with 50% methanol-water (2 runs) as a mobile phase demonstrated no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Because of minute amount, no further investigation was carried out.

Subfraction HB11B2 Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (4 runs) as a mobile phase demonstrated a long tail near the baseline and six major spots with the R_f values of 0.25, 0.50, 0.55, 0.63, 0.75 and 0.85 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was further purified by column chromatography over silica gel. Elution was conducted with 15% methanol-dichloromethane. Subfractions containing similar components were combined and evaporated to dryness under reduced pressure to afford nine subfractions as shown in **Table 86**.

Table 86 Subfractions obtained from **subfraction HB11B2** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HB11B2A	0.8	Yellow gum
HB11B2B	1.1	Colorless gum
HB11B2C	1.2	Colorless gum
HB11B2D	1.4	White solid
HB11B2E	1.2	Colorless gum
HB11B2F	0.7	Colorless gum
HB11B2G	0.4	Colorless gum
HB11B2H	1.3	Colorless gum
HB11B2I	1.9	Yellow gum

Subfraction HB11B2A Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (3 runs) as a mobile phase demonstrated no spots under UV-S. Because of minute quantity, no further investigation was carried out.

Subfraction HB11B2B Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.50 under UV-S. Because of minute quantity, no further investigation was carried out.

Subfraction HB11B2C Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (2 runs) as a mobile phase exhibited two major spots with the R_f values of 0.50 and 0.63 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of adenosine as a major compound. Thus, no attempts were made to purify this subfraction.

Subfraction HB11B2D Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (2 runs) as a mobile phase exhibited one spot with the R_f value of 0.63 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated that this subfraction was adenosine.

Subfraction HB11B2E Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (2 runs) as a mobile phase exhibited one major spot with the R_f value of 0.63 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of adenosine as a major compound. Thus, no attempts were made to purify this subfraction.

Subfraction HB11B2F Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (2 runs) as a mobile phase exhibited one major spot with the R_f value of 0.63 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Because of low quantity, no further purification was performed.

Subfraction HB11B2G Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not investigated.

Subfraction HB11B2H Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (2 runs) as a mobile phase demonstrated two major spots with the R_f values of 0.63 and 0.88 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not investigated.

Subfraction HB11B2I Chromatogram characteristics on normal phase TLC with 15% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Because of low quantity, it was not investigated.

Subfraction HB11B3 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase displayed two major spots with the R_f values of 0.48 and 0.64 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of **H15** as a major compound. Thus, it was not purified.

Subfraction HB11B4 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase displayed one major spot with the R_f value of 0.57 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the presence of **H16** as a major compound. Thus, it was not purified.

Subfraction HB11B5 Chromatogram characteristics on reverse phase TLC with 50% methanol-water (2 runs) as a mobile phase demonstrated a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not investigated.

Fraction HB12 Chromatogram characteristics on reverse phase TLC with 50% methanol-water demonstrated a long tail near the baseline after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Thus, it was not further investigated.

1.2.4.2 The mycelial hexane extract (CH)

The extract (522.3 mg) was chromatographed by column chromatography using 2% methanol-dichloromethane as an eluent. Fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give six fractions as shown in **Table 87**.

Table 87 Fractions obtained from **the mycelial hexane extract** by column chromatography over silica gel

Fraction	Weight (mg)	Physical appearance
HC1	96.6	Yellow gum
HC2	16.0	Yellow gum
HC3	11.4	Yellow gum
HC4	4.0	Yellow gum
HC5	88.2	Yellow gum
HC6	98.1	Dark yellow solid

Fraction HC1 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one major spot with the R_f value of 0.83 under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to the presence of long chain hydrocarbons in the ^{1}H NMR spectrum, no further purification was performed.

Fraction HC2 Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-dichloromethane (2 runs) as a mobile phase showed a long tail and one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.60. Further purification using preparative TLC was performed with 1% ethyl acetate-dichloromethane (3 runs) as a mobile phase to afford one subfraction (**H17**) which was a colorless gum (1.3 mg). Its chromatogram characteristics on normal phase TLC with 1% ethyl acetate-dichloromethane (2 runs) as a mobile phase showed one spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.60.

UV (MeOH) λ_{max} nm (log ε)		:	214 (4.60), 254 (4.12), 313 (3.73)
FT-IR (neat) ν_{max} cm^{-1}		:	3420 (O-H), 1717 (O-C=O)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)		:	10.64 (*s*, 1H), 7.13 (*s*, 1H), 7.05 (*brs*, 1H), 6.52 (*s*, 1H), 5.89, (*s*, 1H), 4.02 (*s*, 3H), 3.98 (*s*, 3H), 3.75 (*s*, 3H), 2.17 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		:	170.1, 164.9, 162.3, 158.0, 153.2, 147.5, 146.6, 136.0, 123.0, 114.9, 112.6, 106.8, 104.2, 101.7, 56.6, 52.8, 52.5, 22.1
DEPT (135^o) ($CDCl_3$)	CH	:	112.6, 106.8, 104.2
	CH_3	:	56.6, 52.8, 52.5, 22.1

Fraction HC3 Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-dichloromethane (2 runs) as a mobile phase showed many spots with one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.60. Further purification using preparative TLC was performed with 1% ethyl acetate-dichloromethane (2 runs) as a mobile phase to afford two subfractions as shown in **Table 88**.

Table 88 Subfractions obtained from **fraction HC3** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HC3A	4.6	Colorless gum
HC3B	5.0	Colorless gum

Subfraction HC3A Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-dichloromethane (2 runs) as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.60. The ^{1}H NMR spectrum revealed the presence of **H17** as a major compound. Thus, it was not further investigated.

Subfraction HC3B Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-dichloromethane (2 runs) as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not investigated.

Fraction HC4 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one major spot with the R_f value of 0.75 under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to the presence of long chain hydrocarbons in the ^{1}H NMR spectrum, no further purification was performed.

Fraction HC5 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to the presence of long chain hydrocarbons in the ^{1}H NMR spectrum, no further purification was conducted.

Fraction HC6 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase demonstrated a long tail and two major spots with the R_f values of 0.30 and 0.88 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to give a chloroform soluble part (**HC6A**) and a chloroform insoluble one (**HC6B**) as shown in **Table 89**.

Table 89 Subfractions obtained from **fraction HC6** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HC6A	3.4	Brown gum
HC6B	80.6	Brown gum

Subfraction HC6A Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail and two major spots with the R_f values of 0.30 and 0.88 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not purified.

Subfraction HC6B Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail and two major spots with the R_f values of 0.30 and 0.88 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

CHAPTER 1.3

RESULTS AND DISCUSSION

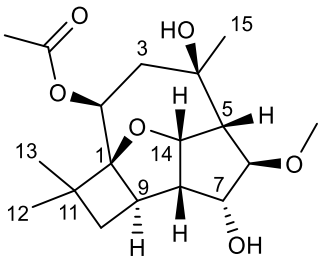
Five new compounds (**H2-H4**, **H10** and **H12**) and twelve known compounds (**H1**, **H5-H9**, **H11** and **H13-H17**) were isolated from the broth and mycelial extracts of *Pseudopestalotiopsis* sp. PSU-AMF45. In addition, compound **H6** was isolated as a natural product for the first time.

1.3.1 Compound H3

H3 was obtained as colorless crystals, melting at 172-173 °C. It had the molecular formula $C_{18}H_{28}O_6$ on the basis of the HRESIMS peak at *m/z* 363.1778 $[M+Na]^+$ (**Figure 4**). The IR spectrum showed absorption bands at 3394 and 1735 cm^{-1} for hydroxy and ester carbonyl functional groups, respectively (Xiao et al., 2017). The ^1H NMR spectroscopic data (**Table 90**) (**Figure 5**) exhibited signals for seven methine protons [δ_H 5.29 (*dd*, *J* = 2.4 and 4.5 Hz, 1H), 5.00 (*dd*, *J* = 7.0 and 9.6 Hz, 1H), 4.18 (*t*, *J* = 7.0 Hz, 1H), 3.27 (*t*, *J* = 7.0 Hz, 1H), 2.78 (*dt*, *J* = 3.6 and 7.0 Hz, 1H), 2.72 (*ddd*, *J* = 3.6, 6.0 and 9.5 Hz, 1H) and 2.30 (*dd*, *J* = 7.0 and 9.6 Hz, 1H)], one methoxy group (δ_H 3.44, *s*, 3H), two sets of nonequivalent methylene protons [δ_H 2.11 (*dd*, *J* = 4.5 and 14.7 Hz, 1H) and 1.98 (*dd*, *J* = 2.4 and 14.7 Hz, 1H); 2.01 (*dd*, *J* = 9.5 and 12.0 Hz, 1H) and 1.42 (*dd*, *J* = 6.0 and 12.0 Hz, 1H)] and four methyl groups [δ_H 2.07 (*s*, 3H), 1.16 (*s*, 3H), 1.06 (*s*, 3H) and 1.03 (*s*, 3H)]. The ^{13}C NMR spectroscopic data (**Table 90**) (**Figure 6**) contained signals for one ester carbonyl carbon (δ_C 170.3), three quaternary carbons (δ_C 94.0, 74.5 and 37.3), seven methine carbons (δ_C 88.7, 84.1, 76.9, 74.0, 59.3, 56.6 and 34.6), two methylene carbons (δ_C 40.4 and 38.1), four methyl carbons (δ_C 29.4, 26.0, 24.3 and 21.5) and one methoxy carbon (δ_C 57.7). The ^1H-^1H COSY correlations of H-7 (δ_H 4.18)/H-6 (δ_H 3.27) and H-8 (δ_H 2.78), H-5 (δ_H 2.30)/H-6 and H-14 (δ_H 5.00) and H-14/H-5 and H-8 (**Table 90**) and the chemical shifts of C-6 (δ_C 88.7), C-7 (δ_C 76.9) and C-14 (δ_C 84.1) constructed a 5-membered ring having oxy

substituents at C-6, C-7 and C-14. Further ^{1}H-^{1}H COSY correlations of H-9 (δ_H 2.72)/H-8 and H_{ab}-10 (δ_H 2.01 and 1.42) together with the HMBC correlations from H_3-12 (δ_H 1.03) and H_3-13 (δ_H 1.06) to C-1 (δ_C 94.0), C-10 (δ_C 40.4) and C-11 (δ_C 37.3) and the same correlation from H-14 to C-1 (**Table 90**) as well as the chemical shift of C-1, established a fused cyclobutane-tetrahydrofuran-cyclopentane skeleton with two methyl groups at C-11 and an ether linkage between C-1 and C-14. The substituent at C-6 was a methoxy group based on the HMBC cross peaks of the methoxy protons resonating at δ_H 3.44 with C-6. The ^{1}H-^{1}H COSY correlations of H-2 (δ_H 5.29) with H_{ab}-3 (δ_H 2.11 and 1.98), the HMBC correlations of H_{ab}-3 with C-4 (δ_H 74.5) and C-15 (δ_C 29.4) and those of H_3-15 (δ_H 1.16) with C-3 (δ_C 38.1) and C-4 established a 1,1,3,3-tetrasubstitutedbutyl unit. C-2 (δ_C 74.0) and C-4 of this unit were connected with C-1 and C-5 (δ_C 56.6) of the fused tricyclic unit, respectively, on the basis of the HMBC correlations of H_{ab}-3 with C-1 and C-5 to form a fused seven-membered ether with the methyl group at C-4. In addition, the chemical shifts of C-4 and C-7 identified that hydroxy groups were attached at both carbons. The acetoxyl group was connected to C-2 based on the HMBC cross peaks of H-2 and 2-CO_2*Me* (δ_H 2.07) with 2-*C*O_2Me (δ_C 170.3).

The relative configuration of **H3** was determined on the basis of the NOEDIFF data (**Table 90**). In the NOEDIFF experiments, when H-9 was irradiated, signal intensity of H-2 and H_3-12 was enhanced, indicating that these protons were located at the same side of the molecule and H-9 was *trans* to H-7, H-8 and H-14. Signal enhancement of H-5, H-8, H-14 and 6-OMe after irradiation of H-7 suggested that these protons were co-facial and *trans* to H-6. Signal enhancement of H_3-15 after irradiation of H-6 located H_3-15 at the same side as H-6. The X-ray data (**Figure 1**) using a graphite-monochromatic CuK_α radiation with the absolute structure parameter value of 0.01(3) established the absolute configuration of **H3** to be 1*S*, 2*S*, 4*R*, 5*S*, 6*R*, 7*R*, 8*S*, 9*R* and 14*S*. Accordingly, **H3** was identified as a new pestaloporinate.



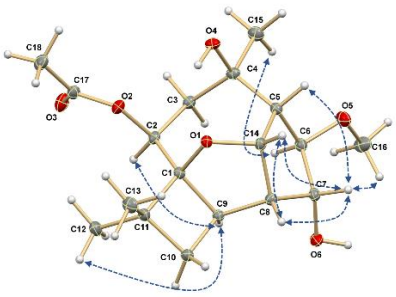


Figure 1 X-ray structure of compound **H3**, = NOEDIFF

Table 90 The ^{1}H and ^{13}C NMR data of compound **H3** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
1	-	94.0 (C)	-	-	-
2	5.29 (*dd*, 2.4, 4.5)	74.0 (CH)	H_{ab}-3	C-3, C-4, 2-*C*O_2Me	H-9, H_3-12
3	a: 2.11 (*dd*, 4.5, 14.7) b: 1.98 (*dd*, 2.4, 14.7)	38.1 (CH_2)	H-2, H_b-3 H-2, H_a-3	C-1, C-2, C-4, C-5, C-15 C-1, C-2, C-4, C-5, C-15	* *
4	-	74.5 (C)	-	-	-
5	2.30 (*dd*, 7.0, 9.6)	56.6 (CH)	H-6, H-14	C-3, C-4, C-6, C-8, C-14, C-15	H-7, H-14, 6-OMe
6	3.27 (*t*, 7.0)	88.7 (CH)	H-5, H-7	C-4, C-5, C-7, C-14, 6-OMe	H_3-15
7	4.18 (*t*, 7.0)	76.9 (CH)	H-6, H-8	C-6, C-8, C-9	H-5, H-8, H-14, 6-OMe
8	2.78 (*dt*, 3.6, 7.0)	59.3 (CH)	H-7, H-9, H-14	C-1, C-5, C-6, C-7, C-9, C-10, C-14,	*
9	2.72 (*ddd*, 3.6, 6.0, 9.5)	34.6 (CH)	H-8, H_{ab}-10	C-2, C-7, C-8, C-10, C-11, C-14	H-2, H_3-12

Table 90 (continued)

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
10	a: 2.01 (*dd*, 9.5, 12.0) b: 1.42 (*dd*, 6.0, 12.0)	40.4 (CH_2)	H-9, H_b-10 H-9, H_a-10	C-1, C-8, C-9, C-11, C-12, C-13 C-1, C-8, C-9, C-11, C-12, C-13	* *
11	-	37.3 (C)	-	-	-
12	1.03 (*s*)	26.0 (CH_3)	-	C-1, C-10, C-11, C-13	*
13	1.06 (*s*)	24.3 (CH_3)	-	C-1, C-10, C-11, C-12	*
14	5.00 (*dd*, 7.0, 9.6)	84.1 (CH)	H-5, H-8	C-1, C-4, C-6, C-8, C-9	H-5, H-7, H-8
15	1.16 (*s*)	29.4 (CH_3)	-	C-3, C-4, C-5	*
2-CO_2*Me*	2.07 (*s*)	21.5 (CH_3)	-	2-*C*O_2Me	*
2-*C*O_2Me	-	170.3 (C=O)	-	-	-
6-OMe	3.44 (*s*)	57.7 (CH_3)	-	C-6	*

*not determined

1.3.2 Compound H1

H1 was obtained as a colorless solid, melting at 209-210 °C. The IR spectrum showed similar absorption bands to those of **H3**. The ^{1}H and ^{13}C NMR spectroscopic data (**Table 91**) (**Figures 7** and **8**) were also similar, except for the presence of an additional signal for a methoxy group (δ_H 3.29, *s* and δ_C 57.3). This methoxy group was attached at C-7 on the basis of the HMBC cross peak of the methoxy protons with C-7 (δ_C 85.8) (**Table 92**). Based on the identical NOEDIFF results (**Table 92**), **H1** had the same relative configuration as **H3**. The absolute configuration of **H1** was assigned based on comparison of specific rotation of **H1**, $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{24}}$: +24.8 (c 0.09, MeOH), with that

of **H3**, $[\alpha]_D^{24}$: +46.7 (c 0.09, MeOH). Accordingly, **H1** was pestalotiopsin D which was previously isolated from the fungus *Sinopodophyllum hexandrum* (Xiao et al., 2017).

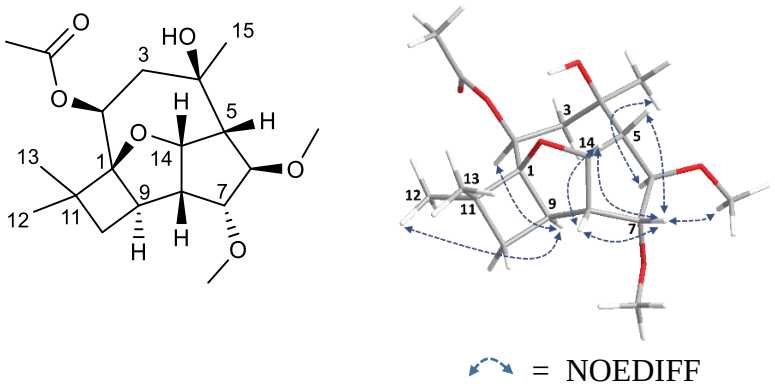


Table 91 The ^{1}H and ^{13}C NMR data of compound **H1** and pestalotiopsin D in $CDCl_3$

Position	**H1**		Pestalotiopsin D	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	93.9 (C)	-	94.0
2	5.28 (*dd*, 2.5, 3.5)	74.0 (CH)	5.25 (*dd*, 2.6, 4.1)	74.0
3	a: 2.03 (*dd*, 3.5, 12.5) b: 1.97 (*dd*, 2.5, 12.5)	37.8 (CH_2)	a: - b: 1.99 (*m*)	37.9
4	-	74.5 (C)	-	74.6
5	2.29 (*dd*, 7.0, 10.0)	56.8 (CH)	2.28 (*dd*, 6.9, 9.9)	56.8
6	3.25 (*t*, 7.0)	87.4 (CH)	3.24 (*t*, 6.6)	87.4
7	3.68 (*t*, 7.0)	85.8 (CH)	3.66 (*t*, 6.7)	85.9
8	2.93 (*dt*, 4.0, 7.0)	56.1 (CH)	2.92 (*dt*, 3.7, 6.9)	56.2
9	2.59 (*ddd*, 4.0, 6.0, 9.5)	34.2 (CH)	2.58 (*ddd*, 9.5, 5.8, 3.7)	34.3

Table 91 (continued)

Position	**H1**		Pestalotiopsin D	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
10	a: 1.99 (*dd*, 9.5, 12.0) b: 1.42 (*dd*, 6.0, 12.0)	40.1 (CH_2)	a: 2.01 (*m*) b: 1.40 (*dd*, 5.9, 11.9)	40.2
11	-	37.4 (C)	-	37.5
12	1.03 (*s*)	26.0 (CH_3)	1.06 (*s*)	24.5
13	1.07 (*s*)	24.3 (CH_3)	1.01 (*s*)	26.1
14	5.03 (*dd*, 7.0, 10.0)	84.4 (CH)	5.02 (*dd*, 6.9, 9.9)	84.5
15	1.15 (*s*)	29.3 (CH_3)	1.13 (*s*)	29.4
2-CO_2*Me*	2.07 (*s*)	21.5 (CH_3)	2.05 (*s*)	21.6
2-*C*O_2Me	-	170.2 (C=O)	-	170.4
4-OH	4.08 (*brs*)	-	-	-
6-OMe	3.37 (*s*)	57.5 (CH_3)	3.36 (*s*)	57.7
7-OMe	3.29 (*s*)	57.3 (CH_3)	3.27 (*s*)	57.4

Table 92 The ^{1}H-^{1}H COSY, HMBC and NOEDIFF data of compound **H1**

Proton	COSY	HMBC	NOEDIFF
H-2	H_{ab}-3	C-1, C-3, C-4, 2-*C*O_2Me	H-9, H_3-12
H_a-3	H-2, H_b-3	C-1, C-2, C-4, C-5, C-15	*
H_b-3	H-2, H_a-3	C-1, C-2, C-4, C-5, C-15	*
H-4	-	-	-
H-5	H-6, H-14	C-3, C-4, C-6, C-7, C-8, C-14, C-15	H-7, H-14, H_3-15, 6-OMe
H-6	H-5, H-7	C-4, C-5, C-7, 6-OMe	H_3-15
H-7	H-6, H-8	C-6, C-8, 7-OMe	H-5, H-8, H-14, 6-OMe, 7-OMe

Table 92 (continued)

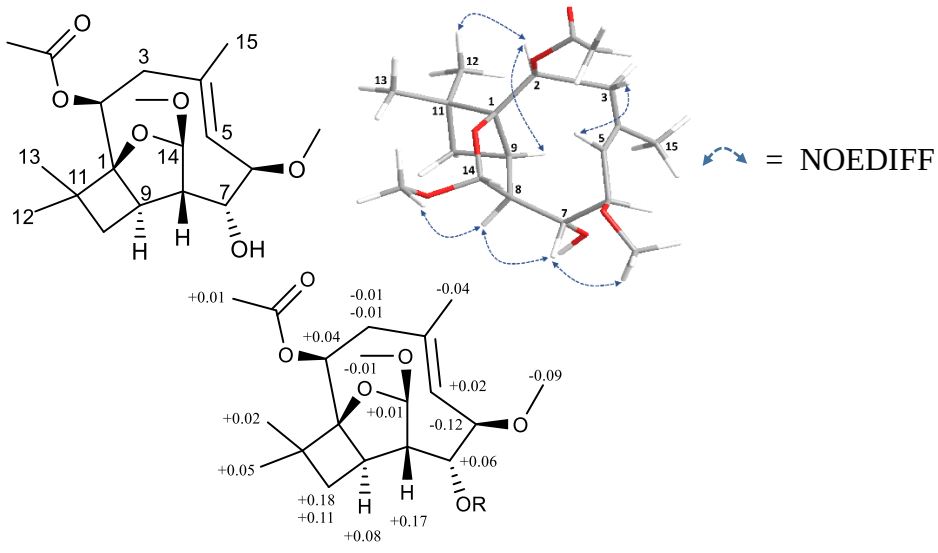
Proton	COSY	HMBC	NOEDIFF
H-8	H-7, H-9, H-14	C-1, C-5, C-6, C-7, C-9 C-10, C-14	*
H-9	H-8, H_{ab}-10	C-1, C-2, C-7, C-8, C-10, C-11, C-14	H-2, H_3-12
H_a-10	H-9, H_b-10	C-1, C-8, C-9, C-11, C-12, C-13	*
H_b-10	H-9, H_a-10	C-1, C-8, C-9, C-11, C-12, C-13	*
H-12	-	C-1, C-10, C-11, C-13	H-2
H-13	-	C-1, C-10, C-11, C-12	*
H-14	H-5, H-8	C-1, C-4, C-6, C-8, C-9	H-5, H-7, H-8
H-15	-	C-3, C-4, C-5	*
2-CO_2*Me*	-	2-*C*O_2Me	*
6-OMe	-	C-6	H-5, H-7, H-14
7-OMe	-	C-7	*

* not determined

1.3.3 Compound H2

H2 was obtained as a colorless gum and had the molecular formula $C_{19}H_{30}O_6$ on the basis of the HRESIMS peak at *m/z* 377.1935 $[M+Na]^+$ (**Figure 9**). The IR spectrum displayed absorption bands at 3387, 1736 and 1673 cm^{-1} for hydroxy, ester carbonyl and alkene moieties, respectively (Liu et al., 2016c). The ^1H NMR spectroscopic data (**Table 93**) (**Figure 10**) were similar to those of **H3** with the replacement of signals for one methine proton (H-5) and one oxymethine proton (H-14) in **H3** with signals for an olefinic proton (δ_H 5.09, *d*, *J* = 11.7 Hz, 1H) and an dioxygenated methine proton (δ_H 5.31, *brd*, *J* = 2.4 Hz, 1Hz) with an additional signal for a methoxy group (δ_H 3.52, *s*, 3H) in **H2**. The ^1H-^1H COSY correlation of H-5 (δ_H 5.09) with only H-6 (δ_H 3.85, *dd*, *J* = 6.3 and 11.7 Hz, 1H) (**Table 93**) together with the HMBC cross

peaks of H-5 with C-3 (δ_C 41.2), C-7 (δ_C 77.9) and C-15 (δ_C 17.8), not with C-14 (δ_C 116.3) (**Table 93**), indicated that a bond between C-5 and C-14 of the cyclopentane unit in **H3** was cleaved. The olefinic proton (δ_H 5.09) was attributed to H-5 according to the HMBC correlations of H_3-15 (δ_H 1.93) with C-3, C-4 (δ_C 138.0) and C-5 (δ_C 123.9) which further established a double bond between C-4 and C-5. Finally, based on the chemical shift of C-14 and the HMBC correlation of the methoxy protons (δ_H 3.52) with this carbon, a methoxy group was attached at C-14. Signal enhancement of H-9 (δ_H 2.44) and H_3-12 (δ_H 1.02), that of H-8 (δ_H 2.58) and 6-OMe (δ_H 3.31) and that of H-7 (δ_H 3.98) and 14-OMe (δ_H 3.52) upon irradiation of H-2 (δ_H 5.24), H-7 and H-8, respectively, in the NOEDIFF experiments (**Table 93**) indicated that H-2 and H-9 were located at opposite side of the molecule to H-7, H-8, 6-OMe and 14-OMe. The *E*-configuration of the C-4/C-5 double bond was established based on signal enhancement of H_b-3 (δ_H 2.49) after irradiation of H-5. The absolute configuration at C-7 was determined as *R* on the basis of the Mosher's method (**Table 94**) (**Figure 2**). Consequently, the remaining absolute configurations at C-1, C-2, C-6, C-8, C-9 and C-14 were assigned as *S*, *S*, *R*, *S*, *R* and *R*, respectively. Therefore, **H2** was identified as a new pestaloporinate.



H2a: R = (*S*)-MTPA
H2b: R = (*R*)-MTPA

Figure 2 $\Delta\delta$ values (δ_S-δ_R) obtained from (*S*)- and (*R*)-MTPA esters (**H2a** and **b**, respectively) of compound **H2**

Table 93 The ^{1}H and ^{13}C NMR data of compound **H2** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
1	-	98.2 (C)	-	-	-
2	5.24 (*dd*, 5.4, 10.8)	73.7 (CH)	H_{ab}-3	C-1, C-3, C-4, 2-*C*O_2Me	H-9, H_3-12
3	a: 2.54 (*dd*, 5.4, 13.2) b: 2.49 (*dd*, 10.8, 13.2)	41.2 (CH_2)	H-2, H_b-3 H-2, H_a-3	C-1, C-2, C-4, C-5, C-15 C-1, C-2, C-4, C-5, C-15	* *
4	-	138.0 (C)	-	-	-
5	5.09 (*d*, 11.7)	123.9 (CH)	H-6	C-3, C-7, C-15	H_b-3, H-7, H-14
6	3.85 (*dd*, 6.3, 11.7)	83.2 (CH)	H-5, H-7	C-4, C-5, C-7, 6-OMe	H_3-15, 6-OMe
7	3.98 (*dd*, 1.5, 6.3)	77.9 (CH)	H-6, H-8	C-6, C-8, C-9, C-14	H-8, H-14, H_3-15, 6-OMe
8	2.58 (*m*)	38.0 (CH)	H-7, H-9, H-14	C-7, C-9, C-10, C-14	H-7, 14-OMe
9	2.44 (*m*)	64.1 (CH)	H-8, H_{ab}-10	C-1, C-2, C-7, C-8, C-10, C-11, C-14	*
10	a: 1.96 (*dd*, 9.6, 12.3) b: 1.60 (*dd*, 6.3, 12.3)	42.3 (CH_2)	H-8, H_b-10 H-8, H_a-10	C-1, C-8, C-9, C-11, C-12, C-13 C-1, C-8, C-9, C-11, C-12, C-13	* *
11	-	40.0 (C)	-	-	-

Table 93 (continued)

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
12	1.02 (*s*)	27.4 (CH_3)	-	C-1, C-10, C-11, C-13	-
13	1.09 (*s*)	23.9 (CH_3)	-	C-1, C-10, C-11, C-12	-
14	5.31 (*brd*, 2.4)	116.3 (CH)	H-8	C-1, C-7, C-8, C-9, 14-OMe	H-5, H-7, 14-OMe
15	1.93 (*s*)	17.8 (CH_3)	-	C-3, C-4, C-5	*
2-CO_2*Me*	2.07 (*s*)	21.6 (CH_3)	-	2-*C*O_2Me	*
2-*C*O_2Me	-	170.6 (C=O)	-	-	-
6-OMe	3.31 (*s*)	56.2 (CH_3)	-	C-6	*
14-OMe	3.52 (*s*)	56.2 (CH_3)	-	C-14	*

* not determined

Table 94 The ^{1}H NMR data of (*S*)-MTPA (**H2a**) and (*R*)-MTPA (**H2b**) esters in $CDCl_3$

Position	**H2a**	**H2b**
	δ_H (*mult.*, J_{Hz})	δ_H (*mult.*, J_{Hz})
2	5.22 (*dd*, 5.4, 10.8)	5.18 (*dd*, 5.4, 10.5)
3	a: 2.54 (*dd*, 5.4, 13.5) b: 2.48 (*dd*, 10.8, 13.5)	a: 2.55 (*dd*, 5.4, 13.5) b: 2.49 (*dd*, 10.8, 13.5)
5	5.17 (*d*, 11.7)	5.15 (*d*, 11.7)
6	3.89 (*dd*, 6.0, 12.0)	4.01 (*dd*, 6.3, 12.0)
7	5.45 (*dd*, 2.7, 6.0)	5.39 (*dd*, 2.4, 6.3)
8	2.57 (*m*)	2.40 (*m*)
9	2.44 (*m*)	2.36 (*m*)
10	a: 1.91 (*dd*, 9.6, 12.3) b: 1.52 (*dd*, 6.0, 12.3)	a: 1.73 (*dd*, 9.6, 12.3) b: 1.41 (*dd*, 6.3, 12.3)
12	1.02 (*s*)	0.97 (*s*)

Table 94 (continued)

Position	**H2a**	**H2b**
	δ_H (*mult.*, J_{Hz})	δ_H (*mult.*, J_{Hz})
13	1.10 (*s*)	1.08 (*s*)
14	5.42 (*d*, 2.4)	5.41 (*d*, 2.4)
15	1.83 (*d*, 1.2)	1.87 (*d*, 0.9)
2-CO_2*Me*	2.07 (*s*)	2.06 (*s*)
6-OMe	3.17 (*s*)	3.26 (*s*)
14-OMe	3.54 (*s*)	3.55 (*s*)

1.3.4 Compound H7

H7 was obtained as a colorless solid, melting at 103-105 °C. The IR absorption bands were similar to those of **H3** except for the absence of a hydroxy absorption band. The ^{1}H and ^{13}C NMR spectroscopic data (**Table 95**) (**Figures 12** and **13**) were also similar to those of **H3**. The HMBC correlation of H-7 (δ_H 3.99) with C-4 (δ_C 79.7) (**Table 96**) established an ether linkage between C-4 and C-7 (δ_C 76.9). The identical NOEDIFF data of **H3** and **H7** (**Table 96**) suggested that they had an identical relative configuration. Comparison of specific rotation of **H7**, $[\alpha]_D^{24}$ = +123.3 (c 0.06, MeOH), with that of pestaloporinate D previously isolated from the fungus *Pestalotiopsis* sp., $[\alpha]_D^{25}$: +139.1 (c 0.06, MeOH), (Liu et al., 2016c), indicated that **H7** had the same absolute configuration as pestaloporinate D. Consequently, **H7** was pestaloporinate D.

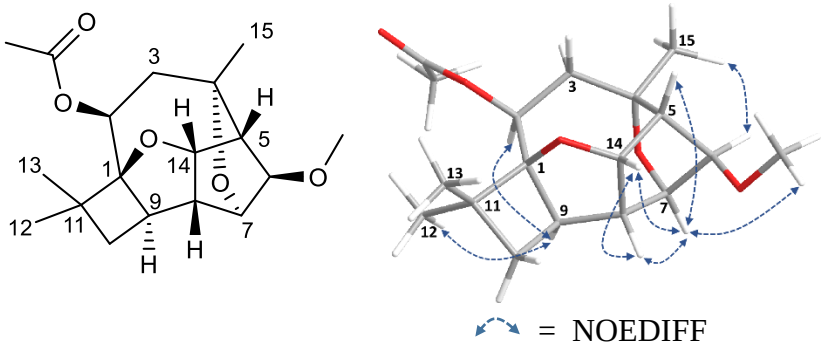


Table 95 The ^{1}H and ^{13}C NMR data of compound **H7** and pestaloporinate D in $CDCl_3$

Position	**H7**		Pestaloporinate D	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	94.5 (C)	-	94.6
2	5.21 (*dd*, 7.2, 8.4)	75.9 (CH)	5.20 (*dd*, 7.0, 8.0)	75.9
3	a: 2.30 (*dd*, 7.2, 14.1) b: 1.87 (*dd*, 8.4, 14.1)	41.3 (CH_2)	a: 2.29 (*dd*, 7.0, 14.1) b: 1.87 (*dd*, 8.0, 14.1)	41.4
4	-	79.7 (C)	-	79.8
5	2.29 (*m*)	51.7 (CH)	2.27 (*m*)	51.7
6	4.27 (*t*, 2.7)	87.9 (CH)	4.26 (*t*, 2.6)	88.0
7	3.99 (*t*, 2.7)	76.9 (CH)	3.98 (*t*, 2.6)	77.0
8	2.59 (*dd*, 2.7, 8.1)	49.9 (CH)	2.58 (*dd*, 2.6, 8.6)	50.0
9	2.74 (*t*, 8.7)	38.6 (CH)	2.73 (*t*, 8.6)	38.6
10	a: 1.74 (*dd*, 8.7, 10.8) b: 1.62 (*dd*, 9.0, 10.8)	37.4 (CH_2)	a: 1.74 (*dd*, 8.6, 10.7) b: 1.61 (*dd*, 8.6, 10.7)	37.5
11	-	38.5 (C)	-	38.7
12	1.13 (*s*)	25.1 (CH_3)	1.13 (*s*)	25.1
13	1.09 (*s*)	23.0 (CH_3)	1.08 (*s*)	23.1
14	5.24 (*dd*, 4.5, 7.8)	88.8 (CH)	5.23 (*dd*, 4.5, 8.6)	88.8
15	1.21 (*s*)	31.5 (CH_3)	1.20 (*s*)	31.5
2-CO_2*Me*	2.03 (*s*)	21.3 (CH_3)	2.02 (*s*)	21.4
2-*C*O_2Me	-	170.3 (C=O)	-	170.3
6-OMe	3.30 (*s*)	57.4 (CH_3)	3.29 (*s*)	57.5

Table 96 The ^{1}H-^{1}H COSY, HMBC and NOEDIFF data of compound **H7**

Proton	COSY	HMBC	NOEDIFF
H-2	H_{ab}-3	C-1, C-3, C-9, 2-*C*O_2Me	H-9, H_3-12
H_a-3	H-2, H_b-3	C-1, C-2, C-4, C-5, C-15	*
H_b-3	H-2, H_a-3	C-1, C-2, C-4, C-5, C-15	*
H-5	H-6, H-14	C-6, C-8	*
H-6	H-5, H-7	C-8, C-14, 6-OMe	H_3-15, 6-OMe
H-7	H-6, H-8	C-4, C-5, C-8, C-14	*
H-8	H-7, H-14	C-1, C-5, C-7, C-9, C-10, C-14	H-7, H_b-10, H-14, 6-OMe
H-9	H_{ab}-10	C-2, C-7, C-8, C-10, C-14	H-2, H_a-10, H_3-12
H_a-10	H-9, H_b-10	C-1, C-2, C-8, C-9, C-11, C-12, C-13	*
H_b-10	H-9, H_a-10	C-1, C-2, C-8, C-9, C-11, C-12, C-13	*
H_3-12	-	C-1, C-10, C-11, C-13	*
H_3-13	-	C-1, C-10, C-11, C-12	*
H-14	H-5, H-8	C-1, C-4, C-7, C-8, C-9	*
H_3-15	-	C-2, C-3, C-4, C-5,	H-5, H-6
2-CO_2*Me*	-	2-*C*O_2Me	*
6-OMe	-	C-6	H-5, H-6, H-7, H-8

* not determined

1.3.5 Compound H11

H11 was isolated as a colorless solid, melting at 130-133 °C. The IR spectrum showed similar absorption bands to those of **H3** with an additional absorption

band at 1675 cm^{-1} for a C=C stretching for an alkene group. The ^{1}H and ^{13}C NMR spectroscopic data (**Table 97**) (**Figure 14** and **15**) were also similar to those of **H3** except for the replacement of one hydroxy quaternary carbon and one methine carbon in **H3** with a tetrasubtituted double bond (δ_C 133.7 and 129.6) in **H11**. This tetrasubtituted double bond was located at C-4 (δ_C 129.6) and C-5 (δ_C 133.7) on the basis of the HMBC correlations from H_3-15 (δ_H 1.81) to C-3 (δ_C 34.4), C-4 and C-5 (**Table 98**). The identical NOEDIFF data of **H11** (**Table 98**) to those of **H3** indicated that **H11** had the same relative configuration as **H3**. Comparison of specific rotation of **H11**, $[\alpha]_D^{24}$ = +90.3 (c 0.14, MeOH), with that of pestaloporinate C, $[\alpha]_D^{25}$ = +85.7 (c 0.14, MeOH), suggested that the absolute configuration of **H11** was the same as that of pestaloporinate C. Therefore, **H11** was pestaloporinate C which was previously isolated from *Pestalotiopsis* sp. (Liu et al., 2016c).

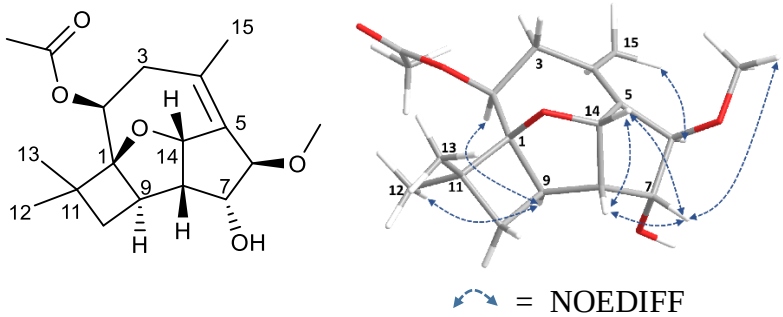


Table 97 The ^{1}H and ^{13}C NMR data of compound **H11** and pestaloporinate C in $CDCl_3$

Position	**H11**		Pestaloporinate C	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	92.7 (C)	-	94.0
2	5.22 (*dd*, 5.1, 10.5)	78.4 (CH)	5.17 (*dd*, 5.0, 11.6)	79.9
3	a: 3.21 (*dd*, 10.5, 13.2) b: 1.97 (*dd*, 5.1, 13.2)	34.4 (CH_2)	a: 3.16 (*dd*, 11.6, 11.8) b: 1.92 (*dd*, 5.0, 11.8)	35.4
4	-	129.6 (C)	-	130.5
5	-	133.7 (C)	-	135.2

Table 97 (continued)

Position	**H11**		Pestaloporinate C	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
6	3.86 (*brs*)	85.3 (CH)	3.86 (*brs*)	86.6
7	4.15 (*d*, 7.8)	73.8 (CH)	4.05 (*brd*, 7.8)	74.4
8	2.60 (*dt*, 3.0, 7.8)	54.6 (CH)	2.53 (*dd*, 2.9, 7.8)	56.4
9	2.98 (*ddd*, 3.0, 6.9, 9.6)	39.4 (CH)	3.00 (*ddd*, 2.9, 5.0, 7.0)	41.0
10	a: 1.96 (*dd*, 6.9, 11.4) b: 1.51 (*dd*, 9.6, 11.4)	42.3 (CH_2)	a: 1.95 (*dd*, 5.0, 11.3) b: 1.48 (*dd*, 7.0, 11.3)	43.5
11	-	38.0 (C)	-	39.0
12	1.11 (*s*)	26.5 (CH_3)	1.11 (*s*)	27.4
13	1.11 (*s*)	23.5 (CH_3)	1.07 (*s*)	24.0
14	5.29 (*brd*, 6.3)	89.5 (CH)	5.20 (*m*)	90.7
15	1.81 (*d*, 1.5)	22.0 (CH_3)	1.79 (*s*)	22.2
2-CO_2*Me*	2.05 (*s*)	21.6 (CH_3)	2.02 (*s*)	21.6
2-*C*O_2Me	-	170.2 (C=O)	-	172.0
6-OMe	3.21 (*s*)	55.2 (CH_3)	3.20 (*s*)	55.6

Table 98 The ^{1}H-^{1}H COSY, HMBC and NOEDIFF data of compound **H11**

Proton	COSY	HMBC	NOEDIFF
H-2	H_{ab}-3	C-1, C-3, C-9, 2-*C*O_2Me	H-9, H_3-12
H_a-3	H-2, H_b-3	C-1, C-2, C-4, C-5, C-15	*
H_b-3	H-2, H_a-3	C-1, C-2, C-4, C-5, C-15	*

Table 98 (continued)

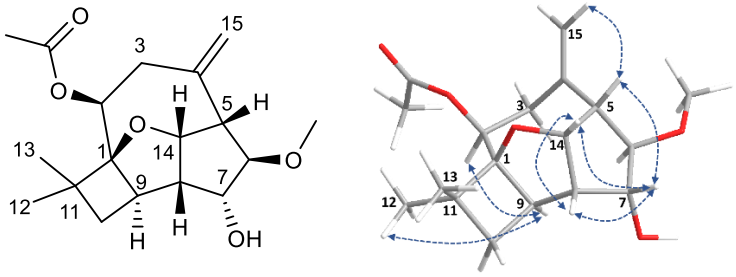
Proton	COSY	HMBC	NOEDIFF
H-6	-	C-4, C-5, C-7, C-8, C-14, 6-OMe	*
H-7	H-8	C-5, C-6, C-9, C-14	H-8, H-14
H-8	H-7, H-9, H-14	C-1, C-5, C-6, C-9, C-10, C-14	H-7, H-14, 6-OMe
H-9	H-8, H_{ab}-10	C-1, C-2, C-7, C-8, C-10, C-11, C-14	H-2, H_{ab}-10, H_3-12
H_a-10	H-9, H_b-10	C-1, C-8, C-9, C-11, C-12, C-13	*
H_b-10	H-9, H_a-10	C-1, C-8, C-9, C-11, C-12, C-13	*
H_3-12	-	C-1, C-10, C-11, C-13	*
H_3-13	-	C-1, C-10, C-11, C-12	*
H-14	H-8	C-1, C-4, C-9	H-7, H-8
H_3-15	-	C-3, C-4, C-5	*
2-CO_2*Me*	-	2-*C*O_2Me	*
6-OMe	-	C-6	*

* not determined

1.3.6 Compound H10

H10 was isolated as a colorless gum. The molecular formula was determined to be $C_{18}H_{26}O_5$ on the basis of the HRESIMS at peak *m*/*z* 345.1686 $[M+Na]^+$ (**Figure 16**). The IR spectrum was similar to that of **H3** with an additional absorption band of a double bond functionality at 1647 cm^{-1}. The ^1H NMR spectroscopic data (**Table 99**) (**Figure 17**) were similar to those of **H3** except for the replacement of one signal for one methyl group in **H3** with two geminal olefinic proton signals [δ_H 4.83 (*d*, *J* = 2.5 Hz, 1H) and 4.72 (*brs*, 1H)] in **H10**. These results together

with the replacement of signals for the hydroxy quaternary (C-4, δ_C 74.5) and the methyl (C-15, δ_C 29.4) carbons in **H3** with one olefinic quaternary carbon (δ_C 144.9) and one olefinic methylene carbon (δ_C 117.0) in the ^{13}C NMR spectrum of **H10** (**Table 99**) (**Figure 18**) suggested the presence of a 1,1-disubstituted alkene in **H10**. The HMBC correlations of both olefinic protons with C-3 (δ_C 35.5), C-4 (δ_C 144.9) and C-5 (δ_C 58.1) (**Table 99**) established a C-4/C-15 double bond. The NOEDIFF data of **H10** (**Table 99**) were similar to those of **H3**, indicating that they had the same relative configuration. The absolute configuration of a secondary alcohol at C-7 could not be assigned on the basis of Mosher's method because of low quantity. Since **H3** and **H10** were cometabolite, their absolute configuration of all chiral centers was proposed to be identical. Therefore, **H10** was identified as a new dehydrated derivative of **H3**.



= NOEDIFF

Table 99 The ^{1}H and ^{13}C NMR data of compound **H10** in acetone-d_6

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
1	-	94.5 (C)	-	-	-
2	5.14 (*t*, 3.5)	72.8 (CH)	H_{ab}-3	C-4, 2-*C*O_2Me	H-9, H_3-12
3	a: 2.75 (*ddd*, 1.0, 3.5, 15.0) b: 2.39 (*dd*, 3.5, 15.0)	35.5 (CH_2)	H-2, H_b-3, H_b-15 H-2, H_a-3	C-1, C-2, C-4, C-5, C-15 C-1, C-2, C-4, C-5, C-15	* *
4	-	144.9 (C)	-	-	-

Table 99 (continued)

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
5	2.95 (*dd*, 5.0, 9.5)	58.1 (CH)	H-6, H-14	C-3, C-4, C-6, C-8, C-14, C-15	*
6	3.58 (*t*, 5.0)	92.7 (CH)	H-5, H-7	C-4, C-7, C-14, 6-OMe	6-OMe
7	4.08 (*m*)	76.5 (CH)	H-6, H-8, 7-OH	C-9	H-5, H-8, 6-OMe
8	2.80 (*dd*, 3.0, 7.5)	59.5 (CH)	H-7, H-14	C-1, C-6, C-7, C-9, C-10, C-14	*
9	2.90 (*ddd*, 3.0, 6.0, 9.5)	35.6 (CH)	H-8, H_{ab}-10	C-2, C-10, C-11, C-14	*
10	a: 1.92 (*dd*, 9.5, 11.5) b: 1.39 (*dd*, 6.0, 11.5)	41.3 (CH_2)	H-9, H_b-10 H-9, H_a-10	C-1, C-8, C-9, C-11, C-12, C-13 C-1, C-8, C-9, C-11, C-12, C-13	* *
11	-	37.4 (C)	-	-	*
12	1.03 (*s*)	26.4 (CH_3)	-	C-1, C-10, C-11, C-13	*
13	0.98 (*s*)	24.3 (CH_3)	-	C-1, C-10, C-11, C-12	*

Table 99 (continued)

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
14	4.93 (*dd*, 7.5, 9.5)	86.4 (CH)	H-5, H-8	C-1, C-4, C-9	H-5, H-7, H-8
15	a: 4.83 (*d*, 2.5) b: 4.72 (*brs*)	117.0 (CH_2)	H_b-15 H_a-15	C-3, C-4, C-5 C-3, C-4, C-5	H-5, 6-OMe *
2-CO_2*Me*	1.99 (*s*)	21.3 (CH_3)	-	2-*C*O_2Me	*
2-*C*O_2Me	-	170.6 (C=O)	-	-	-
6-OMe	3.28 (*s*)	57.5 (CH_3)	-	C-6	*
7-OH	4.37 (*d*, 4.0)	-	H-7	-	*

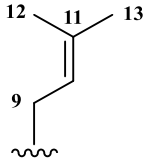
* not determined

1.3.7 Compound H4

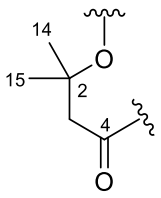
H4 was isolated as a colorless gum and had the molecular formula $C_{16}H_{24}O_5$ on the basis of the HRESIMS peak at *m/z* 319.1530 $[M+Na]^+$ (**Figure 19**). The UV spectrum exhibited an absorption band at 277 nm and the IR spectrum showed absorption bands at 3420 and 1653 cm^{-1} for hydroxy and conjugated ketone carbonyl functional groups, respectively (Sanson et al., 1991). The ^1H NMR spectrum (**Table 100**) (**Figure 20**) displayed signals for one olefinic proton (δ_H 4.94, *septt*, *J* = 1.2 and 8.4 Hz, 1H), two methine protons [δ_H 3.77 (*ddd*, *J* = 5.7, 9.9 and 10.2 Hz, 1H) and 3.42 (*d*, *J* = 9.9 Hz, 1H)], three sets of nonequivalent methylene protons [δ_H 2.93 (*dd*, *J* = 5.7 and 15.9 Hz, 1H) and 1.92 (*dd*, *J* = 10.2 and 15.9 Hz, 1H); 2.65 (*dd*, *J* = 8.4 and 14.4 Hz, 1H) and 2.53 (*dd*, *J* = 8.4 and 14.4 Hz, 1H); 2.57 (*d*, *J* = 16.5 Hz, 1H) and 2.47 (*d*, *J* = 16.5 Hz, 1H)] and four methyl groups [δ_H 1.71 (*brs*, 3H), 1.69 (*brs*, 3H), 1.46 (*s*, 3H) and 1.41 (*s*, 3H)]. The ^{13}C NMR spectrum (**Table 100**) (**Figure 21**) exhibited signals for one ketone carbonyl carbon (δ_C 192.3), one olefinic methine carbon (δ_C 117.4), three olefinic quaternary carbons (δ_C 164.8, 137.0 and 108.2), two oxyquaternary carbons (δ_C 80.6 and 74.8), two oxymethine carbons (δ_C 74.5 and 67.8),

three methylene carbons (δ_C 47.4, 34.5 and 26.5) and four methyl carbons (δ_C 27.4, 26.1, 24.8 and 18.1). Substructure A was constructed based on the fact that the olefinic proton (δ_H 4.94, H-10) was coupled with H_{ab}-9 (δ_H 2.65 and 2.53) as well as H_3-12 (δ_H 1.69) and H_3-13 (δ_H 1.71) with vicinal and allylic coupling constants of 8.4 and 1.2 Hz, respectively. The HMBC correlations of H_{ab}-9 with C-10 (δ_C 117.4) and C-11 (δ_C 137.0) as well as those of H_3-12 and H_3-13 with C-10 and C-11 (**Table 100**) confirmed the assigned substructure A. The HMBC cross peaks of H_3-14 (δ_H 1.41) and H_3-15 (δ_H 1.46) with C-2 (δ_C 80.6) and C-3 (δ_C 47.4) and those of H_{ab}-3 (δ_H 2.57 and 2.47) with C-2, C-4 (δ_C 192.3), C-14 (δ_C 24.8) and C-15 (δ_C 27.4) together with the chemical shift of C-2 afforded substructure B with an oxygen atom at C-2 and a ketone functionality at C-4. Substructure C was established on the basis of the ^{1}H-^{1}H COSY correlations of H-6 (δ_H 3.77) with H_{ab}-5 (δ_H 2.93 and 1.92) and H-7 (δ_H 3.42) (**Table 100**). The substituents at C-6 (δ_C 67.8) and C-7 (δ_C 74.5) were hydroxy groups due to their chemical shifts. The HMBC correlations of H_{ab}-5 of substructure C with C-4a (δ_C 108.2) and C-8a (δ_C 164.8), those of H_{ab}-3 of substructure B with C-4a and those of H_{ab}-9 of substructure A with C-7, C-8 (δ_C 74.8) and C-8a and the chemical shifts of C-2, C-8 and C-8a combined substructures A-C to afford a fused cyclohexene-1,4-pyrone ring.

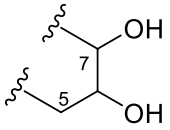
The relative configuration was assigned by the NOEDIFF data (**Table 100**) and the coupling constants. The large coupling constant of 9.9 Hz between H-6 and H-7 suggested that those protons were in *pseudoaxial* positions. Irradiations of H-10, H_3-12 and H_3-13 enhanced signal intensity of H-7, indicating a *cis*-relationship between substructure A and H-7. Therefore, the absolute configuration of **H4** might be either 6*R*, 7*S*, 8*S* or 6*S*, 7*R*, 8*R*. Because the experimental CD data of **H4** were similar to the ECD data of the 6*R*, 7*S*, 8*S* isomer (**Figure 3**), the absolute configuration of **H4** was assigned as 6*R*, 7*S*, 8*S*. Consequently, **H4** was a new natural compound.



Substructure A



Substructure B



Substructure C

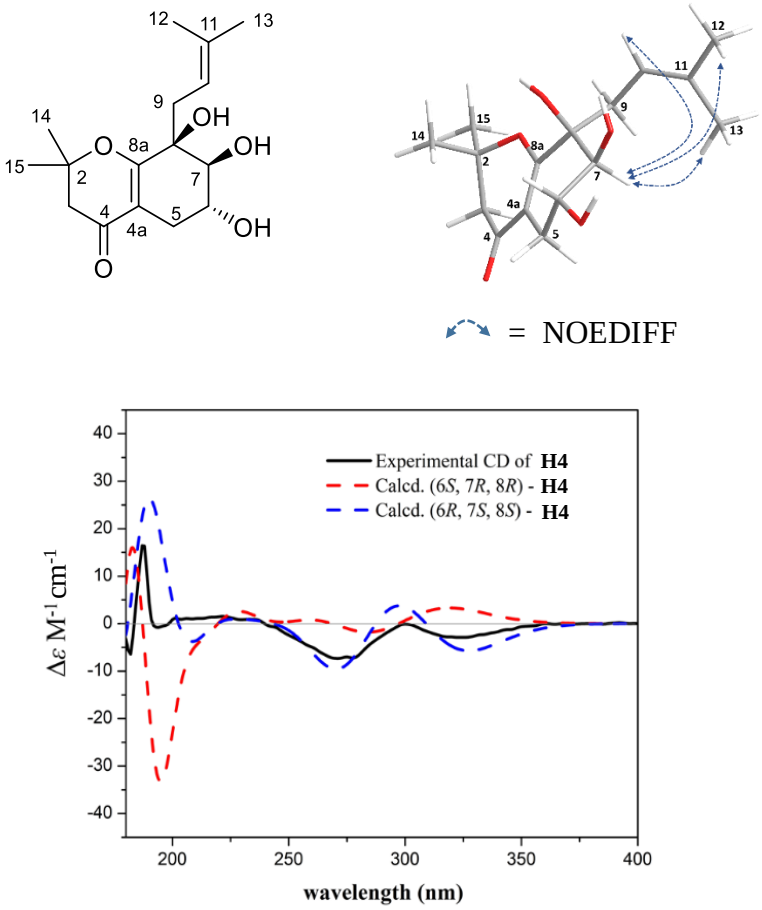


Figure 3 Experimental and calculated ECD spectra of compound **H4**

Table 100 The ^{1}H and ^{13}C NMR data of compound **H4** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
2	-	80.6 (C)	-	-	-
3	a: 2.57 (*d*, 16.5) b: 2.47 (*d*, 16.5)	47.4 (CH_2)	H_b-3 H_a-3	C-2, C-4, C-4a, C-14, C-15 C-2, C-4, C-4a, C-14, C-15	* *
4	-	192.3 (C=O)	-	-	-
4a	-	108.2 (C)	-	-	-

Table 100 (continued)

Position	δ_H (*mult*., J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
5	a: 2.93 (*dd*, 5.7, 15.9) b: 1.92 (*dd*, 10.2, 15.9)	26.5 (CH_2)	H_b-5, H-6 H_a-5, H-6	C-4, C-4a, C-6, C-7, C-8a C-4, C-4a, C-6, C-7, C-8a	H_b-5, H-6 H_a-5, H-7
6	3.77 (*ddd*, 5.7, 9.9, 10.2)	67.8 (CH)	H_{ab}-5, H-7	C-5, C-7	H_a-5, H-7
7	3.42 (*d*, 9.9)	74.5 (CH)	H-6	C-5, C-6, C-9	H_b-5
8	-	74.8 (C)	-	-	-
8a	-	164.8 (C)	-	-	-
9	a: 2.65 (*dd*, 8.4, 14.4) b: 2.53 (*dd*, 8.4, 14.4)	34.5 (CH_2)	H_b-9, H-10 H_a-9, H-10	C-7, C-8, C-8a, C-10, C-11 C-7, C-8, C-8a, C-10, C-11	* *
10	4.94 (*septt*, 1.2, 8.4)	117.4 (CH)	H_{ab}-9, H_3-12, H_3-13	C-12, C-13	H-7, H_{ab}-9, H_3-13, H_3-15
11	-	137.0 (C)	-	-	-
12	1.69 (*brs*)	18.1 (CH_3)	H-10	C-10, C-11, C-13	H-7, H_{ab}-9
13	1.71 (*brs*)	26.1 (CH_3)	H-10	C-10, C-11, C-12	H-7, H-10
14	1.41 (*s*)	24.8 (CH_3)	-	C-2, C-3, C-15	*
15	1.46 (*s*)	27.4 (CH_3)	-	C-2, C-3, C-14	*

*not determined

1.3.8 Compound H8

H8 was obtained as a colorless gum. The IR spectrum revealed absorption bands at 3443, 1746 and 1713 cm^{-1} for hydroxy, ester carbonyl and ketone

carbonyl groups, respectively (Gohrt et al., 1992). The ^{1}H NMR spectrum (**Table 101**) (**Figure 22**) displayed signals for four methine protons [δ_H 5.11 (*dqd*, *J* = 1.2, 6.6 and 11.4 Hz, 1H); 3.74 (*m*, 1H); 3.09 (*ddd*, *J* = 4.2, 4.2 and 11.4 Hz, 1H) and 2.88 (*dd*, *J* = 4.2 and 9.0 Hz, 1H)], one hydroxy group (δ_H 4.31, *brs*, 1H), three sets of nonequivalent methylene protons [δ_H 3.50 (*d*, *J* = 14.4 Hz, 1H) and 3.43 (*d*, *J* = 14.4 Hz, 1H); 2.96 (*dd*, *J* = 5.7 and 13.5 Hz, 1H) and 2.66 (*dd*, *J* = 3.6 and 13.5 Hz, 1H); 2.35 (*ddd*, *J* = 1.2, 4.2 and 14.4 Hz, 1H) and 1.47 (*dt*, *J* = 11.4 and 14.4 Hz, 1H) and one methyl group (δ_H 1.28, *d*, *J* = 6.6 Hz, 3H). The ^{13}C NMR spectrum (**Table 101**) (**Figure 23**) exhibited signals for one ketone carbonyl carbon (δ_C 201.2), one ester carbonyl carbon (δ_C 166.4), four methine carbons (δ_C 69.6, 68.2, 61.4 and 56.1), three methylene carbons (δ_C 52.4, 49.9 and 37.4) and one methyl carbon (δ_C 20.8). The ^{1}H-^{1}H COSY correlations of H-9 (δ_H 5.11)/H_{ab}-8 (δ_H 2.35 and 1.47) and H_3-10 (δ_H 1.28), H-7 (δ_H 3.09)/H-6 (δ_H 2.88) and H_{ab}-8, and H-5 (δ_H 3.74)/H_{ab}-4 (δ_H 2.96 and 2.66), H-6 and 5-OH (δ_H 4.31) (**Table 102**) as well as the HMBC correlations from H-9 to C-1 (δ_C 166.4), from H_{ab}-2 (δ_H 3.50 and 3.43) to C-1 and C-3 (δ_C 201.2), and from H-5 to C-3 (**Table 102**) established a 10-membered lactone with the ketone carbonyl and methyl groups at C-3 and C-9 (δ_C 69.6), respectively. The chemical shifts of C-5 (δ_C 68.2), C-6 (δ_C 61.4) and C-7 (δ_C 56.1) indicated the presence of a hydroxy group at C-5 and an epoxide at C-6 and C-7.

The relative configuration of **H8** was determined on the basis of the coupling constant values. The coupling constants of 1.2 and 12.6 Hz between H_{ab}-8 and H-9 suggested that H-9 was located at an *axial* position. Furthermore, H_b-8 was coupled with H-7 with a large coupling constant of 11.4 Hz, indicating that H-7 was also at an *axial* position. A *cis*-epoxide was established according to the coupling constant of 4.2 Hz between H-6 and H-7. Furthermore, an *axial* position of H-5 was assigned based on a coupling constant of 9.0 Hz between H-5 and H-6. The specific rotation of **H8**, $[\alpha]_D^{24}$ = -40.9 (c 1.00, MeOH), was similar to that of decarestrictine B, $[\alpha]_D^{20}$ = -49.0 (c 1.00, MeOH) (Grabley et al., 1992), indicating that they had the same absolute configuration. Therefore, **H8** was identified as decarestrictine B which was previously isolated from *Penicillium simplicissimum* (Grabley et al., 1992).

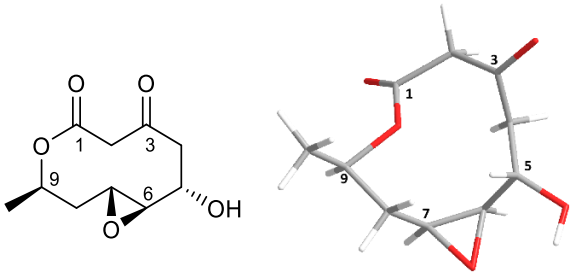


Table 101 The ^{1}H and ^{13}C NMR data of compound **H8** in acetone-d_6 and decarestrictine B in $CDCl_3$

Position	**H8**		Decarestrictine B	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	166.4 (C=O)	-	165.2
2	a: 3.50 (*d*, 14.4) b: 3.43 (*d*, 14.4)	52.4 (CH_2)	a: 3.50 (*ddd*, 0.7, 0.8, 14.4) b: 3.43 (*dd*, 0.4, 14.4)	52.0
3	-	201.2 (C=O)	-	200.1
4	a: 2.96 (*dd*, 5.7, 13.5) b: 2.66 (*dd*, 3.6, 13.5)	49.9 (CH_2)	a: 2.90 (*dddd*, 0.4, 0.8, 6.2, 13.4) b: 2.80 (*ddd*, 0.7, 3.6, 13.4)	48.4
5	3.74 (*m*)	68.2 (CH)	3.83 (*dddd*, 2.6, 3.6, 6.2, 9.1)	67.8
6	2.88 (*dd*, 4.2, 9.0)	61.4 (CH)	2.98 (*dd*, 4.0, 9.0)	60.5
7	3.09 (*ddd*, 4.2, 4.2, 11.4)	56.1 (CH)	3.18 (*ddd*, 4.0, 4.3, 10.4)	56.3
8	a: 2.35 (*ddd*, 1.2, 4.2, 14.4) b: 1.47 (*dt*, 11.4, 14.4)	37.4 (CH_2)	a: 2.35 (*ddd*, 1.4, 4.3, 14.7) b: 1.52 (*ddd*, 10.4, 11.6, 14.7)	36.7
9	5.11 (*dqd*, 1.2, 6.6, 11.4)	69.6 (CH)	5.15 (*dqd*, 1.4, 6.4, 11.6)	69.0

Table 101 (continued)

Position	**H8**		Decarestrictine B	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
10	1.28 (*d*, 6.6)	20.8 (CH_3)	1.34 (*d*, 6.4)	20.6
5-OH	4.31 (*brs*)	-	2.22 (*d*, 2.6)	-

Table 102 The ^{1}H-^{1}H COSY, HMBC and NOEDIFF data of compound **H8**

Proton	COSY	HMBC	NOEDIFF
H_a-2	H_b-2	C-1, C-3, C-4	*
H_b-2	H_a-2	C-1, C-3, C-4	*
H_a-4	H_b-4, H-5	C-2, C-3, C-5, C-6	*
H_b-4	H_a-4, H-5	C-2, C-3, C-5, C-6	*
H-5	H_{ab}-4, H-6, 5-OH	C-3	*
H-6	H-5, H-7	C-4, C-5, C-7	*
H-7	H-6, H_{ab}-8	C-5, C-6, C-8	*
H_a-8	H-7, H_b-8, H-9	C-6, C-7, C-10	H-9, H_3-10
H_b-8	H-7, H_a-8, H-9	C-6, C-7, C-9, C-10	*
H-9	H_{ab}-8, H_3-10	C-1, C-7, C-8, C-10	H_{ab}-8, H_3-10
H_3-10	H-9	C-7, C-8, C-9	H_{ab}-8, H-9
5-OH	H-5	-	*

* not determined

1.3.9 Compound H12

H12 was obtained as a colorless gum and had the molecular formula $C_{10}H_{14}O_5$ on the basis of the HRESIMS peak at *m*/*z* 237.0727 $[M+Na]^+$ (**Figure 24**). The IR spectrum displayed almost identical to those of **H8**. The ^{1}H and ^{13}C NMR data (**Table 103**) (**Figures 25** and **26**) were also similar to those of **H8**. The differences were observed in the ^{1}H-^{1}H COSY spectrum in **H12** which displayed the ^{1}H-^{1}H COSY cross peaks of H-3 (δ_H 4.51) with H_{ab}-2 (δ_H 2.93 and 2.57) and H_{ab}-4 (δ_H 3.16 and 2.77) (**Table 103**). Furthermore, the HMBC correlations of H_{ab}-2 and H-9 (δ_H 5.12) with C-1 (δ_C 168.9) as well as H-3 and H-6 (δ_H 3.74) with C-5 (δ_C 201.6) (**Table 103**) indicated

that the ketone carbonyl group at C-3 and the hydroxy group at C-5 in **H8** were reduced and oxidized to a secondary alcohol and a ketone moiety, respectively, in **H12**. Based on the $J_{\text{H-6, H-7}}$, $J_{\text{H-7, H}_b\text{-8}}$ and $J_{\text{H}_b\text{-8, H-9}}$ values of 4.8, 9.9 and 12.6 Hz, respectively, the relative configuration at C-6, C-7 and C-9 was assigned to be identical to that of **H8**. Due to the large coupling constants of 9.0 and 10.2 Hz between H-3 and H_a-4 as well as H_b-2, respectively, H-3 was located at an *axial* position. These results together with signal enhancement of H_a-4 after irradiation of H-6 in the NOEDIFF data (**Table 103**) deduced that H-3 and H-6 were located at the different side of the molecule. As **H8** and **H12** were cometabolite, the absolute configuration at C-6, C-7 and C-9 of **H12** was proposed to be identical to that of **H8**, resulting in an *R* configuration at C-3 based on the relative configuration. Consequently, **H12** was a new decarestrictine derivative.

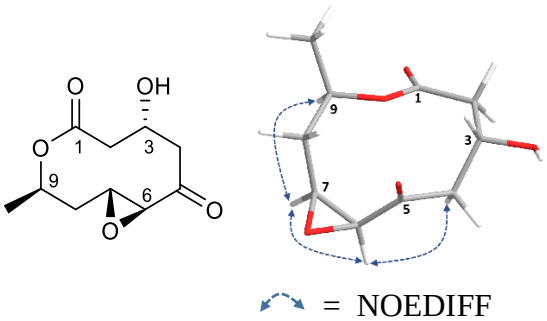


Table 103 The ^{1}H and ^{13}C NMR data of compound **H12** in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
1	-	168.9 (C=O)	-	-	-
2	a: 2.93 (*ddd*, 0.9, 4.2, 15.3) b: 2.57 (*dd*, 10.2, 15.3)	44.2 (CH_2)	H-3, H_b-2 H-3, H_a-2	C-1, C-3, C-4 C-1, C-3, C-4	H-3 *
3	4.51 (*dddd*, 1.2, 4.2, 9.0, 10.2)	66.1 (CH)	H_{ab}-2, H_{ab}-4	C-5	H_a-2, H_b-4
4	a: 3.16 (*dd*, 9.0, 14.4)	50.6 (CH_2)	H-3, H_b-4	C-2, C-3, C-5, C-6	*

Table 103 (continued)

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC	NOEDIFF
	b: 2.77 (*d*, 14.4)		H-3, H_a-4	C-2, C-3, C-5, C-6	H-3
5	-	201.6 (C=O)	-	-	-
6	3.74 (*d*, 4.8)	57.2 (CH)	H-7	C-5, C-7	H_a-4, H-7
7	3.33 (*ddd*, 3.9, 4.8, 9.9)	56.5 (CH)	H-6, H_{ab}-8	C-6, C-8	H-6, H_a-8, H-9
8	a: 2.23 (*ddd*, 1.5, 3.9, 14.7) b: 1.66 (*ddd*, 9.9, 12.6, 14.7)	33.6 (CH_2)	H-7, H_b-8 H-7, H_a-8	C-7, C-9, C-10 C-7, C-9, C-10	H-7, H_b-8, H-9, H_3-10 *
9	5.12 (*dqd*, 1.5, 6.3, 12.6)	67.2 (CH)	H_{ab}-8, H_3-10	C-1, C-7, C-8	H-7, H_a-8, H_3-10
10	1.25 (*d*, 6.3)	20.6 (CH_3)	H-9	C-7, C-8, C-9	H_{ab}-8, H-9

* not determined

1.3.10 Compound H6

H6 was isolated as a yellow gum. The UV spectrum showed absorption bands at 238, 275 and 386 nm while the IR spectrum displayed absorption bands at 3400 and 1637 cm^{-1} for hydroxy and ketone carbonyl functional groups, respectively. The ^1H NMR spectroscopic data (**Table 104**) (**Figure 27**) were similar to those of **H4** with the replacement of signals for the fused 1,2,3-trihydroxy-3-prenylcyclohexene unit in **H4** with those of two *ortho*-coupled aromatic protons [δ_H 7.05 (*d*, *J* = 8.5 Hz, 1H) and 6.31 (*d*, *J* = 8.5 Hz, 1H)] in **H6**. Additional signals for one chelated hydroxy proton (δ_H 11.03, *s*, 1H) and one hydroxy proton (δ_H 7.50, *s*, 1H) were observed in **H6**. The

HMBC cross peaks of the chelated hydroxy proton resonating at δ_H 11.03 with C-4a (δ_C 108.3), C-5 (δ_C 155.0) and C-6 (δ_C 107.9) as well as those of the other hydroxy proton with C-7 (δ_C 125.7), C-8 (δ_C 147.4) and C-8a (δ_C 139.1) (**Table 104**) attached these hydroxy groups at C-5 and C-8, respectively. Accordingly, **H6** was identified as a synthetic chromone derivative (Martínez-Cifuentes et al., 2017) which was isolated as a natural product for the first time.

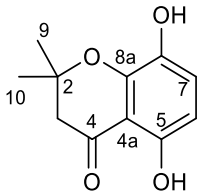


Table 104 The ^{1}H and ^{13}C NMR data of compound **H6** in acetone-d_6

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	COSY	HMBC
2	-	80.5 (C)	-	-
3	2.86 (*s*)	48.8 (CH_2)	-	C-2, C-4, C-4a, C-9, C-10
4	-	199.5 (C=O)	-	-
4a	-	108.3 (C)	-	-
5	-	155.0 (C)	-	-
6	6.31 (*d*, 8.5)	107.9 (CH)	H-7	C-4a, C-5, C-8
7	7.05 (*d*, 8.5)	125.7 (CH)	H-6	C-5, C-8, C-8a
8	-	147.4 (C)	-	-
8a	-	139.1 (C)	-	-
9	1.48 (*s*)	26.6 (CH_3)	-	C-2, C-3, C-10
10	1.48 (*s*)	26.6 (CH_3)	-	C-2, C-3, C-9
5-OH	11.03 (*s*)	-	-	C-4a, C-5, C-6
8-OH	7.50 (*s*)	-	-	C-7, C-8, C-8a

1.3.11 Compound H5

H5 was obtained as a pale yellow solid, melting at 188-190 °C. The UV spectrum exhibited absorption bands at 234, 303 and 352 nm, indicating the presence of a conjugated carbonyl chromophore of a xanthone skeleton (Shimada et al., 2001). Furthermore, the IR spectrum showed absorption bands at 3429 cm^{-1} for a hydroxy group and at 1733 and 1650 cm^{-1} for carbonyl groups of an ester and a ketone of a xanthone, respectively. The ^{1}H NMR spectroscopic data (**Table 105**) (**Figure 29**) contained signals for one chelated hydroxy proton (δ_H 12.28, *s*, 1H), four *meta*-coupled aromatic protons [δ_H 6.90 (*d*, *J* = 2.7 Hz, 1H) and 6.87 (*d*, *J* = 2.7 Hz, 1H); 6.70 (*brs*, 1H) and 6.61 (*brs*, 1H)] and three methyl groups [δ_H 4.02 (*s*, 3H), 3.94 (*s*, 3H) and 2.42 (*s*, 3H)]. The ^{13}C NMR spectrum (**Table 105**) (**Figure 30**) displayed signals for one ketone carbonyl carbon (δ_C 179.7), one ester carbonyl carbon (δ_C 169.3), eight quaternary carbons (δ_C 164.7, 161.6, 158.1, 155.8, 148.6, 135.2, 111.4 and 106.7), four methine carbons (δ_C 112.1, 111.8, 107.2 and 101.5) and three methyl carbons (δ_C 56.2, 53.1 and 22.5). The ^{1}H and ^{13}C NMR spectroscopic data were similar to those of isosulochrin dehydrate (Shimada et al., 2001). Thus, **H5** was isosulochrin dehydrate which was previously isolated from *Pestalotiopsis theae* (Shimada et al., 2001).

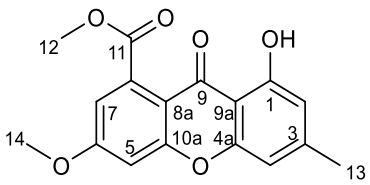


Table 105 The ^{1}H and ^{13}C NMR data of compound **H5** and isosulochrin dehydrate in $CDCl_3$

Position	**H5**		Isosulochrin dehydrate	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	161.6 (C)	-	161.4
2	6.61 (*brs*)	111.8 (CH)	6.59 (*brs*)	111.7
3	-	148.6 (C)	-	148.5

Table 105 (continued)

Position	**H5**		Isosulochrin dehydrate	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
4	6.70 (*brs*)	107.2 (CH)	6.68 (*brs*)	107.1
4a	-	155.8 (C)	-	155.7
5	6.90 (*d*, 2.7)	101.5 (CH)	6.88 (*d*, 2.7)	101.4
6	-	164.7 (C)	-	164.6
7	6.87 (*d*, 2.7)	112.1 (CH)	6.86 (*d*, 2.7)	112.1
8	-	135.2 (C)	-	135.0
8a	-	111.4 (C)	-	111.3
9	-	179.7 (C=O)	-	179.6
9a	-	106.7 (C)	-	106.6
10a	-	158.1 (C)	-	158.0
11	-	169.3 (C=O)	-	169.2
12	3.94 (*s*)	53.1 (CH_3)	3.93 (*s*)	53.1
13	2.42 (*s*)	22.5 (CH_3)	2.41 (*s*)	22.5
14	4.02 (*s*)	56.2 (CH_3)	4.02 (*s*)	56.1
1-OH	12.28 (*s*)	-	12.27 (*s*)	-

1.3.12 Compound H13

H13 was obtained as a pale yellow solid, melting at 198-200 °C. The UV and the IR spectra exhibited similar absorption bands to those of **H5**. The ^{1}H NMR data (**Table 106**) (**Figure 31**) were also similar to those of **H5** except for the absence of one aromatic proton. In addition, the ^{13}C NMR spectrum (**Table 106**) (**Figure 32**) displayed the replacement of one methine carbon with one quaternary carbon (δ_C 111.3). These results together with the presence of only one hydroxy signal in the ^{1}H NMR spectrum indicated that one of four aromatic protons in **H5** was replaced by a chlorine atom. The ^{1}H and ^{13}C NMR spectroscopic data were similar to those of

chloroisosulochrin dehydrate which was previously isolated from *Pestalotiopsis theae* (Shimada et al., 2001).

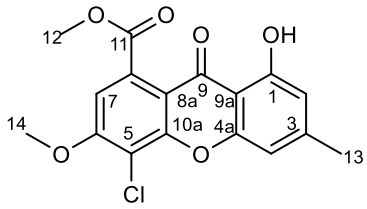


Table 106 The ^{1}H and ^{13}C NMR data of compound **H13** and chloroisosulochrin dehydrate in $CDCl_3$

Position	**H13**		Chloroisosulochrin dehydrate	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	160.0 (C)	-	159.8
2	6.65 (*s*)	112.3 (CH)	6.64 (*brs*)	112.1
3	-	149.4 (C)	-	149.3
4	6.86 (*s*)	107.7 (CH)	6.84 (*brs*)	107.6
4a	-	155.6 (C)	-	155.4
5	-	111.3 (C)	-	111.1
6	-	161.5 (C)	-	161.3
7	6.95 (*s*)	107.0 (CH)	6.94 (*s*)	106.9
8	-	132.9 (C)	-	132.7
8a	-	106.3 (C)	-	106.1
9	-	179.6 (C=O)	-	179.4
9a	-	112.5 (C)	-	112.2
10a	-	153.1 (C)	-	152.9
11	-	169.0 (C=O)	-	169.0
12	4.03 (*s*)	53.3 (CH_3)	4.02 (*s*)	53.2
13	2.44 (*s*)	22.6 (CH_3)	2.43 (*s*)	22.5
14	4.06 (*s*)	57.1 (CH_3)	4.06 (*s*)	57.0
1-OH	12.08 (*s*)	-	12.07 (*s*)	-

1.3.13 Compound H9

Compound **H9** was isolated as a pale yellow gum. The UV spectrum showed absorption bands at 209 and 282 nm while the IR spectrum displayed absorption bands for hydroxy (3367 cm^{-1}), ester carbonyl (1718 cm^{-1}) and ketone carbonyl (1637 cm^{-1}) groups. These results indicated that **H9** had a benzophenone skeleton (Shimada et al., 2001). The ^{1}H NMR spectrum (**Table 107**) (**Figure 33**) showed signals for two *meta*-coupled aromatic protons [δ_H 6.98 (*d*, *J* = 2.5 Hz, 1H) and 6.68 (*d*, *J* = 2.5 Hz, 1H)], two equivalent aromatic protons (δ_H 6.19, *s*, 2H), two methoxy groups [δ_H 3.81 (*s*, 3H) and 3.66 (*s*, 3H)] and one methyl group (δ_H 2.19, *s*, 3H). The ^{13}C NMR spectrum (**Table 107**) (**Figure 34**) displayed signals for one ketone carbonyl carbon (δ_C 200.7), one ester carbonyl carbon (δ_C 167.0), eight quaternary carbons (δ_C 162.8 (x2), 161.1, 156.1, 148.1, 130.7, 127.2 and 110.8), four methine carbons (δ_C 108.8 (x2), 106.7 and 106.5), two methoxy carbons (δ_C 55.9 and 52.2) and one methyl carbon (δ_C 21.9). Two equivalent aromatic protons were assigned as H-3′ (δ_H 6.19) and H-5′ (δ_H 6.19) which showed the HMBC correlations with C-1′ (δ_C 110.8), C-2′ (δ_C 162.8), C-7′ (δ_C 21.9) and C-7 (δ_C 200.7) (**Table 108**). H_3-7′ (δ_H 2.19) exhibited the HMBC correlations with C-3′ (δ_C 108.8), C-4′ (δ_C 148.1) and C-5′ (δ_C 108.8), indicating that the methyl group was placed at C-4′. According to the chemical shifts of C-2′ and C-6′ (δ_C 162.8), the hydroxy groups were attached at these carbons. These results constructed a 2,6-dihydroxy-4-methylbenzoyl moiety. In addition, two *meta*-coupled aromatic protons were assigned as H-4 (δ_H 6.68) and H-6 (δ_H 6.98). The HMBC spectrum displayed the correlations from H-4 to C-2 (δ_C 127.2) and C-6 (δ_C 106.5) whereas H-6 correlated with C-2, C-4 (δ_C 106.7) and C-8 (δ_C 167.0). The HMBC cross peaks of H_3-9 (δ_H 3.66) with C-1 (δ_C 130.7) and C-8 suggested that a methyl ester group was attached at C-1. In addition, H_3-10 (δ_H 3.81) showed the HMBC correlation with C-5 (δ_C 161.1), indicating the attachment of a methoxy group at C-5. The substituent at C-3 (δ_C 156.1) was a hydroxy group based on its chemical shift. The HMBC correlation of H-4 with C-7 suggested that the 2,6-dihydroxy-4-methylbenzoyl unit was attached at C-2. Accordingly, **H9** was identified as isosulochrin, previously isolated from *Pestalotiopsis theae* (Shimada et al., 2001).

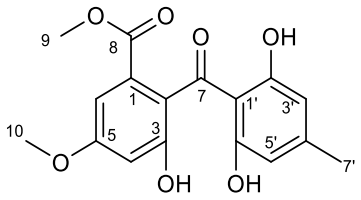


Table 107 The ^{1}H and ^{13}C NMR data of compound **H9** and isosulochrin in acetone-d_6

Position	**H9**		Isosulochrin	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	130.7 (C)	-	131.0
2	-	127.2 (C)	-	127.7
3	-	156.1 (C)	-	156.3
4	6.68 (*d*, 2.5)	106.7 (CH)	6.66 (*d*, 2.7)	106.9
5	-	161.1 (C)	-	161.4
6	6.98 (*d*, 2.5)	106.5 (CH)	6.98 (*d*, 2.7)	106.9
7	-	200.7 (C=O)	-	201.1
8	-	167.0 (C=O)	-	167.3
9	3.66 (*s*)	52.2 (CH_3)	3.66 (*s*)	52.7
10	3.81 (*s*)	55.9 (CH_3)	3.81 (*s*)	56.3
1'	-	110.8 (C)	-	111.1
2'	-	162.8 (C)	-	163.2
3'	6.19 (*s*)	108.8 (CH)	6.19 (*s*)	109.2
4'	-	148.1 (C)	-	148.6
5'	6.19 (*s*)	108.8 (CH)	6.19 (*s*)	109.2
6'	-	162.8 (C)	-	163.2
7'	2.19 (*s*)	21.9 (CH_3)	2.19 (*s*)	22.4

Table 108 The ^{1}H-^{1}H COSY and HMBC data of compound **H9**

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

Table 108 (continued)

Proton	COSY	HMBC
H-3', H-5'	-	C-7, C-1', C-2', C-7'
H_3-7'	-	C-3', C-4', C-5'

1.3.14 Compound H14

H14 was obtained as a pale yellow gum. The UV and IR spectra exhibited similar absorption bands to those of **H9**. The ^{1}H NMR spectroscopic data (**Table 109**) (**Figure 35**) were also similar to those of **H9** except for the presence of only three aromatic protons [δ_H 7.12 (*s*, 1H) and 6.23 (*s*, 2H)] instead of four aromatic protons which were observed in **H9**. The ^{1}H and ^{13}C NMR spectroscopic data (**Table 109**) (**Figures 35** and **36**) were similar to those of chloroisosulochrin which was previously isolated from *Pestalotiopsis* sp. PSU-MA69 (Klaiklay, Doctoral Dissertation, 2013).

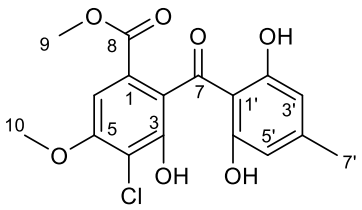


Table 109 The ^{1}H and ^{13}C NMR data of compound **H14** and chloroisosulochrin in $CDCl_3$

Position	**H14**		Chloroisosulochrin	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	123.9 (C)	-	123.8
2	-	127.9 (C)	-	127.9
3	-	149.9 (C)	-	150.0
4	-	113.7 (C)	-	113.6
5	-	155.9 (C)	-	155.9
6	7.12 (*s*)	105.1 (CH)	7.10 (*s*)	104.9
7	-	196.8 (C=O)	-	197.0
8	-	166.3 (C=O)	-	166.5

Table 109 (continued)

Position	**H14**		Chloroisosulochrin	
	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	δ_H (*mult., J_{Hz}*)	δ_C
9	3.71 (*s*)	52.7 (CH_3)	3.70 (*s*)	52.7
10	3.97 (*s*)	56.7 (CH_3)	3.96 (*s*)	56.6
1'	-	109.5 (C)	-	109.5
2'	-	160.3 (C)	-	160.3
3'	6.23 (*s*)	109.4 (CH)	6.23 (*s*)	109.3
4'	-	148.7 (C)	-	148.7
5'	6.23 (*s*)	109.4 (CH)	6.23 (*s*)	109.3
6'	-	160.3 (C)	-	160.3
7'	2.25 (*s*)	22.0 (CH_3)	2.25 (*s*)	22.0
2'-OH	8.88 (*brs*)	-	-	-
6'-OH	6.40 (*brs*)	-	-	-

1.3.15 Compound H15

Compound **H15** was obtained as a colorless gum. The similarity of absorption bands at 212, 247 and 306 nm in the UV spectrum with those of dimethyl-2,3′-dimethylosoate, a biphenyl ether, previously isolated from a marine-derived fungus *Aspergillus* sp. B-F-2 (Liu et al., 2006) indicated the presence of a biphenyl ether skeleton in **H15**. The IR spectrum displayed absorption bands at 3418 cm^{-1} for a hydroxy group and at 1714 and 1629 cm^{-1} for conjugated ester and carboxylic acid carbonyl groups, respectively. The ^{1}H NMR spectroscopic data (**Table 110**) (**Figure 37**) consisted of signals for two *meta*-coupled aromatic protons [δ_H 6.79 (*d, J* = 3.0 Hz, 1H) and 6.62 (*d, J* = 3.0 Hz, 1H)], two aromatic protons [δ_H 6.32 (*s*, 1H) and 6.00 (*s*, 1H)], two methoxy groups (δ_H 3.77, *s*, 6H) and one methyl carboxyl group (δ_H 2.08, *s*, 3H). The ^{13}C NMR spectrum (**Table 110**) (**Figure 38**) displayed signals for one ester carbonyl carbon (δ_C 175.3), one carboxyl carbon (δ_C 167.1), eight quaternary carbons (δ_C 162.4, 159.1, 157.9, 154.0, 142.5, 138.8, 127.4 and 109.7), four methine carbons (δ_C 112.1, 109.0, 107.8 and 105.3), two methoxy carbons (δ_C 55.9 and 52.5) and one

methyl carbon (δ_C 21.7). Two *meta*-coupled aromatic protons were assigned as H-2′ (δ_H 6.79) and H-4′ (δ_H 6.62). H-2′ displayed the HMBC correlations with C-1′ (δ_C 127.4), C-3′ (δ_C 157.9), C-4′ (δ_C 107.8), C-6′ (δ_C 138.8) and C-7′ (δ_C 167.1) whereas H-4′ showed the same correlations with C-2′ (δ_C 105.3), C-3′, C-5′ (δ_C 154.0), and C-6′ (**Table 111**). The HMBC correlation of H_3-9′ (δ_H 3.77) with C-3′ and those of H-2′ and H_3-8′ (δ_H 3.77) with C-7′ suggested the presence of a benzene ring with methoxy and methyl carboxyl groups attached at C-3′ and C-1′, respectively. C-5′ was an oxy carbon on the basis of its chemical shift. The HMBC spectrum showed correlations of H_3-8 (δ_H 2.08) with C-3 (δ_C 112.1), C-4 (δ_C 142.5) and C-5 (δ_C 109.0), those of H-3 (δ_H 6.32) with C-1 (δ_C 109.7), C-2 (δ_C 162.4), C-5, C-7 (δ_C 175.3) and C-8 (δ_C 21.7) and those of H-5 (δ_H 6.00) with C-1, C-3, C-6 (δ_C 159.1), C-7 and C-8. These results established a benzene ring with the carboxyl and methyl groups at C-1 and C-4, respectively. Due to the chemical shift of C-2, a hydroxy group was attached at this carbon. Comparison of the ^{1}H and ^{13}C NMR data with those of dechlorodihydromaldoxin previously isolated from *Xylaria* sp. (Adeboya et al., 1996) indicated that an ether linkage connected C-6′ and C-6 to form a biphenyl ether whereas a hydroxy group was attached at C-5′. Thus, **H15** was dechlorodihydromaldoxin.

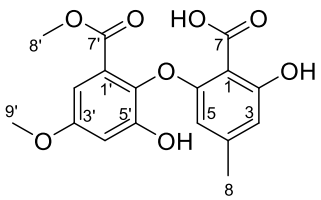


Table 110 The ^{1}H and ^{13}C NMR data of compound **H15** in acetone-d_6 and dechloro-dihydromaldoxin in pyridine-d_5

Position	**H15**		Dechlorodihydromaldoxin	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	109.7 (C)	-	106.2
2	-	162.4 (C)	-	163.6
3	6.32 (*s*)	112.1 (CH)	6.73 (*s*)	112.0
4	-	142.5 (C)	-	153.8
5	6.00 (*s*)	109.0 (CH)	6.58 (*s*)	105.5

Table 110 (continued)

Position	**H15**		Dechlorodihydromaldoxin	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
6	-	159.1 (C)	-	160.3
7	-	175.3 (C=O)	-	174.5
8	2.08 (*s*)	21.7 (CH_3)	2.05 (*s*)	21.9
1'	-	127.4 (C)	-	126.8
2'	6.79 (*d*, 3.0)	105.3 (CH)	7.26 (*d*, 2.6)	108.4
3'	-	157.9 (C)	-	157.8
4'	6.62 (*d*, 3.0)	107.8 (CH)	7.15 (*d*, 2.6)	107.6
5'	-	154.0 (C)	-	144.8
6'	-	138.8 (C)	-	137.6
7'	-	167.1 (C=O)	-	166.3
8'	3.77 (*s*)	52.5 (CH_3)	3.76 (*s*)	52.2
9'	3.77 (*s*)	55.9 (CH_3)	3.74 (*s*)	55.6

Table 111 The ^{1}H-^{1}H COSY, HMBC and NOEDIFF data of compound **H15**

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

1.3.16 Compound H16

Compound **H16** was obtained as a colorless solid, melting at 197-200 °C. It showed identical UV and IR absorption bands to those of **H15**. The ^{1}H NMR spectroscopic data (**Table 112**) (**Figure 39**) were also similar to those of **H15** except for the absence of one aromatic methine proton signal and the replacement of one

aromatic methine carbon with a quaternary aromatic carbon (δ_C 116.4) (**Table 112**) (**Figures 39** and **40**). Comparison of these NMR data with those of pesteic acid indicated that **H16** was pesteic acid previously isolated from *Pestalotiopsis* sp. PSU-MA69 (Klaiklay, Doctoral Dissertation, 2013).

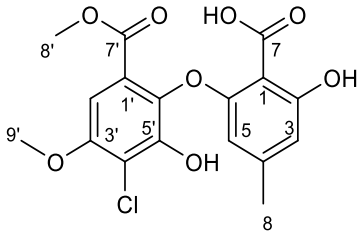


Table 112 The ^{1}H and ^{13}C NMR data of compound **H16** and pesteic acid in CD_3OD

Position	**H16**		Pesteic acid	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	109.0 (C)	-	108.6
2	-	162.3 (C)	-	160.7
3	6.39 (*s*)	112.7 (CH)	6.39 (*d*, 0.6)	111.1
4	-	144.3 (C)	-	142.1
5	5.91 (*s*)	109.0 (CH)	5.93 (*d*, 0.6)	107.9
6	-	159.3 (C)	-	157.6
7	-	176.4 (C=O)	-	173.8
8	2.11 (*s*)	21.8 (CH_3)	2.11 (*s*)	20.4
1'	-	124.5 (C)	-	123.1
2'	7.01 (*s*)	103.5 (CH)	7.00 (*s*)	101.8
3'	-	154.5 (C)	-	152.9
4'	-	116.5 (C)	-	114.9
5'	-	150.8 (C)	-	149.5
6'	-	139.7 (C)	-	138.7
7'	-	167.4 (C=O)	-	166.2
8'	3.76 (*s*)	53.0 (CH_3)	3.78 (*s*)	51.6
9'	3.90 (*s*)	56.9 (CH_3)	3.90 (*s*)	55.5

1.3.17 Compound H17

Compound **H17** was obtained as a colorless gum. The UV and IR spectra were similar to those of **H16**. The ^{1}H and ^{13}C NMR spectroscopic data (**Table 113**) (**Figures 41** and **42**) contained similar signals to those of **H16** except for the presence of signals for an additional methoxy group resonating at δ_H 4.02 (*s*, 3H) and δ_C 52.8 in **H17** along with the replacement of a carboxyl carbon in **H16** with an ester carbonyl carbon (δ_C 170.1) in **H17**. The ^{1}H and ^{13}C NMR spectroscopic data suggested that **H17** was pestalotether A which was previously isolated from *Pestalotiopsis* sp. (Klaiklay et al., 2012).

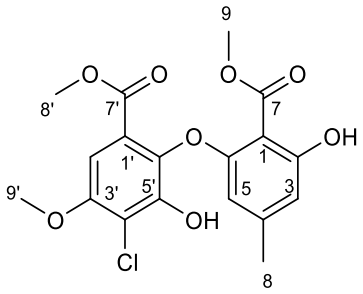


Table 113 The ^{1}H and ^{13}C NMR data of compound **H17** and pestalotether A in $CDCl_3$

Position	**H17**		Pestalotether A	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	101.7 (C)	-	101.6
2	-	162.3 (C)	-	162.2
3	6.52 (*s*)	112.6 (CH)	6.51 (*s*)	112.5
4	-	146.6 (C)	-	146.6
5	5.89 (*s*)	106.8 (CH)	5.88 (*d*, 0.9)	106.4
6	-	158.0 (C)	-	157.9
7	-	170.1 (C=O)	-	170.1
8	2.17 (s)	22.1 (CH_3)	2.17 (*s*)	22.0
9	4.02 (*s*)	52.8 (CH_3)	4.02 (*s*)	52.8
1'	-	123.0 (C)	-	122.9
2'	7.13 (*s*)	104.2 (CH)	7.13 (*s*)	104.1
3'	-	153.2 (C)	-	153.1

Table 113 (continued)

Position	**H17**		Pestalotether A	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
4'	-	114.9 (C)	-	114.9
5'	-	147.5 (C)	-	147.4
6'	-	136.0 (C)	-	136.0
7'	-	164.9 (C=O)	-	164.9
8'	3.75 (*s*)	52.5 (CH_3)	3.74 (*s*)	52.4
9'	3.98 (*s*)	56.6 (CH_3)	3.98 (*s*)	56.6
2-OH	10.64 (*s*)	-	10.65 (*s*)	-
5'-OH	7.05 (*brs*)	-	7.07 (*brs*)	-

CHAPTER 2

METABOLITES FROM THE MARINE-DERIVED FUNGUS *TRICHODERMA LONGIBRACHIATUM* PSU-AMF274

CHAPTER 2.1

INTRODUCTION

2.1.1 Introduction

The genus *Trichoderma* has been a potential source of a lot of bioactive secondary metabolites such as antibiotic, antifungal, and antibacterial agents (Almassi et al., 1991). *T. longibrachiatum* PSU-AMF274 was isolated from a bryozoan, which was collected from the Phuket Coastal Fisheries Research and Development Center, Phuket Province, Thailand. This fungus was cultured at Department of Microbiology, Faculty of Science, Prince of Songkla University. The crude broth (BE) and mycelial (CE) extracts of *T. longibrachiatum* PSU-AMF274 showed interesting antimicrobial and cytotoxic activities, as shown in **Table 114**. Furthermore, the ^{1}H-NMR spectra of the crude extracts displayed signals of aromatic and olefinic protons. Based on SciFinder Scholar Database, secondary metabolites which were isolated from the genus *Trichoderma* were summarized in **Table 115**.

Table 114 Bioactivities of the crude extracts of the fungus PSU-AMF274

Code	Antimicrobial (MIC, μg/mL)					Cytotoxic (IC_{50}, μg/mL)	
	SA	MRSA	CA 3153	CN90113	MG	KB-oral	MCF7-breast
AMF274BE	32	64	-	8	64	15.54	17.19
AMF274CE	-	-	4	-	NA	ND	ND
Control	0.25[a]	0.50[a]	0.25[b]	0.25[b]	0.50[c]	0.53[d]	6.88[d]

MIC = minimum inhibitory concentration (μg/mL), SA = *Staphylococcus aureus* ATCC25923, MRSA = methicillin-resistant *S. aureus*, CA3153 = *Candida albicans* NCPF3153, CN90113 = *Cryptococcus neoformans*, MG = *Microsporum gypseum* clinical isolate, - = no activity, ND = not determined, BE = broth EtOAc extract, CE = mycelial EtOAc extract, CH = mycelial hexane extract, control; [a]Vancomycin, [b]Amphotericin B, [c]Clotrimazole, [d]Doxorubicin

Table 115 Compounds isolated from the genus *Trichoderma*

Scientific name	Compound	Activity	References
Trichoderma asperellum PSU-PSF14	Blennolide L, **1** Blennolide M, **2** Blennolide N, **3** Lachnone C, **4** Endocrocin, **5** Aspergillusidone C, **6** Unguinol, **7** 2-Chlorounguinol, **8**	Antifungal - - - - - - -	Maha et al., 2018
Trichoderma harzianum	Harzianumnone A, **9** Harzianumnone B, **10** Pachybasin, **11** Chrysophanol, **12** Frangulaemodin, **13** Phomarin, **14** (+)-2'*S*-Isorhodoptilometrin, **15** 1-Hydroxy-3-hydroxymethyl-anthraquinone, **16** ω-Hydroxydigitoemodin, **17**	- - - - - - Cytotoxic Cytotoxic -	Shi et al., 2018a
Trichoderma harzianum OUPS-111D-4	Trichodermanin C, **18** Trichodermanin D, **19** Trichodermanin E, **20**	Cytotoxic - -	Yamada et al., 2017
Trichoderma harzianum P1-4	Cyclonerodiol, **21** (10*E*)-12-Acetoxy-10-cycloneren-3,7-diol, **22** 12-Acetoxycycloneran-3,7-diol, **23**	- - -	Fang et al., 2018

Table 115 (continued)

Scientific name	Compound	Activity	References
Trichoderma koningiopsis	Koningiopisin A, **24** Koningiopisin B, **25** Koningiopisin C, **26** Koningiopisin D, **27** Koningiopisin E, **28** Koningiopisin F, **29** Koningiopisin G, **30** Koningiopisin H, **31** Trichodermaketone C, **32** Koninginin A, **33** Koninginin B, **34** Koninginin F, **35**	Antifungal Antifungal Antifungal - Antifungal Antifungal Antifungal Antifungal Antifungal Antifungal -	Liu et al., 2016a
Trichoderma koningiopsis	Koninginin B, **34** Koninginin E, **36** Koninginin J, **37** Koninginin N, **38** Koninginin O, **39** Koninginin P, **40** Koninginin Q, **41** 7-*O*-Methylkoninginin D, **42**	- - - - Antifungal - Antifungal Antifungal	Liu et al., 2016b
Trichoderma longibrachiatum	Trichodimerol, **43** Sorbicillin, **44** Bisvertinol, **45** Bisvertinolone, **46**	- - - -	Andrade et al., 1992
Trichoderma longibrachiatum	Trichodermolide, **47** Sorbiquinol, **48**	- -	Andrade et al., 1996
Trichoderma longibrachiatum	5-Dehydroxyvertinolide, **49** Bislongiquinolide, **50**	- -	Andrade et al., 1997

Table 115 (continued)

Scientific name	Compound	Activity	References
Trichoderma longibrachiatum	Bislongiquinolide, **50** Epoxysorbicilinol, **51**	- -	Sperry et al., 1998
Trichoderma longibrachiatum	Harzianone, **52**	-	Miao et al., 2012
Trichoderma longibrachiatum	10,11-Dihydro-cyclonerotriol, **53** Cateniobblin C, **54** Sohirnone A, **55**	Antifungal Antifungal Antifungal	Xuan et al., 2014
Trichoderma longibrachiatum	Tricholongin BI, **56** Tricholongin BII, **57**	Antibacterial, antifungal Antibacterial, antifungal	Rebuffat et al., 1991
Trichoderma longibrachiatum	Trichogin A IV, **58**	-	Auvin-Guette et al., 1992
Trichoderma longibrachiatum	Longibrachin LGA I, **59** Longibrachin LGA II, **60** Longibrachin LGA III, **61** Longibrachin LGA IV, **62** Longibrachin LGB II, **63** Longibrachin LGB III, **64**	Antibacterial Antibacterial Antibacterial Antibacterial Antibacterial Antibacterial	Leclerc et al., 2001
Trichoderma longibrachiatum Rifai	Trichobrachin A-I, **65** Trichobrachin A-II, **66** Trichobrachin A-III, **67** Trichobrachin A-IV, **68** Trichobrachin B-I, **69** Trichobrachin B-II, **70** Trichobrachin B-III, **71** Trichobrachin B-IV, **72**	- - - - - - - -	Mohamed-Benkada et al., 2006

Table 115 (continued)

Scientific name	Compound	Activity	References
	Trichorovin TV-Ib or IIa, **73**	-	
Trichoderma sp.	6-Demethylsorbicillin, **74** Sohirnone A, **55** Sorbicillin, **44** 2',3'-Dihydrosorbicillin, **75** Bisvertinolone, **46** 10,11-Dihydrobisvertinolone, **76** Trichodimerol, **43** Dihydrotrichodimerol, **77** Bisorbicillinol, **78** Bisvertinoquinol, **79** Bislongiquinolide, **50**	Cytotoxic - Cytotoxic - Cytotoxic Cytotoxic Cytotoxic Cytotoxic - - -	Du et al., 2009
Trichoderma sp.	Trichodermate A, **80** Trichodermate B, **81** Trichodermate C, **82** Trichodermate D, **83** Trichodermate E, **84** Trichodermate F, **85** (-)-Harzianum B, **86**	Cytotoxic Cytotoxic - - - - -	Li et al., 2016
Trichoderma sp. HPQJ-34	5-Hydroxy-cyclopenicillone, **87** *ar*-Turmerone, **88** Citreoisocoumarin, **89** 6-*O*-Methyl-citreoisocoumarin, **90**	Anti-oxidant - - -	Fang et al., 2017
Trichoderma sp. PR-35	Trichoderic acid, **91** 2*β*-Hydroxytrichoacorenol, **92**	Antibacterial Antibacterial	Wu et al., 2011

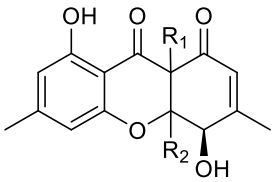
Table 115 (continued)

Scientific name	Compound	Activity	References
	Cyclonerodiol, **21** Cyclonerodiol oxide, **93** Sorbicillin, **44**	Antibacterial Antibacterial Antibacterial	
Trichoderma sp. SCSIO41004	Trichbenzoisochromen A, **94** 5,7-Dihydroxy-3-methyl-2-(2-oxopropyl)naphthalene-1,4-dione, **95** 7-Acetyl-1,3,6-trihydroxy-anthracene-9,10-dione, **96** ZSU-H85 A, **97** 1,3,6-Trihydroxy-8-methytanthra-quinone, **98** 2,5-Dimethyl-7-hydroxy-chromone, **99** 7-Hydroxy-2-(2'*S*-hydroxypropyl)- 5-methylchromone, **100** Cyclonerotriol, **101** Adenosine, **102**	- - - Antiviral - - - - -	Pang et al., 2018
Trichoderma virens	Trichodermamide A, **103** Trichodermamide B, **104**	- Cytotoxic	Garo et al., 2003
Trichoderma virens	Trichocarotin A, **105** Trichocarotin B, **106** Trichocarotin C, **107** Trichocarotin D, **108**	- - Phytoplankton inhibitory Phytoplankton inhibitory	Shi et al., 2018b

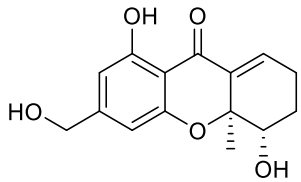
Table 115 (continued)

Scientific name	Compound	Activity	References
	Trichocarotin E, **109**	Phytoplankton inhibitory	
	Trichocarotin F, **110**	-	
	Trichocarotin G, **111**	-	
	Trichocarotin H, **112**	Phytoplankton inhibitory	
	Trichocadinin A, **113**	-	
	CAF-603, **114**	-	
	14-Hydroxy CAF-603, **115**	-	
	7-*β*-Hydroxy CAF-603, **116**	-	
	Trichocarane A, **117**	Phytoplankton inhibitory	
Trichoderma virens Y13-3	Trichorenin A, **118**	Phytoplankton inhibitory	Shi et al., 2018c
	Trichorenin B, **119**	Phytoplankton inhibitory	
	Trichorenin C, **120**	Phytoplankton inhibitory	

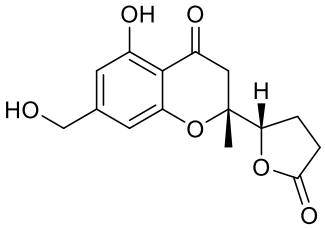
Structures of the metabolites from the genus *Trichoderma*



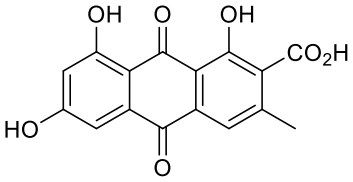
1: R_1 = α-OH, R_2 = β-Me : Blennolide L
2: R_1 = β-OH, R_2 = α-Me : Blennolide M



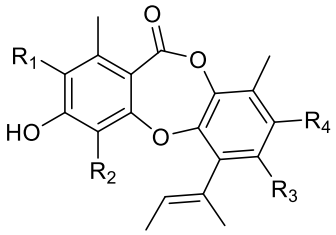
3: Blennolide N



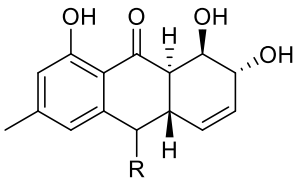
4: Lachnone C



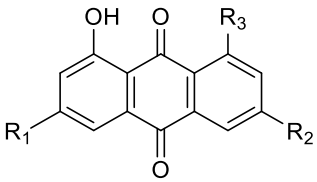
5: Endocrocin



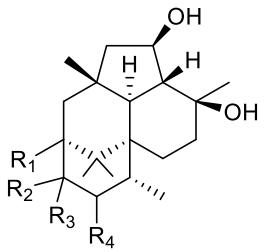
6: R_1 = R_3 = Cl, R_2 = H, R_4 = OH
: Aspergillusidone C
7: R_1 = R_2 = R_3 = H, R_4 = OH
: Unguinol
8: R_1 = Cl, R_2 = R_3 = H, R_4 = OH
: 2-Chlorounguinol



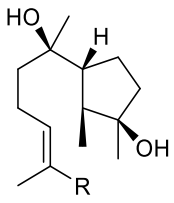
9: R = α-OH : Harzianumnone A
10: R = β-OH : Harzianumnone B



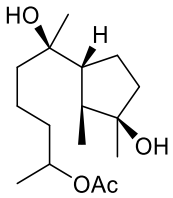
11: R_1 = Me, R_2 = R_3 = H : Pachybasin
12: R_1 = Me, R_2 = H, R_3 = OH : Chrysophanol
13: R_1 = Me, R_2 = R_3 = OH : Frangulaemodin
14: R_1 = Me, R_2 = OH, R_3 = H : Phomarin
15: R_1 = $CH_2CHOHMe$, R_2 = R_3 = OH : (+)-2′*S*-Isorhodoptilometrin
16: R_1 = CH_2OH, R_2 = R_3 = H : 1-Hydroxy-3-methylanthraquinone
17: R_1 = CH_2OH, R_2 = OH, R_3 = H : ω-Hydroxydigitoemodin



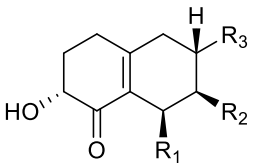
18: $R_1 = R_4 = H$, $R_2 + R_3 = =O$: Trichodermanin C
19: $R_1 = OH$, $R_2 = R_3 = R_4 = H$: Trichodermanin D
20: $R_1 = R_3 = H$, $R_2 = \alpha$-OH, $R_4 = \beta$-OH : Trichodermanin E

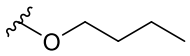


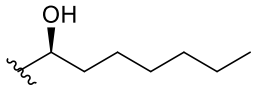
21: R = Me : Cyclonerodiol
22: R = CH_2OAc : (10*E*)-12-Acetoxy-10-cycloneren-3,7-diol



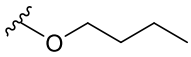
23: 12-Acetoxycycloneran-3,7-diol

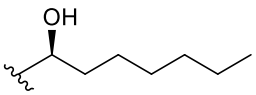


24: R_1 = 

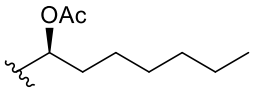
R_2 = H
R_3 = 

Koningiopisin A

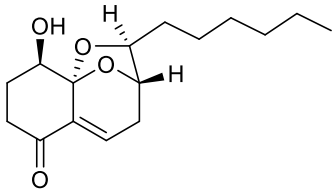
25: R_1 = H
R_2 = 

R_3 = 

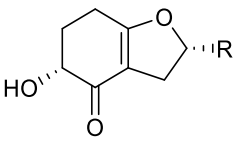
Koningiopisin B

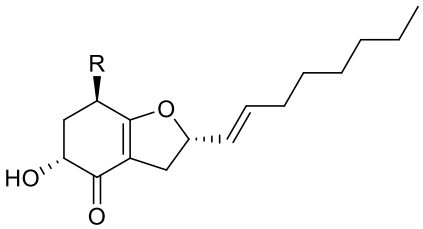
28: $R_1 = R_2$ = H
R_3 = 

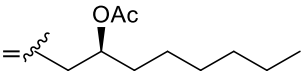
Koningiopisin E



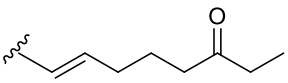
26: Koningiopisin C



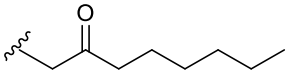


27: R = 

: Koningiopisin D

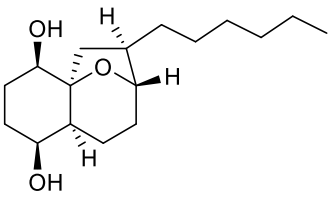
30: R = 

: Koningiopisin G

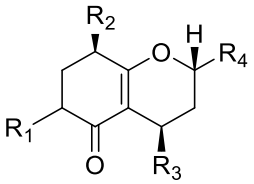
31: R = 

: Koningiopisin H

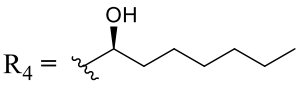
29: R = H : Koningiopisin F
32: R = OH : Trichodermaketone C



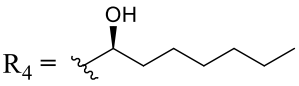
33: Koninginin A



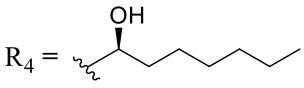
34: R_1 = α-OH, R_2 = R_3 = H **35**: R_1 = α-OH, R_2 = H, R_3 = OH **36**: R_1 = R_3 = H, R_2 = OH



Koninginin B

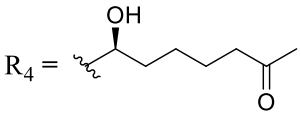


Koninginin F

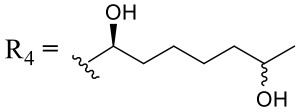


Koninginin E

37: R_1 = β-OH, R_2 = R_3 = H **40**: R_1 = β-OH, R_2 = R_3 = H

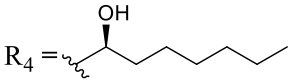


Koninginin J

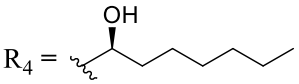


Koninginin P

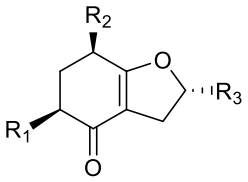
41: R_1 = β-OH, R_2 = H, R_3 = OMe **42**: R_1 = H, R_2 = OH, R_3 = OMe



Koninginin Q

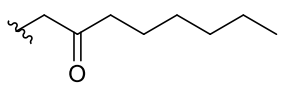


7-*O*-Methylkoninginin D



38: R_1 = H, R_2 = OH

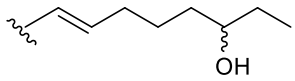
R_3 =



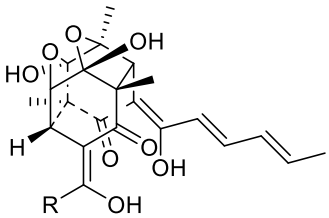
Koninginin N

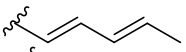
39: R_1 = OH, R_2 = H

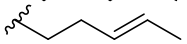
R_3 =

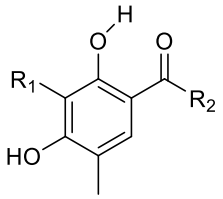


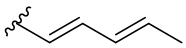
Koninginin O

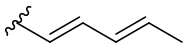


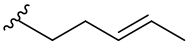
43: R =  : Trichodimerol

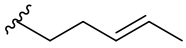
77: R =  : Dihydrotrichodimerol

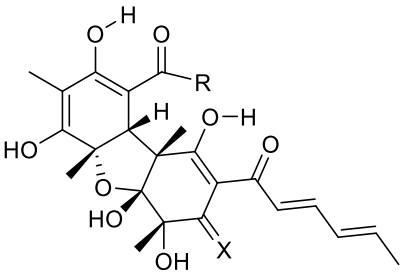


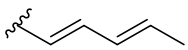
44: R_1 = Me, R_2 = 
: Sorbicillin

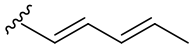
74: R_1 = H, R_2 = 
: 6-Demethylsorbicillin

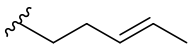
55: R_1 = H, R_2 = 
: Sohirnone A

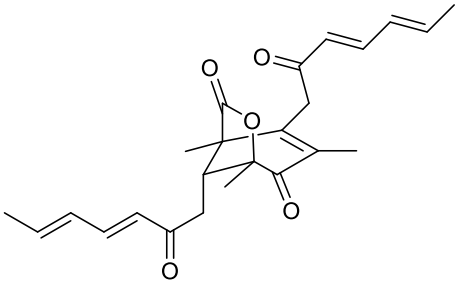
75: R_1 = Me, R_2 = 
: 2',3'-Dihydrosorbicillin



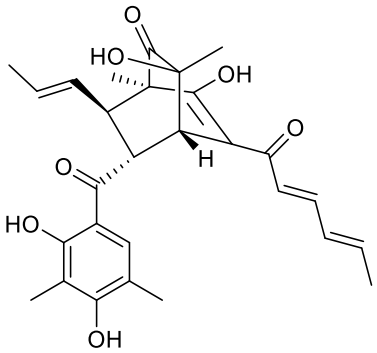
45: X = H_2, R =  : Bisvertinol

46: X = O, R =  : Bisvertinolone

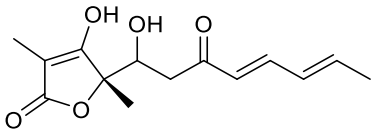
76: X = O, R =  : 10,11-Dihydrobisvertinol-one



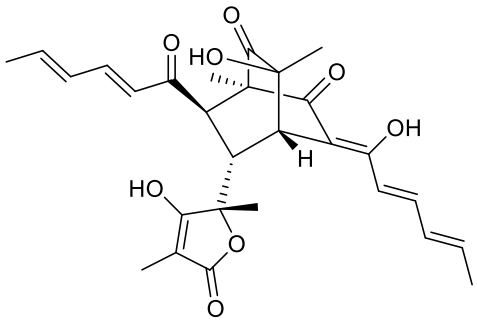
47: Trichodermolide



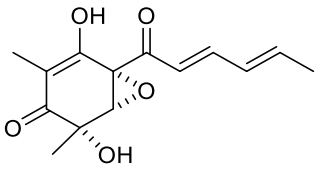
48: Sorbiquinol



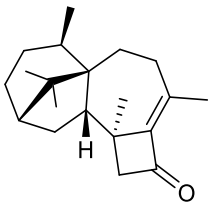
49: 5-Dehydroxyvertinolide



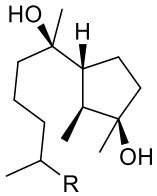
50: Bislongiquinolide



51: Epoxysorbicilinol



52: Harzianone



53: R = CH_2OH : 10,11-Dihydrocyclonerotriol
54: R = COOH : Catenioblin C

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

56: AcAib Gly Phe Aib Aib Gln Aib Aib Aib Ser Leu Aib Pro Val Aib Aib Gln Gln Leuol : Tricholongin BI

57: AcAib Gly Phe Aib Aib Gln Aib Aib Aib Ser Leu Aib Pro Val Aib Iva Gln Gln Leuol : Tricholongin BII

58: OcAib Gly Leu Aib Gly Gly Leu Aib Gly Ile Leuol : Trichogin A IV

59: AcAib Ala Aib Ala Aib Ala Gln Aib Val Aib Gly Leu Aib Pro Val Aib Aib Gln Gln Pheol : Longibrachin LGA I

60: AcAib Ala Aib Ala Aib Ala Gln Aib Val Aib Gly Leu Aib Pro Val Aib Iva Gln Gln Pheol : Longibrachin LGA II

61: AcAib Ala Aib Ala Aib Aib Gln Aib Val Aib Gly Leu Aib Pro Val Aib Aib Gln Gln Pheol : Longibrachin LGA III

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

62: AcAib Ala Aib Ala Aib Aib Gln Aib Val Aib Gly Leu Aib Pro Val Aib Iva Gln Gln Pheol : Longibrachin LGA IV

63: AcAib Ala Aib Ala Aib Ala Gln Aib Val Aib Gly Leu Aib Pro Val Aib Aib Glu Gln Pheol : Longibrachin LGB II

64: AcAib Ala Aib Ala Aib Ala Gln Aib Val Aib Gly Leu Aib Pro Val Aib Iva Glu Gln Pheol : Longibrachin LGB III

1 2 3 4 5 6 7 8 9 10 11

65: AcAib Asn Leu Leu Aib Pro Leu Aib Aib Pro Leuol : Trichobrachin A-I

66: AcAib Asn Leu Leu Aib Pro Val Leu Aib Pro Valol : Trichobrachin A-II

67: AcAib Asn Val Leu Aib Pro Leu Leu Aib Pro Valol : Trichobrachin A-III

68: AcAib Asn Leu Val Aib Pro Leu Leu Aib Pro Valol : Trichobrachin A-IV

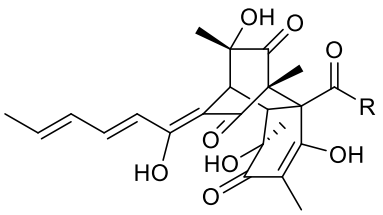
69: AcAib Asn Leu Leu Aib Pro Val Aib Val Pro Leuol : Trichobrachin B-I

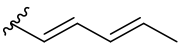
70: AcAib Asn Val Leu Aib Pro Leu Aib Val Pro Leuol : Trichobrachin B-II

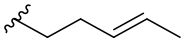
71: AcAib Asn Leu Val Aib Pro Leu Aib Val Pro Leuol : Trichobrachin B-III

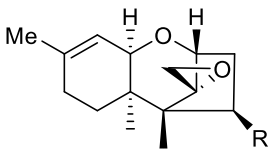
72: AcAib Asn Leu Leu Aib Pro Leu Aib Val Pro Valol : Trichobrachin B-IV

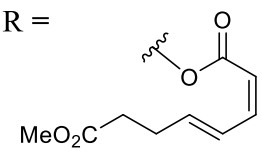
73: AcAib Asn Val Val Aib Pro Leu Leu Aib Pro Leuol : Trichobrachin TV-Ib or IIa



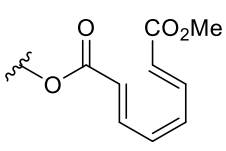
78: R =  : Bisorbicillinol

79: R =  : Bisvertinoquinol

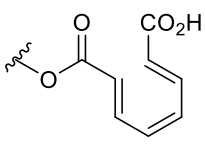


80: R = 

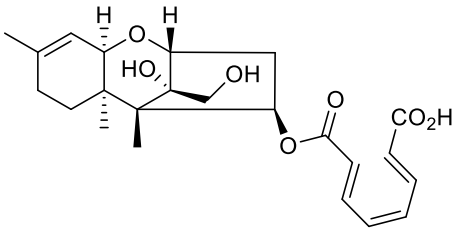
Trichodermate A

81: R = 

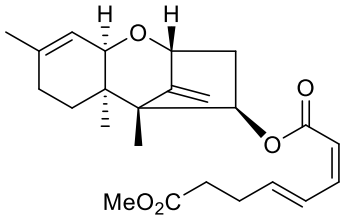
Trichodermate B

86: R = 

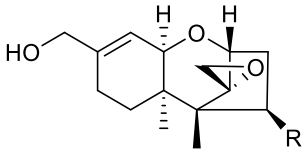
(-)-Harzianum B



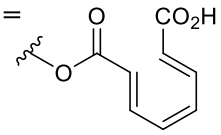
82: Trichodermate C



83: Trichodermate D

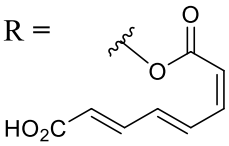


84: R =

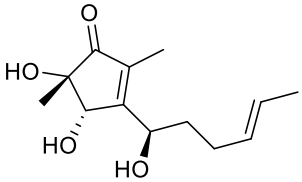


Trichodermate E

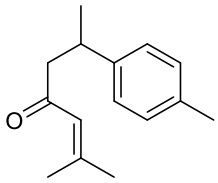
85: R =



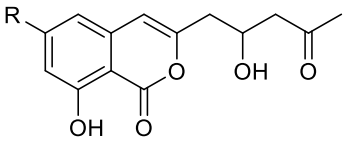
Trichodermate F



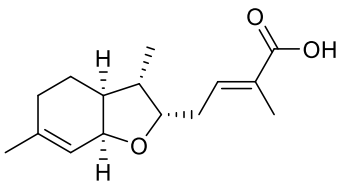
87: 5-Hydroxycyclopenicillone



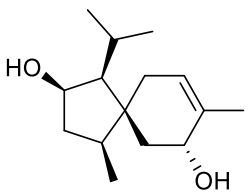
88: *ar*-Turmerone



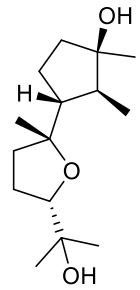
89: R = OH : Citreoisocoumarin
90: R = OMe : 6-*O*-Methylcitreo-isocoumarin



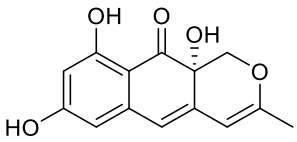
91: Trichoderic acid



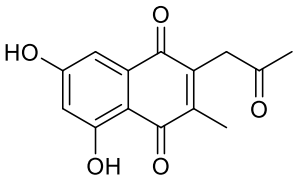
92: 2β-Hydroxytrichoacorenol



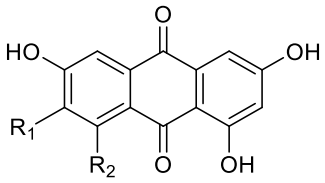
93: Cyclonerodiol oxide



94: Trichbenzoisochromen A



95: 5,7-Dihydroxy-3-methyl-2-(2-oxopropyl)naphthalene-1,4-dione



96: R_1 =  , R_2 = H

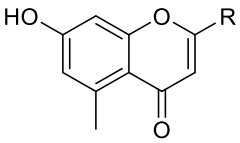
7-Acetyl-1,3,6- trihydroxy-anthracene-9,10-dione

97: R_1 =  , R_2 = H

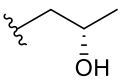
ZSU-H85 A

98: R_1 = H, R_2 = Me

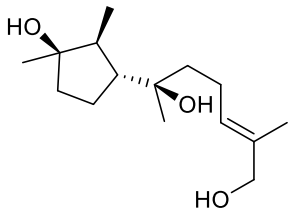
1,3,6-Trihydroxy-8-methytanthraquinone



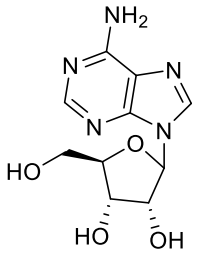
99: R = Me : 2,5-Dimethyl-7-hydroxychromone

100: R = 

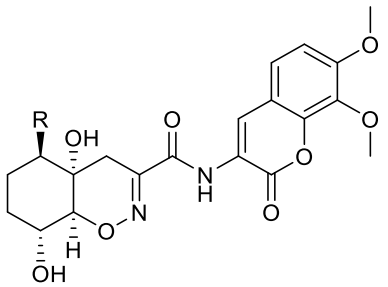
7-Hydroxy-2-(2'*S*-hydroxypropyl)-5-methylchromone



101: Cyclonerotriol

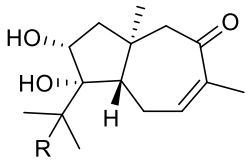


102: Adenosine

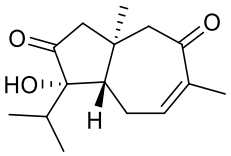


103: R = OH : Trichodermamide A

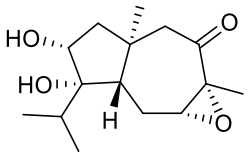
104: R = Cl : Trichodermamide B



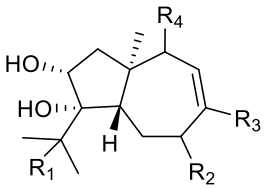
105: R = H : Trichocarotin A
106: R = OH : Trichocarotin B



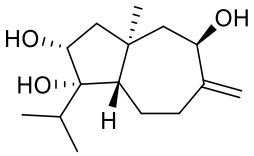
107: Trichocarotin C



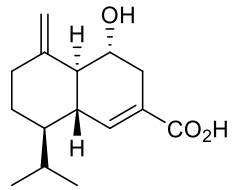
108: Trichocarotin D



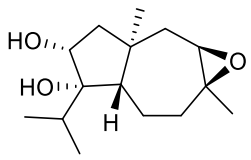
109: R_1 = OH, R_2 = R_4 = H, R_3 = Me : Trichocarotin E
110: R_1 = R_2 = H, R_3 = Me, R_4 = α-OH : Trichocarotin F
111: R_1 = R_2 = R_4 = H, R_3 = CO_2H : Trichocarotin G
114: R_1 = R_2 = R_4 = H, R_3 = Me : CAF-603
115: R_1 = R_2 = R_4 = H, R_3 = CH_2OH : 14-Hydroxy CAF-603
116: R_1 = R_4 = H, R_2 = β-OH, R_3 = Me : 7-β-Hydroxy CAF-603



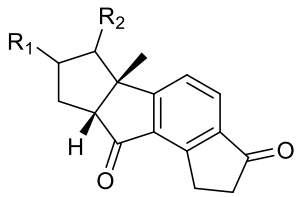
112: Trichocarotin H



113: Trichocadinin A



117: Trichocarane A



118: R_1 = α-OH, R_2 = α-OMe : Trichorenin A
119: R_1 = α-OH, R_2 = β-OMe : Trichorenin B
120: R_1 = β-OH, R_2 = α-OMe : Trichorenin C

2.1.2 The objectives

The objectives are to isolate secondary metabolites from the marine-derived fungus *Trichoderma longibrachiatum* PSU-AMF274 and to identify the structures of the isolated compounds.

CHAPTER 2.2

EXPERIMENTAL

2.2.1 Instruments and chemicals

All instruments and chemicals used for purification of the fungus *T. longibrachiatum* PSU-AMF274 were the same as those reported in Chapter 1.2 with an additional instrument which was Agilent 1200 series HPLC whereas the additional solvents were acetonitrile, trifluoroacetic acid, formic acid and 2-propanol.

2.2.2 Fermentation and extraction of the fungus PSU-AMF274

The fermentation and extraction of the fungus *T. longibrachiatum* PSU-AMF274 were conducted using the same procedure as those of the fungus *Pseudopestalotiopsis* sp. PSU-AMF45. The EtOAc extracts of the culture broth (BE, 3.1 g) and the wet mycelia (CE, 532.9 g) as well as the hexane extract (CH, 32.4 mg) of the wet mycelia were obtained as a dark brown gum. The CE and CH extracts were not investigated because of the presence of major signals in high field region in their ^{1}H NMR spectra.

2.2.3 Purification of the broth extract of the fungus PSU-AMF274

The broth extract of the fungus *T. longibrachiatum* PSU-AMF274 (3.1 g) was subjected to column chromatography over Sephadex LH-20 with 100% methanol as an eluent. All of the obtained fractions were examined by TLC and combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give seven fractions as shown in **Table 116**.

Table 116 Fractions obtained from **the broth EtOAc extract** by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
HR1	932.6	Yellow solid
HR2	206.1	Dark yellow gum
HR3	337.7	Dark yellow gum
HR4	354.0	Dark yellow gum
HR5	514.8	Dark yellow gum
HR6	81.2	Dark yellow gum
HR7	4.0	Dark yellow gum

Fraction HR1 Chromatogram characteristics on reverse phase TLC with 90% methanol-water as a mobile phase exhibited a long tail under UV-S. It was dissolved with chloroform, dichloromethane and methanol to yield a chloroform soluble part (**HR11**), a dichloromethane soluble part (**HR12**) and a methanol soluble part (**HR13**) as shown in **Table 117**.

Table 117 Subfractions obtained from **fraction HR1** by dissolving with methanol, dichloromethane and chloroform

Subfraction	Weight (mg)	Physical appearance
HR11	27.4	Yellow solid
HR12	50.0	Dark yellow gum
HR13	818.0	Dark yellow gum

Subfraction HR11 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of long chain hydrocarbons. Thus, it was not further investigated.

Subfraction HR12 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one major spot under UV-S and after being visualized by anisaldehyde sulfuric acid with the R_f value of 0.90. The ^{1}H NMR spectrum displayed signals of long chain hydrocarbons as a major component. Thus, it was not further purified.

Subfraction HR13 Chromatogram characteristics on reverse phase TLC with 90% methanol-water as a mobile phase showed a long tail under UV-S and after being visualized by ceric ammonium molybdate. **Subfraction HR13** (288 mg) was subjected to column chromatography over reverse phase C_{18} silica gel with 90% methanol-water as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give three subfractions as shown in **Table 118**.

Table 118 Subfractions obtained from **subfraction HR13** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HR131	13.3	Brown gum
HR132	30.5	Brown gum
HR133	181.9	Brown gum

Subfraction HR131 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane (4 runs) as a mobile phase displayed a long tail under UV-S. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HR132 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane (4 runs) as a mobile phase showed a long tail near the baseline and one major spot with the R_f value of 0.48 after being visualized by ceric ammonium molybdate. Purification was conducted by preparative TLC with 10% methanol-dichloromethane as mobile phase to afford two subfractions as shown in **Table 119**.

Table 119 Subfractions obtained from **subfraction HR132** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HR1321	12.5	White solid
HR1322	12.7	White solid

Subfraction HR1321 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed one major spot with the R_f

value of 0.48 after being visualized by ceric ammonium molybdate. The ^{1}H NMR spectrum indicated the presence of sugar as a major component. Therefore, no further investigation was carried out.

Subfraction HR1322 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail after being visualized by ceric ammonium molybdate. The ^{1}H NMR spectrum revealed the presence of sugar as a major component. Therefore, no further investigation was performed.

Subfraction HR133 Chromatogram characteristics on reverse phase TLC with 90% acetonitrile-water as a mobile phase showed a long tail near the baseline after being visualized by ceric ammonium molybdate. The ^{1}H NMR spectrum showed the presence of a mixture of peptides as major components. The ^{13}C NMR spectrum displayed signals for phenylalanine as one of amino acids constructing this peptide structures. Various purification techniques were conducted such as recrystallisation with methanol-chloroform or methanol-acetonitrile, purification based on solubility in acetone, column chromatography over reverse phase C_{18} silica gel with 90% methanol-water, 70% acetonitrile-water + 0.2% formic acid or 7:7:6 acetonitrile:2-propanol:water + 0.2% formic acid, column chromatography over Sephadex LH-20 with 50% methanol-dichloromethane and purification using HPLC over hypersil ODS C_{18} column with 90% acetonitrile-water + 0.1% TFA, column temperature at 40 and 70 °C or 90% acetonitrile-water + 0.1% formic acid, column temperature at 40 and 70 °C with detection at 220 nm. Unfortunately, those attempts were unsuccessful to give any pure compounds from this subfraction. Acetylation and N-methylation as attempts to change the polarity using acetic anhydride and methyl iodide, respectively, were carried out but the reactions resulted in recovery of the starting material and unidentified products, respectively. Therefore, further investigation was not performed.

Fraction HR2 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase exhibited a long tail under UV-S. It was dissolved with chloroform to yield a chloroform soluble part (**HR21**) and a chloroform insoluble one (**HR22**) as shown in **Table 120**.

Table 120 Subfractions obtained from **fraction HR2** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR21	155.0	Brown gum
HR22	49.7	Brown gum

Subfraction HR21 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase showed a long tail under UV-S. The ^{1}H NMR spectrum indicated the presence of aromatic and olefinic protons. Further purification was performed by column chromatography over Sephadex LH-20 with 50% methanol-dichloromethane as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to yield three subfractions as shown in **Table 121**.

Table 121 Subfractions obtained from **subfraction HR21** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR211	7.7	Brown gum
HR212	109.2	Brown gum
HR213	27.6	Brown gum

Subfraction HR211 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further purified.

Subfraction HR212 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane as a mobile phase exhibited a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to give a chloroform soluble part (**HR212A**) and a chloroform insoluble one (**HR212B**) as shown in **Table 122**.

Table 122 Subfractions obtained from **subfraction HR212** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR212A	100.1	Yellow gum
HR212B	5.1	Yellow gum

Subfraction HR212A Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over Sephadex LH-20 with 50% methanol-dichloromethane. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to yield three subfractions as shown in **Table 123**.

Table 123 Subfractions obtained from **subfraction HR212A** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR212A1	76.7	Brown gum
HR212A2	18.2	Brown gum
HR212A3	4.4	Brown gum

Subfraction HR212A1 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HR212A2 Chromatogram characteristics on normal phase TLC with 8:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed a long tail under UV-S and many spots after being visualized by anisaldehyde sulfuric acid. Its ^{1}H NMR spectrum showed many components without major components. Thus, it was not further purified.

Subfraction HR212A3 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane as a mobile phase demonstrated a long tail under UV-S and

after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the absence of major components. Because of low quantity, no further investigation was conducted.

Subfraction HR212B Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane as a mobile phase showed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no attempts were made to purify this subfraction.

Subfraction HR213 Chromatogram characteristics on reverse phase TLC with 75% methanol-water as a mobile phase demonstrated a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HR22 Chromatogram characteristics on normal phase TLC with 60% ethyl acetate-n-hexane as a mobile phase showed a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Fraction HR3 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase showed a long tail and many spots under UV-S and after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over Sephadex LH-20 with 50% methanol-dichloromethane as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give three subfractions as shown in **Table 124**.

Table 124 Subfractions obtained from **fraction HR3** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR31	47.5	Dark yellow gum
HR32	254.6	Dark yellow gum

Table 124 (continued)

Subfraction	Weight (mg)	Physical appearance
HR33	4.0	Yellow gum

Subfraction HR31 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HR32 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Purification was conducted by column chromatography over silica gel with 50% ethyl acetate-n-hexane as an eluent to afford six subfractions as shown in **Table 125**.

Table 125 Subfractions obtained from **subfraction HR32** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HR321	2.3	Yellow gum
HR322	20.3	Yellow gum
HR323	2.0	Yellow gum
HR324	4.2	Yellow gum
HR325	23.1	Yellow gum
HR326	202.0	Dark Yellow gum

Subfraction HR321 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-n-hexane as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of long chain hydrocarbons. Thus, it was not purified.

Subfraction HR322 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-n-hexane as a mobile phase displayed a long tail under UV-S and one major spot after being visualized by anisaldehyde sulfuric acid with the R_f value of

0.55. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HR323 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-n-hexane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed signals in high field region. Therefore, no further purification was carried out.

Subfraction HR324 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-n-hexane as a mobile phase displayed a long tail and many spots near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Because the ^{1}H NMR spectrum showed signals in high field region, no further purification was conducted.

Subfraction HR325 Chromatogram characteristics on reverse phase TLC with 55% methanol-water as a mobile phase demonstrated one major spot with the R_f value of 0.65 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was combined with subfraction **HR424** because they showed similar ^{1}H NMR spectroscopic data. The combined subfraction was purified by column chromatography over reverse phase C_{18} silica gel with 55% methanol-water as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give five subfractions as shown in **Table 126**.

Table 126 Subfractions obtained from **subfraction HR325** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HR3251	7.6	Dark yellow gum
HR3252	12.3	Yellow gum
HR3253	4.9	Yellow gum
HR3254	9.0	Yellow gum
HR3255	15.5	Dark yellow gum

Subfraction HR3251 Chromatogram characteristics on reverse phase TLC with 55% methanol-water as a mobile phase displayed one major spot with the R_f value of 0.83 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HR3252 Chromatogram characteristics on reverse phase TLC with 25% methanol-water as a mobile phase showed one major spot with the R_f value of 0.50 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was purified by column chromatography over reverse phase C_{18} silica gel with 25% methanol-water as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give four subfractions as shown in **Table 127**.

Table 127 Subfractions obtained from **subfraction HR3252** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HR3252A	2.6	Dark yellow gum
HR3252B	6.7	Colorless gum
HR3252C	1.8	Yellow gum
HR3252D	1.0	Yellow gum

Subfraction HR3252A Chromatogram characteristics on reverse phase TLC with 25% methanol-water as a mobile phase demonstrated no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the absence of major components. Thus, no further investigation was conducted.

Subfraction HR3252B (**H18**) Chromatogram characteristics on reverse phase TLC with 25% methanol-water displayed one spot with the R_f value of 0.50 under UV-S and after being visualized by anisaldehyde sulfuric acid.

$[\alpha]_D^{24}$: -50.0 (c 0.05, $CHCl_3$)

UV (MeOH) λ_{max} nm (log ε) : 260 (4.38)

FT-IR (neat) ν_{max} cm^{-1} : 3418 (O-H), 1633 (C=O)

^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)		:	7.16 (*dd*, *J* = 9.5 and 15.5 Hz, 1H), 6.26 (*m*, 2H), 6.04 (*d*, *J* = 15.5 Hz, 1H), 2.46 (*m*, 2H), 1.95 (*m*, 2H), 1.85 (*d*, *J* = 5.0 Hz, 3H), 1.55 (*s*, 3H), 1.33 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		:	203.2, 194.8, 182.7, 145.2, 141.9, 131.6, 128.6, 88.2, 85.5, 35.4, 32.5, 23.9, 18.8, 6.0
DEPT (135^o) ($CDCl_3$)	CH	:	145.2, 141.9, 131.6, 128.6
	CH_2	:	35.4, 32.5
	CH_3	:	23.9, 18.8, 6.0

Subfraction HR3252C Chromatogram characteristics on reverse phase TLC with 25% methanol-water as a mobile phase showed one major spot with the R_f value of 0.50 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed that the major component was **H18**.

Subfraction HR3252D Chromatogram characteristics on reverse phase TLC with 25% methanol-water as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further investigated.

Subfraction HR3253 Chromatogram characteristics on reverse phase TLC with 55% methanol-water as a mobile phase displayed one major spot with the R_f value of 0.65 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity and signals in high field region in the ^{1}H NMR spectrum, it was not further purified.

Subfraction HR3254 Chromatogram characteristics on reverse phase TLC with 55% methanol-water as a mobile phase showed one major spot with the R_f value of 0.65 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was purified by column chromatography over Sephadex LH-20 with 100% methanol as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give two subfractions as shown in **Table 128**.

Table 128 Subfractions obtained from **subfraction HR3254** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR32541	2.9	Yellow gum
HR32542	3.8	Yellow gum

Subfraction HR32541 Chromatogram characteristics on reverse phase TLC with 25% methanol-water as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, no further purification was performed.

Subfraction HR32542 Chromatogram characteristics on reverse phase TLC with 25% methanol-water as a mobile phase showed one major spot with the R_f value of 0.50 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed that the major component was **H18**.

Subfraction HR3255 Chromatogram characteristics on reverse phase TLC with 55% methanol-water as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, no further purification was performed.

Subfraction HR326 Chromatogram characteristics on reverse phase TLC with 60% methanol-water (2 runs) as a mobile phase showed a long tail and two major spots with the R_f values of 0.54 and 0.86 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was subjected to column chromatography over reverse phase C_{18} silica gel with 60% methanol-water as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give four subfractions as shown in **Table 129**.

Table 129 Subfractions obtained from **subfraction HR326** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HR3261	65.0	Brown gum

Table 129 (continued)

Subfraction	Weight (mg)	Physical appearance
HR3262	35.5	Brown gum
HR3263	35.1	Brown gum
HR3264	63.6	Yellow gum

Subfraction HR3261 Chromatogram characteristics on reverse phase TLC with 60% methanol-water (2 runs) as a mobile phase demonstrated a long tail under UV-S and one major spot with the R_f value of 0.86 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the absence of aromatic and olefinic proton signals. Thus, no further investigation was conducted.

Subfraction HR3262 Chromatogram characteristics on reverse phase TLC with 60% methanol-water (2 runs) as a mobile phase showed a long tail and one major spot with the R_f value of 0.54 under UV-S and after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over Sephadex LH-20 with 50% methanol-dichloromethane as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to yield four subfractions as shown in **Table 130**.

Table 130 Subfractions obtained from **subfraction HR3262** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR3262A	4.5	Yellow gum
HR3262B	10.1	Yellow gum
HR3262C	12.7	Yellow gum
HR3262D	4.5	Yellow gum

Subfraction HR3262A Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol (6 runs) as a mobile phase showed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Thus, it was not further purified.

Subfraction HR3262B Chromatogram characteristics on reverse phase TLC with 5:2:13 methanol:acetone:water (3 runs) as a mobile phase exhibited a long tail near the baseline and one major spot with the R_f value of 0.28 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was purified by column chromatography over reverse phase C_{18} silica gel with 5:2:13 methanol:acetone:water as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give two subfractions as shown in **Table 131**.

Table 131 Subfractions obtained from **subfraction HR3262B** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HR3262B1	5.1	Yellow gum
HR3262B2	2.6	Yellow gum

Subfraction HR3262B1 Chromatogram characteristics on reverse phase TLC with 5:2:13 methanol:acetone:water (3 runs) as a mobile phase showed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^1H NMR spectrum exhibited signals in high field region. Therefore, no attempts were made to purify this subfraction.

Subfraction HR3262B2 Chromatogram characteristics on reverse phase TLC with 5:2:13 methanol:acetone:water (3 runs) as a mobile phase displayed one major spot with the R_f value of 0.28 under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to low quantity, it was not further purified.

Subfraction HR3262C Chromatogram characteristics on reverse phase TLC with 55% methanol-water (2 runs) as a mobile phase demonstrated two major spots with the R_f values of 0.36 and 0.42 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was combined with subfraction **HR4252** because they had similar ^1H NMR spectroscopic data.

Subfraction HR3262D Chromatogram characteristics on reverse phase TLC with 55% methanol-water as a mobile phase demonstrated two major spots with the R_f

values of 0.47 and 0.53 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the absence of major components. Because of low quantity, no further investigation was carried out.

Subfraction HR3263 Chromatogram characteristics on reverse phase TLC with 60% methanol-water as a mobile phase displayed a long tail near the baseline and two major spots with the R_f values of 0.39 and 0.30 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was purified by column chromatography over Sephadex LH-20 with 50% methanol-dichloromethane as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford three subfractions as shown in **Table 132**.

Table 132 Subfractions obtained from **subfraction HR3263** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR3263A	12.9	Yellow gum
HR3263B	17.4	Yellow gum
HR3263C	4.5	Yellow gum

Subfraction HR3263A Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase displayed one major spot with the R_f value of 0.33 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region, it was not further purified.

Subfraction HR3263B Chromatogram characteristics on reverse phase TLC with 5:2:13 methanol:acetone:water (3 runs) as a mobile phase displayed a long tail near the baseline and one major spot with the R_f value of 0.28 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was purified by column chromatography over reverse phase C_{18} silica gel with 5:2:13 methanol:acetone:water as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give two subfractions as shown in **Table 133**.

Table 133 Subfractions obtained from **subfraction HR3263B** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HR3263B1	3.1	Yellow gum
HR3263B2	8.5	Yellow gum

Subfraction HR3263B1 Chromatogram characteristics on reverse phase TLC with 5:2:13 methanol:acetone:water (3 runs) as a mobile phase showed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no attempts were made to investigate this subfraction.

Subfraction HR3263B2 Chromatogram characteristics on reverse phase TLC with 5:2:13 methanol:acetone:water (3 runs) as a mobile phase displayed one major spot with the R_f value of 0.28 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, it was not further purified.

Subfraction HR3263C Chromatogram characteristics on normal phase TLC with 18:1:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed a long tail near the baseline and one major spot with the R_f value of 0.33 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not further purified.

Subfraction HR3264 Chromatogram characteristics on reverse phase TLC with 60% methanol-water (2 runs) as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HR33 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the absence of major components. Because of low quantity, no further investigation was carried out.

Fraction HR4 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail under UV-S. The ^{1}H NMR spectrum indicated the presence of aromatic and olefinic proton signals. Further purification was performed by column chromatography over Sephadex LH-20 with 50% methanol-dichloromethane as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give three subfractions as shown in **Table 134**.

Table 134 Subfractions obtained from **fraction HR4** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR41	15.7	Yellow gum
HR42	318.0	Dark yellow gum
HR43	11.2	Yellow gum

Subfraction HR41 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed a long tail and two major spots with the R_f values of 0.53 and 0.70 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HR42 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-n-hexane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over silica gel with 50% n-hexane-ethyl acetate as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford five subfractions as shown in **Table 135**.

Table 135 Subfractions obtained from **subfraction HR42** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HR421	3.1	Yellow gum

Table 135 (continued)

Subfraction	Weight (mg)	Physical appearance
HR422	62.1	Yellow gum
HR423	20.3	Yellow gum
HR424	45.9	Yellow gum
HR425	181.3	Dark yellow gum

Subfraction HR421 Chromatogram characteristics on normal phase TLC with 60% ethyl acetate-n-hexane as a mobile phase demonstrated no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Due to low quantity, it was not further purified.

Subfraction HR422 Chromatogram characteristics on normal phase TLC with 60% ethyl acetate-n-hexane as a mobile phase showed a long tail under UV-S. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HR423 Chromatogram characteristics on reverse phase TLC with 60% methanol-water as a mobile phase showed a long tail and three major spots with the R_f values of 0.58, 0.67 and 0.83 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was subjected to column chromatography over reverse phase C_{18} silica gel with 60% methanol-water as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give six subfractions as shown in **Table 136**.

Table 136 Subfractions obtained from **subfraction HR423** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HR4231	3.5	Yellow gum
HR4232	2.0	Yellow gum
HR4233	2.8	Yellow gum
HR4234	2.2	Yellow gum

Table 136 (continued)

Subfraction	Weight (mg)	Physical appearance
HR4235	2.0	Yellow gum
HR4236	6.9	Yellow gum

Subfraction HR4231 Chromatogram characteristics on reverse phase TLC with 60% methanol-water as a mobile phase demonstrated a long tail under UV-S and one major spot with the R_f value of 0.83 after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no further investigation was conducted.

Subfraction HR4232 Chromatogram characteristics on reverse phase TLC with 60% methanol-water as a mobile phase displayed a long tail under UV-S and one major spot with the R_f value of 0.67 after being visualized by anisaldehyde sulfuric acid. Due to low quantity, no further investigation was conducted.

Subfraction HR4233 Chromatogram characteristics on reverse phase TLC with 60% methanol-water as a mobile phase displayed a long tail under UV-S and two major spots with the R_f values of 0.58 and 0.67 after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no further investigation was performed.

Subfraction HR4234 Chromatogram characteristics on reverse phase TLC with 60% methanol-water as a mobile phase displayed a long tail under UV-S and one major spot with the R_f value of 0.58 after being visualized by anisaldehyde sulfuric acid. Due to low quantity, no attempts were made to investigate this subfraction.

Subfraction HR4235 Chromatogram characteristics on reverse phase TLC with 60% methanol-water as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not further investigated.

Subfraction HR4236 Chromatogram characteristics on reverse phase TLC with 60% methanol-water as a mobile phase demonstrated a long tail near the baseline under UV-S and one major spot with the R_f value of 0.08 after being visualized by

anisaldehyde sulfuric acid. The ^{1}H NMR spectrum revealed the absence of aromatic and olefinic proton signals. Thus, it was not further investigated.

Subfraction HR424 Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase showed one major spot with the R_f value of 0.50 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was combined with subfraction **HR325** because they showed similar ^{1}H NMR spectroscopic data.

Subfraction HR425 Chromatogram characteristics on reverse phase TLC with 55% methanol-water as a mobile phase (2 runs) demonstrated one major spot with the R_f value of 0.42 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was purified by column chromatography over reverse phase C_{18} silica gel with 55% methanol-water as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give three subfractions as shown in **Table 137**.

Table 137 Subfractions obtained from **subfraction HR425** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HR4251	63.9	Yellow gum
HR4252	52.6	Yellow gum
HR4253	55.5	Dark yellow gum

Subfraction HR4251 Chromatogram characteristics on reverse phase TLC with 25% methanol-water (2 runs) as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, it was not further investigated.

Subfraction HR4252 Chromatogram characteristics on reverse phase TLC with 55% methanol-water (2 runs) as a mobile phase showed one major spot with the R_f value of 0.42 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was combined with subfraction **HR3262C** because they showed similar ^{1}H NMR spectroscopic data. It was purified by column chromatography over reverse phase C_{18} silica gel with 55% methanol-water as an eluent. Subfractions were examined by

TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give four subfractions as shown in **Table 138**.

Table 138 Subfractions obtained from **subfraction HR4252** by column chromatography over reverse phase C_{18} silica gel

Subfraction	Weight (mg)	Physical appearance
HR4252A	13.0	Yellow gum
HR4252B	15.0	Yellow gum
HR4252C	10.0	Yellow gum
HR4252D	13.9	Brown gum

Subfraction HR4252A Chromatogram characteristics on reverse phase TLC with 55% methanol-water (2 runs) as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, it was not further purified.

Subfraction HR4252B Chromatogram characteristics on reverse phase TLC with 55% methanol-water (2 runs) as a mobile phase showed one major spot with the R_f value of 0.42 under UV-S and after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over Sephadex LH-20 with 100% methanol. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give three subfractions as shown in **Table 139**.

Table 139 Subfractions obtained from **subfraction HR4252B** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR4252B1	3.1	Brown gum
HR4252B2	7.2	Brown gum
HR4252B3	2.3	Brown gum

Subfraction HR4252B1 Chromatogram characteristics on reverse phase TLC with 55% methanol-water (2 runs) as a mobile phase displayed a long tail under UV-S and

after being visualized by anisaldehyde sulfuric acid. Due to low quantity, no attempts were made to purify this subfraction.

Subfraction HR4252B2 Chromatogram characteristics on reverse phase TLC with 55% methanol-water (2 runs) as a mobile phase displayed one major spot with the R_f value of 0.42 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to afford a chloroform soluble part (**HR4252B21**) and a chloroform insoluble one (**HR4252B22**) as shown in **Table 140**.

Table 140 Subfractions obtained from **subfraction HR4252B2** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR4252B21	3.3	Yellow gum
HR4252B22	2.5	Yellow gum

Subfraction HR4252B21 Chromatogram characteristics on reverse phase TLC with 40% methanol-water (3 runs) as a mobile phase showed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Because of low quantity, it was not further purified.

Subfraction HR4252B22 Chromatogram characteristics on reverse phase TLC with 40% methanol-water (3 runs) as a mobile phase exhibited a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no attempts were made to purify this subfraction.

Subfraction HR4252B3 Chromatogram characteristics on reverse phase TLC with 55% methanol-water (2 runs) as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no further purification was performed.

Subfraction HR4252C Chromatogram characteristics on reverse phase TLC with 35% methanol-water (4 runs) as a mobile phase exhibited a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Accordingly, no attempts were made to investigate this subfraction.

Subfraction HR4252D Chromatogram characteristics on normal phase TLC with 55% methanol-water (2 runs) as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HR4253 Chromatogram characteristics on reverse phase TLC with 55% methanol-water (2 runs) as a mobile phase displayed a long tail near the baseline and two major spots with the R_f values of 0.36 and 0.42 under UV-S and one additional spot with the R_f value of 0.05 after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited major signals in high field region. Therefore, it was not further purified.

Subfraction HR43 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of sugar as a major component. Therefore, no further investigation was carried out.

Fraction HR5 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase exhibited a long tail under UV-S. It was dissolved with chloroform to afford a chloroform soluble part (**HR51**) and a chloroform insoluble one (**HR52**) as shown in **Table 141**.

Table 141 Subfractions obtained from **fraction HR5** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR51	409.5	Brown gum
HR52	104.3	Yellow gum

Subfraction HR51 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase exhibited a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. It was subjected to column chromatography over silica gel using a gradient solvent system, starting from 2% methanol-dichloromethane until pure methanol. Subfractions were examined by TLC,

combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford eleven subfractions as shown in **Table 142**.

Table 142 Subfractions obtained from **subfraction HR51** by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
HR51A	2% methanol-dichloromethane	3.2	Yellow gum
HR51B	2% methanol-dichloromethane	12.2	Yellow gum
HR51C	2% methanol-dichloromethane	6.0	Yellow gum
HR51D	2% methanol-dichloromethane	36.6	Yellow gum
HR51E	2% methanol-dichloromethane	42.3	Yellow gum
HR51F	2% methanol-dichloromethane	24.6	Yellow gum
HR51G	2% methanol-dichloromethane	50.1	Yellow gum
HR51H	2% methanol-dichloromethane	27.0	Yellow gum
HR51I	2% methanol-dichloromethane	21.7	Yellow gum
HR51J	2% methanol-dichloromethane	62.3	Yellow gum
HR51K	30-100% methanol-dichloromethane	103.1	Brown gum

Subfraction HR51A Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed a long tail under UV-S and two major spots with the R_f values of 0.75 and 0.85 after being visualized by anisaldehyde sulfuric acid. Due to low quantity, no attempts were made to investigate this subfraction.

Subfraction HR51B Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed one major spot with the R_f value of 0.45 under UV-S and after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over silica gel with 100% dichloromethane as an eluent. Fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give four subfractions as shown in **Table 143**.

Table 143 Subfractions obtained from **subfraction HR51B** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HR51B1	0.5	Colorless gum
HR51B2	1.8	Colorless gum
HR51B3	0.6	Colorless gum
HR51B4	8.0	Yellow gum

Subfraction HR51B1 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed one major spot with the R_f value of 0.68 under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to minute quantity, it was not further investigated.

Subfraction HR51B2 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed two major spots with the R_f values of 0.55 and 0.68 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not further investigated.

Subfraction HR51B3 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed two major spots with the R_f values of 0.50 and 0.60 under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to minute quantity, it was not further investigated.

Subfraction HR51B4 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HR51C Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed a long tail and two major spots with the R_f values of 0.25 and 0.45 under UV-S and after being visualized by anisaldehyde sulfuric acid. It was subjected to column chromatography over silica gel with 100% dichloromethane as an eluent. Fractions were examined by TLC, combined on the

basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give two subfractions as shown in **Table 144**.

Table 144 Subfractions obtained from **subfraction HR51C** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HR51C1	2.4	Colorless gum
HR51C2	2.5	Yellow gum

Subfraction HR51C1 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed a long tail and two major spots with the R_f values of 0.25 and 0.45 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major compounds. Because of low quantity, it was not further purified.

Subfraction HR51C2 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no attempts were made to purify this subfraction.

Subfraction HR51D Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase exhibited two major spots with the R_f values of 0.30 and 0.65 as well as a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Purification was performed by column chromatography over silica gel. Elution was conducted with 100% dichloromethane. Fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give four subfractions as shown in **Table 145**.

Table 145 Subfractions obtained from **subfraction HR51D** by column chromatography over silica gel

Subfraction	Weight (mg)	Physical appearance
HR51D1	2.2	Colorless gum
HR51D2	1.5	Yellow gum

Table 145 (continued)

Subfraction	Weight (mg)	Physical appearance
HR51D3	20.1	Yellow gum
HR51D4	10.4	Yellow gum

Subfraction HR51D1 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed one major spot with the R_f value of 0.65 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no attempts were made to purify this subfraction.

Subfraction HR51D2 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed three major spots with the R_f values of 0.33, 0.48 and 0.65 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, no further investigation was performed.

Subfraction HR51D3 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed a long tail and one major spot with the R_f value of 0.75 under UV-S and after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over Sephadex LH-20 with 50% methanol-dichloromethane. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to yield three subfractions as shown in **Table 146**.

Table 146 Subfractions obtained from **subfraction HR51D3** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR51D31	5.0	Brown gum
HR51D32	12.0	Brown gum
HR51D33	2.1	Brown gum

Subfraction HR51D31 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum

exhibited many components without major components. Therefore, it was not further investigated.

Subfraction HR51D32 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed a long tail near the baseline and one major spot with the R_f value of 0.75 under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HR51D33 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase showed one major spot with the R_f value of 0.75 under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no further investigation was performed.

Subfraction HR51D4 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed signals in high field region. Thus, no further investigation was conducted.

Subfraction HR51E Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase exhibited four major spots with the R_f values of 0.53, 0.60, 0.65 and 0.73 as well as a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to yield a chloroform soluble part (**HR51E1**) and a chloroform insoluble one (**HR51E2**) as shown in **Table 147**.

Table 147 Subfractions obtained from **subfraction HR51E** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR51E1	37.6	Yellow gum
HR51E2	2.3	Yellow gum

Subfraction HR51E1 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase displayed four major spots with the R_f values of 0.53, 0.60, 0.65 and 0.73 under UV-S and after being visualized by

anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, it was not further investigated.

Subfraction HR51E2 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no further investigation was performed.

Subfraction HR51F Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase exhibited three major spots with the R_f values of 0.53, 0.60 and 0.65 as well as a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to yield a chloroform soluble part (**HR51F1**) and a chloroform insoluble one (**HR51F2**) as shown in **Table 148**.

Table 148 Subfractions obtained from **subfraction HR51F** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR51F1	22.8	Yellow gum
HR51F2	1.5	Yellow gum

Subfraction HR51F1 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase displayed three major spots with the R_f values of 0.53, 0.60 and 0.65 under UV-S and a long tail near the baseline after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum exhibited signals in high field region. Thus, it was not further purified.

Subfraction HR51F2 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of minute quantity, it was not further purified.

Subfraction HR51G Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and after

being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of tyrosol as a major component. Therefore, no further investigation was carried out.

Subfraction HR51H Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase exhibited three major spots with the R_f values of 0.28, 0.38 and 0.45 as well as a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to yield a chloroform soluble part (**HR51H1**) and a chloroform insoluble one (**HR51H2**) as shown in **Table 149**.

Table 149 Subfractions obtained from **subfraction HR51H** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR51H1	25.3	Yellow gum
HR51H2	1.6	Yellow gum

Subfraction HR51H1 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase displayed three major spots with the R_f values of 0.28, 0.38 and 0.45 and a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of tyrosol as a major component. Therefore, no further investigation was carried out.

Subfraction HR51H2 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase displayed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to minute quantity, it was not further purified.

Subfraction HR51I Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase displayed three major spots with the R_f values of 0.28, 0.38 and 0.45 as well as a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to yield a chloroform soluble part (**HR51I1**) and a chloroform insoluble one (**HR51I2**) as shown in **Table 150**.

Table 150 Subfractions obtained from **subfraction HR51I** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR51I1	19.3	Yellow gum
HR51I2	2.0	Yellow gum

Subfraction HR51I1 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase displayed three major spots with the R_f values of 0.28, 0.38 and 0.45 as well as a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further investigated.

Subfraction HR51I2 Chromatogram characteristics on normal phase TLC with 30% acetone-n-hexane (11 runs) as a mobile phase demonstrated no spots under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to low quantity, it was not further purified.

Subfraction HR51J Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase showed a long tail and four major spots with the R_f values of 0.23, 0.30, 0.33 and 0.43 under UV-S and after being visualized by anisaldehyde sulfuric acid. Further purification was performed by column chromatography over Sephadex LH-20 with 50% methanol-dichloromethane as an eluent. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford four subfractions as shown in **Table 151**.

Table 151 Subfractions obtained from **subfraction HR51J** by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
HR51J1	32.6	Yellow gum
HR51J2	16.9	Yellow gum
HR51J3	7.2	Yellow gum
HR51J4	4.6	Yellow gum

Subfraction HR51J1 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase showed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed signals in high field region. Thus, no attempts were made to investigate this subfraction.

Subfraction HR51J2 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase exhibited four major spots with the R_f values of 0.23, 0.30, 0.33 and 0.43 as well as a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to give a chloroform soluble part (**HR51J2A**) and a chloroform insoluble one (**HR51J2B**) as shown in **Table 152**.

Table 152 Subfractions obtained from **subfraction HR51J2** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR51J2A	8.3	Yellow gum
HR51J2B	5.4	Yellow gum

Subfraction HR51J2A Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase displayed four major spots with the R_f values of 0.23, 0.30, 0.33 and 0.43 as well as a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of tyrosol as a major component. Therefore, no further investigation was carried out.

Subfraction HR51J2B Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase displayed three major spots with the R_f values of 0.23, 0.30 and 0.33 as well as a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the absence of major components. Due to low quantity, no further investigation was carried out.

Subfraction HR51J3 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase exhibited three major spots

with the R_f values of 0.18, 0.23 and 0.28 as well as a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of tyrosol as a major component. Therefore, no further investigation was carried out.

Subfraction HR51J4 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase showed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to low quantity, no further purification was conducted.

Subfraction HR51K Chromatogram characteristics on reverse phase TLC with 50% methanol-water as a mobile phase displayed a long tail under UV-S and after being visualized by ceric ammonium molybdate. The ^{1}H NMR spectrum displayed signals in high field region. Therefore, it was not further purified.

Subfraction HR52 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed one major spot near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum indicated the presence of sugar as a major component. Therefore, no further investigation was carried out.

Fraction HR6 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase exhibited a long tail under UV-S. It was dissolved with chloroform to give a chloroform soluble part (**HR61**) and a chloroform insoluble one (**HR62**) as shown in **Table 153**.

Table 153 Subfractions obtained from **fraction HR6** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR61	58.0	Brown gum
HR62	22.0	Brown gum

Subfraction HR61 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed many spots and a long tail under UV-S. Further purification was performed by column chromatography over silica gel. Elution was initially conducted with 2% methanol-dichloromethane, and then gradually enriched

with methanol until pure methanol. Subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to give seven subfractions as shown in **Table 154**.

Table 154 Subfractions obtained from **subfraction HR61** by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
HR61A	2% methanol-dichloromethane	10.8	Yellow gum
HR61B	2% methanol-dichloromethane	4.9	Dark yellow gum
HR61C	2% methanol-dichloromethane	1.1	Dark yellow gum
HR61D	2% methanol-dichloromethane	3.4	Dark yellow gum
HR61E	2% methanol-dichloromethane	9.7	Dark yellow gum
HR61F	60% methanol-dichloromethane	23.0	Dark yellow gum
HR61G	100% methanol	4.9	Dark yellow gum

Subfraction HR61A Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-n-hexane (3 runs) as a mobile phase showed one major spot with the R_f value of 0.48 after being visualized by ceric ammonium molybdate. Purification was conducted by preparative TLC with 5% ethyl acetate-n-hexane as mobile phase to afford two subfractions as shown in **Table 155**.

Table 155 Subfractions obtained from **subfraction HR61A** by preparative TLC

Subfraction	Weight (mg)	Physical appearance
HR61A1	1.4	Colorless gum
HR61A2	9.0	Colorless gum

Subfraction HR61A1 Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-n-hexane (3 runs) as a mobile phase showed no spots under UV-S and after being visualized by ceric ammonium molybdate. The ^{1}H NMR spectrum indicated the absence of major compounds. Thus, it was not further purified.

Subfraction HR61A2 (H19) Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-n-hexane (3 runs) displayed one spot with the R_f value of 0.50 after being visualized by anisaldehyde sulfuric acid.

UV (MeOH) λ_{max} nm (log ε)		:	320 (4.28)
FT-IR (neat) ν_{max} cm^{-1}		:	3331 (O-H), 1638 (C=O)
^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)		:	13.60 (*s*, 1H), 7.46 (*dd*, *J* = 10.0 and 15.0 Hz, 1H), 7.44 (*s*, 1H), 6.95 (*d*, *J* = 15.0 Hz, 1H), 6.30 (*m*, 2H), 5.35 (*s*, 1H), 2.22 (*s*, 3H), 2.15 (*s*, 3H), 1.91 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		:	192.6, 162.6, 158.7, 144.5, 141.0, 130.6, 128.8, 121.9, 114.4, 113.6, 110.4, 18.9, 15.6, 7.5
DEPT (135°) ($CDCl_3$)	CH	:	144.5, 141.0, 130.6, 128.8, 121.9
	CH_3	:	18.9, 15.6, 7.5

Subfraction HR61B Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-n-hexane (3 runs) as a mobile phase showed many spots under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, it was not further purified.

Subfraction HR61C Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. Due to minute quantity, it was not further purified.

Subfraction HR61D Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^1H NMR spectrum indicated the presence of tyrosol as a major component. Therefore, no further investigation was carried out.

Subfraction HR61E Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. The ^{1}H NMR spectrum showed many signals without major components. Thus, it was not further investigated.

Subfraction HR61F Chromatogram characteristics on reverse phase TLC with 75% methanol-water as a mobile phase exhibited a long tail under UV-S and after being visualized by anisaldehyde sulfuric acid. It was dissolved with chloroform to give a chloroform soluble part (**HR61F1**) and a chloroform insoluble one (**HR61F2**) as shown in **Table 156**.

Table 156 Subfractions obtained from **subfraction HR61F** by dissolving with chloroform

Subfraction	Weight (mg)	Physical appearance
HR61F1	17.1	Dark yellow gum
HR61F2	4.7	Yellow gum

Subfraction HR61F1 Chromatogram characteristics on normal phase TLC with 7:2:1 dichloromethane:ethyl acetate:methanol as a mobile phase exhibited a long tail and five major spots with the R_f values of 0.90, 0.85, 0.80, 0.68 and 0.60 under UV-S and after being visualized by ceric ammonium molybdate. Its ^{1}H NMR spectrum showed many components without major components. Thus, it was not further purified.

Subfraction HR61F2 Chromatogram characteristics on normal phase TLC with 7:2:1 dichloromethane:ethyl acetate:methanol as a mobile phase showed no spots under UV-S and after being visualized by ceric ammonium molybdate. The ^{1}H NMR spectrum indicated the absence of major compounds. Thus, it was not further purified.

Subfraction HR61G Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase displayed no spots under UV-S and after being visualized by ceric ammonium molybdate. The ^{1}H NMR spectrum exhibited signals in high field region. Therefore, it was not further investigated.

Subfraction HR62 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase displayed a long tail under UV-S and

many spots after being visualized by anisaldehyde sulfuric acid. Its ^{1}H NMR spectrum showed many components without major components. Thus, it was not further purified.

Fraction HR7 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail near the baseline under UV-S and after being visualized by anisaldehyde sulfuric acid. Because of low quantity, no attempts were made to investigate this subfraction.

CHAPTER 2.3

RESULTS AND DISCUSSION

Two known compounds (**H18** and **H19**) were obtained from the ethyl acetate broth extract of *Trichoderma longibrachiatum* PSU-AMF274. The ethyl acetate and hexane mycelial extracts were not investigated because their ^{1}H NMR spectra displayed major signals in high-field region.

2.3.1 Compound H18

H18 was obtained as a colorless gum. The UV spectrum displayed an absorption band at 260 nm, indicating the presence of a conjugated ketone carbonyl group whereas the IR spectrum showed absorption bands at 3418 and 1633 cm^{-1} for hydroxy and conjugated ketone carbonyl groups, respectively. The ^{1}H NMR spectroscopic data (**Table 157**) (**Figure 43**) showed signals for four olefinic protons [δ_H 7.16 (*dd*, *J* = 9.5 and 15.5 Hz, 1H), 6.26 (*m*, 2H) and 6.04 (*d*, *J* = 15.5 Hz, 1H)], two sets of equivalent methylene protons [δ_H 2.46 (*m*, 2H) and 1.95 (*m*, 2H)] and three methyl groups [δ_H 1.85 (*d*, *J* = 5.0 Hz, 3H), 1.55 (*s*, 3H) and 1.33 (*s*, 3H)]. The ^{13}C NMR spectrum (**Table 157**) (**Figure 44**) displayed signals for two ketone carbonyl carbons (δ_C 203.2 and 194.8), three quaternary carbons (δ_C 182.7, 88.2 and 85.5), four methine carbons (δ_C 145.2, 141.9, 131.6 and 128.6), two methylene carbons (δ_C 35.4 and 32.5) and three methyl carbons (δ_C 23.9, 18.8 and 6.0). The ^{1}H-^{1}H COSY correlations of H-9 (δ_H 7.16) with H-8 (δ_H 6.04) and H-10 (δ_H 6.26), those of H-11 (δ_H 6.26) with H-10 and H_3-12 (δ_H 1.85) and that of H_2-5 (δ_H 1.95) with H_2-6 (δ_H 2.46) (**Table 158**) and the HMBC cross peaks of both H_2-5 and H-9 with C-7 (δ_C 203.2) together with the chemical shift of C-7, (**Table 158**) constructed a 1-substituted-4,6-octadien-3-onyl unit. An *E*-configuration of two alkene groups was assigned based on a coupling constant value of 15.5 Hz between H-8 and H-9, and signal enhancement of H-11 and H-10 after irradiation of H-9 and H_3-12, respectively, in the NOEDIFF

experiments (**Table 158**). Further HMBC correlations of 2-Me (δ_H 1.55) with C-1 (δ_C 182.7), C-2 (δ_C 88.2) and C-3 (δ_C 194.8) and those of 4-Me (δ_H 1.33) with C-3 and C-4 (δ_C 85.5) established a furanone skeleton with a ketone functional group at C-1, a double bond between C-2 and C-3 and a hydroxy group at C-3. In addition, the HMBC correlations of H_2-5 with C-3 and C-4 suggested that C-5 of the dienonyl unit connected to C-4 of the furanone unit. The absolute configuration of C-4 was assigned to be *S* by comparison of the specific rotation of **H18**, $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{24}}$: -50.0 (c 0.05, $CHCl_3$), with that of (-)-vertinolide, $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$: -25.0 (c 0.05, $CHCl_3$) (Trifonov et al., 1981), of which the *S* configuration was established by enantioselective synthesis (Matsuo and Sakaguchi, 1997). Accordingly, **H18** was assigned as (-)-vertinolide, previously isolated from *Verticillium intertextum* (Trifonov et al., 1982).

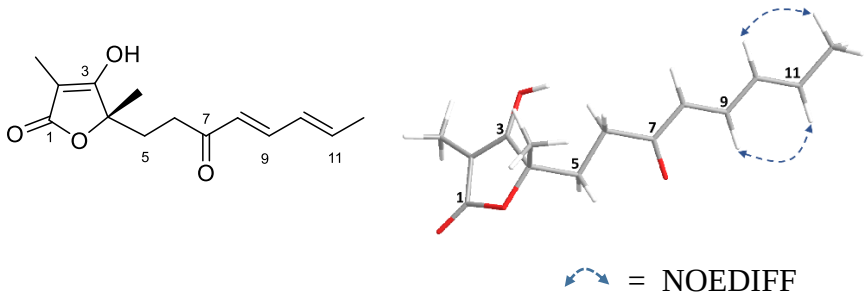


Table 157 The ^1H and ^{13}C NMR data of compound **H18** in CD_3OD and (-)-vertinolide in $CDCl_3$ and acetone-d_6 for ^1H and ^{13}C NMR data, respectively

Position	**H18**		(-)-vertinolide	
	δ_H (*mult.*, J_{Hz})[a]	δ_C (C-type)[a]	δ_H (*mult.*, J_{Hz})[b]	δ_C[c]
1	-	182.7 (C=O)	-	173.9
2	-	88.2 (C)	-	97.2
3	-	194.8 (C)	-	176.6
4	-	85.5 (C)	-	82.6
5	1.95 (*m*)	32.5 (CH_2)	2.10-2.40 (*m*)	31.7
6	2.46 (*m*)	35.4 (CH_2)	2.40-2.80 (*m*)	34.8
7	-	203.2 (C=O)	-	199.1
8	6.04 (*d*, 15.5)	128.6 (CH)	6.06 (*d*, 15.4)	128.6
9	7.16 (*dd*, 9.5, 15.5)	145.2 (CH)	7.19 (*dd*, 9.6, 15.4)	143.5
10	6.26 (*m*)	131.6 (CH)	6.10-6.40 (*m*)	131.4

Table 157 (continued)

Position	**H18**		(-)-vertinolide	
	δ_H (*mult.*, J_{Hz})[a]	δ_C (C-type)[a]	δ_H (*mult.*, J_{Hz})[b]	δ_C[c]
11	6.26 (*m*)	141.9 (CH)	6.10-6.40 (*m*)	140.7
12	1.85 (*d*, 5.0)	18.8 (CH_3)	1.89 (*d*, 5.4)	18.7
2-Me	1.55 (*s*)	6.0 (CH_3)	1.68 (*s*)	6.2
4-Me	1.33 (*s*)	23.9 (CH_3)	1.48 (*s*)	23.6

[a] in CD_3OD
[b] in $CDCl_3$
[c] in acetone-d_6

Table 158 The ^{1}H-^{1}H COSY, HMBC and NOEDIFF data of compound **H18**

Proton	COSY	HMBC	NOEDIFF
H_2-5	H_2-6	C-3, C-4, C-6, C-7, 4-Me	H_3-14
H_2-6	H_2-5	C-4, C-5, C-7	H_2-5, H-8, H-14
H-8	H-9	C-7, C-10	*
H-9	H-8, H-10	C-7, C-8, C-10, C-11	H-11
H-10	H-9, H-11	C-8, C-9, C-12	H_3-12
H-11	H-10, H_3-12	C-9, C-10, C-12	H_3-12
H_3-12	H-11	C-10, C-11	H-10, H-11
2-Me	-	C-1, C-2, C-3	*
4-Me	-	C-3, C-4, C-5	H_2-5

***** not determined

2.3.2 Compound H19

H19 was obtained as a colorless gum. The UV spectrum exhibited an absorption band at 320 nm, indicating the presence of an *α,β*-unsaturated carbonyl chromophore (Trifonov et al., 1983). The IR spectrum showed absorption bands for hydroxy (3331 cm^{-1}) and ketone carbonyl (1638 cm^{-1}) groups. The ^{1}H NMR spectroscopic data (**Table 159**) (**Figure 45**) contained signals for one chelated hydroxy proton (δ_H 13.60, *brs*, 1H), one hydroxy proton (δ_H 5.35, *brs*, 1H), five methine protons [δ_H 7.46 (*dd*, *J* = 10.0 and 15.0 Hz, 1H), 7.44 (*s*, 1H), 6.95 (*d*, *J* = 15.0 Hz, 1H) and

6.30 (*m*, 2H)] and three methyl groups [δ_H 2.22 (*s*, 3H), 2.15 (*s*, 3H) and 1.91 (*d*, *J* = 6.0 Hz, 3H)]. The ^{13}C NMR spectrum (**Table 159**) (**Figure 46**) displayed signals for one ketone carbonyl carbon (δ_C 192.6), five quaternary carbons (δ_C 162.6, 158.7, 114.4, 113.6 and 110.4), five methine carbons (δ_C 144.5, 141.0, 130.6, 128.8 and 121.9) and three methyl carbons (δ_C 18.9, 15.6 and 7.5). According to the ^{1}H and ^{13}C NMR spectroscopic data, **H19** was sorbicillin, previously isolated from *Verticillium intertextum* (Trifonov et al., 1983).

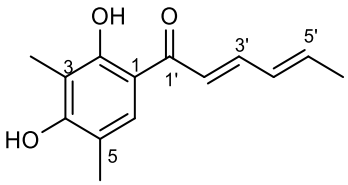


Table 159 The ^{1}H and ^{13}C NMR data of compound **H19** and sorbicillin in $CDCl_3$

Position	**H19**		Sorbicillin	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C
1	-	110.4 (C)	-	110.2
2	-	162.6 (C)	-	162.2
3	-	113.6 (C)	-	113.0
4	-	158.7 (C)	-	158.8
5	-	114.4 (C)	-	114.6
6	7.44 (*s*)	128.8 (CH)	7.43 (*s*)	128.5
1'	-	192.6 (C=O)	-	192.3
2'	6.95 (*d*, 15.0)	121.9 (CH)	6.92 (*d*, 15.0)	121.4
3'	7.46 (*dd*, 10.0, 15.0)	144.5 (CH)	7.46 (*dd*, 10.0, 15.0)	144.4
4'	6.30 (*m*)	130.6 (CH)	6.20-6.40 (*m*)	130.2
5'	6.30 (*m*)	141.0 (CH)	6.20-6.40 (*m*)	141.2
6'	1.91 (*d*, 6.0)	18.9 (CH_3)	1.88 (*d*, 6.0)	18.7
2-OH	13.60 (*brs*)	-	13.61 (*s*)	-
4-OH	5.35 (*brs*)	-	5.30 (*s*)	-
3-Me	2.15 (*s*)	7.5 (CH_3)	2.13 (*s*)	7.3
5-Me	2.22 (*s*)	15.6 (CH_3)	2.20 (*s*)	15.5

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APPENDIX

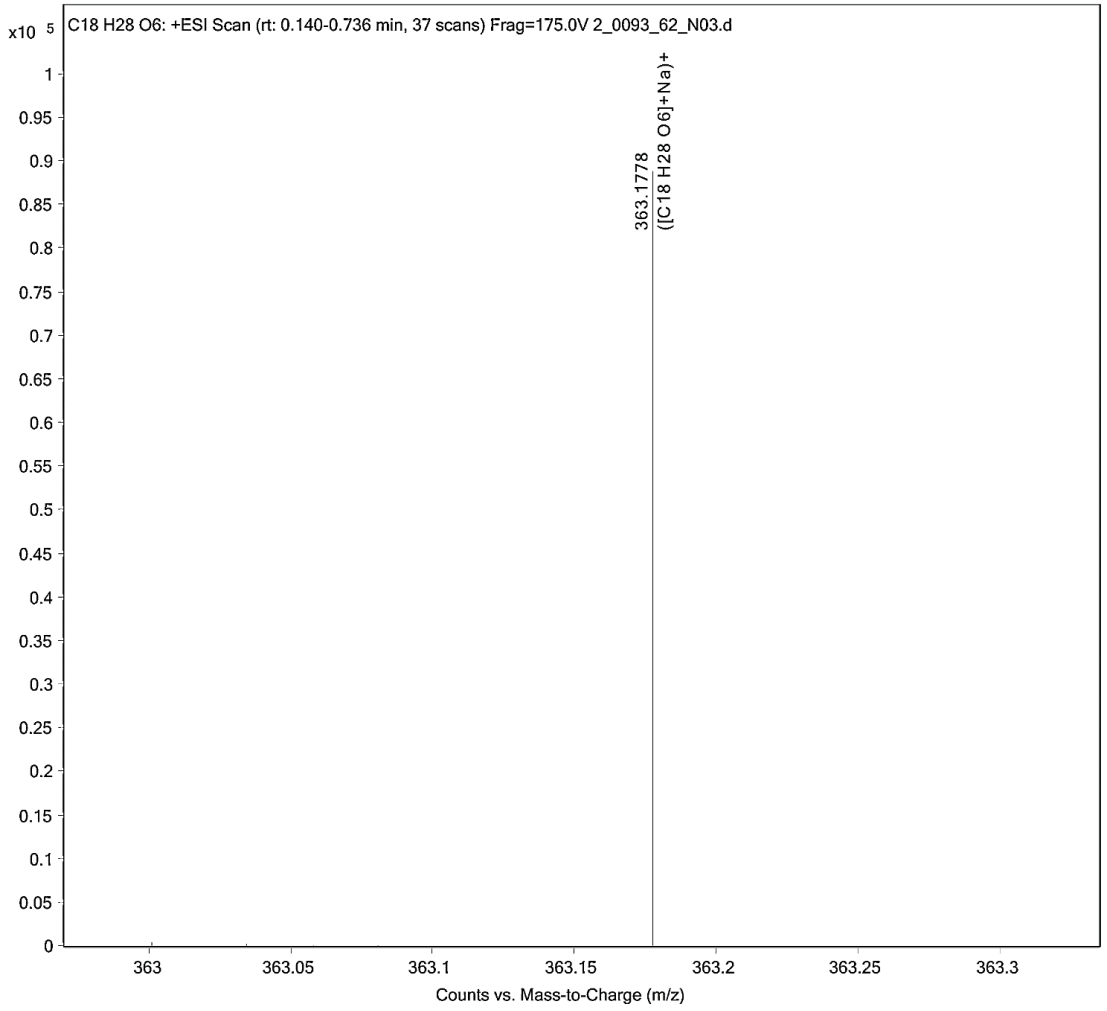

Figure 4 The HRESIMS of compound **H3**

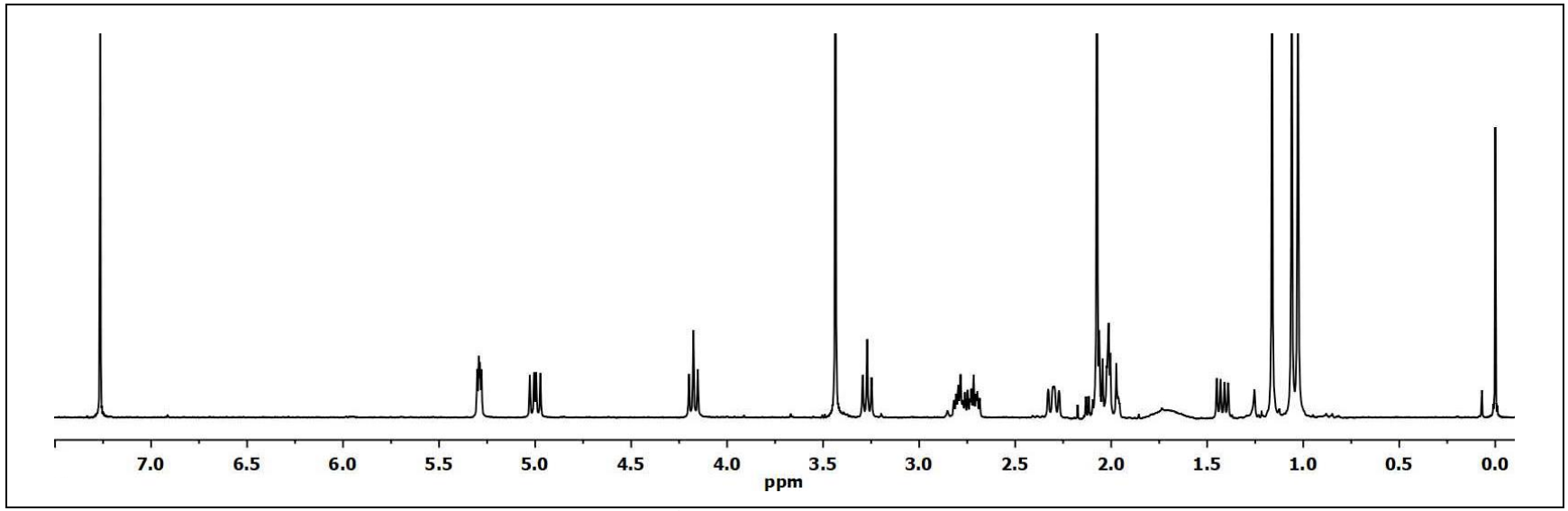

Figure 5 The 300 MHz ^{1}H NMR spectrum of compound **H3** in $CDCl_3$

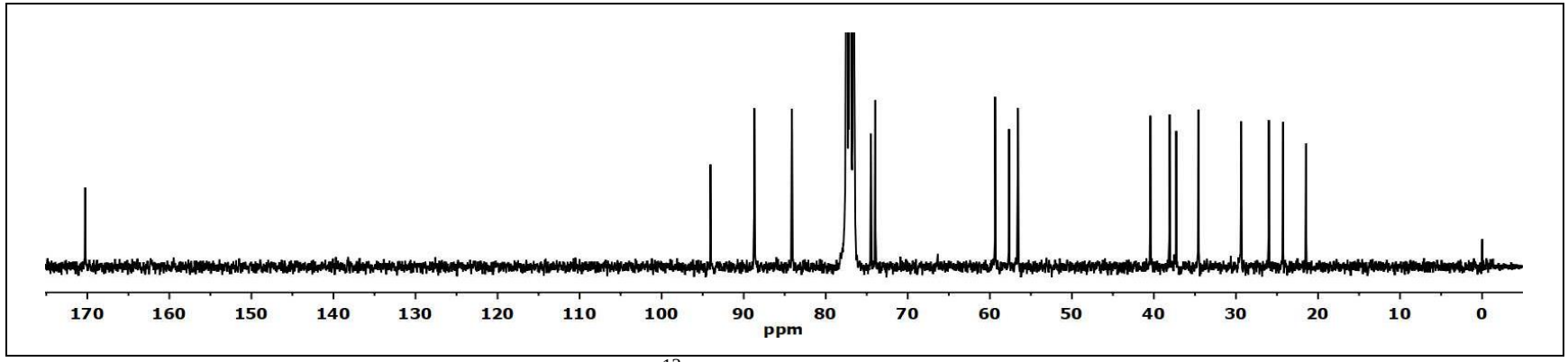

Figure 6 The 75 MHz ^{13}C NMR spectrum of compound **H3** in $CDCl_3$

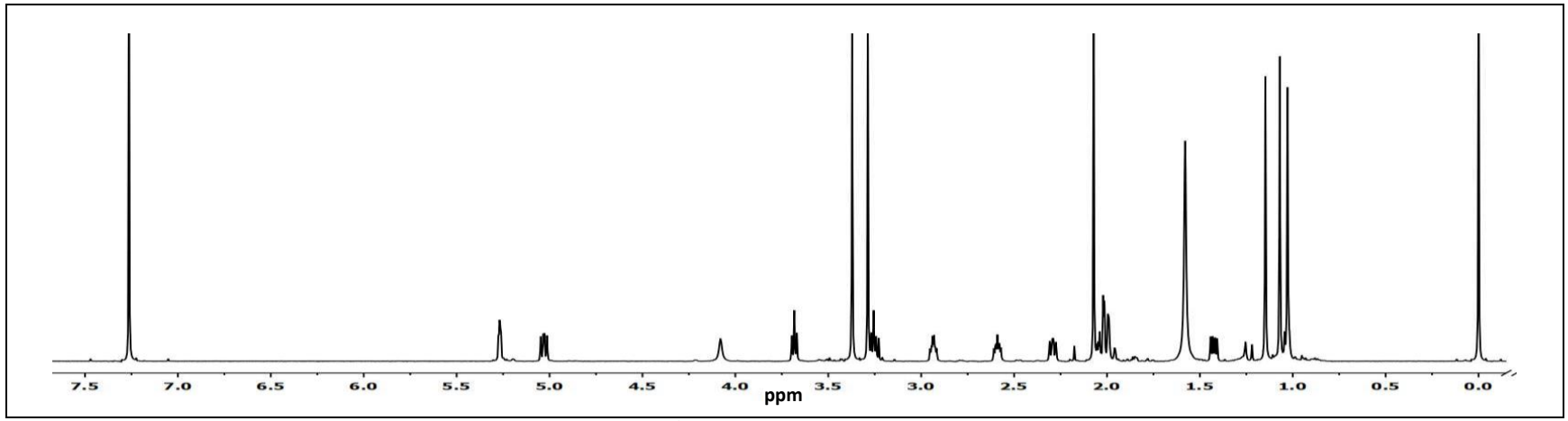

Figure 7 The 500 MHz ^{1}H NMR spectrum of compound **H1** in $CDCl_3$

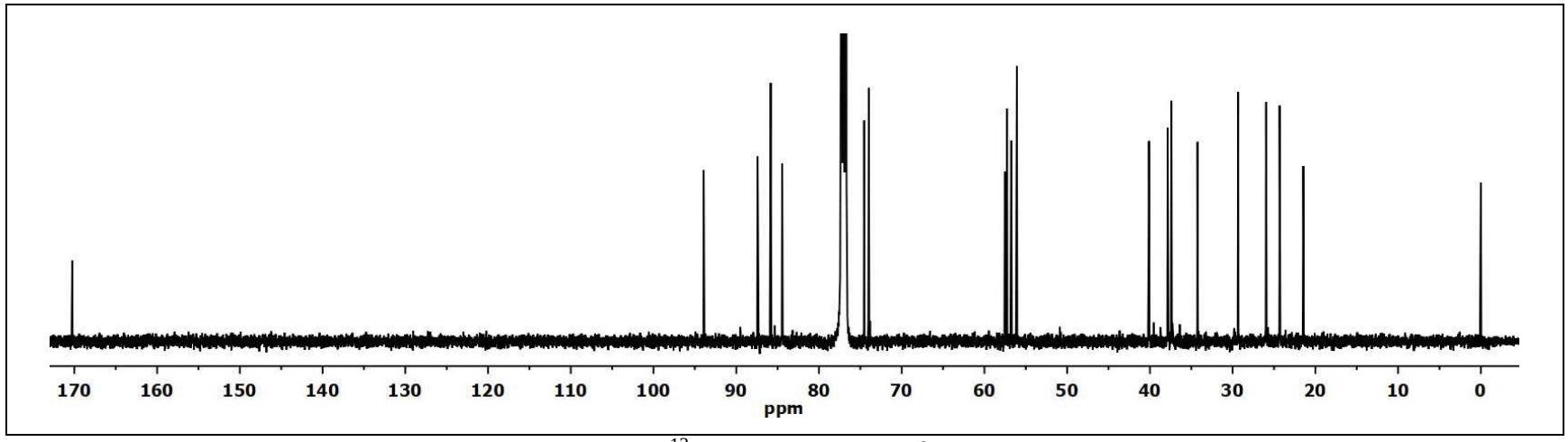

Figure 8 The 125 MHz ^{13}C NMR spectrum of compound **H1** in $CDCl_3$

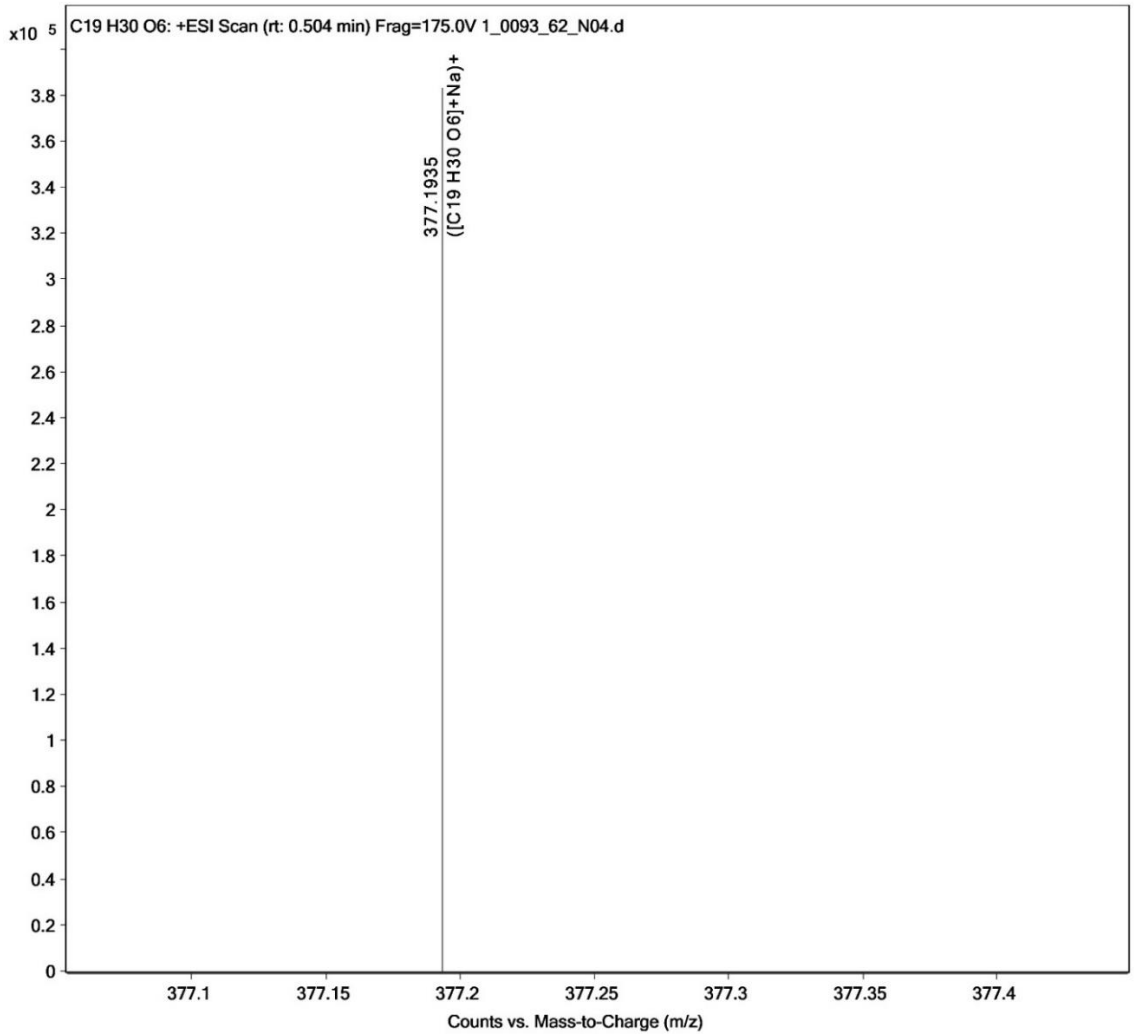

Figure 9 The HRESIMS of compound **H2**

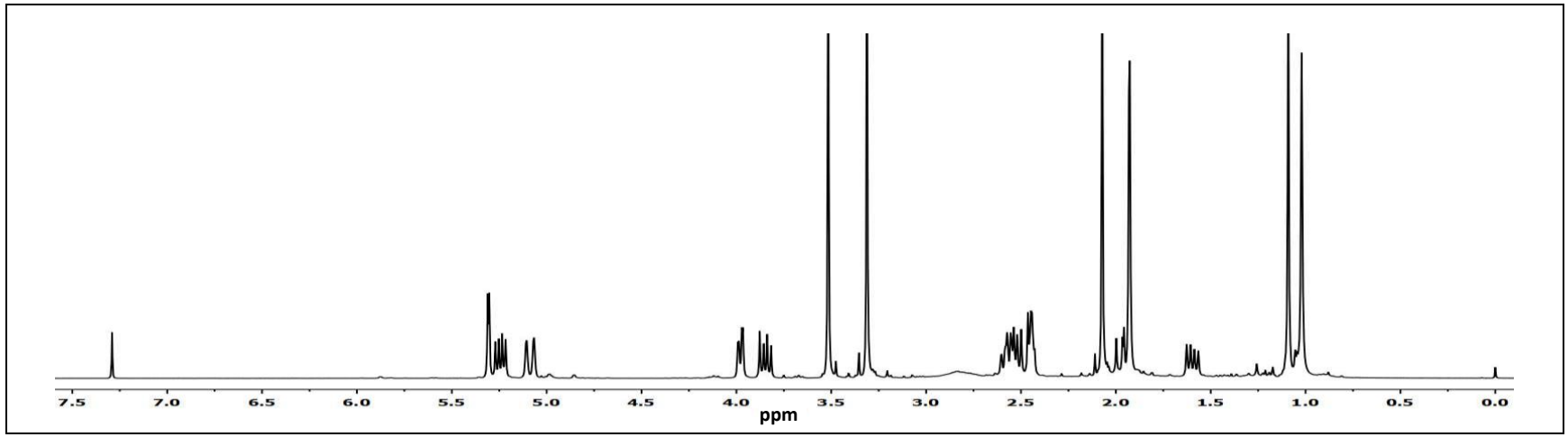

Figure 10 The 300 MHz ^{1}H NMR spectrum of compound **H2** in $CDCl_3$

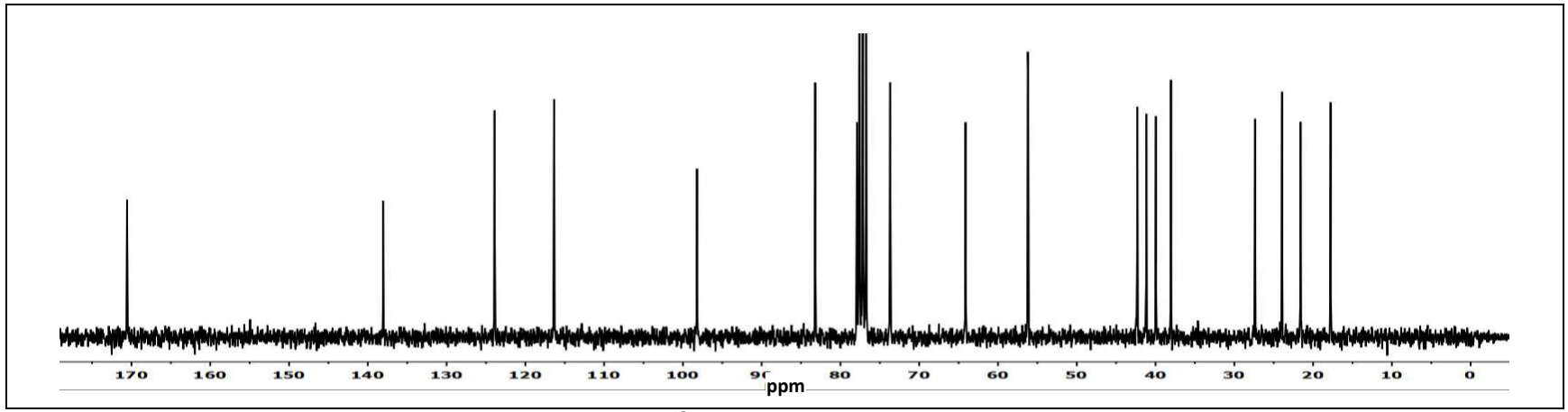

Figure 11 The 75 MHz ^{13}C NMR spectrum of compound **H2** in $CDCl_3$

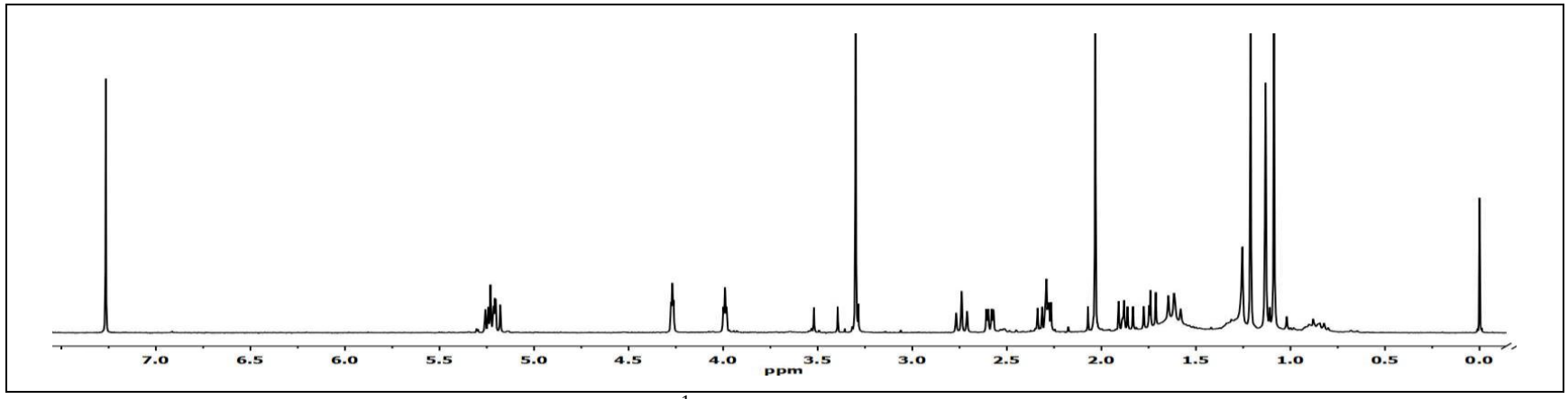

Figure 12 The 300 MHz ^{1}H NMR spectrum of compound **H7** in $CDCl_3$

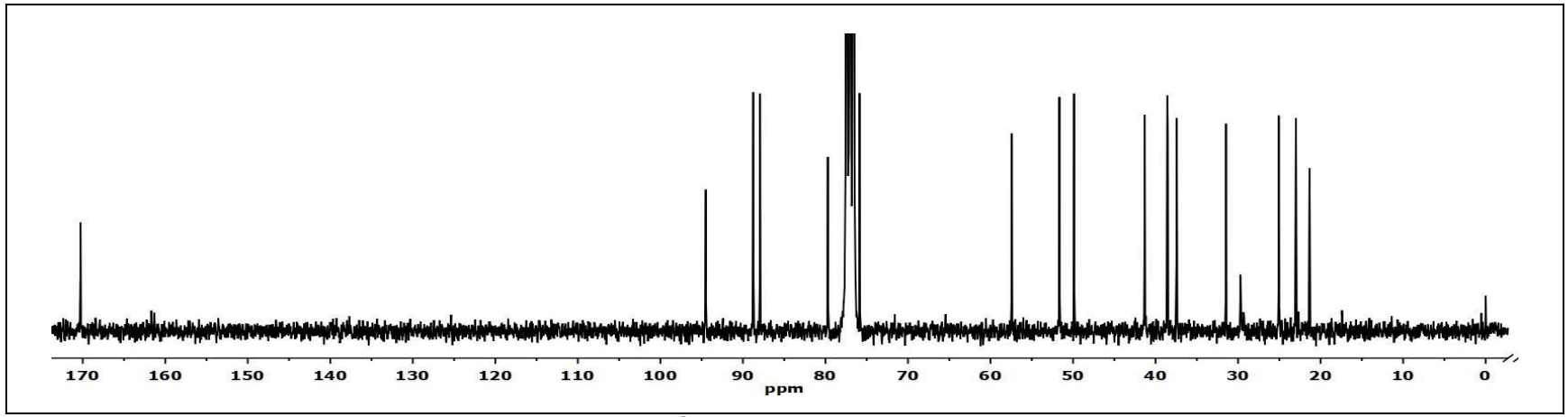

Figure 13 The 75 MHz ^{13}C NMR spectrum of compound **H7** in $CDCl_3$

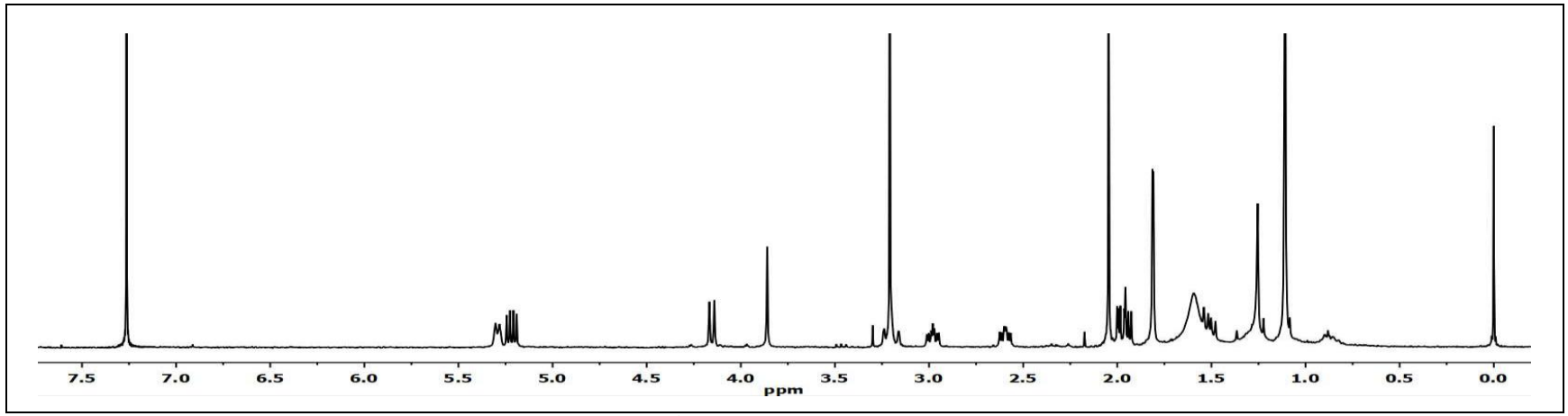

Figure 14 The 300 MHz ^{1}H NMR spectrum of compound **H11** in $CDCl_3$

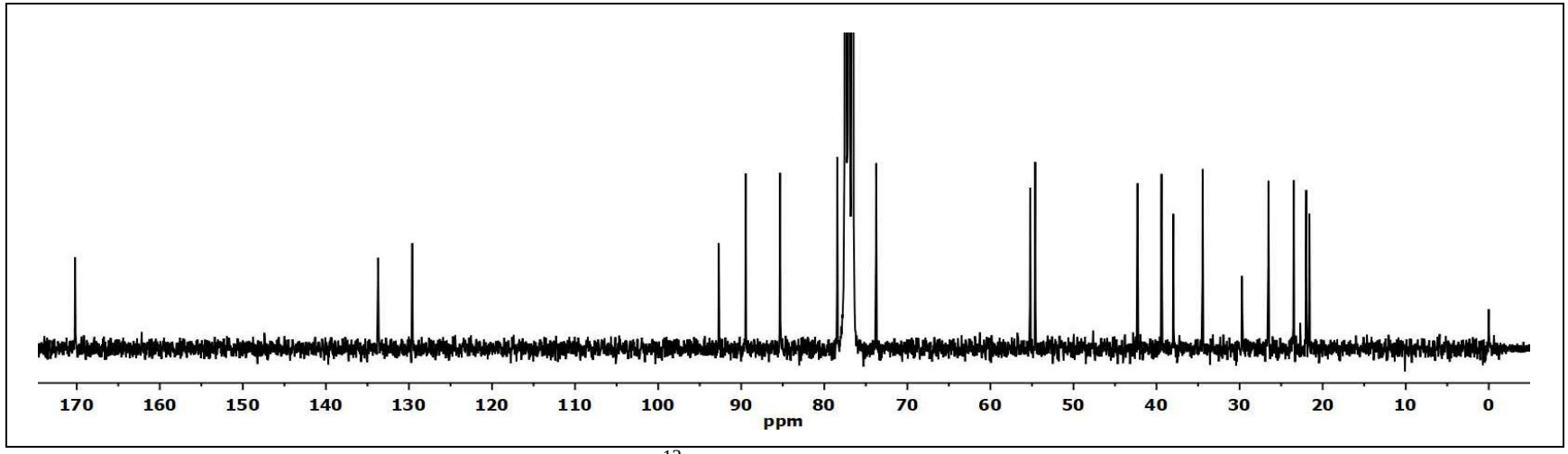

Figure 15 The 75 MHz ^{13}C NMR spectrum of compound **H11** in $CDCl_3$

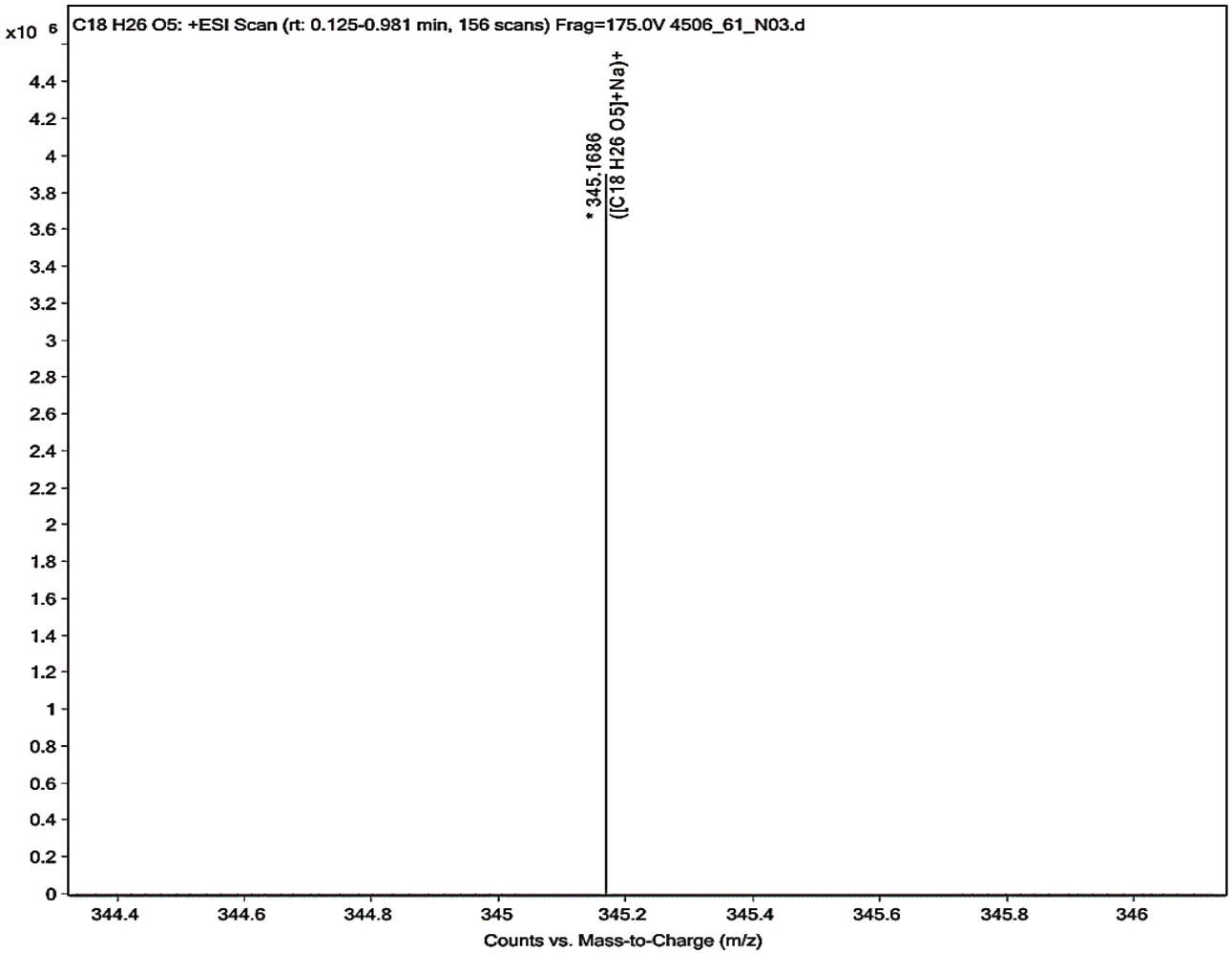

Figure 16 The HRESIMS of compound **H10**

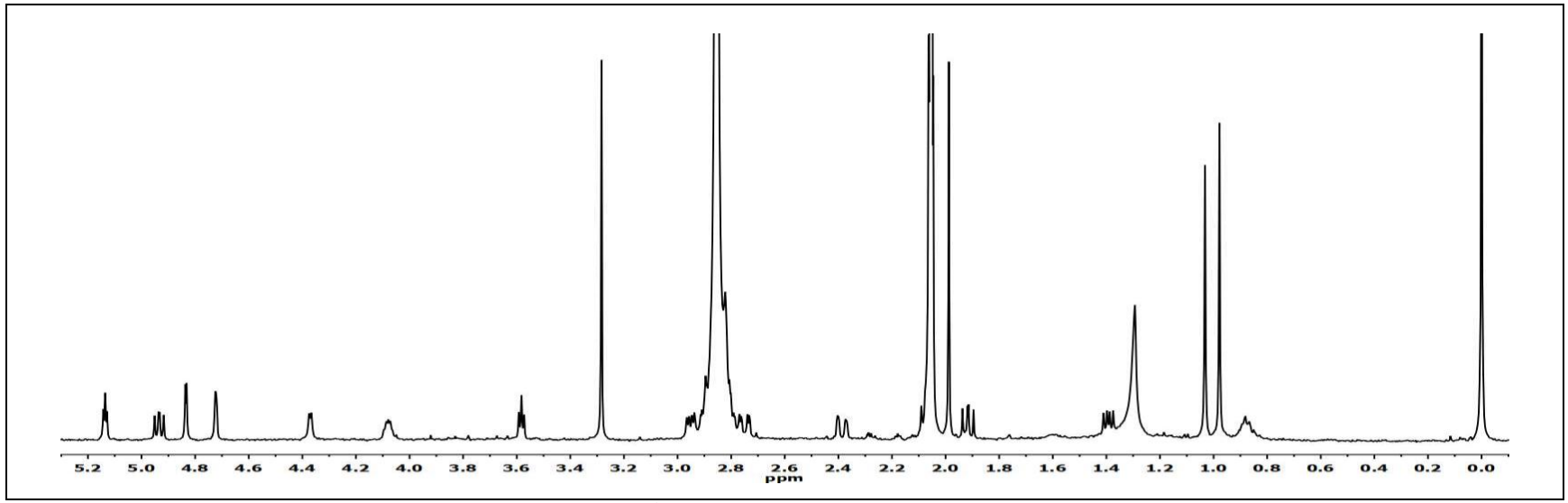

Figure 17 The 500 MHz ^{1}H NMR spectrum of compound **H10** in acetone-d_6

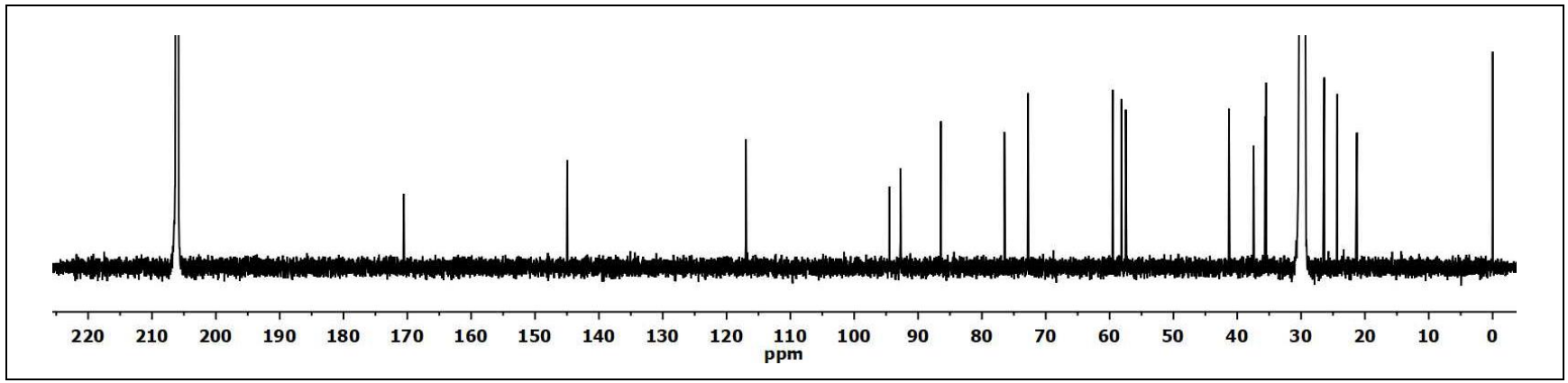

Figure 18 The 125 MHz ^{13}C NMR spectrum of compound **H10** in acetone-d_6

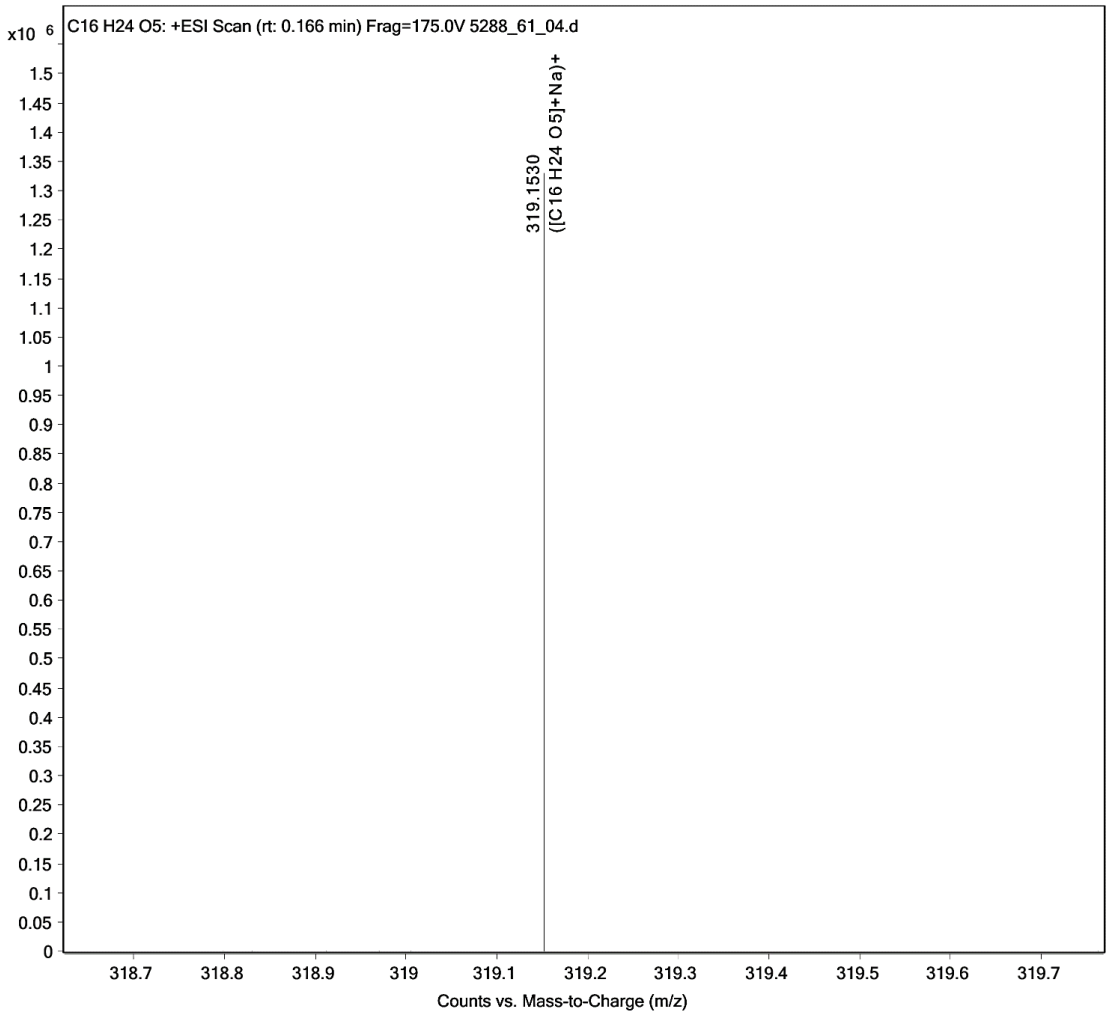

Figure 19 The HRESIMS of compound **H4**

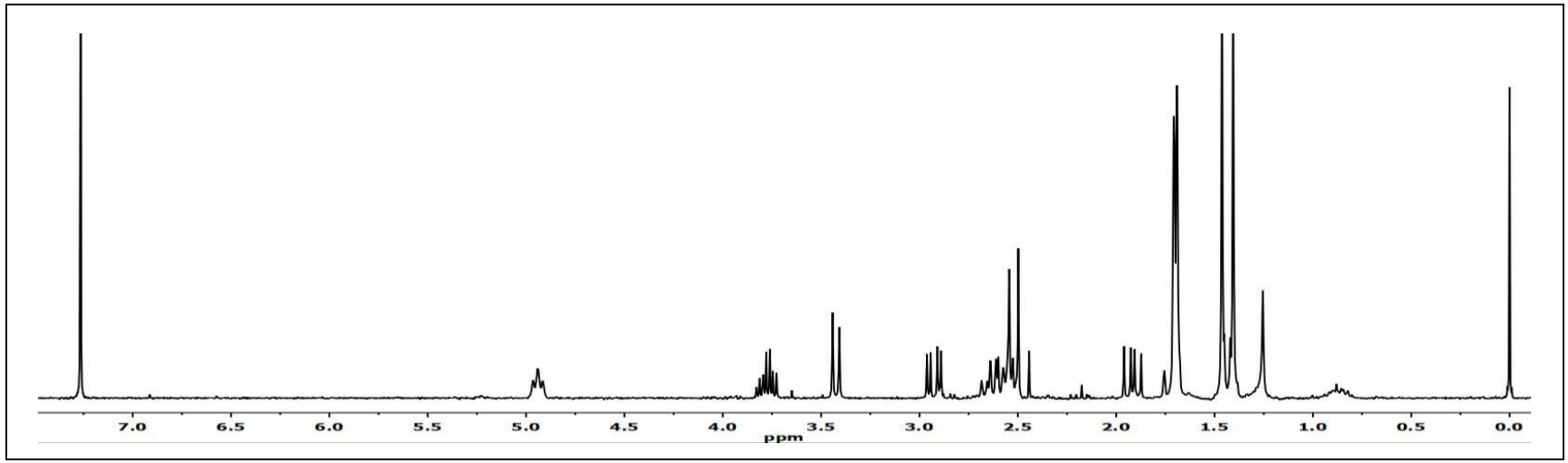

Figure 20 The 300 MHz ^{1}H NMR spectrum of compound **H4** in $CDCl_3$

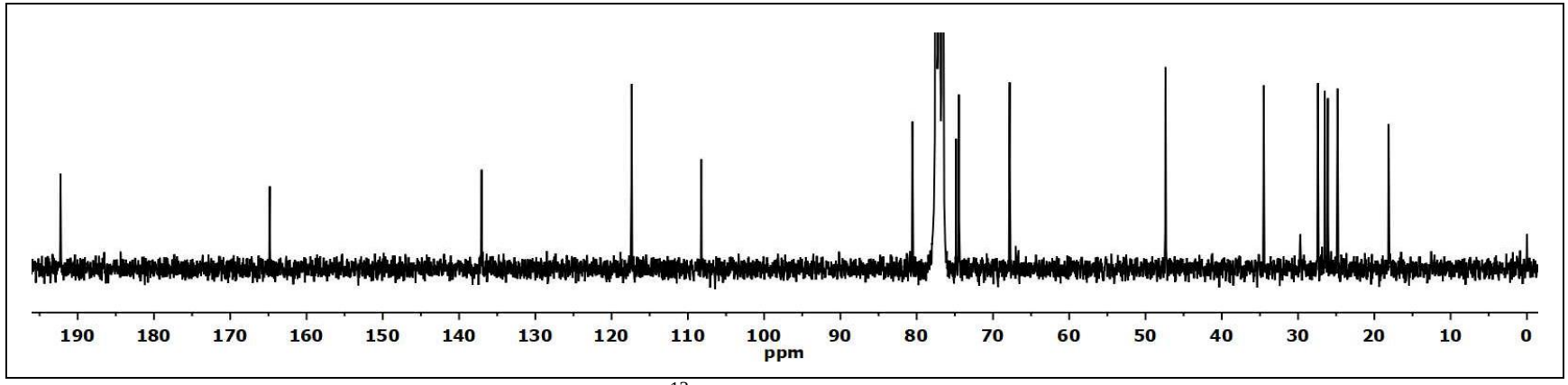

Figure 21 The 75 MHz ^{13}C NMR spectrum of compound **H4** in $CDCl_3$

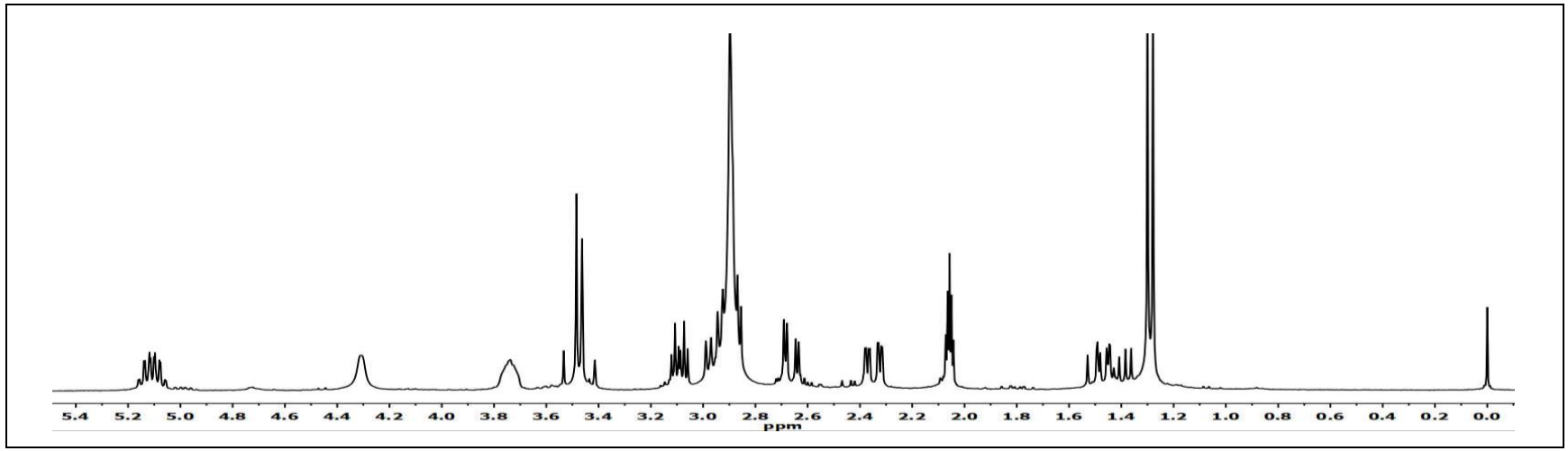

Figure 22 The 300 MHz ^{1}H NMR spectrum of compound **H8** in acetone-d_6

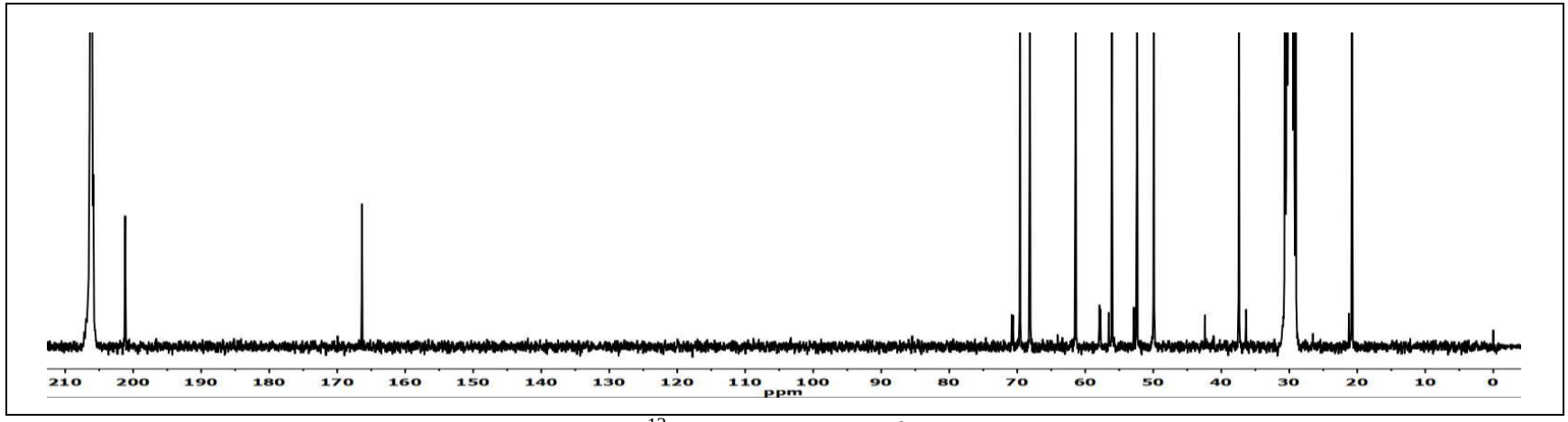

Figure 23 The 75 MHz ^{13}C NMR spectrum of compound **H8** in acetone-d_6

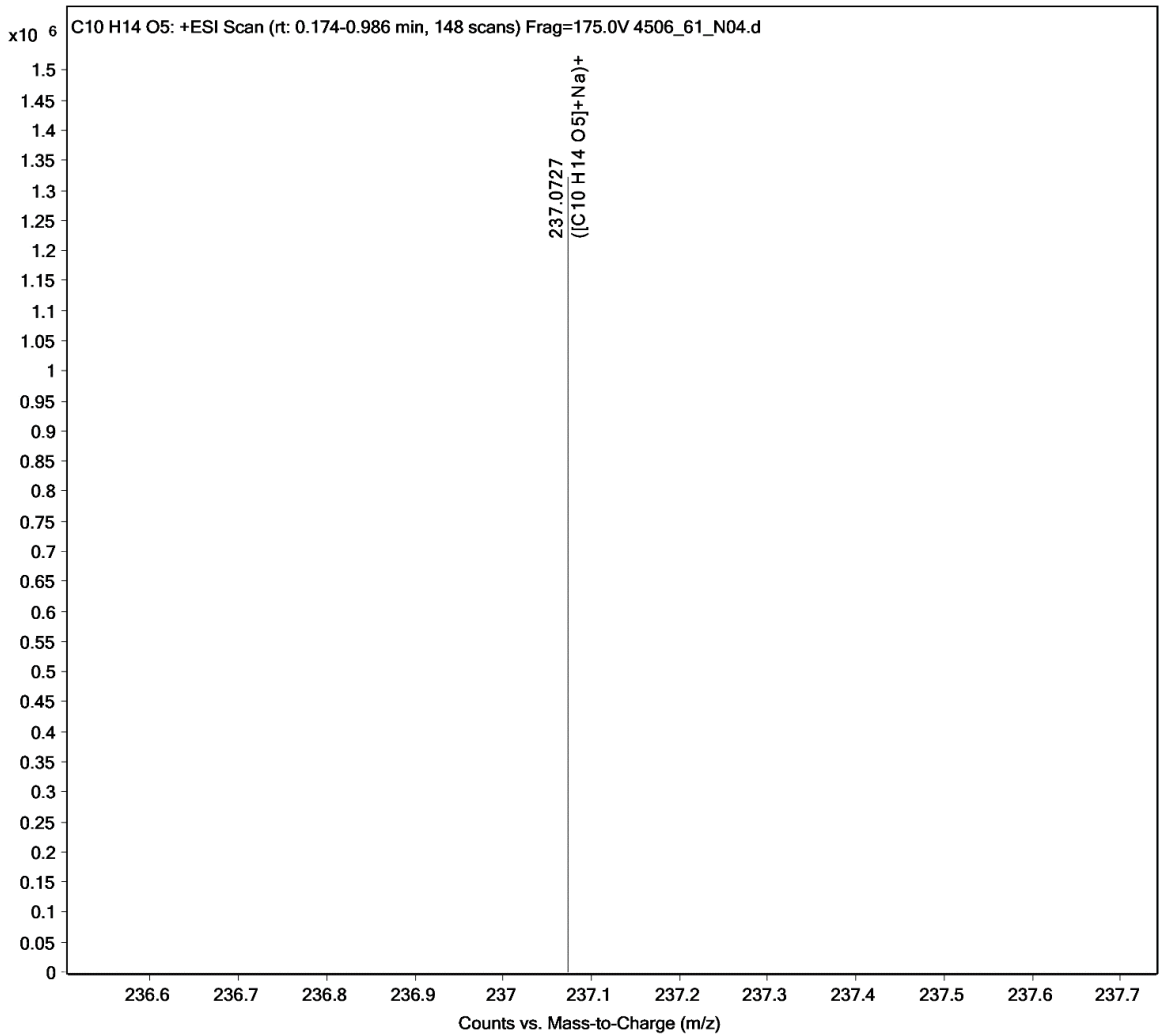

Figure 24 The HRESIMS of compound **H12**

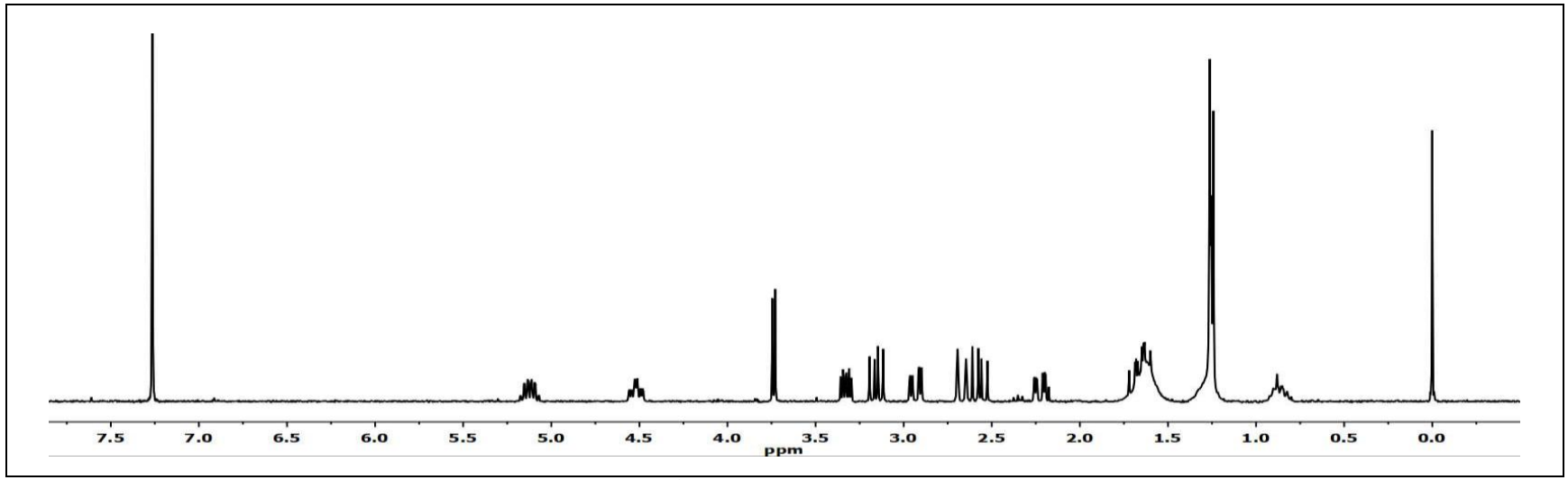

Figure 25 The 300 MHz ^{1}H NMR spectrum of compound **H12** in $CDCl_3$

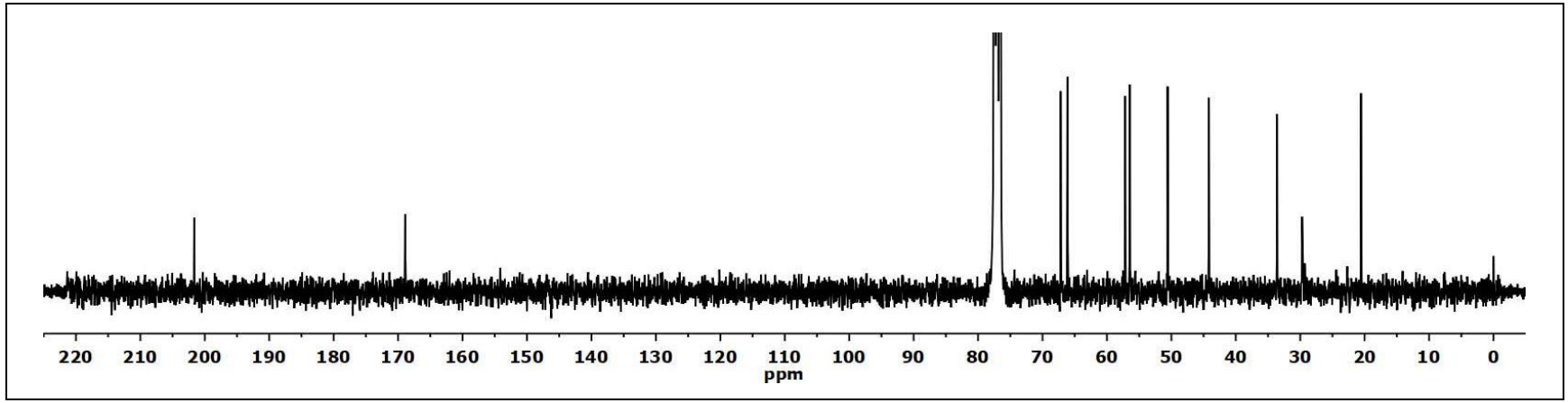

Figure 26 The 75 MHz ^{13}C NMR spectrum of compound **H12** in $CDCl_3$

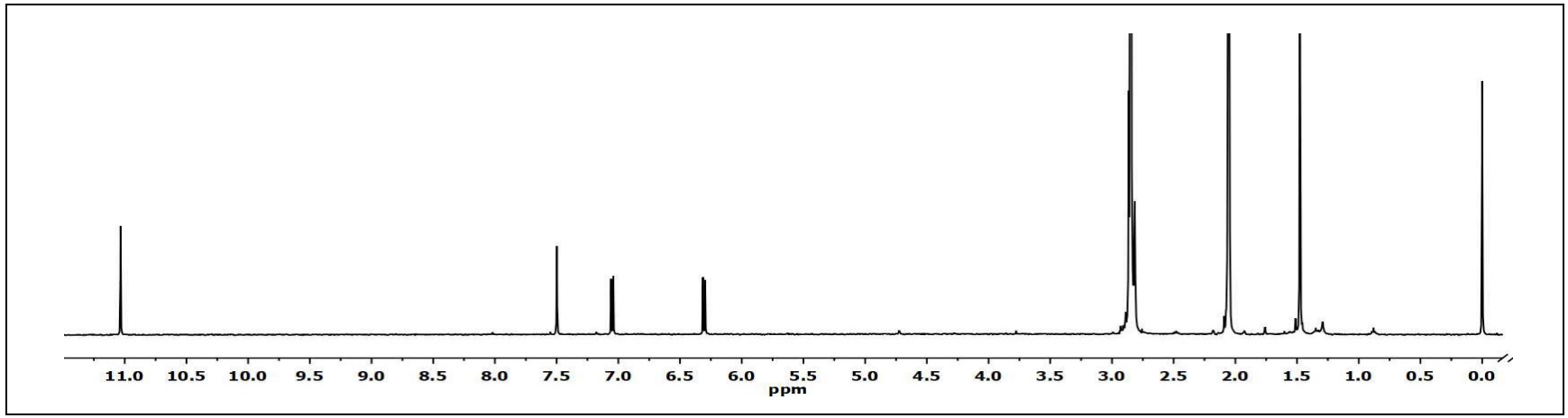

Figure 27 The 500 MHz ^{1}H NMR spectrum of compound **H6** in acetone-d_6

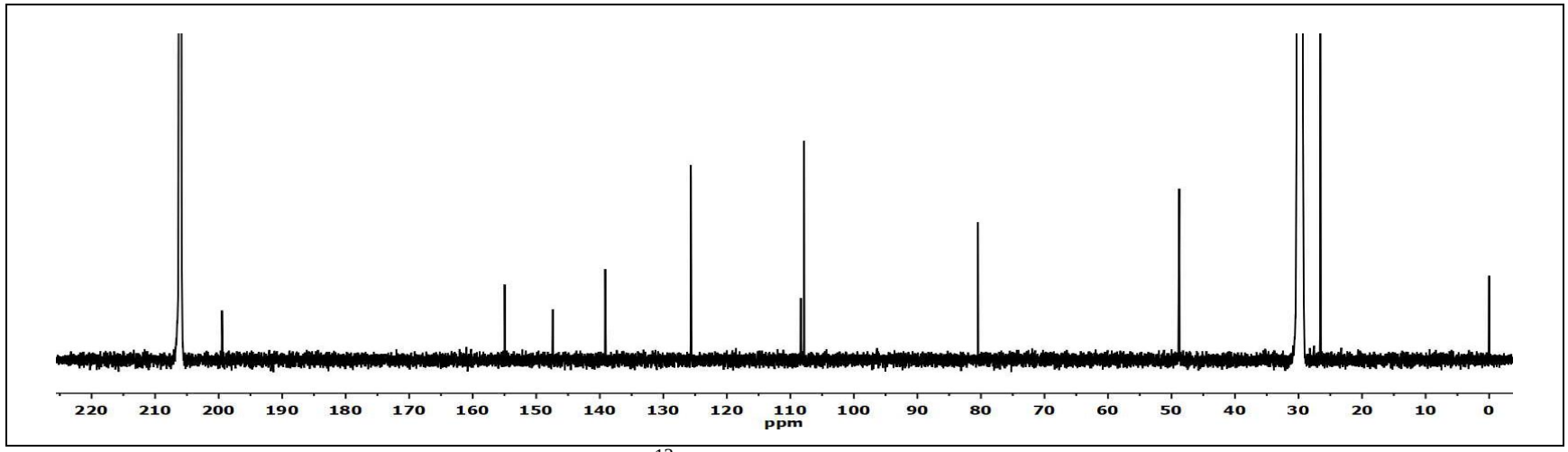

Figure 28 The 125 MHz ^{13}C NMR spectrum of compound **H6** in acetone-d_6

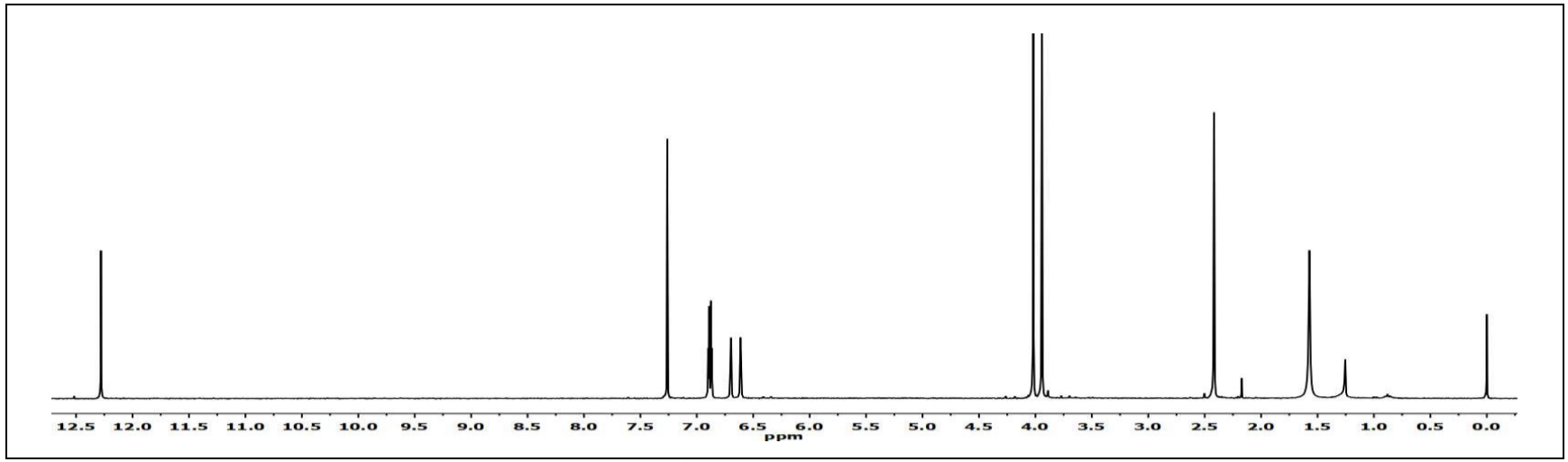

Figure 29 The 300 MHz ^{1}H NMR spectrum of compound **H5** in $CDCl_3$

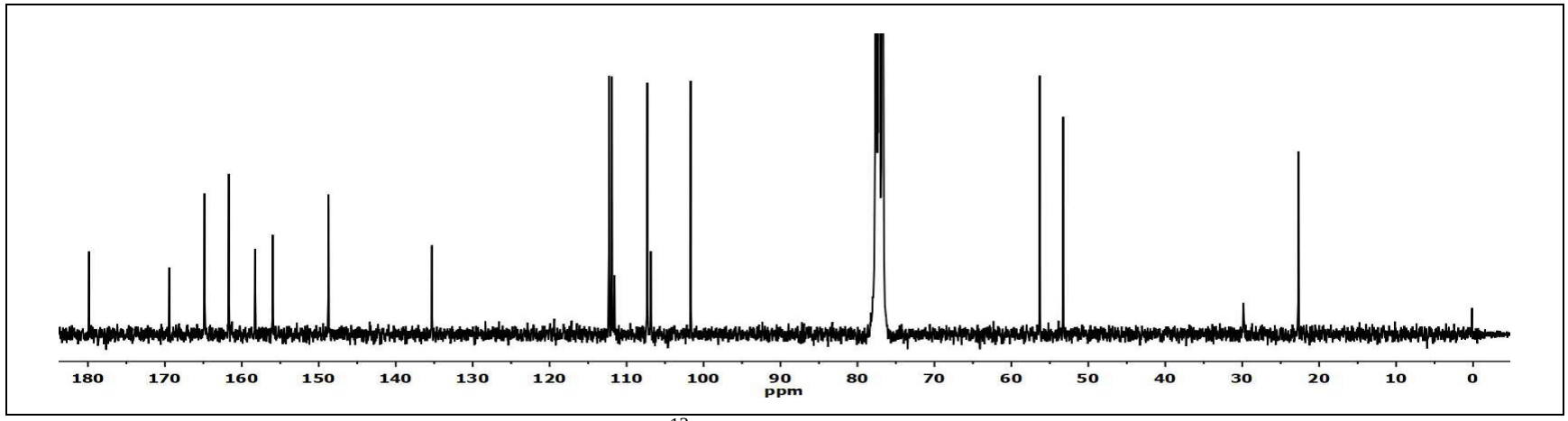

Figure 30 The 75 MHz ^{13}C NMR spectrum of compound **H5** in $CDCl_3$

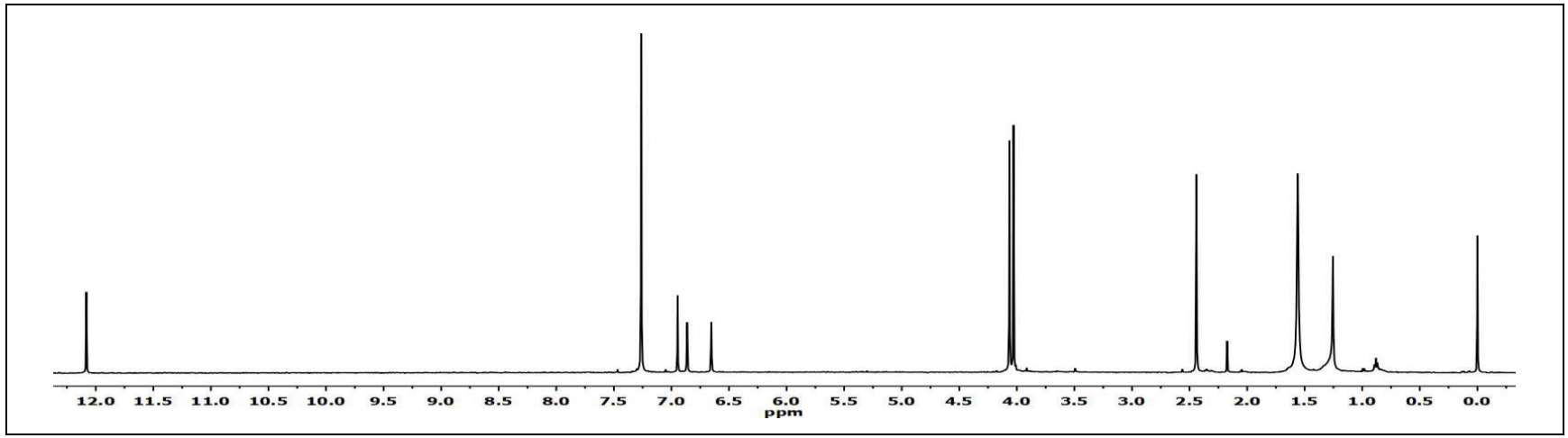

Figure 31 The 500 MHz ^{1}H NMR spectrum of compound **H13** in $CDCl_3$

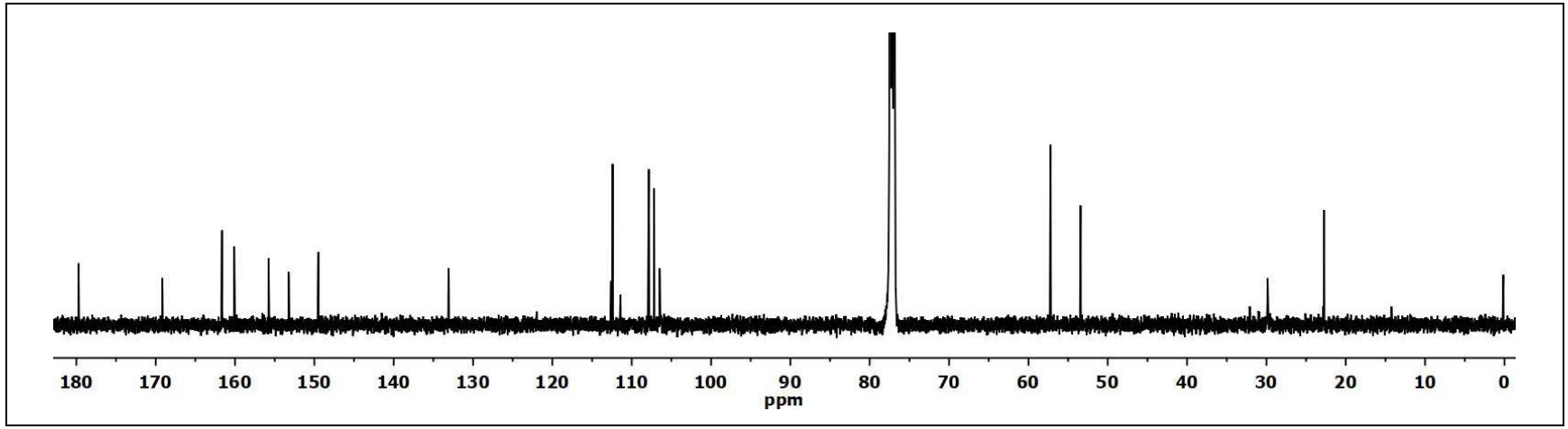

Figure 32 The 125 MHz ^{13}C NMR spectrum of compound **H13** in $CDCl_3$

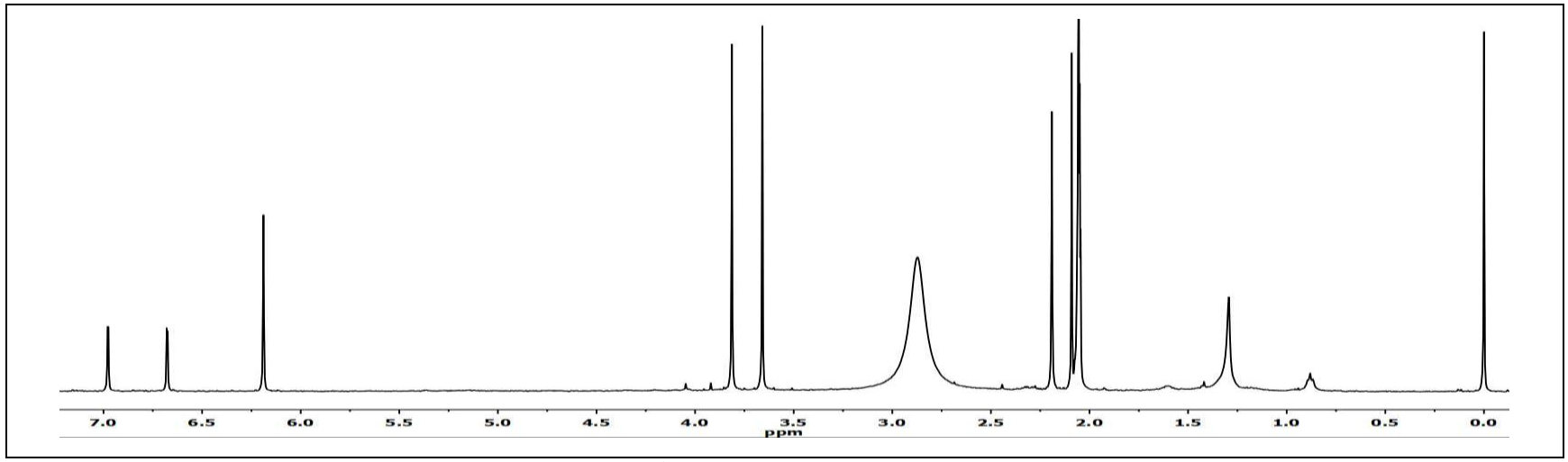

Figure 33 The 500 MHz ^{1}H NMR spectrum of compound **H9** in acetone-d_6

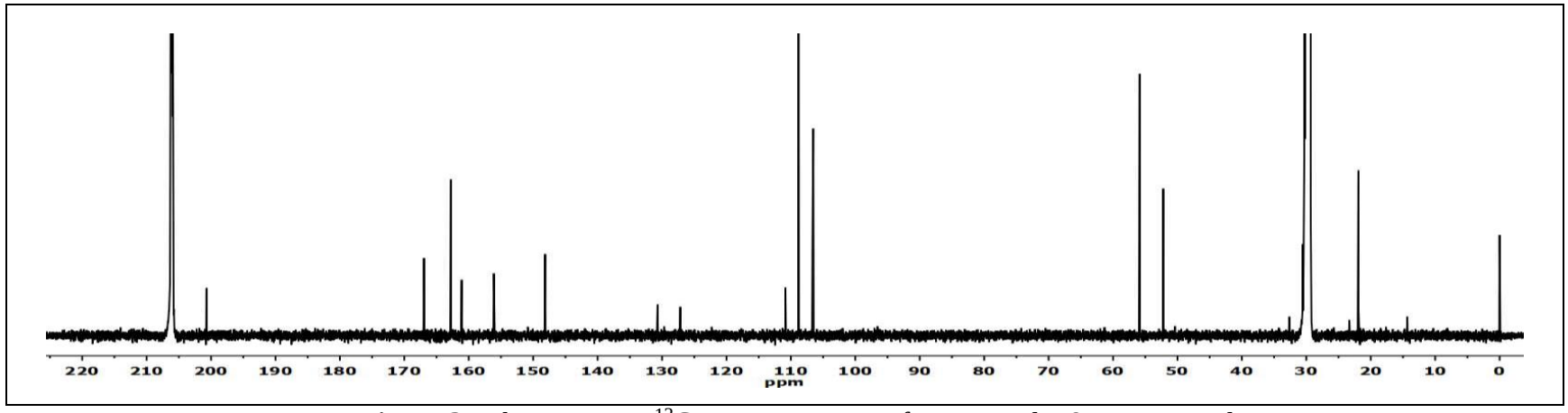

Figure 34 The 125 MHz ^{13}C NMR spectrum of compound **H9** in acetone-d_6

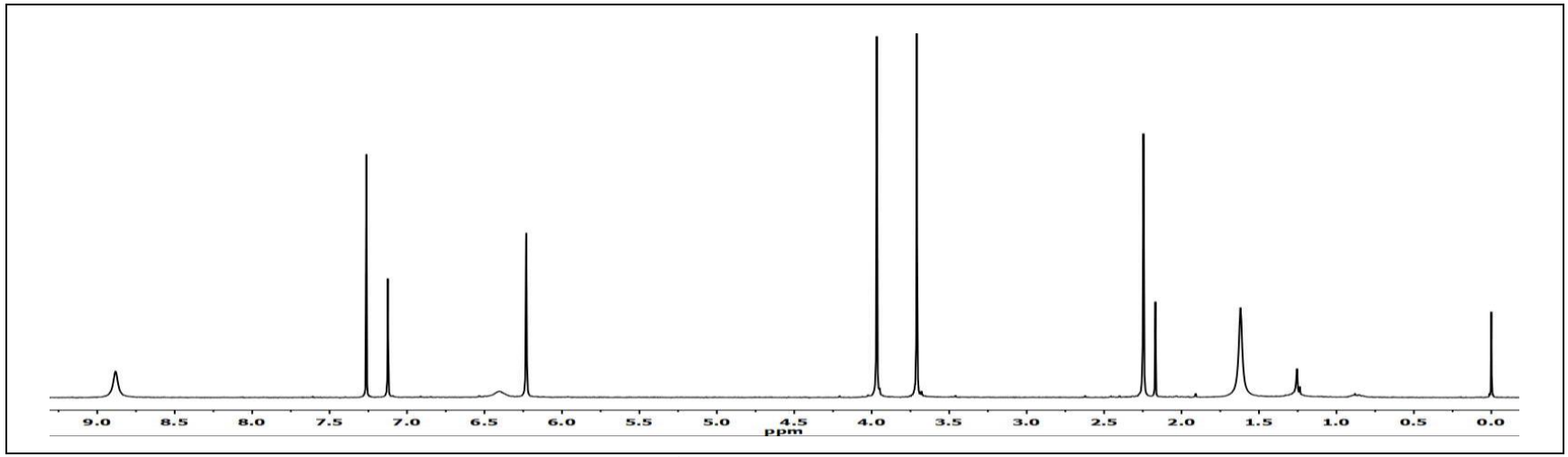

Figure 35 The 300 MHz ^{1}H NMR spectrum of compound **H14** in $CDCl_3$

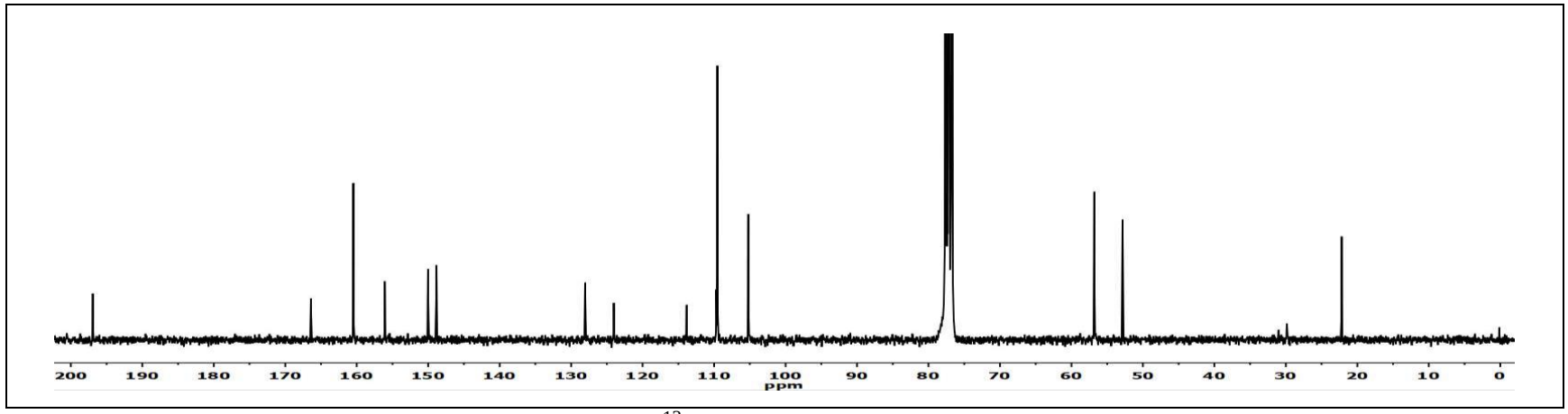

Figure 36 The 75 MHz ^{13}C NMR spectrum of compound **H14** in $CDCl_3$

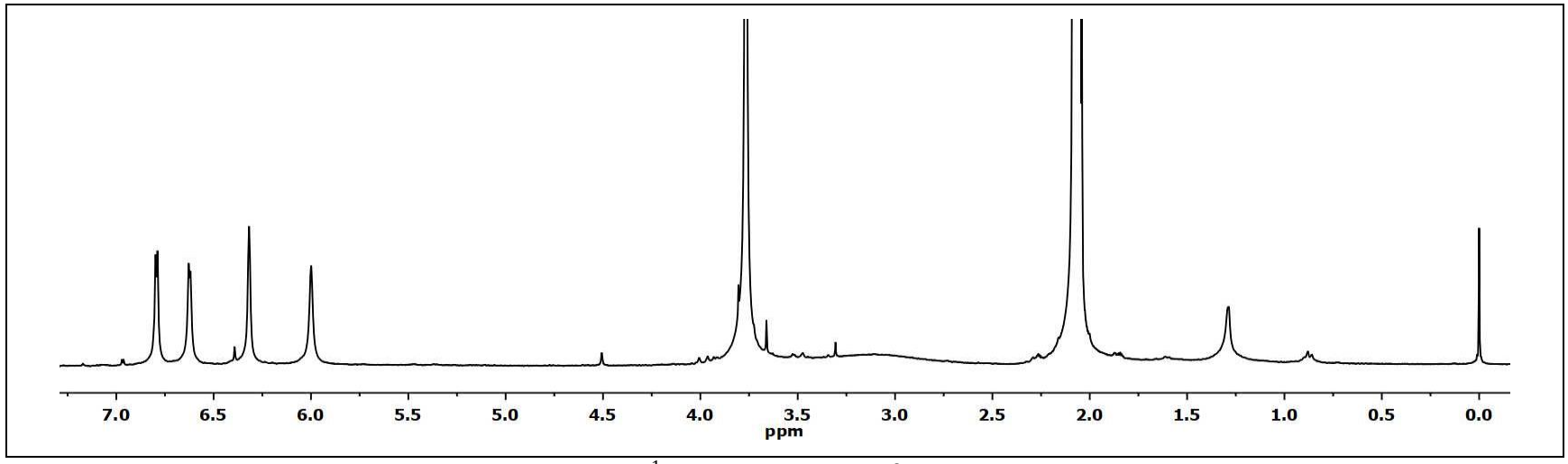

Figure 37 The 300 MHz ^{1}H NMR spectrum of compound **H15** in acetone-d_6

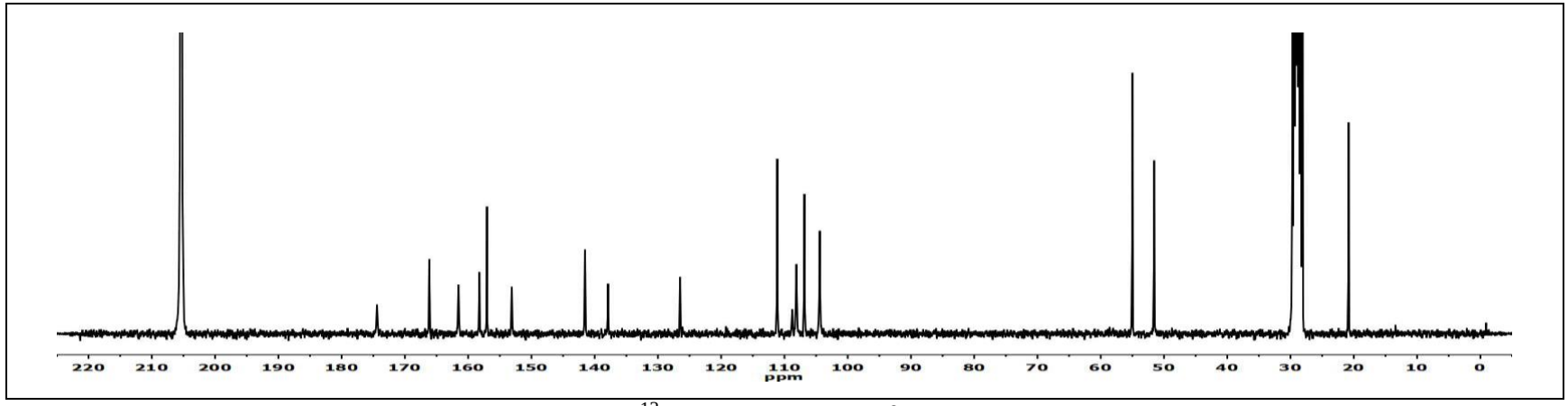

Figure 38 The 75 MHz ^{13}C NMR spectrum of compound **H15** in acetone-d_6

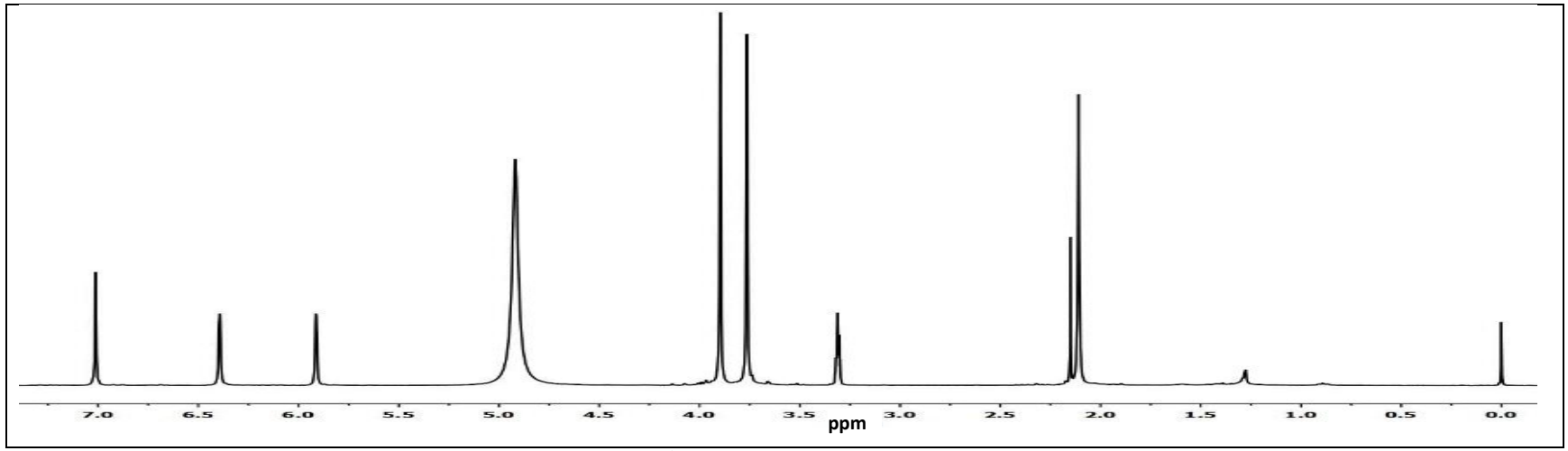

Figure 39 The 300 MHz ^{1}H NMR spectrum of compound **H16** in CD_3OD

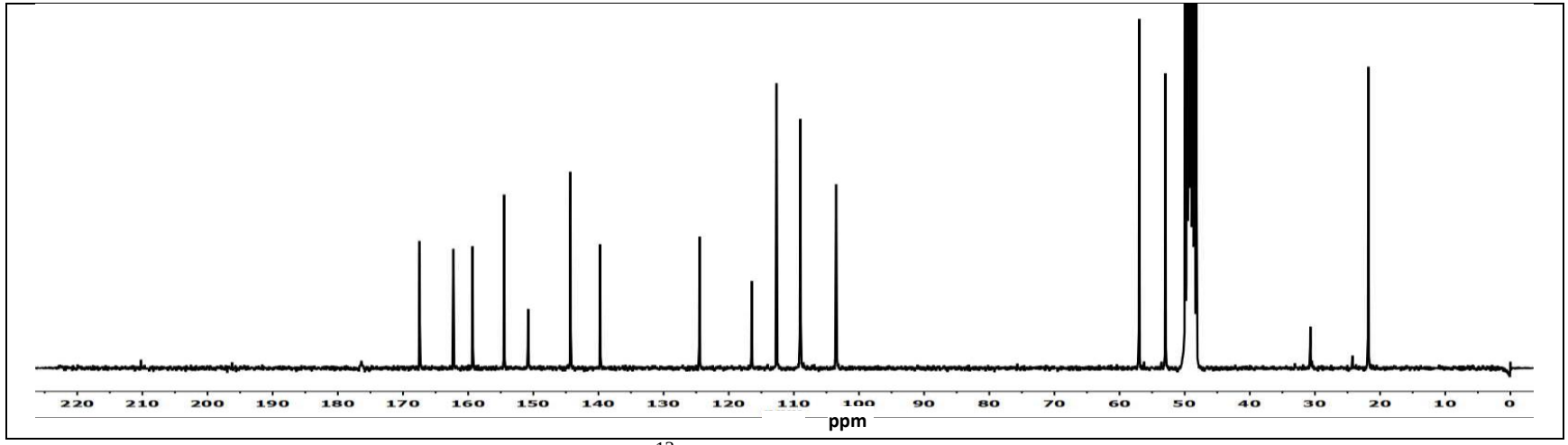

Figure 40 The 75 MHz ^{13}C NMR spectrum of compound **H16** in CD_3OD

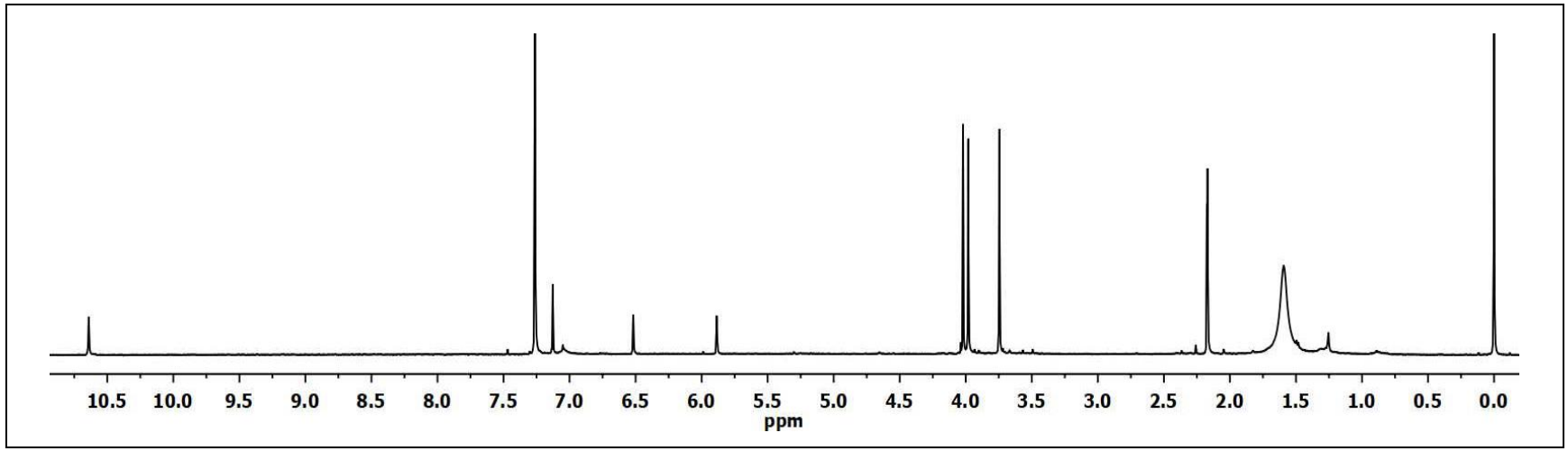

Figure 41 The 500 MHz ^{1}H NMR spectrum of compound **H17** in $CDCl_3$

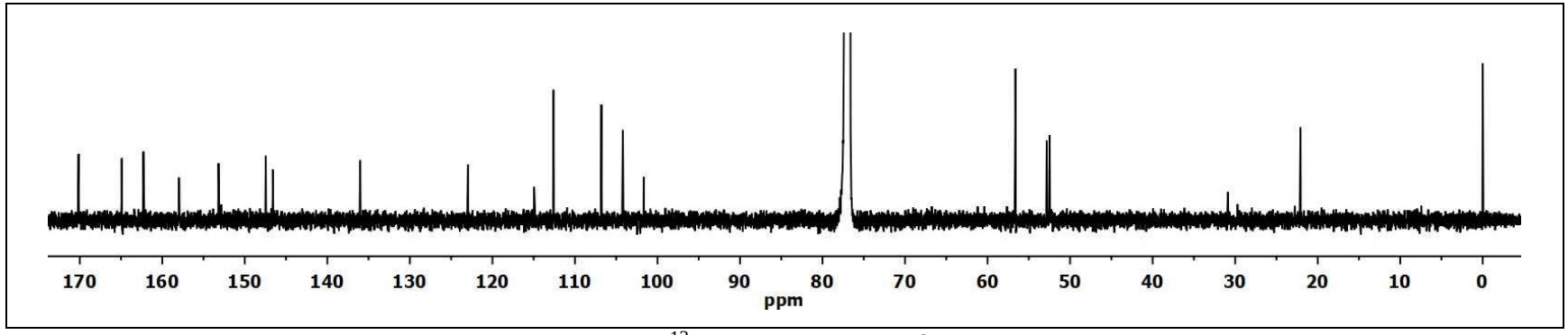

Figure 42 The 125 MHz ^{13}C NMR spectrum of compound **H17** in $CDCl_3$

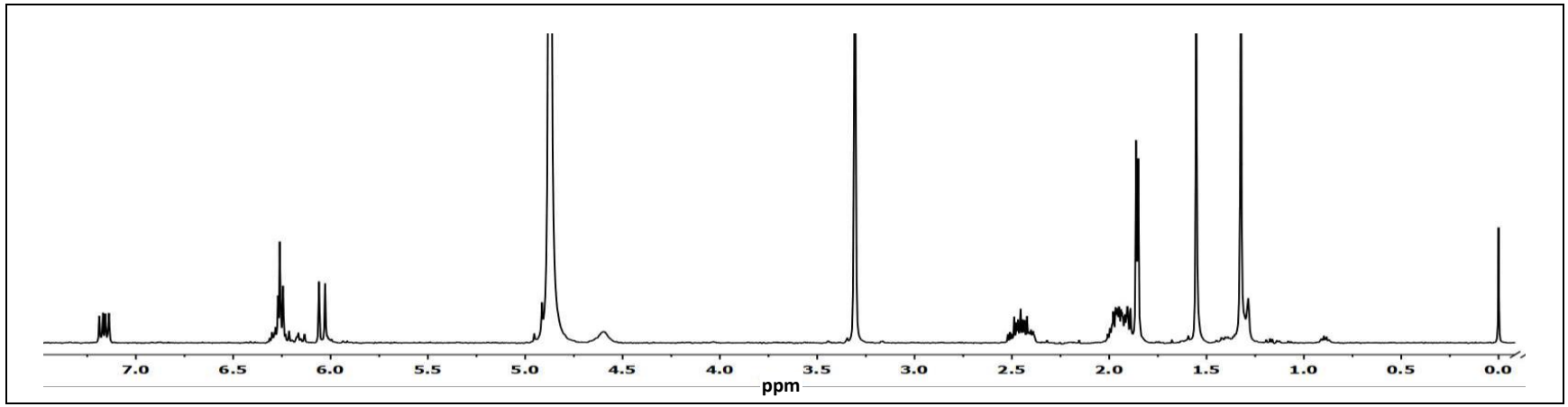

Figure 43 The 500 MHz ^{1}H NMR spectrum of compound **H18** in CD_3OD

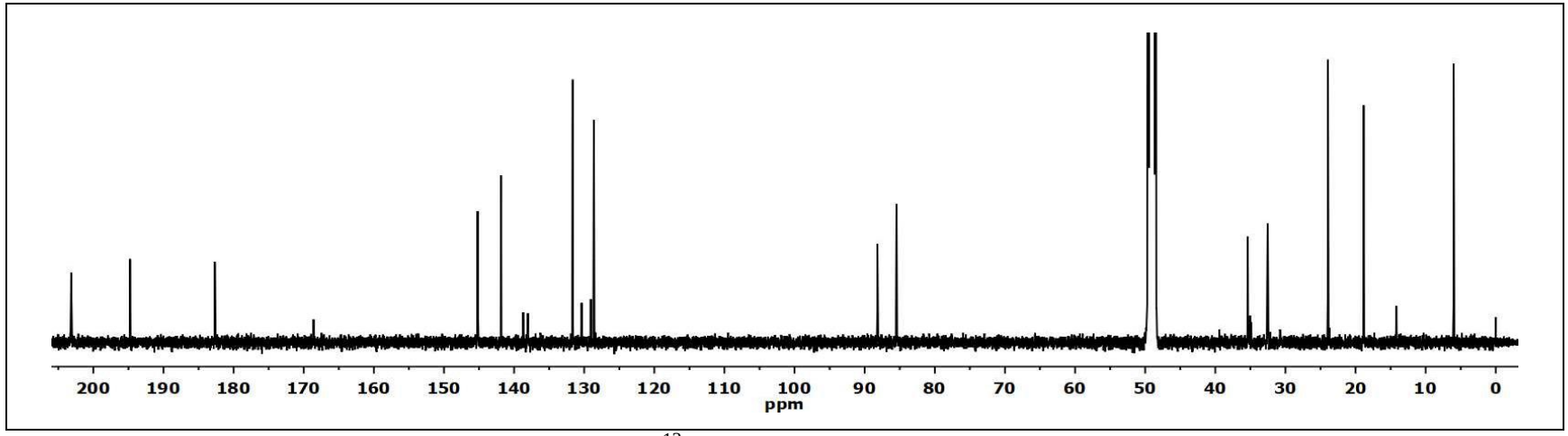

Figure 44 The 125 MHz ^{13}C NMR spectrum of compound **H18** in CD_3OD

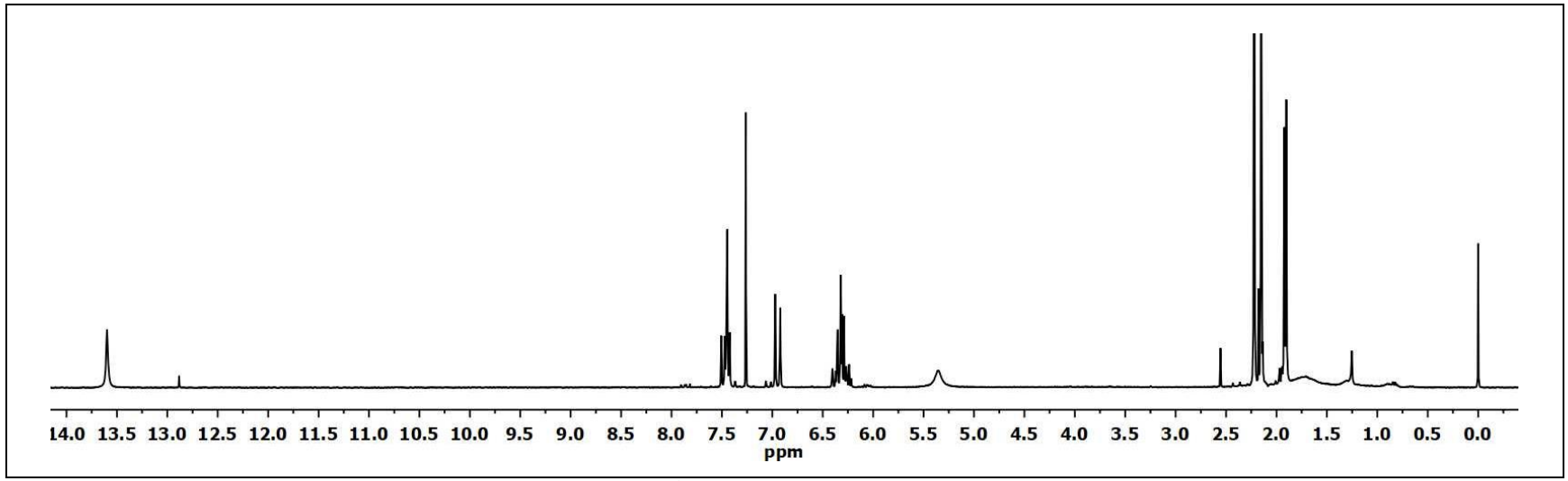

Figure 45 The 300 MHz ^{1}H NMR spectrum of compound **H19** in $CDCl_3$

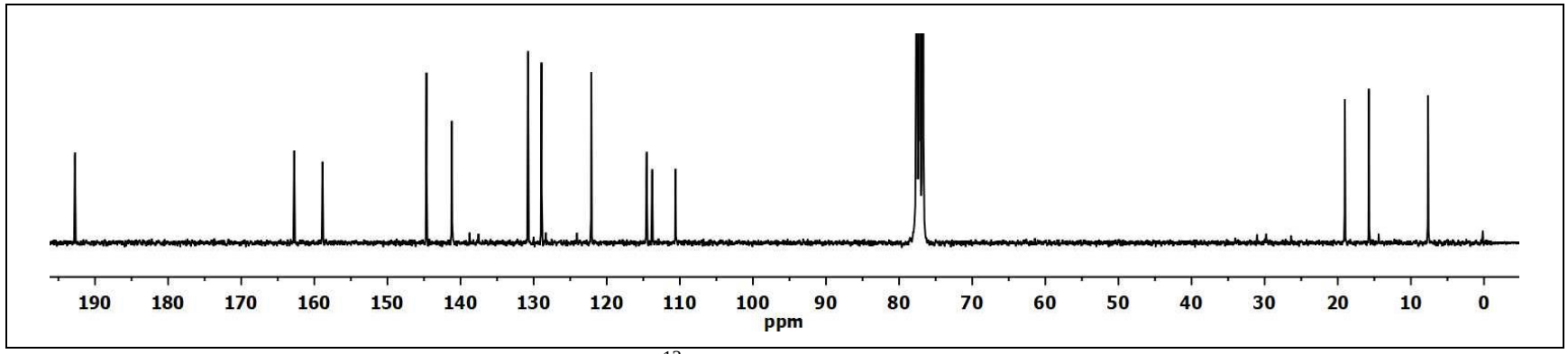

Figure 46 The 75 MHz ^{13}C NMR spectrum of compound **H19** in $CDCl_3$

VITAE

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1. The Higher Education Research Promotion and the Thailand's Education Hub for Southern Region of ASEAN Countries Project Office of the Higher Education Commission.
2. The Center of Excellence for Innovation in Chemistry (PERCH-CIC) for partial support.

List of Publication and Proceedings

Putra, H., Rukachaisirikul, V., Saithong, S., Phongpaichit, S., Preedanon, S., Sakayaroj, J., Hadsadee, S., Jungsuttiwong, S. Caryophyllene sesquiterpenes, chromones and 10-membered macrolides from the marine-derived fungus *Pseudopestalotiopsis* sp. PSU-AMF45 (under review).

Putra, H. N., Phongpaichit, S., Sakayaroj, J., Rukachaisirikul, V., Benzophenone and diphenyl ether derivatives from the marine-derived fungus *Pseudopestalotiopsis* sp. PSU-AMF45. Pure and Applied Chemistry International Conference 2019. Bangkok International trade and exhibition (BITEC), Bangkok, Thailand, 7-8 February 2019 (poster presentation).