Flavonoid Glycosides from *Dendrophthoe pentandra* L. and Isoflavonoid Glycosides from *Derris scandens* Benth

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Thesis Title	Flavonoid Glycosides from *Dendrophthoe pentandra* L. and Isoflavonoid Glycosides from *Derris scandens* Benth
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Major Program	Organic Chemistry
Academic Year	2000

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The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirement for the Master of Science degree in Organic Chemistry.

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(Assoc. Prof. Dr. Piti Trisdikoon)
Dean of the Graduate School

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Abstract

Part I Flavonoid Glycosides from Dendrophthoe pentandra L.

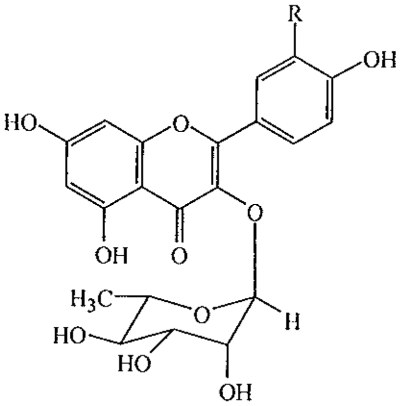
Chemical investigation of constituents of *Dendrophthoe pentandra* was divided into two parts. The first part involved isolation, purification and structure elucidation of compounds from its leaves while the second part dealed with the compounds isolated from twigs. Upon chromatographic separation, the crude extract of leaves yielded three flavone rhamnosides : quercetin 3-*O*-*α*-rhamnoside (**DW1**), kaempferol 3-*O*-*α*-rhamnoside (**DW2**) and isorhamnetin 3-*O*-*α*-rhamnoside (**DW3**) while the crude extract of twigs afforded three known compounds : one steriod [sitosterol (**DT1**)] and two aromatic compounds [4-hydroxy-3-methoxybenzaldehyde (**DT5**) and methyl 4-hydroxy-3-methoxybenzoate (**DT6**)], one new oleanolic acid derivative [oleanoyl palmitate (**DT4**)] and two unidentified compounds [**DT2** and **DT3**].

Part II Isoflavonoid Glycosides from Derris scandens Benth

Phytochemical studies of the crude *n*-butanol extract from stems of *Derris scandens* led to isolation of two known benzoic acid derivatives [4-hydroxy-3-methoxybenzoic acid (**YS9**) and 4-hydroxy-3,5-dimethoxybenzoic acid (**YS12**)], one known isoflavone [7,8-dihydroxy-4′-methoxyisoflavone (**YS15**)], four glucosyl isoflavones : one new glucosyl isoflavone [7-hydroxy-4′-methoxyisoflavone 8-*O*-*β*-glucopyranoside (**YS8**)] and three known ones [4′,7-dimethoxyisoflavone 8-*O*-*β*-glucopyranoside (**YS1**), formonetin 7-*O*-*β*-glucopyranoside (**YS13**) and 4′,8-dimethoxyisoflavone 7-*O*-*β*-glucopyranoside (**YS14**)] and eight rhamnosyl-(1→6)-glucosyl isoflavones : four new compounds [4′,6-dimethoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS6**), 5-hydroxy-4′,6-dimethoxy-

isoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS7**), 8-hydroxy-4′-methoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS10**) and 4′,7-dimethoxyisoflavone 8-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS11**)] and four known ones [4′,8-dimethoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS2**), 4′-methoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS3**), 4′,5-dihydroxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS4**) and 4′-hydroxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS5**)]. Most compounds were isolated and identified as their corresponding acetate derivatives except for **YS1**, **YS2**, **YS4**, **YS9** and **YS12**.

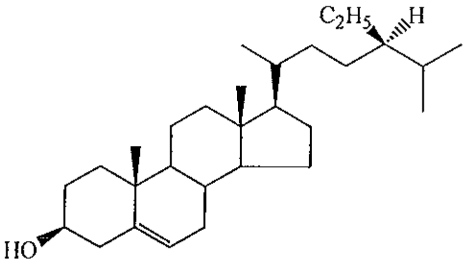
All structures were determined using 1D and 2D NMR spectroscopic data.



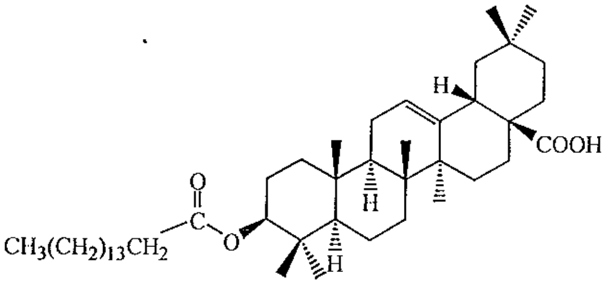
DW1 : $R = OH$

DW2 : $R = H$

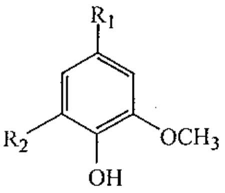
DW3 : $R = OCH_3$



DT1



DT4

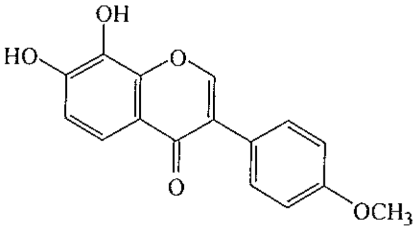


DT5 : $R_1 = CHO, R_2 = H$

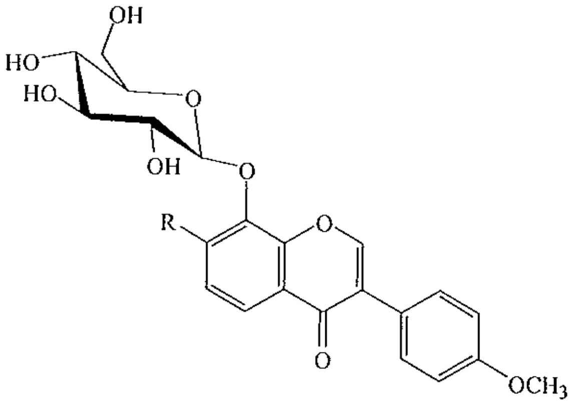
DT6 : $R_1 = CO_2CH_3, R_2 = H$

YS9 : $R_1 = CO_2H, R_2 = H$

YS12: $R_1 = CO_2H, R_2 = OCH_3$

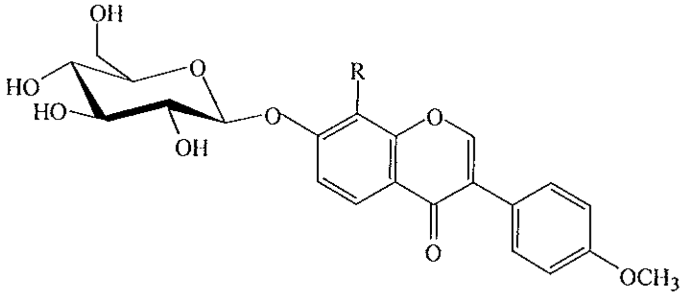


YS15



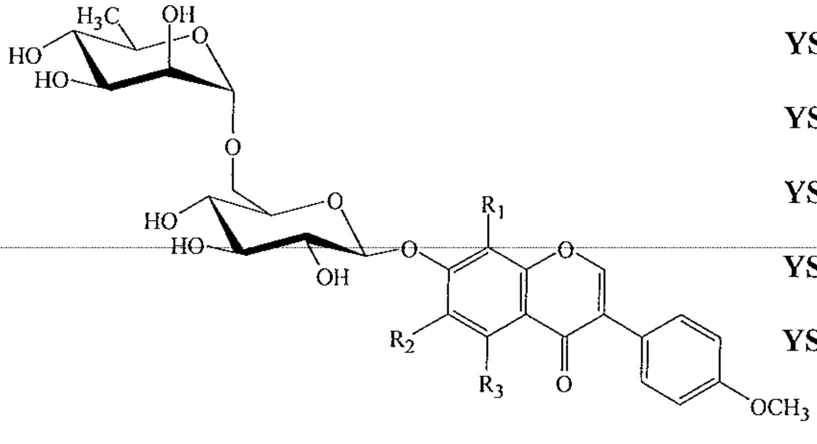
YS1 : R = OCH_3

YS8 : R = OH



YS13 : R = H

YS14 : R = OCH_3



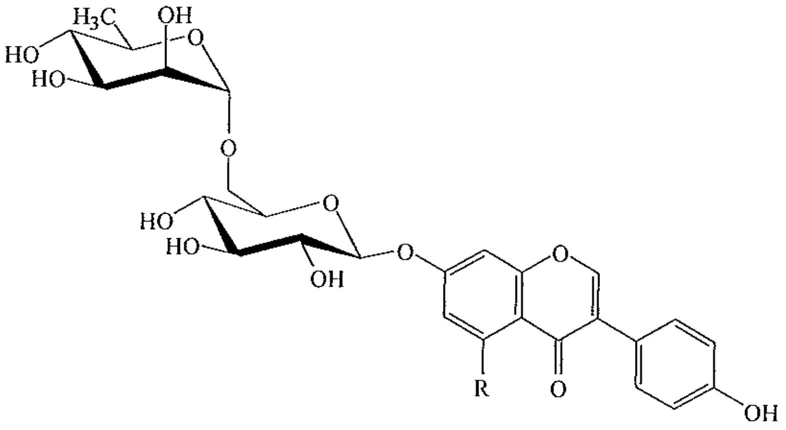
YS2 : $R_1 = OCH_3$, $R_2 = R_3 = H$

YS3 : $R_1 = R_2 = R_3 = H$

YS6 : $R_1 = R_3 = H$, $R_2 = OCH_3$

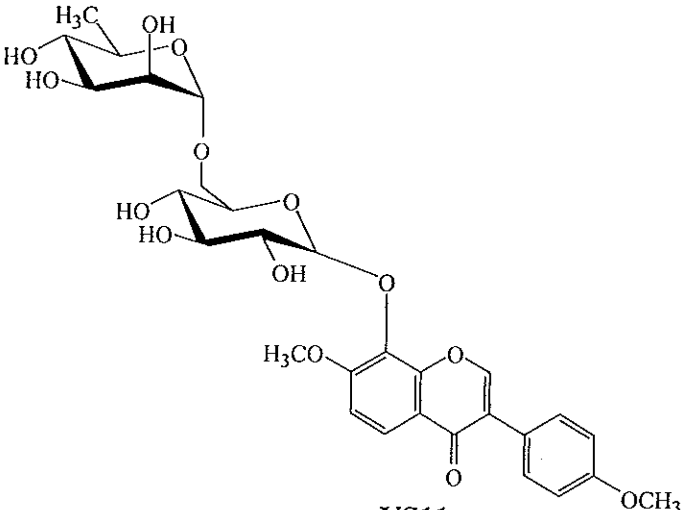
YS7 : $R_1 = H$, $R_2 = OCH_3$, $R_3 = OH$

YS10: $R_1 = OH$, $R_2 = R_3 = H$



YS4 : R = OH

YS5 : R = H



YS11

ชื่อวิทยานิพนธ์	ฟลาโวนอยด์ ไกลโคซายน์ จากกาฝากชนิด *Dendrophthoe pentandra* L. และไอโซฟลาโวนอยด์ ไกลโคซายน์ จากเถาวัลย์เปรียง (*Derris scandens* Benth)
ผู้เขียน	นางสาวเยาวภา สุขพรมา
สาขาวิชา	เคมีอินทรีย์
ปีการศึกษา	2543

บทคัดย่อ

ตอน 1 ฟลาโวนอยด์ไกลโคซายน์ จากกาฝากชนิด Dendrophthoe pentandra L.

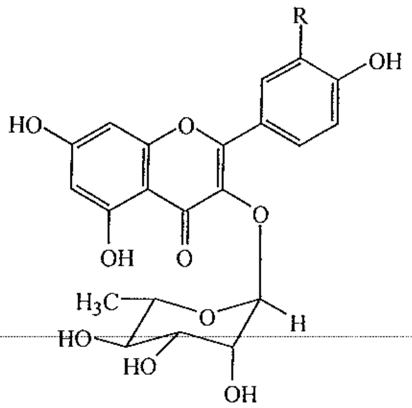
การศึกษานี้แบ่งเป็น 2 ส่วน คือ ส่วนแรกศึกษาส่วนสกัดหยาบจากใบของกาฝากชนิด *Dendrophthoe pentandra* L. เมื่อทำการแยกและทำให้บริสุทธิ์ด้วยวิธีทางโครมาโทกราฟี ได้สารประเภทฟลาโวน แรมโนซายน์ (flavone rhamnoside) จำนวน 3 สาร [quercetin 3-*O*-α-rhamnoside (**DW1**) kaempferol 3-*O*-α-rhamnoside (**DW2**) และ isorhamnetin 3-*O*-α-rhamnoside (**DW3**)] และส่วนที่สองทำการศึกษาส่วนสกัดหยาบจากกิ่งของกาฝากชนิดนี้ หลังจากแยกและทำให้บริสุทธิ์ด้วยวิธีทางโครมาโทกราฟี ได้สารจำนวน 6 สาร ซึ่งเป็นสารประเภทสเตียรอยด์ (steroid) จำนวน 1 สาร [sitosterol (**DT1**)] สารประเภทอะโรมาติก (aromatic compounds) จำนวน 2 สาร [4-hydroxy-3-methoxybenzaldehyde (**DT5**) และ methyl 4-hydroxy-3-methoxybenzoate (**DT6**)] สารใหม่ซึ่งเป็นอนุพันธ์ของกรดโอเลโนลิก (oleanolic acid) จำนวน 1 สาร [oleanoyl palmitate (**DT4**)] และสารประกอบที่ไม่ทราบโครงสร้างจำนวน 2 สาร [**DT2** และ **DT3**]

ตอน 2 ไอโซฟลาโวนอยด์ไกลโคซายน์ จากต้นเถาวัลย์เปรียง (Derris scandens)

ส่วนสกัดหยาบบิวทานอลของลำต้นเถาวัลย์เปรียง เมื่อทำการแยกและทำให้บริสุทธิ์ด้วยวิธีทางโครมาโทกราฟี ได้สารจำนวน 15 สาร ซึ่งเป็นสารประเภทอนุพันธ์ของกรดเบนโซอิก (benzoic acid) จำนวน 2 สาร [4-hydroxy-3-methoxybenzoic acid (**YS9**) และ 4-hydroxy-3,5-dimethoxybenzoic acid (**YS12**)] สารประเภทไอโซฟลาโวน (isoflavone) จำนวน 1 สาร [7,8-dihydroxy-4'-methoxyisoflavone (**YS15**)] สารประเภทไอโซฟลาโวน กลูโคซายน์ (isoflavone glucoside) จำนวน 4 สาร [4',7-dimethoxyisoflavone 8-*O*-β-glucopyranoside

(YS1), 7-hydroxy-4′-methoxyisoflavone 8-*O*-*β*-glucopyranoside **(YS8)**, formonetin 7-*O*-*β*-glucopyranoside **(YS13)** และ 4′,8-dimethoxyisoflavone 7-*O*-*β*-glucopyranoside **(YS14)**] โดย **YS8** จัดเป็นไอโซฟลาโวน กลูโคซายน์สารใหม่ นอกจากนี้ได้สารประเภทแรมโนซิล-(1→6)-กลูโคซิลไอโซฟลาโวน (rhamnosyl-(1→6)-glucosyl isoflavone) จำนวน 8 สาร : สารใหม่จำนวน 4 สาร [4′,6-dimethoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside **(YS6)**, 5-hydroxy-4′,6-dimethoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside **(YS7)**, 8-hydroxy-4′-methoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside **(YS10)** และ 4′,7-dimethoxyisoflavone 8-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside **(YS11)**] และสารที่ทราบโครงสร้างจำนวน 4 สาร [4′,8-dimethoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside **(YS2)**, 4′-methoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside **(YS3)**, 4′,5-dihydroxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside **(YS4)** และ 4′-hydroxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside **(YS5)**] สารบริสุทธิ์ที่แยกได้ส่วนใหญ่ศึกษาในรูปอนุพันธ์อะซิเตด (acetate derivative) ยกเว้น สาร **YS1 YS2 YS4 YS9** และ **YS12**

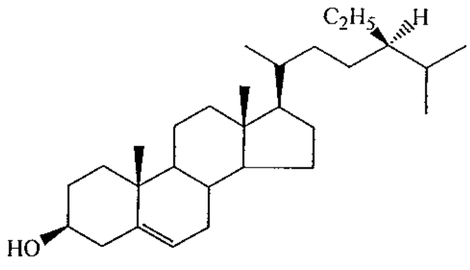
โครงสร้างสารประกอบ วิเคราะห์โดยใช้เทคนิคทางสเปกโทรสโกปี อันได้แก่ 1D และ 2D NMR สเปกโทรสโกปี



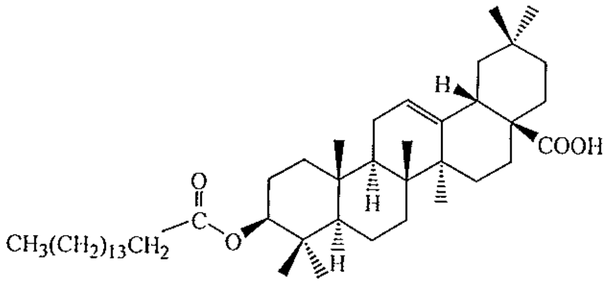
DW1 : R = OH

DW2 : R = H

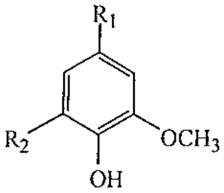
DW3 : R = OCH_3



DT1



DT4

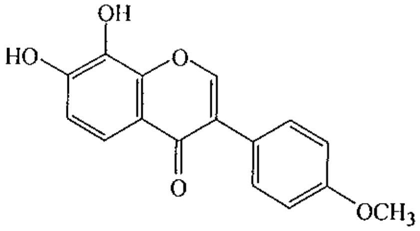


DT5 : $R_1 = CHO, R_2 = H$

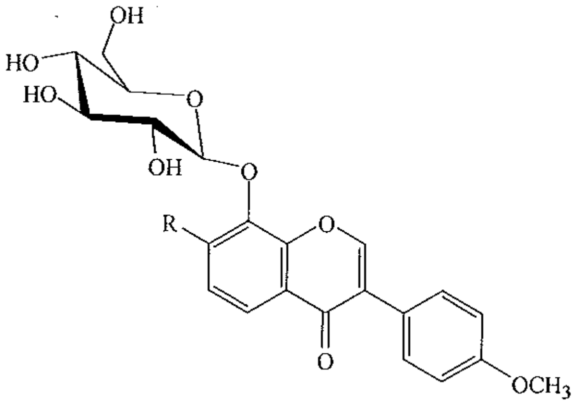
DT6 : $R_1 = CO_2CH_3, R_2 = H$

YS9 : $R_1 = CO_2H, R_2 = H$

YS12: $R_1 = CO_2H, R_2 = OCH_3$

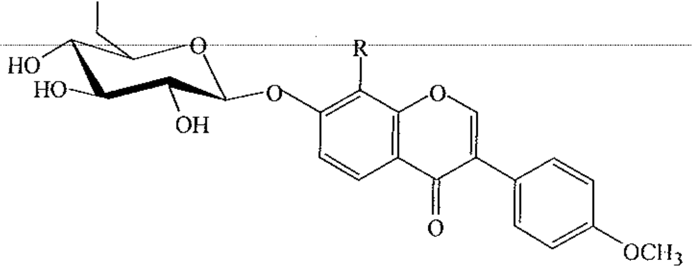


YS15



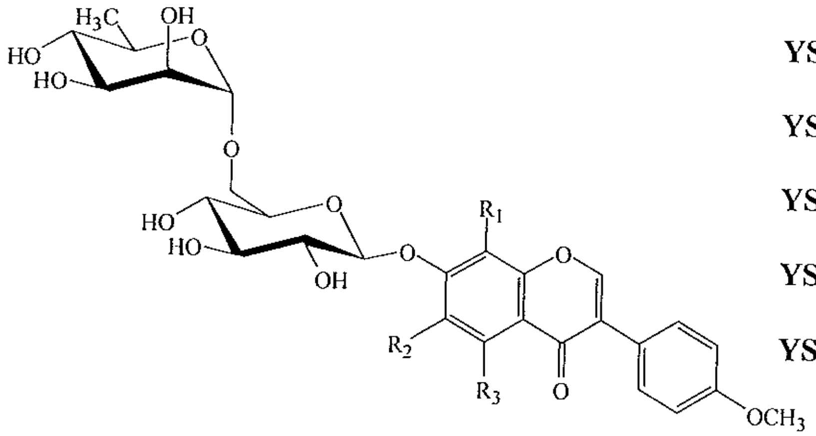
YS1 : $R = OCH_3$

YS8 : $R = OH$



YS13 : $R = H$

YS14 : $R = OCH_3$



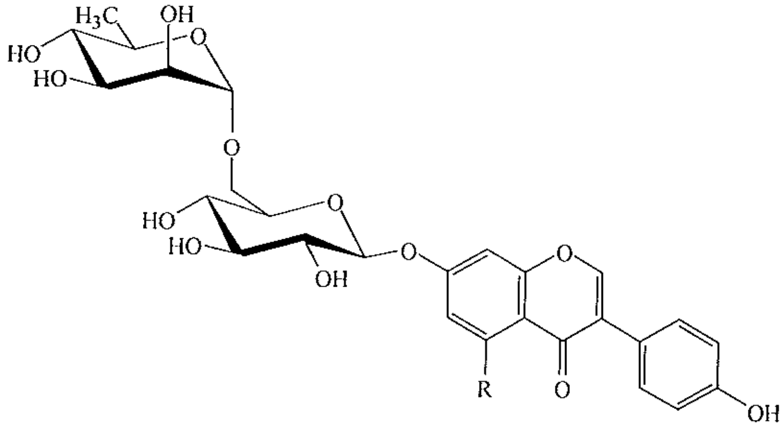
YS2 : $R_1 = OCH_3, R_2 = R_3 = H$

YS3 : $R_1 = R_2 = R_3 = H$

YS6 : $R_1 = R_3 = H, R_2 = OCH_3$

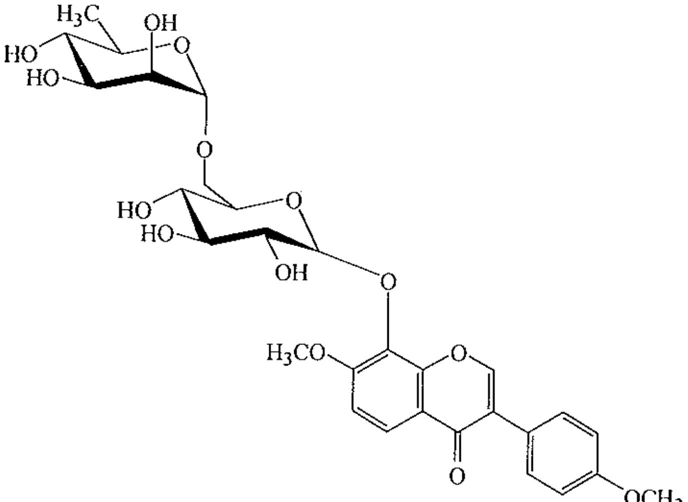
YS7 : $R_1 = H, R_2 = OCH_3, R_3 = OH$

YS10: $R_1 = OH, R_2 = R_3 = H$



YS4 : $R = OH$

YS5 : $R = H$



YS11

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I am very grateful to Associate Professor Dr. Chaweewan Jansakul for providing the crude *n*-butanol extract of *Derris scandens* and Mr. Assadawut Hiranrat for collecting *Dendrophthöe pentandra.* Many thanks go to the staff of the Department of Chemistry, Faculty of Science, Prince of Songkla University for making this thesis possible.

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Finally, none of this would have been possible without love and encouragement of my family and friends. I thank them for their understanding during all of the times when I could not be with them and their steady love that supports me.

All of these are deeply seated in my mind forever.

Yaowapa Sukpondma

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ABBREVIATIONS

s = *singlet*
d = *doublet*
t = *triplet*
dd = *doublet of doublet*
ddd = *doublet of doublet of doublet*
dq = *doublet of quartet*
m = *multiplet*
brs = *broad singlet*
δ = chemical shift
J = coupling constant
g = gram
mg = milligram
ml = milliliter
cm^3 = cubic centimeter
mm = millimeter
$[\alpha]_D$ = specific rotation
λ = wavelength
ε = molar extinction coefficient
EIMS = Electron Impact Mass Spectra
IR = Infrared
UV = Ultraviolet-Visible
UV-S = Ultraviolet-Visible-short wavelength
MS = Mass Spectroscopy
m/z = a value of mass divided by charge
°C = degree celcius
cm^{-1} = wavenumber
TLC = Thin-layer Chromatography
R_f = Retention factor
ANH = Anisaldehyde in sulfuric acid
NMR = Nuclear Magnetic Resonance

ABBREVIATIONS (continued)

2D NMR = Two Dimensional Nuclear Magnetic Resonance
Hz = hertz
MHz = megahertz
ppm = part per million
DEPT = Distortionless Enhancement by Polarization Transfer
COSY = Correlation spectroscopy
NOEDIFF = Nuclear Overhauser Effect Difference Spectroscopy
NOESY = Nuclear Overhauser Enhanced Spectroscopy
HMQC = Heteronuclear Multiple Quantum Coherence
HMBC = Heteronuclear Multiple Bond Coherence

PART I

FLAVONOID GLYCOSIDES
FROM *DENDROPHTHOE PENTANDRA* L.

CHAPTER 1.1

INTRODUCTION

1.1.1 Introduction

Dendrophthoe is one of the genera of the Loranthaceae. Plants of this family have long been recognized as aerial stem-parasitic shrubs. The genus *Dendrophthoe* is found in tropical Africa, southern Asia and southeastwards to Australia about 38 species. In southeast Asia, its distribution in *Malesia* is about 21 species.

- *D. clementis*
- *D. constricta*
- *D. curvata*
- *D. flosculosa*
- *D. gangliiformis*
- *D. gjellerupii*
- *D. glabrescens*
- *D. hallieri*
- *D. incarnata*
- *D. lanosa*
- *D. locellata*
- *D. longituba*
- *D. mearnsii*
- *D. odontocalyx*
- *D. pauciflora*
- *D. pentandra*
- *D. praelonga*
- *D. quadrifida*
- *D. timorana*
- *D. trichanthera*
- *D. xrimituba*

Dendrophthoe pentandra L. is an aerial stem-parasitic shrub. Its leaves are scattered or subopposite, narrowly to broadly elliptic 6-13 cm long and 3-8 cm wide. Inflorescence contains 6-12 flowers, mostly green or yellow or orange, at the node (Barlow, 1997).

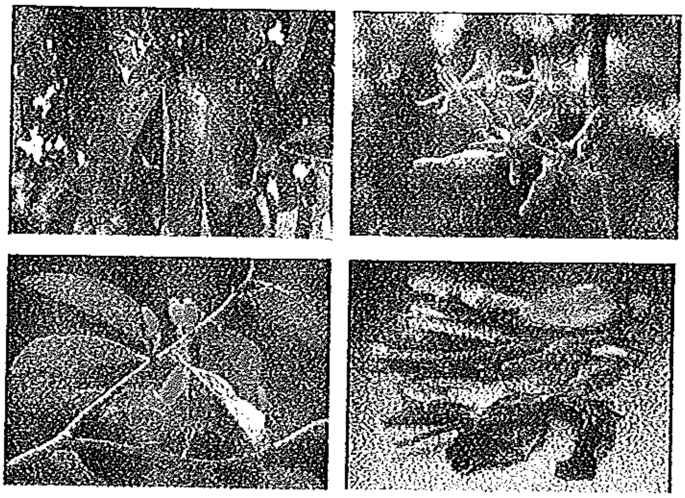
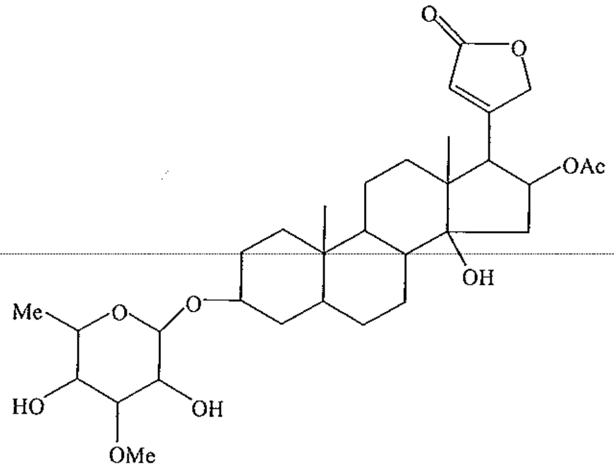


Figure 1 *Dendrophthoe pentandra* L.

(รุ่งระวี เต็มศิริฤกษ์กุล และ ชาติชาย ศันสนียชีวิน, 2541)

Dendrophthoe pentandra L. is found on various trees such as mango, orange and rose apple trees. It is used in folk medicine as it possesses antihypertensive activity. Preliminary examination of methanol extract of its leaves indicated that this crude extract showed a significant hypotensive activity (Jansakul, 1999). According to NAPRALERT database, there was no report on chemical constituents isolated from *Dendrophthoe* species as well as their biological activities. However, the Dictionary of Natural Products has reported isolation of neritaloside from *D. faleata.*



neritaloside

1.1.2 Objectives

Based on NAPRALERT database, there has been no phytochemical examination on *D. pentandra* L. This prompted us to investigate its chemical constituents in order to provide additional information of the plants in this genus. This research involved isolation, purification and structure elucidation of the chemical constituents from both leaves and twigs of *D. pentandra* L.

CHAPTER 1.2

EXPERIMENTAL

1.2.1 Instruments and chemicals

Melting points were recorded in °C and were measured on a digital Electrothermal 9100 Melting Point Apparatus. Infrared spectra were recorded using FTS165 FT-IR spectrometer. Major bands (ν) were recorded in wave numbers (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using UV-160A spectrophotometer (SHIMADZU). Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol or chloroform solution. Nuclear magnetic resonance spectra were recorded on either 400 MHz Brueker AMX 400 spectrometer or 500 MHz Varian UNITY INOVA spectrometer. Spectra were recorded using deuterochloroform, tetradeutero-methanol or hexadeutero-dimethyl sulphoxide solution and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). Optical rotation was measured in methanol or chloroform solution with sodium D line (590 nm) on an AUTOPOL^R II automatic polarimeter. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for petroleum ether (bp. 40-60 ^{0}C), diethyl ether and ethyl acetate which were analytical grade reagents. Quick column chromatography was performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel (Merck) type 100 (70-230 mesh ASTM). Precoated TLC plates of silica gel 60 F_{254} or reversed-phase C_{18} were used for analytical purposes.

1.2.2 Plant material

Leaves and twigs of *D. pentandra* L. were collected from Nakorn Si Thammarat and Songkhla Provinces in Thailand. The plant was identified by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University.

1.2.3 Isolation and extraction

Leaves (1,600 g) and twigs (3,825 g) of *D. pentandra* L. were individually extracted with methanol (12 L) over the period of 7 days at room temperature for 3 times. The filtered solution was then evaporated to dryness under reduced pressure to afford crude methanol extracts as green viscous liquid in 104.33 and 273.49 g, respectively. In addition, leaves (3.0 kg) of *D. pentandra* L. was simmered in hot water for three hours. The solution was then extracted with *n*-butanol. The *n*-butanol phase was evaporated under reduced pressure to yield a crude *n*-butanol extract as a yellow solid in 15.00 g.

1.2.4 Chemical constituents from leaves of D. pentandra **L.**

Investigation of the crude methanol extract

The crude methanol extract of *D. pentandra* L. was tested for solubility in various solvents. The results were shown in Table 1.

Table 1 Solubility of the crude methanol extract of leaves

Solvent	Solubility at room temperature
Petroleum ether	+ yellow solution
CH_2Cl_2	+ yellow solution
Ether	++ green-yellow solution mixed with white solid
$CHCl_3$	++ green-yellow solution mixed with white solid

Table 1 (continued)

Solvent	Solubility at room temperature
EtOAc	++ green-yellow solution mixed with white solid
Acetone	++ green solution mixed with white solid
CH_3OH	+++ green solution
H_2O	++ yellow solution
10%HCl	++ green-yellow solution
10%$NaHCO_3$	+++ red-brown solution
10%NaOH	+++ yellow-green solution

\+ partially soluble ++ moderately soluble +++ well-soluble

It was concluded, based on the solubility results, that the major components in the crude methanol extract was polar and acidic.

The First Investigation

The methanol extract (0.48 g) was chromatographed on silica gel using ethyl acetate, ethyl acetate-methanol gradient and finally pure methanol to afford twenty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford ten fractions as shown in Table 2.

Table 2 Fractions obtained from the crude methanol extract by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
1	0.013	Yellow liquid
2	0.048	Green viscous liquid

Table 2 (continued)

Fraction	Weight (g)	Physical appearance
3	0.030	Green viscous liquid
4	0.018	Dark green viscous liquid
5	0.024	Green viscous liquid
6	0.062	Brown-green viscous liquid
7	0.375	Brown viscous liquid
8	0.206	Brown viscous liquid mixed with yellow solid
9	0.161	Green viscous liquid mixed with yellow solid
10	0.280	Green viscous liquid mixed with yellow solid

Fraction 1 Chromatogram characteristics on normal phase TLC with 10% methanol-ethyl acetate showed one spot under UV-S and vapor of iodine with R_f value of 0.82. Because of low quantity of this fraction, it was not further investigated.

Fraction 2 Chromatogram characteristics on normal phase TLC with 10% methanol-ethyl acetate showed one green spot with R_f value of 0.82. It was not further investigated because it was expected to be chlorophyll.

Fraction 3 Chromatogram characteristics on normal phase TLC with 10% methanol-ethyl acetate demonstrated two well-separated spots with R_f values of 0.77 and 0.83. No further purification was performed because this fraction was obtained in low quantity.

Fraction 4 Chromatogram characteristics on normal phase TLC with 10% methanol-ethyl acetate showed one pale spot under UV-S with R_f value of 0.77. It was therefore not further investigated.

Fraction 5 Chromatogram characteristics on normal phase TLC with 10% methanol-ethyl acetate showed two major UV-active spots with R_f values of 0.39 and 0.71. It was not further investigated because it was obtained in low quantity.

Fraction 6 Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (4 runs) showed two major UV-active spots with R_f values of 0.10 and 0.22. Further separation with precoated TLC on silica gel plates with 10% methanol-chloroform (5 runs) as a mobile phase afforded four bands.

Band 1 was obtained as a yellow viscous liquid in 25.2 mg. Chromatogram characteristics on normal phase TLC with chloroform showed one UV-active spot with R_f value of 0.67. From ^{1}H NMR spectroscopic data, It was found to be plasticizer. It was not further investigated.

Band 2 was obtained as a yellow viscous liquid in 3.0 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one UV-active spot with R_f value of 0.20. Because this fraction was obtained in low quantity, it was not further investigated.

Band 3 was obtained as a yellow viscous liquid in 23.4 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed none of well-separated spots under UV-S. Therefore, no further purification was carried out.

Band 4 was obtained as a brown solid in 8.4 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed none of well-separated spots under UV-S. It was not further investigated.

Fraction 7 Chromatogram characteristics on normal phase TLC with 10% methanol-ethyl acetate showed three major spots under UV-S and vapor of iodine with R_f values of 0.15, 0.24 and 0.45. No further investigation was performed.

Fraction 8 Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (4 runs) and 20% methanol-chloroform (1 run) showed one oval yellow spot with R_f value of 0.34. This fraction (90.9 mg) was further acetylated with acetic anhydride (2.5 ml) and pyridine (0.5 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (3×20 ml) and 10% sodium bicarbonate (3×20 ml). The aqueous bicarbonate layer was then acidified with 10% hydrochloric acid, extracted with ethyl acetate (3×40 ml) and then washed with water. The second ethyl acetate layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to yield a brown viscous liquid in 14.6 mg. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed many unseparated UV-active spots. It was therefore not further investigated.

The first ethyl acetate layer was further washed with water (3×20 ml) to give two portions.

The organic portion was obtained as a yellow solution. It was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to yield a brown viscous liquid in 75.8 mg. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed three well-separated yellow spots with R_f values of 0.45, 0.56 and 0.69. Further acetylation with acetic anhydride (1.5 ml) and pyridine (0.5 ml) was performed. The mixture was stirred at room temperature for 24 hours. The reaction mixture was worked up using the above procedure to afford three fractions.

Fraction 1 was obtained as a yellow viscous liquid in 2.6 mg. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed four well-separated yellow spots with R_f values of 0.35, 0.40, 0.50 and 0.59. Because of low quantity of this fraction, it was not further investigated.

Fraction 2 was obtained as a yellow viscous liquid in 59.2 mg. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed three yellow spots with R_f values of 0.50, 0.59 and 0.67 and the other spot with R_f value of 0.83 under UV-S. It was further separated by precoated TLC on silica gel plates with 3% methanol-chloroform as a mobile phase to afford four bands.

Band 2.1 was obtained as a yellow viscous liquid in 9.7 mg. Chromatogram characteristics on normal phase TLC with 50% dichloromethane-petroleum ether indicated the presence of plasticizer. It was not further investigated.

Band 2.2 was obtained as a yellow viscous liquid in 3.1 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform showed one major yellow spot with R_f value of 0.27.

Band 2.3 was obtained as a yellow viscous liquid in 18.5 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform showed one oval UV-active spot with the same R_f value as that of the **band 2.2**. They were then combined together and separated by precoated TLC on silica gel plates with 5% methanol-chloroform as a mobile phase to afford four bands.

Band 1 was obtained as a yellow viscous liquid in 5.9 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform demonstrated one yellow spot with R_f value of 0.23. The ^{1}H NMR spectrum indicated that it was a **triacetate derivative of DW1**.

^{1}H NMR (CDCl$_3$) (δ ppm) (500 MHz)	7.47 (*s*, 1H), 7.31 (*dd*, *J* = 8.4, 1.5 Hz, 1H), 7.00 (*d*, *J* = 8.4 Hz, 1H), 6.37 (*s*, 1H), 6.26 (*d*, *J* = 1.5 Hz, 1H), 5.64 (*s*,1H), 5.59-5.57 (*m*, 1H), 5.30 (*dd*, *J* = 9.9, 3.0 Hz, 1H), 4.94 (*t*, *J* = 9.9 Hz, 1H), 3.50 (*dq*, *J* = 9.9, 6.5 Hz, 1H), 2.13 (*s*, 3H), 2.03 (*s*, 3H), 2.00 (*s*, 3H), 0.92 (*d*, *J* = 6.5 Hz, 3H)

Band 2 was obtained as a yellow viscous liquid in 4.0 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform demonstrated one major yellow spot with R_f value of 0.24. No further purification was performed because this fraction was obtained in low quantity.

Band 3 was obtained as a yellow viscous liquid in 2.0 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform showed one major yellow spot with R_f value of 0.19. It was not further investigated because of low quantity.

Band 4 was obtained as a yellow viscous liquid in 2.0 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform demonstrated none of well-separated spots under UV-S. No attempted purification was carried out.

Fraction 3 was obtained as a yellow viscous liquid in 7.3 mg. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform demonstrated none of well-separated spots under UV-S. It was not further investigated.

The aqueous portion was obtained as a green solution. It was extracted with *n*-butanol (2×20 ml) and evaporated under reduced pressure to yield a yellow viscous liquid in 55.3 mg. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed one major yellow spot with R_f value of 0.69 and pale spots under UV-S. No further investigation was performed because of limitation of time.

Fraction 8 (106.6 mg) was acetylated under the same reaction conditions as previously mentioned. Upon working up, two fractions were obtained.

Fraction 1 was obtained as a brown-yellow viscous liquid in 90.3 mg. Chromatogram characteristics on normal phase TLC with chloroform showed two major yellow spots with R_f values of 0.17 and 0.29 and two minor spots under UV-S with R_f values of 0.67 and 0.73. Further separation with precoated TLC on silica gel plates with 2% methanol-chloroform as a mobile phase afforded seven bands.

Band 1 was obtained as a yellow viscous liquid in 9.1 mg. Chromatogram characteristics on normal phase TLC with chloroform (2 runs) indicated that it was a plasticizer. It was therefore not further investigated.

Band 2 was obtained as a yellow solid in 6.5 mg. Chromatogram characteristics on normal phase TLC with chloroform (2 runs) showed two major yellow spot with R_f values of 0.24 and 0.36. Because this fraction was obtained in low quantity, it was not further investigated.

Band 3 was obtained as a yellow viscous liquid in 13.4 mg. Chromatogram characteristics on normal phase TLC with chloroform (2 runs) showed two well-separated yellow spots with R_f values of 0.15 and 0.24. No further purification was carried out because of low quantity of this fraction.

Band 4 was obtained as a colorless viscous liquid in 1.9 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform demonstrated one yellow spot with R_f value of 0.59. Because of low quantity, it was not further investigated.

Band 5 was obtained as a yellow viscous liquid in 4.5 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform demonstrated one major yellow spot with R_f value of 0.49. No further investigation was carried out because this fraction was obtained in low quantity.

Band 6 was obtained as a yellow-white solid in 8.7 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform demonstrated two UV-active spots with R_f values of 0.45 and 0.31. From ^{1}H NMR spectroscopic data, it contained **a hexaacetate derivative of DW1** as a major component.

^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	7.73 (*dd*, J = 8.5, 2.0 Hz, 1H), 7.66 (*d*, J = 2.0 Hz, 1H), 7.35 (*d*, J = 8.5 Hz, 1H), 6.59 (*d*, J = 2.0 Hz, 1H), 6.48 (*d*, J = 2.0 Hz, 1H), 5.66 (*dd*, J = 3.5, 1.5 Hz, 1H), 5.56 (*d*, J = 1.5 Hz, 1H), 5.22 (*dd*, J = 10.0, 3.5 Hz, 1H), 4.93 (*t*, J = 10.0 Hz,

1H), 3.36 (*dq*, J = 10.0, 6.0 Hz, 1H), 2.41 (*s*, 3H), 2.33 (*s*, 3H), 2.31 (*s*, 3H), 2.12 (*s*, 3H), 1.99 (*s*, 3H), 1.98 (*s*, 3H), 0.91 (*d*, J = 6.0 Hz, 3H)

Band 7 was obtained as a yellow viscous liquid in 8.9 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform demonstrated three well-separated spots with R_f values of 0.19, 0.24 and 0.28. It was not further investigated because of low quantity.

Fraction 2 was obtained as a yellow viscous liquid in 37.1 mg. Chromatogram characteristics on normal phase TLC with chloroform demonstrated four well-separated spots under UV-S with R_f values of 0.34, 0.39, 0.46 and 0.50. No attempted purification was performed because this fraction was obtained in low quantity.

Fraction 9 Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (4 runs) showed the same pattern as that of **fraction 8**. Therefore, no further purification was performed.

Fraction 10 Chromatogram characteristics on normal phase TLC with 10% methanol-ethyl acetate showed none of well-separated spots under UV-S. It was therefore not further investigated.

The Second Investigation

Attempted purification either by direct chromatography of the crude extract or chromatography of acetate derivatives was unsatisfactory. Only the **triacetate** and **hexaacetate derivatives of DW1** were obtained. The crude extract (0.58 g) was therefore treated with 10% sodium hydroxide (30 ml) and extracted with ethyl acetate (5×60 ml) to give two fractions.

Fraction A (ethyl acetate fraction) was obtained as a yellow viscous liquid in 0.14 g. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed no major UV-active spots. It was therefore not further investigated.

Fraction B (aqueous fraction) was neutralized with 10% hydrochloric acid and then extracted with ethyl acetate (5×60 ml) to provide two subfractions.

Subfraction B1 (ethyl acetate fraction) was obtained as a green viscous liquid in 0.13 g. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed none of well-separated spots under UV-S. No further purification was performed.

Subfraction B2 (aqueous fraction) was acidified with 10% hydrochloric acid and then extracted with ethyl acetate (5×60 ml) into two subfractions.

Subfraction B2.1 (ethyl acetate fraction) was obtained as a brown viscous liquid in 0.26 g. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction B2.2 (aqueous fraction) was extracted with *n*-butanol (4×50 ml). The *n*-butanol layer was evaporated under reduced pressure to yield a brown viscous liquid in 0.03 g. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed none of well-separated spots under UV-S. No further investigation was performed.

The Third Investigation

The crude methanol extract (2.11 g) was separated into two fractions by dissolving in acetone.

Acetone-soluble fraction was obtained as a green viscous liquid in 1.16 g. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed one major UV-active spot with R_f value of 0.19 and the other pale spot with R_f value of 0.33.

Acetone-insoluble fraction was obtained as a brown viscous liquid in 0.38 g. Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed one major UV-active spot with R_f value of 0.19 which was identical to **DW1**.

The Fourth Investigation

The methanol extract (**C**, 570 mg) was dissolved with water 40 ml. The resulting mixture was extracted with *n*-butanol (3×60 ml). The *n*-butanol layer was evaporated under reduced pressure to yield a green viscous liquid in 491.5 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform demonstrated two major yellow spots with R_f values of 0.14 and 0.20. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with chloroform, chloroform-methanol gradient and finally pure methanol to give twenty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six fractions as shown in Table 3.

Table 3 Fractions obtained from **C** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
C1	0.032	Dark green viscous liquid
C2	0.008	Dark green viscous liquid
C3	0.019	Green viscous liquid
C4	0.012	Green viscous liquid
C5	0.067	Green viscous liquid
C6	0.312	Dark green viscous liquid

Fraction C1 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed many UV-active spots. Because of low quantity of this fraction, it was not further investigated.

Fraction C2 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed one green spot with R_f value of 0.09. No further purification was performed.

Fraction C3 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed none of well-separated UV-active spots. It was therefore not further investigated.

Fraction C4 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform demonstrated two well-separated spots under UV-S with R_f values of 0.23 and 0.45. No attempted purification was carried out because this fraction was obtained in low quantity.

Fraction C5 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one yellow spot with R_f value of 0.21 and the other spot at R_f value of 0.33 under UV-S. Further separation with precoated TLC on silica gel plates with 20% methanol-chloroform as a mobile phase afforded two bands.

Band 1 (**DW2**) was obtained as a yellow solid in 8.5 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one pure UV-active spot with R_f value of 0.32.

$[\alpha]_D^{25}$		-104.09° ($c = 9.6 \times 10^{-3}$ g/100 cm^3, CH_3OH)
IR (neat) ν (cm^{-1})		3368 (OH stretching), 2930 (CH stretching), 1657 (C=O stretching), 1611, 1509 (C=C stretching)
UV (CH_3OH) λ_{max} (nm) (log ε)		213 (4.49), 266 (4.51), 348 (4.34)
^{1}H NMR ($CDCl_3$+ DMSO-d_6) (δ ppm) (400 MHz)		12.60 (*s*, 1H), 7.75 (*d*, *J* = 8.7 Hz, 2H), 6.93 (*d*, *J* = 8.7 Hz, 2H), 6.36 (*d*, *J* = 2.1 Hz, 1H), 6.22 (*d*, *J* = 2.1 Hz, 1H), 5.45 (*d*, *J* = 1.8 Hz, 1H), 4.23 (*dd*, *J* = 3.4, 1.8 Hz, 1H), 3.72 (*dd*, *J* = 9.2, 3.4 Hz, 1H), 3.30-3.20 (*m*, 1H), 3.25-3.20 (*m*, 1H), 0.92 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR ($CDCl_3$+ DMSO-d_6) (δ ppm) (100 MHz)		177.95, 164.31, 161.66, 160.01, 157.28 156.74, 134.42, 130.43, 120.88, 115.48 104.56, 101.57, 98.91, 93.75, 71.77, 71.02 70.41, 70.18, 17.30
DEPT (135°) ($CDCl_3$+ DMSO-d_6)	CH	130.43, 115.48, 101.57, 98.91, 93.75,

	71.77, 71.02, 70.41, 70.18
CH_3	17.30

Band 2 (**DW1**) was obtained as a yellow solid in 52.8 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one yellow spot with R_f value of 0.21.

Melting point	178-182 °C
$[\alpha]_D^{25}$	-119.04° (c = 8.4 x 10^{-3} g/100 cm^3, CH_3OH)
IR (KBr) ν (cm^{-1})	3300 (O-H stretching), 1651 (C=O stretching) 1600, 1560 (C=C stretching)
UV (CH_3OH) λ_{max} (nm) (log ε)	213 (4.33), 257 (4.32), 347 (4.20)
^1H NMR ($CDCl_3$+ DMSO-d_6) (δ ppm) (400 MHz)	12.70 (*s*, 1H), 7.33 (*d*, *J* = 2.4 Hz, 1H), 7.28 (*dd*, *J* = 8.4, 2.4 Hz, 1H), 6.90 (*d*, *J* = 8.4 Hz, 1H), 6.42 (*d*, *J* = 2.4 Hz, 1H), 6.22 (*d*, *J* = 2.4 Hz, 1H), 5.38 (*d*, *J* = 1.8 Hz, 1H), 4.24 (*dd*, *J* = 3.4, 1.8 Hz, 1H), 3.77 (*dd*, *J* = 9.4, 3.4 Hz, 1H), 3.38-3.32 (*m*, 1H), 3.47-3.41 (*m*, 1H), 0.85 (*d*, *J* = 6.1 Hz, 3H)
^{13}C NMR ($CDCl_3$+ DMSO-d_6) (δ ppm) (100 MHz)	177.46, 163.88, 161.00, 157.04, 156.19 148.12, 144.87, 133.93, 120.85, 120.49 115.37, 115.17, 103.83, 101.48, 98.43 93.38, 70.92, 70.28, 70.10, 69.76, 17.14
DEPT (135°) ($CDCl_3$+ DMSO-d_6) CH	120.85, 115.37, 115.17, 101.48, 98.43, 93.38, 70.92, 70.28, 70.10, 69.76
CH_3	17.14

Fraction C6 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one major yellow spot of **DW1**.

Investigation of the crude *n*-butanol extract

The First Investigation

The crude *n*-butanol extract (5.01 g) was recrystallized several times in a mixture of methanol-chloroform to afford three fractions.

Fraction 1 was obtained as a yellow solid in 300 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** as a pure component.

Fraction 2 was obtained as a brown solid. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed **DW1** as a major component and the other component with R_f value of 0.37. This fraction (**D**, 217.5 mg) was separated by column chromatography over silica gel. Elution was conducted initially with 5% methanol-chloroform and gradually enriched with pure methanol to give twenty nine fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness under reduced pressure to afford four fractions as shown in Table 4.

Table 4 Fractions obtained from **D** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
D1	0.007	Yellow solid
D2	0.008	Yellow solid
D3	0.039	Yellow solid
D4	0.187	Yellow-brown solid

Fraction D1 Chromatogram characteristics on normal phase TLC with chloroform showed one pale spot under UV-S with R_f value of 0.42. It was therefore not further investigated.

Fraction D2 Chromatogram characteristics on normal phase TLC with chloroform showed none of well-separated spots under UV-S. No further purification was performed.

Fraction D3 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated that this fraction contained **DW2**. Further separation with precoated TLC on silica gel plates with 20% methanol-chloroform as a mobile phase afforded three bands.

Band 1 was obtained as a yellow viscous liquid in 8.4 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed the presence of **DW2** as a major component. Chromatography on reversed-phase TLC with 70% methanol-water as a mobile phase afforded two bands.

Band 1.1 was obtained as a yellow viscous liquid in 2.6 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW2** as a pure component.

Band 1.2 was obtained as a yellow viscous liquid in 1.1 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one pure spot under UV-S with R_f value of 0.28. Because of low quantity of this fraction, it was not further investigated.

Band 2 was obtained as a yellow viscous liquid in 5.0 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1**.

Band 3 was obtained as a yellow solid in 11.0 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one major yellow spot with the same R_f value as that of **DW1**.

Fraction 3 The brown filtrate was evaporated to dryness under reduced pressure to afford a brown viscous liquid. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform demonstrated many well-separated spots and contained **DW2** as a component. This fraction (166.4 mg) was purified by precoated TLC on silica gel plates with 20% methanol-chloroform as a mobile phase to afford seven bands.

Band 1 was obtained as a yellow viscous liquid mixed with a brown solid in 2.9 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed the absence of UV-active spots. It was not further investigated.

Band 2 was obtained as a brown viscous liquid in 4.0 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform demonstrated pale spots under UV-S. No attempted purification was performed.

<u>Band 3</u> was obtained as a brown viscous liquid in 6.0 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed none of well-separated spots under UV-S. No further investigation was performed.

<u>Band 4</u> was obtained as a yellow viscous liquid in 23.4 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW2**.

<u>Band 5</u> was obtained as a brown viscous liquid in 7.0 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed two major spots under UV-S which were identical to **DW1** and **DW2**.

<u>Band 6</u> was obtained as a yellow-brown viscous liquid in 25.7 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one major spot with the same R_f value as that of **DW1**.

<u>Band 7</u> was obtained as a red-brown viscous liquid in 68.2 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated that it contained **DW1** and the other component with R_f value of 0.09.

In order to obtain enough **DW2** for structural determination. The crude *n*-butanol extract (**E**, 1.06 g) was purified by column chromatography over silica gel. Elution was conducted initially with 5% methanol-chloroform and gradually enriched with methanol to give forty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford seven fractions as shown in Table 5.

Table 5 Fractions obtained from **E** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
E1	0.010	Brown-yellow viscous liquid
E2	0.003	Brown-yellow viscous liquid
E3	0.007	Brown viscous liquid
E4	0.013	Yellow-brown viscous liquid

Table 5 (continued)

Fraction	Weight (g)	Physical appearance
E5	0.276	Yellow solid
E6	0.446	Yellow solid
E7	0.280	Dark brown viscous liquid

Fraction E1 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed many UV-active spots. No attempted purification was carried out.

Fraction E2 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed none of UV-active spots. Therefore, no further purification was performed.

Fraction E3 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one pale spot under UV-S with R_f value of 0.32. Its ^1H NMR (500 MHz) showed the absence of aromatic proton signals. It was therefore not further investigated.

Fraction E4 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** and **DW2** as major components and the other brown spot with R_f value of 0.28. Because of low quantity of this fraction, it was not further investigated.

Fraction E5 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed two major spots with R_f values of 0.35 and 0.19, indicating the presence of **DW1** and **DW2**.

Fraction E6 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** as a major component.

Fraction E7 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform demonstrated **DW1** as a major component and a brown spot near baseline. It was separated into two fractions by dissolving in methanol.

<u>Methanol-soluble fraction</u> afforded a yellow-brown solid in 246.2 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated that it contained **DW1** as a major component.

Methanol-insoluble fraction afforded a brown solid mixed with a red-brown viscous liquid in 54.1 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed no spot from baseline. Its ^{1}H NMR spectrum showed none of aromatic-proton signals. No further purification was carried out.

The Second Investigation

All fractions obtained from recrystallization of the crude *n*-butanol extract were combined together. Further purification of combined fraction (**F**, 1.04 g) by column chromatography over silica gel was performed. Elution was conducted initially with 1% methanol-chloroform and gradually enriched with methanol to afford thirteen fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to give three fractions as shown in Table 6.

Table 6 Fractions obtained from **F** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
F1	0.012	Yellow viscous liquid
F2	0.784	Yellow viscous liquid
F3	0.304	Yellow viscous liquid

Fraction F1 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed none of major spots. It was therefore not further investigated.

Fraction F2 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** and **DW2** as major components. It was further purified by column chromatography over silica gel. Elution was conducted initially with 20% methanol-chloroform and gradually enriched with pure methanol to give twelve fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and evaporated to dryness under reduced pressure to afford five fractions as shown in Table 7.

Table 7 Fractions obtained from **F2** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
F2.1	0.009	Yellow viscous liquid
F2.2	0.012	Yellow viscous liquid
F2.3	0.045	Yellow viscous liquid
F2.4	0.480	Yellow solid
F2.5	0.289	Brown-yellow viscous liquid

Fraction F2.1 Chromatogram characteristics on normal phase TLC with chloroform showed two well-separated spots under UV-S with R_f values of 0.26 and 0.73. Because this fraction was obtained in low quantity, no further investigation was carried out.

Fraction F2.2 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform demonstrated one yellow spot with the same R_f value as that of **DW2**.

Fraction F2.3 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** and **DW2** as major components. This fraction was further investigated together with **F2.5**.

Fraction F2.4 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed two major spots belonging to **DW1** and **DW2**. It was further separated by the same procedure as **F2** to give seventeen fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and evaporated to dryness *in vacuo* to afford four subfractions as shown in Table 8.

Table 8 Fractions obtained from **F2.4** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
F2.4-a	0.013	Yellow viscous liquid
F2.4-b	0.006	Yellow viscous liquid

Table 8 (continued)

Fraction	Weight (g)	Physical appearance
F2.4-c	0.143	Yellow viscous liquid
F2.4-d	0.286	Yellow viscous liquid

Subfraction F2.4-a Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed no major spots under UV-S. It was therefore not further investigated.

Subfraction F2.4-b Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW2** as a major component.

Subfraction F2.4-c Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** and **DW2** as major components. It was further purified together with **F2.5-c**.

Subfraction F2.4-d Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed **DW1** as a major component.

Fraction F2.5 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** as a major component and **DW2** as a minor component. Therefore, it was combined with **F2.3** and further separated by the same procedure as **F2** to give nineteen fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness under reduced pressure to afford four subfractions as shown in Table 9.

Table 9 Fractions obtained from **F2.5** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
F2.5-a	0.016	Yellow viscous liquid
F2.5-b	0.009	Yellow viscous liquid
F2.5-c	0.043	Yellow viscous liquid
F2.5-d	0.363	Yellow viscous liquid

<u>Subfraction F2.5-a</u> Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed no major UV-active spots. It was therefore not further investigated.

<u>Subfraction F2.5-b</u> Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW2** as a major component. It was further purified together with **fraction 3**.

<u>Subfraction F2.5-c</u> Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform was similar to **F2.4-c**. They were then combined and further purified by precoated TLC on silica gel plates with 20% methanol-chloroform as a mobile phase afforded two bands.

Band 1 was obtained as a yellow solid in 3.5 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW2** as a major component. It was further investigated together with **fraction 3**.

Band 2 was obtained as a yellow viscous liquid in 122.0 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform demonstrated two major spots of **DW1** and **DW2**.

<u>Subfraction F2.5-d</u> Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** as a major component.

Fraction F3 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** as a major component. It was crystallized in a mixture of methanol-chloroform to yield a yellow solid (**DW1**) in 100 mg.

The Third Investigation

The crude *n*-butanol extract (5.0 g) was purified by flash column chromatography over silica gel. Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with pure methanol to afford fourteen fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four fractions as shown in Table 10.

Table 10 Fractions obtained from the crude *n*-butanol extract by flash column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
1	0.020	Red-brown viscous liquid
2	0.880	Yellow solid mixed with yellow viscous liquid
3	3.300	Brownish-yellow solid
4	0.360	Red-brown solid

Fraction 1 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one pale spot under UV-S. It was therefore not further investigated.

Fraction 2 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** and **DW2** as major components. Further purification by chromatotron with silica gel plate (4 mm thickness). Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with pure methanol to give eleven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two fractions as shown in Table 11.

Table 11 Fractions obtained from **fraction 2** by chromatotron

Fraction	Weight (g)	Physical appearance
2.1	0.478	Yellow-brown solid
2.2	0.111	Yellow viscous liquid

Fraction 2.1 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** as a major component.

Fraction 2.2 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** and **DW2** as major components. Further purification by chromatotron with silica gel plate (1 mm thickness) was performed. Elution was conducted initially with chloroform and gradually enriched with methanol to afford two fractions.

Fraction 2.2a was obtained as a yellow viscous liquid in 15.5 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW2** as a major component.

Fraction 2.2b was obtained as a yellow viscous liquid in 15.5 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** as a major component.

Fraction 3 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** and **DW2** as major components. It was purified by chromatotron with silica gel plate (2 mm thickness). Fractions containing **DW2** were combined and evaporated to dryness to yield a yellow solid in 97.3 mg. It was then combined with other previously fractions which contained **DW2** as a major component. Further separation of the mixture (168.6 mg) by chromatotron with silica gel plate (2 mm thickness) yielded two fractions.

Fraction 3.1 was obtained as a yellow viscous liquid in 116.9 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW2** as a major component. It was further purified by precoated TLC on silica gel plates with 15% methanol-chloroform (9 runs) to yield four bands.

Band 1 was obtained as a yellow solid in 1.0 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed no major spots under UV-S. It was therefore not further investigated.

Band 2 (**DW3**) was obtained as a yellow viscous liquid in 2.4 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one pure yellow spot with R_f value of 0.20.

$[\alpha]_D^{29}$	-20.41° ($c = 4.9 \times 10^{-2}$ g/ 100 cm^3, CH_3OH)
IR (neat) ν (cm^{-1})	3419 (OH stretching), 2927 (CH stretching), 1652 (C=O stretching), 1599 (C=C stretching)
UV (CH_3OH) λ_{max} (nm) (log ε)	213 (4.05), 265 (3.90), 348 (3.77)

^{1}H NMR (CD$_3$OD) (δ ppm) (500 MHz)		7.45 (*d*, J = 2.0 Hz, 1H), 7.42 (*dd*, J = 8.5, 2.0 Hz, 1H), 6.96 (*d*, J = 8.5 Hz, 1H), 6.41 (*d*, J = 2.0 Hz, 1H), 6.22 (*d*, J = 2.0 Hz, 1H), 5.40 (*d*, J = 1.7 Hz, 1H), 4.20 (*dd*, J = 3.6, 1.7 Hz, 1H), 3.95 (*s*, 3H), 3.74 (*dd*, J = 9.1, 3.6 Hz, 1H), 0.92 (*d*, J = 6.0 Hz, 3H)
^{13}C NMR (CD$_3$OD) (δ ppm) (125 MHz)		159.40, 158.80, 150.95, 149.00, 136.40, 124.22, 123.20, 116.50, 113.45, 106.00, 103.40, 100.00, 95.26, 73.12, 72.12, 72.00, 71.85, 56.74, 17.69
DEPT (135°) (CD$_3$OD)	CH	124.22, 116.50, 113.45, 103.40, 100.00, 95.26, 73.12, 72.12, 72.00, 71.85
	CH$_3$	56.74, 17.69

Band 3 was obtained as a yellow solid in 35.0 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one pure yellow spot with R_f value of 0.18 (**DW2**).

Band 4 was obtained as a yellow viscous liquid in 3.6 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed none of well-separated spots under UV-S. It was therefore not further investigated.

Fraction 4 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform indicated the presence of **DW1** as a major component.

1.2.5 *Chemical constituents from twigs of D. pentandra* L.

The First Investigation

Twigs (675 g) of *D. pentandra* L. were extracted with methanol (3 L) over the period of 7 days at room temperature for 3 times. The filtered solutions were combined and evaporated to dryness under reduced pressure to afford a green viscous liquid in 22.55 g. The crude methanol extract was tested for solubility in various solvents. The results were shown in Table 12.

Table 12 Solubility of the crude methanol extract of twigs

Solvent	Solubility at room temperature
Petroleum ether	-
CH_2Cl_2	-
Ether	+ yellow solution
$CHCl_3$	+ yellow solution
EtOAc	+ yellow solution
Acetone	++ yellow-brown solution
CH_3OH	+++ yellow-brown solution
H_2O	++ brown solution

-	insoluble	+	partially soluble
++	moderately soluble	+++	well-soluble

Chromatogram characteristics of the crude methanol extract dissolved in ethyl acetate and methanol on normal phase TLC with dichloromethane and ethyl acetate as eluants showed none of well-separated spots. However, the crude methanol extract which was partially soluble in ether showed one well-separated spot with R_f value of 0.61. Therefore, the crude methanol extract (6.04 g) was separated in two parts by dissolving in ether.

Ether-insoluble part was obtained as a brown solid in 5.72 g. Chromatogram characteristics on normal and reversed-phase TLC with 50% methanol-chloroform and 50% methanol-water, respectively, showed none of well-separated spots under UV-S. It was therefore not further investigated.

Ether-soluble part (G) was obtained as a yellow-brown liquid in 0.32 g. Chromatogram characteristics on normal phase TLC with chloroform showed three well-separated spots under UV-S with R_f values of 0.55, 0.61 and 0.67. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with chloroform and gradually enriched with pure methanol to give fourteen fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three fractions as shown in Table 13.

Table 13 Fractions obtained from **G** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
G1	0.306	Dark green viscous liquid
G2	0.085	Yellow-brown viscous liquid
G3	0.070	Yellow-brown viscous liquid

Fraction G1 Chromatogram characteristics on normal phase TLC with 80% chloroform-petroleum ether (3 runs) showed four major spots with R_f values of 0.67, 0.72, 0.82 and 0.86. It was further separated by column chromatography over silica gel. Elution was conducted initially with 50%chloroform-petroleum ether and gradually enriched with pure chloroform to give twenty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions as shown in Table 14.

Table 14 Fractions obtained from **G1** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
G1.1	0.031	Yellow viscous liquid
G1.2	0.055	Pale yellow solid
G1.3	0.032	Yellow viscous liquid
G1.4	0.002	White solid
G1.5	0.132	Green viscous liquid
G1.6	0.054	Green solid

Subfraction G1.1 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed three pale well-separated spots under UV-S with R_f values of 0.41, 0.51 and 0.65. Because of limitation of time, it was not further investigated.

Subfraction G1.2 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed many pale spots under UV-S. No further purification was carried out.

Subfraction G1.3 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether demonstrated one pale UV-active spot with R_f value of 0.26. No attempted purification was carried out.

Subfraction G1.4 Chromatogram characteristics on normal phase TLC with 80% chloroform-petroleum ether showed one UV-active spot with R_f value of 0.42 and the other spot with R_f value of 0.36 as a violet spot after dipping in ANH and subsequently heating the TLC plate. Because of low quantity of this fraction, it was not further investigated.

Subfraction G1.5 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed one major UV-active spot with R_f value of 0.14. Further separation with precoated TLC on silica gel plates with 50% chloroform-petroleum ether as a mobile phase afforded four bands.

Band 1 was obtained as a colorless viscous liquid in 16.9 mg. Chromatogram characteristics on normal phase TLC with 80% chloroform-petroleum ether showed one UV-active spot with R_f value of 0.39 and a violet spot with R_f value of 0.66 after dipping in ANH and subsequently heating the TLC plate. It was shown by ^{1}H NMR spectrum to be a mixture of compounds.

Band 2 was obtained as a yellow viscous liquid in 5.2 mg. Chromatogram characteristics on normal phase TLC with 80% chloroform-petroleum ether demonstrated one spot under UV-S with R_f value of 0.35 and the other spot with R_f value of 0.27 as a violet spot after dipping in ANH and subsequently heating the TLC plate. Because of low quantity of this fraction, no further purification was carried out.

Band 3 was obtained as a yellow solid in 16.7 mg. Chromatogram characteristics on normal phase TLC with 80% chloroform-petroleum ether showed one major violet spot with R_f value of 0.20 after dipping in ANH and subsequently heating the TLC plate.

Band 4 was obtained as a green viscous liquid in 33.0 mg. Chromatogram characteristics on normal phase TLC with 80% chloroform-petroleum ether showed two well-separated spots under UV-S with R_f values of 0.25 and 0.44. Because of low quantity, it was not further investigated.

Subfraction G1.6 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed none of well-separated spots under UV-S. Therefore, no further purification was performed.

Fraction G2 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed no major spots under UV-S. It was therefore not further investigated.

Fraction G3 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed none of well-separated spots. No attempted investigation was carried out.

The Second Investigation

Twigs (3,150 g) of *D. pentandra* L. were extracted with methanol (3 L) over the period of 7 days at room temperature for 3 times. The filtered solutions were combined and evaporated to dryness under reduced pressure to afford a green viscous liquid in 72.95 g. It was combined with the remainder of the first crude extract (15.51 g) and then separated into two fractions by dissolving in ether.

Ether-insoluble fraction was obtained as a brown solid in 58.37 g. No further investigation was performed.

Ether-soluble fraction (**H**) was obtained as a green viscous liquid in 12.15 g. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 50% chloroform-petroleum ether, gradually enriched with chloroform and followed by increasing amount of methanol in chloroform and finally with pure methanol to give thirty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford seven fractions as shown in Table 15.

Table 15 Fractions obtained from **H** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
H1	0.380	Orange-yellow liquid
H2	1.920	Green viscous liquid
H3	2.700	Green viscous liquid
H4	0.430	Green viscous liquid
H5	2.010	Green viscous liquid
H6	1.490	Yellow-brown viscous liquid
H7	0.850	Brown viscous liquid

Fraction H1 Chromatogram characteristics on normal phase TLC with chloroform showed many yellow spots. No further purification was carried out.

Fraction H2 Chromatogram characteristics on normal phase TLC with 70% chloroform-petroleum ether showed three major spots under UV-S with R_f values of 0.24, 0.37 and 0.48. It was separated by column chromatography over silica gel. Elution was conducted initially with 50% chloroform-petroleum ether, gradually enriched with chloroform and finally with pure chloroform to give thirty two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine subfractions as shown in Table 16.

Table 16 Fractions obtained from **H2** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
H2.1	0.360	Orange-yellow liquid
H2.2	0.062	Orange-yellow solid
H2.3	0.141	Red-violet solid
H2.4	0.213	Orange-yellow solid
H2.5	0.229	Orange-red viscous liquid

Table 16 (continued)

Fraction	Weight (g)	Physical appearance
H2.6	0.146	Yellow-orange viscous liquid
H2.7	0.109	Yellow-green viscous liquid
H2.8	0.729	Green viscous liquid
H2.9	0.036	Green viscous liquid

Subfraction H2.1 Chromatogram characteristics on normal phase TLC with 20% chloroform-petroleum ether showed no spots under UV-S. It was therefore not further investigated.

Subfraction H2.2 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether demonstrated three pale spots under UV-S with R_f values of 0.34, 0.41 and 0.48 and the other spot with R_f value of 0.65 which appeared as a blue spot after dipping in ANH and subsequently heating the TLC plate. Because of low quantity of this fraction, it was not further investigated.

Subfraction H2.3 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether demonstrated one violet spot with R_f value of 0.29, one UV-active spot with R_f value of 0.36 and one spot with R_f value of 0.51 which appeared as a violet spot after dipping in ANH and subsequently heating the TLC plate. Because of limitation of time, it was not further investigated.

Subfraction H2.4 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether demonstrated many spots under UV-S. Its ^1H NMR spectroscopic data showed majority of signals at high field. No attempted purification was performed.

Subfraction H2.5 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed many spots under UV-S. However, this fraction gave majority of signals at high field in ^1H NMR spectrum.

Subfraction H2.6 Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether demonstrated one major spot under UV-S with R_f value of 0.19. It was further separated by precoated TLC on silica gel plates with 60% chloroform-petroleum ether as a mobile phase to afford three bands.

Band 1 was obtained as a violet solid in 3.0 mg. Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether (3 runs) showed one violet spot with R_f value of 0.24. Because of low quantity, it was not further investigated.

Band 2 was obtained as a yellow viscous liquid in 27.9 mg. Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether (3 runs) showed one major UV-active spot with R_f value of 0.13 and many spots after dipping in ANH and subsequently heating the TLC plate. It was therefore not further investigated.

Band 3 was obtained as a yellow solid in 4.3 mg. Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether (3 runs) showed two major UV-active spots with R_f values of 0.08 and 0.15. Because this fraction was obtained in low quantity, it was not further investigated.

Subfraction H2.7 Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether demonstrated one major UV-active spot with R_f value of 0.19. Further purification by precoated TLC on silica gel plates with 60% chloroform-petroleum ether as a mobile phase afforded three bands.

Band 1 was obtained as a yellow viscous liquid in 27.1 mg. Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether (3 runs) showed one major UV-active spot with R_f value of 0.11 which was identical to **DT3**.

Band 2 was obtained as a yellow viscous liquid in 4.2 mg. Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether (3 runs) showed two well-separated spots under UV-S with R_f values of 0.05 and 0.13. No further investigation was performed because it was obtained in low quantity.

Band 3 was obtained as a yellow viscous liquid in 2.2 mg. Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether (3 runs) showed one major yellow spot with R_f value of 0.07. No attempted purification was carried out because of low quantity.

Subfraction H2.8 Chromatogram characteristics on normal phase TLC with 80% chloroform-petroleum ether showed two green spots with R_f values of 0.14 and 0.41. It was therefore not further investigated.

Subfraction H2.9 Chromatogram characteristics on normal phase TLC with 80% chloroform-petroleum ether showed one green spot with R_f value of 0.15. ^1H NMR (60 MHz) spectrum demonstrated majority of signals at high field. Therefore, no further purification was carried out.

Fraction H3 Chromatogram characteristics on normal phase TLC with 70% chloroform-petroleum ether demonstrated many well-separated spots under UV-S. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 50% chloroform-petroleum ether, gradually enriched with chloroform and followed by increasing amount of methanol in chloroform and finally with pure methanol to give sixty four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine fractions as shown in Table 17.

Table 17 Fractions obtained from **H3** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
H3.1	0.034	Yellow viscous liquid
H3.2	0.041	Yellow viscous liquid
H3.3	0.012	Yellow solid
H3.4	0.113	Red-violet solid
H3.5	0.061	Yellow solid
H3.6	0.364	Orange-yellow viscous liquid
H3.7	0.465	Green viscous liquid
H3.8	0.845	Green viscous liquid
H3.9	0.813	Green viscous liquid

Subfraction H3.1 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed two pale spots under UV-S with R_f values of 0.58 and 0.72. It was therefore not further investigated.

Subfraction H3.2 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether showed one pale spot under UV-S with R_f value of 0.48. Therefore, no further purification was carried out.

Subfraction H3.3 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether (3 runs) showed many spots under UV-S. Because of low quantity of this fraction, it was not further investigated.

Subfraction H3.4 Chromatogram characteristics on normal phase TLC with 50% chloroform-petroleum ether (3 runs) showed one UV-active and one violet spot with R_f values of 0.51 and 0.47, respectively. Therefore, no further investigation was performed because of limitation of time.

Subfraction H3.5 Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether showed one major spot under UV-S with R_f value of 0.37. It was not further investigated because of limitation of time.

Subfraction H3.6 Chromatogram characteristics on normal phase TLC with 60% chloroform-petroleum ether showed one UV-active spot with R_f value of 0.26. This fraction became solid upon standing at room temperature. It was then washed with methanol to afford two portions.

Portion 1 (MeOH-insoluble fraction) was obtained as a white solid in 205.2 mg. Chromatogram characteristics on normal phase TLC with 70% chloroform-petroleum ether (3 runs) showed one UV-active spot with R_f value of 0.27 and the other component with R_f value of 0.21 as a blue spot after dipping in ANH and subsequently heating the TLC plate. It was further separated by chromatotron with silica gel plate (2mm thickness). Elution was conducted with 1% methanol-chloroform to 10% methanol-chloroform to afford three fractions as shown in Table 18.

Table 18 Fractions obtained from **portion 1** by chromatotron

Fraction	Weight (g)	Physical appearance
1.1	0.012	White solid
1.2	0.182	Pale yellow solid
1.3	0.005	Pale yellow solid

Fraction 1.1 (DT3) Chromatogram characteristics on normal phase TLC with chloroform (3 runs) showed one pure spot under UV-S with R_f value of 0.29.

IR (neat) ν (cm^{-1})	3451 (OH stretching), 2935 (CH stretching), 1676 (C=C stretching)
^{1}H NMR (CDCl$_3$) (δ ppm) (500 MHz)	5.74 (*m*, 1H), 2.47-2.35 (*m*, 3H), 2.29 (*ddt*, *J* = 15.0, 4.0, 2.0, 1H), 2.07-2.00 (*m*, 2H), 1.91-1.83 (*m*, 2H), 1.74-1.68 (*m*, 2H), 1.68-1.58 (*m*, 3H), 1.58-1.50 (*m*, 3H), 1.50-1.40 (*m*, 1H), 1.40-1.34 (*m*, 2H), 1.34-1.21 (*m*, 3H), 1.20 (*s*, 3H), 1.18-1.10 (*m*, 4H), 1.10-1.00 (*m*, 3H), 1.00-0.90 (*m*, 1H), 0.94 (*d*, *J* = 6.3 Hz, 3H), 0.88 (*d*, *J* = 2.1 Hz, 3H), 0.84 (*d*, *J* = 4.2 Hz, 3H), 0.82 (*s*, 3H), 0.72 (*s*, 3H)

Fraction 1.2 (DT1) Chromatogram characteristics on normal phase TLC with chloroform (3 runs) showed one blue spot with R_f value of 0.26 after dipping in ANH and subsequently heating the TLC plate.

Melting point	135.0-136.0 °C
IR (neat) ν (cm^{-1})	3286 (OH stretching), 2943 (CH stretching), 1664 (C=C stretching)
^{1}H NMR (CDCl$_3$) (δ ppm) (500 MHz)	5.35 (*m*, 1H), 3.56-3.49 (*m*, 1H), 2.30 (*ddd*, *J* = 12.9, 5.3, 2.1 Hz, 1H), 2.28-2.21 (*m*, 1H), 2.05-1.95 (*m*, 3H), 1.88- 1.82 (*m*, 2H), 1.70-1.62 (*m*, 3H), 1.60-1.41 (*m*, 11H), 1.30-1.04 (*m*, 11H), 1.02 (*s*, 3H), 0.94 (*d*, *J* = 6.5 Hz, 3H), 0.86 (*d*, *J* = 2.2 Hz, 3H), 0.84 (*d*, *J* = 4.4 Hz, 3H), 0.82 (*s*, 3H), 0.70 (*s*, 3H)
^{13}C NMR (CDCl$_3$) (δ ppm) (125 MHz)	141.51, 139.01, 129.98, 122.32, 72.17, 57.10, 56.40, 50.45, 46.12, 42.56, 40.02, 37.49, 36.72, 36.36, 34.17, 32.11, 31.86, 29.37, 28.41, 26.30, 24.45, 23.23, 21.23, 19.93, 19.50, 19.17, 18.90, 12.06, 11.94

DEPT (135 °) ($CDCl_3$)	CH	122.32, 72.17, 57.10, 56.40, 50.45, 46.12, 36.36, 31.86, 29.37
	CH_2	42.56, 40.02, 37.49, 34.17, 32.11, 29.37, 26.30, 24.45, 23.23, 21.23
	CH_3	19.93, 19.50, 19.17, 18.90, 12.06, 11.94

Fraction 1.3 Chromatogram characteristics on normal phase TLC with chloroform (3 runs) showed none of well-separated spots under UV-S. No attempted purification was performed.

Portion 2 (MeOH soluble fraction) was obtained as a yellow-white solid. Chromatogram characteristics on normal phase TLC with 70% chloroform-petroleum ether (3 runs) indicated the presence of **DT1** and **DT3** as major components.

Subfraction H3.7 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction H3.8 Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform showed many well-separated spots under UV-S. Further separation with column chromatography over silica gel was carried out. Elution was conducted initially with 50% chloroform-petroleum ether, gradually enriched with chloroform and followed by increasing amount of methanol in chloroform and finally with 10% methanol-chloroform to give fifteen fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions.

Subfraction 1 was obtained as a green-yellow viscous liquid in 11.4 mg. Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform showed one green spot with R_f value of 0.52 which was expected to be chlorophyll. It was therefore not further investigated.

Subfraction 2 (I) was obtained as a green viscous liquid in 913.4 mg. Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform showed three well-separated spots under UV-S with R_f values of 0.32, 0.41 and 0.75. Further separation with column chromatography over silica gel was performed. Elution was conducted with chloroform and gradually enriched with 10% methanol-chloroform to give eighty nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford eight subfractions as shown in Table 19.

Table 19 Fractions obtained from **I** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
I1	0.015	Green viscous liquid
I2	0.028	Green viscous liquid
I3	0.062	Green viscous liquid
I4	0.002	Dark green solid
I5	0.108	Green solid
I6	0.066	Green solid
I7	0.140	Green solid
I8	0.384	Dark green viscous liquid

Subfraction I1 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed many spots under UV-S. It was therefore not further investigated.

Subfraction I2 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) demonstrated many spots without major spots under UV-S. No further purification was performed.

Subfraction I3 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed two major spots under UV-S with R_f values of 0.16 and 0.39. Further purification by precoated TLC on silica gel plates with 1% methanol-chloroform as a mobile phase afforded two bands.

Band 1 was obtained as a green solid in 30.4 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed one major UV-active spot with R_f value of 0.48 and the other component with R_f value of 0.54 as a green spot after dipping in ANH and subsequently heating the TLC plate. It was not further investigated because of limitation of time.

Band 2 was obtained as a yellow viscous liquid in 11.3 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed one major spot with R_f value of 0.20 under UV-S and as a brown spot after dipping in ANH and subsequently heating the TLC plate. Because this component was obtained in low quantity, it was not further investigated.

IR (neat) ν (cm^{-1})	3407 (OH stretching), 2923, 2852 (CH stretching), 1667 (C=O stretching), 1630, 1602 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm)	328
^1H NMR ($CDCl_3$) (δ ppm) (500 MHz)	11.00 (*s*, 1H), 7.55 (*dd*, J = 8.5, 7.5 Hz, 1H), 7.04 (*d*, J = 7.5 Hz, 1H), 7.00 (*d*, J = 8.5 Hz, 1H), 4.64-4.57 (*m*, 2H), 1.52 (*d*, J = 6.2 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	168.42, 162.04, 141.11, 136.86, 117.85, 116.16, 106.65, 79.90, 69.18, 31.91, 22.67, 17.91

Subfraction I4 Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform showed one green spot. It was not further investigated.

Subfraction I5 Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed under UV-S one major spot with R_f value of 0.35 and other two spots with R_f values of 0.08 and 0.15. Because of limitation of time, no further purification was performed.

Subfraction I6 Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed one major spot with the same R_f value as that of **I5**. It was not further investigated.

Subfraction I7 Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed two well-separated spots under UV-S with R_f values of 0.30 and 0.40. It was separated by precoated TLC on silica gel plates with 2% methanol-chloroform as a mobile phase to yield two bands.

Band 1 was obtained as a green viscous liquid in 22.6 mg. Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform

(2 runs) showed one major spot under UV-S with R_f value of 0.44. No further investigation was carried out.

Band 2 was obtained as a green viscous liquid in 14.3 mg. Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed one major UV-active spot with R_f value of 0.36. No further investigation was performed.

Subfraction I8 Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform showed none of well-separated spots under UV-S. Therefore, no further purification was carried out.

Subfraction H3.9 Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform and 4% methanol-chloroform demonstrated none of well-separated spots under UV-S. No attempted purification was carried out.

Fraction H4 Chromatogram characteristics on normal phase TLC with chloroform showed one major yellow spot under UV-S with R_f value of 0.27. Further purification by column chromatography over silica gel was carried out. Elution was conducted with chloroform and gradually enriched with methanol in chloroform and finally with pure methanol to give twenty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions as shown in Table 20.

Table 20 Fractions obtained from **H4** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
H4.1	0.013	Green-yellow viscous liquid
H4.2	0.008	Green viscous liquid
H4.3	0.037	Brown-green viscous liquid
H4.4	0.030	Yellow-green solid
H4.5	0.428	Brown-yellow viscous liquid

Subfraction H4.1 Chromatogram characteristics on normal phase TLC with chloroform showed no major spots under UV-S. It was therefore not further investigated.

Subfraction H4.2 Chromatogram characteristics on normal phase TLC with chloroform showed one major green spot. It was therefore not further investigated.

Subfraction H4.3 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform demonstrated three well-separated spots under UV-S with R_f values of 0.08, 0.18 and 0.38. Further separation with precoated TLC on silica gel plates with 1% methanol-chloroform afforded four bands.

<u>Band 1</u> (**DT2**) was obtained as a yellow viscous liquid in 7.8 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed one yellow spot with R_f value of 0.38. However, It was decomposed at room temperature.

^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	6.83 (*s*, 1H), 6.30 (*s*, 1H), 5.28 (*m*, 2H), 4.10 (*d*, J = 6.0 Hz, 2H), 3.45 (*d*, J = 8.0 Hz, 2H), 3.81 (*s*, 3H), 1.84 (*s*, 3H), 1.83 (*s*, 3H), 1.77 (*s*, 3H), 1.69 (*s*, 3H)

<u>Band 2</u> was obtained as a yellow solid in 0.8 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed one UV-active spot with R_f value of 0.18. Because it was obtained in low quantity, it was not further investigated.

<u>Band 3</u> was obtained as a yellow viscous liquid in 3.0 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed one UV-active spot with R_f value of 0.08. No further investigation was carried out because of low quantity of this fraction.

<u>Band 4</u> was obtained as a yellow viscous liquid in 1.1 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed no major spots under UV-S. It was not further investigated.

Subfraction H4.4 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (2 runs) showed three pale spots under UV-S with R_f values of 0.26, 0.30 and 0.36. Because of low quantity of this fraction, it was not further investigated.

Subfraction H4.5 Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed none of well-separated spots under UV-S. No attempted purification was performed.

Fraction H5 Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform showed three major spots under UV-S with R_f values of 0.02, 0.04 and 0.12. Because of limitation of time, it was not further investigated.

Fraction H6 Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform demonstrated three major spots under UV-S with R_f values of 0.02, 0.14 and 0.21. No further purification was carried out because of limitation of time.

Fraction H7 Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform showed none of well-separated spots under UV-S. It was not further investigated because of limitation of time.

The Third Investigation

The crude methanol extract (127.86 g) was dissolved with ether. The ether-soluble fraction (**J**) was evaporated to dryness under reduced pressure to yield a green viscous liquid in 23.00 g. Further separation with column chromatography over silica gel was performed. Elution was conducted with 50% chloroform-petroleum ether, gradually enriched with chloroform and followed by increasing amount of methanol in chloroform and finally with 40% methanol-chloroform to give thirty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford eight subfractions as shown in Table 21.

Table 21 Fractions obtained from **J** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
J1	2.880	Orange-yellow liquid
J2	1.260	Red-orange viscous liquid
J3	0.440	Orange viscous liquid

Table 21 (continued)

Fraction	Weight (g)	Physical appearance
J4	0.960	Yellow-orange viscous liquid
J5	2.260	Yellow-orange viscous liquid
J6	8.890	Yellow-orange viscous liquid
J7	1.340	Green viscous liquid
J8	2.330	Black-green viscous liquid

Fraction J1 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform showed one major UV-active spot with R_f value of 0.68 and a violet spot with R_f value of 0.76 after dipping in ANH and subsequently heating the TLC plate. It was therefore not further investigated because of limitation of time.

Fraction J2 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform showed one major spot under UV-S with R_f value of 0.52 and a violet spot with R_f value of 0.32 after dipping in ANH and subsequently heating the TLC plate. No further purification was carried out because of limitation of time.

Fraction J3 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform showed one UV-active spot with R_f value of 0.62 and other two spots with R_f values of 0.38 as a violet spot and 0.68 as a blue spot after dipping in ANH and subsequently heating the TLC plate. It was therefore not further investigated.

Fraction J4 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform demonstrated three UV-active spots with R_f values of 0.36, 0.46 and 0.60. In addition, it showed other three spots with R_f values of 0.25 (violet), 0.38 (violet) and 0.45 (yellow) after dipping in ANH and subsequently heating the TLC plate. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 10% ethyl acetate-petroleum ether and gradually enriched with 15% ethyl acetate-petroleum ether to give eleven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions as shown in Table 22.

Table 22 Fractions obtained from **J4** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
J4.1	0.183	Yellow-viscous liquid
J4.2	0.150	Red-orange viscous liquid
J4.3	0.069	Red-orange viscous liquid
J4.4	0.036	Yellow viscous liquid
J4.5	0.111	Pale yellow solid
J4.6	0.004	Yellow viscous liquid

Subfraction J4.1 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed three UV-active spots with R_f values of 0.64, 0.72 and 0.78. In addition, it showed other two spots with R_f values of 0.62 (yellow) and 0.75 (violet) after dipping in ANH and subsequently heating the TLC plate. No further purification was carried out because of limitation of time.

Subfraction J4.2 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) was similar to that of **J4.1**. It showed two additional spots with R_f values of 0.56 (violet) and 0.68 (violet) after dipping in ANH and subsequently heating the TLC plate. It was not further investigated because of limitation of time.

Subfraction J4.3 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) demonstrated one UV-active spot with R_f value of 0.65. In addition, it showed other two spots with R_f values of 0.49 (violet) and 0.58 (violet) after dipping in ANH and subsequently heating the TLC plate. Because of low quantity, no further purification was performed.

Subfraction J4.4 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed two UV-active spots with R_f values of 0.46 and 0.51. In addition, it showed other two spots with R_f values of 0.34 (violet) and 0.58 (violet) after dipping in ANH and subsequently heating the TLC plate. Because of low quantity, no further purification was performed.

Subfraction J4.5 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed one UV-active spot with R_f value of 0.28. In addition, it showed other two spots with R_f values of 0.35 and 0.75 as violet after dipping in ANH and subsequently heating the TLC plate. Further separation with precoated TLC on silica gel plates with 10% ethyl acetate-petroleum ether (3 runs) as a mobile phase afforded three bands.

Band 1 was obtained as a yellow viscous liquid in 4.0 mg. Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed one UV-active spot with R_f value of 0.26 and the other spot with R_f value of 0.33 as a blue spot after dipping in ANH and subsequently heating the TLC plate.

Band 2 was obtained as a yellow viscous liquid in 11.1 mg. Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) indicated the presence of **DT1** as a pure component.

Band 3 (**DT4**) was obtained as a yellow viscous liquid in 36.9 mg. Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed one pure spot with R_f value of 0.79 which appeared as a blue-violet spot after dipping in ANH and subsequently heating the TLC plate.

$[\alpha]_D^{29}$	+29.85° (c = 13.4 x 10^{-2} g/100 cm^3, $CHCl_3$)
IR (neat) ν (cm^{-1})	2920 (CH stretching), 1727, 1694 (C=O stretching)
^1H NMR ($CDCl_3$) (δ ppm) (500 MHz)	5.28 (*t*, J = 4.5 Hz, 1H), 4.50 (*dd*, J = 10.0, 7.0 Hz, 1H), 2.82 (*dd*, J = 14.0, 4.5 Hz, 1H), 2.28 (*t*, J = 7.0 Hz, 2H), 2.00-1.95 (*m*, 2H), 1.94-1.83 (*m*, 2H), 1.82-1.70 (*m*, 1H), 1.75-1.64 (*m*, 1H), 1.67-1.55 (*m*, 2H), 1.66-1.56 (*m*, 1H), 1.66-1.54 (*m*, 2H), 1.64-1.52 (*m*, 2H), 1.62-1.51 (*m*, 2H), 1.62-1.52 (*m*, 1H), 1.57-1.47 (*m*, 1H), 1.50-1.40 (*m*; 1H), 1.44-1.32 (*m*, 1H), 1.40-1.28 (*m*, 1H), 1.32-1.30 (*m*, 1H), 1.30-1.21 (*m*, 2H), 1.28-1.24 (*m*, 2H), 1.26-1.18 (*m*, 1H), 1.25 (*brs*, 20H), 1.20-1.10 (*m*, 1H), 1.14-1.02 (*m*, 1H), 1.13 (*s*, 3H), 0.94 (*s*, 3H). 0.93 (*s*, 3H), 0.91 (*s*, 3H), 0.90-0.78

		(*m*, 1H), 0.88 (*t*, *J* = 7.0 Hz, 3H), 0.86 (*s*, 3H), 0.85 (*s*, 3H), 0.76 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		183.75, 173.83, 143.69, 122.67, 80.66, 55.40, 47.65, 46.64, 45.93, 41.66, 41.05, 39.39, 38.17, 37.84, 37.09, 34.97, 33.89, 33.16, 32.64, 32.53, 32.04, 30.77, 29.82, 29.80, 29.78, 29.77, 29.75, 29.70, 29.59, 29.48, 29.37, 29.29, 28.16, 27.77, 26.02, 25.29, 23.68, 23.66, 23.50, 23.00, 22.81, 18.28, 17.23, 16.83, 15.48, 14.23
DEPT (135 °) ($CDCl_3$)	CH	122.67, 80.66, 55.40, 47.65, 41.05
	CH_2	45.93, 38.17, 34.97, 33.89, 32.64, 32.53, 32.04, 29.82, 29.80, 29.78, 29.77, 29.75, 29.70, 29.59, 29.48, 29.37, 29.29, 27.77, 25.29, 23.66, 23.50, 23.00, 22.81, 18.28
	CH_3	33.16, 28.16, 26.02, 23.68, 17.23, 16.83, 15.48, 14.23

Subfraction J4.6 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed two violet spots with R_f values of 0.56 and 0.70 after dipping in ANH and subsequently heating the TLC plate. Because of low quantity of this fraction, it was not further investigated.

Fraction J5 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane showed five well-separated spots under UV-S with R_f values of 0.44, 0.50, 0.58, 0.65 and 0.73. In addition, it showed the other spot with R_f value of 0.34 as a violet spot after dipping in ANH and subsequently heating the TLC plate. Further separation with column chromatography over silica gel was performed. Elution was conducted with 1% methanol-dichloromethane to afford eleven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford ten subfractions as shown in Table 23.

Table 23 Fractions obtained from **J5** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
J5.1	0.007	Yellow viscous liquid
J5.2	0.023	Green viscous liquid
J5.3	0.030	Red-violet viscous liquid
J5.4	0.058	Brown-green viscous liquid
J5.5	0.374	Brown-green viscous liquid
J5.6	0.258	Yellow-green viscous liquid
J5.7	0.656	Yellow solid
J5.8	0.300	Green viscous liquid
J5.9	0.137	Brown-green viscous liquid
J5.10	0.008	Yellow viscous liquid

Subfraction J5.1 Chromatogram characteristics on normal phase TLC with chloroform showed one violet spot with R_f value of 0.83 after dipping in ANH and subsequently heating the TLC plate. Because of low quantity of this fraction, it was not further investigated.

Subfraction J5.2 Chromatogram characteristics on normal phase TLC with chloroform showed two violet spots with R_f values of 0.73 and 0.86 after dipping in ANH and subsequently heating the TLC plate. Therefore, no further purification was carried out.

Subfraction J5.3 Chromatogram characteristics on normal phase TLC with chloroform showed one oval violet spot with R_f value of 0.64 after dipping in ANH and subsequently heating the TLC plate. It was therefore not further investigated.

Subfraction J5.4 Chromatogram characteristics on normal phase TLC with chloroform showed three pale UV-active spots with R_f values of 0.45, 0.51 and 0.60. The highest R_f spot became a violet spot when TLC plate was dipped in ANH and subsequently heated. No attempted purification was carried out.

Subfraction J5.5 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether demonstrated two UV-active spots with R_f values of 0.21 and 0.59. In addition, TLC plate showed other violet spot with R_f value of

0.54 after dipping in ANH and subsequently heating the TLC plate. It was further purified by column chromatography over silica gel. Elution was conducted with 10% ethyl acetate-petroleum ether to 40% ethyl acetate-petroleum ether afforded forty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford ten subfractions as shown in Table 24.

Table 24 Fractions obtained from **J5.5** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
J5.5.1	0.018	Green viscous liquid
J5.5.2	0.040	Yellow viscous liquid
J5.5.3	0.129	Orange viscous liquid
J5.5.4	0.047	Orange viscous liquid
J5.5.5	0.027	Brown viscous liquid
J5.5.6	0.039	Green viscous liquid
J5.5.7	0.012	Green viscous liquid
J5.5.8	0.022	Green viscous liquid
J5.5.9	0.013	Green viscous liquid
J5.5.10	0.020	Green viscous liquid

Subfraction J5.5.1 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one major UV-active spot with R_f value of 0.63. It was not further investigated because of limitation of time.

Subfraction J5.5.2 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether demonstrated one pale UV-active spot with R_f value of 0.55. It was not further investigated because of limitation of time.

Subfraction J5.5.3 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one pale UV-active spot with R_f value of 0.50. It was not further investigated because of limitation of time.

Subfraction J5.5.4 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one pale UV-active spot with R_f value of 0.55. It was not further investigated because of limitation of time.

Subfraction J5.5.5 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed two pale UV-active spots with R_f values of 0.50 and 0.54. It was not further investigated because of limitation of time.

Subfraction J5.5.6 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one pale UV-active spot with R_f value of 0.50. It was not further investigated because of limitation of time.

Subfraction J5.5.7 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed two pale UV-active spots with R_f values of 0.19 and 0.26. It was not further investigated because of limitation of time.

Subfraction J5.5.8 (**DT6**) Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether demonstrated one major UV-active spot with R_f value of 0.23 and the other green spot with R_f value of 0.39. It was further purified by precoated TLC on silica gel plates with 20% ethyl acetate-petroleum ether (2 runs) to yield a yellow viscous liquid in 6.2 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one UV-active spot with R_f value of 0.38.

IR (neat) ν (cm^{-1})	3413 (OH stretching), 2954 (CH stretching), 1714 (C=O stretching), 1597, 1515 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)	304 (2.97)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	7.64 (*dd*, J = 7.7, 1.4 Hz, 1H), 7.55 (*d*, J = 1.4 Hz, 1H), 6.93 (*d*, J = 7.7 Hz, 1H), 6.00 (*brs*, 1H), 3.94 (*s*, 3H), 3.88 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	166.98, 150.10, 146.25, 124.30, 122.39, 114.16, 111.82, 56.21, 52.08

Subfraction J5.5.9 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one major UV-active spot with R_f

value of 0.19. Further separation with precoated TLC on silica gel plates with 20% ethyl acetate-petroleum ether (2 runs) afforded a yellow-green viscous liquid in 1.8 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one pale spot under UV-S with R_f value of 0.38. It was not further investigated because it was obtained in low quantity.

Subfraction J5.5.10 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one major UV-active spot with R_f value of 0.15. It was therefore not further investigated because of limitation of time.

Subfraction J5.6 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed two UV-active spots with R_f values of 0.14 and 0.23. In addition, it revealed a mixture of **DT1**, **DT3** and **DT4** as major components after dipping in ANH and subsequently heating the TLC plate. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 15% ethyl acetate-petroleum ether and gradually enriched with 40% ethyl acetate-petroleum ether to give sixty nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford ten subfractions as shown in Table 25.

Table 25 Fractions obtained from **J5.6** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
J5.6.1	0.006	Yellow viscous liquid
J5.6.2	0.080	Yellow viscous liquid
J5.6.3	0.024	Yellow viscous liquid
J5.6.4	0.022	Yellow viscous liquid
J5.6.5	0.038	Yellow viscous liquid mixed with white solid
J5.6.6	0.010	Colorless viscous liquid
J5.6.7	0.008	Yellow viscous liquid
J5.6.8	0.036	Green viscous liquid
J5.6.9	0.019	Green viscous liquid
J5.6.10	0.012	Yellow viscous liquid

Subfraction J5.6.1 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed a few pale UV-active spots. Therefore, no further purification was performed.

Subfraction J5.6.2 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether indicated the presence of **DT3** as a major component.

Subfraction J5.6.3 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed three major spots after dipping in ANH and subsequently heating the TLC plate with R_f values of 0.44 (violet), 0.51 (pink) and 0.58 (UV-active) indicating that it was a mixture of **DT1** (R_f value of 0.44) and **DT3** (R_f value of 0.58).

Subfraction J5.6.4 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed one major UV-active spot (violet) with R_f value of 0.49 and it became a violet spot when TLC plate was dipped in ANH and subsequently heated. Because of limitation of time, it was not further investigated.

Subfraction J5.6.5 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed three major spots with R_f values of 0.42 as a violet spot, 0.51 as a blue spot and 0.73 as a violet spot after dipping in ANH and subsequently heating the TLC plate. Therefore, no further purification was carried out.

Subfraction J5.6.6 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether demonstrated one major component with R_f value of 0.73 as a violet spot after dipping in ANH and subsequently heating the TLC plate. Because of low quantity of this fraction, no further purification was performed.

Subfraction J5.6.7 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one pale UV-active spot with R_f value of 0.27. It was not further investigated.

Subfraction J5.6.8 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether indicated the presence of **DT4** as a major component.

Subfraction J5.6.9 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether indicated the presence of **DT4** as a major component and the other yellow spot with R_f value of 0.18. Further separation with precoated TLC on silica gel plates with 20% ethyl acetate-petroleum ether (2 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a yellow viscous liquid in 4.6 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether indicated the presence of **DT4** as a pure component.

Band 2 (**DT5**) was obtained as a yellow-white solid in 5.1 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one yellow spot with R_f value of 0.15.

IR (neat) ν (cm^{-1})	3362 (OH stretching), 2849 (CH stretching), 1674 (C=O stretching), 1592, 1515 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)	324 (2.85)
^1H NMR ($CDCl_3$) (δ ppm) (500 MHz)	9.80 (*s*, 1H), 7.43 (*dd*, J = 8.5, 1.5 Hz, 1H), 7.42 (*d*, J = 1.5 Hz, 1H), 7.04 (*d*, J = 8.5 Hz, 1H), 6.20 (*brs*, 1H), 3.96 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	190.87, 151.65, 147.12, 129.89, 127.53, 114.35, 108.72, 56.11

Subfraction J5.6.10 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed no spots under UV-S and after dipping in ANH and subsequently heating the TLC plate. It was not further investigated.

Subfraction J5.7 and J5.8 Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed two major spots of **DT1** and **DT4**.

Subfraction J5.9 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform showed two major spots with R_f values of 0.65 and 0.78 as violet spots after dipping in ANH and subsequently heating the TLC plate. It was therefore not further investigated.

Subfraction J5.10 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform showed no major spots under UV-S and after dipping in ANH and subsequently heating the TLC plate. Therefore, no attempted further purification was carried out.

Fraction J6 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform demonstrated many spots under UV-S. No attempted investigation was performed.

Fraction J7 and J8 Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform showed no spots from baseline. It was not further investigated.

CHAPTER 1.3

RESULTS AND DISCUSSION

Chemical investigation of *Dendrophthoe pentandra* was divided into two parts. The first part involved isolation, purification and structure elucidation of compounds from its leaves while the second part dealed with the compounds isolated from its twigs. Upon chromatographic separation, the crude extract of leaves yielded three known flavone rhamnosides (**DW1**, **DW2** and **DW3**) while the crude extract of twigs afforded three known compounds : one steroid (**DT1**) and two aromatic compounds (**DT5** and **DT6**), one new oleanolic acid derivative (**DT4**) and two unidentified compounds (**DT2** and **DT3**). **DT2** was decomposed upon standing at room temperature while **DT3** was a mixture of a triterpene and stigmasterol. The structures were established by spectroscopic evidence, especially 1D and 2D NMR spectral data. The ^{13}C NMR signals were assigned from DEPT, HMQC and HMBC spectra.

1.3.1 Structural determination of compounds isolated from leaves of D. pentandra L.

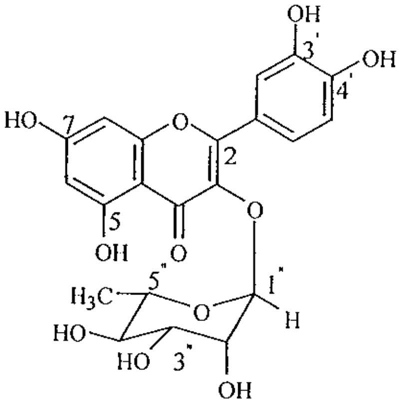
Compound DW1

DW1 was obtained as a yellow solid, melting at 178-182 °C with $[\alpha]_D^{25}$ -119.04°. The UV spectrum (Figure 2) with absorption bands at 347, 257 and 213 nm was typical for a compound of flavone type (Markham, 1982). The IR spectrum (Figure 3) exhibited absorption bands for a hydroxyl functional group (3300 cm^{-1}), a conjugated and chelated carbonyl group (1651 cm^{-1}) and an aromatic ring (1600, 1560 cm^{-1}). The aromatic region of the ^{1}H NMR spectrum (Table 26) (Figure 4) showed an ABX system at δ_H 7.33 (*d*, J = 2.4 Hz, H-2$'$), δ_H 6.90 (*d*, J = 8.4 Hz, H-5$'$) and δ_H 7.28 (*dd*, J = 8.4 and 2.4 Hz, H-6$'$) and an AX system at δ_H 6.22 (*d*, J = 2.4 Hz, H-6) and δ_H 6.42 (*d*, J = 2.4 Hz, H-8). It also revealed the presence of the chelated hydroxyl group at δ_H 12.70 (5-OH). The proton chemical shifts and splitting patterns of aromatic protons indicated the presence of 3′, 4′, 5, 7-tetraoxyflavone moiety while the characteristic *doublet* signal at δ_H 0.85 together with five oxymethine protons [δ_H 5.38 (*d*, J = 1.8 Hz, 1H), 4.24 (*dd*, J = 3.4, 1.8 Hz, 1H), 3.77 (*dd*, J = 9.4, 3.4 Hz, 1H), 3.47-3.41 (*m*, 1H) and 3.38-3.32 (*m*, 1H)] revealed the presence of one rhamnose unit. A *doublet* with coupling constant of 1.8 Hz at δ_H 5.38 was attributed to the H-1″ of the rhamnose unit. Furthermore, this small coupling constant for the anomeric proton suggested α-configuration for the rhamnose unit (Agrawal, 1992).

Table 26 The NMR spectral data of **DW1**

Position	δ_H	δ_C	Type of C	HMBC
2	-	157.04	C	
3	-	133.93	C	
4	-	177.46	C	
4a	-	103.83	C	
5	-	161.00	C	
6	6.22 (*d*, 2.4)	98.43	CH	C-4, C-5, C-7, C-8, C-8a
7	-	163.88	C	
8	6.42 (*d*, 2.4)	93.38	CH	C-4, C-4a, C-6, C-7, C-8a
8a	-	156.19	C	
1′	-	120.49	C	
2′	7.33 (*d*, 2.4)	115.37	CH	C-2, C-3′, C-4′, C-6′
3′	-	144.87	C	
4′	-	148.12	C	
5′	6.90 (*d*, 8.4)	115.17	CH	C-2, C-2′, C-3′, C-4′, C-6′
6′	7.28 (*dd*, 8.4, 2.4)	120.85	CH	C-2, C-2′, C-3′, C-4′
Rha-1″	5.38 (*d*, 1.8)	101.48	CH	C-3, C-3″, C-5″
2″	4.24 (*dd*, 3.4, 1.8)	70.10	CH	
3″	3.77 (*dd*, 9.4, 3.4)	69.76	CH	
4″	3.38-3.32 (*m*)	70.92	CH	C-2″, C-4″
5″	3.47-3.41 (*m*)	70.28	CH	
6″	0.85 (*d*, 6.1)	17.14	CH_3	C-4″, C-5″
5-OH	12.70 (*s*)	-	-	

The HMBC data (Table 26) (Figure 8) showed a correlation of the H-1″ (δ_H 5.38) of the rhamnose unit with the C-3 (δ_C 133.93) of the flavone moiety, indicating that the rhamnose unit was linked to the hydroxyl group on the C-3. **DW1** was therefore assigned as quercetin 3-*α*-rhamnoside which was isolated from blue flowers of *Nymphaea caerulea* (Fossen, *et al.*, 1999).



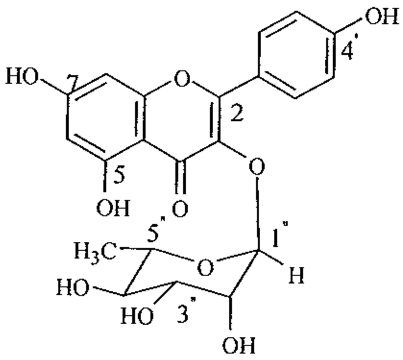
Compound DW2

DW2 was obtained as a yellow solid with $[\alpha]_D^{25}$ −104.09^0. The UV spectrum (Figure 9) with absorption bands at 348, 266 and 213 nm was characteristic of a flavone skeleton (Markham, 1982). The IR spectrum (Figure 10) exhibited absorption bands for a hydroxy functional group (3368 cm^{-1}), a conjugated and chelated carbonyl group (1657 cm^{-1}) and an aromatic ring (1611, 1509 cm^{-1}). The aromatic region of the ^{1}H NMR spectrum (Table 27) (Figure 11) showed an AA′XX′ system at δ_H 7.75 (*d, J* = 8.7 Hz, H-2′, H-6′) and δ_H 6.93 (*d, J* = 8.7 Hz, H-3′, H-5′) and an AX system at δ_H 6.36 (*d, J* = 2.1 Hz, H-8) and δ_H 6.22 (*d, J* = 2.1 Hz, H-6) in accordance with a kaempferol derivative. The sugar region in the ^{1}H NMR spectrum was similar to that of the *α*-rhamnosyl region of **DW1**. The presence of the *α*-rhamnose unit was confirmed by a *doublet* signal of the 6″-methyl proton at δ_H 0.92 and a *doublet* signal of the anomeric H-1″ at δ_H 5.45 (*J* = 1.8 Hz).

Table 27 The NMR spectral data of **DW2**

Position	δ_H	δ_C	Type of C	HMBC
2	-	157.28	C	
3	-	134.42	C	
4	-	177.95	C	
4a	-	104.56	C	
5	-	161.66	C	
6	6.22 (*d*, 2.1)	98.91	CH	C-4, C-4a, C-5, C-7, C-8, C-8a
7	-	164.31	C	
8	6.36 (*d*, 2.1)	93.75	CH	C-4, C-4a, C-6, C-7, C-8a
8a	-	156.74	C	
1′	-	120.88	C	
2′, 6′	7.75 (*d*, 8.7)	130.43	CH	C-2, C-3, C-1′, C-2′, 6′, C-3′, 5′, C-4′
3′, 5′	6.93 (*d*, 8.7)	115.48	CH	C-2, C-1′, C-2′, 6′, C-3′, 5′, C-4′
4′	-	160.01	C	
Rha-1″	5.45 (*d*, 1.8)	101.57	CH	C-3, C-2″, C-3″, C-5″
2″	4.23 (*dd*, 3.4, 1.8)	70.18	CH	C-1″, C-3″, C-4″
3″	3.72 (*dd*, 9.2, 3.4)	71.02	CH	C-4″, C-5″
4″	3.30-3.20 (*m*)	71.77	CH	C-2″, C-3″, C-5″
5″	3.25-3.20 (*m*)	70.41	CH	C-1″, C-3″, C-4″
6″	0.92 (*d*, 6.0)	17.30	CH$_3$	C-4″, C-5″
5-OH	12.60 (*s*)	-	-	

The connectivity between the kaempferol and the rhamnose unit was established by HMBC spectral data (Figure 15) as shown in Table 27 to be attached to the hydroxyl group on the C-3, the same position as found in **DW1**. Thus, **DW2** was assigned as kaempferol 3-*O*-α-rhamnoside. This compound has previously been isolated from the blue flowers of *Nymphaea caerulea* (Fossen, *et al*., 1999).



Compound DW3

DW3 was obtained as a yellow solid with [α $]_D^{29}$ –20.41^O. The UV spectrum (Figure 16) showed absorption bands at 348, 265 and 213 nm which were typical of a flavone type (Markham, 1982). The IR spectrum (Figure 17) exhibited absorption bands for a hydroxy functional group (3419 cm^{-1}), a conjugated and chelated carbonyl group (1652 cm^{-1}) and an aromatic ring (1599 cm^{-1}). The aromatic region of the ^1H NMR spectrum (Table 28) (Figure 18) showed an ABX system in B ring at δ_H 7.45 (*d*, *J* = 2.0 Hz, H-2$'$), δ_H 6.96 (*d*, *J* = 8.5 Hz, H-5$'$) and δ_H 7.42 (*dd*, *J* = 8.5 and 2.0 Hz, H-6$'$) and an AX system in A ring at δ_H 6.41 (*d*, *J* = 2.0 Hz, H-8) and δ_H 6.22 (*d*, *J* = 2.2 Hz, H-6) which were similar to those of **DW1**. However, an additional signal of methoxy proton at δ_H 3.95 was observed. A *doublet* signal of the 6$''$-methyl group at δ_H 0.92 with coupling constant of 6.0 Hz and a *doublet* of the anomeric proton (H-1$''$) at δ_H 5.40 with coupling constant of 1.7 Hz revealed the presence of one α-rhamnose unit.

Table 28 The NMR spectral data of **DW3**

Position	δ_H	δ_C	Type of C	HMBC
2	-	159.40	C	
3	-	136.40	C	
4	-	**	C	
4a	-	106.00	C	
5	-	**	C	
6	6.22 (*d*, 2.0)	100.00	CH	C-4a
7	-	**	C	

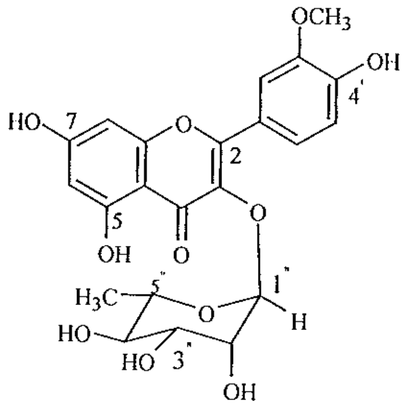
Table 28 (continued)

Position	δ_H (C_n)	δ_C (C_n)	Type of C	HMBC
8	6.41 (*d*, 2.0)	95.26	CH	C-4a, C-6, C-8a
8a	-	158.80	C	
1′	-	123.20	C	
2′	7.45 (*d*, 2.0)	113.45	CH	C-2, C-4′, C-6′
3′	-	149.00	C	
4′	-	150.95	C	
5′	6.96 (*d*, 8.5)	116.50	CH	C-1′, C-3′
6′	7.42 (*dd*, 8.5, 2.0)	124.22	CH	C-2′, C-4′
Rha-1″	5.40 (*d*, 1.7)	103.40	CH	C-3, C-3″
2″	4.20 (*dd*, 3.6, 1.7)	71.85	CH	
3″	3.74 (*dd*, 9.1, 3.6)	72.12	CH	
4″	*	73.12	CH	
5″	*	72.00	CH	
6″	0.92 (*d*, 6.0)	17.69	CH_3	C-4″, C-5″
3′-OMe	3.95 (*s*)	56.74	CH_3	

* appeared at the same position as methanol in CD_3OD

** not observed due to the low concentration of the NMR sample

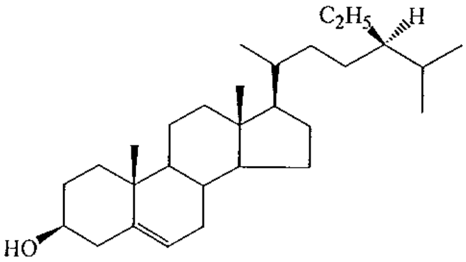
The location of rhamnose unit was established by HMBC correlation data (Table 28) (Figure 22). The anomeric proton (H-1″) of rhamnose residue showed a cross peak with the C-3 (δ_C 136.40) of the aglycone, indicating that the rhamnose unit was attached to the hydroxyl group on the C-3, the same position as found in **DW1** and **DW2**. The methoxy proton at δ_H 3.95 showed a HMBC correlation with the C-3′ (δ_C 149.00). From these data, **DW3** was identified as 5,7,4′-trihydroxy-3′-methoxyflavone 3-*O*-α-rhamnoside isolated from *Cicer mogoltavicum* (Yuldashev, 1998).



1.3.2 Structural determination of compounds isolated from twigs of D. pentandra L.

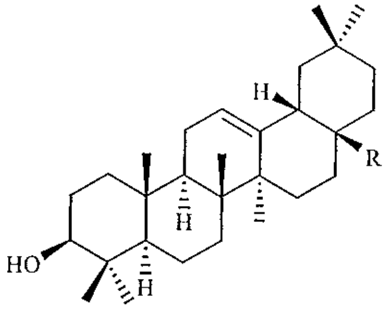
Compound DT1

DT1 was obtained as a white solid with melting point 135.0-136.0 °C. The IR spectrum (Figure 23) showed absorption bands at 3286 (O-H stretching) and 1664 cm^{-1} (C=C stretching). The ^1H and ^{13}C NMR spectra (Figures 24 and 25) were identical to those of *β*-sitosterol. **DT1** was then identified as *β*-sitosterol.



Compound DT4

DT4 was obtained as a colorless viscous liquid with $[\alpha]_D^{29}$ +29.85°. The IR spectrum (Figure 30) showed absorption bands at 1727 and 1694 cm^{-1} for carbonyl groups of ester and carboxylic acid, respectively. The presence of the carbonyl carbons at δ_C 173.83 and 183.75 in the ^{13}C NMR spectrum (Figure 32) supported the IR spectral results. The ^1H NMR spectrum (Table 29) (Figure 31) consisted of seven *singlet* methyl groups at δ_H 1.13, 0.94, 0.93, 0.91, 0.86, 0.85 and 0.76, one *triplet* methyl group at δ_H 0.88 (J = 7.0 Hz), one oxymethine proton at δ_H 4.50 (*dd*, J = 10.0 and 7.0 Hz) and one olefinic proton at δ_H 5.28 (*t*, J = 4.5 Hz). The ^{13}C NMR spectrum (Table 29) (Figure 32) showed forty six carbon atoms which were classified by DEPT experiment (Figure 33) to be eight methyl carbons, twenty four methylene carbons, five methine carbons, seven quaternary carbons and two carbonyl carbons. These data indicated that **DT4** should have an amyrin skeleton which consisted of eight tertiary methyl groups. It was then concluded that one of the eight methyl groups generally present in the amyrin moiety was converted to a carboxyl group. Furthermore, the DEPT data supported that the olefinic proton at δ_H 5.28 also belonged to a trisubstituted double bond.



R = Me : β-amyrin
R = COOH : Oleanolic acid

Table 29 The NMR spectral data of **DT4**

Position	δ_H	δ_C	Type of C	^1H-^1H COSY	HMBC
1	1.50-1.40 (*m*) 1.32-1.30 (*m*)	32.64*	CH_2		C-6
2	1.67-1.55 (*m*)	23.66	CH_2	H-3	C-31
3	4.50 (*dd*, 10.0, 7.0)	80.66	CH	H-2	C-23, C-24, C-31
4	-	37.84	C		
5	0.90-0.78 (*m*)	55.40	CH		
6	1.57-1.47 (*m*) 1.44-1.32 (*m*)	18.28	CH_2		
7	1.64-1.52 (*m*)	38.17	CH_2		C-14
8	-	39.39	C		
9	1.62-1.52 (*m*)	47.65	CH		
10	-	37.09	C		
11	1.94-1.83 (*m*)	23.50	CH_2	H-12	C-12, C-13

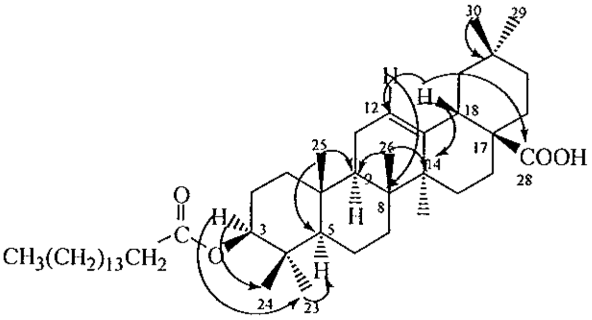
Table 29 (continued)

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
12	5.28 (*t*, 4.5)	122.67	CH	H-11	C-9, C-14
13	-	143.69	C		
14	-	41.66	C		
15	1.75-1.64 (*m*) 1.14-1.02 (*m*)	27.77	CH_2	H-16	
16	2.00-1.95 (*m*)	23.00	CH_2	H-15	C-28
17	-	46.64	C		
18	2.82 (*dd*, 14.0, 4.5)	41.05	CH	H-19	C-12, C-13, C-14, C-17, C-28
19	1.66-1.56 (*m*) 1.20-1.10 (*m*)	45.93	CH_2	H-18	C-20, C-29, C-30
20	-	30.77	C		
21	1.82-1.70 (*m*) 1.62-1.51 (*m*)	32.53*	CH_2	H-22	
22	1.40-1.28 (*m*) 1.26-1.18 (*m*)	33.89	CH_2	H-21	C-16, C-20, C-30
23	0.86 (*s*)	28.16	CH_3		C-4, C-5, C-6, C-24
24	0.85 (*s*)	16.83	CH_3		C-3, C-23
25	0.94 (*s*)	15.48	CH_3		C-5, C-9, C-10
26	0.76 (*s*)	17.23	CH_3		C-8, C-9, C-14
27	1.13 (*s*)	26.02	CH_3		C-8, C-14, C-15
28	-	183.75	C		
29	0.91 (*s*)	33.16	CH_3		C-19, C-20
30	0.93 (*s*)	23.68	CH_3		C-22
31	-	173.83	C		
32	2.28 (*t*, 7.0)	34.97	CH_2		C-31, C-34
33	1.62-1.51 (*m*) 1.28-1.24 (*m*)	32.04	CH_2		C-3
34	1.66-1.54 (*m*)	25.29	CH_2		C-31
35-44	1.25 (*brs*)	29.82, 29.80, 29.78, 29.77, 29.75,	10xCH_2		

Table 29 (continued)

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
		29.70, 29.59, 29.48, 29.37, 29.29			
45	1.67-1.55 (*m*)	23.66	CH_2		
46	0.88 (*d*, 7.0)	14.23	CH_3		

* Assignments with the same superscripts may be interchanged.



The location of the carboxyl group at the C-17 and the trisubstituted double bond at C-12/C-13 were established as shown using HMBC correlation data (Table 29) (Figure 37) starting from the observed correlations between H-3 (δ_H 4.50) and the Me-23 (δ_C 28.16) and the Me-24 (δ_C 16.83). These suggested that **DT4** contained an oleanolic acid moiety. The similarity of its ^{13}C NMR spectral data with those of the oleanolic acid (Maillard, *et al.*, 1992) (Table 30) supported this conclusion. In addition, the H-3 of **DT4** was assigned to have the same spatial rearrangement as an oleanolic acid due to its appearance as a *doublet of doublet* with large coupling constant of 10.0 and 7.0 Hz.

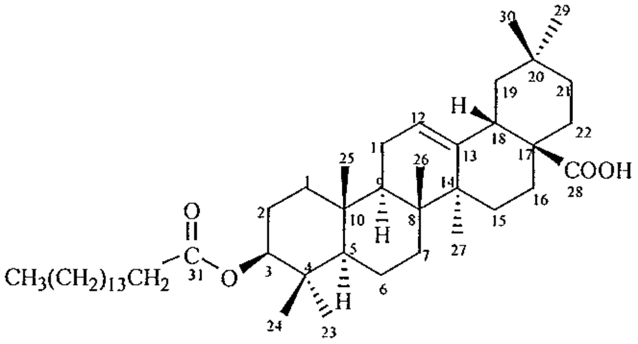
Table 30 The ^{13}C NMR spectra of **DT4** and oleanolic acid

C_n	DT4	Oleanolic acid	C_n	DT4	Oleanolic acid
1	32.64*	38.5	16	23.00	23.4
2	23.66	27.4	17	46.64	46.6
3	80.66	78.7	18	41.05	41.3
4	37.84	38.7	19	45.93	45.8
5	55.40	55.2	20	30.77	30.6
6	18.28	18.3	21	32.53*	33.8
7	38.17	32.6	22	33.89	32.3
8	39.39	39.3	23	28.16	28.1
9	47.65	47.6	24	16.83	15.6
10	37.09	37.0	25	15.48	15.3
11	23.50	23.1	26	17.23	16.8
12	122.67	122.1	27	26.02	26.0
13	143.69	143.4	28	183.75	181.0
14	41.66	41.6	29	33.16	33.1
15	27.77	27.7	30	23.68	23.6

* Assignments with the same superscripts may be interchanged.

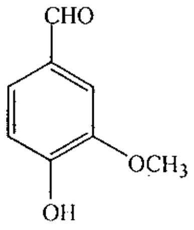
The chemical shift of the H-3 at δ_H 4.50 together with the signal of the carbonyl carbon of an ester at δ_C 173.83 suggested the presence of an ester functionality at the C-3. In the HMBC spectrum (Table 29) (Figure 37), a cross peak between the methylene proton, H-34, of the ester chain with the oxymethine carbon, C-3, also supported the attachment of ester functionality at the C-3. As the ^{13}C NMR spectra (Table 29) (Figure 32) contained, apart from the carbon skeleton of oleanolic acid, an additional fourteen methylene carbons (δ_C 34.97, 32.04, 29.82, 29.80, 29.78, 29.77, 29.75, 29.70, 29.59, 29.48, 29.37, 29.29, 25.29 and 23.66), one methyl carbon (δ_C 14.23) and a carbonyl carbon (δ_C 173.83). The ester moiety was therefore derived from palmitic acid, a natural fatty acid.

Only the relative stereochemistry between the H-18 (δ_H 2.82) and Me-30 (δ_H 0.93) was found to *cis* by the NOEDIFF results as the enhancement of the Me-30 was observed after irradiation at the H-18 (Figure 35). A 2D NOESY experiment will be required in order to establish completely relative stereochemistry. However, it is believed that the triterpene moiety of **DT4** possessed the same relative stereochemistry as oleanolic acid. Thus, **DT4** was assigned as oleanoyl palmitate, a new natural product.



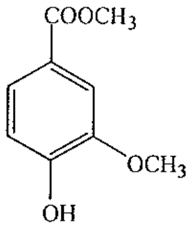
Compound DT5

DT5 was obtained as a yellow viscous liquid. The UV spectrum (Figure 38) showed an absorption band at 324 nm. Its IR spectrum (Figure 39) contained strong absorption bands at 3362 cm^{-1} (a hydroxyl group), 1674 cm^{-1} (a carbonyl group of an aromatic aldehyde) and 1592, 1515 cm^{-1} (an aromatic ring). The ^1H NMR spectrum (Figure 40) showed two *singlets* of an aldehyde proton at δ_H 9.80 and a methoxyl group at δ_H 3.96. Its also showed aromatic proton signals of 3,4-dioxybenzaldehyde as a *doublet of doublet* at δ_H 7.43 (J = 8.5, 1.5 Hz, H-6) and two sets of *doublet* at δ_H 7.42 (J = 1.5 Hz, H-2) and δ_H 7.04 (J = 8.5 Hz, H-5). An additional *broad singlet* signal at δ_H 6.20 was assigned to be a hydroxy proton. The ^{13}C NMR spectrum (Figure 41) revealed the presence of the carbonyl carbon of an aldehyde at δ_C 190.87. In NOEDIFF experiment, irradiation of the methoxy proton at δ_H 3.96 (Figure 42) enhanced only the aromatic proton signal at δ_H 7.42 (H-2). This result indicated the attachment of the methoxyl group at the C-3 which was *ortho* to the aromatic proton H-2 and the 4-hydroxyl group. Therefore, **DT5** was 4-hydroxy-3-methoxybenzaldehyde.



Compound DT6

DT6 was obtained as a yellow viscous liquid. The UV spectrum (Figure 43) showed an absorption band at 304 nm. Its IR spectrum (Figure 44) showed an absorption bands at 3413 cm^{-1} (a hydroxyl group), 1714 cm^{-1} (a carbonyl group of an ester) and 1597, 1515 cm^{-1} (an aromatic ring). The aromatic region of the ^1H NMR spectrum (Figure 45) displayed three aromatic protons at δ_H 7.64 (*dd*, J = 7.7, 1.4 Hz, H-6), δ_H 7.55 (*d*, J = 1.4 Hz, H-2) and δ_H 6.93 (*d*, J = 7.7 Hz, H-5) which were characteristic signals of a 3,4-dioxysubstituted benzene. In addition, its showed signals of two methoxyl groups and one hydroxyl group at δ_H 3.94, 3.88 and 6.00, respectively. The ^{13}C NMR spectrum (Figure 46) exhibited a carbonyl carbon of an ester group at δ_C 166.98. These indicated that **DT6** was a methyl benzoate derivative. NOEDIFF data established the location of the other methoxyl group at the C-3 because irradiation of the methoxy proton at δ_H 3.94 (Figure 47) enhanced only the aromatic proton signal at δ_H 7.55 (H-2) while irradiation of the methyl proton of an ester group at δ_H 3.88 (Figure 48) enhanced both *ortho*-proton signals at δ_H 7.55 (H-2) and 7.64 (H-6). Thus, **DT6** was identified as methyl 4-hydroxy-3-methoxybenzoate.



PART II

ISOFLAVONOID GLYCOSIDES FROM *DERRIS SCANDENS*

CHAPTER 2.1

INTRODUCTION

2.1.1 Introduction

Derris scandens Benth., a plant belonging to the Leguminosae family, is a wide-climbing shrub. Leaves are leaflets 9-18 obtuse or acute equal at the base. Flowers are small white and in very long racemes with distant nodes. About 40 species of the genus *Derris* are abundant in tropical area of the world (Hooker, 1879).

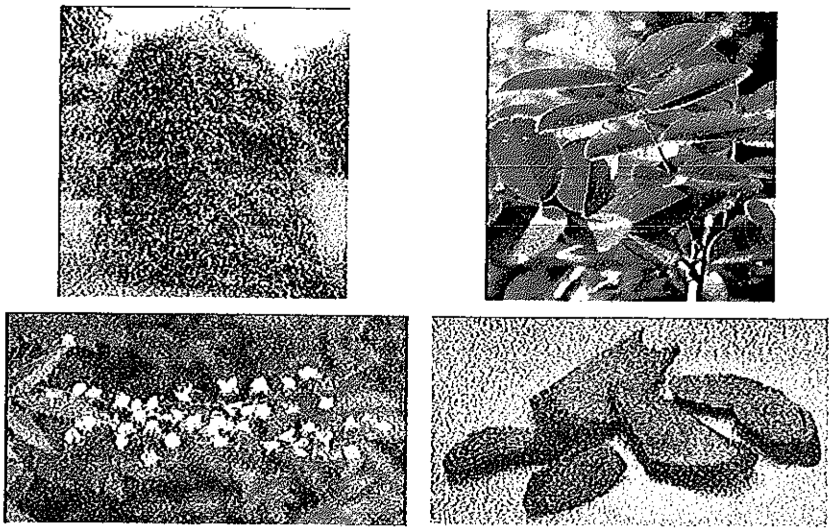


Figure 49 *Derris scandens* Benth.

(รุ่งระวี เต็มศิริฤกษ์กุล และ ชาดิชาย ศันสนียชีวิน, 2541)

D. scandens has various local names in Thai : "Thao-Wan-Priang" (เถาวัลย์เปรียง) in middle part region, "Yan-Mho" (ย่านเหมาะ) in Nakhon Si Thammarat and "Khrua-Ta-Pla" (เครือตาปลา) in Nakhon Ratchasima (วุฒิธรรมเวช, 2540).

D. scandens is a medicinal plant. Its dried stem is used in Thai traditional medicine as expectorant, antitussive, diuretic, antidysentery and for the treatment of muscle ache and pain, while the root is used as a fish poison (Chavalittumrong, *et al.*, 1999). It was recently found that warangalone, robustic acid, 8-γ,γ-dimethylallylwighteone, 3′-γ,γ-dimethylallylwighteone and nallanin, isolated from *D. scandens*, were selective and potent inhibitors of rat liver cyclic AMP-dependent protein kinase catalytic subunit (cAK) (Wang, *et al.*, 1997). In addition, 50% ethanolic extract of *D. scandens* showed marked *in vitro* immunomodulating activity in mouse splenic lymphocytes (Chuthaputti, *et al.*, 1998) while the *n*-butanol extract had hypotensive activity in the rats (Jansakul, *et al.*, 1997). Phytochemical investigation of stem and root of *D. scandens* revealed the isolation of various compounds as summarized in Table 31.

Information from NAPRALERT database, Chemical Abstracts and Dictionary of Natural Products indicated that several types of compounds have been isolated from plants belonging to *Derris* genus, as shown in Table 31.

Table 31 Compounds isolated from *Derris* species

Scientific name	Part	Compound	Structure	Bibliography
Derris sp.	-	Alpinumisoflavone	12a.1	Rocha and Zoghbi, 1982
		Alpinumisoflavone -4′-methyl-ether	12a.3	
		4′-*O*-Dimethylallylalpinumisoflavone	12b	
		α-Amyrin	17d.1	Zoghbi, *et al.*, 1988
		Coumestrol	5m	
		α-Toxicarol	7d	
	Roots	6a, 12a-Dehydrodeguelin	12c.1	Clark and Keenan, 1933
		Dehydrotoxicarol	17c	

Table 31 (continued)

Scientific name	Part	Compound	Structure	Bibliography
D. amazonica	Wood	(6aS, 11aS)-Demethylhomopterocarpin	13b	Braz Filho, *et al.*, 1975b
		(3S)-2′-*O*-methylvestitol	9b	
		Lupenone	17a	
		Lupenol	17b	
		Rotenone	7g	Moretti and Grenand, 1982
D. araripensis	Roots	Methylenedioxy-(3, 4)-2′-hydroxy-5′, 6′-dimethoxy-furano-(3′, 4′, 2″, 3″)-dihydrochalcone	4a	Nascimento and Mors, 1981
		3, 6-Dimethoxy-6″, 6″-dimethylchromeno-(7, 8, 2″, 3″)-flavone	10a	
		Methylenedioxy-(3, 4)-3, 6-dimethoxy-6″, 6″-dimethyl-chromeno-(7, 8, 2″, 3″)-flavone	10b	
		3, 5, 6-Trimethoxyfurano-(7, 8, 2″, 3″)-flavone	10d	
		Methylenedioxy-(3′, 4′)-3, 5, 6-trimethoxyfurano-(7, 8, 2″, 3″)-flavone	10e	
		Methylenedioxy-(3′, 4′)-5, 6-dimethoxyfurano-(7, 8, 2″, 3″)-flavone	10f	

Table 31 (continued)

Scientific name	Part	Compound	Structure	Bibliography
		Methylenedioxy-(3′, 4′)-5-hydroxy-6-methoxyfurano-(7, 8, 2″, 3″)-flavanone	8a	
		3, 5, 6-Trimethoxyfurano-(7, 8, 2″, 3″)-flavanonol	8b	
		3, 4, 5, 6-Tetramethoxyfurano-(7, 8, 2″, 3″)-flavan	9a	
D. brevipes	Stems	Damnacanthal	2a	Desai, *et al.*, 1977
		Rotenone	7g	
		β-Sitosterol	15a	
D. elliptica	Leaves	2S-Carboxy-4R, 5S-dihydroxy-piperidine	1a	Marlier, *et al.*, 1976
		2S-Carboxy-4S, 5S-dihydroxy-piperidine	1b	
		2, 5-Dihydroxymethyl-3, 4-dihydroxypyrrolidine	1c	Welter, *et al.*, 1976
	Roots	Deguelin	7a	Kodama, *et al.*, 1980
		6a, 12a-Dehydrorotenone	12d.1	
		Elliptinol	7f	Ahmed, *et al.*, 1989
		Elliptone	7e	
		Tephrosin	7b	
		β-Tubaic acid	18.1a	Obara, *et al.*, 1976
		Tubaic acid	18.1b	
		(+)-Maackiain	13c	Obara and Matsubara, 1981
		(-)-Maackiain	13c	
		Rotenone	7g	Crombie, *et al.*, 1968a ; 1968b
		α-Toxicarol	7d	

Table 31 (continued)

Scientific name	Part	Compound	Structure	Bibliography
D. ferruginea		6a, 12a-Dehydrorotenone	12d.1	
D. floribunda	Roots	Derricidin	4h	Braz Filho, *et al.*, 1975b
		3, 4-Dihydroxylonchocarpin	4c	
		5, 7-Dihydroxy-6-prenyl-flavanone	8j	
		3, 5-Dimethoxy-4-prenyl-stilbene	16b	
		Isobavachromene	4d	
		Isocordoin	4f	
		Lonchocarpin	4e	
		3, 4′, 5-Trimethoxy-4-prenyl-stilbene	16c	
D. glabrescens	Seeds	Derrusnin	5a	Delle Monache, *et al.*, 1977
		Glabrescin	5b	
		Glabrescione A	12e	
		Glabrescione B	12f	
D. laxiflora	Roots	*β*-Amyrin	17d.2	Lin, *et al.*, 1991a ; 1992
		Flemichapparin B	13d	
		Isolaxifolin	10o	
		Laxifolin	10n	
		Lupenol	17b	
		Lupinifolin	8d	
		3′-Methoxylupinifolin	8c	
		Derrichalcone	4k	Lin, *et al.*, 1992
		Derriflavanone	8e or 8f	
		Epiderriflavanone	8f or 8e	
		Laxichalcone	4i	
		Prunetin	12g.1	

Table 31 (continued)

Scientific name	Part	Compound	Structure	Bibliography
	Twigs and leaves	Laxiflorin 6, 8-Diprenyleriodictyol Hiravanone Lonchocarpol A Lupinofolin	8o 8i 8g 8h 8d	Kim, *et al.*, 1995
D. malaccensis	Roots	Rotenone	7g	Yoxopeus, 1952
D. mollis	Roots	Betulinic acid	17e	Lyra, *et al.*, 1979
		3, 4′-Dimethoxyfurano-(4″, 5″, 8, 7)-flavone	10m	
		Karanjin	10i	
		Lanceolatin B	10h	
		Lupenol	17b	
		4′-Methoxyfurano-(4″, 5″, 8, 7)-flavone	10l	
		Pongaglabrone	10j	
		Pongapin	10k	
D. negrensis	Entire plant	6a, 12a-Dehydrorotenone Rotenone	12d.1 7g	Vasconcelos, *et al.*, 1976
D. nicou	-	Rotenone	7g	Mors, *et al.*, 1973
D. oblonga	Roots	6a, 12a-Dehydro-α-toxicarol Derricarpin 6a, 12a-Dehydro-β-toxicarol Villosol	12c.2 13a 12w 12d.2	Lin and Kuo, 1993a
		12-Deoxo-12α-acetoxy-elliptone	7j	Lin, *et al.*, 1993b

Table 31 (continued)

Scientific name	Part	Compound	Structure	Bibliography
		Oblongin	6a	Lin and Kuo, 1993c
		Oblonginol	6b	
		β-Amyrin	17d.2	Lin and Kuo, 1995
		8-Methoxycoumestrol	5n	
		Daidzein	12v.1	
		6a, 12a-Dehydrodeguelin	12c.1	
		Emodin	2b	
		Formononetin	12v.2	
		Lupenol	17b	
		Lupenone	17a	
		Maackiain	13c	
		12a-Hydroxyrotenone	7h	
		6a, 12a-Dehydrorotenone	12d.1	
		Sucrose	-	
		Sumatrol	7i	
		Tephrosin	7b	
		11-Hydroxytephrosin	7c	
		Toxicarol isoflavone	12h	
		6-Oxo-6a, 12a-Dehydro-*α*-toxicarol	12c.4	
		6-Hydroxy-6a, 12a-Dehydro-*α*-toxicarol	12c.3	
		Physcion	2c	
D. obtusa	Roots barks	3′, 4′-Methylenedioxyfurano-(6, 7, 2″, 3″)-aurone	3d	Nascimento, *et al.*, 1976
		4-Hydroxyfurano-(6, 7, 2″, 3″)-aurone	3b	

Table 31 (continued)

Scientific name	Part	Compound	Structure	Bibliography
		4-Methoxyfurano-(6, 7, 2″, 3″)-aurone	3c	
		5-Hydroxy-6″, 6″-dimethyl-chromeno-(7, 8, 2″, 3″)-flavone	10c	
		3, 6-Dimethoxy-6″, 6″-dimethyl-chromeno-(7, 8, 2″, 3″)-flavone	10a	
		Furano-(6, 7, 2″, 3″)-aurone	3a	
		Methylenedioxy-(3, 4)-5′-hydroxy-2′-methoxyfurano-(3′, 4′, 2″, 3″)-chalcone	4b	
		Derriobtusone A	3e	
		Derriobtusone B	3f	
		Heptacosan-1-ol	18.2c	
		β-Sitosterol	15a	
D. rariflora	Wood	*β*-Sitosterol	15a	Braz Filho, *et al.*, 1975a
		5, 7-Dihydroxy-6-prenyl-flavanone	8j	
		3, 5-Dimethoxy-4-prenyl-stilbene	16b	
		5-Hydroxy-7-methoxy-6-prenylflavanone	8k	
D. reticulata	Stems	2‴, 3‴-Epoxylupinifolin	8l	Mahidol, *et al.*, 1997
		Dereticulatin	8m	
		Lupinifolin	8d	

Table 31 (continued)

Scientific name	Part	Compound	Structure	Bibliography
D. robusta	Seed shells	Robustigenin	12i.1	Chibber and Sharma, 1979a
		Derrugenin	12i.3	Chibber and Sharma, 1979b ; Tsukayama, *et al*., 1980
		Rubone	4j	Chibber, *et al*., 1979c
		5-Hydroxy-7-methoxy-isoflavone	12g.2	Chibber and Sharma, 1979d
		Robustigenin-5-*O*-methyl ether	12i.2	Chibber and Sharma, 1979e
		O, *O*-Dimethylalpinum-isoflavone	12a.2	Chibber and Sharma, 1980a
		Daucosterol	11.3a	
		Derrusnin	5a	
		Robustin methyl ether	5c	
		Robustone	12j.1	
		Robustone methyl ether	12j.2	
		β-Sitosterol	15a	
		Derrone-4′-*O*-methyl ether	12l.2	Chibber, *et al*., 1981
		4′-Hydroxy-3′, 5, 6′, 7-tetra-methoxyflavone	10g	Gupta, *et al*., 1998
		6-Hydroxy-2′, 4′, 7-trimethoxy-isoflavone	12x	

Table 31 (continued)

Scientific name	Part	Compound	Structure	Bibliography
		23-Hydroxyoctacos-5-ene-3-one	18.2a	Gupta, *et al.*, 1999
		Octacosan-3-one	-	
	Roots	Robustin	5d	Johnson and Pelter, 1966b
		Robustic acid	5e	Johnson and Pelter, 1966c
		Robustic acid methyl ether	5f	
		Derrubone	12k.1	East, *et al.*, 1969
		Derrusnin	5a	
		Derrustone	12k.2	
		Robustin	5d	
		Robustin methyl ether	5c	
		Robustone	12j.1	
		Robustone methyl ether	12j.2	
		Derrone	12l.1	Chibber and Sharma, 1980b
D. scandens	Roots	Scandenin	5g	Johnson, *et al.*, 1966a
		Lonchocarpic acid	5i	
		Osajin	12m.1	Pelter, *et al.*, 1966
		Scandenone	12n.1	
		Scandinone	12m.2	
		Lonchocarpic acid	5i	
		Chandalone	12n.2	Falshaw, *et al.*, 1969
		Lonchocarpenin	5j	
		Scandenin	5g	Sengupta, *et al.*, 1971
		Lupenol	17b	

Table 31 (continued)

Scientific name	Part	Compound	Structure	Bibliography
		Dehydrotoxicarol	17c	Rao, *et al.*, 1994
		β-Sitosterol	15a	
		3′-*γ*,*γ*-Dimethylallylwighteone	12p.2	
		Robustic acid	5e	
		4, 4′-Di-*O*-methyl scandenin	5h	
	Stems	Derrisisoflavone A	12p.3	Sekine, *et al.*, 1999
		Derrisisoflavone B	12r.1	
		Derrisisoflavone C	12s	
		Derrisisoflavone D	12q.1	
		Derrisisoflavone E	12q.2	
		Derrisisoflavone F	12t	
		Erysenegalensein E	12q.3	
		Lupinisol A	12r.3	
		Lupalbigenin	12r.2	
		Lupinisoflavone G	12u	
		Scandinone	12m.2	
		5, 7, 4′-Trimethoxy-6, 8-diprenylisoflavone	12g.3	
		Derriscanoside A	11.2a	Dianpeng, *et al.*, 1999
		Derriscanoside B	11.2b	
		Genistein-7-*O*-rhamnosyl-(1→6)-glucoside	11.2c	Suwannaroj, *et al.*, 2000
		Daidzein-7-*O*-rhamnosyl-(1→6)-glucoside	11.2d	
		Orobol-7-*O*-rhamnosyl-(1→6)-glucoside	11.2e	

Table 31 (continued)

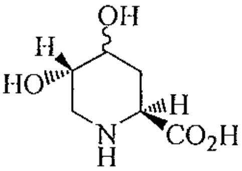
Scientific name	Part	Compound	Structure	Bibliography
D. sericea	Root barks	Lonchocarpin	4e	Nascimento, *et al.*, 1970
		Isolonchocarpin	8n	
		2′-Hydroxy-3′-dimethylallyl-4′-methoxychalcone	4f	
		Derricidin	4h	Nascimento, *et al.*, 1972
		Derricin	4g	
D. spruceana	Roots	Deguelin	7a	Menichini, *et al.*, 1982
		Rotenone	7g	
		12a-Hydroxyrotenone	7h	
		Tephrosin	7b	
		2, 4-Dimethoxy-2″, 2″-dimethylchromeno-(5″, 6″, 3′, 4′)-stilbene	16a	Garcia, *et al.*, 1986
		3-Methylenedioxy-(3′, 4′)-phenyl-4-hydroxy-5-methoxy-2″, 2″-dimethylchromeno-(5″, 6″, 7, 8)-coumarin	5k	
		3-Methylenedioxy-(3′, 4′)-phenyl-4, 5-dimethoxy-2″, 2″-dimethylchromeno-(5″, 6″, 7, 8)-coumarin	5l	
		Methylenedioxy-(3′, 4′)-5-hydroxy-2″, 2″-dimethyl-chromeno-(5″, 6″, 7, 8)-isoflavone	12l.3	
		Scandenin	5g	
		Sitosterol	15a	

Table 31 (continued)

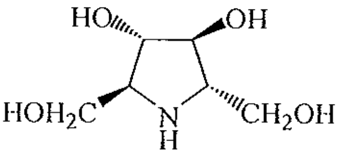
Scientific name	Part	Compound	Structure	Bibliography
D. trifoliata	Leaves	Ceryl alcohol	18.2b	Sudachan, 1967
		β-Sitosterol	15a	
		Lupenol	17b	
		α-Amyrin	17d.1	Ghosh, *et al.*, 1985
		β-Amyrin	17d.2	
		Campesterol	15b	
		Cholesterol	15c	
		Stigmasterol	15e	
		Stigmast-7-en-3-*β*-ol	15d	
		Quercetin-3-*O*-*β*-neohesperidoside	11.1b	Nair and Seetharaman, 1986
		Rhamnetin-3-*O*-*β*-neohesperidoside	11.1a	
D. uliginosa	Roots	Rotenone	7g	Milsum, 1938 ; Petard, 1951 ; Gaudin and Vacherat, 1938
		Lupenol	17b	Bose, *et al.*, 1976
		6a, 12a-Dehydrorotenone	12d.1	
D. urucu	Roots	12a-Hydroxyrotenone	7h	Braz Filho, *et al.*, 1975b
		Flemichapparin B	13d	
		6a, 12a-Dehydrorotenone	12d.1	
		Rotenone	7g	
		Tephrosin	7b	
		Derrissaponin	14a	Parente and Mors, 1980

2.1.2 Structures of compounds isolated from Derris spieces

1. Alkaloids

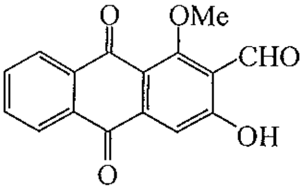


1a : 2S-Carboxy-4R,5S-dihydroxypiperidine
1b : 2S-Carboxy-4S,5S-dihydroxypiperidine

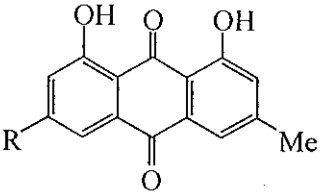


1c : 2,5-Dihydroxymethyl-3,4-dihydroxy-pyrrolidine

2. Anthraquinones

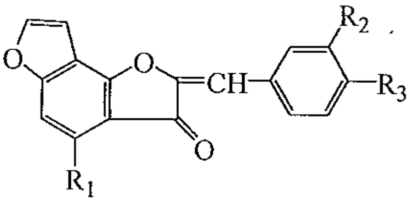


2a : Damnacanthal

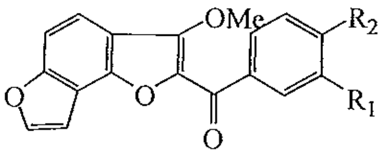


2b : R = OH : Emodin
2c : R = OMe : Physcion

3. Aurones and auronols

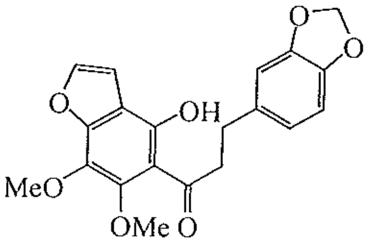


3a : $R_1 = R_2 = R_3 = H$: Furano-(6, 7, $2''$, $3''$)-aurone
3b : $R_1 = OH$, $R_2 = R_3 = H$: 4-Hydroxyfurano-(6, 7, $2''$,$3''$)-aurone
3c : $R_1 = OMe$, $R_2 = R_3 = H$: 4-methoxyfurano-(6, 7, $2''$,$3''$)-aurone
3d : $R_1 = H$, $R_2 + R_3 = O\text{-}CH_2\text{-}O$: $3'$, $4'$ -Methylenedioxyfurano-(6, 7, $2''$,$3''$)-aurone

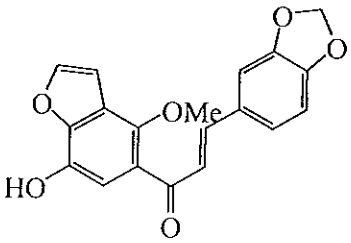


3e : $R_1 = R_2 = H$: Derriobtusone A
3f : $R_1 + R_2 = O\text{-}CH_2\text{-}O$: Derriobtusone B

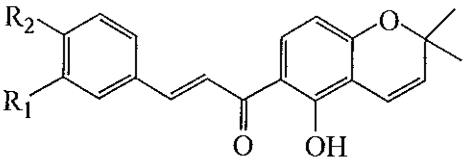
4. Chalcones



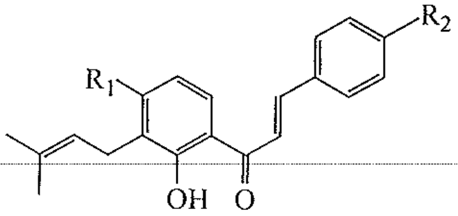
4a : Methylenedioxy-(3, 4)-2'-hydroxy-5', 6'-dimethoxyfurano-(3', 4', 2'', 3'')-dihydrochalcone



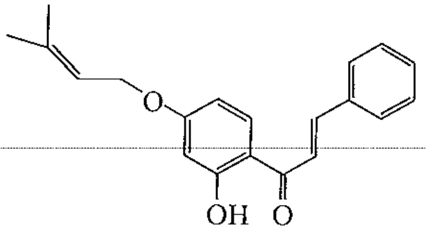
4b : Methylenedioxy-(3, 4)-5'-hydroxy-2'-methoxyfurano-(3', 4', 2'', 3'')-chalcone



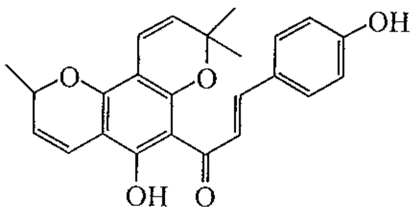
4c : $R_1 = R_2 = OH$: 3,4-Dihydroxylonchocarpin
4d : $R_1 = H, R_2 = OH$: Isobavachromene
4e : $R_1 = R_2 = H$: Lonchocarpin



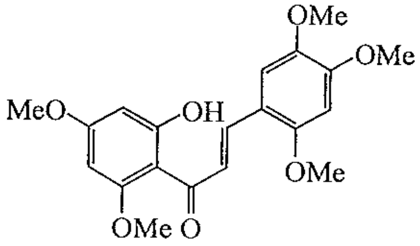
4f : $R_1 = H, R_2 = OMe$: 2'-Hydroxy-3'-dimethyl allyl-4'-methoxychalcone
4g : $R_1 = OMe, R_2 = H$: Derricin



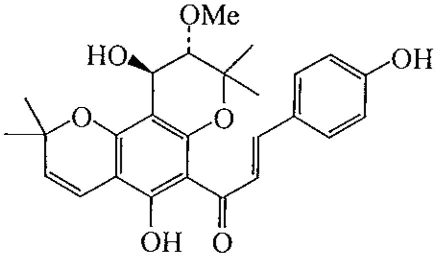
4h : Derricidin



4i : Laxichalcone

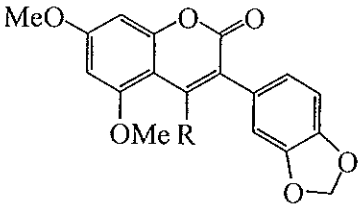


4j : Rubone

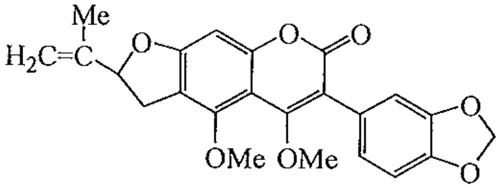


4k : Derrichalcone

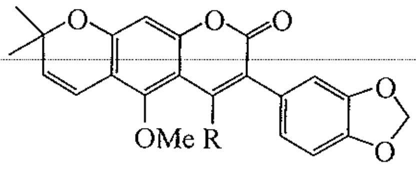
5. Coumarins



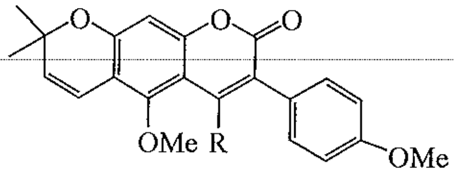
5a : R = OMe : Derrusnin



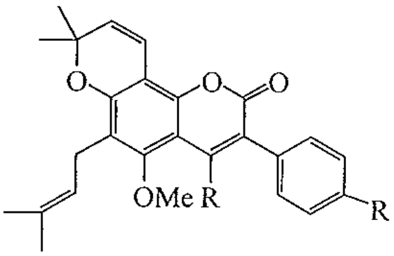
5b : Glabrescin



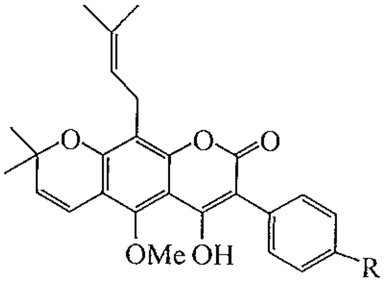
5c : R = OMe : Robustin methyl ether
5d : R = OH : Robustin



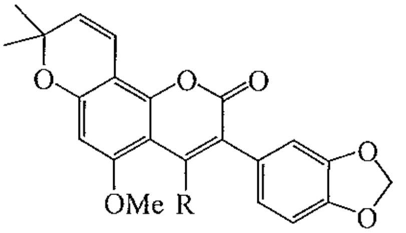
5e : R = OH : Robustic acid
5f : R = OMe : Robustic methyl ether



5g : R = OH : Scandenin
5h : R = OMe : 4,4'-Di-*O*-methylscandenin

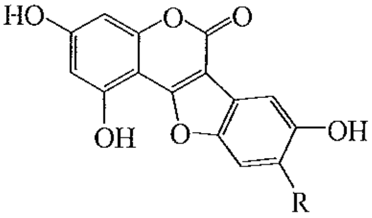


5i : R = OH : Lonchocarpic acid
5j : R = OMe : Lonchocarpenin



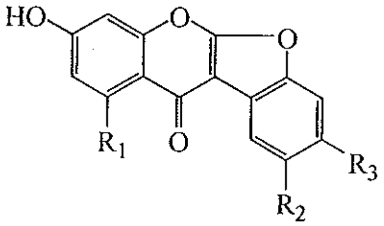
5k : R = OH : 3-Methylenedioxy-(3', 4')-phenyl-4-hydroxy-5-methoxy-2",2"-dimethylchromeno-(5", 6", 7, 8)-coumarin

5l : R = OMe : 3-Methylenedioxy-(3', 4')-phenyl-4, 5-dimethoxy-2", 2"-dimethylchromeno-(5", 6", 7, 8)-coumarin



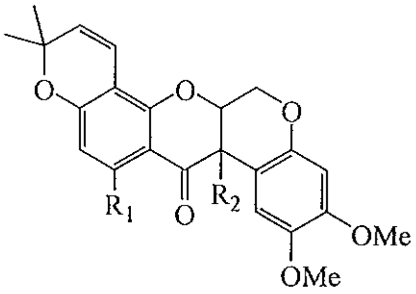
5m : R = OH : Coumestrol
5n : R = OMe : 8-Methoxycoumestrol

6. Coumaronochromones

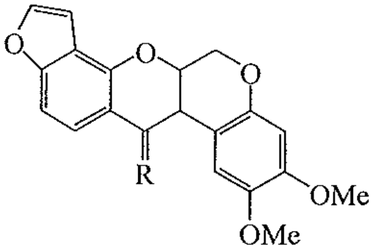


6a : R_1 = H, R_2 = OMe, R_3 = OH : Oblongin
6b : R_1 = OH, R_2 = OH, R_3 = OMe : Oblonginol

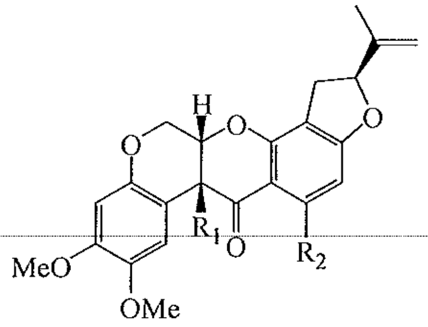
7. Dihydroisoflavones and Dihydroflavones



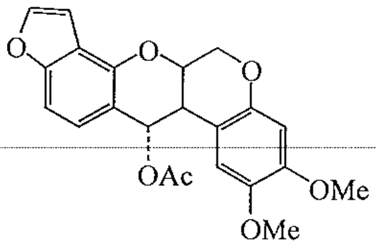
7a : R_1 = R_2 = H : Deguelin
7b : R_1 = H, R_2 = OH : Tephrosin
7c : R_1 = R_2 = OH : 11-Hydroxytephrosin
7d : R_1 = OH, R_2 = H : *a*-Toxicarol



7e : R = O : Elliptone
7f : R = H, α-OH : Elliptinol

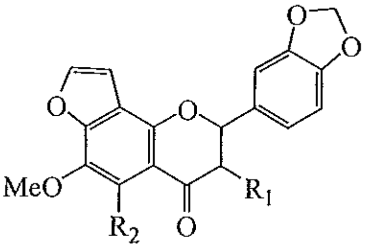


7g : R_1 = R_2 = H : Rotenone
7h : R_1 = OH, R_2 = H : 12a-Hydroxyrotenone
7i : R_1 = H, R_2 = OH : Sumatrol



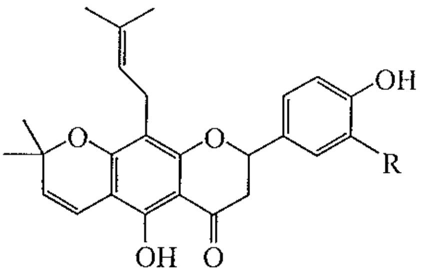
7j : R = OAc : 12-Deoxo-12*a*-acetoxyelliptone

8. Flavanones

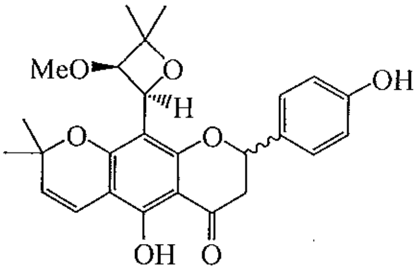


8a : R_1 = H, R_2 = OH : Methylenedioxy-(3′, 4′)-5-hydroxy-6-methoxyfurano-(7, 8, 2″, 3″)-flavanone

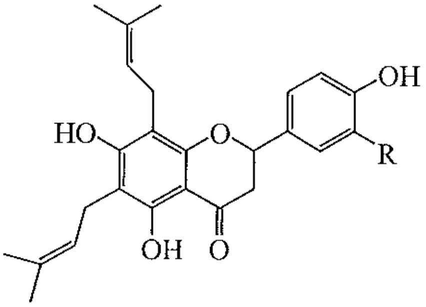
8b : R_1 = R_2 = OMe : 3, 5, 6-Trimethoxyfurano-(7, 8, 2″, 3″)-flavanonol



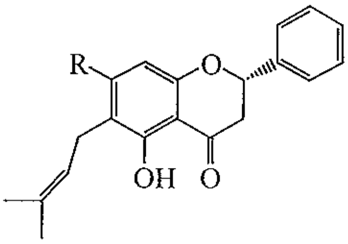
8c : R = OMe : 3′-Methoxylupinifolin
8d : R = H : Lupinifolin



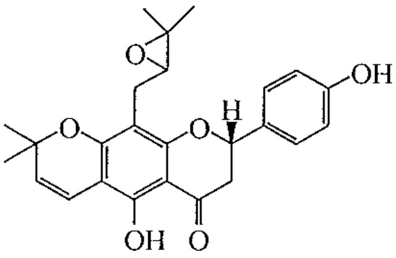
8e : Derriflavanone
8f : Epiderriflavanone



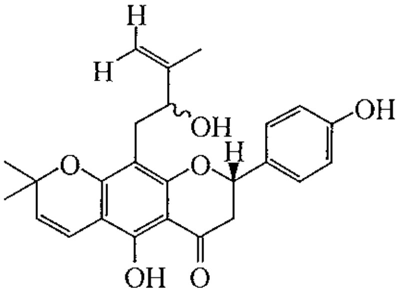
8g : R = OMe : Hiravanone
8h : R = H : Lonchocarpol A
8i : R = OH : 6,8-Diprenyleriodictyol



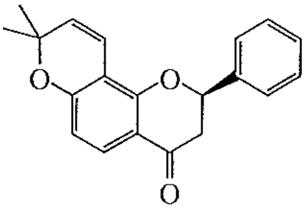
8j : R = OH : 5,7-Dihydroxy-6-prenylflavanone
8k : R = OMe : 5-Hydroxy-7-methoxy-6-prenylflavanone



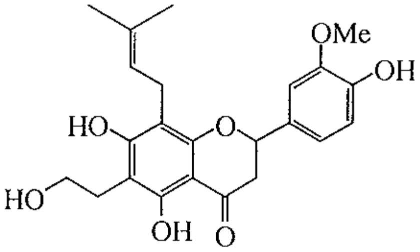
8l : 2",3"-Epoxylupinifolin



8m : Dereticulatin

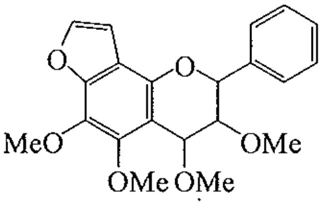


8n : Isolonchocarpin

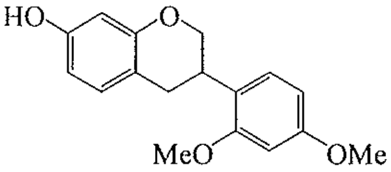


8o : Laxiflorin

9. Flavan and isoflavan

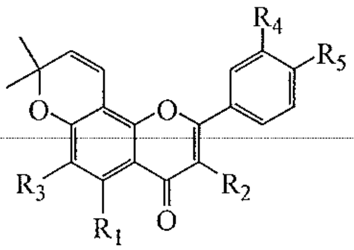


9a : 3, 4, 5, 6-Tetramethoxyfurano-(7, 8, 2", 3")-flavan



9b : (3S)-2'-*O*-Methylvestitol

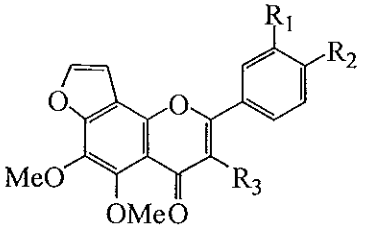
10. Flavones



10a : $R_1 = R_4 = R_5 = H$, $R_2 = R_3 = OMe$: 3, 6-Dimethoxy-6", 6"-dimethylchromeno-(7, 8, 2", 3")-flavone

10b : $R_1 = H$, $R_2 = R_3 = OMe$, $R_4 + R_5 =$ O-CH_2-O : Methylenedioxy-(3, 4)-3, 6-dimethoxy-6", 6"-dimethylchromeno-(7, 8, 2", 3")-flavone

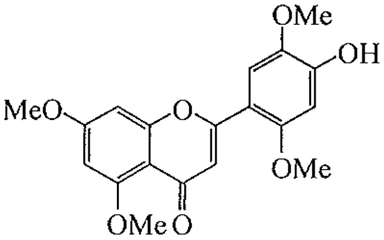
10c : $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$: 5-Hydroxy-6", 6"-dimethylchromeno-(7, 8, 2", 3")-flavone



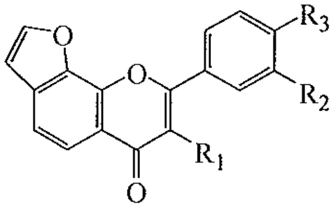
10d : $R_1 = R_2 = H$, $R_3 = OMe$: 3, 5, 6-Trimethoxyfurano-(7, 8, $2''$, $3''$)-flavone

10e : $R_1 + R_2 = O\text{-}CH_2\text{-}O$, $R_3 = OMe$: Methylenedioxy-($3'$, $4'$)-3, 5, 6-trimethoxyfurano-(7, 8, $2''$, $3''$)-flavone

10f : $R_1 + R_2 = O\text{-}CH_2\text{-}O$, $R_3 = H$: Methylenedioxy-($3'$, $4'$)-5, 6-dimethoxyfurano-(7, 8, $2''$, $3''$)-flavone



10g : $4'$-Hydroxy-$3'$, 5, $6'$, 7-tetramethoxyflavone



10h : $R_1 = R_2 = R_3 = H$: Lanceolatin B

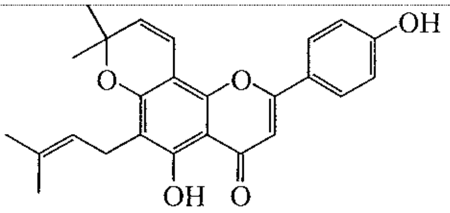
10i : $R_1 = OMe$, $R_2 = R_3 = H$: Karanjin

10j : $R_1 = H$, $R_2 + R_3 = O\text{-}CH_2\text{-}O$: Pongaglabrone

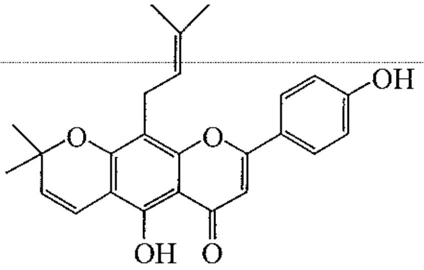
10k : $R_1 = OMe$, $R_2 + R_3 = O\text{-}CH_2\text{-}O$: Pongapin

10l : $R_1 = R_2 = H$, $R_3 = OMe$: $4'$-Methoxyfurano-($4''$, $5''$, 8, 7)- flavone

10m : $R_1 = OMe$, $R_2 = H$, $R_3 = OMe$: 3,$4'$-Dimethoxyfurano-($4''$, $5''$, 8, 7)- flavone



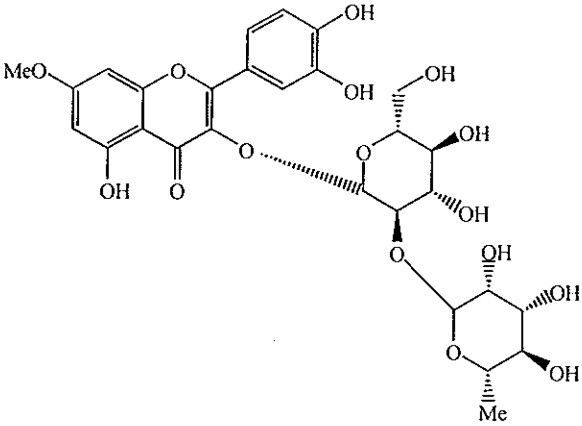
10n : Laxifolin



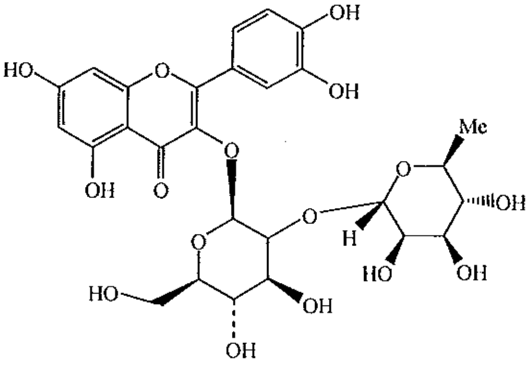
10o : Isolaxifolin

11. Glycosides

11.1 Flavone glycosides

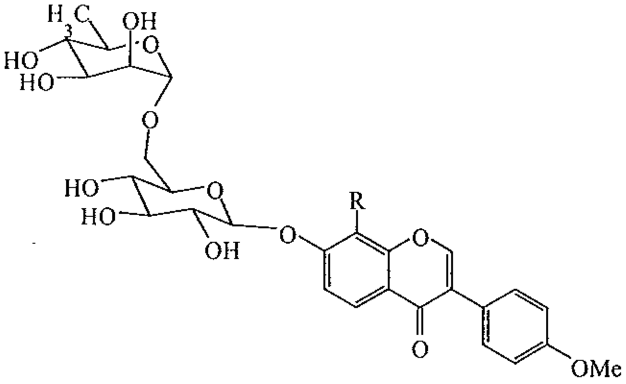


11.1a : Rhamnetin -3-*O*-*β*- neohesperidoside

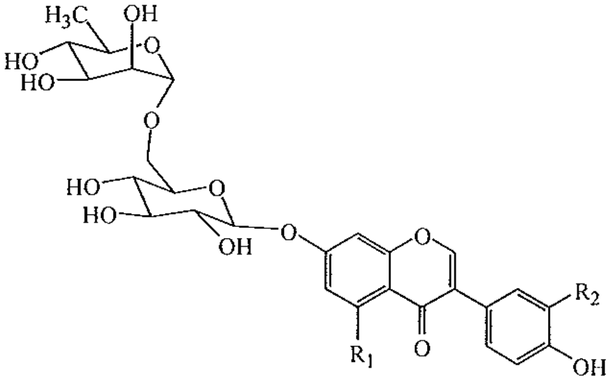


11.1b : Quercetin-3-*O*-*β*- neohesperidoside

11.2 Isoflavone glycosides

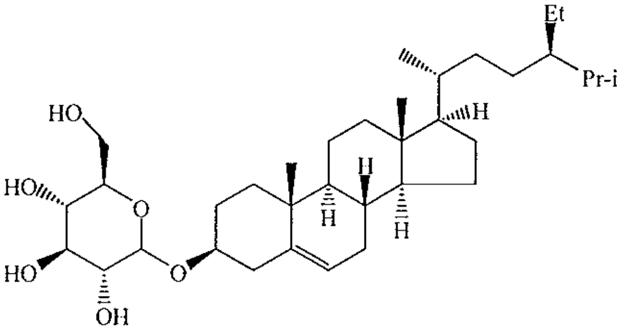


11.2a : R = H : Derriscanoside A
11.2b : R = OMe : Derriscanoside B



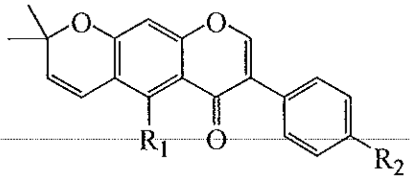
11.2c : R_1 = OH, R_2 = H : Genistein-7-*O*-rhamnosyl-(1 → 6)-glucoside
11.2d : R_1 = R_2 = H : Daidzein-7-*O*-rhamnosyl-(1 → 6)-glucoside
11.2e : R_1 = R_2 = OH : Orobol-7-*O*-rhamnosyl-(1 → 6)-glucoside

11.3 Steriod glycosides

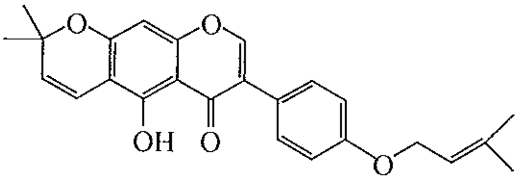


11.3a : Daucosterol

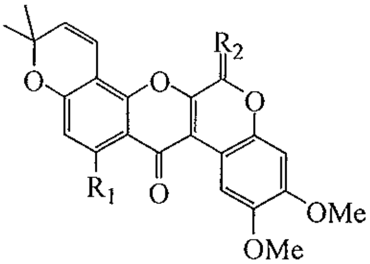
12. Isoflavones



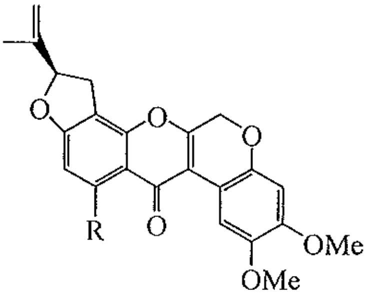
12a.1 : R_1 = R_2 = OH : Alpinumisoflavone
12a.2 : R_1 = R_2 = OMe : *O*, *O*-Dimethylalpinumisoflavone
12a.3 : R_1 = OH, R_2 = OMe : Alpinumisoflavone-4′-methyl ether



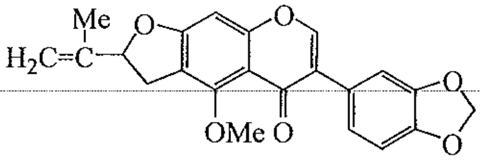
12b : 4'-*O*-Dimethylallylalpinumisoflavone



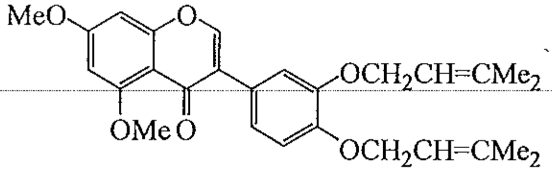
12c.1 : R_1 = H, R_2 = H_2 : 6a, 12a- Dehydrodeguelin
12c.2 : R_1 = OH, R_2 = H_2 : 6a, 12a-Dehydro-*α*-toxicarol
12c.3 : R_1 = OH, R_2 = H, OH : 6-Hydroxy-6a, 12a-Dehydro-*α*-toxicarol
12c.4 : R_1 = OH, R_2 = O : 6-Oxo-6a, 12a-Dehydro-*α*-toxicarol



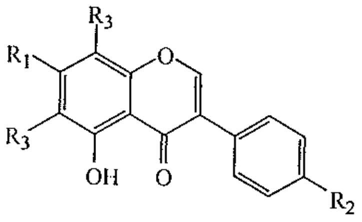
12d.1 : R = H : 6a, 12a- Dehydrorotenone
12d.2 : R = OH : Villosol



12e : Glabrescione A



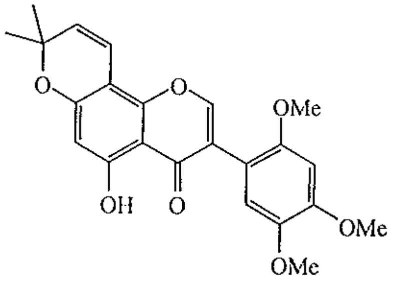
12f : Glabrescione B



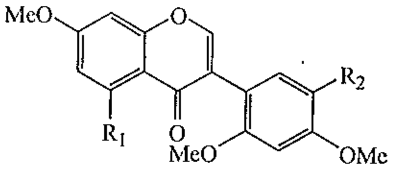
12g.1 : R_1 = OMe, R_2 = OH, R_3 = H : Prunetin

12g.2 : R_1 = OMe, R_2 = R_3 = H : 5-Hydroxy-7-methoxyisoflavone

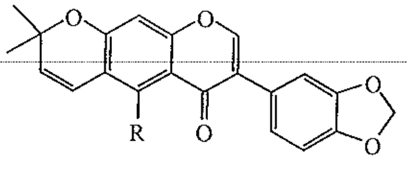
12g.3 : R_1 = OH, R_2 = OH, R_3 = isoprenyl : 5, 7, 4'-Trihydroxy-6, 8-diprenylisoflavone



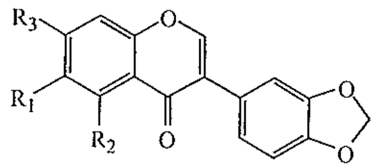
12h : Toxicarol isoflavone



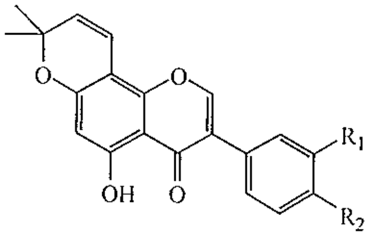
12i.1 : R_1 = OH, R_2 = OMe : Robustigenin
12i.2 : R_1 = R_2 = OMe : Robustigenin-5-*O*-methyl ether
12i.3 : R_1 = R_2 = OH : Derrugenin



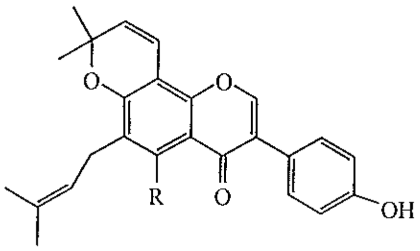
12j.1 : R = OH : Robustone
12j.2 : R = OMe : Robustone methyl ether



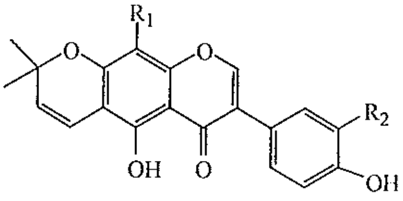
12k.1 : R_1 = isoprenyl, R_2 = OH, R_3 = OH : Derrubone
12k.2 : R_1 = H, R_2 = R_3= OMe : Derrustone



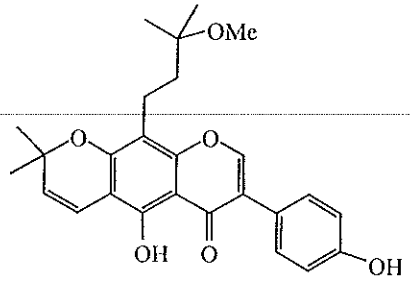
12l.1 : R_1 = H, R_2 = OH : Derrone
12l.2 : R_1 = H, R_2 = OMe : Derrone-4'-*O*-methyl ether
12l.3 : R_1 + R_2= O-CH_2-O : 3', 4'-Methylenedioxy-5-hydroxy-2'', 2''-dimethylchromeno-(5'', 6'', 7, 8)-isoflavone



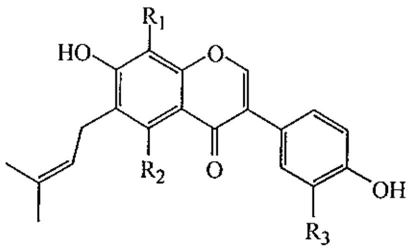
12m.1 : R = OH : Osajin
12m.2 : R = OMe : Scandinone



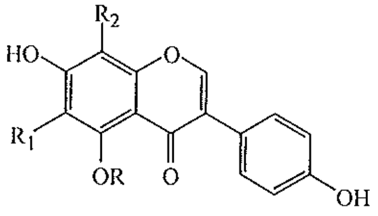
12n.1 : R_1 = isoprenyl, R_2 = H : Scandenone
12n.2 : R_1 = H, R_2 = isoprenyl : Chandalone



12o : Eturunagarone



12p.1 : R_1 = isoprenyl, R_2 = OH, R_3 = H : 8-γ,γ-Dimethylallylwighteone
12p.2 : R_1 = H, R_2 = OH, R_3 = isoprenyl : 3'-γ,γ-Dimethylallylwighteone
12p.3 : R_1 = isoprenyl, R_2 = OMe, R_3 = H : Derrisisoflavone A .



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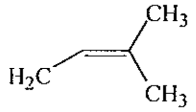

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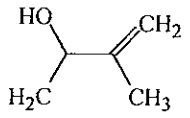
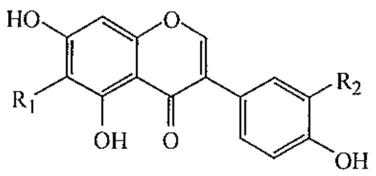
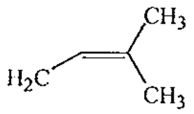
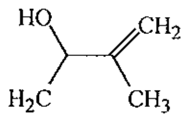


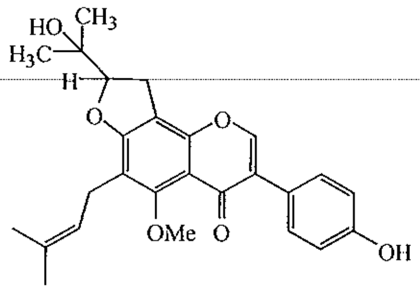
12q.1 : R_1 = b, R_2 = a, R = CH_3 : Derrisisoflavone D
12q.2 : R_1 = a, R_2 = b, R = CH_3 : Derrisisoflavone E
12q.3 : R_1 = a, R_2 = b, R = H : Erysenegalensein E



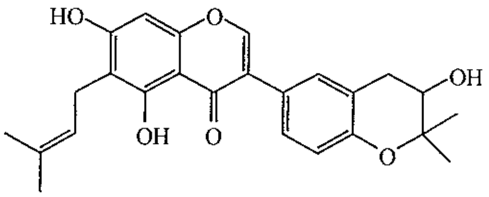
a =


b =


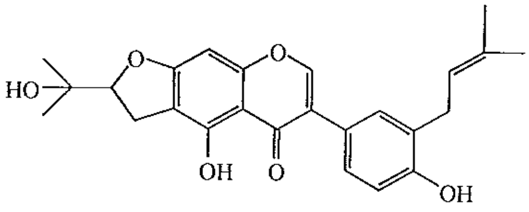
12r.1 : R_1 = a, R_2 = b : Derrisisoflavone B
12r.2 : R_1 = R_2 = a : Lupalbigenin
12r.3 : R_1 = b, R_2 = a : Lupinisol A



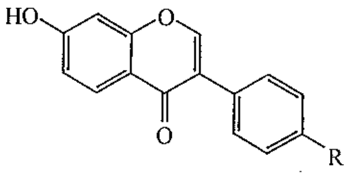
12s : Derrisisoflavone C



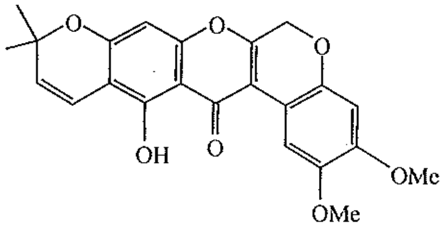
12t : Derrisisoflavone F



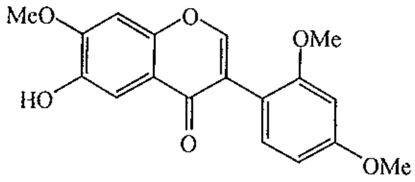
12u : Lupinisoflavone G



12v.1 : R = OH : Daidzein
12v.2 : R = OMe : Formononetin

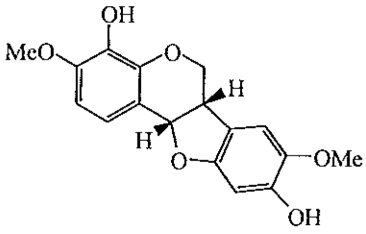


12w : 6a, 12a-Dehydro-*β*-toxicarol

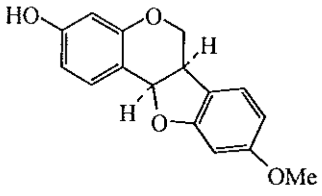


12x : 6-Hydroxy-2', 4', 7-trimethoxyisoflavone

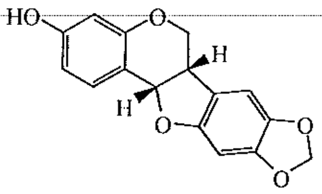
13. Pterocarpan



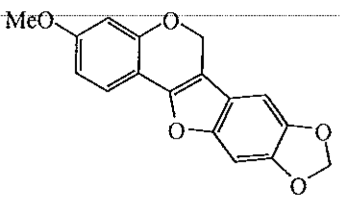
13a : Derricarpin



13b : 6aS,11aS-Demethylhomopterocarpin

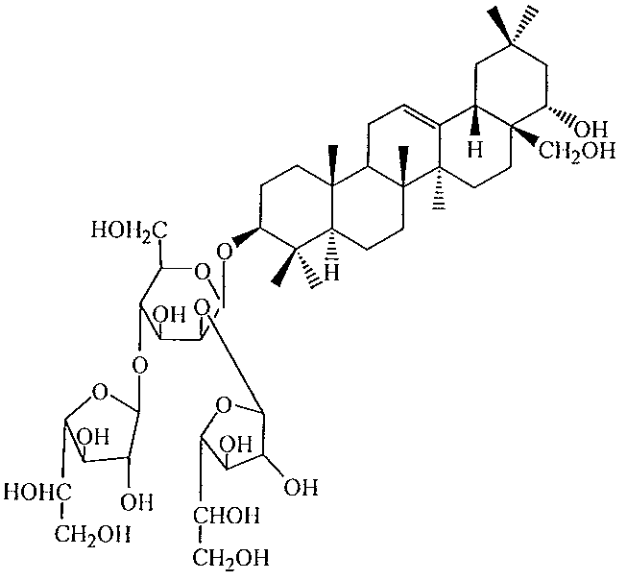


13c : Maackiain



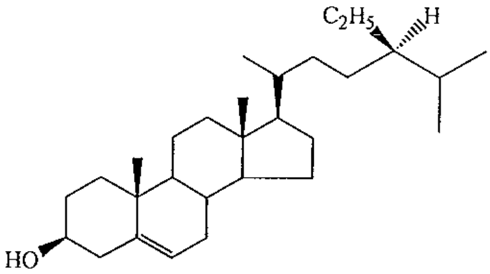
13d : Flemichapparin B

14. Saponin

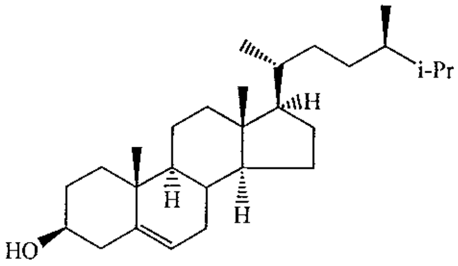


14a : Derrissaponin

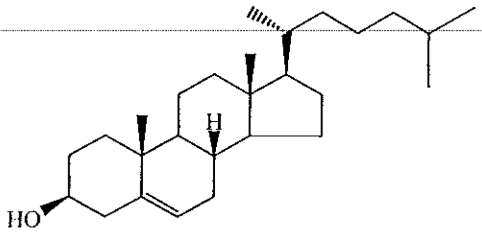
15. Steroids



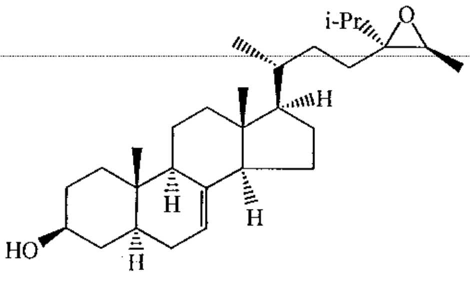
15a : β-Sitosterol



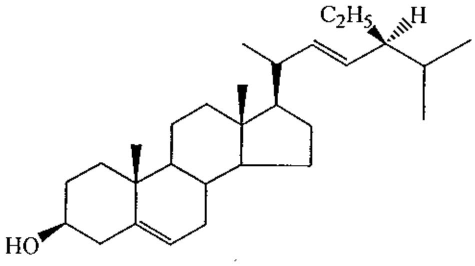
15b : Campesterol



15c : Cholesterol

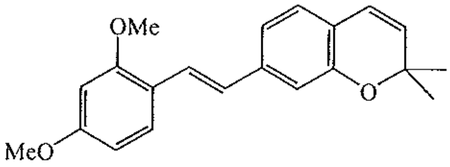


15d : Stigmast-7-en-3-β-ol

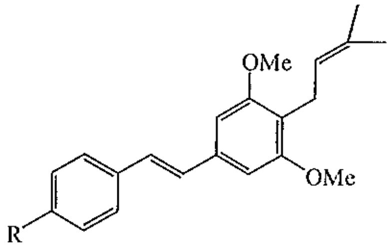


15e : Stigmasterol

16. Stilbenes

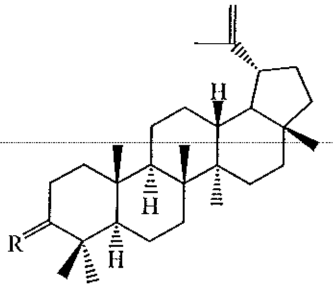


16a : 2,4-Dimethoxy-2″,2″-dimethylchromene-(5″,6″,3′,4′) stilbene

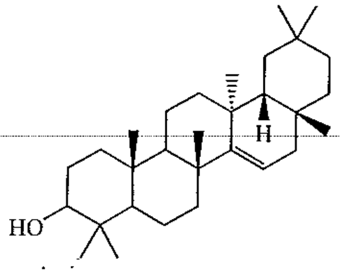


16b : R = H : 3,5-Dimethoxy-4-prenylstilbene
16c : R = OMe : 3,4′,5-Trimethoxy-4-prenylstilbene

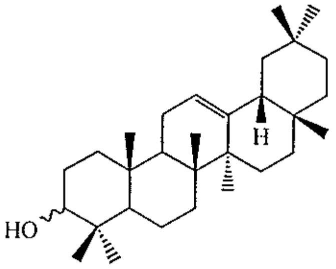
17. Triterpenes



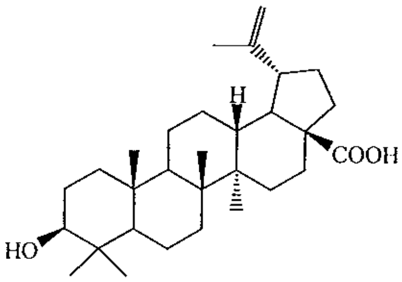
17a : R = O : Lupenone
17b : R = β-OH, H : Lupenol



17c : Dehydrotoxicarol

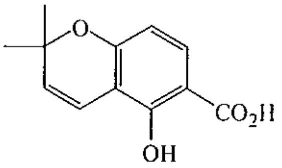


17d.1 : α-3-OH : α -Amyrin
17d.2 : β-3-OH : β -Amyrin

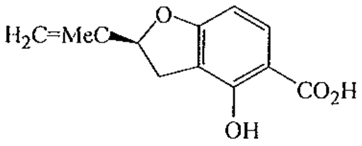


17e : Betulinic acid

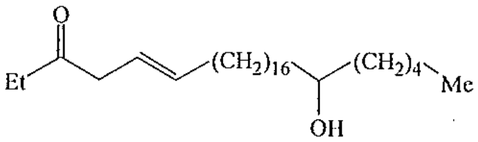
18. Miscellaneous



18.1a : β-Tubaic acid



18.1b : Tubaic acid



18.2a : 23-Hydroxyoctacos-5-ene-3-one

$HO\text{-}(CH_2)_{25}Me$

18.2b : Ceryl alcohol

$CH_3(CH_2)_{25}CH_2OH$

18.2c : Heptacosan-1-ol

2.1.3 Objectives

This research work involved chemical investigation of stems of *Derris scandens*. As demonstrated in **Table 31**, the stem of *Derris scandens* consisted of mainly non-polar constituents which were separated from either chloroform or ethanol extract. However, *D. scandens* has been used in Thai folk medicine in the form of decoction. In addition, *n*-butanol extract, obtained by extracting its stem decoction with *n*-butanol, showed marked decrease in blood pressure and heart rate in a dose-dependant manner (Jansakul, *et al.*, 1997) Thus, it is of interest to isolate, purify and structural elucidation of polar chemical constituents from the crude *n*-butanol extract of the stems.

CHAPTER 2.2

EXPERIMENTAL

2.2.1 Instruments and chemicals

Melting points were measured in centigrade (°C) on a digital Electrothermal 9100 Melting Point Apparatus. Infrared spectra were recorded using FTS165 FT-IR spectrometer. Major band (ν) were recorded in wave numbers (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using UV-160A spectrophotometer (SHIMADZU). Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol or chloroform solution. Nuclear magnetic resonance spectra were recorded on either 400 MHz Brueker AMX 400 spectrometer or 500 MHz Varian UNITY INOVA spectrometer. Spectra were recorded using deuterochloroform, tetradeutero-methanol or hexadeutero-dimethyl sulphoxide solution and δ value in ppm downfield from TMS (internal standard δ 0.00). Optical rotation was measured in methanol or chloroform solution with sodium D line (590 nm) on an AUTOPOL^{R} II automatic polarimeter. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for petroleum ether (bp. 40-60 ^{0}C), diethyl ether and ethyl acetate which were analytical grade reagent. Quick column chromatography was performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel (Merck) type 100 (70-230 mesh ASTM) or reversed-phase C_{18}. Precoated TLC plates of silica gel 60 F_{254} or reversed-phase C_{18} were used for analytical purposes.

2.2.2 *Plant material*

Stems of *Derris scandens* Benth. were collected from Phang-nga province in Thailand. Proper identification was made by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University, and the specimen deposited at the Prince of Songkla University Herbarium.

2.2.3 *Isolation and extraction*

Stems of *D. scandens* were chopped into small pieces and air dried. Dried stem of the plant (≈ 10 kg) was simmered in hot water twice for a three hour period. Clear solution of the two extracts was mixed, simmered at 50 °C to reduced volume to 50%, and followed by partition extraction with water-saturated *n*-butanol. The *n*-butanol phase was evaporated to dryness under reduced pressure to give 170 g of a yellow powder.

2.2.4 *Chemical investigation*

The First Investigation

Previous studies on the stems of *D. scandens* by Miss Morakot Kaewpet (Kaewpet, 2000) led to the isolation of compounds which showed hypotensive activity. Examination of remaining fractions obtained from above investigation on both silica gel and reversed-phase TLC indicated that these fractions contained many UV-active compounds. Therefore, the preliminary investigation involved the purification of these remaining fractions :

Fraction A Chromatogram characteristics on normal phase TLC revealed the presence of high polar compounds which were previously obtained in low quantity by silica gel column chromatography. To avoid loss of these compounds on silica gel column chromatography, **A** (126.2 mg) was chromatographed on acid-washed silica gel. Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with methanol, afforded fifty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and evaporated to dryness under reduced pressure to afford four fractions as shown in following Table 32.

Table 32 Fractions obtained from **A** by column chromatography over acid-washed silica gel

Fraction	Weight (g)	Physical appearance
A1	0.019	Yellow viscous liquid
A2	0.036	Yellow viscous liquid
A3	0.042	Yellow viscous liquid
A4	0.019	Brown-yellow solid

Fraction A1 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) and reversed-phase TLC (50% methanol-water) showed one spot under UV-S with different R_f values of 0.40 and 0.67, respectively. No further investigation was performed.

Fraction A2 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) and reversed-phase TLC (50% methanol-water) showed one major UV-active spot with R_f values of 0.29 and 0.57, respectively. Therefore, no further investigation was performed.

Fraction A3 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) and reversed-phase TLC (50% methanol-water) showed two major UV-active spots with R_f values of 0.12, 0.22 and 0.34, 0.48, respectively. No attempted purification was carried out because of low quantity of this fraction.

Fraction A4 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) and reversed-phase TLC (50% methanol-water) showed one major spot under UV-S with different R_f values of 0.26 and 0.56, respectively. No further investigation was performed.

Fraction B It (455.3 mg) was separated by chromatotron. Elution was conducted initially with chloroform and gradually with methanol to give twenty eight fractions. All fractions were examined by TLC with normal and reversed phase silica gel, combined on the basis of their chromatogram characteristics and evaporated to dryness *in vacuo* to afford ten fractions as shown in Table 33.

Table 33 Fractions obtained from **B** by chromatotron

Fraction	Weight (g)	Physical appearance
B1	0.008	Brown viscous liquid
B2	0.022	Yellow viscous liquid
B3	0.026	White solid mixed with yellow viscous liquid
B4	0.049	White solid mixed with yellow viscous liquid
B5	0.028	Yellow viscous liquid
B6	0.056	Pale yellow solid
B7	0.112	Pale yellow solid
B8	0.051	Yellow viscous liquid
B9	0.036	Yellow viscous liquid
B10	0.062	Yellow viscous liquid

Fraction B1 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) and reversed-phase TLC (50% methanol-water) showed none of well-separated spots under UV-S. It was therefore not further investigated.

Fraction B2 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) and reversed-phase TLC (50% methanol-water) showed no major spots. It was therefore not further investigated.

Fraction B3 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) and reversed-phase TLC (50% methanol-water) showed only one spot with different R_f values of 0.41 and 0.28, respectively. It was recrystallized in a mixture of methanol-chloroform. White solid (**YS1**), melting at 199.4-201.0 °C, was obtained in 3 mg.

$[\alpha]_D^{29}$		-16.81° (c = 11.9 x 10^{-2} g/100 cm^3, CH_3OH)
IR (KBr) ν (cm^{-1})		3426 (OH stretching), 2937 (CH stretching), 1617 (C=O stretching), 1605 (C=C stretching)
UV (CH_3OH) λ_{max} (nm) (log ε)		254 (4.10), 209 (3.91)
^{1}H NMR (CD_3OD) (δ ppm) (500 MHz)		8.24 (*s*, 1H), 8.00 (*d*, *J* = 9.0 Hz, 1H), 7.49 (*d*, *J* = 9.0 Hz, 2H), 7.27 (*d*, *J* = 9.0 Hz, 1H), 6.99 (*d*, *J* = 9.0 Hz, 2H), 5.17 (*d*, *J* = 7.5 Hz, 1H), 4.02 (*s*, 3H), 3.84 (*s*, 3H), 3.83 (*dd*, *J* = 12.0, 2.5 Hz, 1H), 3.64 (*dd*, *J* = 12.0, 5.5 Hz, 1H), 3.57 (*dd*, *J* = 9.0, 7.5 Hz, 1H), 3.46 (*t*, *J* = 9.0 Hz, 1H), 3.41 (*t*, *J* = 9.0 Hz, 1H), 3.25 (*ddd*, *J* = 9.0, 5.5, 2.5 Hz, 1H)
^{13}C NMR ($CDCl_3$+ DMSO-d_6) (δ ppm) (100 MHz)		175.50, 159.28, 155.80, 152.79, 150.50, 133.00, 129.97, 124.08, 123.57, 121.74, 118.80, 113.69, 110.47, 103.95, 76.80, 72.50, 74.23, 70.25, 61.59, 56.66, 55.14
DEPT (135°) (CD_3OD)	CH	152.79, 129.97, 121.74, 113.69, 110.47, 103.95, 76.80, 72.50, 74.23, 70.25
	CH_2	61.59
	CH_3	56.66, 55.14

The yellow filtrate was shown to contain **YS1** by normal and reversed-phase TLC. Purification with precoated TLC on silica gel plates with 10% methanol-chloroform afforded an additional **YS1** (3.3 mg).

Fraction B4 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water revealed the presence of **YS1** together with a compound with R_f value of 0.34. No further purification was performed.

Fraction B5 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one major spot under UV-S with R_f value of 0.24 which was expected to be **YS2**.

Fraction B6 This fraction crystallized at room temperature to yield a white solid (**YS2**) in 28.5 mg, melting at 205-207 °C. Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed one pure spot under UV-S with R_f values of 0.17 and 0.24, respectively.

$[\alpha]_D^{29}$	-84.07° ($c = 22.6 \times 10^{-2}$ g/100 cm^3, CH_3OH)
IR (KBr) ν (cm^{-1})	3407 (OH stretching), 2944, 2886 (CH stretching), 1621 (C=O stretching), 1605, 1578 (C=C stretching)
UV (CH_3OH) λ_{max} (nm) (log ε)	255 (4.58)
^{1}H NMR ($CDCl_3$+ DMSO-d_6) (δ ppm) (400 MHz)	8.24 (*s*, 1H), 7.88 (*d*, *J* = 8.8 Hz, 1H), 7.50 (*d*, *J* = 8.8 Hz, 2H), 7.29 (*d*, *J* = 8.8 Hz, 1H), 6.95 (*d*, *J* = 8.8 Hz, 2H), 5.40 (*d*, *J* = 5.4 Hz, 1H), 5.13 (*d*, *J* = 2.7 Hz, 1H), 5.11 (*d*, *J* = 1.8 Hz, 1H), 5.00 (*d*, *J* = 7.2 Hz, 1H), 4.65 (*s*, 1H), 4.59 (*d*, *J* = 5.4 Hz, 1H), 4.53 (*d*, *J* = 3.6 Hz, 1H), 4.39 (*d*, *J* = 5.9 Hz, 1H), 4.01 (*s*, 3H), 4.00-3.98 (*m*, 1H), 3.82 (*s*, 3H), 3.78-3.73 (*m*, 1H), 3.62-3.55 (*m*, 1H), 3.58-3.53 (*m*, 1H), 3.55-3.51 (*m*, 1H), 3.54-3.48 (*m*, 2H), 3.43 (*t*, *J* = 8.4 Hz, 1H), 3.31 (*t*, *J* = 8.4 Hz, 1H), 3.30-3.25 (*m*, 1H), 1.16 (*d*, *J* = 6.1 Hz, 3H)
^{13}C NMR ($CDCl_3$+ DMSO-d_6) (δ ppm) (100 MHz)	175.16, 159.19, 154.08, 152.80, 150.27, 137.34, 129.99, 123.96, 123.68, 120.62, 119.89, 114.19, 113.60, 101.18, 100.63, 76.90, 75.91, 73.29, 72.36, 71.17, 70.49, 69.92, 68.28, 66.56, 61.42, 55.06, 17.71

DEPT (135°) (CDCl$_3$ + DMSO-d_6)	CH	152.80, 129.99, 120.62, 114.19, 113.60, 101.18, 100.63, 76.90, 75.91, 73.29, 72.36, 71.17, 70.49, 69.62, 68.28
	CH$_2$	66.56
	CH$_3$	61.42, 55.06, 17.71
EIMS *m/z* (% relative intensity)		298 (33), 283 (5), 255 (5), 166 (5), 138 (31), 123 (42), 117 (19), 89 (28), 73 (18), 60 (61), 44 (100)

Chromatogram characteristics of the yellow filtrate on normal and reversed-phase TLC indicated the presence of **YS2** as a major component.

Fraction B7 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform and reversed-phase TLC with 50% methanol-water were similar to that of **A3**.

Fraction B8 It consisted of **YS4** as a major component and two minor components with R$_f$ values of 0.49 and 0.35 according to chromatogram characteristics on reversed-phase TLC with 50% methanol-water.

Fraction B9 Chromatogram characteristics on normal phase and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed two UV-active spots with R$_f$ values of 0.11, 0.42 and 0.30, 0.63, respectively.

Fraction B10 Chromatogram characteristics on normal phase and reversed-phase TLC with above mobile phases demonstrated two spots under UV-S with R$_f$ values of 0.14, 0.21 and 0.34, 0.64, respectively.

Fraction C It (607.4 mg) was further separated by chromatotron using silica-gel plate with 2 mm thickness. Elution was conducted initially with chloroform and finally with methanol to give thirty eight fractions. All fractions were examined by TLC with normal and reversed-phase silica gel plates, combined on the basis of their chromatogram characteristics and evaporated to dryness under reduced pressure to afford eight fractions as shown in Table 34.

Table 34 Fractions obtained from **C** by chromatotron

Fraction	Weight (g)	Physical appearance
C1	0.014	Yellow viscous liquid
C2	0.037	Yellow viscous liquid
C3	0.009	Yellow solid
C4	0.055	White solid
C5	0.064	Yellow viscous liquid
C6	0.085	Yellow viscous liquid
C7	0.264	Yellow viscous liquid
C8	0.103	Yellow viscous liquid

Fraction C1 Chromatogram characteristics on normal phase and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed no definite spots on the TLC plate. No further purification was performed.

Fraction C2 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water were identical to **A3**.

Fraction C3 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water and normal phase TLC with 20% methanol-chloroform showed one major spot under UV-S with R_f values of 0.29 and 0.15, respectively.

Fraction C4 It was recrystallized in a mixture of methanol-chloroform to give a white solid (**YS4**). Chromatogram characteristics on normal phase (20% methanol-chloroform) and reversed-phase (50% methanol-water) showed one spot under UV-S with R_f values of 0.09 and 0.43, respectively.

Melting point	195.5-198.5 °C
$[\alpha]_D^{29}$	-52.63°
	($c = 3.8 \times 10^{-2}$ g/100 cm^3, CH_3OH)
IR (KBr) ν (cm^{-1})	3369 (OH stretching),
	2902 (CH stretching)
	1655 (C=O stretching),

		1609, 1572 (C=C stretching)
UV (CH_3OH) λ_{max} (nm) (log ε)		261 (4.58), 210 (4.28)
^{1}H NMR ($CDCl_3$ + DMSO-d_6) (δ ppm) (400 MHz)		12.85 (*s*, 1H), 9.45 (*s*, 1H), 8.25 (*s*, 1H), 7.38 (*d*, *J* = 8.8 Hz, 2H), 6.85 (*d*, *J* = 8.8 Hz, 2H), 6.70 (*d*, *J* = 2.2 Hz, 1H), 6.43 (*d*, *J* = 2.2 Hz, 1H), 5.45 (*d*, *J* = 3.5 Hz, 1H), 5.17 (*d*, *J* = 5.0 Hz, 1H), 5.16 (*d*, *J* = 3.0 Hz, 1H), 4.93 (*d*, *J* = 7.8 Hz, 1H), 4.64 (*d*, *J* = 5.0 Hz, 1H), 4.62 (*s*, 1H), 4.54 (*d*, *J* = 4.0 Hz, 1H), 4.43 (*d*, *J* = 6.0 Hz, 1H), 4.00 (*d*, *J* = 9.8 Hz, 1H), 3.80-3.76 (*m*, 1H), 3.70-3.64 (*m*, 1H), 3.62-3.52 (*m*, 1H), 3.58-3.50 (*m*, 1H), 3.45-3.38 (*m*, 1H), 3.45-3.36 (*m*, 2H), 3.27 (*dd*, *J* = 9.1, 4.9 Hz, 1H), 3.23 (*t*, *J* = 8.4 Hz, 1H), 1.19 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR ($CDCl_3$ + DMSO-d_6) (δ ppm) (100 MHz)		180.65, 162.87, 161.71, 157.55, 157.39, 154.17, 129.96, 122.80, 121.17, 115.20, 106.49, 100.68, 100.41, 99.96, 94.64, 76.84, 75.90, 73.06, 72.48, 71.08, 70.36, 70.15, 68.34, 66.60, 17.79
DEPT (135°) ($CDCl_3$ + DMSO-d_6)	CH	154.17, 129.96, 115.20, 100.68, 100.41, 99.96, 94.64, 76.84, 75.90, 73.06, 72.48, 71.08, 70.36, 70.15, 68.34
	CH_2	66.60
	CH_3	17.79
EIMS *m/z* (% relative intensity)		298 (10), 270 (62), 254 (3), 241 (8), 213 (4), 153 (59), 135 (55), 124 (52), 118 (72), 96 (23), 89 (57), 69 (100), 60 (63), 43 (68)

YS4 (19.3 mg) was acetylated with acetic anhydride (0.5 ml) in the presence of pyridine (0.2 ml). The mixture was stirred at room temperature overnight. The

reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield a white solid in 29.8 mg. Further separation with precoated TLC on silica gel plates with 45% ethyl acetate-petroleum ether (5 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a colorless viscous liquid in 4.5 mg. Chromatogram characteristics on normal phase TLC with 8% acetone-chloroform showed two UV-active spots with R_f values of 0.34 and 0.42. Because of low quantity of this band, it was not further investigated.

Band 2 (**AcYS4**) was obtained as a colorless viscous liquid in 22.0 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (3 runs) showed one pure UV-active spot with R_f value of 0.46.

IR (neat) ν (cm^{-1})	2940 (CH stretching) 1755, 1653 (C=O stretching), 1633, 1569 (C=C stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	7.89 (*s*, 1H), 7.50 (*d*, *J* = 8.5 Hz, 2H), 7.14 (*d*, *J* = 8.5 Hz, 2H), 6.96 (*d*, *J* = 2.7 Hz, 1H), 6.66 (*d*, *J* = 2.7 Hz, 1H), 5.34 (*d*, *J* = 9.0 Hz, 1H), 5.32 (*dd*, *J* = 3.5, 1.8 Hz, 1H), 5.30 (*d*, *J* = 7.5 Hz, 1H), 5.26 (*dd*, *J* = 10.0, 3.5 Hz, 1H), 5.21 (*d*, *J* = 7.5 Hz, 1H), 5.09 (*dd*, *J* = 10.0, 9.0 Hz, 1H), 5.05 (*t*, *J* = 10.0 Hz, 1H), 4.73 (*d*, *J* = 1.8 Hz, 1H), 3.95 (*ddd*, *J* = 10.0, 7.0, 2.5 Hz, 1H), 3.83 (*dd*, *J* = 9.5, 6.0 Hz, 1H), 3.76 (*dd*, *J* = 12.0, 2.0 Hz, 1H), 3.62 (*dd*, *J* = 12.0, 7.0 Hz, 1H), 2.40 (*s*, 3H), 2.31 (*s*, 3H), 2.09 (*s*, 3H), 2.08 (*s*, 3H), 2.06 (*s*, 3H), 2.05 (*s*, 6H), 1.97 (*s*, 3H), 1.19 (*d*, *J* = 6.5 Hz, 3H)

^{13}C NMR (CDCl$_3$) (δ ppm) (125 MHz)	174.27, 170.14, 169.96, 169.89, 169.73, 169.65, 169.44, 169.22, 159.71, 158.59, 152.43, 151.01, 150.62, 130.27, 129.22, 125.15, 121.64, 113.44, 110.04, 101.89, 98.25, 97.97, 73.73, 72.50, 70.80, 70.70, 69.16, 69.10, 68.70, 66.70, 66.24, 21.14, 21.12, 20.78, 20.75, 20.63, 20.59, 20.56, 17.32

Fraction C5 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, indicated the presence of **YS5** as a major component. It (4.2 mg) was acetylated with acetic anhydride (0.20 ml) in the presence of pyridine (0.05 ml). The mixture was stirred at room temperature overnight. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield a yellow viscous liquid in 7.1 mg. Further separation with precoated TLC on silica gel plates with 45% ethyl acetate-petroleum ether (7 runs) as a mobile phase afforded a colorless viscous liquid in 3.3 mg (**AcYS5**). Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (3 runs) showed one pure UV-active spot with R$_f$ value of 0.33.

$[\alpha]_D^{29}$	-33.33° (c = 3.0 x 10^{-2} g/100 cm^3, CHCl$_3$)
IR (neat) ν (cm^{-1})	2926 (CH stretching), 1756, 1750, 1659, 1653 (C=O stretching), 1622, 1579 (C=C stretching)
UV (CHCl$_3$) λ_{max} (nm) (log ε)	306 (3.63)
^{1}H NMR (CDCl$_3$) (δ ppm) (500 MHz)	8.27 (*d*, *J* = 9.0 Hz, 1H), 8.02 (*s*, 1H), 7.61 (*d*, *J* = 9.0 Hz, 2H), 7.18 (*d*, *J* = 9.0 Hz, 2H), 7.08 (*d*, *J* = 2.0 Hz, 1H), 7.06 (*dd*, *J* = 9.0, 2.0 Hz, 1H), 5.36 (*t*, *J* = 9.0 Hz, 1H), 5.34 (*dd*, *J* = 9.0, 7.5 Hz,

		1H), 5.33 (*dd*, *J* = 3.5, 1.5 Hz, 1H), 5.31 (*dd*, *J* = 10.0, 3.5 Hz, 1H), 5.24 (*d*, *J* = 7.5 Hz, 1H), 5.10 (*dd*, *J* = 10.0, 9.1 Hz, 1H), 5.07 (*t*, *J* = 10.0 Hz, 1H), 4.75 (*d*, *J* = 1.5 Hz, 1H), 3.96 (*ddd*, *J* = 10.0, 7.5, 2.5 Hz, 1H), 3.84 (*dq*, *J* = 10.0, 6.5 Hz, 1H), 3.77 (*dd*, *J* = 12.0, 2.5 Hz, 1H), 3.65 (*dd*, *J* = 12.0, 7.5 Hz, 1H), 2.34 (*s*, 3H), 2.18 (*s*, 3H), 2.10 (*s*, 3H), 2.09 (*s*, 3H), 2.07 (*s*, 3H), 2.06 (*s*, 3H), 2.00 (*s*, 3H), 1.19 (*d*, *J* = 6.5 Hz, 3H)
^{13}C NMR (CDCl$_3$) (δ ppm) (125 MHz)		175.43, 170.18, 169.98, 169.91, 169.74, 169.47, 169.25, 160.47, 157.40, 153.33, 150.57, 130.04, 129.57, 128.24, 124.33, 121.64, 120.16, 115.43, 104.10, 98.21, 77.20, 73.80, 72.55, 70.74, 69.21, 69.06, 68.79, 66.73, 66.36, 21.16, 20.80, 20.77, 20.76, 20.63, 20.62, 20.59, 17.33
DEPT (135°) (CDCl$_3$)	CH	153.33, 130.04, 128.24, 121.64, 115.43, 104.10, 98.21, 77.20, 73.80, 72.55, 70.74, 69.21, 69.06, 68.79, 66.73
	CH$_2$	66.36
	CH$_3$	21.16, 20.80, 20.77, 20.76, 20.63, 20.62, 20.59, 17.33

Fraction C6 Chromatogram characteristics on normal phase and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed that it contained **YS4** and three minor UV-active components, two of which were found in **A3**.

Fraction C7 Chromatogram characteristics on normal phase and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, indicated that this fraction contained **YS4** and other two components as found in **A3**.

Fraction C8 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed no definite spots. However, reversed-phase TLC with

50% methanol-water showed one major spot with R_f value of 0.68. Further purification was not attempted.

Fraction D It (436.3 mg) was separated into forty eight fractions by chromatotron using silica gel plate with 2 mm thickness. Elution was conducted initially with chloroform, followed by increasing amount of methanol and finally with pure methanol. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and evaporated to dryness under reduced pressure to afford subfractions **D1-D7** as shown in Table 35.

Table 35 Fractions obtained from **D** by chromatotron

Fraction	Weight (g)	Physical appearance
D1	0.002	White solid
D2	0.002	White solid
D3	0.002	White solid
D4	0.019	Colorless viscous liquid
D5	0.141	White solid
D6	0.199	Colorless viscous liquid
D7	0.044	Yellow viscous liquid

Fraction D1 Chromatogram characteristics on normal phase (20% methanol-chloroform) and reversed-phase TLC (50% methanol-water) showed no major components. It was not further investigated.

Fraction D2 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) showed two spots under UV-S with R_f values of 0.33 and 0.47. However, chromatogram characteristics on reversed-phase TLC (50% methanol-water) showed only one spot with R_f value of 0.51.

Fraction D3 Chromatogram characteristics on reversed-phase TLC (50% methanol-water) showed two spots under UV-S with R_f values of 0.52 and 0.61 which were not identical to any of the previously isolated compounds.

Fraction D4 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) showed three UV-active components with R_f values of 0.15, 0.22 and 0.27. It was not further investigated.

Fraction D5 Chromatogram characteristics on reversed-phase TLC (50% methanol-water) showed one spot with the same R_f value as that of **YS2**. It was recrystallized in a mixture of methanol-chloroform to give **YS2** (27.0 mg).

Fraction D6 This fraction contained **YS2** and **YS3** according to their R_f values of 0.17, 0.15 (normal phase TLC with 20% methanol-chloroform) and 0.23, 0.29 (reversed-phase TLC with 50% methanol-water). It was then separated by chromatotron with silica gel plate (2 mm thickness). Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with pure methanol to give eighty nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and evaporated to dryness *in vacuo* to afford four fractions as shown in Table 36.

Table 36 Fractions separated from **D6** by chromatotron

Fraction	Weight (g)	Physical appearance
D6.1	0.013	Yellow-brown solid
D6.2	0.013	White solid
D6.3	0.194	Colorless solid
D6.4	0.018	Yellow viscous liquid

Fraction D6.1 Chromatogram characteristics on reversed-phase TLC (50% methanol-water) indicated the presence of **YS2** mixed with **YS3** and the other minor component with R_f value of 0.49.

Fraction D6.2 Chromatogram characteristics on reversed-phase TLC (50% methanol-water) indicated the presence of **YS2**.

Fraction D6.3 Chromatogram characteristics on reversed-phase TLC (50% methanol-water) indicated the presence of **YS2** and **YS3**. Attempted recrystallization in a mixture of methanol-chloroform was unsuccessful.

Fraction D6.4 Chromatogram characteristics on reversed-phase TLC (50% methanol-water) showed no major spot under UV-S. It was then not further investigated.

Fraction D7 Chromatogram characteristics on reversed-phase TLC (50% methanol-water) showed no major spot under UV-S. It was therefore not further investigated.

Fraction E It (528.9 mg) was separated into two fractions by dissolving in methanol. The methanol-soluble fraction, after evaporation to dryness, afforded **YS4** as a white-yellow solid. The methanol-insoluble fraction showed the similar chromatogram characteristics as **C**. It was then combined with the remainder of **C** and further separated by column chromatography over acid-washed silica gel. Elution was conducted initially with chloroform and finally with pure methanol to give sixty seven fractions. All fractions were examined by TLC with normal and reversed-phase silica gel plates, combined on the basis of their chromatogram characteristics and evaporated to dryness under reduced pressure to afford eleven fractions as shown in Table 37.

Table 37 Fractions obtained from **E** by column chromatography over acid-washed silica gel

Fraction	Weight (g)	Physical appearance
E1	0.008	Yellow solid
E2	0.004	White solid
E3	0.005	Yellow solid
E4	0.007	Yellow solid
E5	0.034	Yellow viscous liquid
E6	0.049	Yellow viscous liquid
E7	0.153	Pale yellow solid
E8	0.020	Yellow viscous liquid
E9	0.114	Pale yellow solid

Table 37 (continued)

Fraction	Weight (g)	Physical appearance
E10	0.027	Yellow viscous liquid
E11	0.030	Yellow viscous liquid

Fraction E1 Chromatogram characteristics on normal phase and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of UV-active spots. It was therefore not further investigated.

Fraction E2 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one major spot with R_f value of 0.66. Because of low quantity of this fraction, it was not further investigated.

Fraction E3 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed light spot under UV-S. It was therefore not further investigated.

Fraction E4 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water indicated the presence of **YS2** mixed with **YS3**.

Fraction E5 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water were similar to those of **A3**.

Fraction E6 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed the presence of **YS4** as a major component and other two components with R_f values of 0.29 and 0.66. Chromatography on reversed phase TLC with 50% methanol-water afforded four bands

Band 1 was obtained as a white solid in 3.3 mg. Chromatogram characteristics on normal phase with 20% methanol-chloroform showed one spot under UV-S with R_f value of 0.32 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one spot under UV-S with R_f value of 0.70. It was not further investigated because of low quantity of this fraction.

Band 2 was obtained as a white solid in 8.8 mg. It was shown to be **YS4** by chromatogram characteristics on normal phase and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively.

Band 3 was obtained as a white solid in 17.5 mg. Chromatogram characteristics on reversed-phase TLC with 50% methanol-water indicated the presence of **YS2** (R_f 0.25) and the other less polar compound with R_f value of 0.14.

Band 4 was obtained as a yellow viscous liquid mixed with white solid in 3.9 mg. Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two spots under UV-S with the same R_f values as **YS2** and **YS3** and the other non-polar compound with R_f value of 0.74. It was therefore not further investigated.

Fraction E7 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed the presence of **YS4** as a major spot. It crystallized at room temperature to give **YS4** as a white solid in 27.8 mg.

Fraction E8 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water indicated the presence of **YS2, YS3** and **YS4.**

Fraction E9 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water were similar to those of **A3**.

Fraction E10 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water indicated the presence of **YS4** and other two spots as found in **A3**.

Fraction E11 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water indicated the presence of **YS2, YS3** and **YS4.**

Fraction F Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one spot with R_f value of 0.78 under UV-S. Therefore, It (278.0 mg) was separated by column chromatography over reversed phase silica gel. Elution was conducted initially with 30% methanol-water and gradually enriched with methanol to give thirty nine fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and evaporated to dryness to afford six fractions as shown in Table 38.

Table 38 Fractions obtained from **F** by column chromatography over reversed-phase silica gel

Fraction	Weight (g)	Physical appearance
F1	0.156	Yellow-brown viscous liquid
F2	0.044	Yellow viscous liquid
F3	0.006	Yellow viscous liquid
F4	0.010	Yellow viscous liquid
F5	0.017	Yellow viscous liquid
F6	0.040	Brown solid

Fraction F1 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed three major spots under UV-S with R_f values of 0.33, 0.42 and 0.78. It was further separated by column chromatography over reversed-phase silica gel. Elution was conducted initially with 10% methanol-water, followed by increasing amount of methanol and finally with pure methanol to give sixty five fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and evaporated to dryness to afford seven fractions as shown in Table 39.

Table 39 Fractions obtained from **F1** by column chromatography over reversed-phase silica gel

Fraction	Weight (g)	Physical appearance
F1.1	0.031	Colorless viscous liquid
F1.2	0.009	Colorless viscous liquid
F1.3	0.009	White solid
F1.4	0.005	Colorless viscous liquid
F1.5	0.002	White solid
F1.6	0.009	White solid
F1.7	0.055	Yellow viscous liquid

Fraction F1.1 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one oval spot under UV-S with R_f value of 0.39 whereas chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one spot under UV-S with R_f value of 0.70. It was not further investigated.

Fraction F1.2 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed no definite spots under UV-S whereas chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two well-separated spots under UV-S with R_f values of 0.66 and 0.79. No further investigation was performed.

Fraction F1.3 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed two well-separated spots under UV-S with R_f values of 0.05, 0.45 and 0.66, 0.74, respectively. It was not further investigated because of low quantity of this fraction.

Fractions F1.4 and F1.5 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one spot with R_f value of 0.45. However, chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two well-separated spots under UV-S with R_f values of 0.66 and 0.74. It was not further investigated.

Fraction F1.6 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed one spot under UV-S with R_f values of 0.44 and 0.62, respectively. No further investigation was performed.

Fraction F1.7 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed pale spots under UV-S. It was therefore not further investigated.

Fraction F2 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water indicated the presence of **YS3** and other two spots as found in **A3**. It was not further investigated.

Fraction F3 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two spots under UV-S with the same R_f values as **YS2** and **YS3**.

Fraction F4 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed the presence of **YS2** as a major spot.

Fraction F5 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed the presence of **YS2** as a major spot together with the other spot under UV-S with R_f value of 0.09.

Fraction F6 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed pale spots under UV-S. It was therefore not further investigated.

Fraction G Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (6 runs) showed four well-separated spots under UV-S with R_f values of 0.20, 0.27, 0.34 and 0.42. In addition, chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed three major spots under UV-S with R_f values of 0.38, 0.49 and 0.65. This fraction (903.1 mg) was further separated by column chromatography over reversed-phase silica gel. Elution was conducted with 50% methanol-water to give thirty two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford three fractions.

Fraction G1 was obtained as a yellow solid in 148.4 mg. Chromatogram characteristics on normal and reversed-phase TLC with 14% methanol-dichloromethane and 50% methanol-water, respectively, showed no major spots under UV-S. It was therefore not further investigated.

Fraction G2 was obtained as a yellow solid mixed with yellow viscous liquid in 226.2 mg. Chromatogram characteristics on normal and reversed-phase TLC with 14% methanol-dichloromethane and 50% methanol-water, respectively, indicated the presence of **YS4** as a major component.

Fraction G3 was obtained as a yellow viscous liquid in 490.7 mg. Chromatogram characteristics on normal and reversed-phase TLC with 14% methanol-dichloromethane (5 runs) and 50% methanol-water, respectively, showed four major spots of **YS2**, **YS3**, **YS4** and one UV-active spot with R_f values of 0.49 and 0.35, respectively. Further separation by column chromatography over silica gel was carried out. Elution was conducted with 14% methanol-dichloromethane to give eighty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four fractions.

Fraction G3.1 was obtained as a colorless viscous liquid in 13.2 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane indicated that it contained **YS2** as a major component.

Fraction G3.2 was obtained as a yellow solid in 202.6 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane indicated the presence of **YS2** and **YS3** as major components.

Fraction G3.3 was obtained as a yellow viscous liquid in 98.9 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (3 runs) showed one major UV-active spot with R_f value of 0.15. Further separation with precoated TLC on silica gel plates with the same solvent system (7 runs) afforded **YS10** as a yellow viscous liquid in 30.1 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (4 runs) showed one UV-active spot with R_f value of 0.22.

^{1}H NMR (CD_3OD) (δ ppm) (500 MHz)	8.27 (*s*, 1H), 7.70 (*d*, *J* = 9.5 Hz, 1H), 7.50 (*d*, *J* = 9.0 Hz, 2H), 7.35 (*d*, *J* = 9.5 Hz, 1H), 7.00 (*d*, *J* = 9.0 Hz, 1H), 4.99 (*d*, *J* = 7.5 Hz, 1H), 4.72 (*d*, *J* = 1.5 Hz, 1H), 4.05 (*d*, *J* = 9.2 Hz, 1H) , 3.86 (*dd*, *J* = 3.5, 1.5 Hz, 1H), 3.71 (*dd*, *J* = 9.2, 3.5 Hz, 1H), 3.68-3.63 (*m*, 3H), 3.59 (*dd*, *J* = 9.5, 7.5 Hz, 1H), 3.52 (*t*, *J* = 9.2 Hz, 1H), 3.41 (*t*, *J* = 9.2 Hz, 1H), 3.36 (*t*, *J* = 9.2 Hz, 1H), 1.20 (*d*, *J* = 6.5 Hz, 3H)

It (20.1 mg) was further acetylated with acetic anhydride (0.8 ml) in the presence of pyridine (0.2 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield a yellow-white solid in 29.7 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform (3 runs) showed two well-separated spots under UV-S with R_f values of 0.42 and 0.50. Further purification on precoated TLC with 1% methanol-chloroform (7 runs) as a mobile phase afforded **AcYS10** as a pale yellow viscous liquid in 14.0 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-chloroform showed one pure spot under UV-S with R_f value of 0.52.

$[\alpha]_D^{29}$	-51.47° (c = 13.6 x 10^{-2} g/100 cm^3, $CHCl_3$)
IR (neat) ν (cm^{-1})	2940 (CH stretching), 1732, 1659 (C=O stretching), 1613, 1573 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)	257 (4.46)
^1H NMR ($CDCl_3$) (δ ppm) (500 MHz)	8.20 (*d*, J = 9.0 Hz, 1H), 7.90 (*s*, 1H), 7.46 (*d*, J = 8.5 Hz, 2H), 7.12 (*d*, J = 9.0 Hz, 1H), 6.96 (*d*, J = 8.5 Hz, 2H), 5.35-5.31 (*m*, 1H), 5.34-5.30 (*m*, 1H), 5.30 (*dd*, J = 10.0, 3.5 Hz, 1H), 5.24 (*dd*, J = 3.5, 1.5 Hz, 1H), 5.21 (*d*, J = 7.5 Hz, 1H), 5.09 (*t*, J = 10.0 Hz, 1H), 5.06 (*t*, J = 10.0 Hz, 1H), 4.74 (*d*, J = 1.5 Hz, 1H), 3.93 (*ddd*, J = 10.0, 6.5, 2.5 Hz, 1H), 3.86 (*dq*, J = 10.0, 6.5 Hz, 1H), 3.84 (*s*, 3H), 3.79 (*dd*, J = 11.5, 2.5 Hz, 1H), 3.67 (*dd*, J = 11.5, 6.5 Hz, 1H), 2.40 (*s*, 3H), 2.09 (*s*, 3H), 2.08 (*s*, 3H), 2.07 (*s*, 3H), 2.06 (*s*, 3H), 2.04 (*s*, 3H), 2.01 (*s*, 3H), 1.17 (*d*, J = 6.5 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	175.28, 170.05, 170.03, 169.98, 169.89, 169.47, 169.45, 168.09, 159.72, 152.00, 152.15, 151.95, 149.59, 130.13, 125.07, 124.96, 123.90, 120.08, 114.00, 112.00, 98.00, 97.00, 73.79, 72.26, 70.78, 70.53, 69.39, 68.78, 68.68, 66.83, 66.39, 55.31, 20.79, 20.72, 20.60, 20.55, 20.15, 17.33

DEPT (135°) ($CDCl_3$)	CH	152.15, 151.95, 130.13, 125.07, 124.96, 114.00, 98.00, 73.79, 72.26, 70.78, 70.53, 69.39, 68.78, 68.68, 66.83
	CH_2	66.39
	CH_3	55.31, 20.79, 20.72, 20.60, 20.55, 20.15, 17.33

Fraction G3.4 was obtained as a yellow viscous liquid in 11.4 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane indicated the presence of **YS4** as major component.

The Second Investigation

The crude *n*-butanol extract of *D. scandens* Benth. was tested for solubility in various solvents. The results were shown in Table 40.

Table 40 Solubility of the crude *n*-butanol extract

Solvent	Solubility at room temperature
Petrol	-
CH_2Cl_2	-
$CHCl_3$	-
Ether	-
EtOAc	-
Acetone	+ yellow solution
CH_3OH	+++ brown solution
H_2O	+++ brown solution
10% HCl	+++ yellow solution
10% NaOH	+++ brown solution
10% $NaHCO_3$	+++ brown solution
BuOH	++ yellow solution

- insoluble + slightly soluble

++ moderately soluble +++ well-soluble

The above results revealed that the crude *n*-butanol extract consisted of highly polar and acidic compounds.

The *n*-butanol extract (122.48 g) was chromatographed on silica gel using chloroform, chloroform-methanol gradient and finally pure methanol to give eighty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford seven fractions as shown in Table 41.

Table 41 Fractions obtained from the crude *n*-butanol extract by column chromatograghy over silica gel

Fraction	Weight (g)	Physical appearance
1	4.35	Yellow-brown viscous liquid
2	0.69	Yellow-brown solid
3	1.57	Yellow-brown viscous liquid
4	3.08	Yellow-brown viscous liquid
5	4.38	Yellow-brown viscous liquid
6	2.85	Pale yellow solid mixed with yellow-brown viscous liquid
7	4.89	Yellow-brown solid

Fraction 1 Chromatogram characteristics on normal phase TLC (20% methanol-chloroform) and reversed-phase TLC (50% methanol-water) showed many spots under UV-S. This fraction was further investigated by Mrs. Supamas Intarit.

Fraction 2 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed three major UV-active spots with R_f values of 0.55, 0.76 and 0.83 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one major UV-active spot with R_f value of 0.77. Therefore, It was further investigated by Miss Morakot Kaewpet.

Fraction 3 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed many spots. This fraction was further investigated by Miss Morakot Kaewpet.

Fraction 4 Chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two major UV-active spots with R_f values of 0.55 and 0.73. This fraction was divided into three portions for further investigation.

The first portion (**H**, 280.2 mg) was further separated by column chromatography over reversed-phase silica gel. Elution was conducted initially with 30% methanol-water and gradually enriched with pure methanol to afford one hundred and two fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness under reduced pressure to afford ten subfractions as shown in Table 42.

Table 42 Fractions obtained from **H** by column chromatography over reversed-phase silica gel

Fraction	Weight (g)	Physical appearance
H1	0.002	White solid
H2	0.015	Yellow viscous liquid
H3	0.011	Yellow viscous liquid
H4	0.023	Pale yellow solid
H5	0.004	White solid
H6	0.011	Brownish solid
H7	0.009	Pale yellow solid
H8	0.061	Yellow viscous liquid
H9	0.044	Yellow viscous liquid
H10	0.099	Yellow viscous liquid

Subfraction H1 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of UV-active spots. It was therefore not further investigated.

Subfraction H2 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed two UV-active spots with R_f values of 0.34 and 0.38 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one major UV-active spot with R_f value of 0.80.

Subfraction H3 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed two of well-separated spots with R_f values of 0.37, 0.44 and 0.68, 0.78, respectively. Because it was obtained in low quantity, it was not further investigated.

Subfraction H4 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed two of well-separated spots under UV-S with R_f values of 0.27, 0.44 and 0.46, 0.70, respectively. It was not further investigated.

Subfraction H5 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, were similar to those of **H4**. It was not further investigated.

Subfraction H6 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform demonstrated two pale and well-separated spots under UV-S with R_f values of 0.42 and 0.48 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one UV-active spot with R_f value of 0.66. Because of low quantity of this fraction, it was not further investigated.

Subfraction H7 (YS9) Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed one UV-active spot with R_f values of 0.49 and 0.66, respectively. However, this fraction was obtained as a pure compound but in low quantity.

Melting point	145^0 (decomposed)
IR (neat) ν (cm^{-1})	3445 (OH stretching), 1668 (C=O stretching), 1591 (C=C stretching)
UV (CH_3OH) λ_{max} (nm) (log ε)	291 (4.04), 259 (4.32), 220 (4.32)
^{1}H NMR (CD_3COCD_3) (δ ppm) (500 MHz)	7.58 (*dd*, J = 7.7, 1.4 Hz, 1H), 7.55 (*d*, J = 1.4 Hz, 1H), 6.90 (*d*, J = 7.7 Hz, 1H), 3.90 (*s*, 3H)
^{13}C NMR (CD_3COCD_3) (δ ppm) (125 MHz)	167.29, 151.79, 147.79, 124.56, 122.64, 115.24, 113.23, 56.03

Subfraction H8 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction H9 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed three major spots under UV-S with R_f values of 0.19, 0.34 and 0.43. Further separation with precoated TLC on silica gel plates with 20% methanol-chloroform (2 runs) as a mobile phase afforded four bands.

Band 1 was obtained as a white solid in 5.9 mg. Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, indicated that it was **YS1**.

Band 2 was obtained as a white solid in 3.7 mg. Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, demonstrated one major UV-active spot with equal R_f value of 0.38. Because of low quantity of this fraction, it was not further investigated.

Band 3 was obtained as a yellow solid in 1.2 mg. Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed one pure UV-active spot with R_f values of 0.30 and 0.37, respectively. Therefore, no further investigation was performed because this fraction was obtained in low quantity.

Band 4 was obtained as a white solid in 1.7 mg. Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, indicated the presence of **YS2** as a major spot.

Subfraction H10 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of well-separated spots under UV-S. It was therefore not further investigated.

The second portion (**I**, 227.7 mg) was chromatographed using the same procedure as that of **H** to give ninety one fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine subfractions as shown in Table 43.

Table 43 Fractions obtained from **I** by column chromatography over reversed-phase silica gel

Fraction	Weight (g)	Physical appearance
I1	0.019	Yellow viscous liquid mixed with white solid
I2	0.025	Pale yellow solid
I3	0.014	Yellow viscous liquid
I4	0.015	Yellow viscous liquid
I5	0.031	Yellow viscous liquid
I6	0.017	Yellow viscous liquid
I7	0.023	Yellow viscous liquid
I8	0.031	Yellow viscous liquid
I9	0.044	Yellow viscous liquid

Subfraction I1 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed three major UV-active spots with R_f values of 0.40, 0.49 and 0.54. In addition, chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two UV-active spots with R_f values of 0.69 and 0.82. Because it was obtained in low quantity, no further purification was performed.

Subfraction I2 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of well-separated spots. It was not further investigated.

Subfraction I3 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed three major UV-active spots with R_f values of 0.27, 0.47 and 0.56 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one oval UV-active spot with R_f value of 0.69. Finally, it was combined with other fractions which had the same chromatogram characteristics.

Subfraction I4 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed two well-separated spots under UV-S with R_f values of 0.26 and 0.39. In addition, chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one major UV-active spot with R_f value of 0.59. Because it was obtained in low quantity, it was not further investigated.

Subfraction I5 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, demonstrated none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction I6 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed two well-separated spots under UV-S with R_f values of 0.33 and 0.43 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one major UV-active spot with R_f value of 0.25. No further purification was performed because it was obtained in low quantity.

Subfraction I7 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed four well-separated spots under UV-S with R_f values of 0.16, 0.33, 0.38 and 0.42. In addition, chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one oval UV-active spot with R_f value of 0.23. It was not further investigated because it was obtained in low quantity.

Subfraction I8 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed three major UV-active spots with R_f values of 0.16, 0.38 and 0.44. In addition, chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one oval UV-active spot with R_f value of 0.16. It was not further investigated because it was obtained in low quantity.

Subfraction I9 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed no major spots under UV-S. It was therefore not further investigated.

The third portion (**J**, 1.97 g) was chromatographed using the same procedure as that of **H** to afford eighty five fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness *in vacuo* to afford eleven subfractions as shown in Table 44.

Table 44 Fractions obtained from **J** by column chromatography over reversed-phase silica gel

Fraction	Weight (g)	Physical appearance
J1	0.143	Yellow viscous liquid
J2	0.150	Pale yellow solid
J3	0.013	Yellow viscous liquid
J4	0.115	Yellow viscous liquid
J5	0.059	Yellow viscous liquid
J6	0.153	Yellow viscous liquid
J7	0.204	Yellow viscous liquid
J8	0.242	Brownish solid
J9	0.341	Yellow solid
J10	0.163	Yellow solid mixed with yellow viscous liquid
J11	0.423	Yellow viscous liquid

Subfraction J1 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed two spots under UV-S with R_f values of 0.40 and 0.45 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one major spot under UV-S with R_f value of 0.76. No attempted purification was carried out.

Subfraction J2 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed one major UV-active spot with R_f values of 0.28 and 0.35, respectively. It (56.2 mg) was further separated by column chromatography over reversed-phase silica gel. Elution was conducted initially with 10% methanol-water and gradually enriched with pure methanol to afford one hundred and one fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness under reduced pressure to give three subfractions as shown in Table 45.

Table 45 Fractions obtained from **J2** by column chromatography over reversed-phase silica gel

Fraction	Weight (g)	Physical appearance
J2.1	0.001	Yellow viscous liquid
J2.2	0.018	White solid mixed with yellow viscous liquid
J2.3	0.045	Pale yellow solid

Subfraction J2.1 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of UV-active spots. It was therefore not further investigated.

Subfraction J2.2 Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed three well-separated spots under UV-S with R_f values of 0.09, 0.18 and 0.30 whereas chromatogram characteristics on reversed-phase TLC with 10% methanol-water showed two well-separated spots under UV-S with R_f values of 0.13 and 0.65. Because it was obtained in low quantity, it was not further investigated.

Subfraction J2.3 Chromatogram characteristics on normal and reversed-phase TLC with 10% methanol-chloroform and 10% methanol-water, respectively, showed one major UV-active spot with R_f values of 0.18 and 0.14, respectively. It was not further investigated.

Subfraction J3 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed one pale major spot under UV-S with R_f values of 0.44 and 0.65, respectively. It was therefore not further investigated.

Subfraction J4 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, indicated the presence of **YS9** as a major component.

Subfraction J5 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, indicated that this fraction contained **YS9** as a major component. Further separation with column chromatography over reversed-phase silica gel was performed. Elution was conducted with 30% methanol-water to afford fifty one fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions as shown in Table 46.

Table 46 Fractions obtained from **J5** by column chromatography over reversed-phase silica gel

Fraction	Weight (g)	Physical appearance
J5.1	0.008	Brownish solid
J5.2	0.005	White solid mixed with yellow viscous liquid
J5.3	0.010	Yellow viscous liquid
J5.4	0.049	Yellow solid
J5.5	0.006	Yellow-brown viscous liquid

Subfraction J5.1 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one oval spot under UV-S with R_f value of 0.50. No attempted purification was performed because of low quantity of this fraction.

Subfraction J5.2 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed three UV-active spots with R_f values of 0.19, 0.38 and 0.44. No further purification was performed because this fraction was obtained in low quantity.

Subfraction J5.3 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed four well-separated spots under UV-S with R_f values of 0.14, 0.19, 0.39 and 0.46. Because it was obtained in low quantity, no further purification was carried out.

Subfraction J5.4 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform as a mobile phase showed one oval spot under UV-S with R_f value of 0.53 whereas that with 60% ethyl acetate-petroleum ether as a mobile phase showed two UV-active spots with R_f values of 0.14 and 0.29. Further separation with precoated TLC on silica gel plates with 60% ethyl acetate-petroleum ether (3 runs) as a mobile phase to afford three bands.

Band 1 was obtained as a yellow solid in 5.3 mg. Chromatogram characteristics on normal phase TLC with 60% ethyl acetate-petroleum ether showed one major spot with R_f value of 0.48, indicating the presence of **YS9,** and the other component with R_f value of 0.60.

Band 2 was obtained as a yellow solid in 10.8 mg. Chromatogram characteristics on normal phase with 50% ethyl acetate-petroleum ether showed one pure UV-active spot with the same R_f value as **YS9**.

Band 3 (**YS12**) was obtained as a pale yellow solid in 8.2 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed one pure UV-active spot with R_f value of 0.19.

IR (neat) ν (cm^{-1})	3472 (OH stretching), 2925 (CH stretching), 1683 (C=O stretching),1598 (C=C stretching)
UV (CH_3OH) λ_{max} (nm) (log ε)	270 (3.68), 217 (4.07)
^{1}H NMR (CD_3COCD_3) (δ ppm) (500 MHz)	7.30 (*s*, 2H), 3.90 (*s*, 6H)
^{13}C NMR (CD_3COCD_3) (δ ppm) (125 MHz)	168.54, 149.04, 142.18, 122.16, 108.53, 56.76

Subfraction J5.5 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed no UV-active spot. It was therefore not further investigated.

Subfraction J6 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (3 runs) showed the same major component as **I6** with R_f value of 0.31.

Subfraction J7 Chromatogram characteristics on normal and reversed-phase TLC with 15% methanol-chloroform and 50% methanol-water, respectively, showed none of well-separated spots under UV-S. It was then acetylated using acetic anhydride (5.0 ml) and pyridine (1.5 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a yellow viscous liquid in 235.9 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction J8 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed four spots under UV-S with R_f values of 0.12, 0.19, 0.24 and 0.32. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 10% methanol-chloroform and gradually enriched with pure methanol to afford one hundred and four fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions as shown in Table 47.

Table 47 Fractions obtained from **J8** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
J8.1	0.015	Yellow viscous liquid
J8.2	0.037	Pale yellow solid
J8.3	0.015	Colorless viscous liquid
J8.4	0.079	Yellow viscous liquid
J8.5	0.086	Yellow viscous liquid
J8.6	0.026	Yellow viscous liquid

Subfraction J8.1 Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction J8.2 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed the presence of **YS1** as a major component.

Subfraction J8.3 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform indicated the presence of **YS1** as a major component. It was further acetylated using acetic anhydride (0.8 ml) and pyridine (0.2 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a yellow viscous liquid in 40.2 mg. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (4 runs) showed one major spot under UV-S with R_f value of 0.15. Further purification on precoated TLC with 45% ethyl acetate-petroleum ether (4 runs) as a mobile phase afforded a yellow viscous liquid in 11.9 mg (**AcYS1**). Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed one pure UV-active spot with R_f value of 0.15.

IR (neat) ν (cm^{-1})		2937, 2849 (CH stretching), 1755, 1645 (C=O stretching), 1609, 1567 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)		253 (4.45)
^1H NMR ($CDCl_3$) (δ ppm) (500 MHz)		8.10 (*d*, *J* = 9.0 Hz, 1H), 7.99 (*s*, 1H), 7.52 (*d*, = 9.0 Hz, 2H), 7.06 (*d*, *J* = 9.0 Hz, 1H), 6.98 *d*, *J* = 9.0 Hz, 2H), 5.38 (*dd*, *J* = 8.5, 7.0 Hz, 1H), 5.31 (*d*, *J* = 8.5 Hz, 1H), 5.28 (*dd*, *J* = 9.5, 8.5 Hz, 1H), 5.19 (*d*, *J* = 7.0 Hz, 1H), 4.29 (*dd*, *J* = 12.0, 5.5 Hz, 1H), 4.08 (*dd*, *J* = 12.0, 2.5 Hz, 1H), 3.99 (*s*, 3H), 3.84 (*s*, 3H), 3.70 (*ddd*, *J* = 9.5, 5.5, 2.5 Hz, 1H), 2.09 (*s*, 3H), 2.05 (*s*, 3H), 2.04 (*s*, 3H), 1.94 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		176.17, 170.74, 170.71, 169.81, 169.69, 159.98, 156.06, 152.70, 150.92, 132.52, 130.29, 124.54, 124.04, 123.41, 119.24, 114.12, 110.03, 101.28, 72.63, 72.02, 71.69, 68.35, 61.78, 56.37, 55.16, 20.39, 20.32, 20.27
DEPT (135°) ($CDCl_3$)	CH	152.70, 130.29, 123.41, 114.12, 110.03, 101.28,

	72.63, 72.02, 71.69, 68.35
CH_2	61.78
CH_3	56.37, 55.16, 20.39, 20.32, 20.27

Subfraction J8.4 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed three major UV-active spots with R_f values of 0.27, 0.32 and 0.42. Further separation with column chromatography over silica gel was carried out. Elution was conducted with 15% methanol-chloroform to afford forty seven fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness under reduced pressure to give six subfractions as shown in Table 48.

Table 48 Fractions obtained from **J8.4** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
1	0.007	Yellow viscous liquid
2	0.014	Yellow viscous liquid
3	0.086	Yellow viscous liquid
4	0.022	Yellow viscous liquid
5	0.019	Yellow viscous liquid
6	0.022	Yellow viscous liquid

Subfraction 1 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) indicated the presence of **YS1** as a major component.

Subfraction 2 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) under UV-S indicated that this fraction contained **YS1** and the other component with R_f value of 0.49. It was acetylated using acetic anhydride (0.4 ml) and pyridine (0.1 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a yellow viscous liquid in 17.5 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed two well-separated spots under UV-S with R_f values of 0.28 and 0.46. Further separation with precoated TLC on silica gel plates with 50% ethyl acetate-petroleum ether (2 runs) afforded two bands.

Band 2.1 (**AcYS14**) was obtained as a white solid in 4.5 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one pure UV-active spot with R_f value of 0.49.

$[\alpha]_D^{29}$	-45.45° (c = 2.2 x 10^{-2} g/100 cm^3, $CHCl_3$)
IR (neat) ν (cm^{-1})	2938, 2851 (CH stretching), 1756, 1645 (C=O stretching), 1609, 1569 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)	258 (4.19)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	8.01 (*s*, 1H), 8.00 (*d*, *J* = 9.0 Hz, 1H), 7.50 (*d*, *J* = 9.0 Hz, 2H), 7.18 (*d*, *J* = 9.0 Hz, 1H), 6.98 (*d*, *J* = 9.0 Hz, 2H), 5.39 (*dd*, *J* = 9.0, 7.2 Hz, 1H), 5.33 (*t*, *J* = 9.0 Hz, 1H), 5.20 (*dd*, *J* = 9.6, 9.0 Hz, 1H), 5.16 (*d*, *J* = 7.2 Hz, 1H), 4.31 (*dd*, *J* = 12.0, 6.0 Hz, 1H), 4.20 (*dd*, *J* = 12.0, 2.2 Hz, 1H), 3.96 (*s*, 3H), 3.90 (*ddd*, *J* = 9.6, 6.0, 2.2 Hz, 1H), 3.85 (*s*, 3H), 2.10 (*s*, 3H), 2.09 (*s*, 3H), 2.06 (*s*, 3H), 2.05 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	176.36, 171.01, 170.67, 169.91, 169.81, 160.20 153.80, 152.72, 151.15, 138.85, 130.42, 125.14 123.97, 121.75, 115.11, 114.24, 99.98, 77.17 72.38, 70.90, 68.13, 61.95, 61.78, 55.22, 20.39 20.32, 20.30, 20.27
DEPT (135°) ($CDCl_3$) CH	152.72, 130.42, 121.45, 115.11, 114.24, 99.98, 77.17, 72.38, 70.90, 68.13

CH_2	61.78
CH_3	61.95, 55.22, 20.39, 20.32, 20.30, 20.27

Band 2.2 was obtained as a yellow viscous liquid in 2.6 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether indicated the presence of pure **AcYS1**.

Subfraction 3 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed three well-separated spots under UV-S with R_f values of 0.41, 0.48 and 0.55. Further purification by precoated TLC on silica gel plates with 15% methanol-chloroform (3 runs) as a mobile phase afforded three bands.

Band 3.1 was obtained as a yellow viscous liquid in 1.6 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) indicated the presence of pure **YS1**.

Band 3.2 was obtained as a yellow viscous liquid in 6.6 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one UV-active spot with R_f value of 0.44. It was then acetylated using acetic anhydride (0.10 ml) and pyridine (0.03 ml). The mixture was stirred at room temperature for 24 hours. It was then extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a yellow viscous liquid in 8.9 mg. Further purification on precoated TLC with 50% ethyl acetate-petroleum ether (3 runs) as a mobile phase afforded a white solid in 1.9 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) with R_f value of 0.45 indicated that it was identical to **AcYS14**.

Band 3.3 was obtained as a yellow viscous liquid in 13.0 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major UV-active spot with R_f value of 0.38. It was then acetylated using acetic anhydride (0.40 ml) and pyridine (0.10 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10%sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to yield a yellow viscous liquid in 16.7 mg. Further purification on precoated TLC with 45% ethyl acetate-petroleum ether (3 runs) as a mobile phase afforded a yellow viscous liquid (**AcYS13**) in 2.3 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one UV-active spot with R_f value of 0.49.

$[\alpha]_D^{29}$		-38.46° (c = 2.6 x 10^{-2} g/100 cm^3, $CHCl_3$)
IR (neat) ν (cm^{-1})		2924, 2852 (CH stretching), 1756, 1642 (C=O stretching), 1622, 1591 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)		263 (4.47)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)		8.25 (*d*, *J* = 8.1 Hz, 1H), 7.94 (*s*, 1H), 7.50 (*d*, *J* = 8.1 Hz, 2H), 7.05 (*dd*, *J* = 8.1, 2.5 Hz, 1H), 7.03 (*d*, *J* = 2.5 Hz, 1H), 6.98 (*d*, *J* = 8.1 Hz, 2H), 5.36-5.33 (*m*, 1H), 5.34-5.30 (*m*, 1H), 5.23 (*d*, *J* = 7.5 Hz, 1H), 5.18 (*dd*, *J* = 9.0, 8.4 Hz, 1H), 4.29 (*dd*, *J* = 12.0, 6.0 Hz, 1H), 4.22 (*dd*, *J* = 12.0, 2.2 Hz, 1H), 3.96 (*ddd*, *J* = 9.0, 6.0, 2.2 Hz, 1H), 3.85 (*s*, 3H), 2.10 (*s*, 3H), 2.08 (*s*, 3H), 2.07 (*s*, 3H), 2.05 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		176.09, 170.84, 170.53, 169.74, 169.57, 160.83, 160.07, 157.69, 152.54, 130.29, 128.40, 125.31, 124.03, 120.43, 115.37, 114.13, 104.36, 98.43, 72.45, 72.34, 70.92, 68.07, 61.78, 55.13, 20.24, 20.21, 20.18, 20.16
DEPT (135°) ($CDCl_3$)	CH	152.54, 130.29, 128.40, 115.37, 114.13, 104.36, 98.43, 72.45, 72.34, 70.92, 68.07
	CH_2	61.78

CH_3	55.13, 20.24, 20.21, 20.18, 20.16

Subfraction 4 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed none of well-separated spots under UV-S. It was then acetylated using acetic anhydride (0.60 ml) and pyridine (0.15 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a yellow viscous liquid in 26.4 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed no major spots under UV-S. It was therefore not further investigated.

Subfraction 5 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed three well-separated spots under UV-S with R_f values of 0.22, 0.31 and 0.38. Further acetylation with acetic anhydride (0.55 ml) and pyridine (0.14 ml) was performed. The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure to yield a yellow viscous liquid in 22.8 mg. Further separation with precoated TLC on silica gel plates with 50% ethyl acetate-petroleum ether (2 runs) as a mobile phase afforded a white solid (**AcYS6**) in 2.8 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether demonstrated one pure component under UV-S with R_f value of 0.45.

$[\alpha]_D^{29}$	-68.18° ($c = 4.4 \times 10^{-2}$ g/100 cm^3, $CHCl_3$)
IR (neat) ν (cm^{-1})	2938 (CH stretching), 1750, 1634 (C=O stretching), 1602, 1579 (C=C stretching)

UV ($CHCl_3$) λ_{max} (nm) (log ε)		260 (4.47)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)		7.97 (*s*, 1H), 7.65 (*s*, 1H), 7.51 (*d*, *J* = 9.3 Hz, 2H), 7.20 (*s*, 1H), 6.98 (*d*, *J* = 9.3 Hz, 2H), 5.35-5.32 (*m*, 2H), 5.31(*dd*, *J* = 3.4, 1.7 Hz, 1H), 5.26 (*dd*, *J* = 9.9, 3.4 Hz, 1H), 5.13 (*d*, *J* = 7.6 Hz, 1H), 5.09 (*t*, *J* = 9.9 Hz, 1H), 5.04 (*t*, *J* = 9.9 Hz, 1H), 4.74 (*d*, *J* = 1.7 Hz, 1H), 3.92 (*s*, 3H), 3.89 (*ddd*, *J* = 9.9, 7.0, 2.2 Hz, 1H), 3.84 (*s*, 3H), 3.83 (*dd*, *J* = 9.9, 5.9 Hz, 1H), 3.77 (*dd*, *J* = 11.2, 2.4 Hz, 1H), 3.65 (*dd*, *J* = 11.5, 7.0 Hz, 1H), 2.09 (*s*, 3H), 2.08 (*s*, 3H), 2.07 (*s*, 3H), 2.05 (*s*, 3H), 2.04 (*s*, 3H), 1.96 (*s*, 3H), 1.19 (*d*, *J* = 5.9 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		175.84, 170.43, 170.14, 170.05, 169.96, 169.68 169.49, 159.79, 152.94, 151.52, 150.99, 148.82 130.33, 124.69, 124.32, 120.71, 114.21, 107.19 106.57, 100.03, 98.44, 73.90, 72.64, 71.24, 71.07 69.55, 69.36, 69.25, 66.93, 66.80, 56.62, 55.55, 21.00 20.93, 20.83, 17.58
DEPT (135°) ($CDCl_3$)	CH	152.94, 130.33, 114.21, 107.19, 106.57, 100.03, 98.44, 73.90, 72.64, 71.24, 71.07, 69.55, 69.36, 69.25, 66.93
	CH_2	66.80
	CH_3	56.62, 55.55, 21.00, 20.93, 20.83, 17.58

Subfraction 6 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major spot under UV-S with R_f value of 0.37. Because it was obtained in low quantity, it was not further investigated.

Subfraction J8.5 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one major UV-active spot with R_f value of 0.31. Further purification by precoated TLC on silica gel plates with 15% methanol-chloroform (4 runs) as a mobile phase afforded three bands.

Band J8.5-a (**Acetate of AcYS8**) was obtained as a white solid in 9.5 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major component under UV-S with R_f value of 0.32. Further acetylation with acetic anhydride (0.2 ml) and pyridine (0.06 ml) was carried out. The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield a yellow viscous liquid in 10.8 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one pure compound under UV-S with R_f value of 0.43.

$[\alpha]_D^{29}$	-55.56° ($c = 3.6 \times 10^{-2}$ g/100 cm^3, CHCl$_3$)
IR (neat) ν (cm^{-1})	2925, 2851 (CH stretching), 1756, 1645 (C=O stretching), 1609, 1581 (C=C stretching)
UV (CHCl$_3$) λ_{max} (nm) (log ε)	255 (4.93), 213 (4.87)
^{1}H NMR (CDCl$_3$) (δ ppm) (500 MHz)	8.14 (*d*, J = 8.5 Hz, 1H), 8.00 (*s*, 1H), 7.52 (*d*, J = 7.0 Hz, 2H), 7.16 (*d*, J = 8.5 Hz, 1H), 7.00 (*d*, J = 7.0 Hz, 2H), 5.37 (*dd*, J = 9.5, 7.5 Hz, 1H), 5.31 (*t*, J = 9.5 Hz, 1H), 5.23 (*dd*, J = 9.5, 9.0 Hz, 1H), 5.18 (*d*, J = 7.5 Hz, 1H), 4.36 (*dd*, J = 11.3, 4.2 Hz, 1H), 4.00 (*dd*, J = 11.3, 2.5 Hz, 1H), 3.87 (*s*, 3H), 3.70 (*ddd*, J = 9.0, 4.2, 2.5 Hz, 1H), 2.38 (*s*, 3H), 2.15 (*s*, 3H), 2.07 (*s*, 3H), 2.05 (*s*, 3H), 2.02 (*s*, 3H)
^{13}C NMR (CDCl$_3$) (δ ppm) (125 MHz)	175.63, 170.59, 170.48, 169.61, 169.42, 168.42 160.18, 152.14, 150.40, 147.82, 136.47, 130.36 125.65, 123.76, 123.58, 122.95, 121.00, 114.39 101.69, 72.88, 72.56, 71.84, 68.23, 61.72, 55.59

		20.97, 20.96, 20.82, 20.77
DEPT (135°) ($CDCl_3$)	CH	152.14, 130.36, 122.95, 121.00, 114.39, 101.69 72.88, 72.56, 71.84, 68.23
	CH_2	61.72
	CH_3	55.92, 20.97, 20.96, 20.82, 20.77

Band J8.5-b was obtained as a white solid in 1.1 mg. Chromatogram characteristics on normal phase with 15% methanol-chloroform (2 runs) showed two major UV-active spots with R_f values of 0.14 and 0.17. It was therefore not further investigated because it was obtained in low quantity.

Band J8.5-c was obtained as a white solid in 1.2 mg. Chromatogram characteristics on normal phase with 15% methanol-chloroform (2 runs) showed one UV-active spot with R_f value of 0.24. It was therefore not further investigated because it was obtained in low quantity.

Subfraction J8.6 Chromatogram characteristics on normal phase with 15% methanol-chloroform showed none of well-separated spots. It was therefore not further investigated.

Subfraction J9 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed five spots under UV-S with R_f values of 0.13, 0.20, 0.26, 0.30 and 0.34. It was further chromatographed on silica gel column chromatography using 10% methanol-chloroform, chloroform-methanol gradient and finally pure methanol to give one hundred and forty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford six subfractions as shown in Table 49.

Table 49 Fractions obtained from **J9** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
J9.1	0.025	Yellow viscous liquid
J9.2	0.015	Pale yellow solid

Table 49 (continued)

Fraction	Weight (g)	Physical appearance
J9.3	0.105	Yellow viscous liquid
J9.4	0.061	White solid mixed with yellow viscous liquid
J9.5	0.058	Yellow viscous liquid
J9.6	0.072	Pale yellow solid mixed with yellow viscous liquid

Subfraction J9.1 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed no UV-active spots. Therefore, no further purification was performed.

Subfraction J9.2 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) indicated the presence of pure **YS1**.

Subfraction J9.3 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed two major spots of **YS1** and **YS6**. This fraction was combined with **J8.4**.

Subfraction J9.4 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major spot of **YS2**.

Subfraction J9.5 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) under UV-S indicated that this fraction contained **YS2** and the other component with R_f value of 0.26. Further separation with column chromatography over silica gel was carried out. Elution was conducted with 15% methanol-chloroform to afford seventy eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions as shown in Table 50.

Table 50 Fractions obtained from **J9.5** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
J9.5-a	0.003	Colorless viscous liquid
J9.5-b	0.018	Colorless viscous liquid

Table 50 (continued)

Fraction	Weight (g)	Physical appearance
J9.5-c	0.008	Colorless viscous liquid
J9.5-d	0.006	Colorless viscous liquid

Subfraction J9.5-a Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major UV-active spot with R_f value of 0.30. No further purification was performed because this fraction was obtained in low quantity.

Subfraction J9.5-b Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) under UV-S indicated that this fraction contained **YS2** and the other component with R_f value of 0.31. It was not further investigated because of low quantity of this fraction.

Subfraction J9.5-c and J9.5-d Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) indicated the presence of **YS2** as a major component.

Subfraction J9.6 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) indicated the presence of **YS2** as a major component.

Subfraction J10 Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed none of well-separated spots under UV-S. Further acetylation with acetic anhydride (4.00 ml) and pyridine (1.20 ml) was carried out. The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield a brownish viscous liquid in 187.3 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed one major UV-active spot with R_f value of 0.48. It was then chromatographed on silica gel column. Elution was conducted initially with 40% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with 10% methanol-ethyl acetate to give forty four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford seven subfractions as shown in Table 51.

Table 51 Fractions obtained from **J10** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
J10.1	0.008	Yellow viscous liquid
J10.2	0.023	Yellow viscous liquid
J10.3	0.017	Yellow viscous liquid
J10.4	0.014	Yellow viscous liquid
J10.5	0.034	Yellow viscous liquid
J10.6	0.064	Yellow viscous liquid
J10.7	0.038	Yellow viscous liquid

Subfraction J10.1 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed one major spot under UV-S with R_f value of 0.45. Further purification by precoated TLC on silica gel plates with 30% ethyl acetate-petroleum ether (8 runs) as a mobile phase afforded a yellow viscous liquid (**AcYS15**) in 2.2 mg. Chromatogram characteristics on normal phase TLC with chloroform showed one pure spot under UV-S with R_f value of 0.12.

IR (neat) ν (cm^{-1})	2924, 2852 (CH stretching), 1782, 1730, 1649 (C=O stretching), 1611, 1568 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)	257 (4.12)

^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	8.20 (*d*, J = 8.8 Hz, 1H), 7.94 (*s*, 1H), 7.48 (*d*, J = 8.8 Hz, 2H), 7.26 (*d*, J = 8.8 Hz, 1H), 6.98 (*d*, J = 8.8 Hz, 2H), 3.84 (*s*, 3H), 2.42 (*s*, 3H), 2.36 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	175.34, 167.54, 167.21, 159.85, 152.03, 149.46, 146.42, 130.14, 125.42, 124.04, 123.38, 119.97, 114.08, 55.35, 20.64, 20.26

Subfraction J10.2 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed two well-separated spots under UV-S with R_f values of 0.31 and 0.36. It was therefore not further investigated because it was obtained in low quantity.

Subfraction J10.3 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed two well-separated spots under UV-S with R_f values of 0.19 and 0.27. It was therefore not further investigated because it was obtained in low quantity.

Subfraction J10.4 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed two well-separated spots under UV-S with R_f values of 0.15 and 0.20. It was not further investigated because it was obtained in low quantity.

Subfraction J10.5 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed four spots under UV-S with R_f values of 0.06, 0.12, 0.15 and 0.25. It was not further investigated because it was obtained in low quantity.

Subfraction J10.6 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction J10.7 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed three UV-active spots with R_f values of 0.05, 0.10 and 0.25. It was not further investigated because it was obtained in low quantity.

Subfraction J11 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed no major spots under UV-S. It was therefore not further investigated.

Fraction 5 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed four well-separated spots under UV-S with R_f values of 0.42, 0.56, 0.72 and 0.90. In addition, it contained **YS2** (R_f 0.28) and **YS3** (R_f 0.20) as major components. This fraction was divided into two portions.

The first portion (**K**, 2.09 g) was further separated by column chromatography over reversed-phase silica gel. Elution was conducted initially with 30% methanol-water and gradually enriched with pure methanol to afford one hundred and four fractions. All fractions were examined by TLC, combined on the basis of chromatogram characteristics and then evaporated to dryness *in vacuo* to afford twelve subfractions as shown in Table 52.

Table 52 Fractions obtained from **K** by column chromatography over reversed-phase silica gel

Fraction	Weight (g)	Physical appearance
K1	0.062	Yellow viscous liquid
K2	0.032	Yellow viscous liquid
K3	0.030	Yellow viscous liquid
K4	0.032	Pale yellow solid
K5	0.013	Yellow viscous liquid
K6	0.044	Yellow viscous liquid
K7	0.082	Yellow viscous liquid
K8	0.138	Yellow viscous liquid
K9	0.107	Yellow viscous liquid
K10	0.150	Yellow viscous liquid
K11	0.883	Pale yellow solid
K12	0.343	Yellow viscous liquid

Subfraction K1 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of separated spots under UV-S. It was therefore not further investigated.

Subfraction K2 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one oval spot under UV-S with R_f value of 0.17. In addition, chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed one major spot under UV-S with R_f value of 0.84. No further purification was performed because this fraction was obtained in low quantity.

Subfraction K3 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one major spot under UV-S with R_f value of 0.26 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two UV-active spots with R_f values of 0.56 and 0.66. Chromatography on reversed-phase TLC with 33% methanol-water afforded four bands.

Band 1 was obtained as a white solid in 1.6 mg. Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 33% methanol-water demonstrated one pale spot under UV-S with R_f values of 0.08 and 0.84, respectively. This fraction was obtained in low quantity. It was therefore not further investigated.

Band 2 was obtained as a yellow solid in 4.9 mg. Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed two well-separated spots under UV-S with R_f values of 0.25 and 0.45. In addition, chromatogram characteristics on reversed-phase TLC with 33% methanol-water showed only one spot under UV-S with R_f value of 0.82. Further acetylation with acetic anhydride (1.00 ml) and pyridine (0.10 ml) was performed. The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield a yellow viscous liquid in 3.4 mg. Chromatogram characteristics on normal phase TLC with chloroform, followed by 10% methanol-chloroform, showed two pale well-separated spots under UV-S with R_f values of 0.53 and 0.80. It was therefore not further investigated.

<u>Band 3</u> was obtained as a white solid in 4.1 mg Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 33% methanol-water, respectively, showed two well-separated spots under UV-S with R_f values of 0.23, 0.39 and 0.64, 0.84, respectively. It was obtained in low quantity. Therefore, no further purification was performed.

<u>Band 4</u> was obtained as a yellow solid in 1.9 mg. Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 33% methanol-water, respectively, showed only one spot under UV-S with R_f values of 0.23 and 0.84, respectively. It was therefore not further investigated because this fraction was obtained in low quantity.

Subfraction K4 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one major UV-active spot with R_f value of 0.49 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two pale UV-active spots with R_f values of 0.55 and 0.67. It was not further investigated.

Subfraction K5 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction K6 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed three spots with R_f values of 0.15, 0.22, 0.33 and 0.60, 0.68, 0.73, respectively. This fraction (18.4 mg) was further acetylated with acetic anhydride (0.46 ml) and pyridine (0.14 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield a yellow viscous liquid in 8.7 mg. Chromatogram characteristics on normal phase TLC with 8% acetone-chloroform showed none of well-separated spots under UV-S. The remainder of **K6** was then dissolved with methanol. Two fractions were obtained as follow :

0.20, 0.35 and 0.42. In addition, chromatogram characteristics on reversed-phase TLC with 30% methanol-water showed two major spots under UV-S with R_f values of 0.62 and 0.72. Because of low quantity, it was not further investigated.

Subfraction K6.3 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed three major UV-active spots with R_f values of 0.22, 0.26 and 0.38 while chromatogram characteristics on reversed-phase TLC with 30% methanol-water showed two well-separated spots with R_f values of 0.34 and 0.62. Because of low quantity, it was not further investigated.

Subfraction K6.4 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 30% methanol-water, respectively, showed two well-separated spots under UV-S with R_f values of 0.27, 0.39 and 0.34, 0.61, respectively. It was further separated into two fractions by dissolving in methanol.

<u>Methanol-insoluble fraction</u> was obtained as a white solid in 2.3 mg. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major UV-active spot with R_f value of 0.22. It was not further investigated as it was obtained in low quantity.

<u>Methanol-soluble fraction</u> was obtained as a pale yellow solid. Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed many spots under UV-S. It was therefore not further investigated.

Subfraction K6.5 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed three major spots under UV-S with R_f values of 0.25, 0.35 and 0.40 while chromatogram characteristics on reversed-phase TLC with 30% methanol-water showed two well-separated spots under UV-S with R_f values of 0.34 and 0.62. No further purification was performed because of low quantity.

Subfraction K6.6 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed two well-separated spots under UV-S with R_f values of 0.26 and 0.40. In addition, chromatogram characteristics on reversed-phase TLC with 30% methanol-water showed one major UV-active spot with R_f value of 0.60. It was therefore not further investigated because it was obtained in low quantity.

Subfraction K6.7 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 30% methanol-water, respectively, showed one major UV-active spot with R_f values of 0.27 and 0.60. Further investigation was not performed because of low quantity.

Subfraction K6.8 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one pale UV-active spot with R_f value of 0.26. In addition, chromatogram characteristics on reversed-phase TLC with 30% methanol-water showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction K6.9 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 30% methanol-water, respectively, showed no major spots under UV-S. Therefore, no further purification was carried out.

Subfraction K7 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction K8 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed no major UV-active spots. It was therefore not further investigated.

Subfraction K9 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed one major UV-active spot with R_f values of 0.39 and 0.31, respectively. This subfraction was divided into three portions.

The first portion (31.5 mg) was further separated by column chromatography over silica gel. Elution was conducted with 14% methanol-dichloromethane to afford forty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford two subfrations.

<u>Subfraction 1</u> was obtained as a colorless viscous liquid in 14.8 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (3 runs) demonstrated one major spot under UV-S with R_f value of 0.23. Further purification by precoated TLC with 14% methanol-dichloromethane (6 runs) as a mobile phase afforded a yellow viscous liquid in 1.4 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (3 runs) and 20% methanol-chloroform showed one UV-active spot with R_f values of 0.46 and

0.32, respectively. Therefore, no further purification was performed because this fraction was obtained in low quantity.

Subfraction 2 was obtained as a colorless viscous liquid in 6.1 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (3 runs) showed none of UV-active spots. It was therefore not further investigated.

The second portion (16.1 mg) was further separated by precoated TLC on silica gel plates with 14% methanol-dichloromethane as a mobile phase afforded two bands.

Band 1 (**YS11**) was obtained as a yellow-white solid in 3.2 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (2 runs) showed one UV-active spot with R_f value of 0.25.

Band 2 was obtained as a white solid in 0.8 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (2 runs) demonstrated one UV-active spot with R_f value of 0.22. No further purification was performed because this fraction was obtained in low quantity.

The third portion (50.5 mg) was further separated by column chromatography over silica gel. Elution was conducted with 14% methanol-dichloromethane to afford sixty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions.

Subfraction 1 was obtained as a colorless viscous liquid in 1.3 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (3 runs) showed no UV-active spots. It was therefore not further investigated.

Subfraction 2 was obtained as a white solid mixed with yellow viscous liquid in 37.5 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (3 runs) indicated the presence of **YS11** as a major component. Further separation with precoated TLC on silica gel plates with 14% methanol-dichloromethane (5 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a yellow-white solid in 13.0 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (4 runs) indicated that the major component was **YS11**. Further acetylation with acetic anhydride (0.40 ml) and pyridine (0.10 ml) was performed. The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to give a yellow viscous liquid in 13.5 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed well-separated spots with R_f values of 0.19 and 0.27. Further separation with precoated TLC on silica gel plates with 50% ethyl acetate-petroleum ether (4 runs) as a mobile phase afforded two bands.

Band 1.1 was obtained as a white viscous liquid in 1.5 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed two UV-active spots with R_f values of 0.20 and 0.28. No further purification was performed because this band was obtained in low quantity.

Band 1.2 (**AcYS11**) was obtained as a colorless viscous liquid in 7.8 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one pure spot with R_f value of 0.20.

$[\alpha]_D^{29}$	-40.54° (c = 7.4 x 10^{-2} g/100 cm^3, $CHCl_3$)
IR (neat) ν (cm^{-1})	2939, 2848 (CH stretching), 1748, 1645 (C=O stretching), 1609, 1567 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)	253 (4.50)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	8.06 (*d*, *J* = 9.1 Hz, 1H), 7.99 (*s*, 1H), 7.52 (*d*, *J* = 9.1 Hz, 1H), 7.04 (*d*, *J* = 9.1 Hz, 1H), 6.98 (*d*, *J* = 9.1 Hz, 1H), 5.36 (*dd*, *J* = 9.2, 6.8 Hz, 1H), 5.31 (*t*, *J* = 9.2 Hz, 1H), 5.22 (*d*, *J* = 6.8 Hz, 1H), 5.15 (*t*, *J* = 9.2 Hz, 1H), 5.12 (*dd*, *J* = 9.2, 3.2 Hz, 1H), 5.06 (*dd*, *J* = 3.2, 1.6 Hz, 1H), 4.98 (*t*, *J* = 9.2 Hz, 1H), 4.56 (*d*, *J* = 1.6 Hz, 1H), 3.99 (*s*, 3H), 3.84 (*s*, 3H), 3.75 (*ddd*, *J* = 9.2, 6.8, 2.4 Hz, 1H), 3.70 (*dq*, *J* = 9.2, 6.3 Hz, 1H),

		3.69 (*dd, J* = 12.0, 6.8 Hz, 1H), 3.62 (*dd, J* = 12.0, 2.4 Hz, 1H), 2.11 (*s*, 3H), 2.08 (*s*, 3H), 2.06 (*s*, 3H), 1.99 (*s*, 3H), 1.94 (*s*, 3H), 1.11 (*d, J* = 6.3 Hz, 3H)
^{13}C NMR (CDCl$_3$) (δ ppm) (125 MHz)		175.95, 170.49, 170.25, 170.15, 170.09, 169.88, 169.56, 159.92, 156.05, 152.71, 150.82, 132.35, 130.49, 124.72, 124.24, 123.41,119.35,114.21, 110.30, 101.15, 97.92, 74.38, 72.89, 72.12, 70.91, 69.56, 69.26, 69.18, 66.94, 66.53, 56.80, 55.56, 20.95, 20.93, 20.87, 20.86, 20.85, 17.52
DEPT (135°) (CDCl$_3$)	CH	152.71, 130.49, 123.41, 114.21, 110.30, 101.15, 97.92, 74.38, 72.89, 72.12, 70.91, 69.56, 69.26, 69.18, 66.94
	CH$_2$	66.53
	CH$_3$	56.80, 55.56, 20.95, 20.93, 20.87, 20.86, 20.85, 17.52

Band 2 was obtained as a yellow viscous liquid in 3.0 mg. Chromatogram characteristics on normal phase TLC with 14% methanol-dichloromethane (4 runs) showed one major UV-active spot with R_f value of 0.30. No further purification was performed because it was obtained in low quantity.

Subfraction K10 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, indicated the presence of **YS3** and **YS11** as major components.

Subfraction K11 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, indicated that the major component was **YS2**.

Subfraction K12 Chromatogram characteristics on normal and reversed-phase TLC with 20% methanol-chloroform and 50% methanol-water, respectively, showed none of well-separated spots under UV-S. It was therefore not further investigated.

The second portion (**L**, 1.65 g) was further acetylated with acetic anhydride (43.75 ml) in the presence of pyridine (13.02 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid

(2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield a yellow viscous liquid in 1.81 g. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed three major spots under UV-S with R_f values of 0.25, 0.32 and 0.39. Further separation with column chromatography over silica gel was carried out. Elution was conducted initially with 10% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate, ethyl acetate-acetone gradient and followed by increasing amount of methanol in acetone and finally with pure methanol to give one hundred and fifty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford fourteen subfractions as shown in Table 54.

Table 54 Fractions obtained from **L** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
L1	0.082	Yellow viscous liquid
L2	0.013	Yellow viscous liquid
L3	0.147	Yellow viscous liquid
L4	0.062	Yellow viscous liquid
L5	0.139	Yellow viscous liquid
L6	0.364	Colorless viscous liquid mixed with white solid
L7	0.156	Yellow viscous liquid
L8	0.104	Yellow viscous liquid
L9	0.085	Yellow viscous liquid
L10	0.006	Colorless viscous liquid
L11	0.041	Yellow viscous liquid
L12	0.008	Orange-yellow viscous liquid
L13	0.390	Yellow viscous liquid
L14	0.211	Yellow viscous liquid

Subfraction L1 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed none of UV-active spots. It was therefore not further investigated.

Subfraction L2 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (3 runs) showed one pale UV-active spot with R_f value of 0.28. It was therefore not further investigated.

Subfraction L3 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (3 runs) showed many UV-active spots. No further purification was performed.

Subfraction L4 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (3 runs) showed two major spots under UV-S with R_f values of 0.41 and 0.50. Further separation with precoated TLC with 45% ethyl acetate-petroleum ether as a mobile phase afforded five bands.

Band 1 was obtained as a colorless viscous liquid in 7.2 mg. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with R_f value of 0.42. Therefore, no further purification was performed because of low quantity.

Band 2 was obtained as a colorless viscous liquid in 13.9 mg. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with R_f value of 0.36. Because of low quantity, it was not further investigated.

Band 3 was obtained as a colorless viscous liquid in 3.7 mg. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with R_f value of 0.50. No further purification was carried out because this fraction was obtained in low quantity.

Band 4 was obtained as a colorless viscous liquid in 1.9 mg. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed two pale UV-active spots with R_f values of 0.38 and 0.46. It was therefore not further investigated.

Band 5 was obtained as a colorless viscous liquid in 0.8 mg. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with R_f value of 0.38. Because this fraction was obtained in low quantity, it was not further investigated.

Subfraction L5 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (3 runs) demonstrated two major spots under UV-S with R_f values of 0.35 and 0.45. It was not further investigated because it was obtained in low quantity.

Subfraction L6 (AcYS2) Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (3 runs) showed one pure spot under UV-S with R_f value of 0.28.

IR (neat) ν (cm^{-1})	2940 (CH stretching), 1755, 1645 (C=O stretching), 1609, 1570 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)	255 (4.10)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	8.04 (*d*, *J* = 9.5 Hz, 1H), 7.99 (*s*, 1H), 7.49 (*d*, *J* = 9.5 Hz, 2H), 7.18 (*d*, *J* = 9.5 Hz, 1H), 6.97 (*d*, *J* = 9.5 Hz, 2H), 5.37 (*dd*, *J* = 9.7, 7.4 Hz, 1H), 5.33 (*t*, *J* = 9.7 Hz, 1H), 5.27 (*dd*, *J* = 10.0, 3.7 Hz, 1H), 5.23 (*dd*, *J* = 3.7, 1.5 Hz, 1H), 5.18 (*d*, *J* = 7.4 Hz, 1H), 5.12 (*dd*, *J* = 10.5, 9.7 Hz, 1H), 5.06 (*t*, *J* = 10.0 Hz, 1H), 4.78 (*d*, *J* = 1.5 Hz, 1H), 3.96 (*s*, 3H), 3.90 (*ddd*, *J* = 10.5, 6.3, 2.5 Hz, 1H), 3.87 (*dq*, *J* = 10.0, 6.5 Hz, 1H), 3.79 (*dd*, *J* = 11.3, 2.5 Hz, 1H), 3.68 (*dd*, *J* = 11.3, 6.3 Hz, 1H), 2.11 (*s*, 3H), 2.10 (*s*, 3H), 2.08 (*s*, 3H), 2.06 (*s*, 3H), 2.05 (*s*, 3H), 2.00 (*s*, 3H), 1.19 (*d*, *J* = 6.5 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	175.75, 170.17, 169.98, 169.97, 169.91, 169.46, 169.29, 159.68, 153.29, 152.21, 150.72, 138.48, 130.11, 124.82, 123.83, 121.87, 121.55, 114.99, 113.99, 99.77, 98.09, 73.66, 72.40, 71.03, 70.79, 69.43, 68.79, 66.80, 66.59, 62.00, 55.32, 20.80,

20.70, 20.63, 20.61, 20.60, 17.36

Subfraction L7 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (3 runs) demonstrated the presence of **AcYS2** as a major component and the other UV-active spot with R_f value of 0.22. It was further separated by precoated TLC with 40% ethyl acetate-petroleum ether (8 runs) as a mobile phase to afford two bands.

Band 1 was obtained as a colorless viscous liquid in 55.3 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) indicated the presence of pure **AcYS2**.

Band 2 was obtained as a yellow viscous liquid in 46.2 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one UV-active spot with R_f value of 0.32. However, ^{1}H NMR spectrum indicated that it was a mixture. Further separation with precoated TLC on silica gel plates with 50% ethyl acetate-petroleum ether (3 runs), followed by 1% methanol-chloroform (3 runs) and then 6% acetone-chloroform afforded a yellow viscous liquid (**AcYS6**) in 11.3 mg. Chromatogram characteristics on normal phase TLC with 8% acetone-chloroform (2 runs) showed one pure spot under UV-S with R_f value of 0.32.

Subfraction L8 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (3 runs) showed one major UV-active spot with R_f value of 0.16. Further separation with precoated TLC on silica gel plates with 45% ethyl acetate-petroleum ether (7 runs) and then 50% ethyl acetate-petroleum ether (3 runs) afforded **AcYS11**, a yellow viscous liquid in 21.5 mg.

Subfraction L9 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (3 runs) showed two well-separated spots under UV-S with R_f values of 0.15 and 0.40. It was further separated by precoated TLC on silica gel plates with 50% ethyl acetate-petroleum ether (3 runs) to afford **AcYS8** as a yellow viscous liquid in 5.6 mg. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (3 runs) demonstrated one pure spot under UV-S with R_f value of 0.35.

$[\alpha]_D^{29}$		-55.56° (c = 3.6 x 10^{-2} g/100 cm^3, $CHCl_3$)
IR (neat) ν (cm^{-1})		3443 (OH stretching) 2920, 2850 (CH stretching), 1755, 1630 (C=O stretching), 1609 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)		254 (4.07)
^1H NMR ($CDCl_3$) (δ ppm) (400 MHz)		8.05 (*d*, *J* = 9.0 Hz, 1H), 7.85 (*s*, 1H), 7.47 (*d*, *J* = 9.0 Hz, 2H), 7.05 (*d*, *J* = 9.0 Hz, 1H), 6.98 (*d*, *J* = 9.0 Hz, 2H), 5.40 (*dd*, *J* = 9.8, 8.1 Hz, 1H), 5.33 (*t*, *J* = 9.8 Hz, 1H), 5.20 (*t*, *J* = 9.8 Hz, 1H), 4.99 (*d*, *J* = 8.1 Hz, 1H), 4.30 (*dd*, *J* = 12.6, 5.6 Hz, 1H), 4.20 (*dd*, *J* = 12.6, 2.8 Hz, 1H), 3.84 (*s*, 3H), 3.82 (*ddd*, *J* = 9.8, 5.6, 2.8 Hz, 1H), 2.20 (*s*, 3H), 2.12 (*s*, 3H), 2.07 (*s*, 3H), 2.06 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (100 MHz)		175.59, 170.57, 170.14, 169.30, 169.17, 159.85, 154.37, 151.34, 150.19, 131.07, 130.21, 125.37, 124.26, 123.49, 118.66, 115.29, 114.08, 103.36, 72.68, 72.06, 70.98, 67.93, 61.34, 55.35, 20.74, 20.57
DEPT (135°) ($CDCl_3$)	CH	151.34, 130.21, 124.26, 115.29, 114.08, 103.36, 72.68, 72.06, 70.98, 67.93
	CH_2	61.34
	CH_3	55.35, 20.74, 20.57

Subfraction L10 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed two well-separated spots under UV-S with R_f values of 0.19 and 0.23. It was further separated by precoated TLC on silica gel plates with 45% ethyl acetate-petroleum ether to afford two bands.

Band 1 was obtained as a colorless viscous liquid in 0.8 mg. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed one UV-active spot with R_f value of 0.32. Because this fraction was obtained in low quantity, it was not further investigated.

Band 2 was obtained as a colorless viscous liquid in 1.6 mg. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with R_f value of 0.22. Because this fraction was obtained in low quantity, it was not further investigated.

Subfraction L11 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (3 runs) showed one major UV-active spot with R_f value of 0.38. It was further separated by precoated TLC with 45% ethyl acetate-petroleum ether as a mobile phase afforded four bands.

Band 1 was obtained as a colorless viscous liquid in 0.6 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with R_f value of 0.58. No attempted purification was carried out because of low quantity.

Band 2 was obtained as a colorless viscous liquid in 2.9 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one UV-active spot with R_f value of 0.48 which was identical to that of **AcYS7**.

Band 3 was obtained as a colorless viscous liquid in 1.3 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one UV-active spot with R_f value of 0.19. No further investigation was performed because it was obtained in low quantity.

Band 4 was obtained as a white solid in 1.1 mg. Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one pale spot under UV-S with R_f value of 0.12. It was therefore not further investigated.

Subfraction L12 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (3 runs) showed one oval spot under UV-S with R_f value of 0.19. No further investigation was carried out because it was obtained in low quantity.

Subfraction L13 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed none of well-separated spots under UV-S. No attempted purification was carried out.

Subfraction L14 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (3 runs) showed one major spot under UV-S with R_f value of 0.48. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 45% ethyl acetate-petroleum ether and gradually enriched with 50% ethyl acetate-petroleum ether to give fifty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine subfractions as shown in Table 55.

Table 55 Fractions obtained from **L14** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
L14.1	0.001	Colorless viscous liquid
L14.2	0.001	Yellow viscous liquid
L14.3	0.001	Yellow viscous liquid
L14.4	0.002	Yellow viscous liquid
L14.5	0.001	Yellow viscous liquid
L14.6	0.0001	Yellow viscous liquid
L14.7	0.001	Yellow viscous liquid
L14.8	0.006	Yellow viscous liquid
L14.9	0.110	Yellow viscous liquid

Subfraction L14.1 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed none of UV-active spots. It was therefore not further investigated.

Subfraction L14.2 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one pale UV-active spot with R_f value of 0.73. No attempted purification was carried out.

Subfraction L14.3 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed no UV-active spots. It was therefore not further investigated.

Subfraction L14.4 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed three well-separated spots under UV-S with R_f values of 0.49, 0.58 and 0.67. No further purification was performed because it was obtained in low quantity.

Subfraction L14.5 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) demonstrated one pale UV-active spot with R_f value of 0.69. It was therefore not further investigated.

Subfraction L14.6 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed no UV-active spots. No further investigation was carried out.

Subfraction L14.7 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed three well-separated spots under UV-S with R_f values of 0.27, 0.38 and 0.68. Because this fraction was obtained in low quantity, it was not further investigated.

Subfraction L14.8 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) demonstrated two pale well-separated spots under UV-S with R_f values of 0.55 and 0.69. No attempted investigation was performed because this fraction was obtained in low quantity.

Subfraction L14.9 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one major spot under UV-S with R_f value of 0.67, indicating the presence of **AcYS7**. Further separation with precoated TLC on silica gel plates with 50% ethyl acetate-petroleum ether and 8% acetone-chloroform (4 runs) afforded **AcYS7** as a yellow viscous liquid in 9.0 mg. Chromatogram characteristics on normal phase TLC with 8% acetone-chloroform showed one pure spot under UV-S with R_f value of 0.56.

$[\alpha]_D^{29}$	-90.90^o ($c = 1.1 \times 10^{-2}$ g/100 cm^3, $CHCl_3$)
IR (neat) ν (cm^{-1})	2923 (CH stretching), 1756, 1659 (C=O stretching), 1613, 1591 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)	265 (4.33)

^{1}H NMR (CDCl$_3$) (δ ppm) (400 MHz)		12.90 (*brs*, 1H), 7.95 (*s*, 1H), 7.48 (*d*, J = 9.0 Hz, 2H), 6.99 (*d*, J = 9.0 Hz, 2H), 6.71 (*s*, 1H), 5.36 (*t*, J = 9.0 Hz, 1H), 5.34 (*dd*, J = 3.0, 1.5 Hz, 1H), 5.33 (*t*, J = 9.0 Hz, 1H), 5.27 (*dd*, J = 9.0, 3.0 Hz, 1H), 5.12 (*d*, J = 7.5 Hz, 1H), 5.07 (*t*, J = 9.0 Hz, 1H), 5.05 (*t*, J = 9.0 Hz, 1H), 4.76 (*d*, J = 1.5 Hz, 1H), 3.93-3.86 (*m*, 1H), 3.86-3.81 (*m*, 1H), 3.84 (*s*, 3H), 3.83 (*s*, 3H), 3.76 (*dd*, J = 12.0, 3.0 Hz, 1H), 3.63 (*dd*, J = 12.0, 9.0 Hz, 1H), 2.10 (*s*, 3H), 2.08 (*s*, 3H), 2.07 (*s*, 3H), 2.05 (*s*, 3H), 2.04 (*s*, 3H), 1.96 (*s*, 3H), 1.20 (*d*, J = 6.4 Hz, 3H)
^{13}C NMR (CDCl$_3$) (δ ppm) (100 MHz)		181.36, 170.18, 169.89, 169.76, 169.48, 169.29, 159.78, 155.68, 154.28, 153.73, 152.74, 133.72, 130.09, 123.02, 122.93, 114.21, 108.39, 100.89, 99.45, 98.29, 95.41, 73.67, 72.37, 70.74, 69.19, 68.93, 66.67, 66.46, 61.08, 55.36, 20.80, 20.62, 17.34
DEPT (135°) (CDCl$_3$)	CH	153.73, 130.09, 114.21, 99.45, 98.29, 95.41, 73.67, 72.37, 70.74, 69.19, 68.93, 66.67
	CH$_2$	66.46
	CH$_3$	61.08, 55.36, 20.80, 20.62, 17.34

Fraction 6 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one oval major spot under UV-S with R$_f$ value of 0.24. In addition, chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two pale spots under UV-S with R$_f$ values of 0.71 and 0.89 and two major spots under UV-S with R$_f$ values of 0.06 and 0.20. It was further acetylated with acetic anhydride (27.90 ml) in the presence of pyridine (8.40 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3×20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2×20 ml), 10% sodium bicarbonate (3×20 ml) and water (2×20 ml). The organic phase was dried over anhydrous sodium sulfate,

filtered and evaporated to dryness *in vacuo* to yield a brown-yellow solid in 1.49 g. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether demonstrated three major UV-active spots with R_f values of 0.26, 0.39 and 0.60. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 20% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of acetone in ethyl acetate and finally with pure acetone to give eight four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine subfractions as shown in Table 56.

Table 56 Fractions obtained from **fraction 6** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
M1	0.052	Yellow viscous liquid
M2	0.037	Yellow viscous liquid
M3	0.127	Yellow viscous liquid
M4	0.364	White solid
M5	0.165	Yellow viscous liquid
M6	0.159	Yellow viscous liquid
M7	0.103	Yellow viscous liquid
M8	0.099	Yellow viscous liquid
M9	0.069	Yellow viscous liquid

Subfraction M1 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed none of well-separated spots under UV-S. It was therefore not further investigated.

Subfraction M2 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed two well-separated spots under UV-S with R_f values of 0.39 and 0.54. It was further separated by precoated TLC on silica gel plates with 45% ethyl acetate-petroleum ether (4 runs) to afford **AcYS3** as a colorless viscous liquid in 29.2 mg. Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (4 runs) showed one pure UV-active spot with R_f value of 0.60.

$[\alpha]_D^{29}$		-41.52° (c = 28.9 x 10^{-2} g/100 cm^3, $CHCl_3$)
IR (neat) ν (cm^{-1})		2939 (CH stretching), 1748, 1645 (C=O stretching), 1622, 1569 (C=C stretching)
UV ($CHCl_3$) λ_{max} (nm) (log ε)		260 (4.39)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)		8.24 (*d*, *J* = 8.0 Hz, 1H), 7.95 (*s*, 1H), 7.51 (*d*, *J* = 8.0 Hz, 2H), 7.04 (*s*, 1H), 7.03 (*d*, *J* = 8.0 Hz, 1H), 6.97 (*d*, *J* = 8.0 Hz, 2H), 5.34 (*t*, *J* = 9.0 Hz, 1H), 5.31 (*dd*, *J* = 9.0, 7.6 Hz, 1H), 5.30 (*d*, *J* = 3.5 Hz, 1H), 5.28 (*dd*, *J* = 9.7, 3.5 Hz, 1H), 5.21 (*d*, *J* = 7.6 Hz, 1H), 5.09 (*t*, *J* = 9.0 Hz, 1H), 5.04 (*t*, *J* = 9.7 Hz, 1H), 4.74 (*s*, 1H), 3.94 (*ddd*, *J* = 9.0, 7.0, 2.3 Hz, 1H), 3.85 (*dq*, *J* = 9.7, 6.1 Hz, 1H), 3.84 (*s*, 3H), 3.77 (*dd*, *J* = 12.0, 2.3 Hz, 1H), 3.65 (*dd*, *J* = 12.0, 7.0 Hz, 1H), 2.09 (*s*, 3H), 2.08 (*s*, 3H), 2.06 (*s*, 3H), 2.05 (*s*, 6H), 1.99 (*s*, 3H), 1.18 (*d*, *J* = 6.1 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		175.75, 170.17, 169.94, 169.92, 169.75, 169.47, 169.25, 160.42, 159.66, 157.43, 152.69, 130.14, 128.29, 124.81, 124.23, 120.27, 115.26, 114.02, 104.19, 98.33, 98.24, 73.80, 72.62, 71.08, 70.85, 69.34, 69.10, 68.88, 66.78, 66.45, 55.34, 20.79, 20.76, 20.74, 20.63, 20.62, 20.60, 17.35
DEPT (135°) ($CDCl_3$)	CH	152.69, 130.14, 128.29, 115.26, 114.02, 104.19, 98.33, 98.24, 73.80, 72.62, 71.08, 70.83, 69.34, 69.10, 68.88, 66.78
	CH_2	66.45

CH_3	55.34, 20.79, 20.76, 20.74, 20.63, 20.62, 20.60, 17.35
EIMS *m/z* (% relative intensity)	829 (52), 708 (2), 664 (4), 621 (4), 576 (4), 532 (3), 488 (5), 434 (8), 426 (2), 331 (2), 273 (9), 211 (2), 153 (2), 143 (10), 111 (15)

Subfraction M3 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (4 runs) indicated the presence of **AcYS3** as a major component.

Subfraction M4 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (4 runs) showed two major spots of **AcYS2** and **AcYS3**.

Subfraction M5 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (4 runs) showed three major spots of **AcYS2**, **AcYS3** and **AcYS6**.

Subfraction M6 Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (4 runs) indicated the presence of **AcYS2**, **AcYS6** and **AcYS11** as the major components.

Subfraction M7 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed no major spots under UV-S. It was therefore not further investigated.

Subfraction M8 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed two major spots under UV-S with R_f values of 0.58 and 0.63. Because of limitation of time, it was not further investigated.

Subfraction M9 Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed two major spots under UV-S with R_f values of 0.52 and 0.60. No further purification was carried out because of limitation of time.

Fraction 7 Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one pale spot under UV-S with R_f value of 0.25 while chromatogram characteristics on reversed-phase TLC with 50% methanol-water showed two major spots under UV-S with R_f values of 0.06 and 0.20. Because of limitation of time, it was not further purified.

CHAPTER 2.3

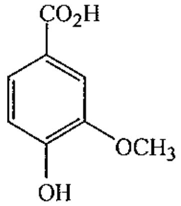
RESULTS AND DISCUSSION

Chromatographic separation of the crude *n*-butanol extract from stems of *D. scandens* afforded 15 compounds : two known benzoic acid derivatives (**YS9** and **YS12**), one known isoflavone (**YS15**), four glucosyl isoflavones [one new (**YS8**) and three known ones (**YS1**, **YS13** and **YS14**)] and eight rhamnosyl-(1→6)-glucosyl isoflavones [four new (**YS6**, **YS7**, **YS10** and **YS11**) and four known ones (**YS2**, **YS3**, **YS4** and **YS5**)]. Most of the compounds were isolated and identified as their corresponding acetates except for **YS1**, **YS2**, **YS4**, **YS9** and **YS12**. Their structures were determined using 1D and 2D NMR spectroscopic data. All carbons of an aglycone unit were assigned by ^{13}C NMR, HMQC and HMBC data. The ^{1}H chemical shift of a sugar moiety were determined using 1D ^{1}H NMR and ^{1}H-^{1}H COSY spectroscopic data.

2.3.1 Benzoic acid derivatives

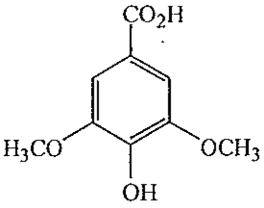
Compound YS9

YS9 was obtained as a pale yellow solid, melting at 145 °C. The UV spectrum (Figure 50) showed absorption bands at 291, 259 and 220 nm, indicating the presence of a conjugated system. The IR spectrum (Figure 51) showed absorption bands at 3445 cm^{-1} (a hydroxyl group) and 1668 cm^{-1} (a carbonyl group). The ^{1}H NMR spectrum (Figure 52) displayed signals of aromatic protons at δ_H 7.58 (J = 7.7 and 1.4 Hz, H-6) as a *doublet of doublet*, δ_H 7.55 (J = 1.4 Hz, H-2) and δ_H 6.90 (J = 7.7 Hz, H-5) as two sets of *doublet* integrating for single proton for each signal. The splitting pattern of aromatic protons indicated that **YS9** was a 1, 3, 4-trisubstituted benzene. Its ^{1}H NMR spectrum also showed an additional *singlet* signal for one methoxyl group at δ_H 3.90. The ^{13}C NMR spectrum (Figure 53) revealed the presence of a carbonyl carbon of an acid at δ_C 167.29. The following NOEDIFF data established the location of the methoxyl group at the C-3. Irradiation of the methoxy proton at δ_H 3.90 (Figure 54) enhanced the aromatic proton signal at δ_H 7.55 (H-2). Thus, **YS9** was 4-hydroxy-3-methoxybenzoic acid which was isolated from *Gardenia sootepensis* (Nauvanit, 1998).



Compound YS12

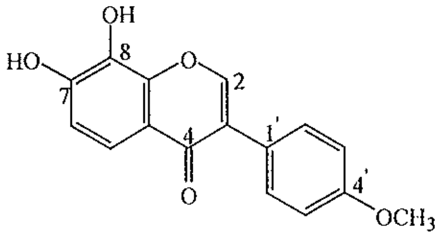
YS12 was obtained as a pale yellow solid. In the UV spectrum (Figure 55), absorption bands at 270 and 217 nm revealed the presence of a conjugated system. The IR spectrum (Figure 56) showed absorption bands at 3472 cm^{-1} (a hydroxyl group) and 1683 cm^{-1} (a carbonyl group). The ^{1}H NMR spectrum (Figure 57) showed a sharp *singlet* at δ_H 7.30 integrating for two protons and a sharp *singlet* at δ_H 3.90 for two methoxyl groups. The ^{13}C NMR spectrum (Figure 58) revealed the presence of a carbonyl carbon of an acid at δ_C 168.54. **YS12** was therefore identified as 4-hydroxy-3,5-dimethoxybenzoic acid. This proposed structure was confirmed by comparison of its ^{1}H and ^{13}C NMR spectra with those of 4-hydroxy-3,5-dimethoxybenzoic acid isolated from *Gardenia sootepensis* (Nauvanit, 1998).



2.3.2 *Isoflavone*

The diacetate of YS15 (AcYS15)

AcYS15 was obtained as a yellow viscous liquid. The UV spectrum (Figure 59) exhibited a characteristic absorption band of an isoflavone at 257 nm (Markham, 1982). The IR spectrum (Figure 60) showed absorption bands for acetyl carbonyl groups (1782 and 1730 cm^{-1}), a carbonyl group of an isoflavone (1649 cm^{-1}) and an aromatic ring (1611 and 1568 cm^{-1}). The ^{1}H NMR spectrum (Figure 61) revealed the presence of the isoflavone skeleton according to a characteristic sharp *singlet* of the H-2 at δ_H 7.94. Two *doublets* at δ_H 8.20 and 7.26 with *ortho* coupling constant of 8.8 Hz were attributed to the H-5 and H-6, respectively. It also showed a pair of *doublets* at δ_H 7.48 and 6.98 (2H for each signal, $J = 8.8$ Hz), characteristic of a *p*-disubstituted benzene ring carrying a methoxyl group as a substituent (Intarit, 1999), and protons of two acetyl groups at δ_H 2.42 and 2.36. The ^{13}C NMR spectrum (Figure 62) displayed 16 signals for 20 carbons (δ_C 175.34, 167.54, 167.21, 159.85, 152.03, 149.46, 146.42, 130.14, 125.42, 124.04, 123.38, 119.97, 114.08, 55.35, 20.64, 20.26). Thus, **AcYS15** was identified as the diacetate of 7, 8-Dihydroxy-4$'$-methoxyisoflavone (**YS15**), isolated from *Prosopis juliflora* (Shukla, *et al.*, 1981).



2.3.3 *Glucosyl isoflavones*

The tetraacetate of YS13 (AcYS13)

AcYS13 was isolated as a yellow viscous liquid with $[\alpha]_D^{29}$ −38.46^{o}. The UV spectrum (Figure 63) with an absorption band at 263 nm represented an isoflavone skeleton. The IR spectrum (Figure 64) showed absorption bands for an acetyl carbonyl group (1756 cm^{-1}), a carbonyl group of an isoflavone (1642 cm^{-1}) and an aromatic ring (1622 and 1591 cm^{-1}). The ^{1}H NMR spectrum (Table 57) (Figure 65) displayed a characteristic *singlet* at δ_H 7.94 for the H-2 of an isoflavonoid nucleus and a *singlet* for one methoxyl group at δ_H 3.85. Its also exhibited two *doublets* at δ_H 8.25 (J = 8.1 Hz, H-5) and δ_H 7.03 (J = 2.5 Hz, H-8) as well as a *doublet of doublet* at δ_H 7.05 (J = 8.1 and 2.5 Hz, H-6), indicating the presence of an oxysubstituent at the C-7 of A-ring. An $AA'XX'$ pattern of B-ring, typical of 1,4-disubstituted benzene with two *doublets* at δ_H 7.50 (J = 8.1 Hz, H-2$'$, H-6$'$) and δ_H 6.98 (J = 8.1 Hz, H-3$'$, H-5$'$) was also detected. The NOEDIFF experiment revealed the attachment of the methoxyl group at the C-4$'$ of B-ring due to the enhancement of the proton signal of the H-3$'$ and H-5$'$ after irradiation at the methoxy proton (Figure 68). In addition, it exhibited typical signals of glucose moiety : an anomeric proton signal [δ_H 5.23 (*d*, J = 7.5 Hz)], four oxymethine protons [δ_H 5.36-5.33 (*m*), 5.34-5.30 (*m*), 5.18 (*dd*, J = 9.0 and 8.4 Hz) and 3.96 (*ddd*, J = 9.0, 6.0 and 2.2 Hz)] and one oxymethylene group [δ_H 4.29 (*dd*, J = 12.0 and 6.0 Hz) and 4.22 (*dd*, J = 12.0 and 2.2 Hz)] together with methyl proton of four acetyl groups [δ_H 2.10, 2.08, 2.07 and 2.05]. The appearance of the anomeric proton as a *doublet* with large coupling constant of 7.5 Hz indicated that glucose must have a β-glucopyranose form (Agrawal, 1992). From these results, the β-glucosyl unit was attached to the 7-hydroxyl of the isoflavone moiety through a glycosidic bond, although the HMBC spectrum (Table 57) (Figure 70) showed no correlation between the anomeric proton of glucose residue with the C-7 of the isoflavone moiety. **AcYS13** was identified as the tetraacetate of formonetin 7-*O*-β-glucopyranoside (**YS13**), a known isoflavone glucoside isolated from *Astragali semen* (Cui, *et al.*, 1993).

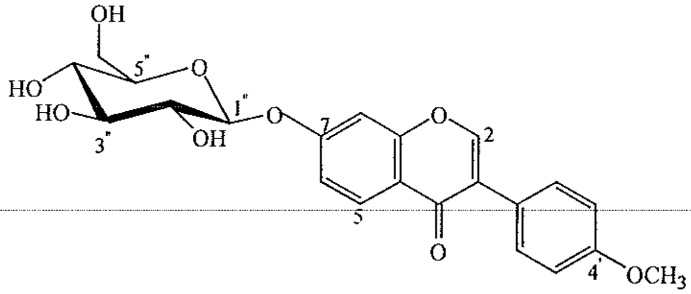


Table 57 The NMR spectral data of **AcYS13**

Position	δ_H	δ_C	Type of C	HMBC
2	7.94 (*s*)	152.54	CH	C-3, C-4, C-8a, C-1′
3	-	125.31	C	
4	-	176.09	C	
4a	-	120.43	C	
5	8.25 (*d*, 8.1)	128.40	CH	C-4, C-7, C-8a
6	7.05 (*dd*, 8.1, 2.5)	115.37	CH	C-4a, C-8
7	-	160.83	C	
8	7.03 (*d*, 2.5)	104.36	CH	C-4a, C-6, C-7, C-8a
8a	-	157.69	C	
1′	-	124.03	C	
2′, 6′	7.50 (*d*, 8.1)	130.29	CH	C-3, C-2′, 6′, C-4′
3′, 5′	6.98 (*d*, 8.1)	114.13	CH	C-1′, C-3′, 5′, C-4′
4′	-	160.07	C	
Glc-1″	5.23 (*d*, 7.5)	98.43	CH	
2″	5.34-5.30 (*m*)	70.92	CH	
3″	5.36-5.33 (*m*)	72.34	CH	
4″	5.18 (*dd*, 9.0, 8.4)	68.07	CH	
5″	3.96 (*ddd*, 9.0, 6.0, 2.2)	72.45	CH	
6″	4.22 (*dd*, 12.0, 2.2)	61.78	CH_2	
	4.29 (*dd*, 12.0, 6.0)			
4′-OMe	3.85 (*s*)	55.13	CH_3	C-4′
$OCOCH_3$	2.10, 2.08, 2.07, 2.05	20.24	CH_3	
		20.21	CH_3	
		20.18	CH_3	
		20.16	CH_3	
$OCOCH_3$		170.84	C	
		170.53	C	
		169.74	C	
		169.57	C	

The tetraacetate of YS8 (AcYS8)

AcYS8 was obtained as a yellow viscous liquid with $[\alpha]_D^{29}$ −55.56°. The UV spectrum (Figure 71) with an absorption band at 254 nm suggested an isoflavone skeleton. The IR spectrum (Figure 72) showed absorption bands for a hydroxyl group (3443 cm^{-1}), an acetyl carbonyl group (1755 cm^{-1}), a carbonyl group of an isoflavone (1630 cm^{-1}) and an aromatic ring (1609 cm^{-1}). The ^1H NMR spectrum (Table 58) (Figure 73) displayed a characteristic *singlet* at δ_H 7.85 for the H-2 of an isoflavone, two *doublets* with *ortho* coupling constant of 9.0 Hz at δ_H 8.05 and 7.05, the H-5 and H-6, respectively, and a pair of *doublets* at δ_H 7.47 and 6.98 with coupling constant of 9.0 Hz, characteristic of a *p*-disubstituted benzene ring. This results implied that **AcYS8** had a 4′,7,8-trioxygenated framework. The methoxyl group at δ_H 3.84 was assigned to C-4′ in B-ring as irradiation at δ_H 6.98 (H-3′, H-5′) enhanced the signals of the methoxyl group and the H-2′, H-6′ in the NOEDIFF spectrum (Figure 76). This was further confirmed by the HMBC correlation (Table 58) (Figure 78) between the methoxy proton (δ_H 3.84) with the C-4′ (δ_C 159.85). In addition, the signal of protons of glucose unit were observed together with methyl proton of four acetyl groups [δ_H 2.20, 2.12, 2.07 and 2.06]. The anomeric proton of glucose at δ_H 4.99 with large coupling constant of 8.1 Hz implied that this glucose moiety must have a *β*-glucopyranose form (Ozden, *et al.*, 1998). In order to determine the attachment of glucose unit, the HMBC spectrum (Table 58) (Figure 78) was measured. A cross peak between the H-1″ (δ_H 4.99) with the C-8 (δ_C 131.07) indicated that the glucose moiety was attached to the 8-hydroxyl of the isoflavone moiety. Therefore, **AcYS8** was determined to be the tetraacetate of 7-hydroxy-4′-methoxyisoflavone 8-*O*-*β*-glucopyranoside (**YS8**), a new isoflavone glucoside.

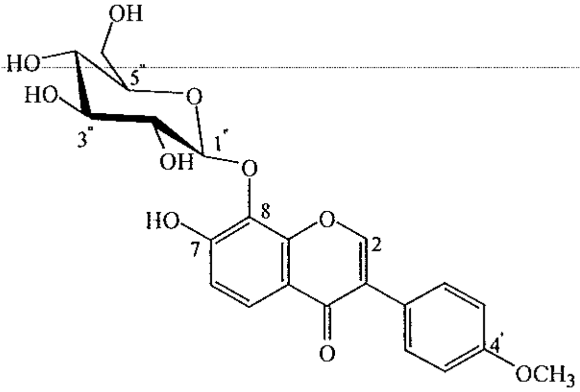


Table 58 The NMR spectral data of **AcYS8**

Position	δ_H	δ_C	Type of C	HMBC
2	7.85 (*s*)	151.34	CH	C-3, C-4, C-8a, C-1′
3	-	125.32	C	
4	-	175.59	C	
4a	-	118.66	C	
5	8.05 (*d*, 9.0)	124.26	CH	C-4, C-7, C-8, C-8a
6	7.05 (*d*, 9.0)	115.29	CH	C-4a, C-7, C-8, C-8a
7	-	154.37	C	
8	-	131.07	C	
8a	-	150.19	C	
1′	-	123.49	C	
2′, 6′	7.47 (*d*, 9.0)	130.21	CH	C-3, C-2′, 6′, C-4′
3′, 5′	6.98 (*d*, 9.0)	114.08	CH	C-1′, C-3′, 5′, C-4′
4′	-	159.85	C	
Glc-1″	4.99 (*d*, 8.1)	103.36	CH	C-8
2″	5.40 (*dd*, 9.8, 8.1)	70.98	CH	C-3″, 169.17 (C=O)
3″	5.33 (*t*, 9.8)	72.06	CH	C-2″, C-4″, 170.14 (C=O)
4″	5.20 (*t*, 9.8)	67.93	CH	C-3″, C-5″, C-6″, 169.30 (C=O)
5″	3.82 (*ddd*, 9.8, 5.6, 2.8)	72.68	CH	
6″	4.30 (*dd*, 12.6, 5.6)	61.34	CH_2	C-5″, 170.57 (C=O)
	4.20 (*dd*, 12.6, 2.8)			
4′-OMe	3.84 (*s*)	55.35	CH_3	C-4′
$OCOCH_3$	2.20, 2.12, 2.07, 2.06	20.74	CH_3	
		20.57	CH_3	
$OCOCH_3$		170.57	C	
		170.14	C	
		169.30	C	
		169.17	C	

Compound YS1

YS1 was obtained as a white solid, melting at 199.4-201.0 °C with $[\alpha]_D^{29}$ –16.81°. The UV spectrum (Figure 79) with an absorption band at 254 nm was typical for a compound of an isoflavone type. The IR spectrum (Figure 80) exhibited absorption bands for a hydroxyl group (3426 cm^{-1}), a carbonyl group of an isoflavone (1617 cm^{-1}) and an aromatic ring (1605 cm^{-1}). A *singlet* proton signal at δ_H 8.24 in the ^1H NMR spectrum (Table 59) (Figure 81) was a characteristic signal of the H-2 of an isoflavone. It exhibited two *singlets* integrating three protons for each signal at δ_H 4.02 and 3.84 for two methoxyl groups. Two *doublets* with coupling constant of 9.0 Hz at δ_H 8.00 and 7.27 were assigned to the H-5 and H-6, respectively, due to a chemical-shift characteristic of the H-5. It also displayed an $AA'XX'$ pattern, typical of $4'$-substituted B-ring, with two *doublets* at δ_H 7.49 and 6.99 (2H for each signal, J = 9.0 Hz), which were assigned to the H-$2'$, H-$6'$ and H-$3'$, H-$5'$, respectively. An anomeric proton signal at δ_H 5.17 with coupling constant of 7.5 Hz suggested the presence of glucose residue with β-configuration. The location of the methoxyl group at the C-$4'$ and C-7 was determined by NOEDIFF experiments. Irradiation of the methoxy proton at δ_H 3.84 (Figure 85) enhanced the signal for the H-$3'$, H-$5'$ (δ_H 6.99) and irradiation of the methoxy proton at δ_H 4.02 (Figure 86) showed an enhancement of the H-6 (δ_H 7.27). The low-field methoxyl group (δ_H 4.02) was then assigned to the C-7. Supporting evidence for these assignments was provided by HMBC correlation data (Table 59) (Figure 88) which showed the polarization transfer between the methoxyl groups at δ_H 3.84 and 4.02 and the C-$4'$ (δ_C 159.28) and the C-7 (δ_C 155.80), respectively. Complete ^1H and ^{13}C assignments were reported in Table 59, as results from DEPT (Figure 84), HMQC (Figure 87) and HMBC (Figure 88) experiments. The location of β-glucose residue was then assigned to the C-8 using a correlation between the sugar H-$1''$ (δ_H 5.17) and the C-8 (δ_C 133.00) of an isoflavone in the HMBC correlation spectrum (Table 59) (Figure 88). Therefore, **YS1** was characterized as $4'$,7-dimethoxyisoflavone 8-*O*-β-glucopyranoside, a known isoflavone glucoside (Fujita, *et al.*, 1982).

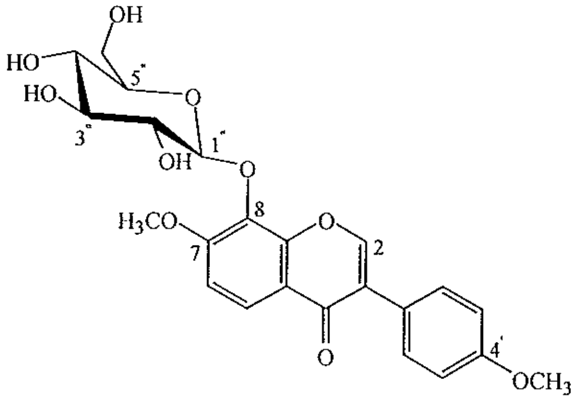


Table 59 The NMR spectral data of **YS1**

Position	δ_H	δ_C	Type of C	HMBC
2	8.24 (*s*)	152.79	CH	C-3, C-4, C-8a, C-1′
3	-	123.57	C	
4	-	175.50	C	
4a	-	118.80	C	
5	8.00 (*d*, 9.0)	121.74	CH	C-4, C-7, C-8, C-8a
6	7.27 (*d*, 9.0)	110.47	CH	C-4a, C-7, C-8, C-8a
7	-	155.80	C	
8	-	133.00	C	
8a	-	150.50	C	
1′	-	124.08	C	
2′, 6′	7.49 (*d*, 9.0)	129.97	CH	C-3, C-2′, 6′, C-4′
3′, 5′	6.99 (*d*, 9.0)	113.69	CH	C-1′, C-3′, 5′, C-4′
4′	-	159.28	C	
Glc-1″	5.17 (*d*, 7.5)	103.95	CH	C-8
2″	3.57 (*dd*, 9.0, 7.5)	72.50	CH	C-2″
3″	3.46 (*t*, 9.0)	76.80	CH	C-4″, C-5″
4″	3.41 (*t*, 9.0)	70.25	CH	C-4″, C-5″
5″	3.25 (*ddd*, 9.0, 5.5, 2.5)	74.23	CH	C-3″
6″	3.83 (*dd*, 12.0, 2.5) 3.64 (*dd*, 12.0, 5.5)	61.59	CH_2	C-4″
4′-OMe	3.84 (*s*)	55.14	CH_3	C-4′
7-OMe	4.02 (*s*)	56.66	CH_3	C-7

The tetraacetate of YS14 (AcYS14)

AcYS14 was isolated as a white solid with $[\alpha]_D^{29}$ –45.45°. The UV spectrum (Figure 94) with an absorption band at 258 nm suggested an isoflavone skeleton. The IR spectrum (Figure 95) showed absorption bands for an acetyl group (1756 cm^{-1}), a carbonyl group of an isoflavone (1645 cm^{-1}) and an aromatic ring (1609 and 1569 cm^{-1}). Comparison of its ^1H NMR spectrum (Figure 96) with that of the tetraacetate of **YS1 (AcYS1)** (Table 60) (Figure 91) revealed that **AcYS14** was also a tetraacetate derivative of an isoflavone glucoside with the same substitution pattern on the isoflavone moiety, 4$'$,7,8-trioxygenated framework. **AcYS14** also consisted of two methoxyl groups at δ_H 3.85 and 3.96. Their location was assigned to the C-4$'$ and C-8 by NOEDIFF. Irradiation of the methoxy proton at δ_H 3.85 (Figure 99) enhanced the aromatic proton the H-3$'$ and H-5$'$ (δ_H 6.98) while irradiation of the other methoxy proton (δ_H 3.96) (Figure 100) caused no enhancement of any aromatic protons. A large coupling constant of 7.2 Hz for the anomeric proton (H-1$''$) at δ_H 5.16 in the ^1H NMR spectrum suggested a β-configuration for the glucose unit. The HMBC correlation data (Table 61) (Figure 102) between the H-1$''$ (δ_H 5.16) of glucose residue with the C-7 (δ_C 153.80) established the linkage between the sugar and isoflavone moiety. Thus, **AcYS14** was identified as the tetraacetate of 4$'$,8-dimethoxyisoflavone 7-*O*-β-glucopyranoside (**YS14**), a known isoflavone glucoside isolated from *Ebenus cretica* (Mitrocotsa, *et al.*, 1999).

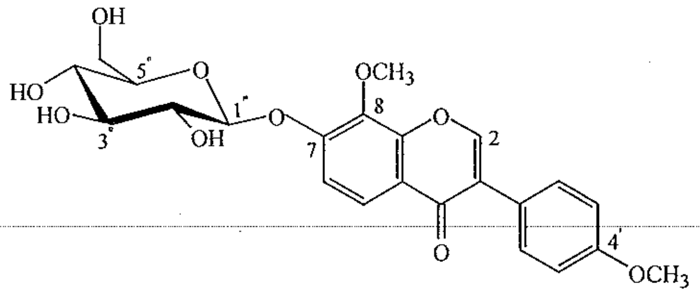


Table 60 Comparison of ^{1}H NMR data of **AcYS1** with **AcYS14**

^{1}H NMR of AcYS1	^{1}H NMR of AcYS14
8.10 (*d*, J = 9.0 Hz, 1H)	8.00 (*d*, J = 9.0 Hz, 1H)
7.99 (*s*, 1H)	8.01 (*s*, 1H)
7.52 (*d*, J = 9.0 Hz, 2H)	7.50 (*d*, J = 9.0 Hz, 2H)
7.06 (*d*, J = 9.0 Hz, 1H)	7.18 (*d*, J = 9.0 Hz, 1H)
6.98 (*d*, J = 9.0 Hz, 2H)	6.98 (*d*, J = 9.0 Hz, 2H)
5.38 (*dd*, J = 8.5, 7.0 Hz, 1H)	5.39 (*dd*, J = 9.0, 7.2 Hz, 1H)
5.31 (*t*, J = 8.5 Hz, 1H)	5.33 (*t*, J = 9.0 Hz, 1H)
5.28 (*dd*, J = 9.5, 8.5 Hz, 1H)	5.20 (*dd*, J = 9.6, 9.0 Hz, 1H)
5.19 (*d*, J = 7.0 Hz, 1H)	5.16 (*d*, J = 7.2 Hz, 1H)
4.29 (*dd*, J = 12.0, 5.5 Hz, 1H)	4.31 (*dd*, J = 12.0, 6.0 Hz, 1H)
4.08 (*dd*, J = 12.0, 2.5 Hz, 1H)	4.20 (*dd*, J = 12.0, 2.2 Hz, 1H)
3.99 (*s*, 3H)	3.96 (*s*, 3H)
3.84 (*s*, 3H)	3.85 (*s*, 3H)
3.70 (*ddd*, J = 9.5, 5.5, 2.5 Hz, 1H)	3.90 (*ddd*, J = 9.6, 6.0, 2.2 Hz, 1H)
2.09 (*s*, 3H)	2.10 (*s*, 3H)
2.05 (*s*, 3H)	2.09 (*s*, 3H)
2.04 (*s*, 3H)	2.06 (*s*, 3H)
1.94 (*s*, 3H)	2.05 (*s*, 3H)

Table 61 The NMR spectral data of **AcYS14**

Position	δ_H	δ_C	Type of C	HMBC
2	8.01 (*s*)	152.72	CH	C-3, C-4, C-8a, C-1′
3	-	125.14	C	
4	-	176.36	C	
4a	-	115.11	C	
5	8.00 (*d*, 9.0)	121.75	CH	C-7, C-8a
6	7.18 (*d*, 9.0)	115.11	CH	C-5, C-7, C-8
7	-	153.80	C	
8	-	138.85	C	
8a	-	151.15	C	
1′	-	123.97	C	
2′, 6′	7.50 (*d*, 9.0)	130.42	CH	C-3, C-2′, 6′, C-4′

Table 61 (continued)

Position	δ_H	δ_C	Type of C	HMBC
3', 5'	6.98 (*d*, 9.0)	114.24	CH	C-1', C-3', 5', C-4'
4'	-	160.20	C	
Glc-1''	5.16 (*d*, 7.2)	99.98	CH	C-7
2''	5.39 (*dd*, 9.0, 7.2)	70.90	CH	C-1'', C-3''
3''	5.33 (*t*, 9.0)	72.38	CH	C-2'', C-4''
4''	5.20 (*dd*, 9.6, 9.0)	68.13	CH	C-3''
5''	3.90 (*ddd*, 9.6, 6.0, 2.2)	77.17	CH	
6''	4.20 (*dd*, 12.0, 2.2) 4.31 (*dd*, 12.0, 6.0)	61.78	CH_2	
4'-OMe	3.85 (*s*)	55.22	CH_3	C-4'
8-OMe	3.96 (*s*)	61.95	CH_3	C-8
$OCOCH_3$	2.10, 2.09, 2.06, 2.05	20.39	CH_3	
		20.32	CH_3	
		20.30	CH_3	
		20.27	CH_3	
$OCOCH_3$		171.01	C	
		170.67	C	
		169.91	C	
		169.81	C	

2.3.4 Rhamnosyl-(1→6)-glucosyl isoflavones

The heptaacetate of YS5 (AcYS5)

AcYS5 was obtained as a colorless viscous liquid with $[\alpha]_D^{29}$ –33.33°. Its spectroscopic behavior including the UV absorption band at 306 nm (Figure 103), IR absorption bands (Figure 104) for an acetyl carbonyl group (1756 cm^{-1}), a carbonyl group of an isoflavone (1659 cm^{-1}) and an aromatic ring (1622 and 1579 cm^{-1}), and the *singlet* signal at δ_H 8.02 (H-2) in the ^1H NMR spectrum (Table 62) (Figure 105) indicated that **AcYS5** was a compound of an isoflavone type. The 4'-oxysubstitution pattern of ring-B and 7-oxysubstitution pattern of ring-A were readily deduced from the proton signals forming an AA'XX' system (δ_H 7.61, J = 9.0 Hz, H-2', H-6' and δ_H 7.18, J = 9.0 Hz, H-3', H-5') and an ABX-type [δ_H 7.06 (1H, *dd*, J = 9.0, 2.0 Hz, H-6),

δ_H 8.27 (1H, *d*, J = 9.0 Hz, H-5) and δ_H 7.08 (1H, *d*, J = 2.0 Hz, H-8)]. In addition, ^{1}H NMR signals (Table 62) (Figure 105) belonging to two sugar residues and seven acetyl groups were observed. According to chemical shifts and splitting pattern of these protons as well as the presence a methyl *doublet* at δ_H 1.19 (J = 6.5 Hz), a characteristic signal of the H-6$'''$ of rhamnose unit, two sugar residues were determined as glucose and rhamnose units. Two *doublets* at δ_H 5.24 (J = 7.5 Hz) and δ_H 4.75 (J = 1.5 Hz) integrating for single proton for each signal were attributed to the H-1$''$ of glucose unit and the H-1$'''$ of rhamnose unit, respectively. A large coupling constant (J = 7.5 Hz) of the H-1$''$ and a small coupling constant of the H-1$'''$ established the presence of glucose and rhamnose units in β-glucopyranose and α-rhamnopyranose forms (Agrawal, 1992). The appearance of the C-6$''$ signal of glucose unit in the ^{13}C NMR spectrum (Table 62) (Figure 106) at δ_C 66.36 revealed that the rhamnose unit was attached to the C-6$''$ of glucose unit. The HMBC correlation experiments (Table 62) (Figure 110) exhibited a correlation between the H-1$'''$ (δ_H 4.75) of rhamnose unit and the C-6$''$ (δ_C 66.36) of glucose unit. These results confirmed that the C-1$'''$ of rhamnose unit was attached to the C-6$''$ of glucose unit through a glycosidic bond. Furthermore, a cross peak between the H-1$''$ (δ_H 5.24) of glucose unit and the C-7 (δ_C 160.47) established the attachment of glucose unit to the 7-hydroxyl of the isoflavone aglycone. The proton signals of glucose and rhamnose units were assigned by ^{1}H-^{1}H COSY spectrum (Table 62) (Figure 108). Thus, **AcYS5** was identified as the heptaacetate of 4$'$-hydroxyisoflavone 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (**YS5**), which has been isolated from stems of *Derris scandens* (Suwannaroj, *et al.*, 2000).

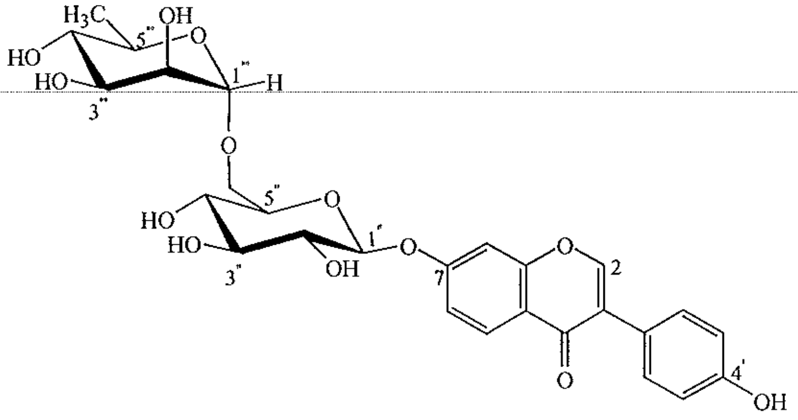


Table 62 The NMR spectral data of **AcYS5**

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
2	8.02 (*s*)	153.33	CH		C-3, C-4, C-8a
3	-	124.33	C		
4	-	175.43	C		
4a	-	120.16	C		
5	8.27 (*d*, 9.0)	128.24	CH	H-6	C-7, C-8a
6	7.06 (*dd*, 9.0, 2.0)	115.43	CH	H-5	C-4a, C-7, C-8
7	-	160.47	C		
8	7.08 (*d*, 2.0)	104.10	CH		C-4a, C-6, C-8a
8a	-	157.40	C		
1′	-	129.57	C		
2′, 6′	7.61 (*d*, 9.0)	130.04	CH	H-3′, 5′	C-3, C-2′, 6′, C-4′
3′, 5′	7.18 (*d*, 9.0)	121.64	CH	H-2′, 6′	C-1′, C-3′, 5′, C-4′
4′	-	150.57	C		
Glc-1″	5.24 (*d*, 7.5)	98.21	CH	H-2″	C-7
2″	5.34 (*dd*, 9.0, 7.5)	70.74	CH	H-1″, H-3″	C-3″
3″	5.36 (*t*, 9.0)	72.55	CH	H-2″, H-4″	C-2″
4″	5.10 (*dd*, 10.0, 9.0)	68.79	CH	H-3″, H-5″	C-3″
5″	3.96 (*ddd*, 10.0, 7.5, 2.5)	73.80	CH	H-4″, H-6″	
6″	3.77 (*dd*, 12.0, 2.5)	66.36	CH_2	H-5″, H-6″	C-5″
	3.65 (*dd*, 12.0, 7.5)				
Rha-1‴	4.75 (*d*, 1.5)	98.21	CH	H-2‴	C-6″
2‴	5.33 (*dd*, 3.5, 1.5)	70.74	CH	H-1‴, H-3‴	
3‴	5.31 (*dd*, 10.0, 3.5)	69.21	CH	H-2‴, H-4‴	
4‴	5.07 (*t*, 10.0)	69.06	CH	H-3‴, H-5‴	
5‴	3.84 (*dq*, 10.0, 6.5)	66.73	CH	H-4‴, H-6‴	
6‴	1.19 (*d*, 6.5)	17.33	CH_3	H-5‴	C-4‴, C-5‴
$OCOCH_3$	2.34, 2.18, 2.10, 2.09, 2.07, 2.06, 2.00	21.16	CH_3		
		20.80	CH_3		
		20.77	CH_3		

Table 62 (continued)

Position	δ_H	δ_C	Type of C	^1H-^1H COSY	HMBC
$OCOCH_3$		20.76	CH_3		
		20.63	CH_3		
		20.62	CH_3		
		20.59	CH_3		
		170.18	C		
		169.98	C		
		169.91	C		
		169.74	C		
		169.47	C		
		169.25	C		

The hexaacetate of YS3 (AcYS3)

AcYS3 was obtained as a colorless viscous liquid with $[\alpha]_D^{29}$ –41.52°. The UV spectrum (Figure 111) showed an absorption band at 260 nm while the IR spectrum (Figure 112) revealed absorption bands for an acetyl carbonyl group (1748 cm^{-1}), a carbonyl group of an isoflavone (1645 cm^{-1}) and an aromatic ring (1622 and 1569 cm^{-1}). These results together with the presence of a *singlet* signal at δ_H 7.95 in the ^1H NMR spectrum (Table 63) (Figure 113) established an isoflavone framework for **AcYS3**. Its ^1H NMR spectrum data was similar to that of **AcYS13**, except for additional proton signals of the other sugar unit, apart from glucose residue, and additional protons of other two acetyl groups. A *doublet* of a methyl group at δ_H 1.18 (J = 6.1 Hz) suggested that the additional sugar unit was rhamnose moiety. The 4′-methoxy substitution pattern of B-ring and 7-oxysubstitution pattern of A-ring were confirmed by the proton signals forming an AA′XX′ system (δ_H 7.51, J = 8.0 Hz, H-2′, H-6′ and δ_H 6.97, J = 8.0 Hz, H-3′, H-5′) and an ABX-type [δ_H 8.24 (1H, *d*, J = 8.0 Hz, H-5), δ_H 7.04 (1H, *d*, J = 2.5 Hz, H-8) and δ_H 7.03 (1H, *dd*, J = 8.0 and 2.5 Hz, H-6)]. A *doublet* at δ_H 5.21 (J = 7.6 Hz) and a *singlet* at δ_H 4.74 integrating for single proton for each signal were attributed to the H-1″ of β-glucose unit and the H-1‴ of α-rhamnose unit, respectively. The chemical shift of the C-6″ carbon signal at

δ_C 66.45 of glucose unit in the ^{13}C NMR spectrum (Table 63) (Figure 114) revealed that the rhamnose unit was attached to the C-6$''$ of glucose residue. The HMBC correlation experiments (Table 63) (Figure 118) exhibited a correlation between the H-1$'''$ (δ_H 4.74) of rhamnose unit and the C-6$''$ (δ_C 66.45) of glucose unit as well as a correlation between the H-1$''$ (δ_H 5.21) of glucose unit and the C-7 (δ_C 160.42) of the isoflavone aglycone. These results indicated that the rhamnose unit formed a (1→6) glycosidic bond with the glucose unit of which the C-1$''$ was attached to the 7-hydroxyl of the aglycone. The proton signals of the glucose and rhamnose units were assigned by ^{1}H-^{1}H COSY spectrum (Figure 116) as shown in Table 63. Therefore, **AcYS3** was identified as the hexaacetate of 4$'$-methoxyisoflavone 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside **(YS3)**, isolated from *Dalbergia paniculata* (Parthasarathy, *et al.*, 1976).

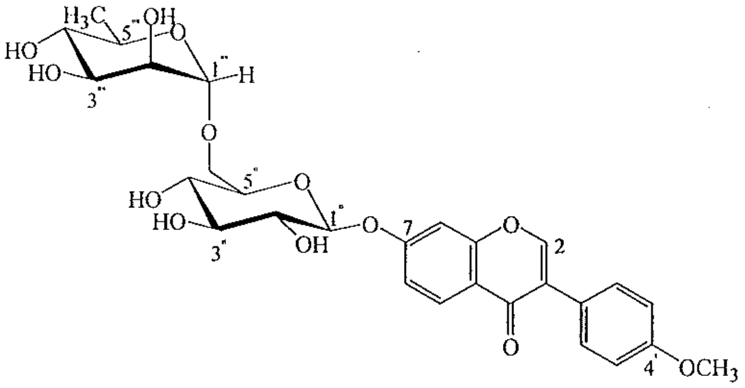


Table 63 The NMR spectral data of **AcYS3**

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
2	7.95 (*s*)	152.69	CH		C-3, C-4, C-8a
3	-	124.81	C		
4	-	175.75	C		
4a	-	120.27	C		
5	8.24 (*d*, 8.0)	128.29	CH	H-6	C-4, C-7, C-8a
6	7.03 (*dd*, 8.0, 2.5)	114.02	CH	H-5	C-4a, C-7, C-8a
7	-	160.42	C		
8	7.04 (*d*, 2.5)	115.26	CH		C-4, C-7, C-8
8a	-	157.43	C		

Table 63 (continued)

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
1′	-	124.23	C		
2′, 6′	7.51 (*d*, 8.0)	130.14	CH	H-3′, 5′	C-3, C-2′, 6′, C-4′
3′, 5′	6.97 (*d*, 8.0)	104.19	CH	H-2′, 6′	C-1′, C-3′, 5′, C-4′
4′	-	159.66	C		
Glc-1″	5.21 (*d*, 7.6)	98.33	CH	H-2″	C-7, C-3″
2″	5.31 (*dd*, 9.0, 7.6)	71.08	CH	H-1″, H-3″	C-2″, C-4″
3″	5.34 (*t*, 9.0)	72.62	CH	H-2″, H-4″	C-2″, C-4″
4″	5.09 (*t*, 9.0)	69.10	CH	H-3″, H-5″	C-3″
5″	3.94 (*ddd*, 9.0, 7.0, 2.3)	73.80	CH	H-4″, H-6″	
6″	3.77 (*dd*, 12.0, 2.3)	66.45	CH$_2$	H-5″, H-6″	C-5″
	3.65 (*dd*, 12.0, 7.0)				
Rha-1‴	4.74 (*s*)	98.24	CH		C-6″
2‴	5.30 (*d*, 3.5)	69.34*	CH	H-3‴	C-4‴
3‴	5.28 (*dd*, 9.7, 3.5)	68.88*	CH	H-2‴, H-4‴	C-4‴
4‴	5.04 (*t*, 9.7)	70.85	CH	H-3‴, H-5‴	
5‴	3.85 (*dq*, 9.7, 6.1)	66.78	CH	H-4‴, H-6‴	
6‴	1.18 (*d*, 6.1)	17.35	CH$_3$	H-5‴	
4′-OMe	3.84 (*s*)	55.34	CH$_3$		
OCOCH$_3$	2.09, 2.08, 2.06, 2.05 (6H),	20.79	CH$_3$		
	1.99	20.76	CH$_3$		
		20.74	CH$_3$		
		20.63	CH$_3$		
		20.62	CH$_3$		
		20.60	CH$_3$		
OCOCH$_3$		170.17	C		
		169.94	C		
		169.92	C		
		169.75	C		
		169.47	C		
		169.25	C		

* interchangeable

The heptaacetate of YS10 (AcYS10)

AcYS10 was obtained as a pale yellow viscous liquid with $[\alpha]_D^{29}$ –51.47°. The IR spectrum (Figure 122) showed absorption bands for an acetyl carbonyl group (1732 cm^{-1}), a carbonyl group of an isoflavone (1659 cm^{-1}) and an aromatic ring (1613 and 1573 cm^{-1}). The UV absorption band (Figure 121) at 257 nm was typical for an isoflavone. Its ^1H NMR spectrum (Table 64) (Figure 123) was similar to that of **AcYS8**. It consisted of the H-2 signal at δ_H 7.90, two protons *ortho*-related with coupling constant of 9.0 Hz of the H-5 (δ_H 8.20) and H-6 (δ_H 7.12) and a pair of *doublets* at δ_H 7.46 and 6.96, characteristic of a *p*-disubstituted benzene ring (2H each, J = 8.5 Hz). Thus, **AcYS10** was a $4'$,7,8-trioxygenated isoflavone. The appearance of two anomeric protons as two *doublets* at δ_H 5.21 (J = 7.5 Hz) and δ_H 4.74 (J = 1.5 Hz) together with methyl proton of six acetyl groups [δ_H 2.09, 2.08, 2.07, 2.06, 2.04 and 2.01] indicated the presence of acetylated β-glucose and acetylated α-rhamnose units. The presence of seven carbonyl carbons of acetyl groups apart from the carbonyl carbon of the isoflavone suggested that **AcYS10** contained one hydroxyl substituent on the isoflavone skeleton apart from six hydroxyl groups of two sugar units. The methoxyl group at δ_H 3.84 was assigned to the C-$4'$ in B-ring due to the ^1H chemical shift values of the H-$2'$, H-$6'$ and H-$3'$, H-$5'$ (Intarit, 1999). Enhancement of the aromatic proton H-$3'$ and H-$5'$ (δ_H 6.96) after irradiation of the methoxy proton at δ_H 3.84 (Figure 127) confirmed the location of the methoxyl group at the C-$4'$. In the HMBC spectrum (Table 64) (Figure 129), a cross peak between the methoxy proton (δ_H 3.84) and the C-$4'$ (δ_C 159.72) also supported above conclusion. The location of two sugar units was also established by the HMBC spectrum. The H-$1''$ signal (δ_H 5.21) of glucose unit showed a cross peak with the C-7 (δ_C 151.95) while the H-$1'''$ signal at δ_H 4.74 of rhamnose unit showed a correlation with the C-$6''$ (δ_C 66.39) of glucose unit. These results suggested that the C-$1'''$ of rhamnose unit formed a (1→6)-glycosidic bond with the glucose unit of which the C-$1''$ was linked with the 7-hydroxyl of the isoflavone aglycone. Other protons of the sugar residues were assigned by analysis of the ^1H-^1H COSY spectrum (Table 64) (Figure 126) using the anomeric protons as starting points. These data permitted the identification of

AcYS10 as the heptaacetate of 8-hydroxy-4'-methoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS10**), a new isoflavone glycoside.

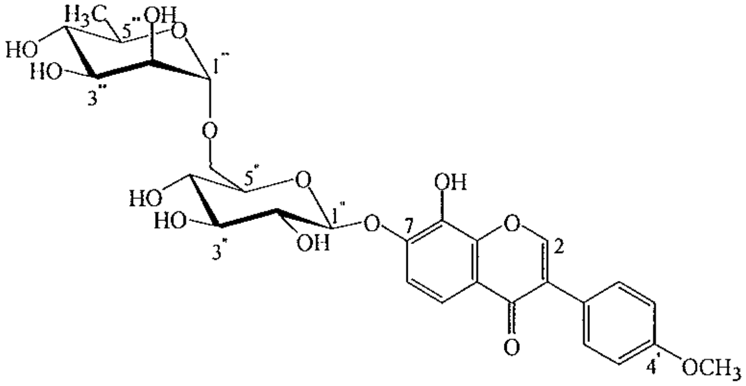


Table 64 The NMR spectral data of **AcYS10**

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
2	7.90 (*s*)	152.15	CH		C-3, C-4, C-8a
3	-	125.07	C		
4	-	175.28	C		
4a	-	120.08	C		
5	8.20 (*d*, 9.0)	123.90	CH	H-6	C-4, C-7, C-8a
6	7.12 (*d*, 9.0)	112.00	CH	H-5	C-8
7	-	151.95	C		
8	-	152.00	C		
8a	-	149.59	C		
1'	-	124.96	C		
2', 6'	7.46 (*d*, 8.5)	130.13	CH	H-3', 5'	C-3, C-2', 6', C-4'
3', 5'	6.96 (*d*, 8.5)	114.00	CH	H-2', 6'	C-1', C-3', 5', C-4'
4'	-	159.72	C		
Glc-1''	5.21 (*d*, 7.5)	98.00	CH	H-2''	C-7,
2''	5.35-5.31 (*m*)	70.53	CH	H-1'', H-3''	C-3''
3''	5.34-5.30 (*m*)	72.26	CH	H-2'', H-4''	C-3''
4''	5.09 (*t*, 10.0)	68.68*	CH	H-3'', H-5''	C-3''
5''	3.93 (*ddd*, 10.0, 6.5, 2.5)	73.79	CH	H-6''	
6''	3.79 (*dd*, 11.5, 2.5)	66.39	CH_2	H-5'', H-6''	C-1'''

Table 64 (continued)

Position	δ_H	δ_C	Type of C	^1H-^1H COSY	HMBC
	3.67 (*dd*, 11.5, 6.5)				
Rha-1'''	4.74 (*d*, 1.5)	97.00	CH	H-2'''	C-6'', C-3'''
2'''	5.24 (*dd*, 3.5, 1.5)	69.39	CH	H-3'''	
3'''	5.30 (*dd*, 10.0, 3.5)	68.78*	CH	H-2''', H-4'''	
4'''	5.06 (*t*, 10.0)	70.78	CH	H-3''', H-5'''	
5'''	3.86 (*dq*, 10.0, 6.5)	66.83	CH	H-4''', H-6'''	
6'''	1.17 (*d*, 6.5)	17.33	CH_3		C-4''', C-5'''
4'-OMe	3.84 (*s*)	55.31	CH_3		C-4'
$OCOCH_3$	2.40, 2.09, 2.08, 2.07,	20.79	CH_3		
	2.06, 2.04, 2.01	20.72	CH_3		
		20.60	CH_3		
		20.55	CH_3		
		20.15	CH_3		
$OCOCH_3$		170.05	C		
		170.03	C		
		169.98	C		
		169.89	C		
		169.47	C		
		169.45	C		
		168.09	C		

* interchangeable

Compound YS2

YS2 was obtained as a white solid, melting at 205-207 °C with $[\alpha]_D^{29}$ −84.07°. The UV spectrum (Figure 130) with an absorption band at 255 nm was typical for a compound of an isoflavone type. The IR spectrum (Figure 131) showed absorption bands for a hydroxyl group (3407 cm^{-1}), a carbonyl group of an isoflavone (1621 cm^{-1}) and an aromatic ring (1605, 1578 cm^{-1}). The ^1H NMR spectrum (Table 65) (Figure 132) indicated an isoflavonoid nucleus by the appearance of the H-2 signal at δ_H 8.24. It also displayed signals for the methoxyl groups at δ_H 4.01 and 3.82 and two *doublets* signals for the H-5 and H-6 at δ_H 7.88 (1H, J = 8.8 Hz) and δ_H 7.29 (1H, J = 8.8 Hz), respectively. A pair of *ortho doublets* (J = 8.8 Hz) each integrating for two protons centered at δ_H 7.50 and 6.95 were assigned to the H-2′, H-6′ and H-3′, H-5′ of 4′-methoxysubstituted B-ring, respectively. In addition, the two anomeric protons at δ_H 5.00 as a *doublet* with coupling constant of 7.2 Hz and δ_H 4.65 as a *singlet* suggested the presence of one β-glucosyl unit and one α-rhamnosyl unit. The ^{13}C NMR spectrum (Table 65) (Figure 133) showed two methoxyl carbons at δ_C 61.42 and 55.06 and one carbonyl carbon at δ_C 175.16. It also revealed a set of signals belonging to the sugar moieties which showed the C-1″ of glucose unit and the C-1‴ of rhamnose unit at δ_C 101.18 and 100.63, respectively. The ^1H-^1H COSY spectrum (Figure 135) exhibited coupling of nearby protons as shown in Table 65. In the HMBC spectrum (Table 65) (Figure 137), the methoxy proton (δ_H 4.01) showed a correlation with the C-8 (δ_C 137.34) of the aglycone, indicating the attachment of this methoxyl group at the C-8 of the aglycone. The proton signal at δ_H 7.50 (H-2′, H-6′) showed a correlation with the C-4′ (δ_C 159.19), which in turn correlated with the proton signal of the methoxyl group at δ_H 3.82. These results revealed the presence of the methoxyl group (δ_H 3.82) at the C-4′ of the aglycone and the other methoxyl group (δ_H 4.01) at the C-8. The glycosidic linkage at the 7-hydroxyl of the aglycone was confirmed by the HMBC correlation of the H-1″ (δ_H 5.00) of β-glucosyl unit with the C-7 (δ_C 154.08). In addition, the H-1‴ (δ_H 4.65) of α-rhamnosyl moiety correlated with the C-6″ (δ_C 66.56) of glucose unit, suggesting an interglycosidic linkage to be rhamnose-(1→6)-glucose. From the above data, **YS2** can be identified as 4′,8-

dimethoxyisoflavone 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (Derriscanoside B), a known isoflavone glycoside isolated from *Derris scandens* (Dianpeng, *et al.*, 1999).

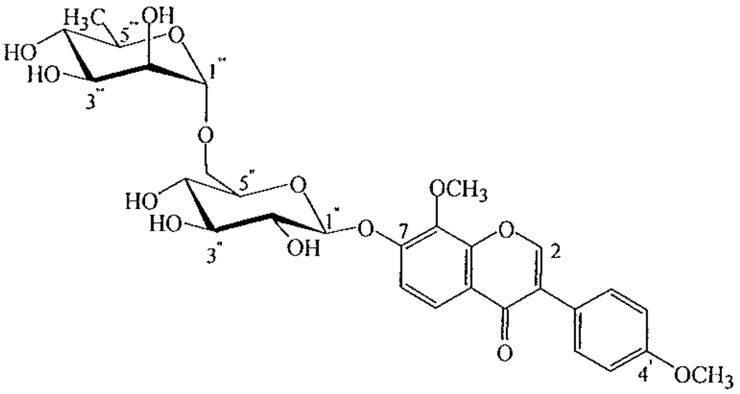


Table 65 The NMR spectral data of **YS2**

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
2	8.24 (*s*)	152.80	CH		C-4, C-8a, C-2′, 6′
3	-	123.68	C		
4	-	175.16	C		
4a	-	119.89	C		
5	7.88 (*d*, 8.8)	120.62	CH	H-6	C-4, C-6, C-7, C-8, C-8a
6	7.29 (*d*, 8.8)	114.19	CH	H-5	C-4a, C-7, C-8, C-8a
7	-	154.08	C		
8	-	137.34	C		
8a	-	150.27	C		
1′	-	123.96	C		
2′, 6′	7.50 (*d*, 8.8)	129.99	CH	H-3′, 5′	C-3, C-2′, 6′, C-4′
3′, 5′	6.95 (*d*, 8.8)	113.60	CH	H-2′, 6′	C-1′, C-2′, 6′, C-3′, 5′, C-4′
4′	-	159.19	C		
Glc-1″	5.00 (*d*, 7.2)	101.18	CH	H-2″	C-7, C-3″, C-5″
2″	3.54-3.48 (*m*)	73.29	CH	H-1″	C-3″
3″	3.43 (*t*, 8.4)	76.90	CH		C-2″, C-4″
4″	3.31 (*t*, 8.4)	69.92	CH		C-3″, C-5″, C-6″
5″	3.58-3.53 (*m*)	75.91	CH	H-6″	C-3″, C-4″, C-5″, C-6″

Table 65 (continued)

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
6''	4.00-3.98 (*m*) 3.54-3.48 (*m*)	66.56	CH_2	H-5''	C-1''', C-4''
Rha-1'''	4.65 (*s*)	100.63	CH		C-6'', C-2''', C-3''', C-5'''
2'''	3.78-3.73 (*m*)	70.49	CH	H-3'''	C-2''', C-4'''
3'''	3.62-3.55 (*m*)	71.17	CH	H-2'''	C-1''', C-4'''
4'''	3.30-3.25 (*m*)	72.36	CH		C-3''', C-5'''
5'''	3.55-3.51 (*m*)	68.28	CH		C-3''', C-4'''
6'''	1.16 (*d*, 6.1)	17.71	CH_3		C-4''', C-5'''
4'-OMe	3.82 (*s*)	55.06	CH_3		C-4'
8-OMe	4.01 (*s*)	61.42	CH_3		C-8
2''-OH	5.40 (*d*, 5.4)			H-2''	C-1'', C-2'', C-3''
3''-OH	5.11 (*d*, 1.8)			H-3''	C-2'', C-3'', C-4'', C-5''
4''-OH	5.13 (*d*, 2.7)			H-4''	C-2'', C-3'', C-4'', C-5''
2'''-OH	4.53 (*d*, 3.6)			H-2'''	C-1''', C-2''', C-3'''
3'''-OH	4.39 (*d*, 5.9)			H-3'''	C-2''', C-3''', C-4'''
4'''-OH	4.59 (*d*, 5.4)			H-4'''	C-3''', C-4''', C-5'''

The hexaacetate of YS11 (AcYS11)

AcYS11 was obtained as a colorless viscous liquid with $[\alpha]_D^{29}$ −40.54°. This compound exhibited an UV absorption band at 253 nm (Figure 143), characteristic of an isoflavonoid. IR absorption bands (Figure 144) were found at 1748 cm^{-1} (an acetyl carbonyl group), 1645 cm^{-1} (a carbonyl group of an isoflavone) and 1609, 1567 cm^{-1} (an aromatic ring). The ^{1}H NMR spectrum (Table 66) (Figure 145) was similar to that of **the hexaacetate of YS2 (AcYS2)** (Figure 141) : a sharp *singlet* integrating for one proton at δ_H 7.99 for the H-2, two *doublets* with *ortho* coupling constant (J = 9.1 Hz) at δ_H 8.06 and 7.04 for the H-5 and H-6, respectively, an AA'XX' system with two *doublets* at δ_H 7.52 and 6.98 (2H each, J = 9.1 Hz), typical of 4'-substituted B-ring, and two methoxyl groups at δ_H 3.84 and 3.99. In NOESY spectrum (Figure 149), two cross peaks between the H-6 (δ_H 7.04) and the H-3', H-5' (δ_H 6.98) with the methoxy signals at δ_H 3.99 and δ_H 3.84, respectively, established the attachment of the methoxyl groups at the C-7 of A-ring and the C-4′ of B-ring. In addition, **AcYS11** also contained two sugar units, one glucose and one rhamnose, as found in **AcYS2** due to the presence of two anomeric protons at δ_H 5.22 (*d*, J = 6.8 Hz) and δ_H 4.56 (*d*, J = 1.6 Hz) which were attributed to the H-1″ of glucose unit and the H-1‴ of rhamnose unit, respectively. The presence of α-rhamnosyl residue was confirmed by the presence of a methyl *doublet* at δ_H 1.11 (J = 6.3 Hz). By analysis of ^{1}H-^{1}H COSY spectrum (Figure 148), seven protons of glucose residue and eight protons of rhamnose residue were assigned as shown in Table 66. Complete ^{1}H and ^{13}C assignments were reported in Table 66, as results from DEPT (Figure 147), HMQC (Figure 150) and HMBC (Figure 151) experiments. The HMBC correlation between the H-1‴ (δ_H 4.56) of rhamnose unit and the C-6″ (δ_C 66.53) of glucose unit revealed that the rhamnose unit formed a (1→6) glycosidic linkage with the glucose unit of which the C-1″ was connected to the C-8 of the aglycone due to a correlation between the H-1″ (δ_H 5.22) of glucose unit with the C-8 (δ_C 132.35) of the aglycone. **AcYS11** was therefore identified as the hexaacetate of 7,4′-dimethoxyisoflavone 8-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (**YS11**), a new isoflavone glycoside. Thus, the structural differences between **AcYS2** and **AcYS11** were the location of the methoxyl group and the sugar moiety on the A-ring.

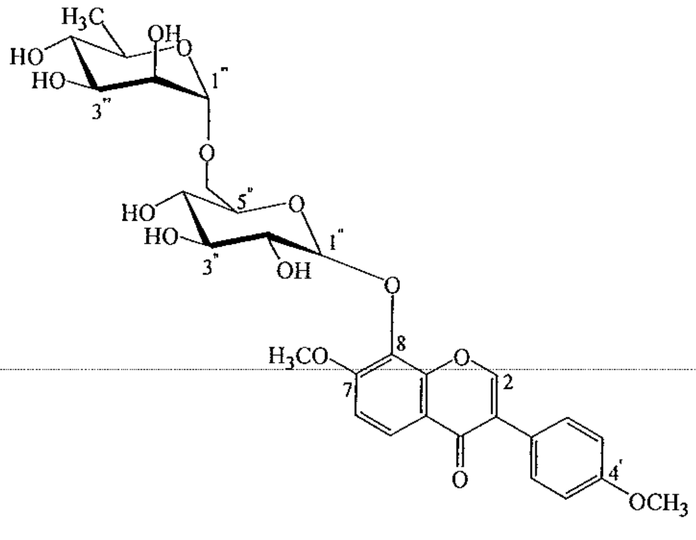


Table 66 The NMR spectral data of **AcYS11**

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
2	7.99 (*s*)	152.71	CH		C-4, C-7, C-8a
3	-	124.72	C		
4	-	175.95	C		
4a	-	119.35	C		
5	8.06 (*d*, 9.1)	123.41	CH	H-6	C-4, C-7, C-8a
6	7.04 (*d*, 9.1)	110.30	CH	H-5	C-4a, C-7, C-8
7	-	156.05	C		
8	-	132.35	C		
8a	-	150.82	C		
1′	-	124.24	C		
2′, 6′	7.52 (*d*, 9.1)	130.49	CH	H-3′, 5′	C-3, C-2′, 6′, C-4′
3′, 5′	6.98 (*d*, 9.1)	114.21	CH	H-2′, 6′	C-1′, C-2′, 6′, C-4′
4′	-	159.92	C		
Glc-1″	5.22 (*d*, 6.8)	101.15	CH	H-2″	C-8, C-3″, C-5″, C-6″
2″	5.36 (*dd*, 9.2, 6.8)	72.12	CH	H-1″, H-3″	C-2″, C-3″, C-4″
3″	5.31 (*t*, 9.2)	72.89	CH	H-2″, H-4″	C-2″, C-4″, C-5″
4″	5.15 (*t*, 9.2)	69.18	CH	H-3″, H-5″	C-4″, C-5″
5″	3.75 (*ddd*, 9.2, 6.8, 2.4)	74.38	CH	H-4″, H-6″	
6″	3.62 (*dd*, 12.0, 2.4) 3.69 (*dd*, 12.0, 6.8)	66.53	CH$_2$	H-5″	C-5″
Rha-1‴	4.56 (*d*, 1.6)	97.92	CH	H-2‴	C-6″, C-3‴, C-5‴
2‴	5.06 (*dd*, 3.2, 1.6)	69.56	CH	H-1‴, H-3‴	C-3‴, C-4‴
3‴	5.12 (*dd*, 9.2, 3.2)	69.26	CH	H-2‴, H-4‴	C-3‴, C-4‴
4‴	4.98 (*t*, 9.2)	70.91	CH	H-3‴, H-5‴	C-2‴, C-3‴, C-5‴
5‴	3.70 (*dq*, 9.2, 6.3)	66.94	CH	H-4‴, H-6‴	C-1‴
6‴	1.11 (*d*, 6.3)	17.52	CH$_3$	H-5‴	C-4‴, C-5‴
4′-OMe	3.84 (*s*)	55.56	CH$_3$		C-4′
7-OMe	3.99 (*s*)	56.80	CH$_3$		C-7

Table 66 (continued)

Position	δ_H	δ_C	Type of C	^1H-^1H COSY	HMBC
$OCOCH_3$	2.11, 2.08, 2.06, 1.99 (6H),	20.95	CH_3		
	1.94	20.93	CH_3		
		20.87	CH_3		
		20.86	CH_3		
		20.85	CH_3		
$OCOCH_3$		170.49	C		
		170.25	C		
		170.15	C		
		170.09	C		
		169.88	C		
		169.56	C		

The hexaacatete of YS6 (AcYS6)

AcYS6 was obtained as a white solid with $[\alpha]_D^{29}$ –68.18°. The UV spectrum (Figure 152) with an absorption band at 260 nm suggested an isoflavone skeleton. The IR spectrum (Figure 153) exhibited absorption bands for an acetyl carbonyl group (1750 cm^{-1}), a carbonyl group of an isoflavone (1634 cm^{-1}) and an aromatic ring (1602 and 1579 cm^{-1}). The ^1H NMR spectrum (Table 67) (Figure 154) displayed a characteristic one proton *singlet* at δ_H 7.97 for the H-2 of an isoflavonoid nucleus. Two *singlets* at δ_H 7.65 and 7.20 were assigned to the H-5 and H-8, respectively, due to a chemical shift characteristic of the H-5 and value of coupling constant (J = 0 Hz). It also displayed an AA$'$XX$'$ system, typical of 4$'$-substituted B-ring, with two *doublets* at δ_H 7.51 and 6.98 (2H each, J = 9.3 Hz) which were assigned to the H-2$'$, H-6$'$ and H-3$'$, H-5$'$, respectively. This compound also composed of two methoxyl groups at δ_H 3.92 and 3.84. The location of the methoxyl groups at the C-6 and C-4$'$ were determined by NOESY experiment (Figure 158). The aromatic proton (H-5) at δ_H 7.65 correlated with the methoxy proton at δ_H 3.92 while the aromatic proton at δ_H 6.98 (H-3$'$, H-5$'$) correlated with the methoxy proton at δ_H 3.84. Furthermore, HMBC correlations (Table 67) (Figure 160) between the methoxy protons (δ_H 3.92

and 3.84) with the C-6 (δ_C 148.82) and the C-4′ (δ_C 159.79), respectively, of the aglycone supported above conclusion. The two anomeric protons of two sugar units (δ_H 5.13, *d*, *J* = 7.6 Hz, H-1″ and δ_H 4.74, *d*, *J* = 1.7 Hz, H-1‴) suggested a *β*-configuration for the glucose unit and a *α*-configuration for the rhamnose unit. The ^{13}C NMR spectrum (Table 67) (Figure 155) revealed a set of signals belonging to the sugar units which showed the C-1″ of glucose unit and the C-1‴ of rhamnose unit at δ_C 100.03 and 98.44, respectively, and the C-6″ of glucose residue and the C-6‴ of rhamnose residue at δ_C 66.80 and 17.58, respectively. The obvious downfield shift (> 6.5 ppm) of the C-6″ of glucose unit in the ^{13}C NMR spectrum indicated that rhamnose was linked to the 6-hydroxyl of glucose unit. This (1→6)-glycosidic linkage was confirmed by a HMBC correlation between the H-1‴ (δ_H 4.74) of rhamnose unit with the C-6″ (δ_C 66.80) of glucose unit. The attachment of the disaccharide to the 7-hydroxyl of the aglycone was directly deduced from a correlation observed between the anomeric proton, H-1″ (δ_H 5.13), of glucose moiety and the C-7 (δ_C 150.99) of the aglycone in the HMBC experiment. The location of the sugars was further supported by a cross peak between the methoxy proton at the C-6 (δ_H 3.92) with the H-1″ of glucose unit in the NOESY spectrum (Figure 158). The presence of six methyl signals of acetyl groups in the ^{1}H NMR spectrum indicated that all of the hydroxyl groups of glucose and rhamnose units were protected in a form of an acetate ester. Therefore, **AcYS6** was identified as the hexaacetate of 4′,6-dimethoxyisoflavone 7-*O*-[*α*-rhamnopyranosyl-(1→6)]-*β*-glucopyranoside (**YS6**), a new isoflavone glycoside.

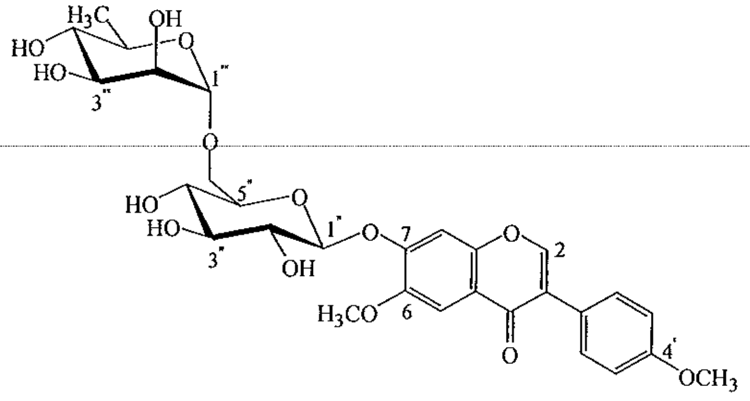


Table 67 The NMR spectral data of **AcYS6**

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
2	7.97 (*s*)	152.94	CH		C-4, C-8a, C-1′
3	-	124.32	C		
4	-	175.84	C		
4a	-	120.71	C		
5	7.65 (*s*)	106.57	CH		C-4, C-4a, C-6, C-8a
6	-	148.82	C		
7	-	150.99	C		C-4, C-4a, C-6, C-8a
8	7.20 (*s*)	107.19	CH		
8a	-	151.52	C		
1′	-	124.69	C		
2′, 6′	7.51 (*d*, 9.3)	130.33	CH	H-3′, 5′	C-3, C-2′, 6′, C-4′
3′, 5′	6.98 (*d*, 9.3)	114.21	CH	H-2′, 6′	C-1′, C-3′, 5′, C-4′
4′	-	159.79	C		
Glc-1″	5.13 (*d*, 7.6)	100.03	CH	H-2″	C-7
2″	5.35-5.32 (*m*)	71.24	CH	H-1″, H-3″	C-2″
3″	5.35-5.32 (*m*)	72.64	CH	H-2″, H-4″	C-2″
4″	5.09 (*t*, 9.9)	69.25	CH	H-3″, H-5″	C-3″
5″	3.89 (*ddd*, 9.9, 7.0, 2.5)	73.90	CH	H-6″	C-4″
6″	3.77 (*dd*, 11.5, 2.5) 3.65 (*dd*, 11.5, 7.0)	66.80	CH_2	H-5″	C-5″, C-1‴
Rha-1‴	4.74 (*d*, 1.7)	98.44	CH	H-2‴	C-6″, C-3‴
2‴	5.31 (*dd*, 3.4, 1.7)	69.55	CH	H-3‴	C-3‴, C-4‴
3‴	5.26 (*dd*, 9.9, 3.4)	69.36	CH	H-2‴, H-4‴	C-4‴
4‴	5.04 (*t*, 9.9)	71.07	CH	H-3‴, H-5‴	C-3‴, C-5‴
5‴	3.83 (*dq*, 9.9, 5.9)	66.93	CH	H-4‴, H-6‴	C-4‴
6‴	1.19 (*d*, 5.9)	17.58	CH_3		C-4‴, C-5‴
4′-OMe	3.84 (*s*)	55.55	CH_3		C-4′
6-OMe	3.92 (*s*)	56.62	CH_3		C-6

Table 67 (continued)

Position	δ_H	δ_C	Type of C	^1H-^1H COSY	HMBC
$OCOCH_3$	2.09, 2.08, 2.07, 2.05, 2.04, 1.96	21.00	CH_3		
		20.93	CH_3		
		20.83	CH_3		
$OCOCH_3$		170.43	C		
		170.14	C		
		170.05	C		
		169.96	C		
		169.68	C		
		169.49	C		

The hexaacetate of YS7 (AcYS7)

AcYS7 was obtained as a yellow viscous liquid with $[\alpha]_D^{29}$ –90.90°. This compound exhibited an UV absorption band at 265 nm (Figure 161), characteristic of an isoflavonoid. The IR absorption bands (Figure 162) were found at 1756 cm^{-1} (an acetyl carbonyl group) , 1659 cm^{-1} (a carbonyl group of an isoflavone) and 1613, 1590 cm^{-1} (an aromatic ring). The ^1H NMR spectrum (Table 68) (Figure 163) revealed the presence of a chelated hydroxyl group (δ_H 12.90, *s*, 5-OH) and a sharp *singlet* integrating for one proton at δ_H 7.95 assigned to the H-2 of an isoflavonoid nucleus. It exhibited two *singlets* integrating for three protons for each signal at δ_H 3.84 and 3.83 for two methoxyl groups and a one proton *singlet* at δ_H 6.71 for the H-8. It also displayed an $AA'XX'$ pattern, typical of 4′-substituted B-ring with two *doublets* at δ_H 7.48 and 6.99 (2H each, J = 9.0 Hz), which were assigned to the H-2′, H-6′ and H-3′, H-5′, respectively. Two anomeric proton signals at δ_H 5.12 (J = 7.5 Hz) and δ_H 4.76 (J = 1.5 Hz) were attributed to the H-1″ and H-1‴ of β-glucose and α-rhamnose units, respectively. The presence of α-rhamnose unit was confirmed by a methyl *doublet* at δ_H 1.20 (J = 6.4 Hz). Moreover, the C-6″ carbon signal of glucose unit in the ^{13}C NMR spectrum (Table 68) (Figure 164) was shifted downfield (δ_C 66.46) which suggested the attachment of rhamnose unit at the C-6″ of glucose unit. The HMBC correlation experiments (Table 68) (Figure 168) revealed a correlation between the H-1‴ (δ_H 4.76) of rhamnose unit and the C-6″ (δ_C 66.46) of glucose unit as well as a correlation between the H-1″ (δ_H 5.12) of glucose unit and the C-7 (δ_C 155.68) of the aglycone. These results indicated that the rhamnose unit was attached to C-6″ of glucose unit of which the C-1″ was attached to the C-7 of the aglycone. The location of the methoxyl groups at the C-4′ and C-6 was determined by HMBC correlation data (Table 68)(Figure 168) which showed polarization transfer between the methoxyl groups at δ_H 3.84 and 3.83 with the C-4′ and C-6, respectively. The presence of six methyl groups of acetyl group at δ_H 2.10, 2.08, 2.07, 2.05, 2.04 and 1.96 suggested that all of hydroxyl groups of rhamnose and glucose units were acetylated while the 5-chelated hydroxyl group of the aglycone was intact. Therefore, **AcYS7** was characterized as the hexaacetate of 5-hydroxy-4′,6-dimethoxyisoflavone 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside (**YS7**), a new isoflavone glycoside.

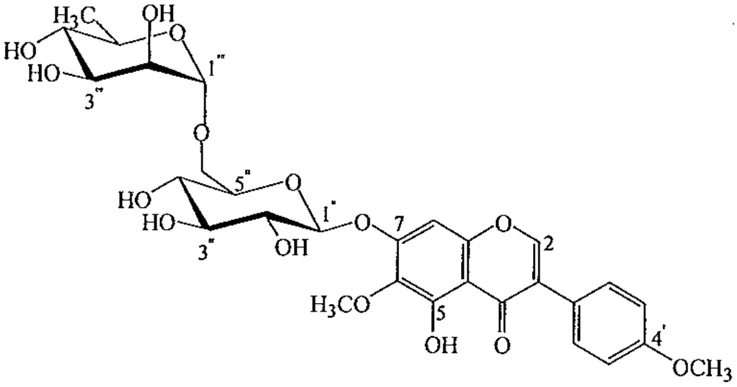


Table 68 The NMR spectral data of **AcYS7**

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
2	7.95 (*s*)	153.73	CH		C-3, C-4, C-8a
3	-	122.93	C		
4	-	181.36	C		
4a	-	108.39	C		
5	-	154.28	C		
6	-	133.72	C		
7	-	155.68	C		

Table 68 (continued)

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
8	6.71 (*s*)	95.41	CH		C-4, C-4a, C-6, C-7, C-8a
8a	-	152.74	C		
1′	-	123.02	C		
2′, 6′	7.48 (*d*, 9.0)	130.09	CH	H-3′, 5′	C-1′, C-2′, 6′, C-4′
3′, 5′	6.99 (*d*, 9.0)	114.21	CH	H-2′, 6′	C-3, C-3′, 5′, C-4′
4′	-	159.78	C		
Glc-1″	5.12 (*d*, 7.5)	99.45	CH	H-2″	C-7, C-3″
2″	5.36 (*t*, 9.0)	70.74	CH	H-1″, H-3″	C-3″, C-4″
3″	5.33 (*t*, 9.0)	72.37	CH	H-2″, H-4″	C-2″, C-4″
4″	5.07 (*t*, 9.0)	68.93	CH	H-3″, H-5″	C-3″, C-5″
5″	3.93-3.86 (*m*)	73.67	CH	H-6″	C-1″, C-4″
6″	3.76 (*dd*, 12.0, 3.0) 3.63 (*dd*, 12.0, 9.0)	66.46	CH_2	H-5″	C-4″, C-6″
Rha-1‴	4.76 (*d*, 1.5)	98.29	CH	H-2‴	C-6″, C-3‴
2‴	5.34 (*dd*, 3.0, 1.5)	69.19	CH	H-1‴, H-3‴	C-3‴, C-4‴
3‴	5.27 (*dd*, 9.0, 3.0)	69.19	CH	H-2‴, H-4‴	C-2‴, C-4‴, C-5‴
4‴	5.05 (*t*, 9.0)	70.74	CH	H-3‴, H-5‴	C-2‴, C-3‴, C-5‴
5‴	3.86-3.81 (*m*)	66.67	CH	H-4‴, H-6‴	C-3‴, C-4‴
6‴	1.20 (*d*, 6.4)	17.34	CH_3	H-5‴	C-4‴, C-5‴
4′-OMe	3.84 (*s*)	55.36	CH_3		C-4′
6-OMe	3.83 (*s*)	61.08	CH_3		C-6
5-OH	12.90 (*brs*)				
$OCOCH_3$	2.10, 2.08, 2.07, 2.05, 2.04, 1.96	20.80 20.62	CH_3 CH_3		
$OCOCH_3$		170.18	C		
		169.89	C		
		169.76	C		
		169.48	C		
		169.29	C		

Compound YS4

YS4 was obtained as a white solid, melting at 195.5-198.5 °C with $[\alpha]_D^{29}$ -52.63°. This compound exhibited an UV absorption band at 262 nm (Figure 169), characteristic of an isoflavonoid nucleus and IR absorption bands (Figure 170) at 3369 cm^{-1} (a hydroxyl group), 1655 cm^{-1} (a carbonyl group of an isoflavone) and 1609, 1572 cm^{-1} (an aromatic ring). The ^1H NMR spectrum (Table 69) (Figure 171) revealed the presence of a chelated hydroxyl group (δ_H 12.85, *s*, 5-OH) and a sharp *singlet* integrating for one proton at δ_H 8.25, assigned to the H-2 of an isoflavonoid nucleus. The $4'$-oxysubstituted pattern of B-ring and 5,7-dioxysubstituted pattern of A-ring were readily deduced from the proton signals forming an $AA'XX'$ system (δ_H 7.38, J = 8.8 Hz, H-$2'$, H-$6'$ and δ_H 6.85, J = 8.8 Hz, H-$3'$, H-$5'$) and two protons *meta*-related with coupling constant of 2.2 Hz, representing the H-8 (δ_H 6.43) and H-6 (δ_H 6.70). A *doublet* at δ_H 4.93 (J = 7.8 Hz) and a *broad singlet* at δ_H 4.62 were attributed to the H-$1''$ of β-glucose unit and the H-$1'''$ of α-rhamnose unit. The presence of the α-rhamnosyl unit was confirmed by a methyl signal at δ_H 1.19 as a *doublet*. Moreover, the C-$6''$ carbon signal of glucose unit in the ^{13}C NMR spectrum (Table 69) (Figure 172) was shifted downfield (δ_C 66.60), indicating that the rhamnosyl moiety was attached to the C-$6''$ of glucose residue with a (1→6)-glycosidic bond. The HMBC correlation experiments (Table 69) (Figure 176) revealed a correlation between the H-$1'''$ (δ_H 4.62) of rhamnose unit and the C-$6''$ (δ_C 66.60) of glucose unit as well as a correlation between the H-$1''$ (δ_H 4.93) of glucosyl unit and the C-7 (δ_C 162.87) of the aglycone. This suggested that the rhamnosyl unit was attached to the C-$6''$ of glucosyl moiety of which the C-$1''$ was attached to the 7-hydroxyl of the aglycone. These data permitted the identification of **YS4** as $4'$,5-dihydroxyisoflavone 7-*O*-[α-rhamnopyranosyl-(1→6)]-β-glucopyranoside, isolated from *Eriosema tuberosum* and *Derris scandens* (Guang, *et al.*, 1998 ; Suwannaroj, *et al.*, 2000).

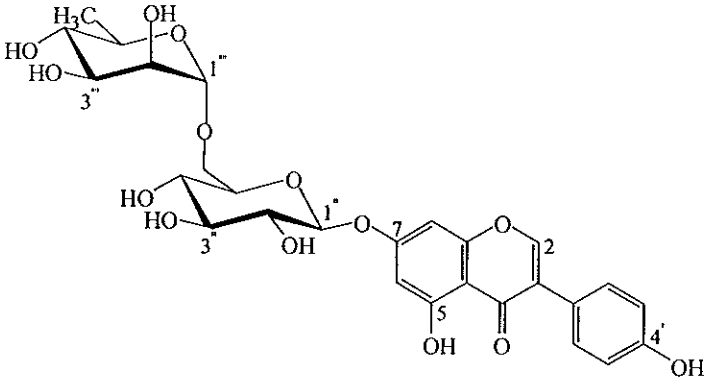


Table 69 The NMR spectral data of **YS4**

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
2	8.25 (*s*)	154.17	CH		C-3, C-4, C-1′, C-2′, 6′
3	-	122.80	C		
4	-	180.65	C		
4a	-	106.49	C		
5	-	157.39	C		
6	6.70 (*d*, 2.2)	94.64	CH	H-8	C-4, C-4a, C-6, C-7, C-8a
7	-	162.87	C		
8	6.43 (*d*, 2.2)	99.96	CH	H-6	C-4, C-4a, C-5, C-7, C-8
8a	-	161.71	C		
1′	-	121.17	C		
2′, 6′	7.38 (*d*, 8.8)	129.96	CH	H-3′, 5′	C-3, C-2′, 6′, C-3′, 5′, C-4′
3′, 5′	6.85 (*d*, 8.8)	115.20	CH	H-2′, 6′	C-1′, C-2′, 6′, C-3′, 5′, C-4′
4′	-	157.55	C		
Glc-1″	4.93 (*d*, 7.8)	100.41	CH	H-2″	C-7, C-3″, C-4″, C-5″
2″	3.45-3.36 (*m*)	73.06	CH	H-1″, H-3″, 2″-OH	C-2″, C-3″, C-4″, C-5″
3″	3.45-3.36 (*m*)	76.84	CH	H-2″, H-4″, 3″-OH	C-2″, C-3″, C-4″, C-5″
4″	3.23 (*t*, 8.4)	70.15	CH	H-3″, H-5″, 4″-OH	C-3″, C-5″, C-6″
5″	3.62-3.52 (*m*)	75.90	CH	H-6″	C-5″, C-6″
6″	4.00 (*d*, 9.8) 3.45-3.38 (*m*)	66.60	CH_2	H-5″, H-6″	C-4″, C-1‴
Rha-1‴	4.62 (*s*)	100.68	CH	H-2‴	C-6″, C-2‴, C-3‴, C-5‴
2‴	3.80-3.76 (*m*)	70.36	CH	H-3‴, 2‴-OH	C-1‴, C-3‴, C-4‴
3‴	3.70-3.64 (*m*)	71.08	CH	H-2‴, H-4‴	C-4‴, C-5‴

Table 69 (continued)

Position	δ_H	δ_C	Type of C	^{1}H-^{1}H COSY	HMBC
4'''	3.27 (*dd*, 9.1, 4.9)	72.48	CH	H-3''', 4'''-OH	C-2''', C-3''', C-5'''
5'''	3.58-3.50 (*m*)	68.34	CH	H-4''', H-6'''	C-1''', C-3''', C-4'''
6'''	1.19 (*d*, 6.0)	17.79	CH$_3$		C-4''', C-5'''
5-OH	12.85 (*s*)	-			C-4, C-7, C-8a
4'-OH	9.45 (*s*)	-			C-4'
2''-OH	5.45 (*d*, 3.5)	-		H-2''	C-1'', C-2'', C-3''
3''-OH	5.16 (*d*, 3.0)	-		H-3''	C-2'', C-3'', C-4'', C-5''
4''-OH	5.17 (*d*, 5.0)	-		H-4''	C-2'', C-3'', C-4'', C-5''
2'''-OH	4.54 (*d*, 4.0)	-		H-2'''	C-1''', C-2''', C-3'''
3'''-OH	4.43 (*d*, 6.0)	-		H-3'''	C-1''', C-2''', C-3''', C-4'''
4'''-OH	4.64 (*d*, 5.0)	-		H-4'''	C-3''', C-4''', C-5'''

APPENDIX

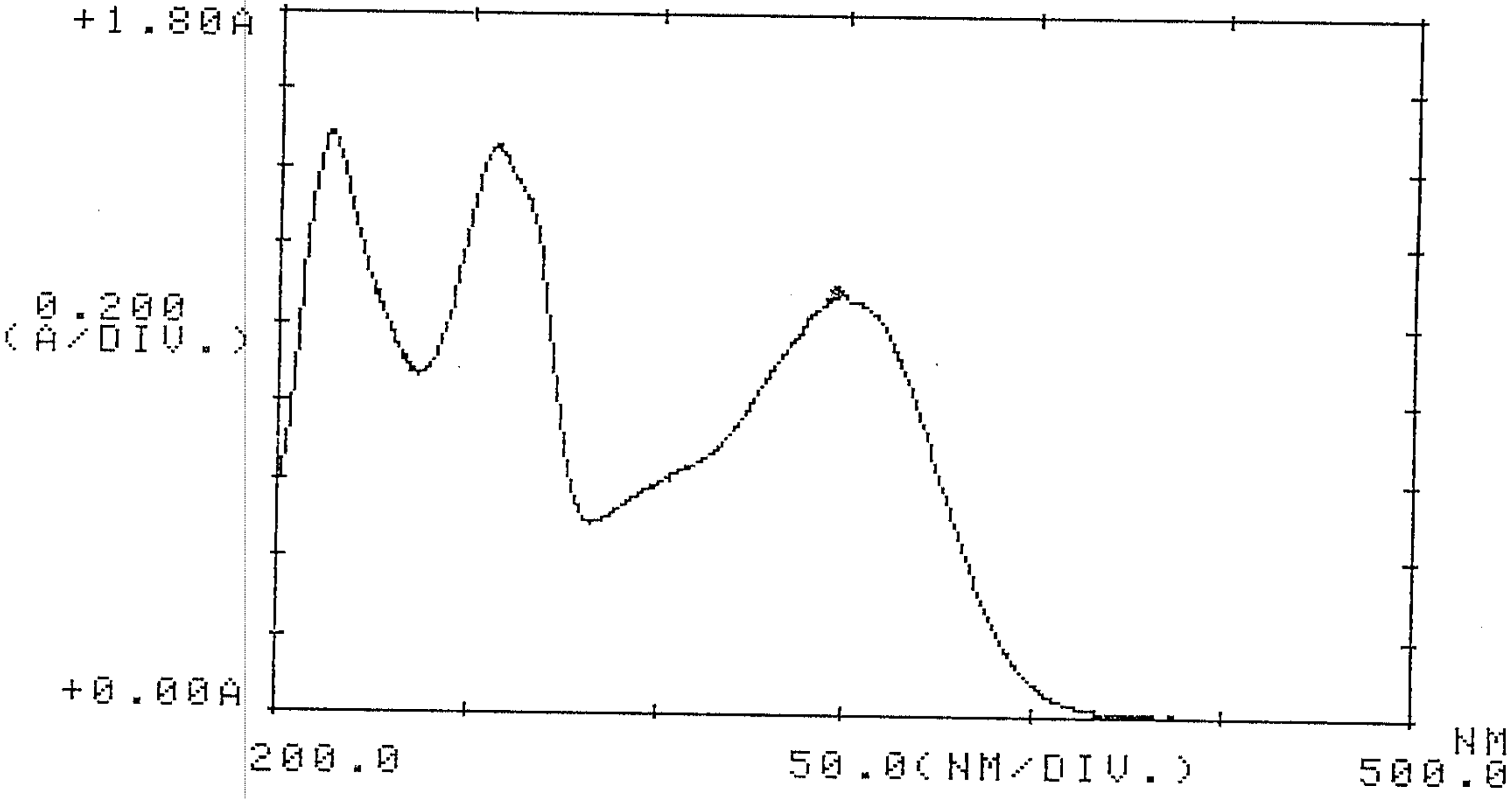


Figure 2 UV (CH_3OH) spectrum of **DW1**

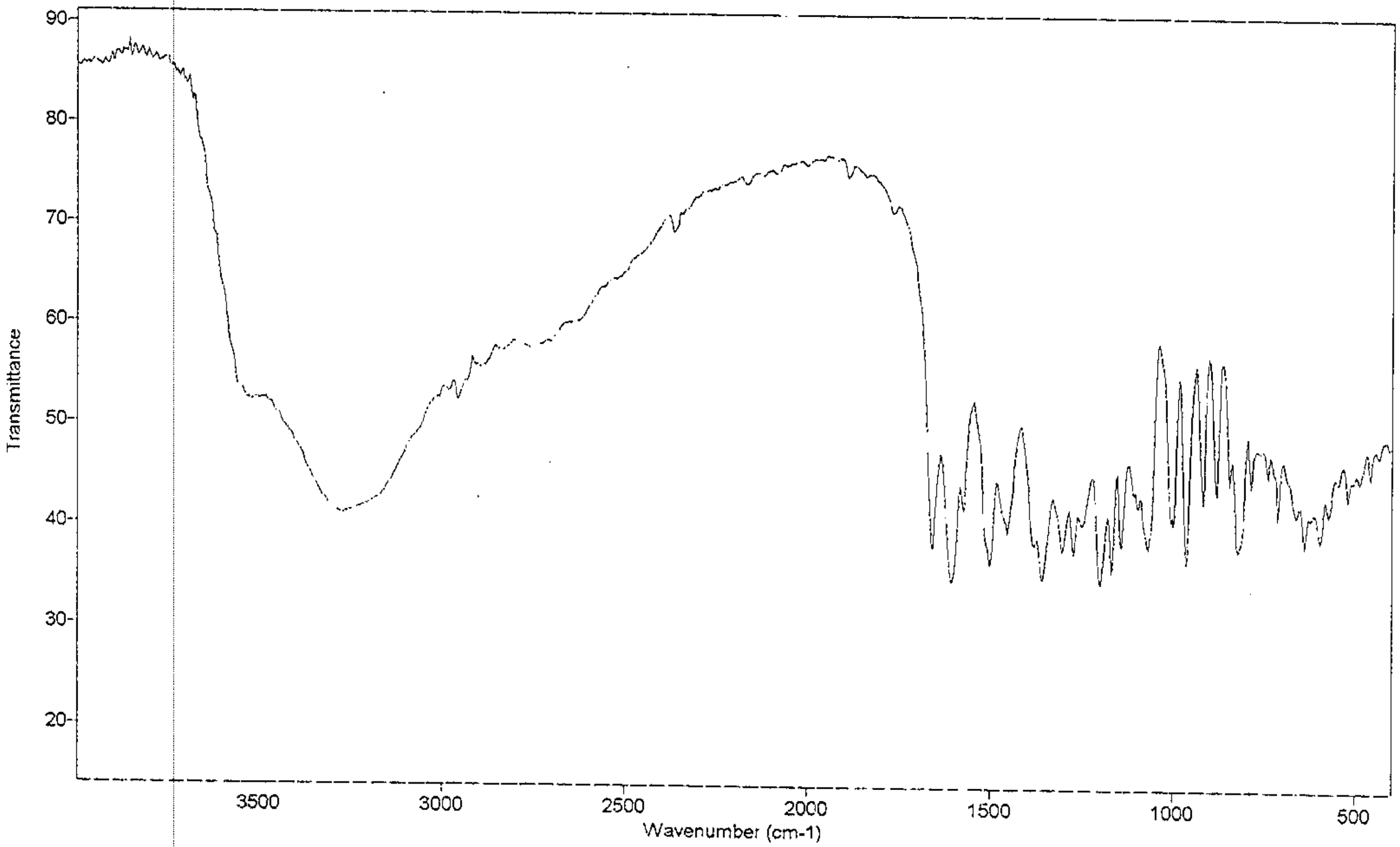


Figure 3 IR (KBr) spectrum of **DW1**

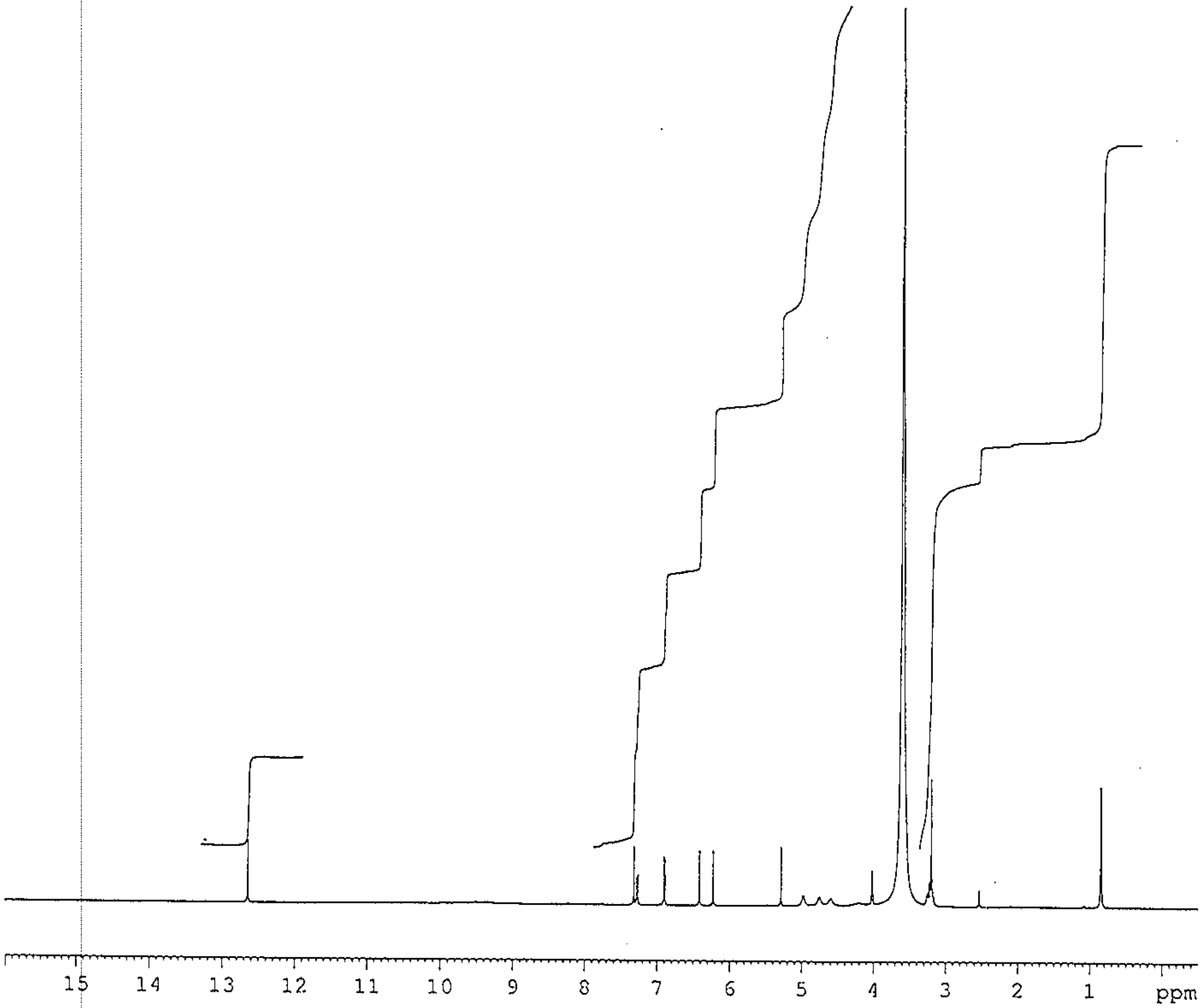


Figure 4 ^{1}H NMR (400 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of **DW1**

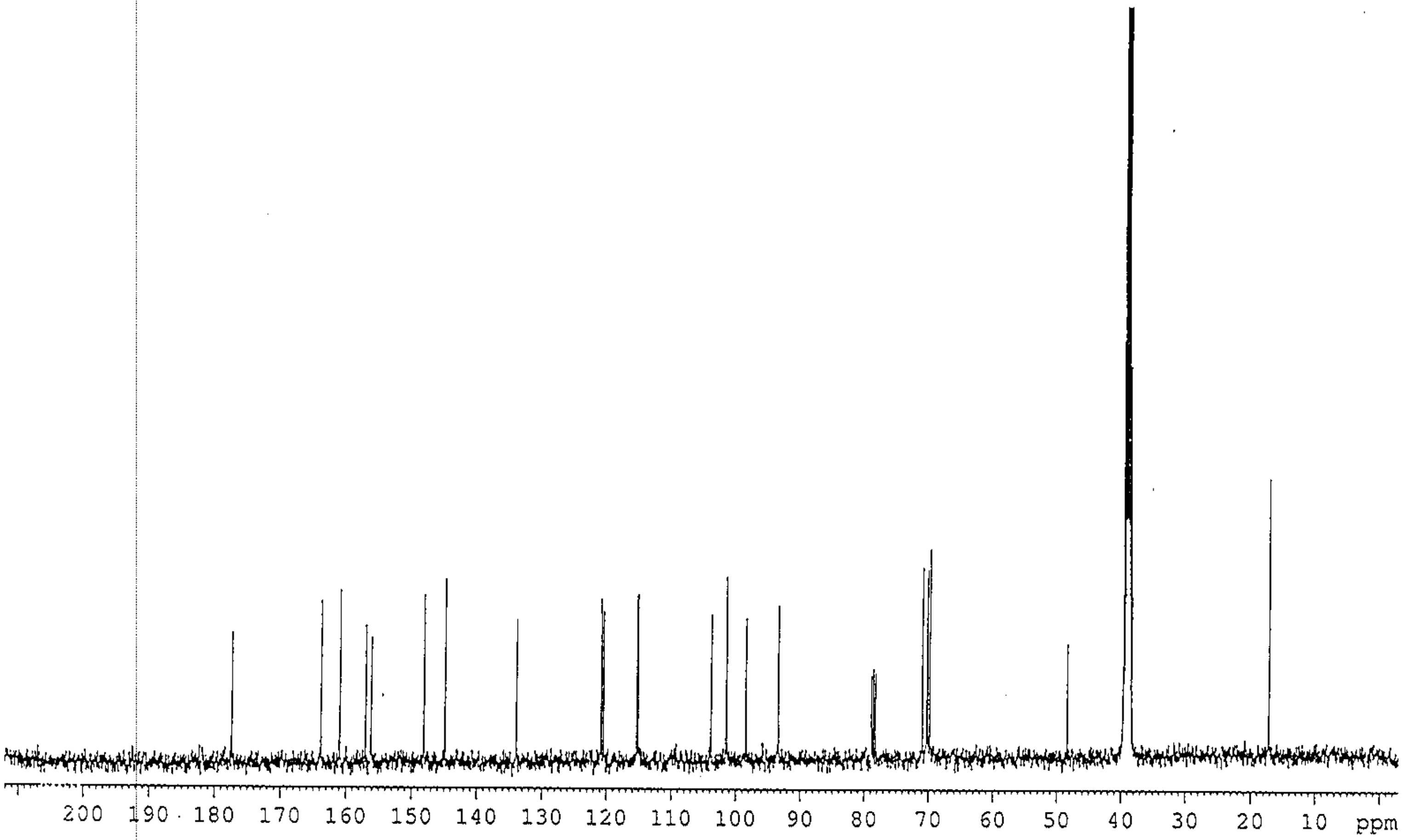


Figure 5 ^{13}C NMR (100 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of **DW1**

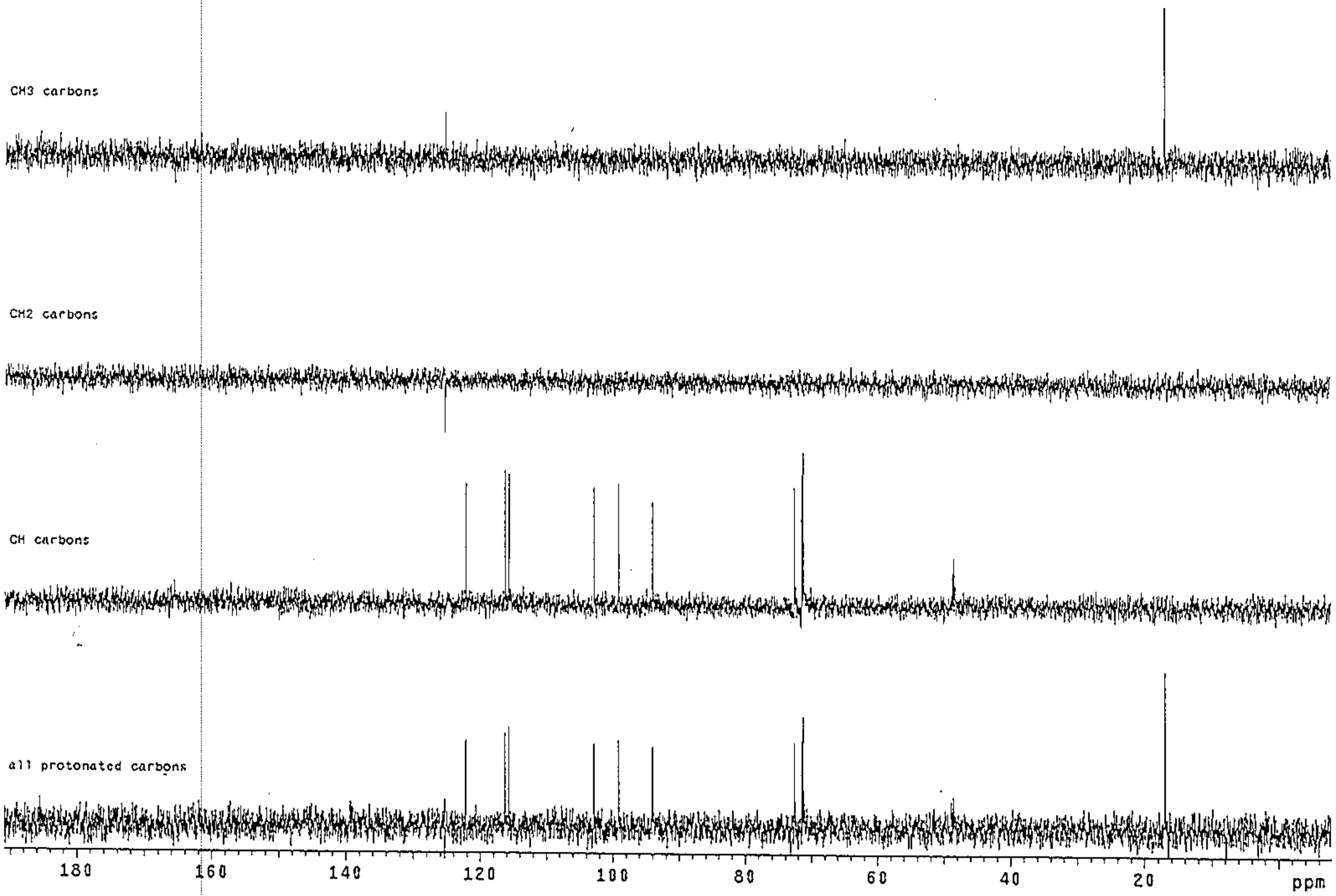


Figure 6 DEPT (135°) ($CDCl_3$ +DMSO-d_6) spectrum of **DW1**

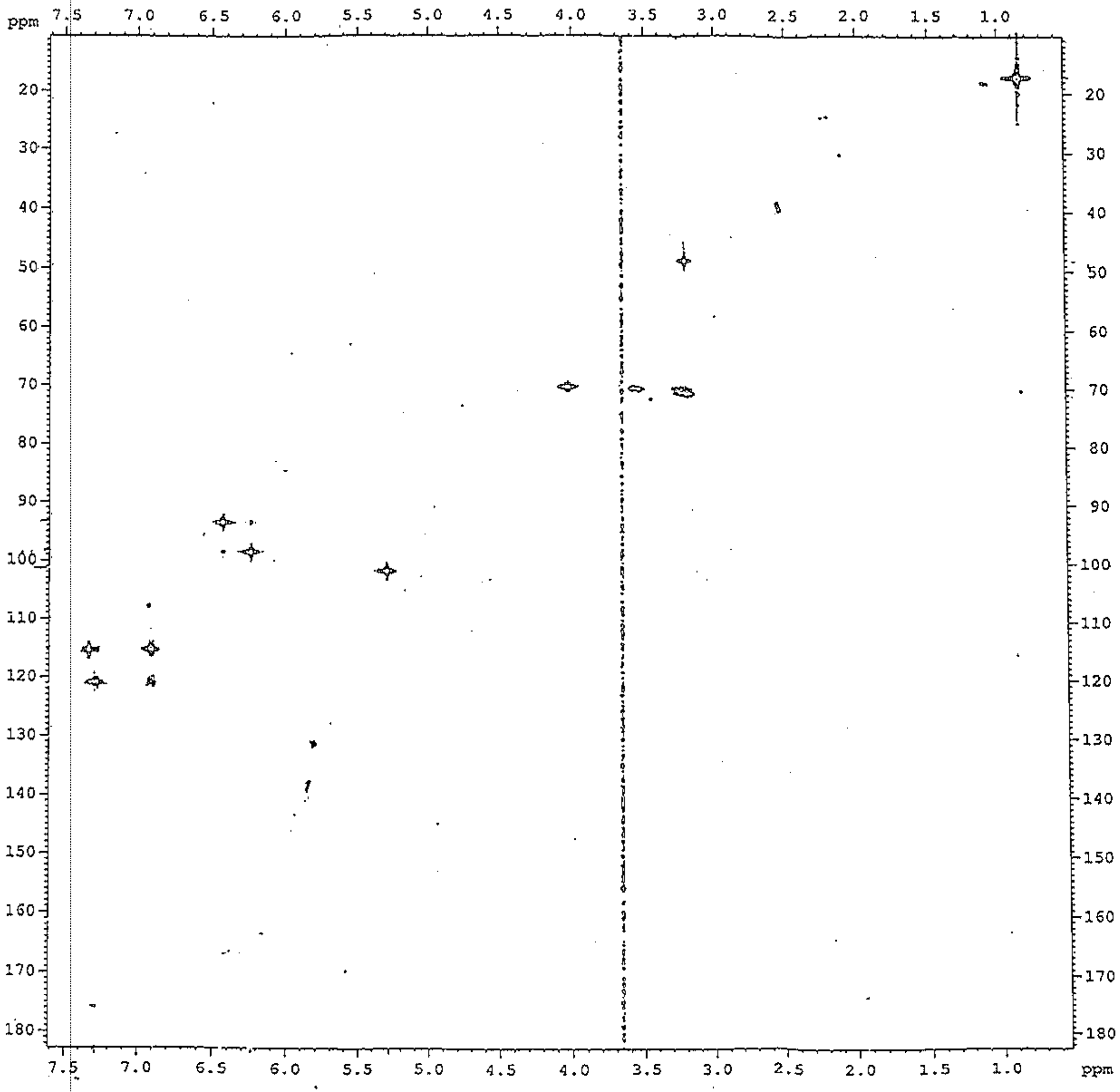


Figure 7 2D HMQC spectrum of **DW1**

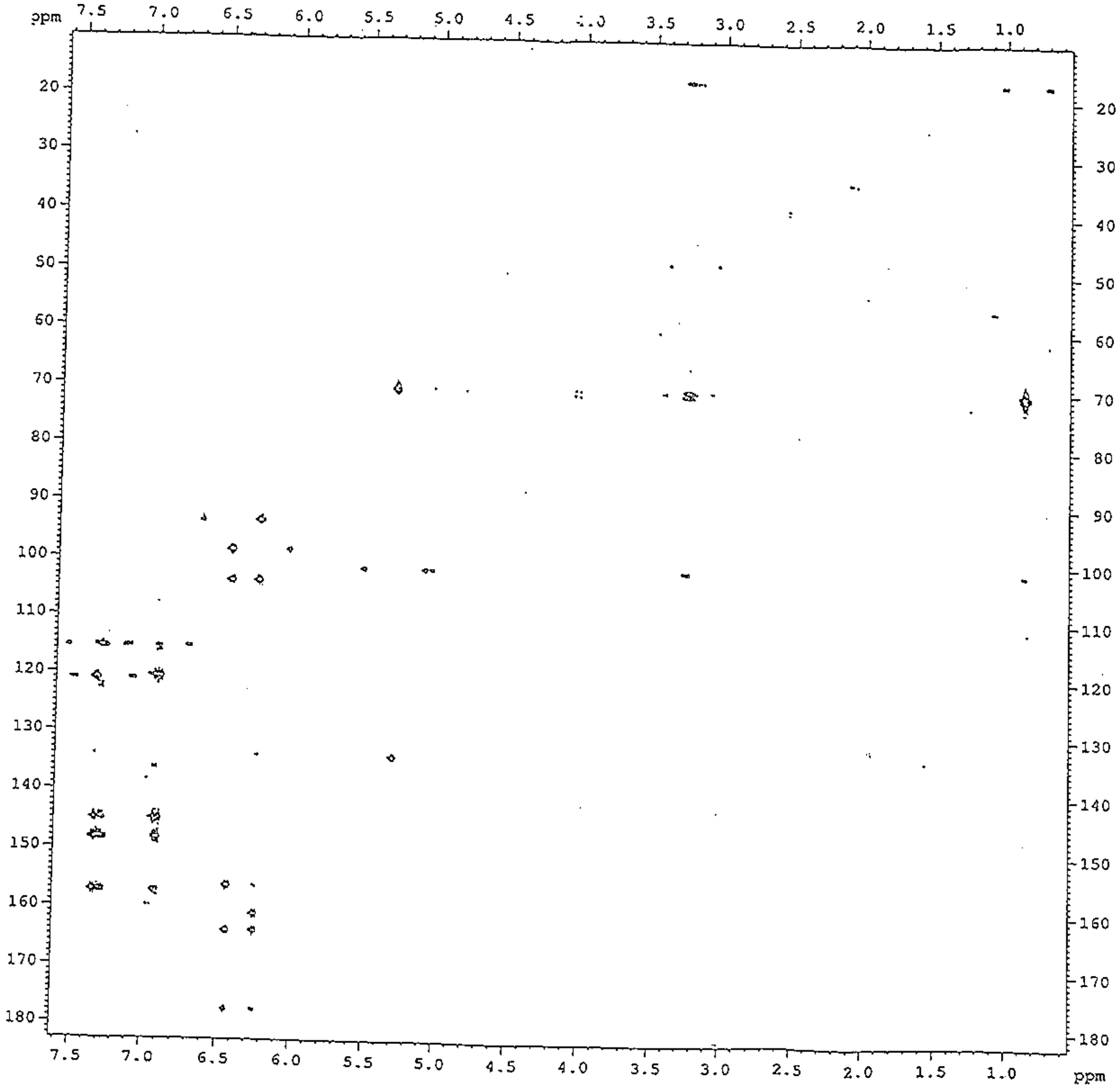


Figure 8 2D HMBC spectrum of **DW1**

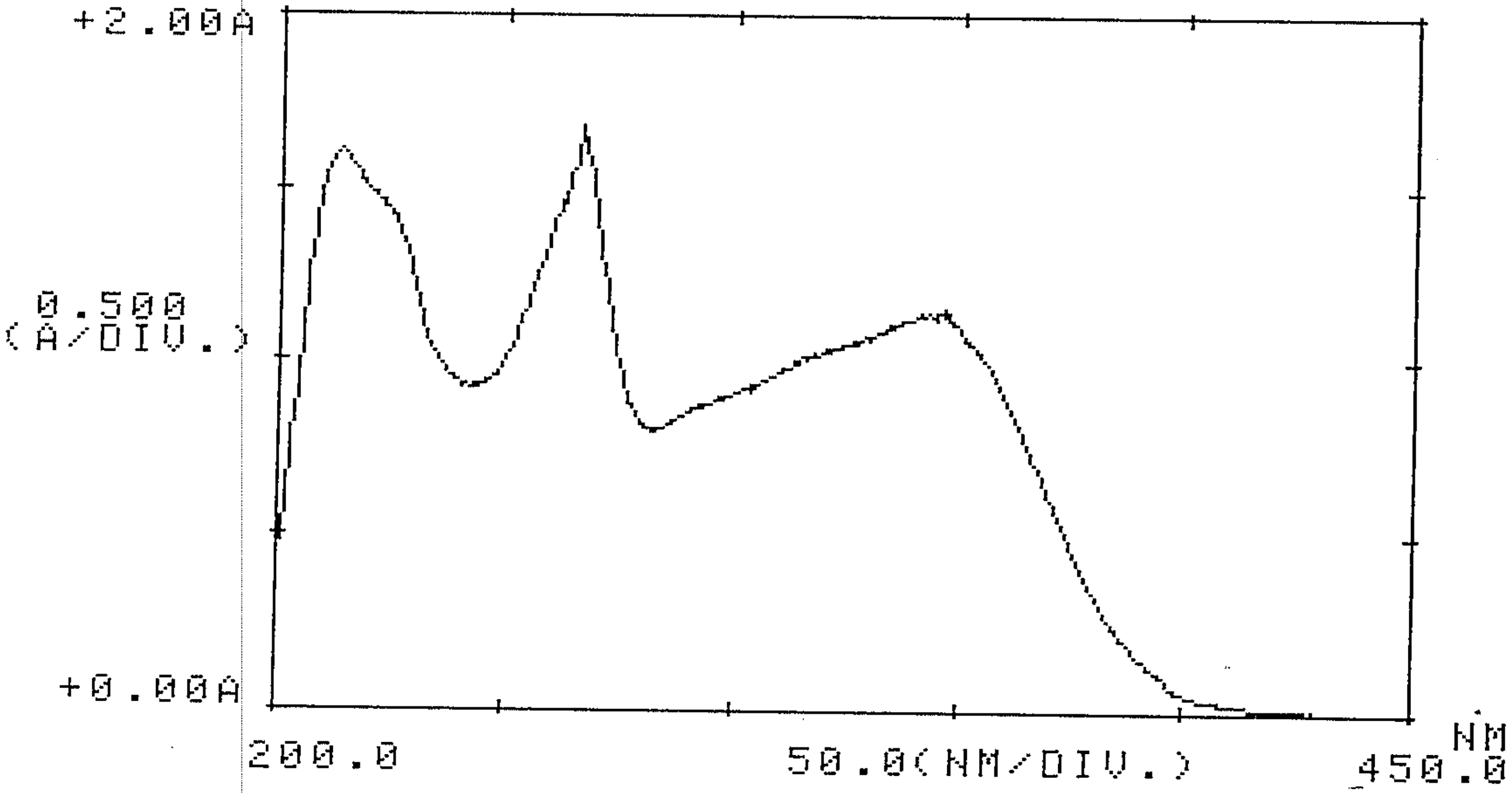


Figure 9 UV (CH_3OH) spectrum of **DW2**

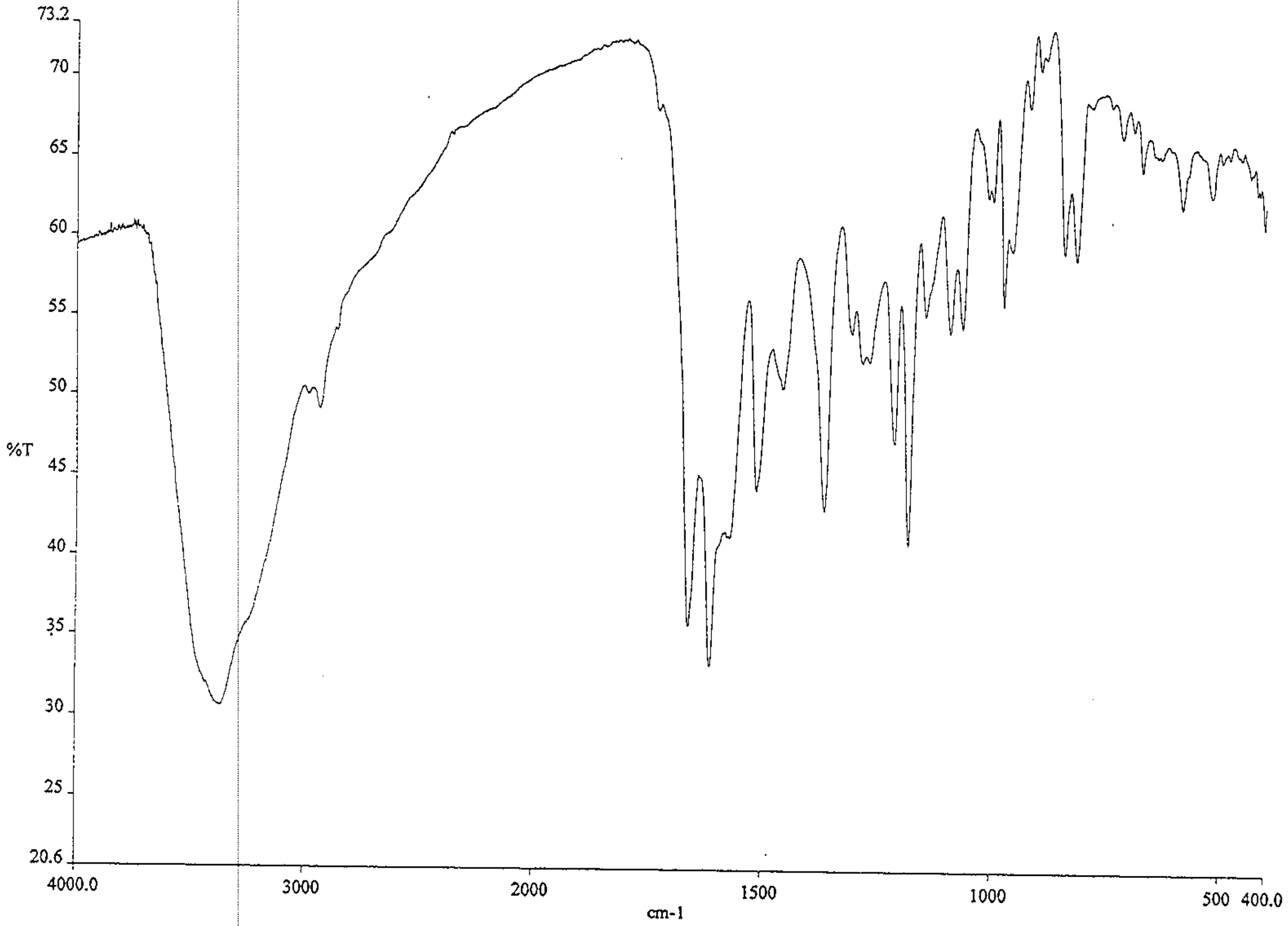


Figure 10 IR (neat) spectrum of **DW2**

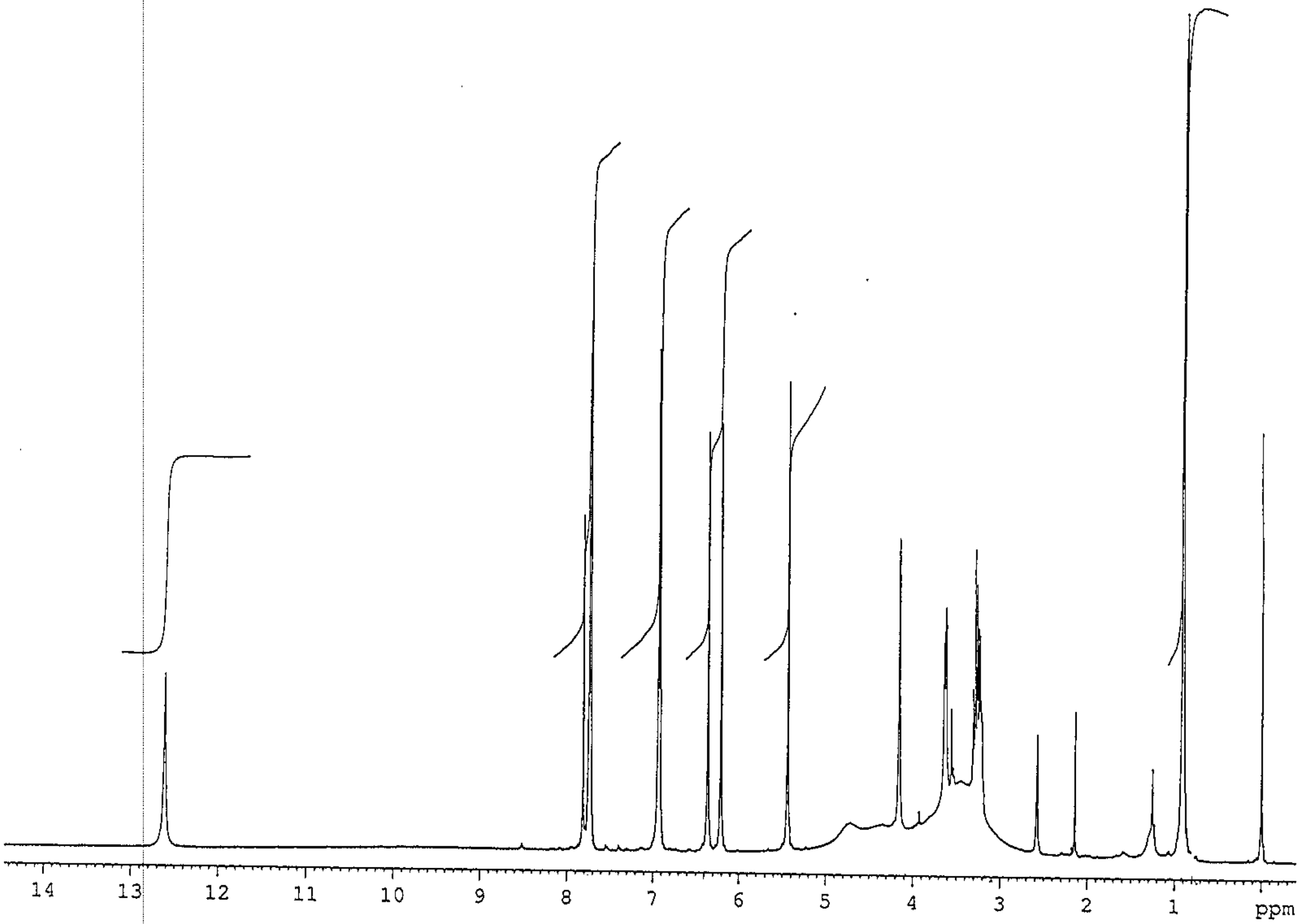


Figure 11 ^{1}H NMR (400 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of **DW2**

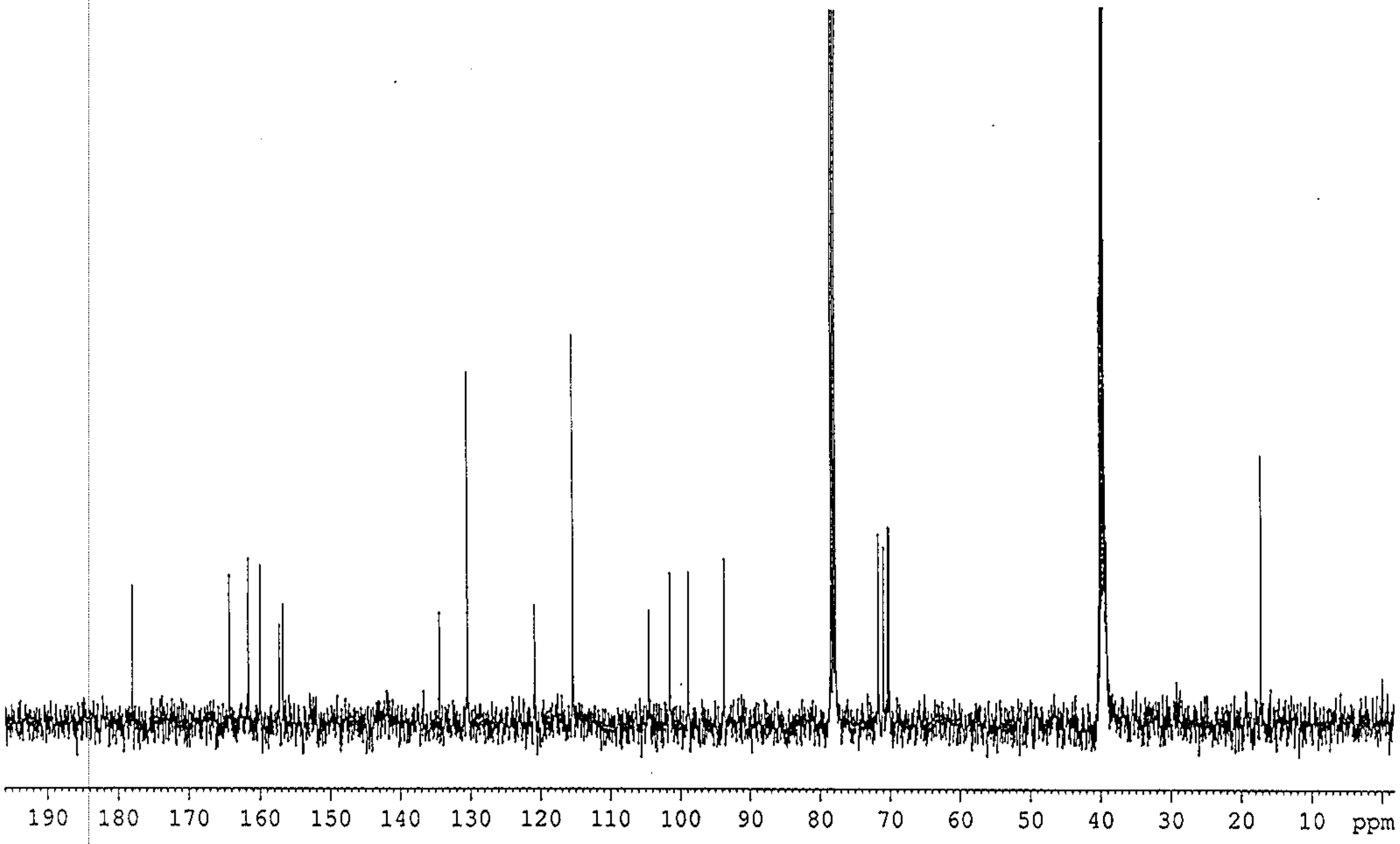


Figure 12 ^{13}C NMR (100 MHz) ($CDCl_3$ +DMSO-d_6) spectrum of **DW2**

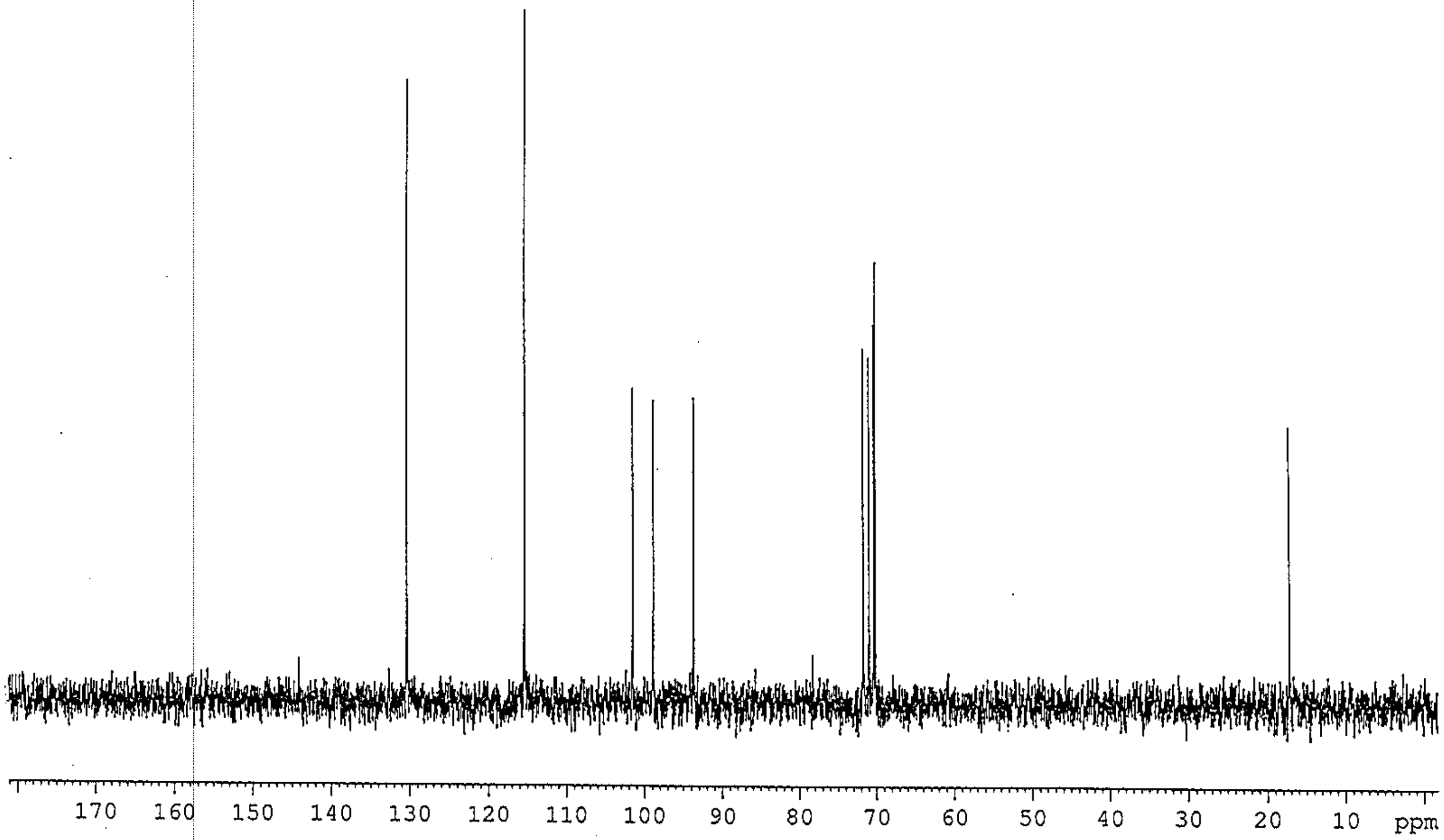


Figure 13 DEPT (135°) ($CDCl_3$ +DMSO-d_6) spectrum of **DW2**

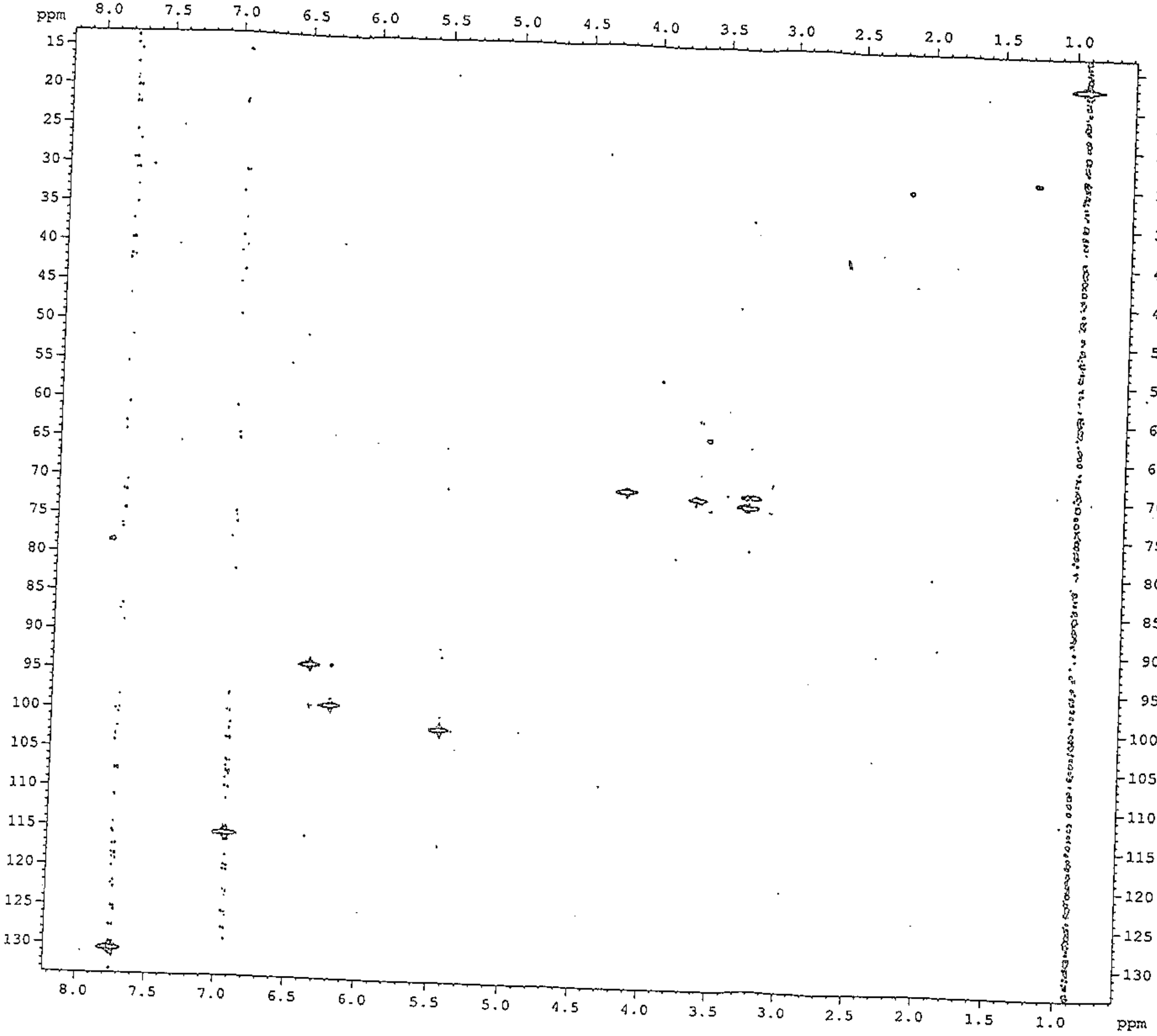


Figure 14 2D HMQC spectrum of **DW2**

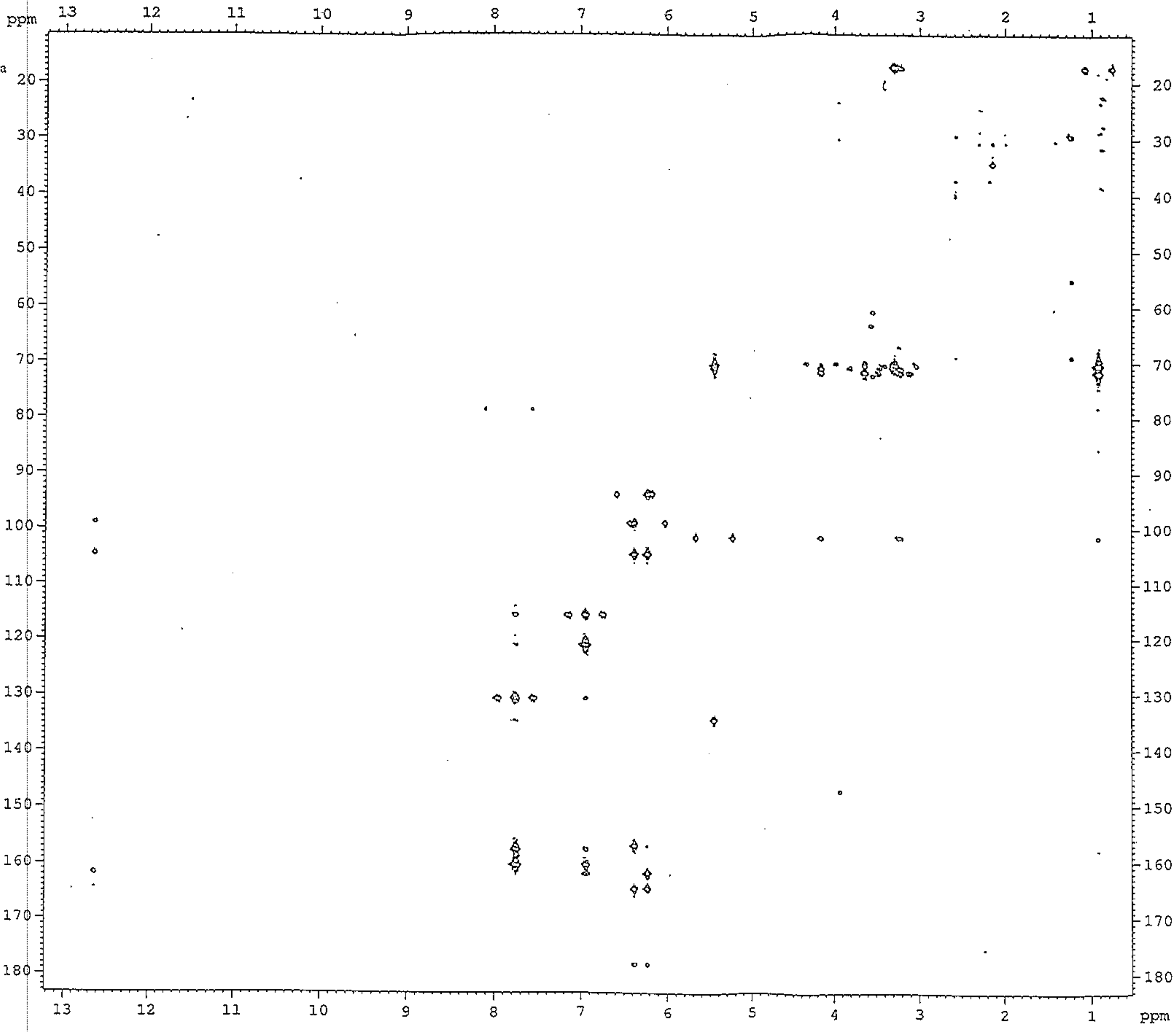


Figure 15 2D HMBC spectrum of **DW2**

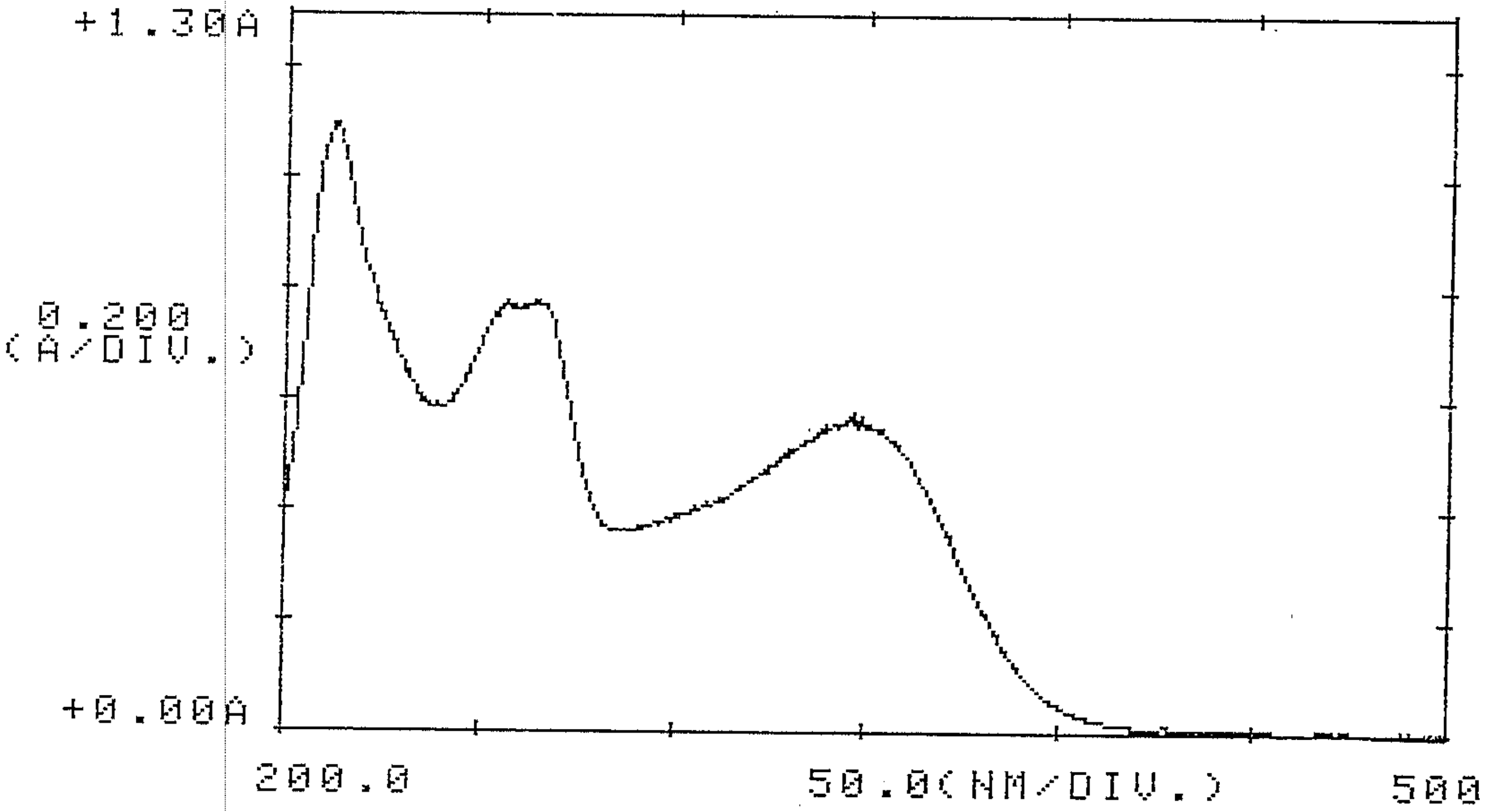


Figure 16 UV (CH_3OH) spectrum of **DW3**

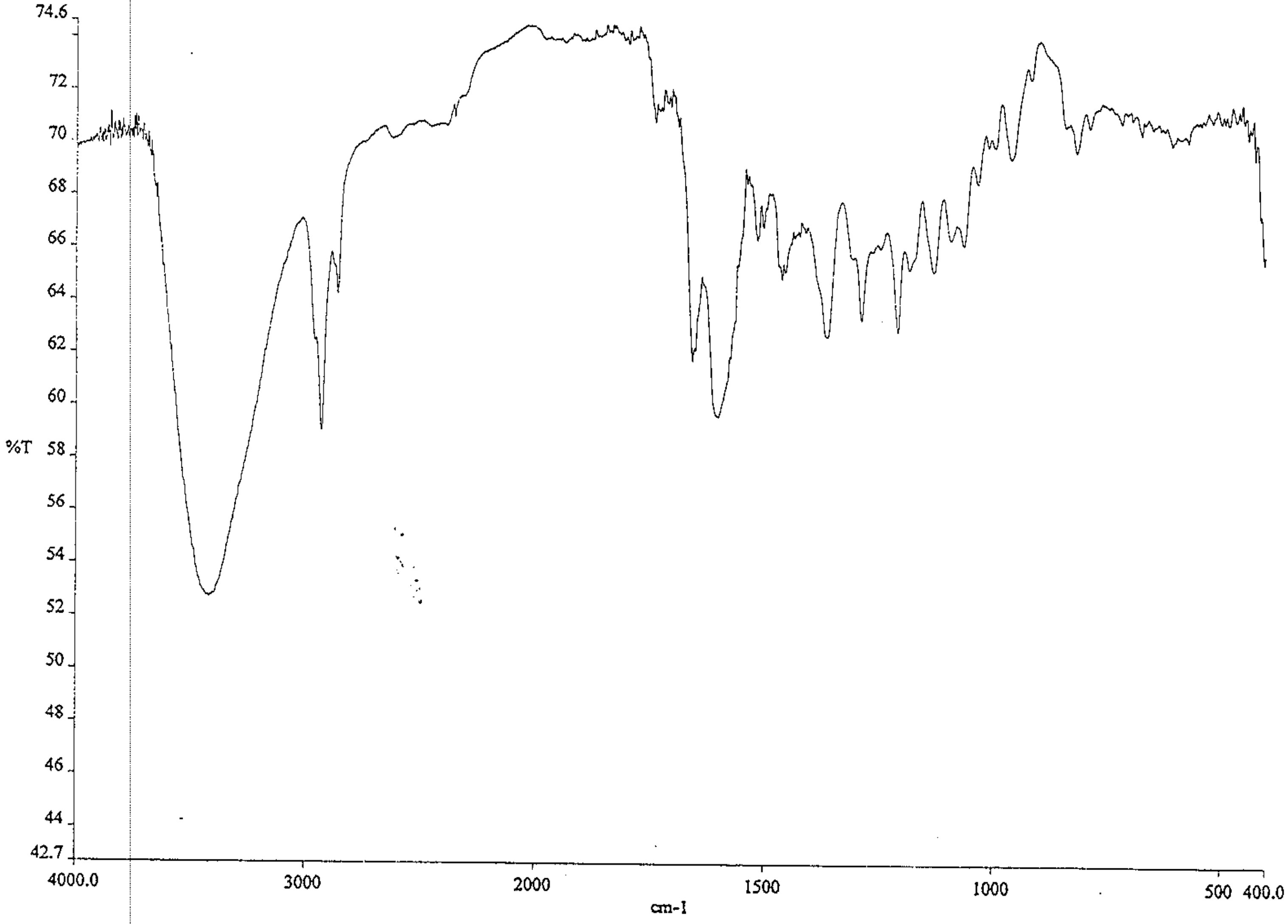


Figure 17 IR (neat) spectrum of **DW3**

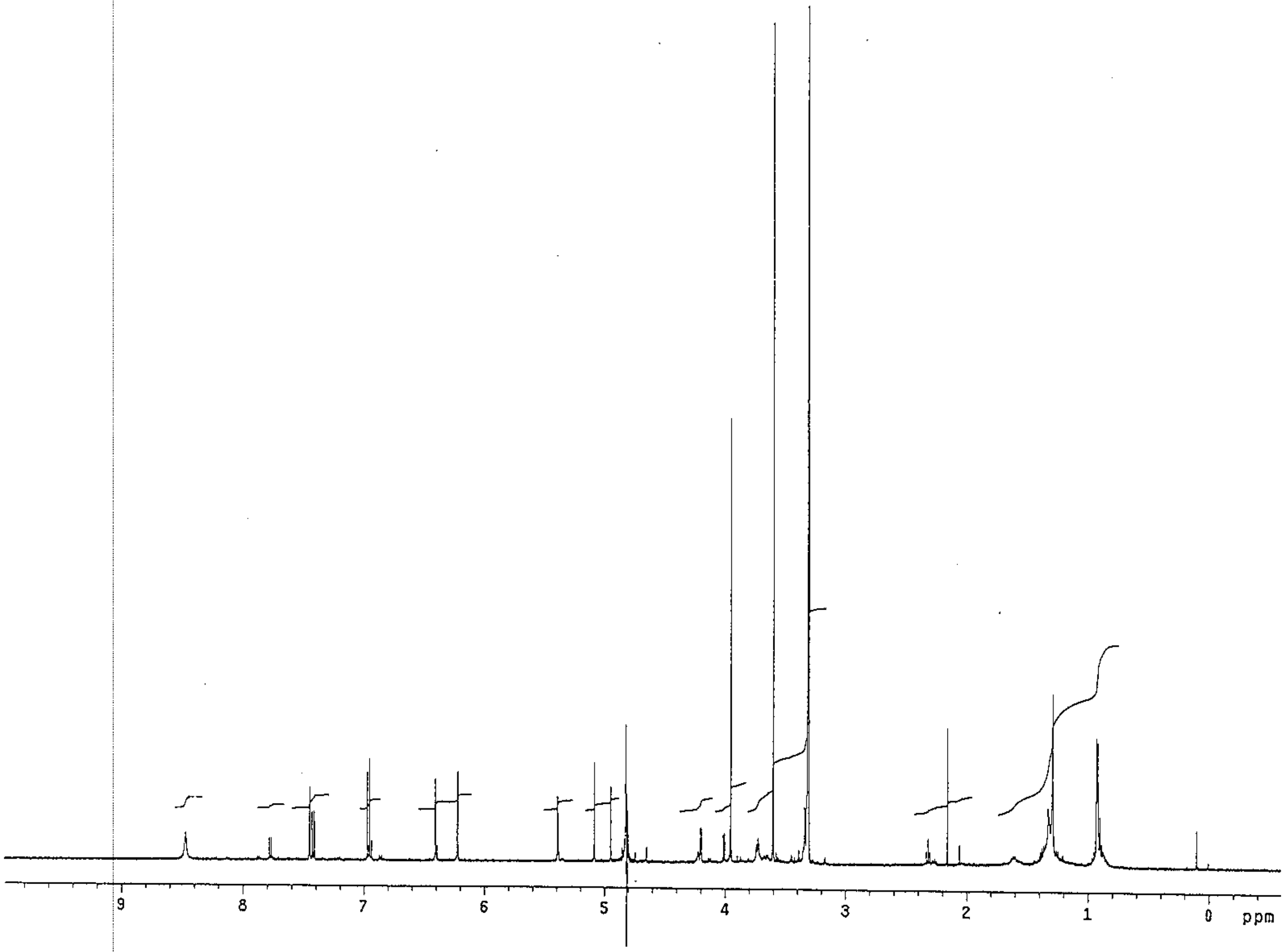


Figure 18 ^{1}H NMR (500 MHz) (CD$_3$OD) spectrum of **DW3**

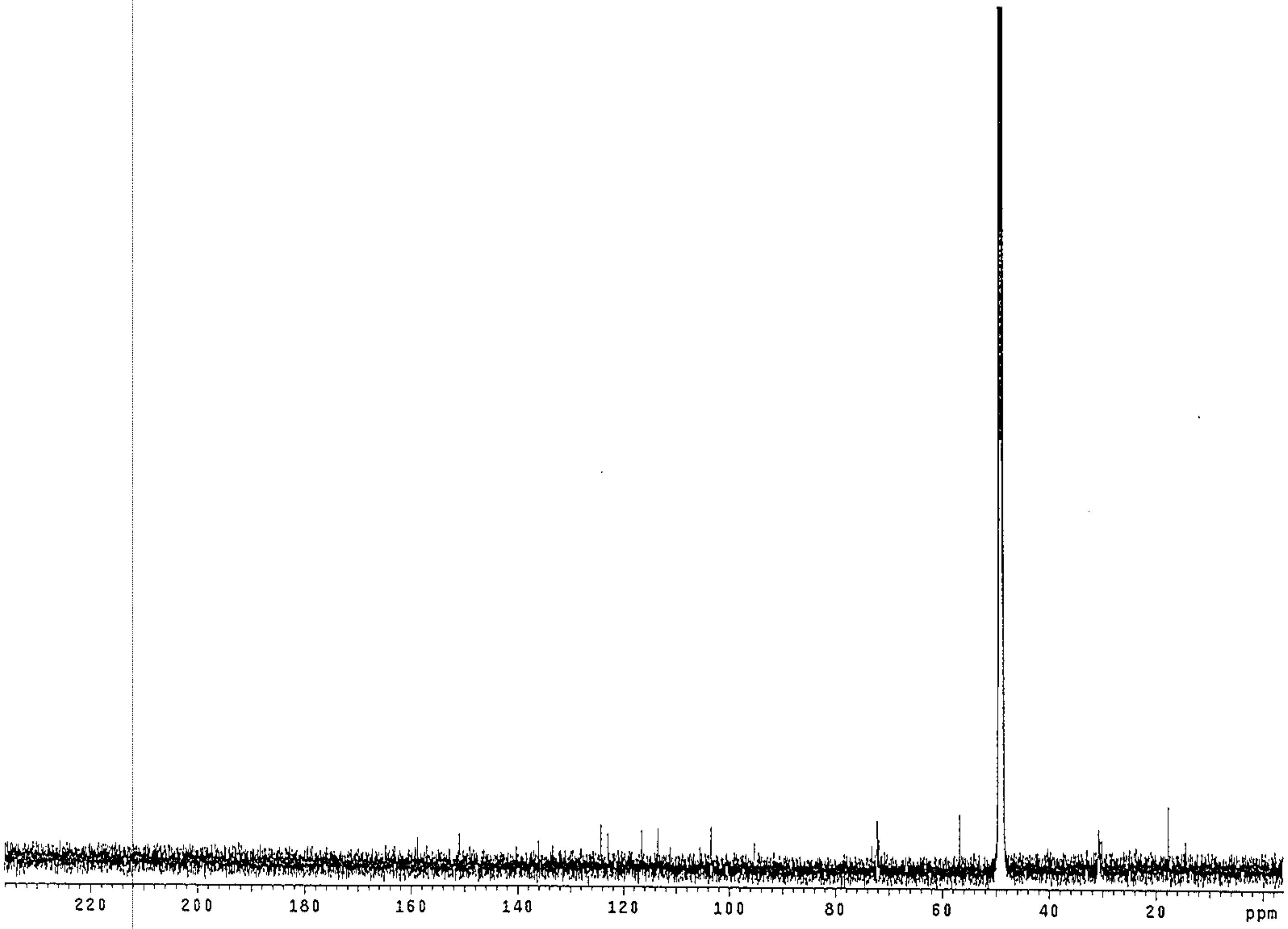


Figure 19 ^{13}C NMR (125 MHz) (CD_3OD) spectrum of **DW3**

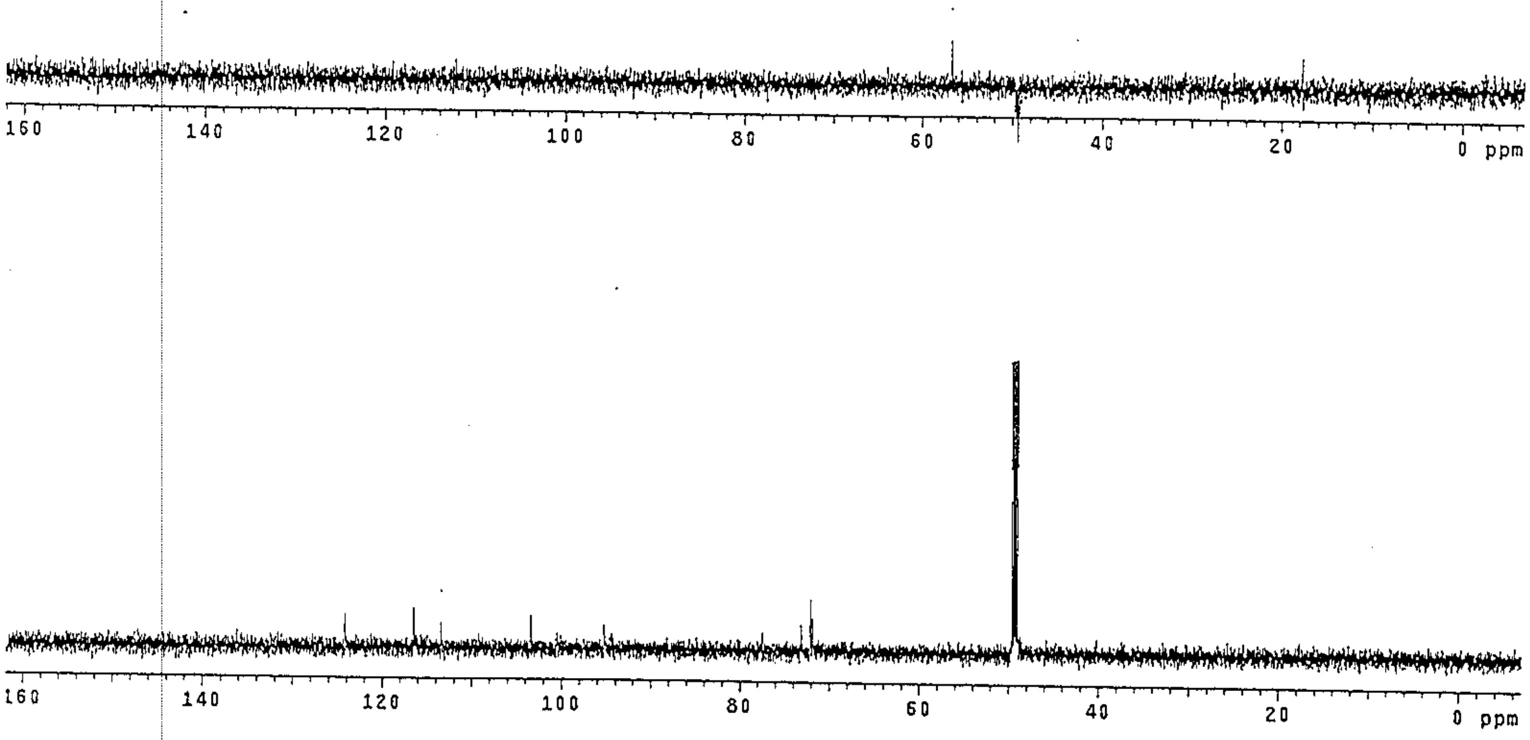


Figure 20 DEPT (135°) (CD_3OD) spectrum of **DW3**

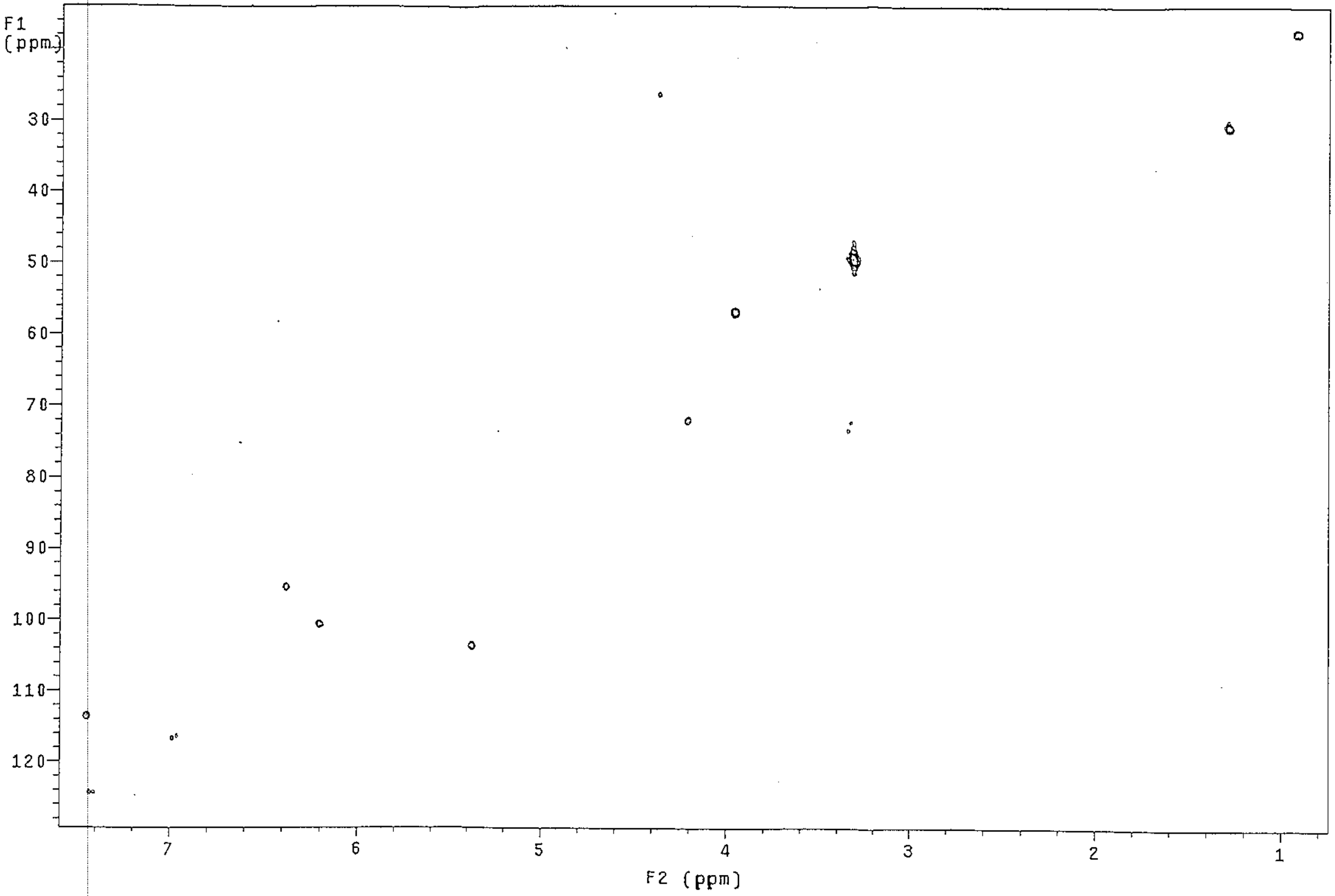


Figure 21 2D HMQC spectrum of **DW3**

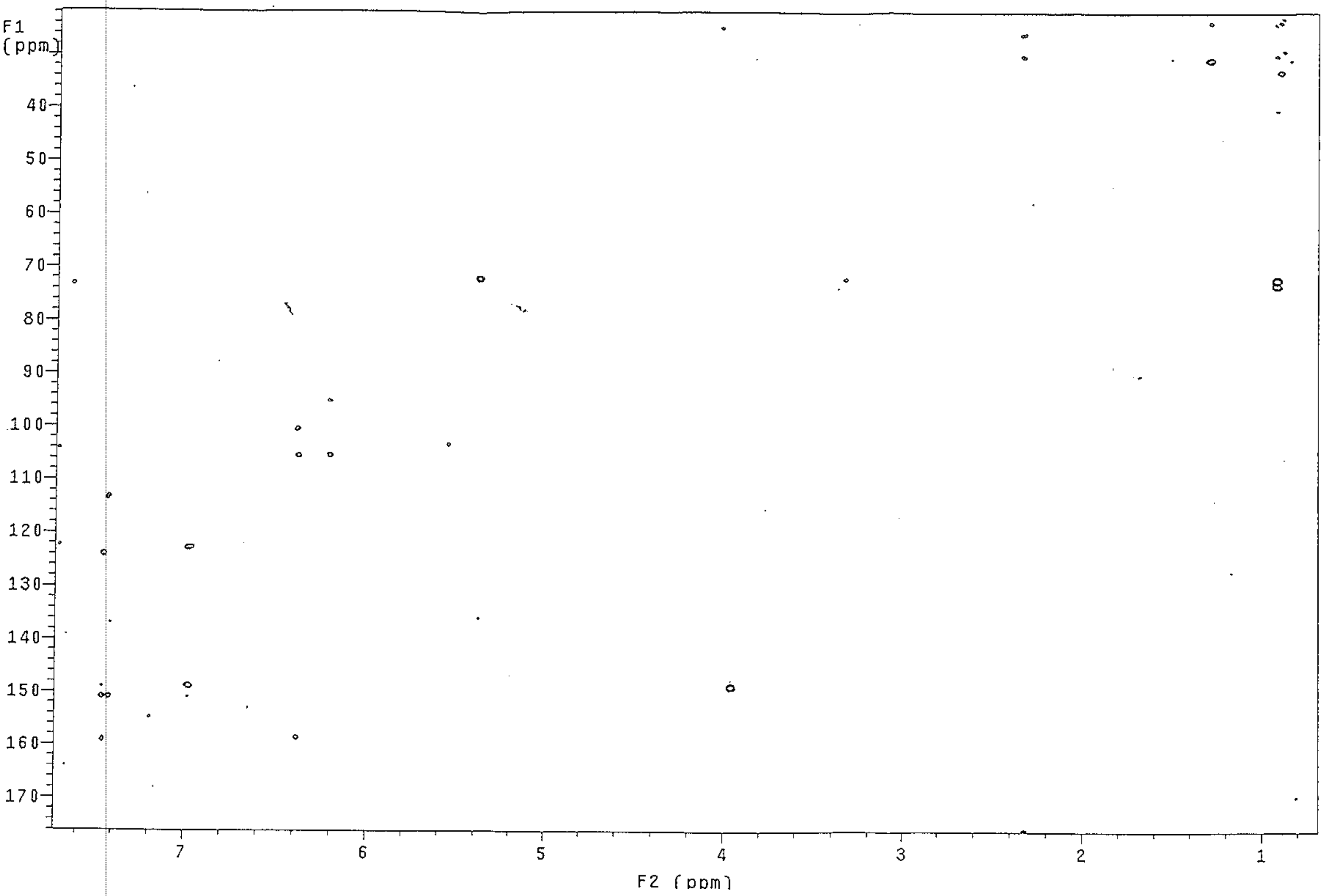


Figure 22 2D HMBC spectrum of **DW3**

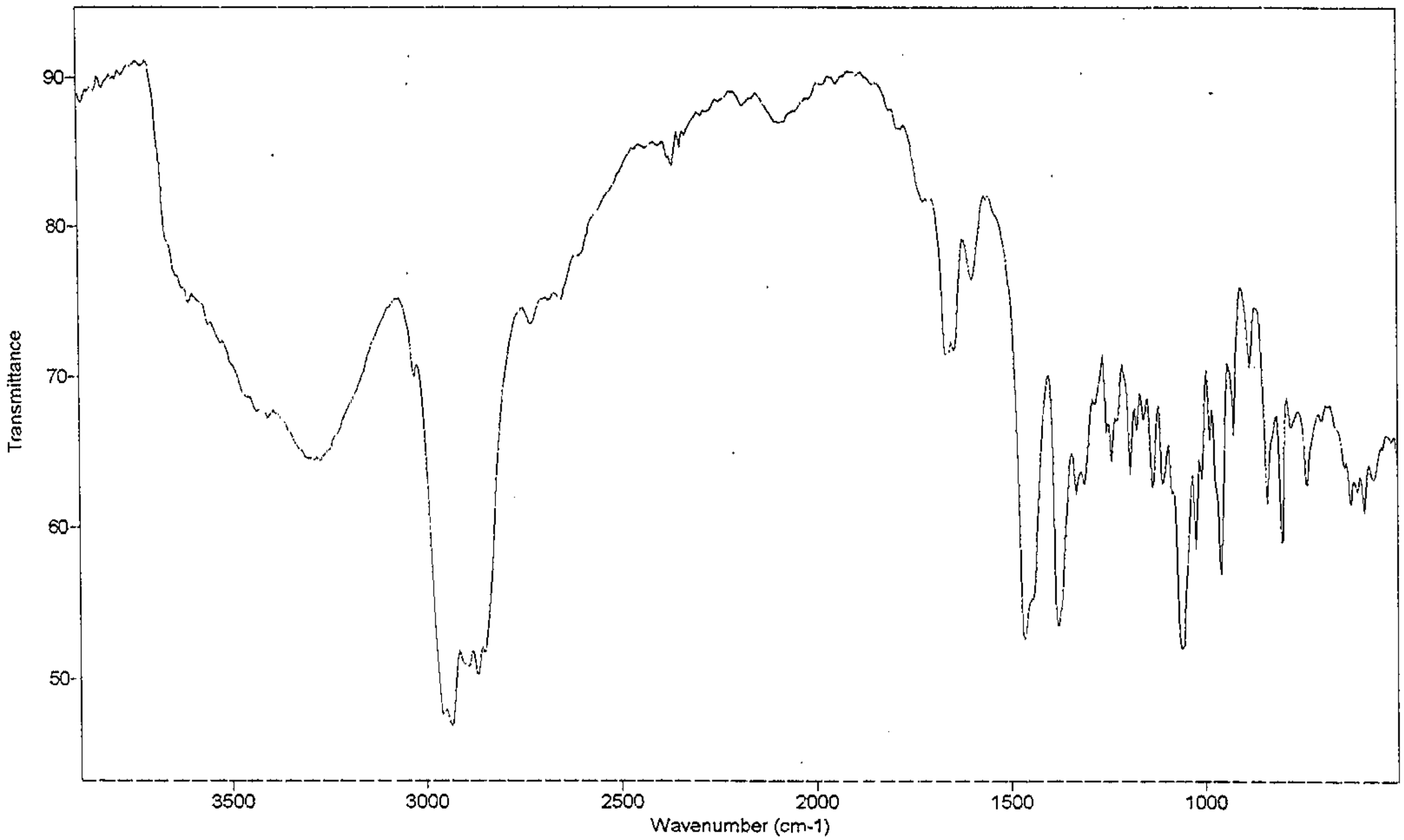


Figure 23 IR (KBr) spectrum of **DT1**

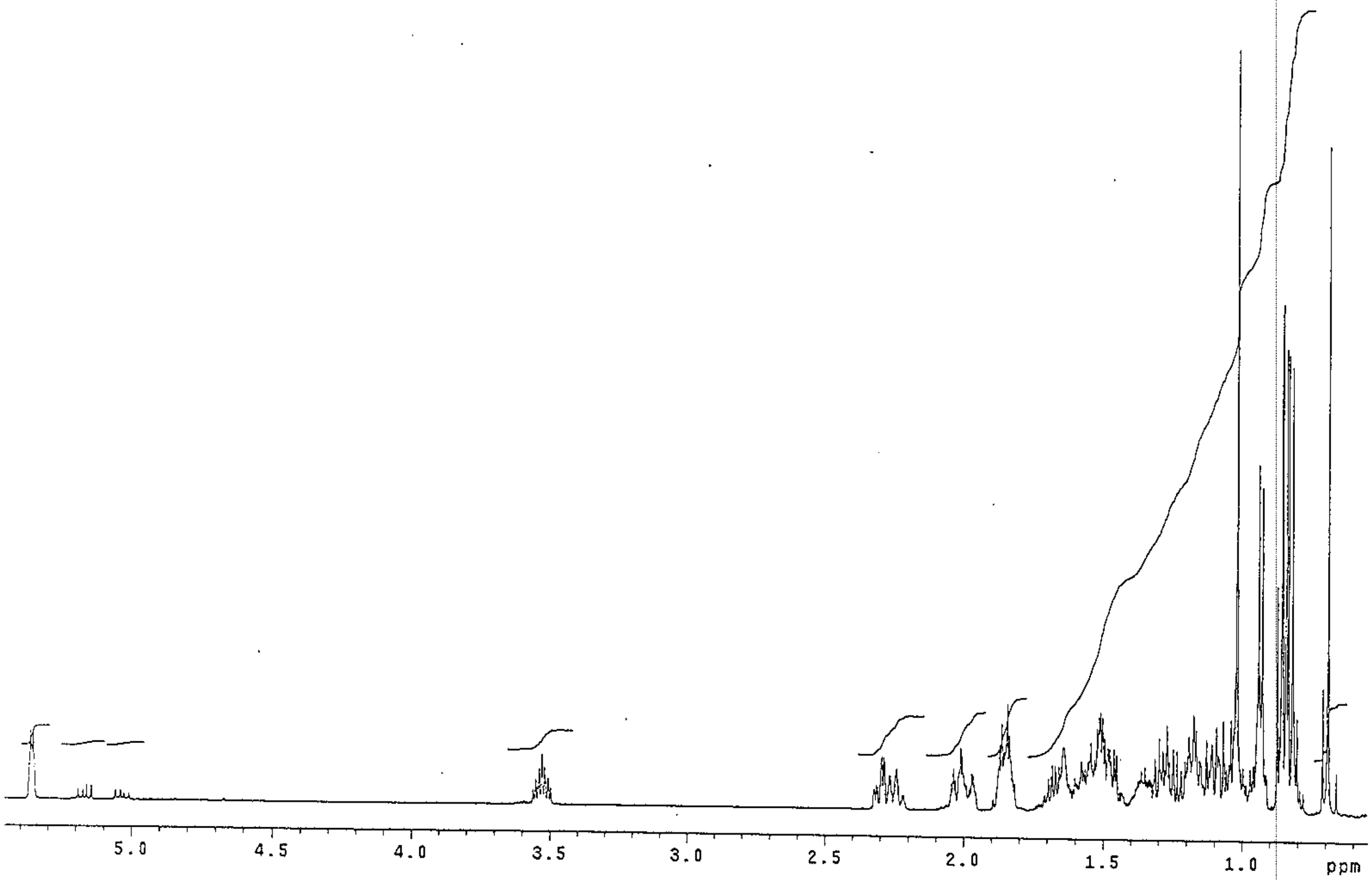


Figure 24 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **DT1**

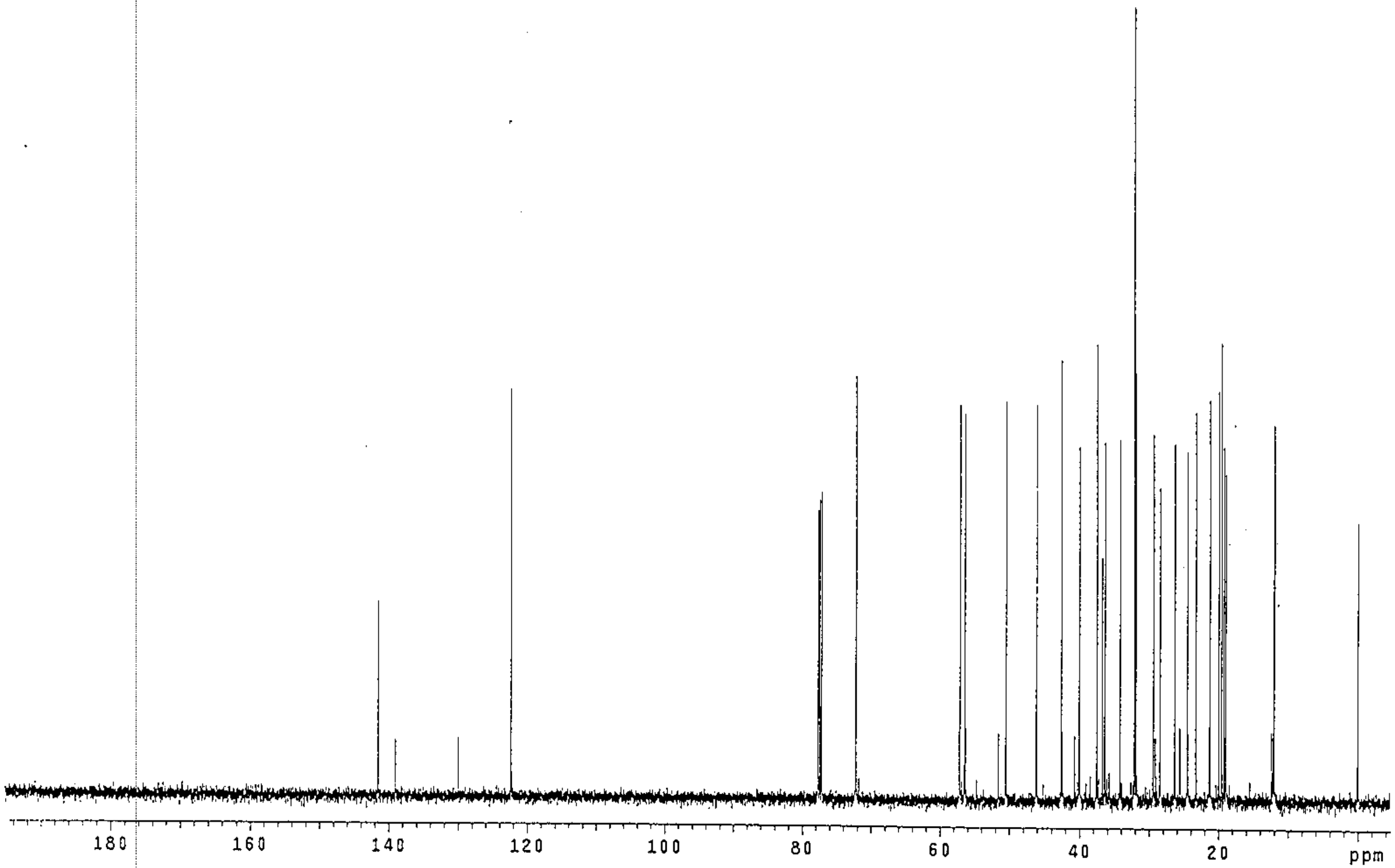


Figure 25 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **DT1**

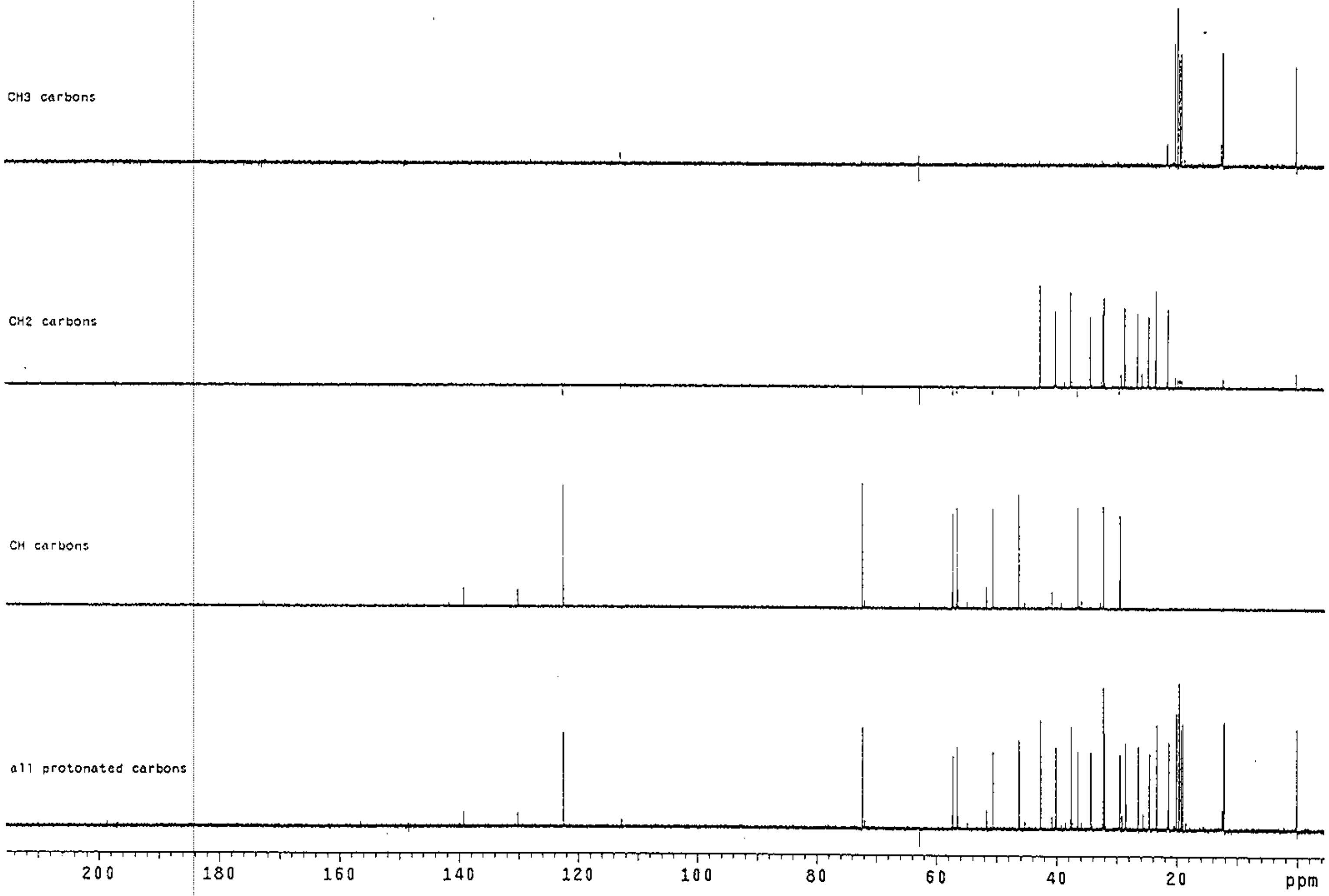


Figure 26 DEPT (135°) (CDCl$_3$) spectrum of **DT1**

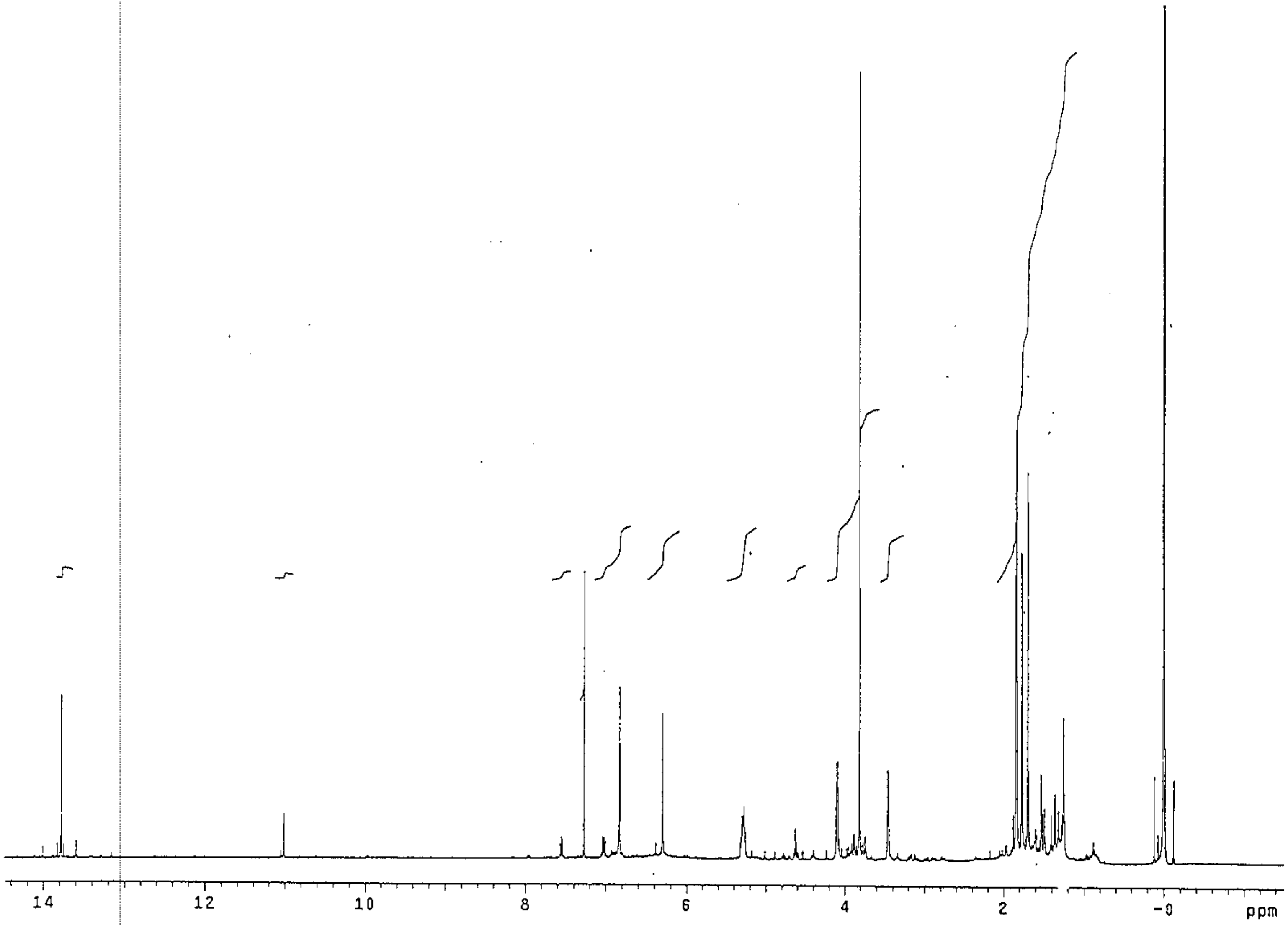


Figure 27 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **DT2**

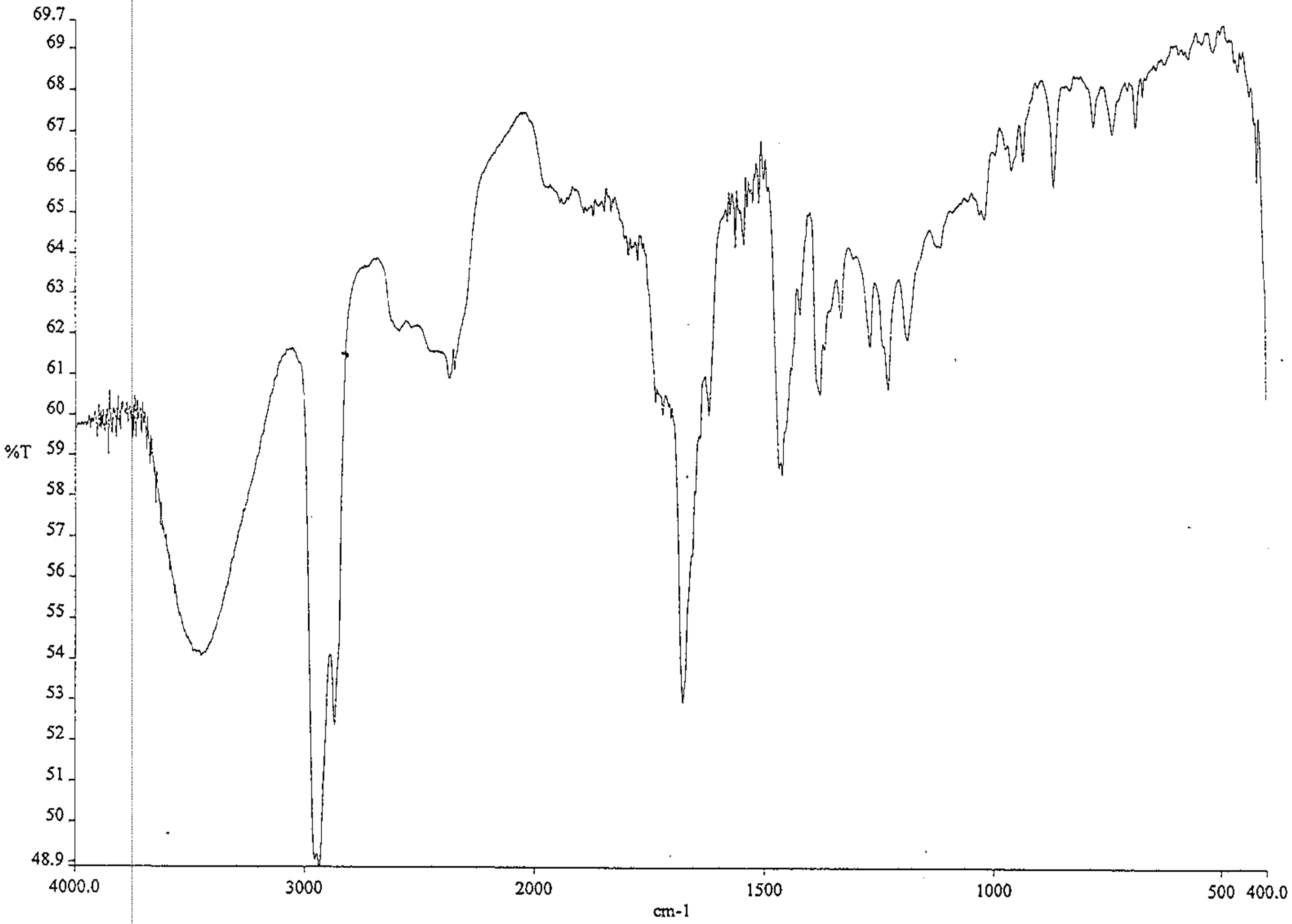


Figure 28 IR (KBr) spectrum of **DT3**

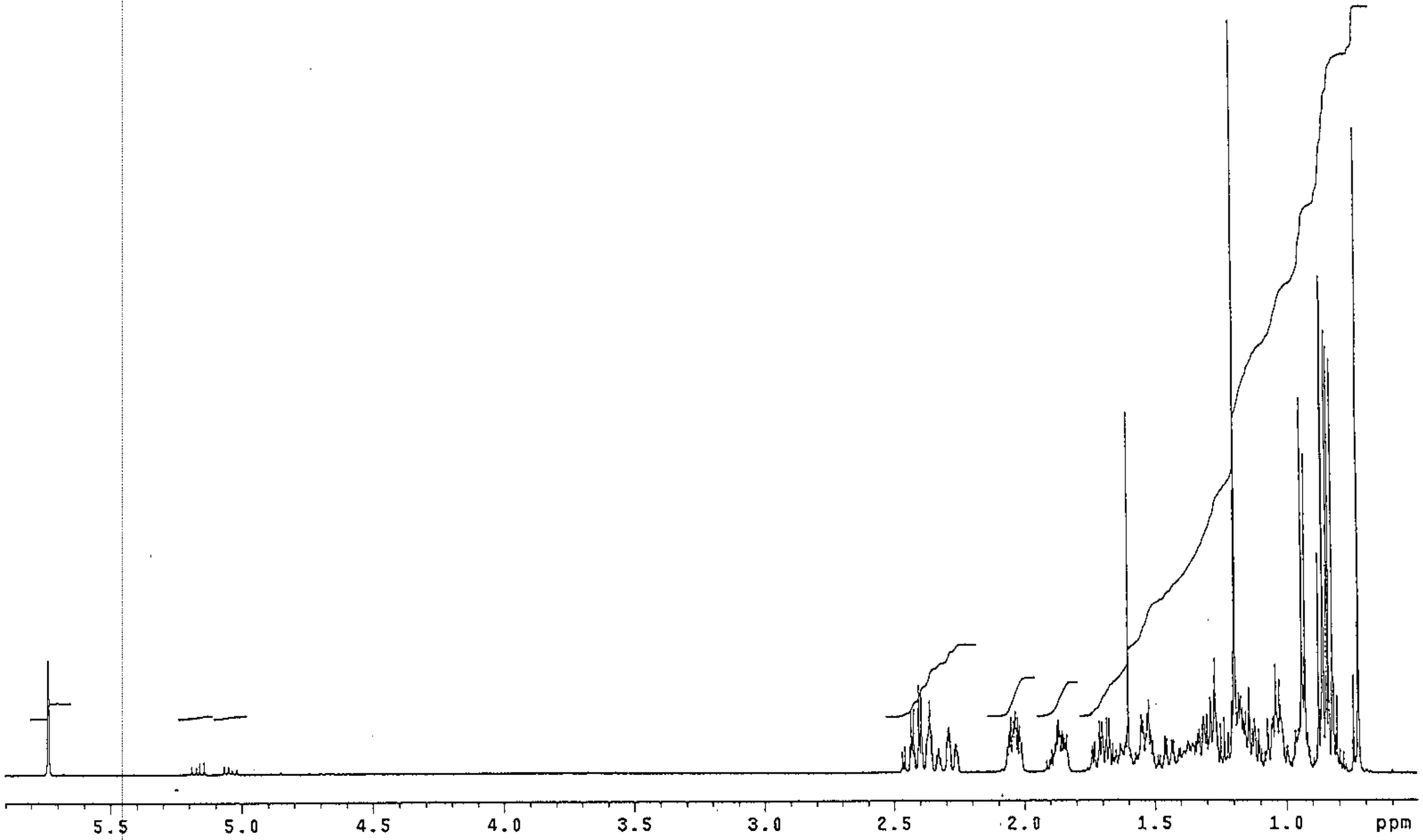


Figure 29 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **DT3**

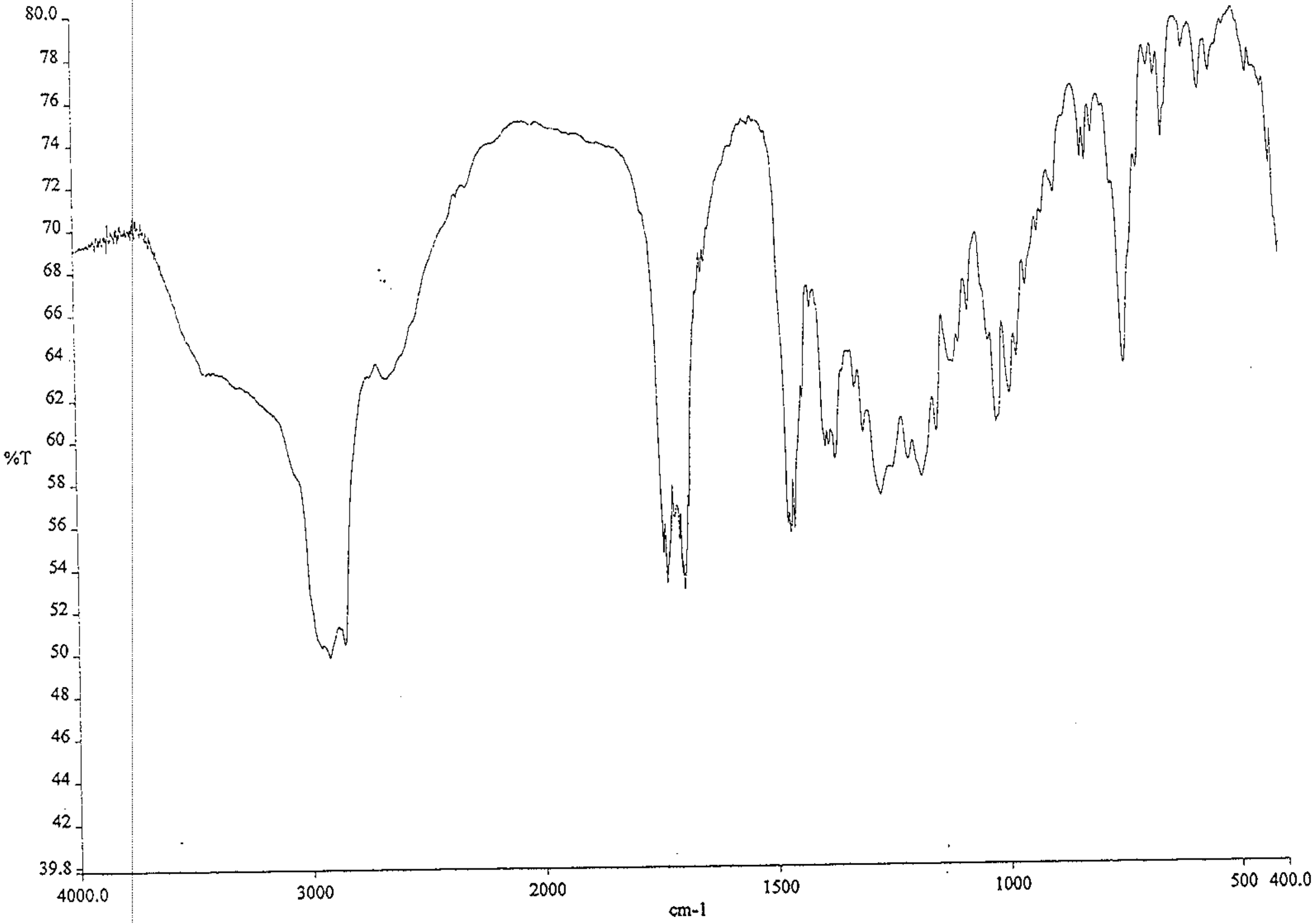


Figure 30 IR (neat) spectrum of **DT4**

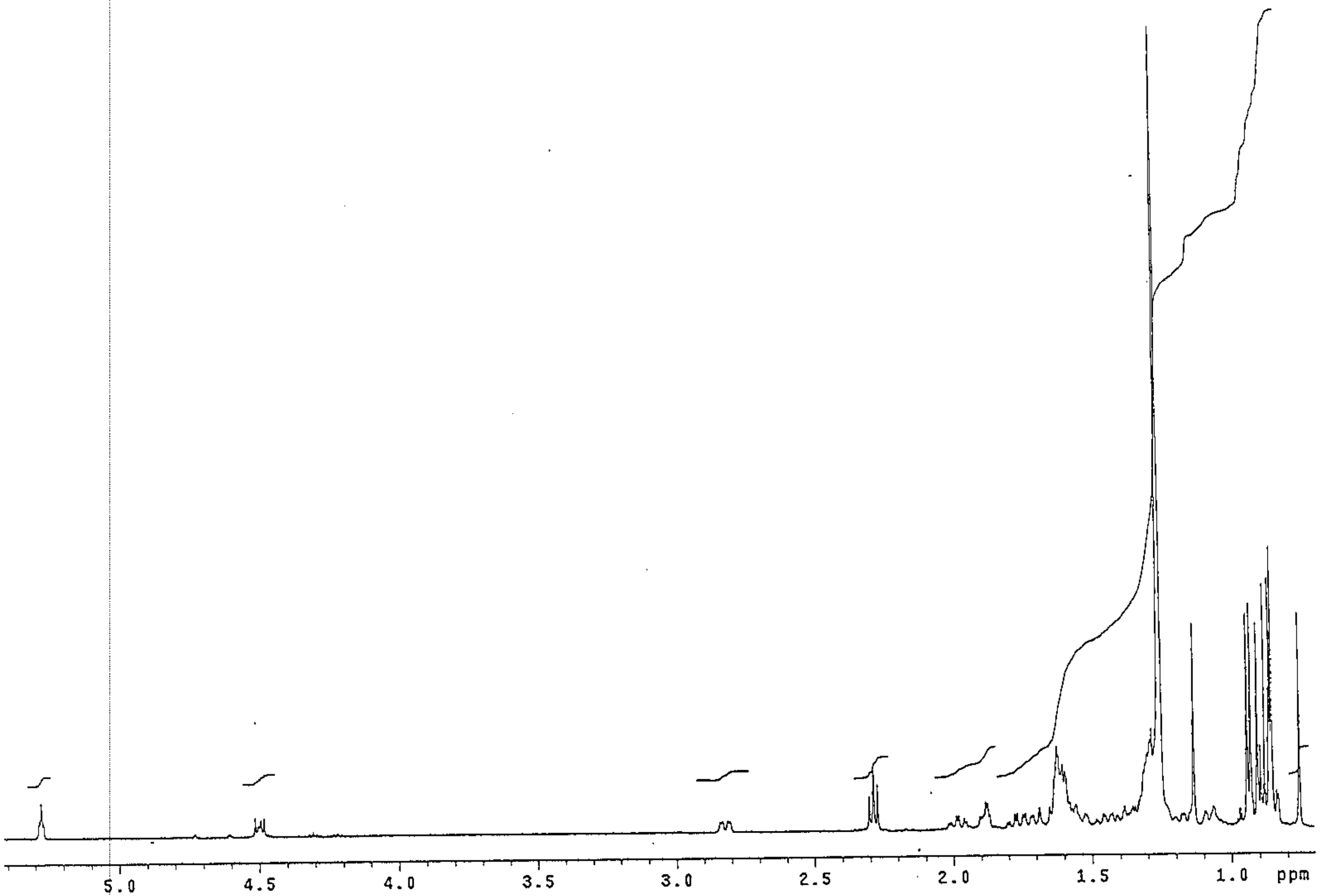


Figure 31 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **DT4**

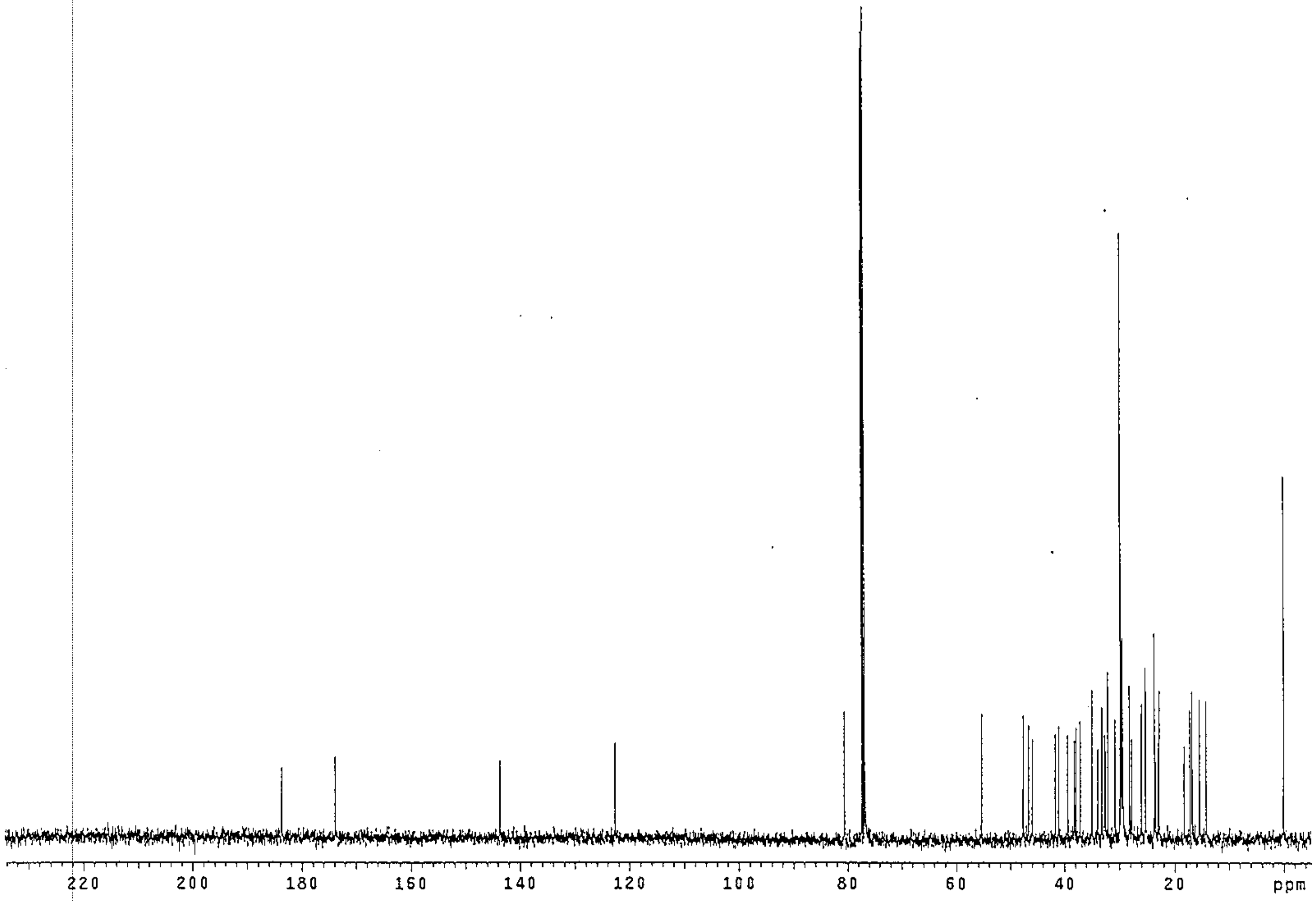


Figure 32 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DT4**

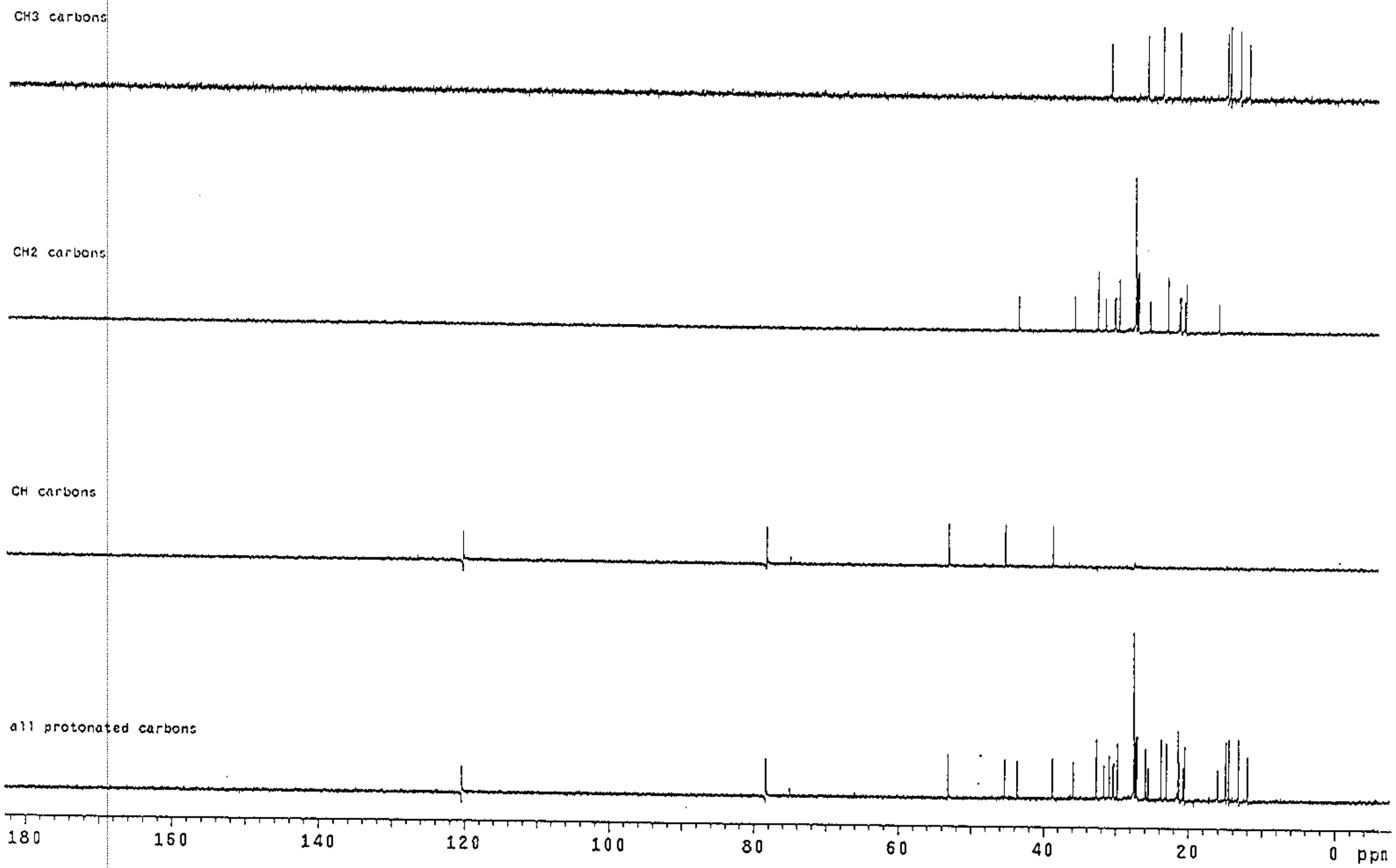


Figure 33 DEPT (135°) ($CDCl_3$) spectrum of **DT4**

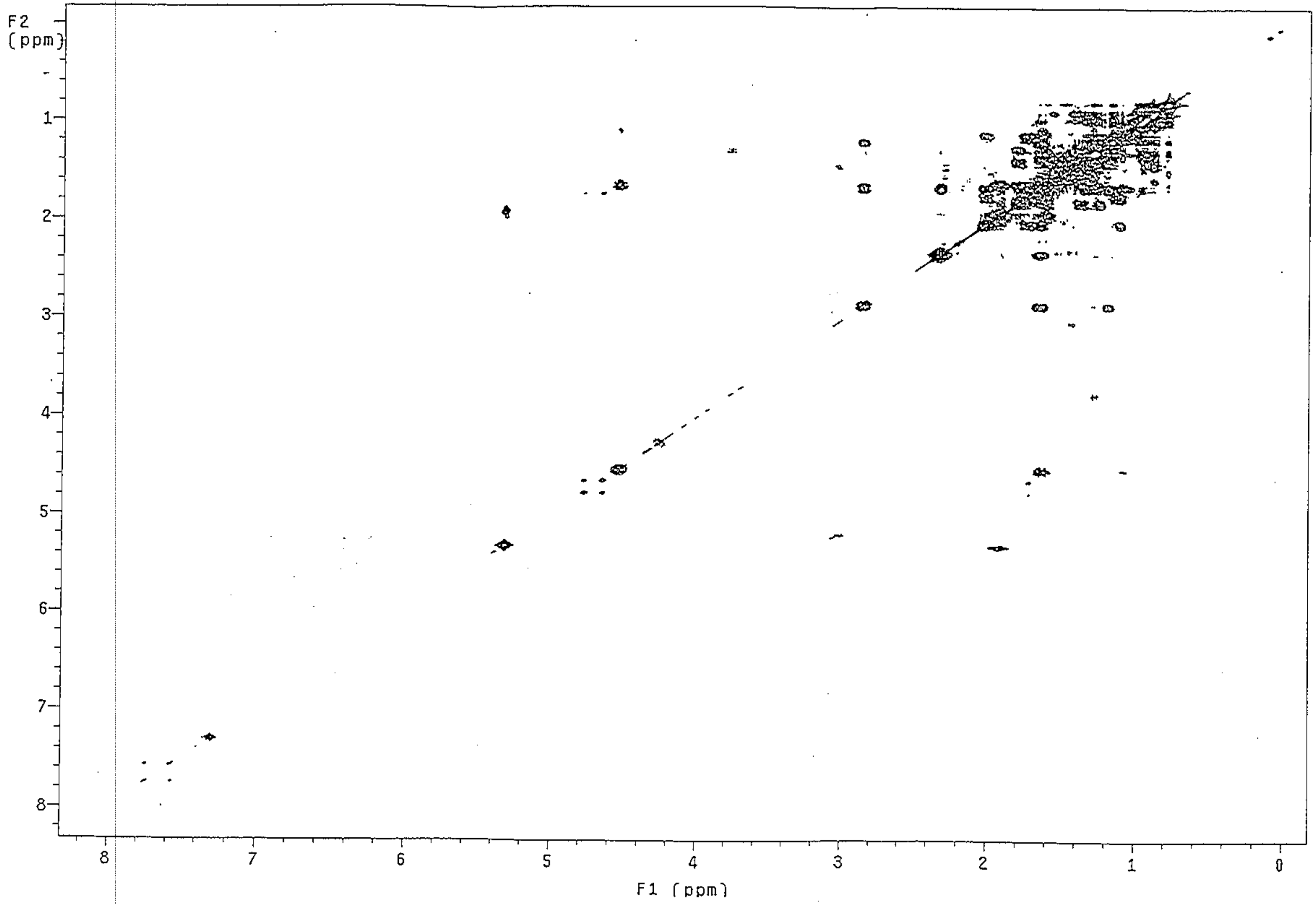


Figure 34 ^{1}H-^{1}H COSY spectrum of **DT4**

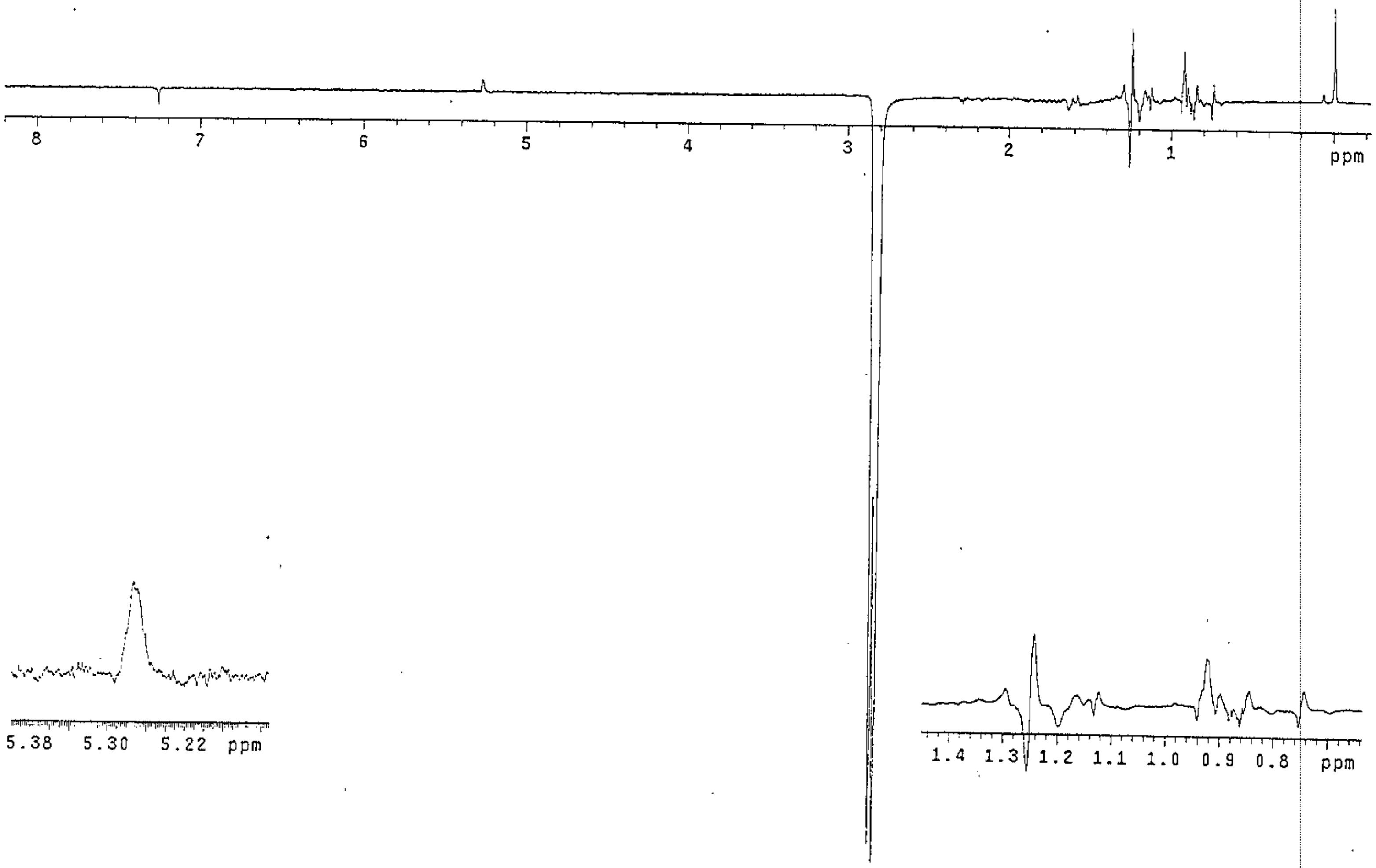


Figure 35 NOEDIFF spectrum of **DT4** after irradiation at δ_H 2.82

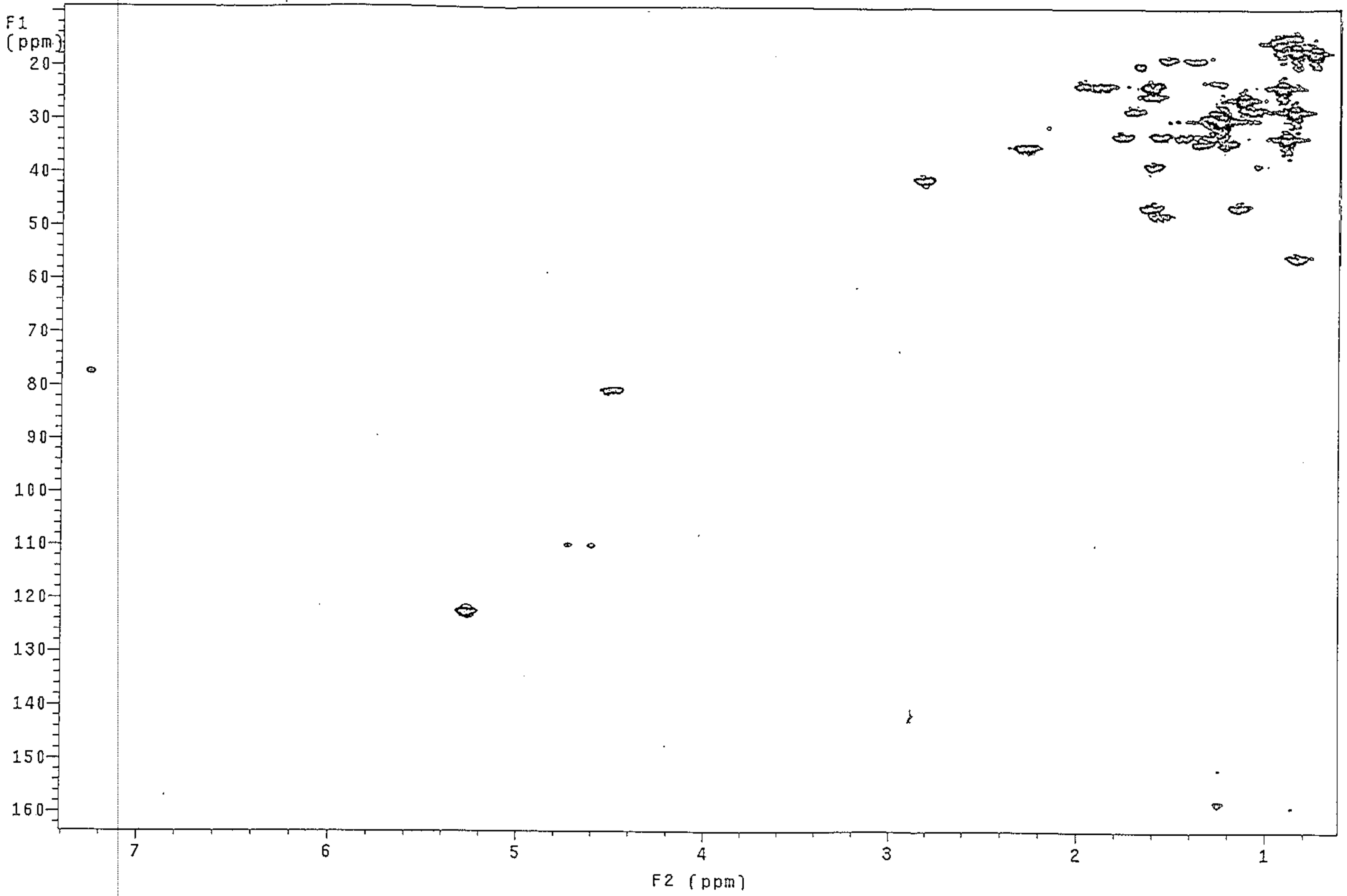


Figure 36 2D HMQC spectrum of **DT4**

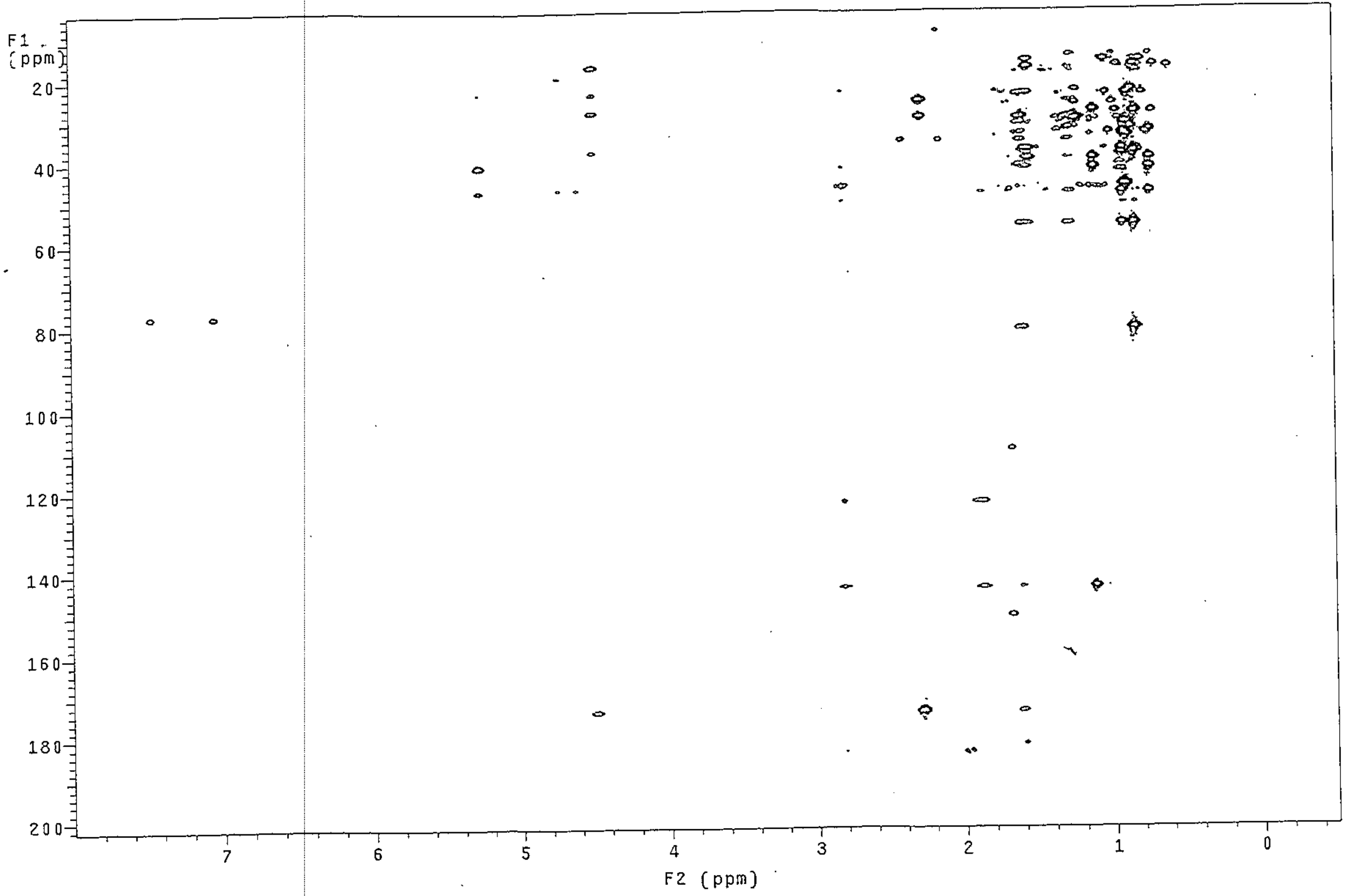


Figure 37 2D HMBC spectrum of **DT4**

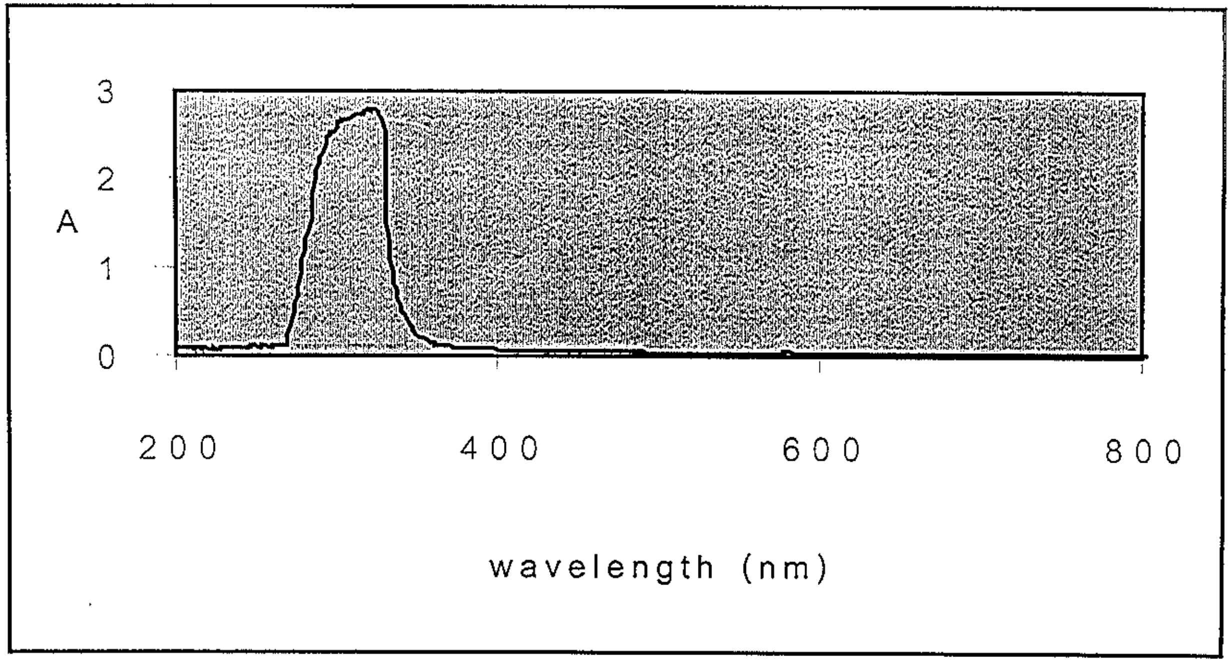


Figure 38 UV ($CHCl_3$) spectrum of **DT5**

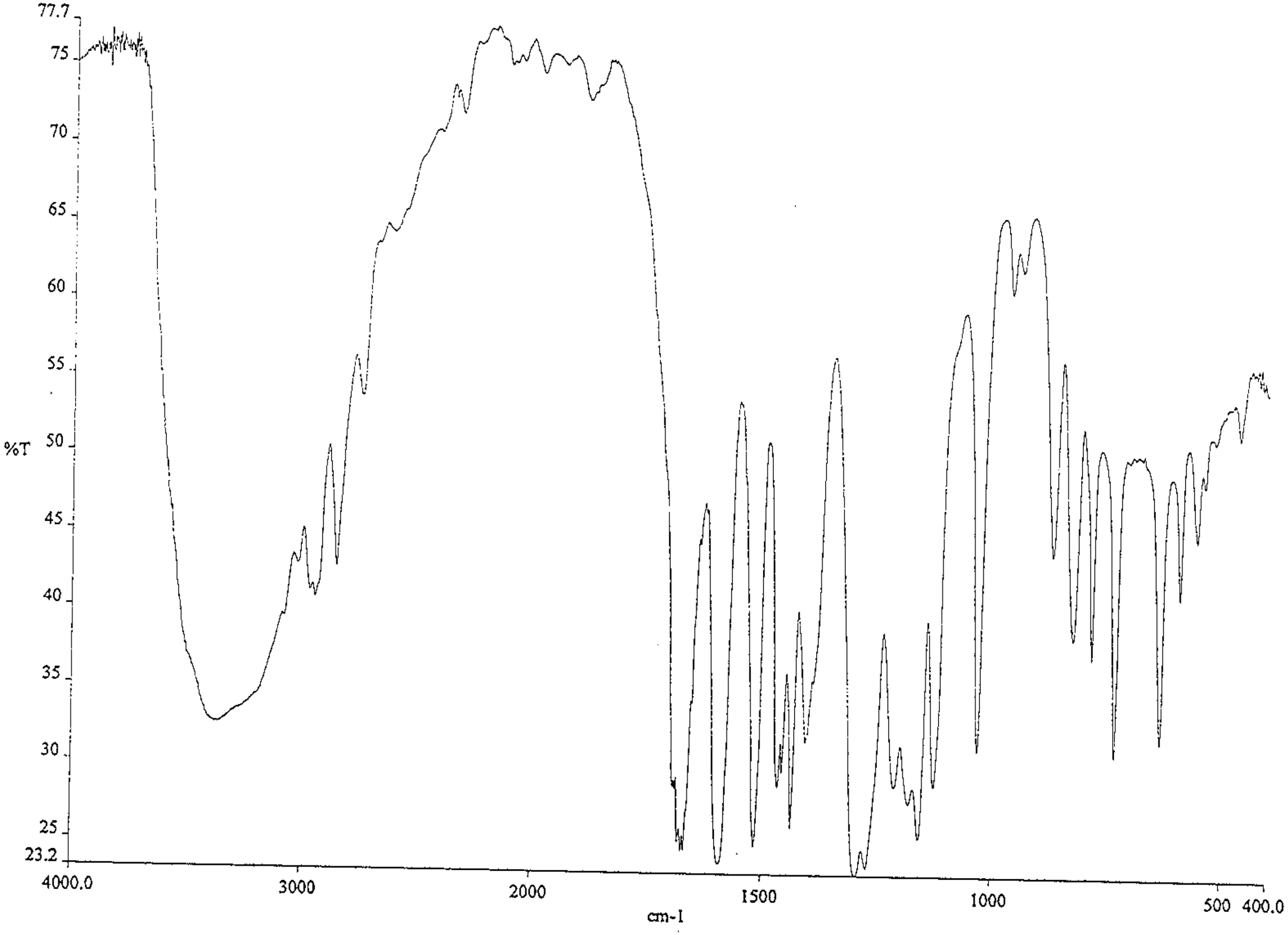


Figure 39 IR (neat) spectrum of **DT5**

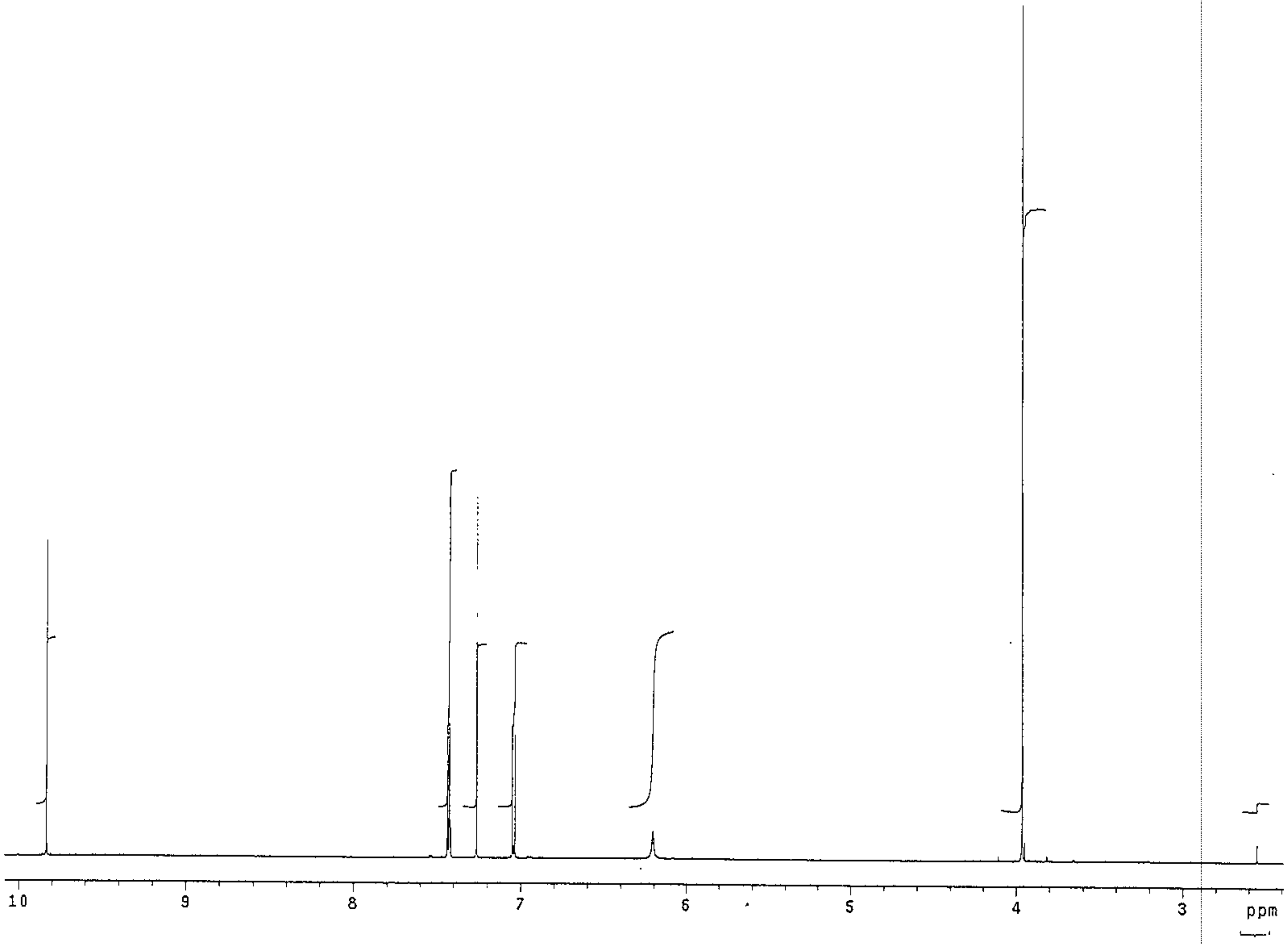


Figure 40 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **DT5**

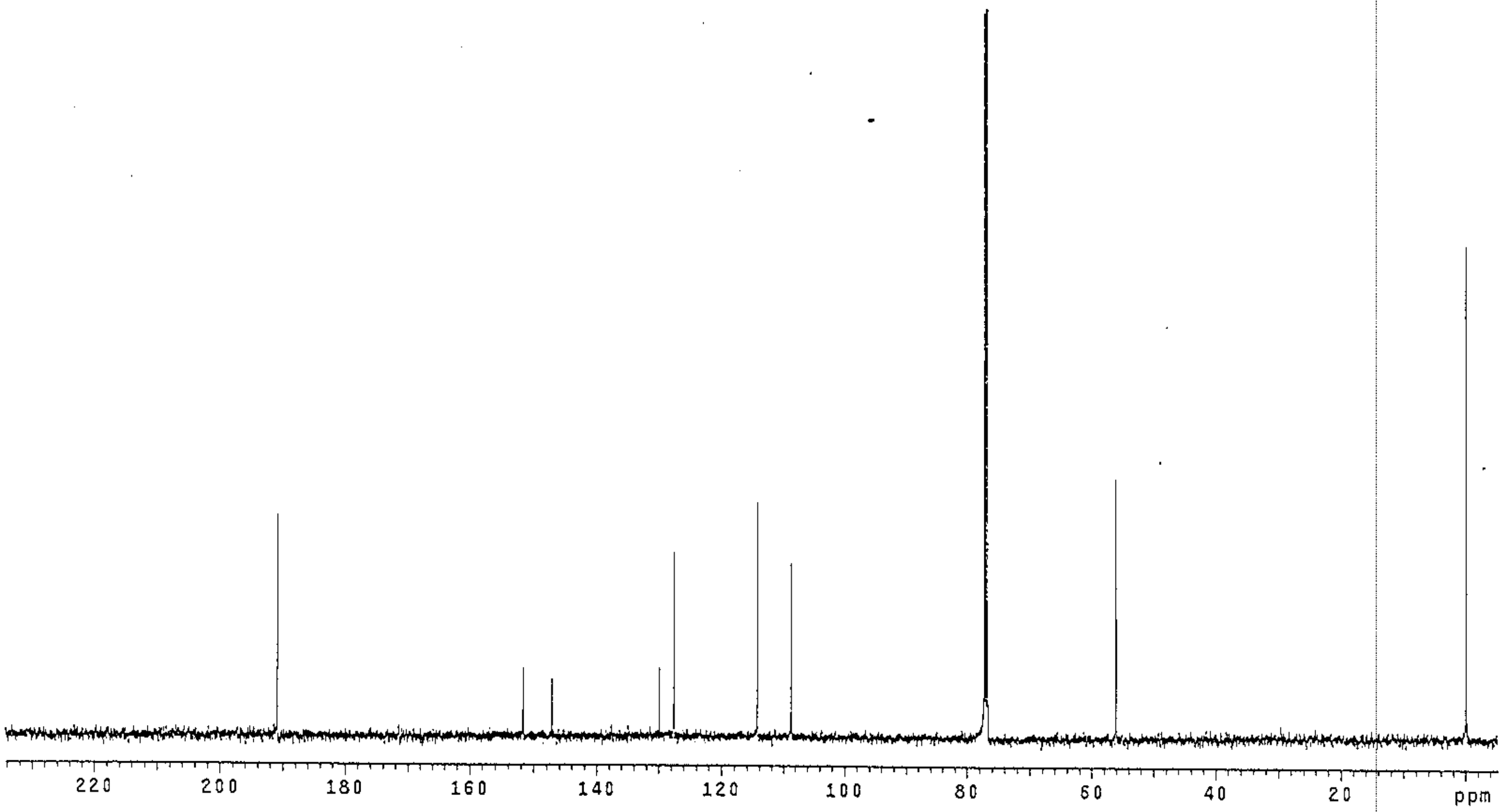


Figure 41 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **DT5**

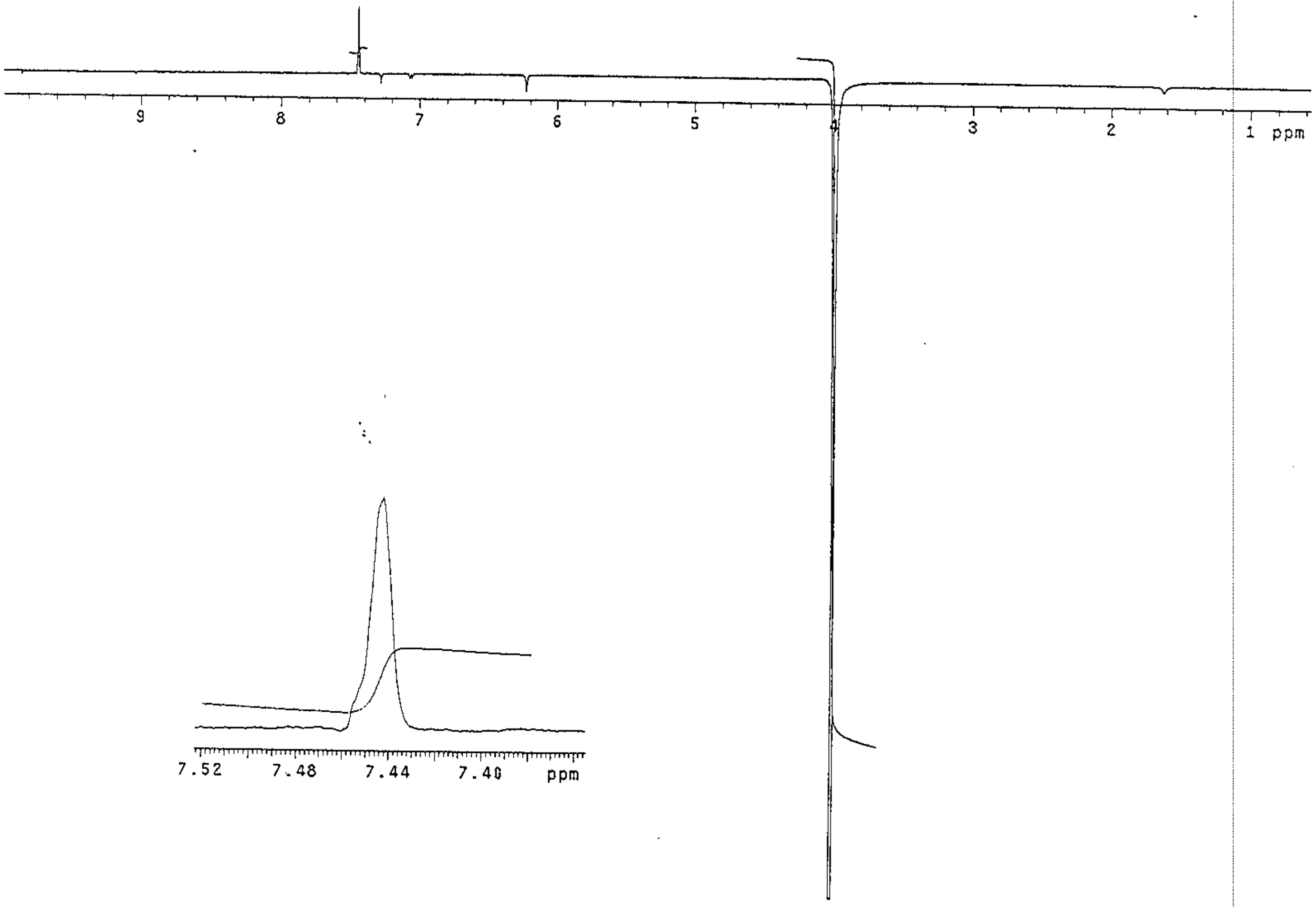


Figure 42 NOEDIFF spectrum of **DT5** after irradiation at δ_H 3.96

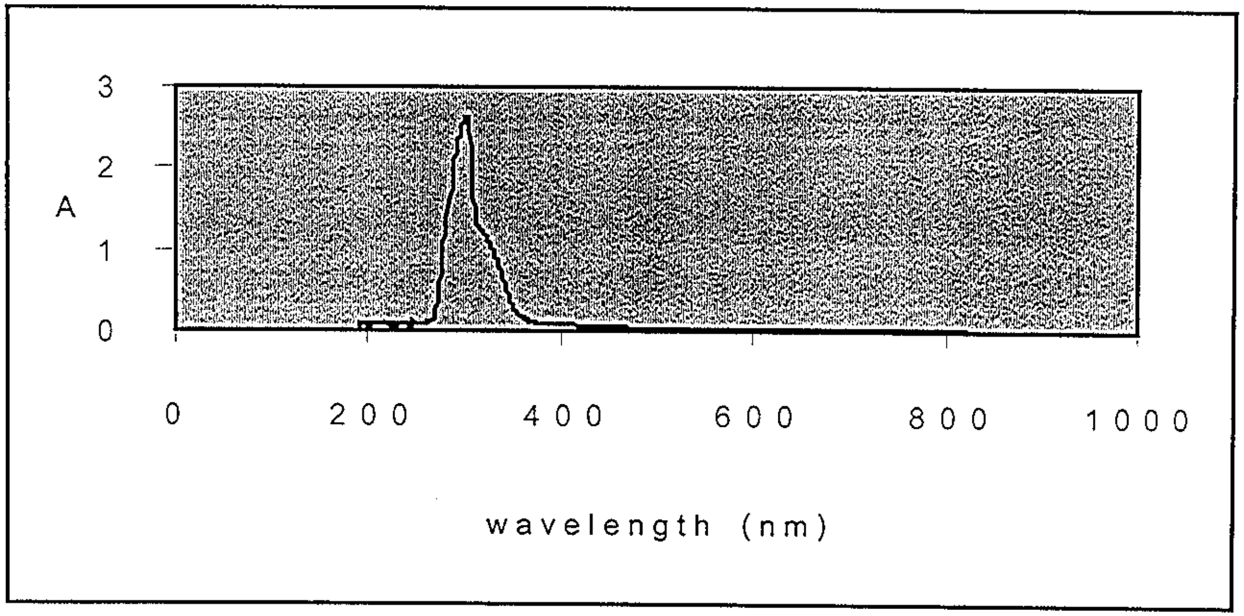


Figure 43 UV ($CHCl_3$) spectrum of **DT6**

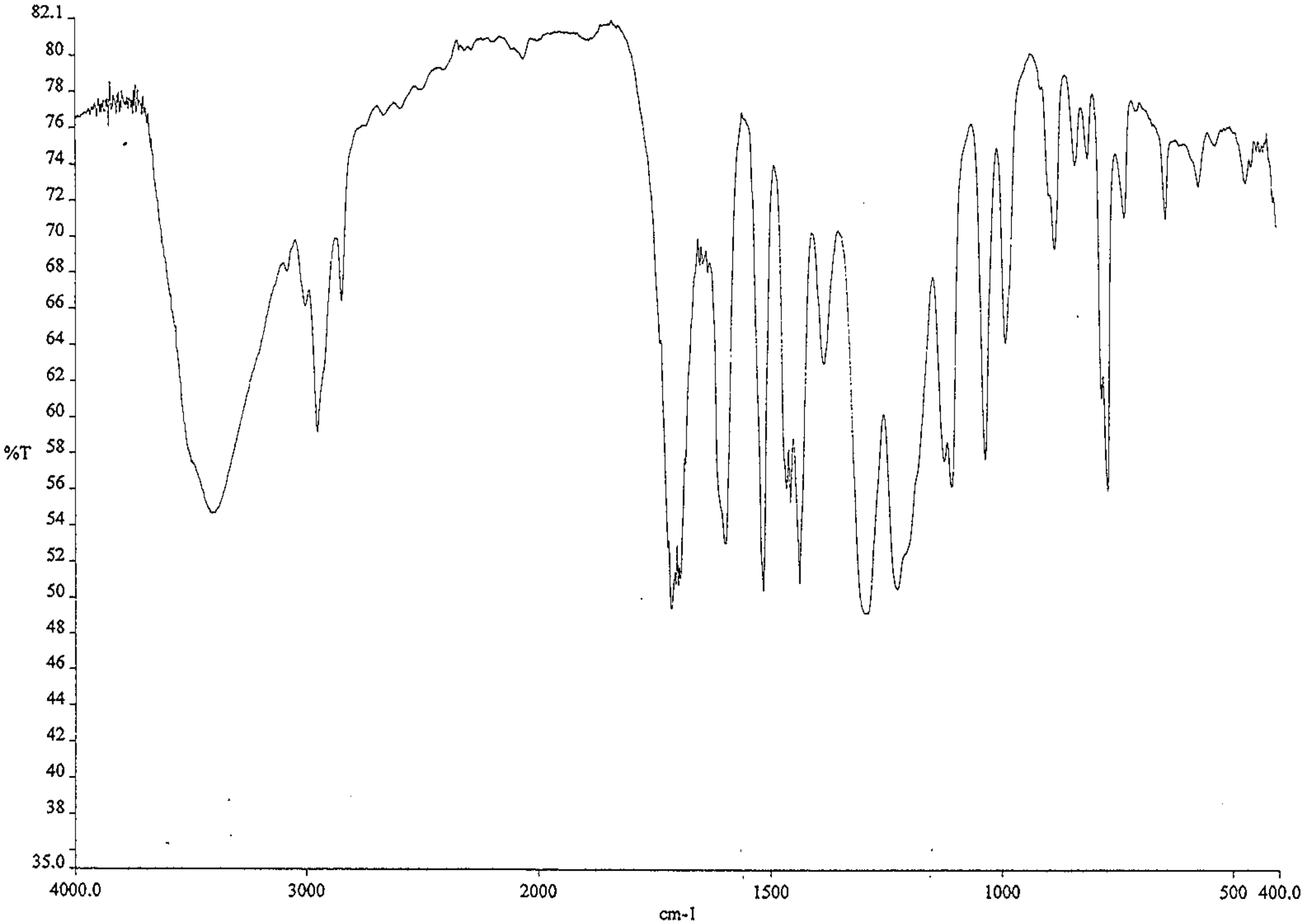


Figure 44 IR (neat) spectrum of **DT6**

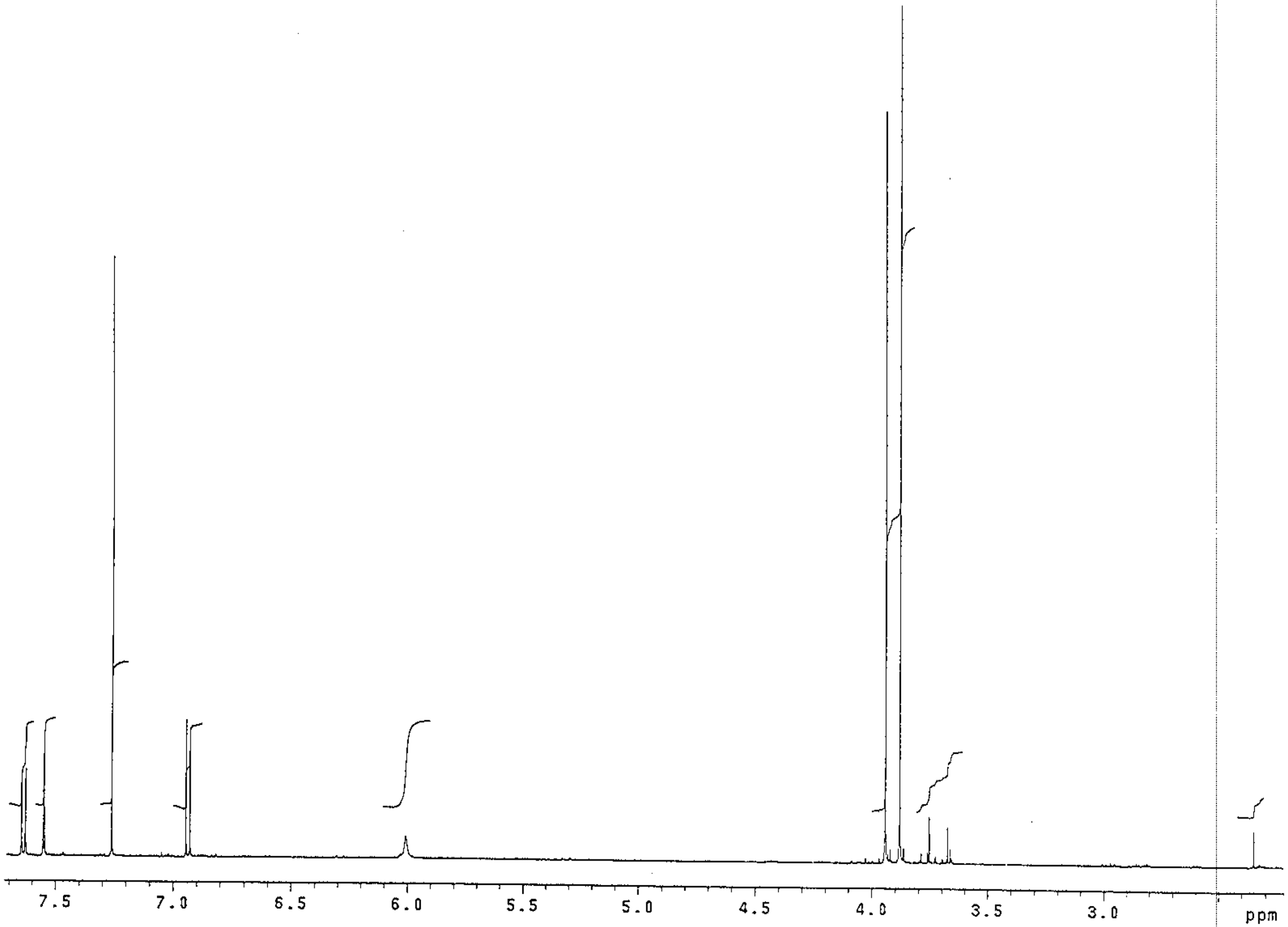


Figure 45 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **DT6**

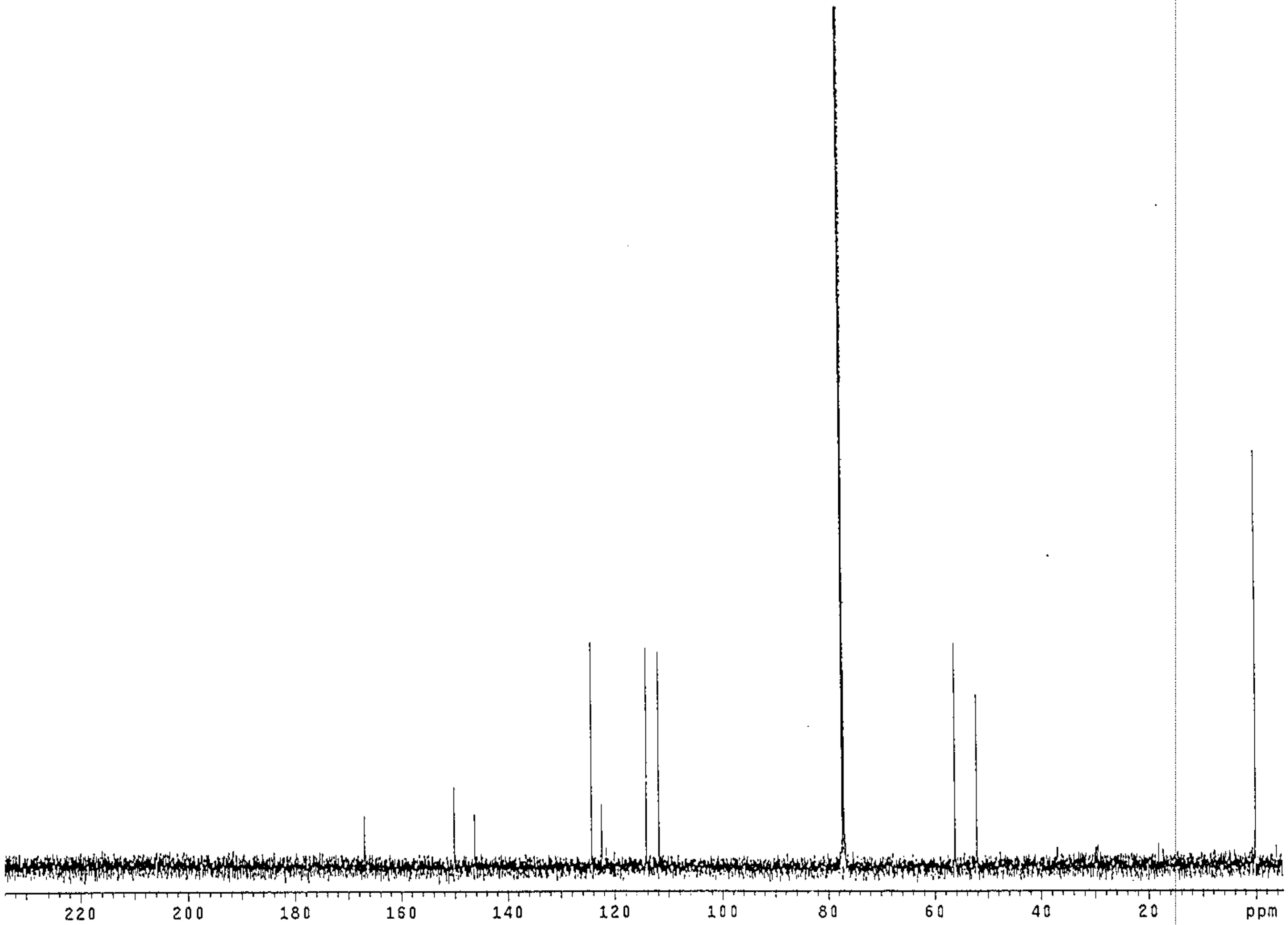


Figure 46 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **DT6**

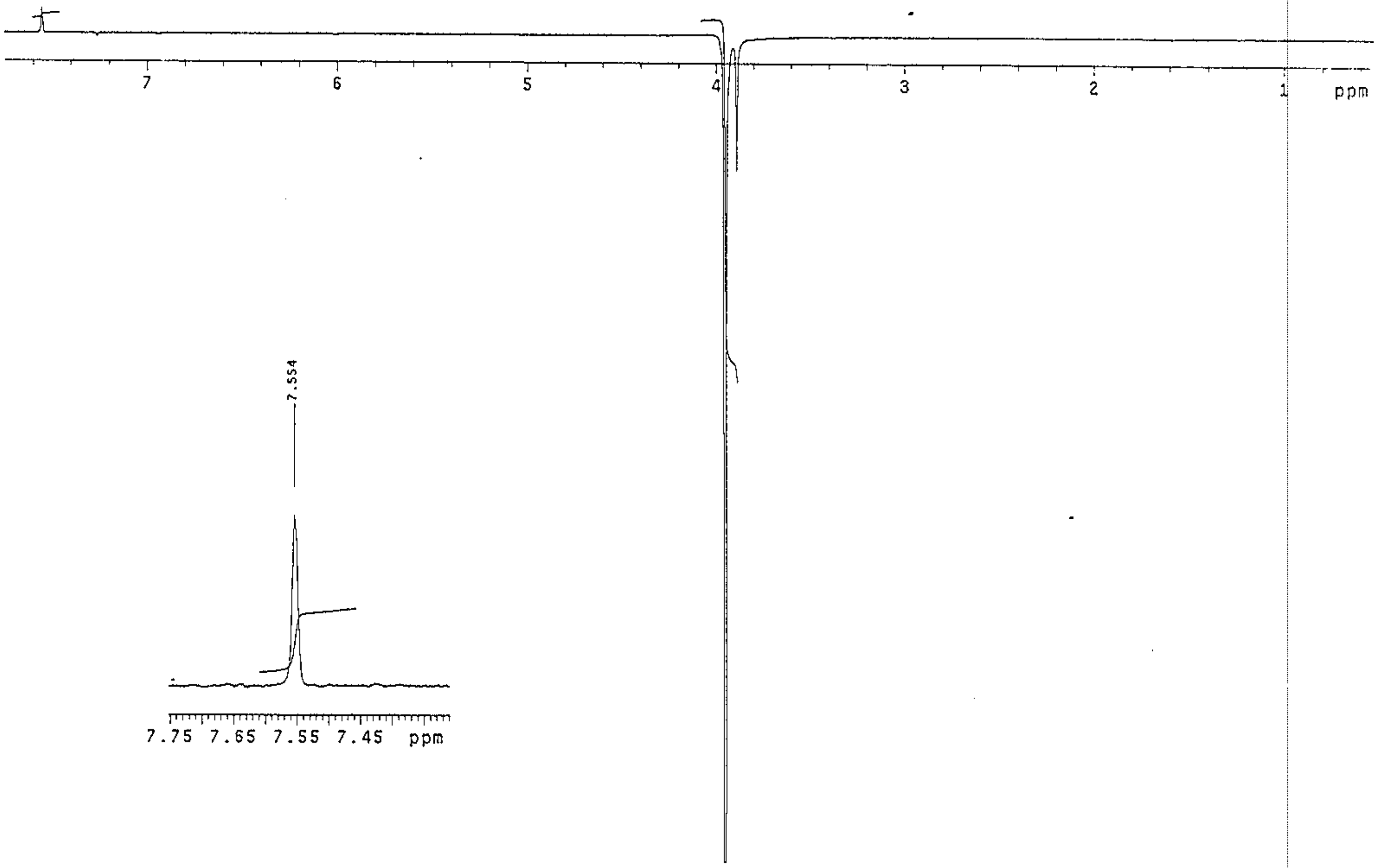


Figure 47 NOEDIFF spectrum of **DT6** after irradiation at δ_H 3.94

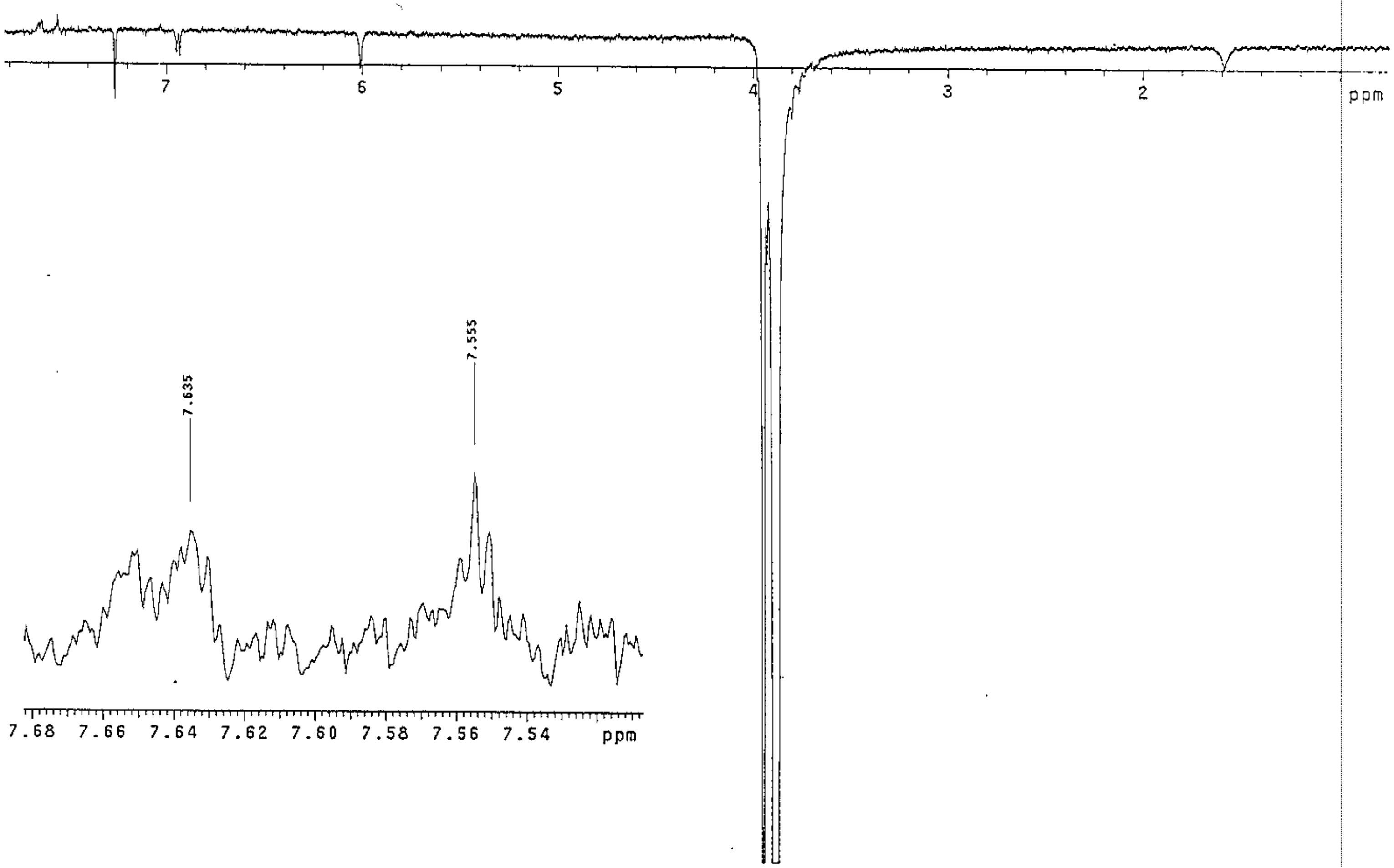


Figure 48 NOEDIFF spectrum of **DT6** after irradiation at δ_H 3.88

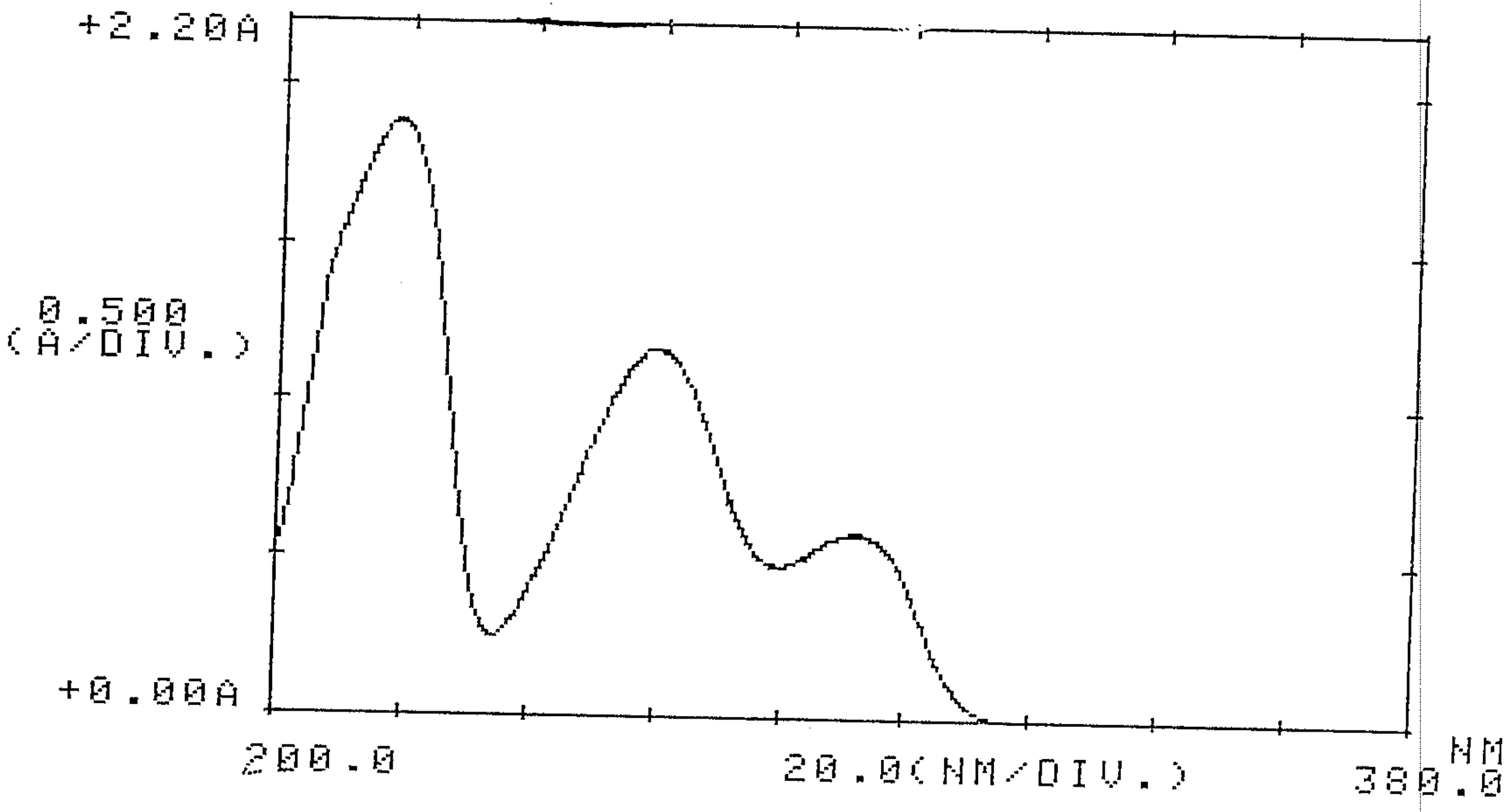


Figure 50 UV (CH_3OH) spectrum of **YS9**

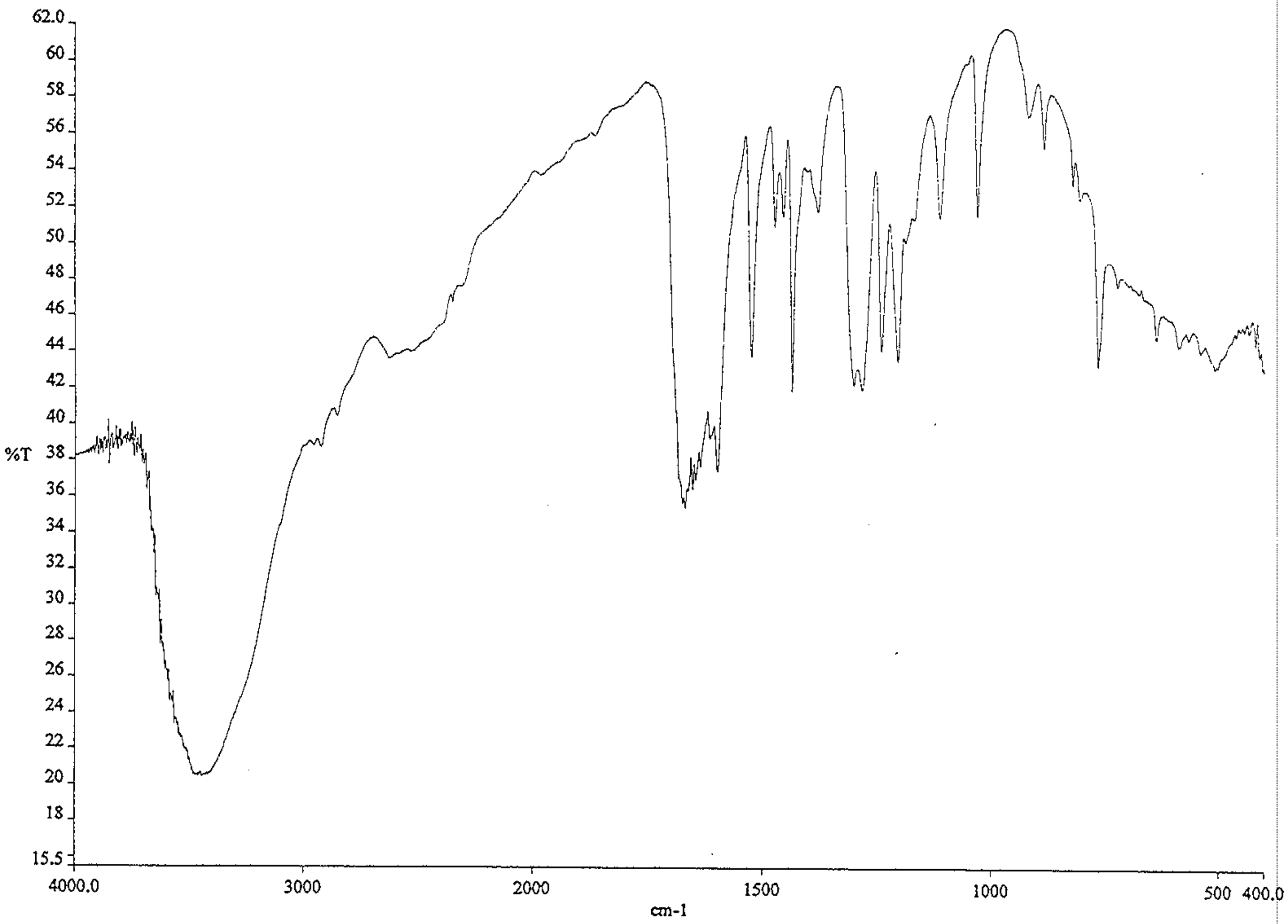


Figure 51 IR (neat) spectrum of **YS9**

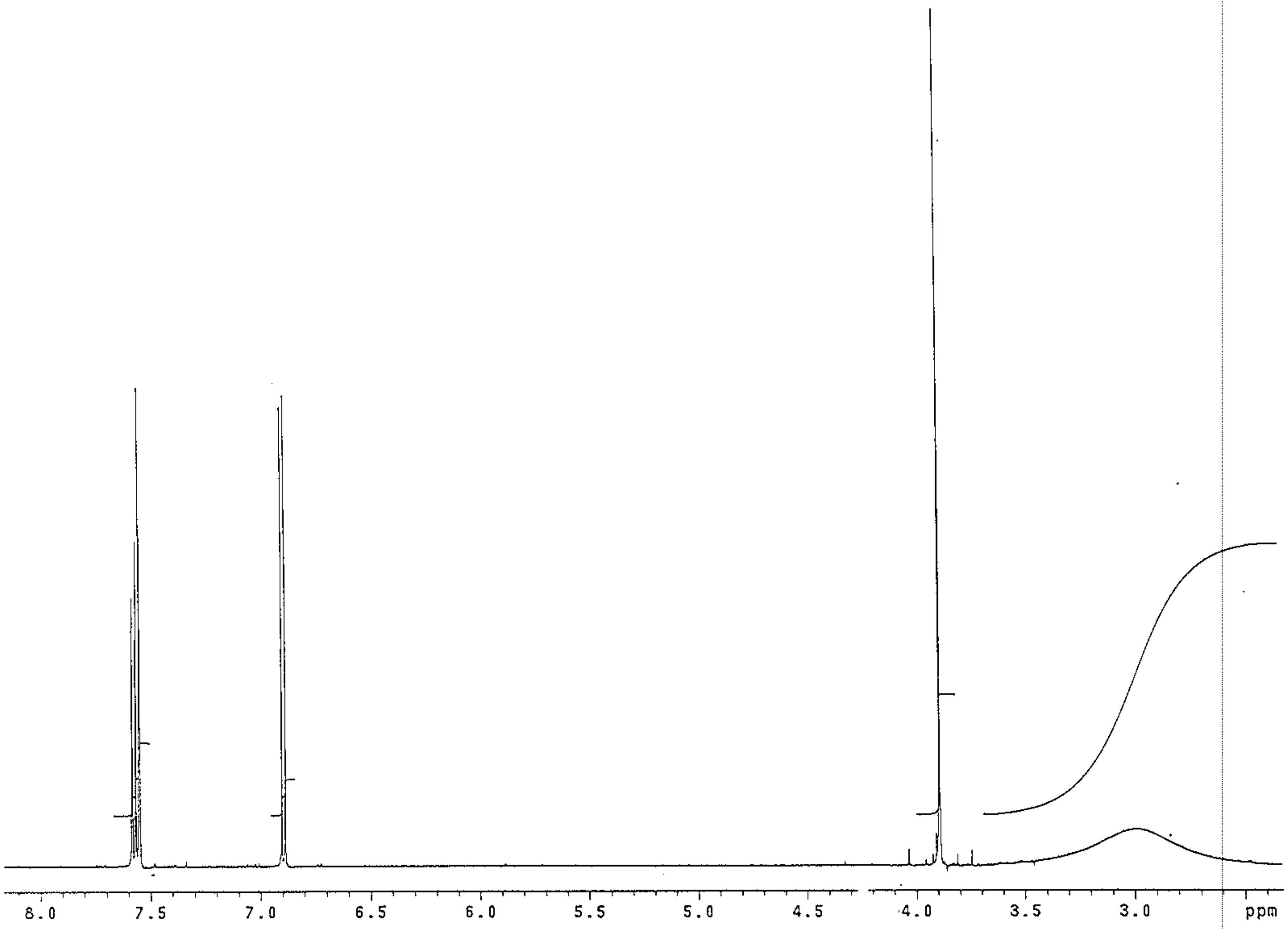


Figure 52 ^{1}H NMR (500 MHz) (CD_3COCD_3) spectrum of **YS9**

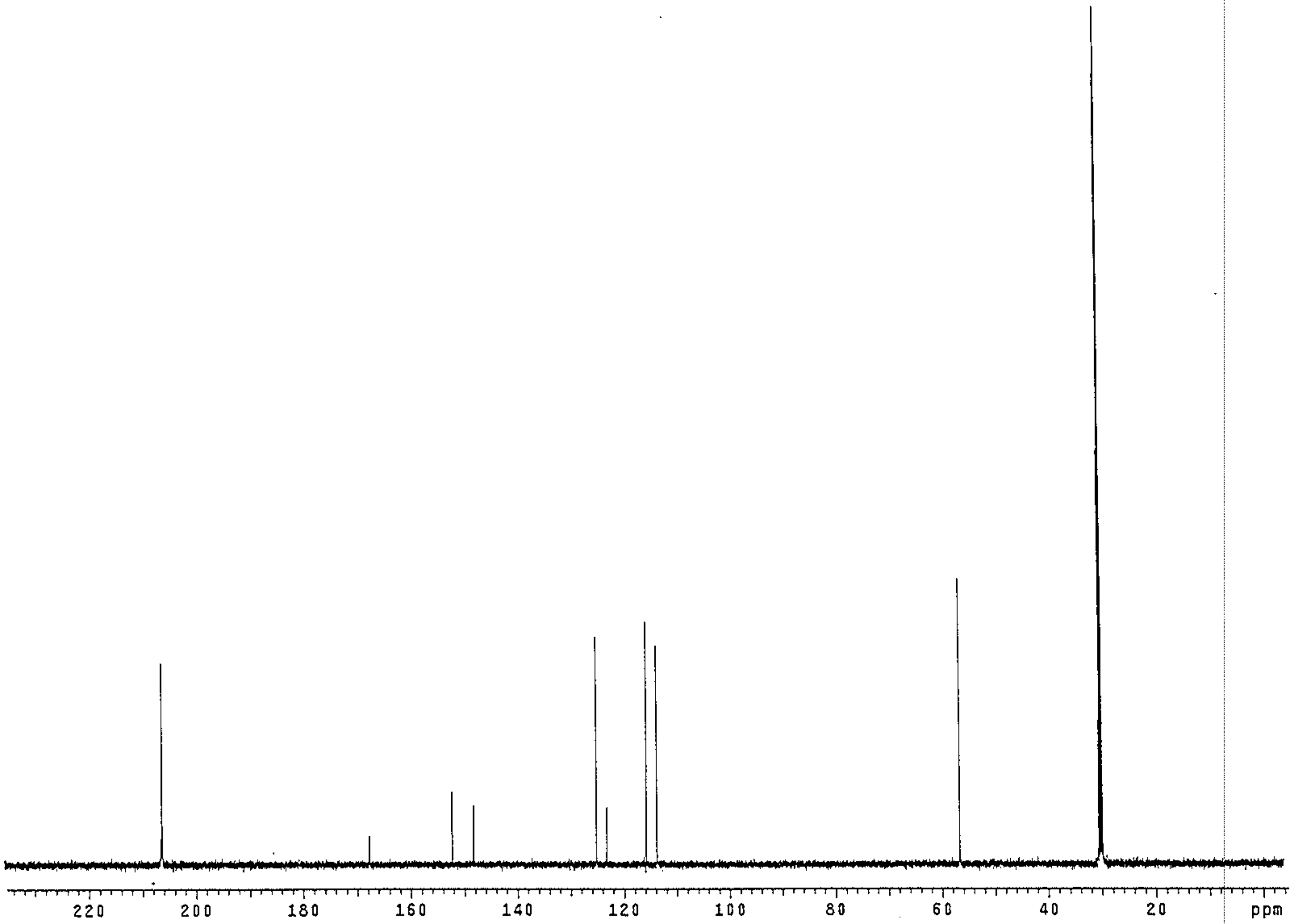


Figure 53 ^{13}C NMR (125 MHz) (CD_3COCD_3) spectrum of **YS9**

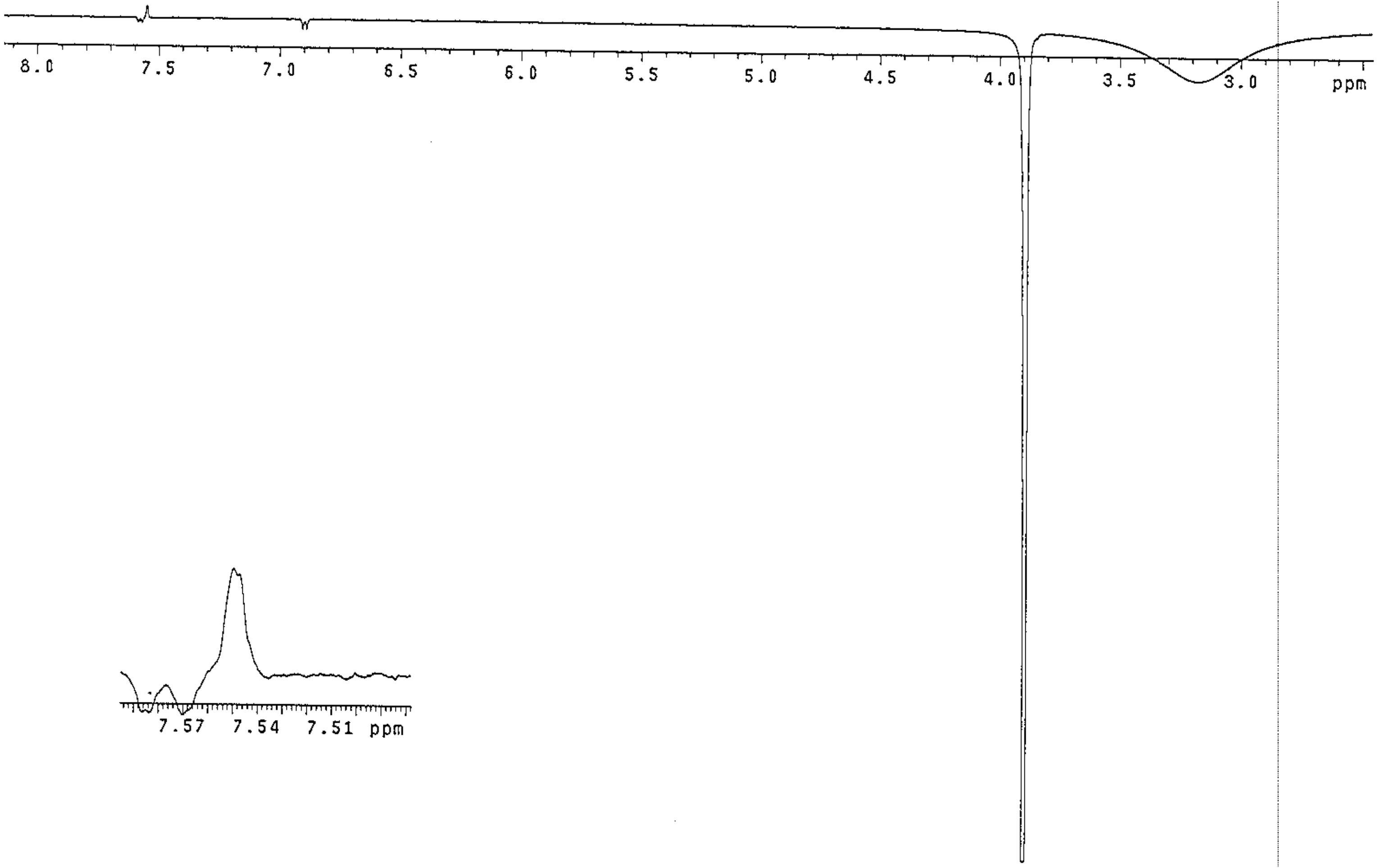


Figure 54 NOEDIFF spectrum of **YS9** after irradiation at δ_H 3.90

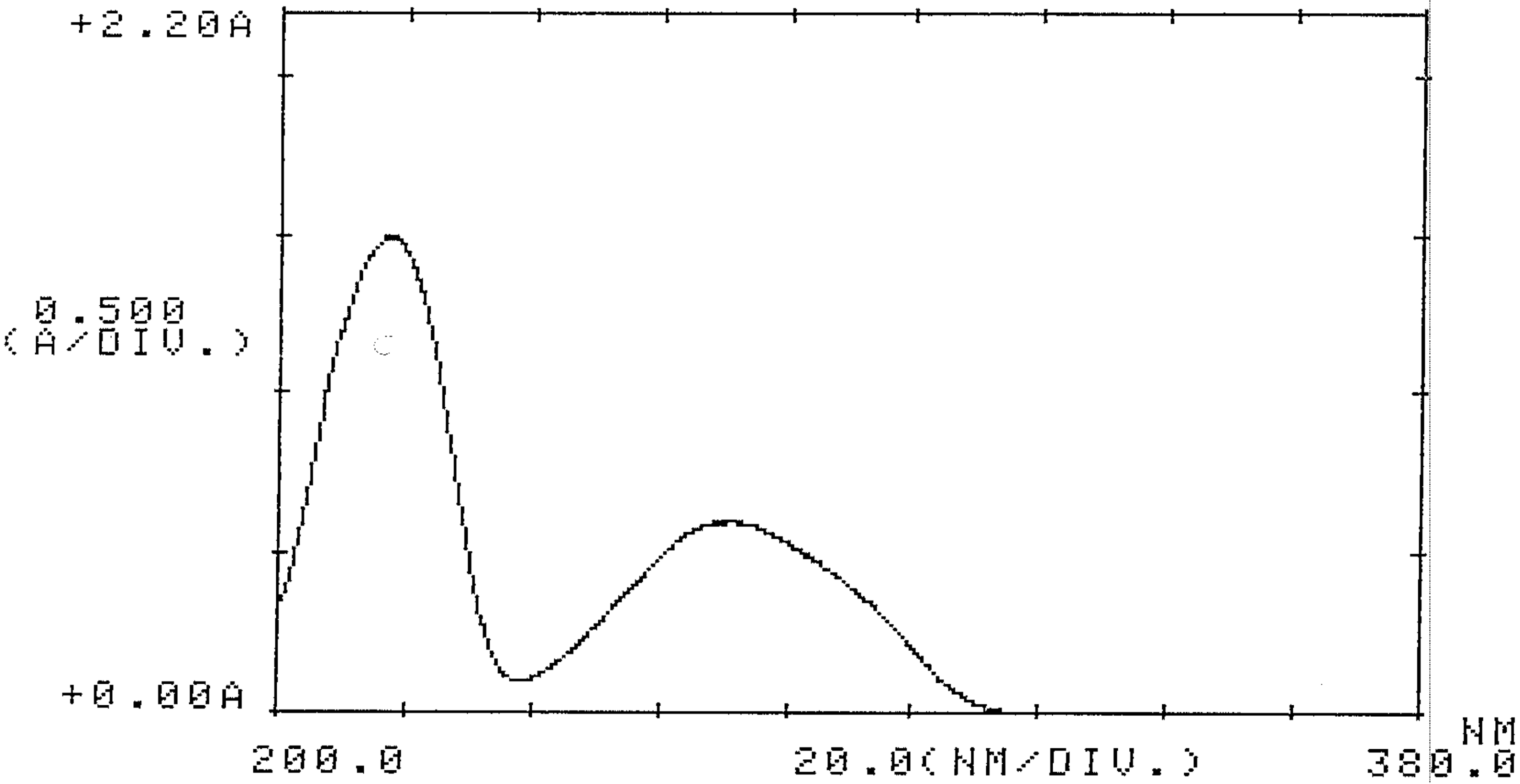


Figure 55 UV (CH_3OH) spectrum of **YS12**

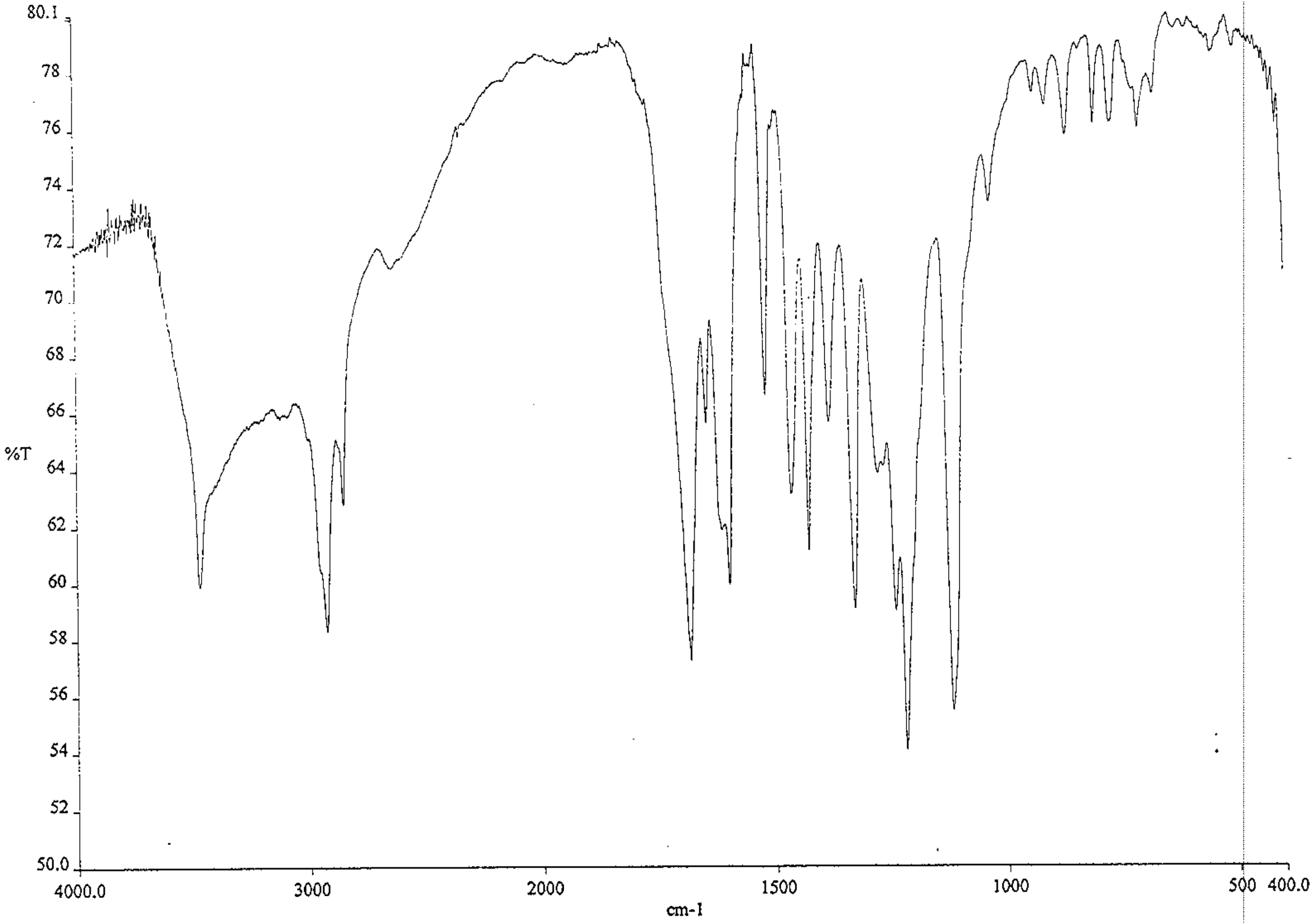


Figure 56 IR (neat) spectrum of **YS12**

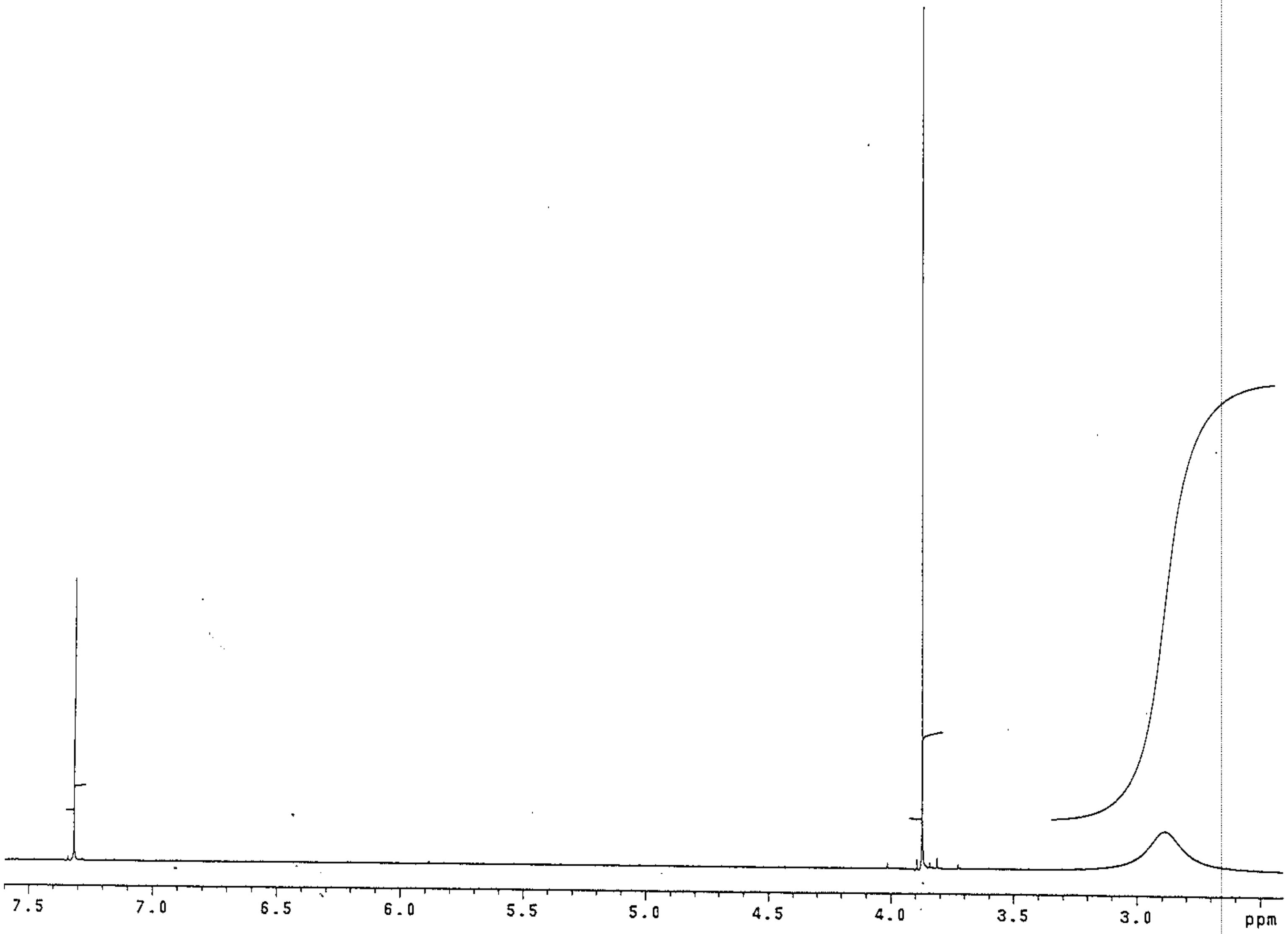


Figure 57 ^{1}H NMR (500 MHz) (CD_3COCD_3) spectrum of **YS12**

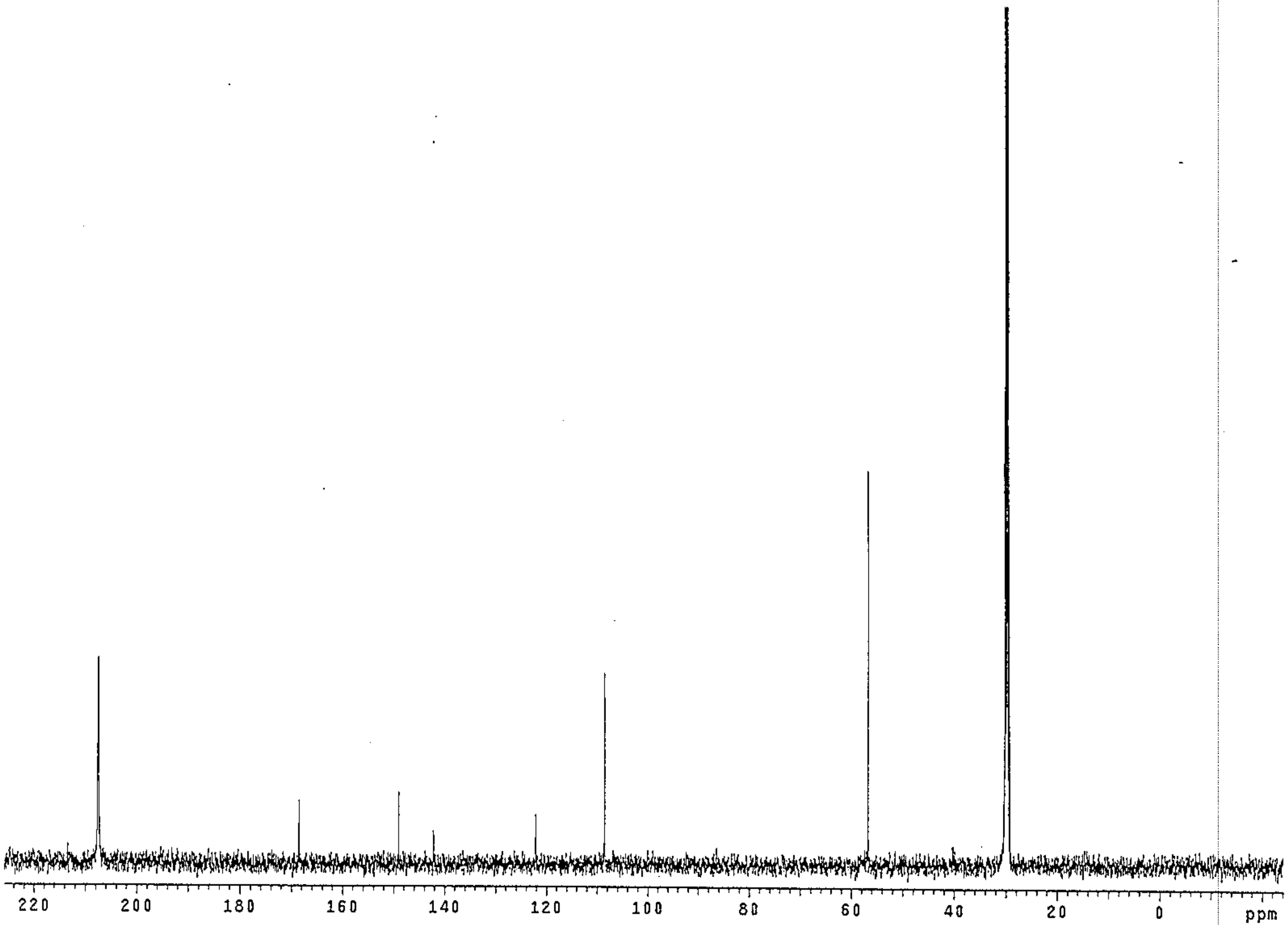


Figure 58 ^{13}C NMR (125 MHz) (CD_3COCD_3) spectrum of **YS12**

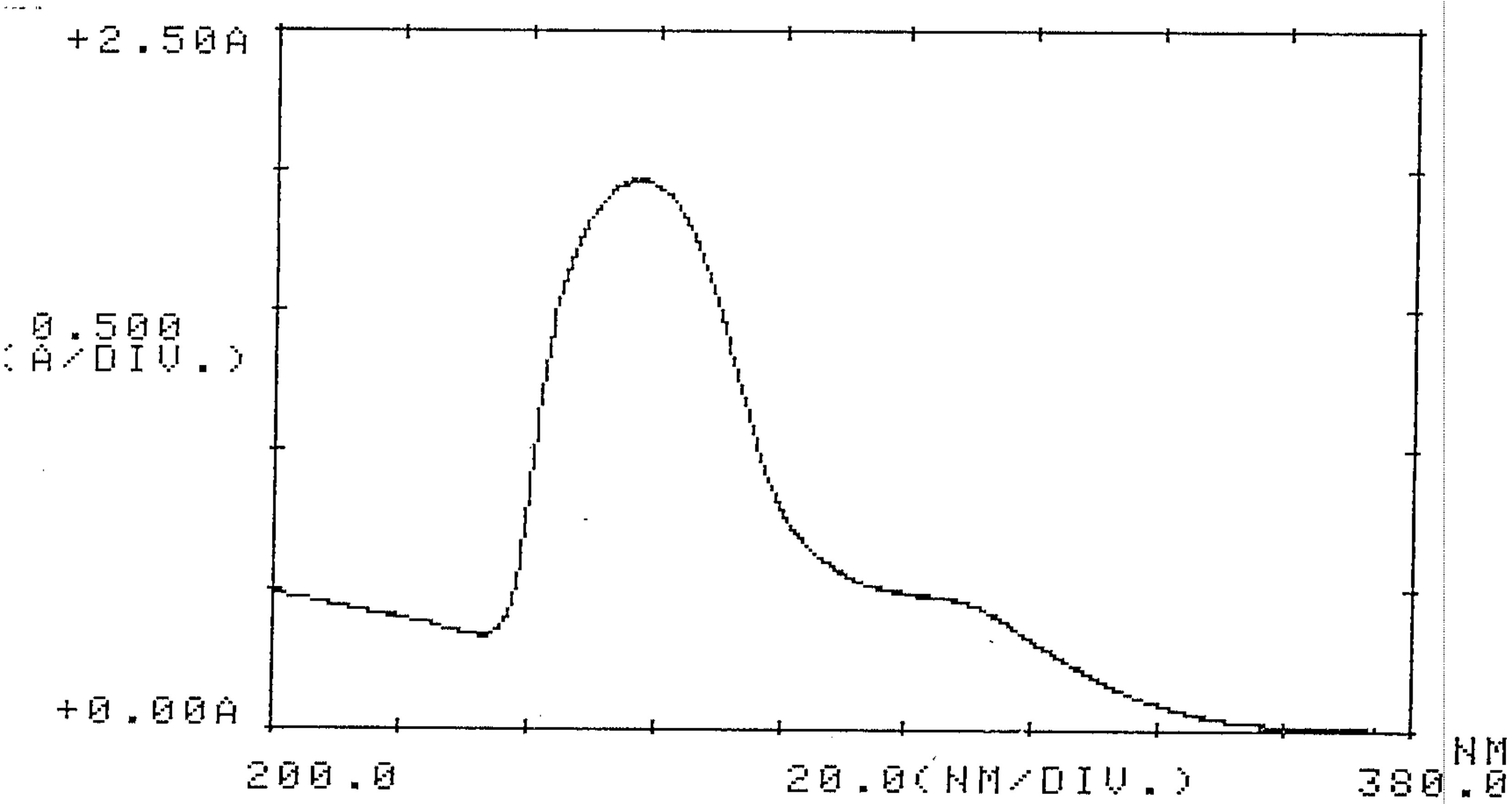


Figure 59 UV (CHCl$_3$) spectrum of **AcYS15**

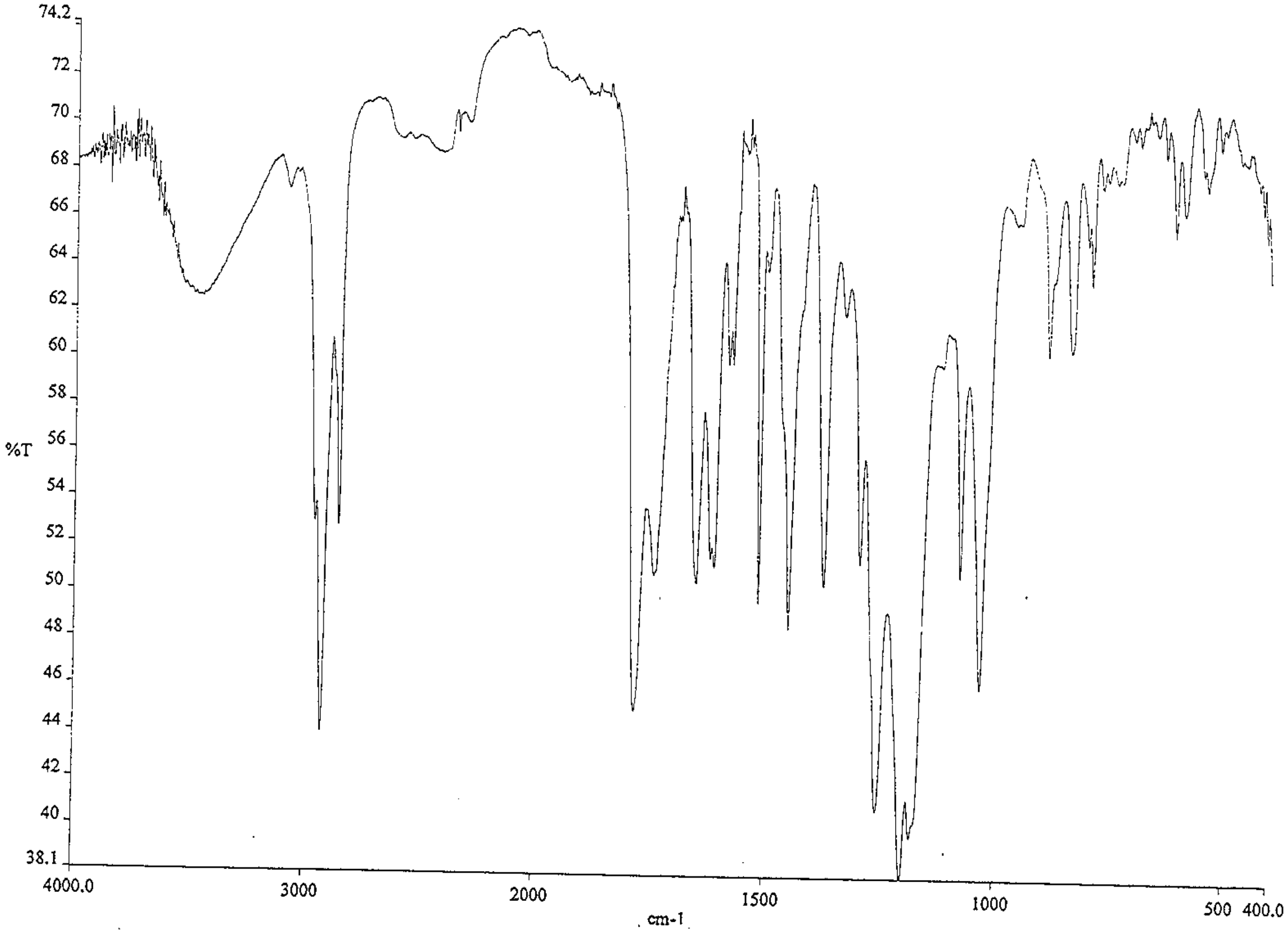


Figure 60 IR (neat) spectrum of **AcYS15**

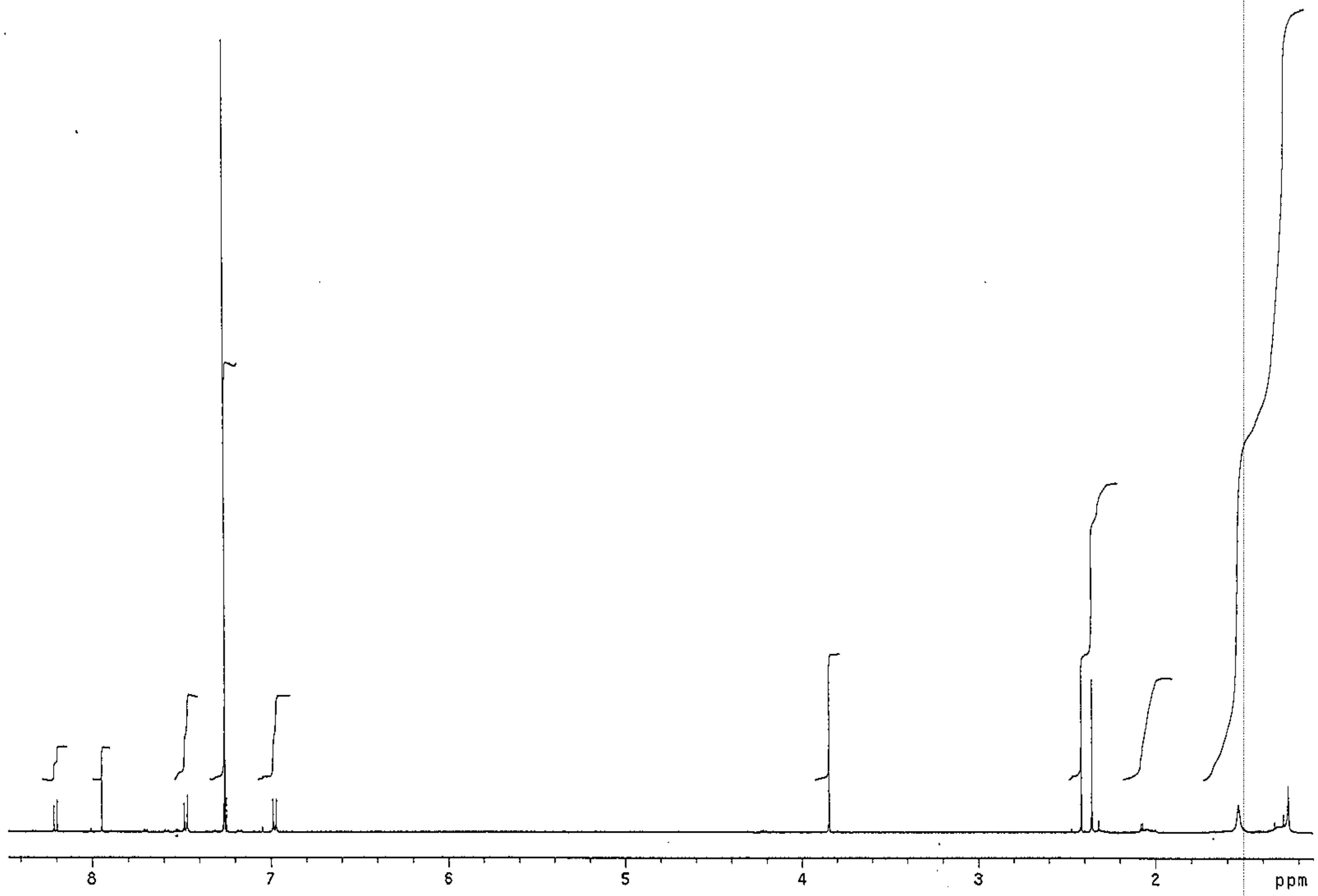


Figure 61 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **AcYS15**

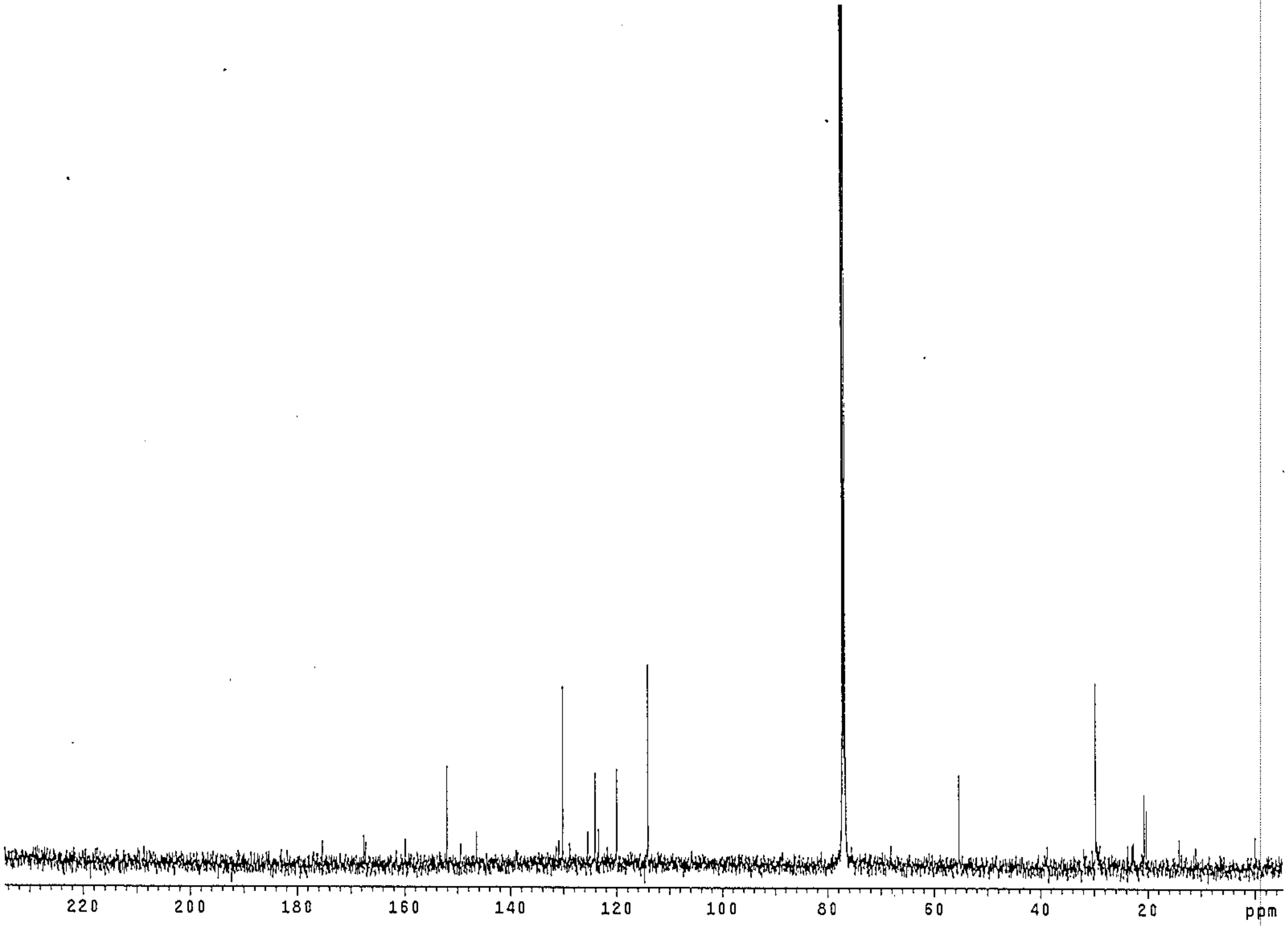


Figure 62 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **AcYS15**

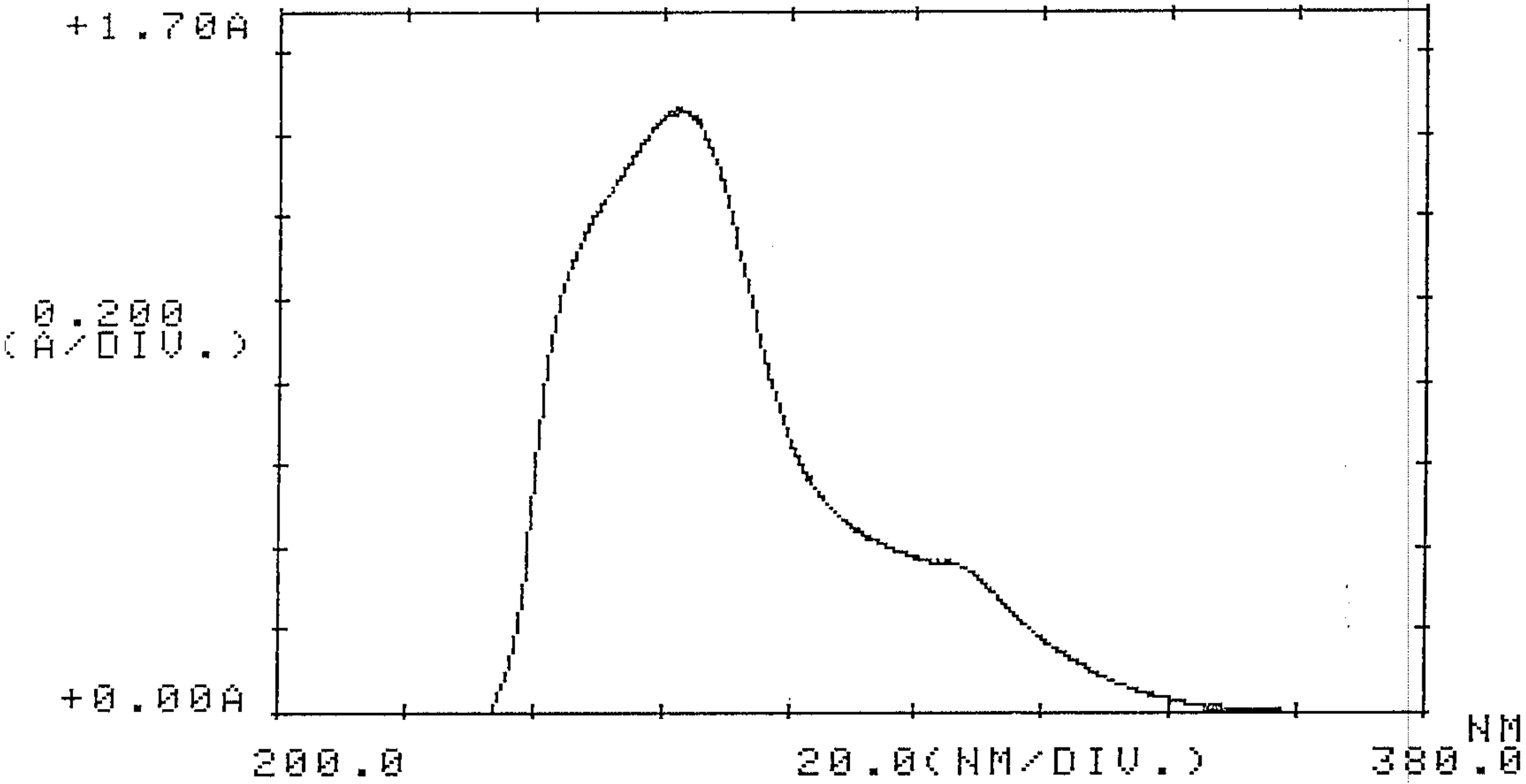


Figure 63 UV ($CHCl_3$) spectrum of **AcYS13**

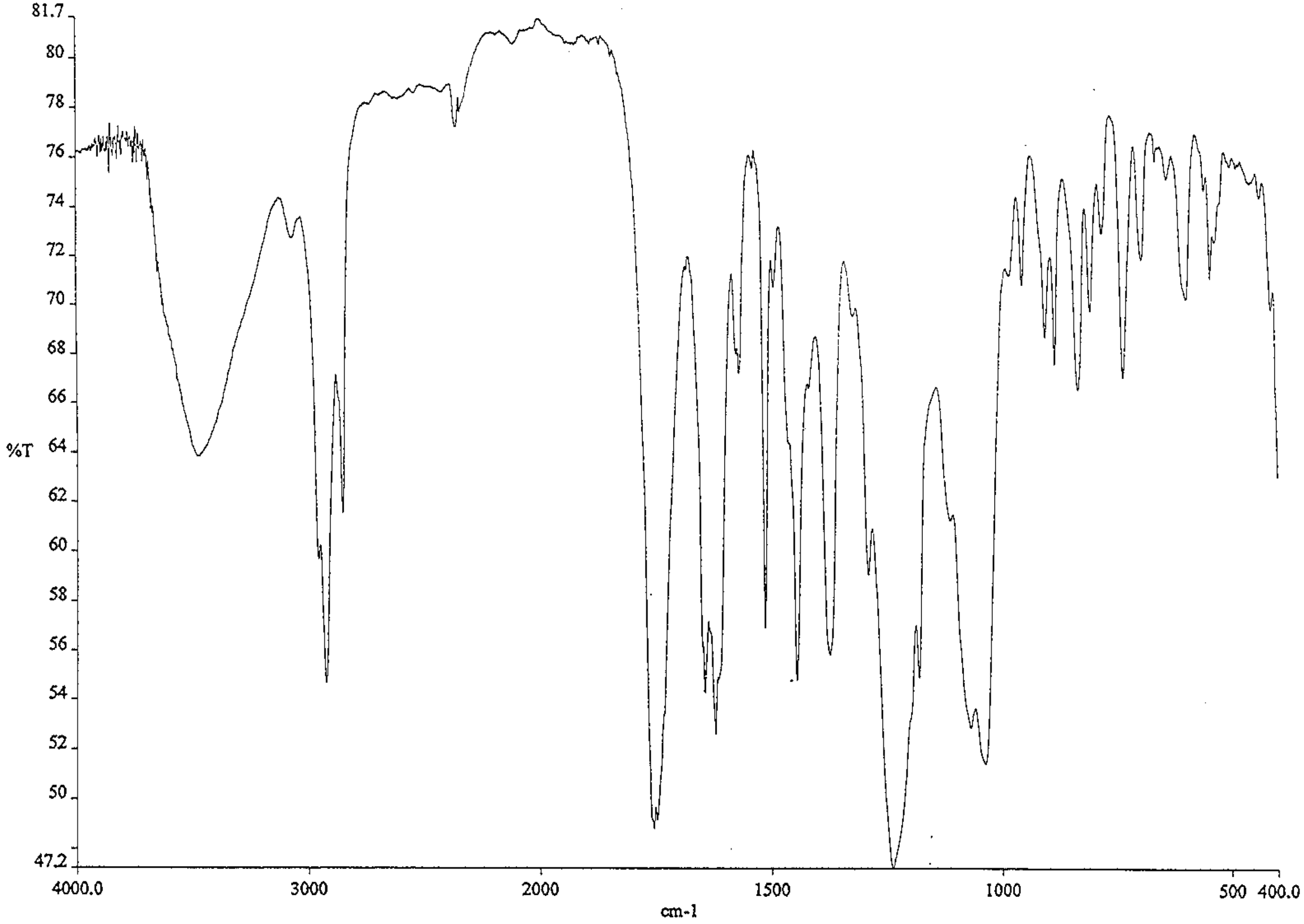


Figure 64 IR (neat) spectrum of **AcYS13**

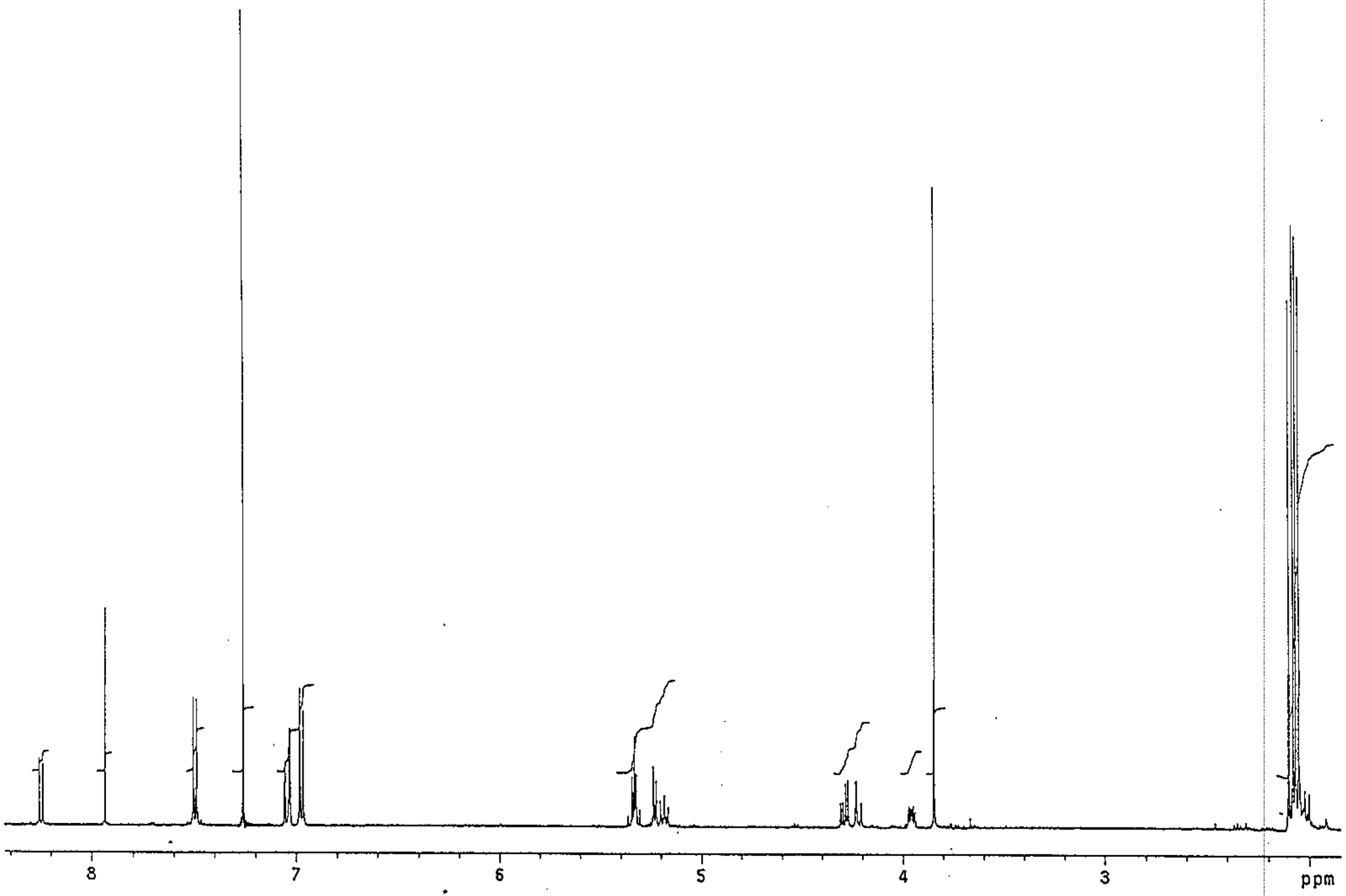


Figure 65 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **AcYS13**

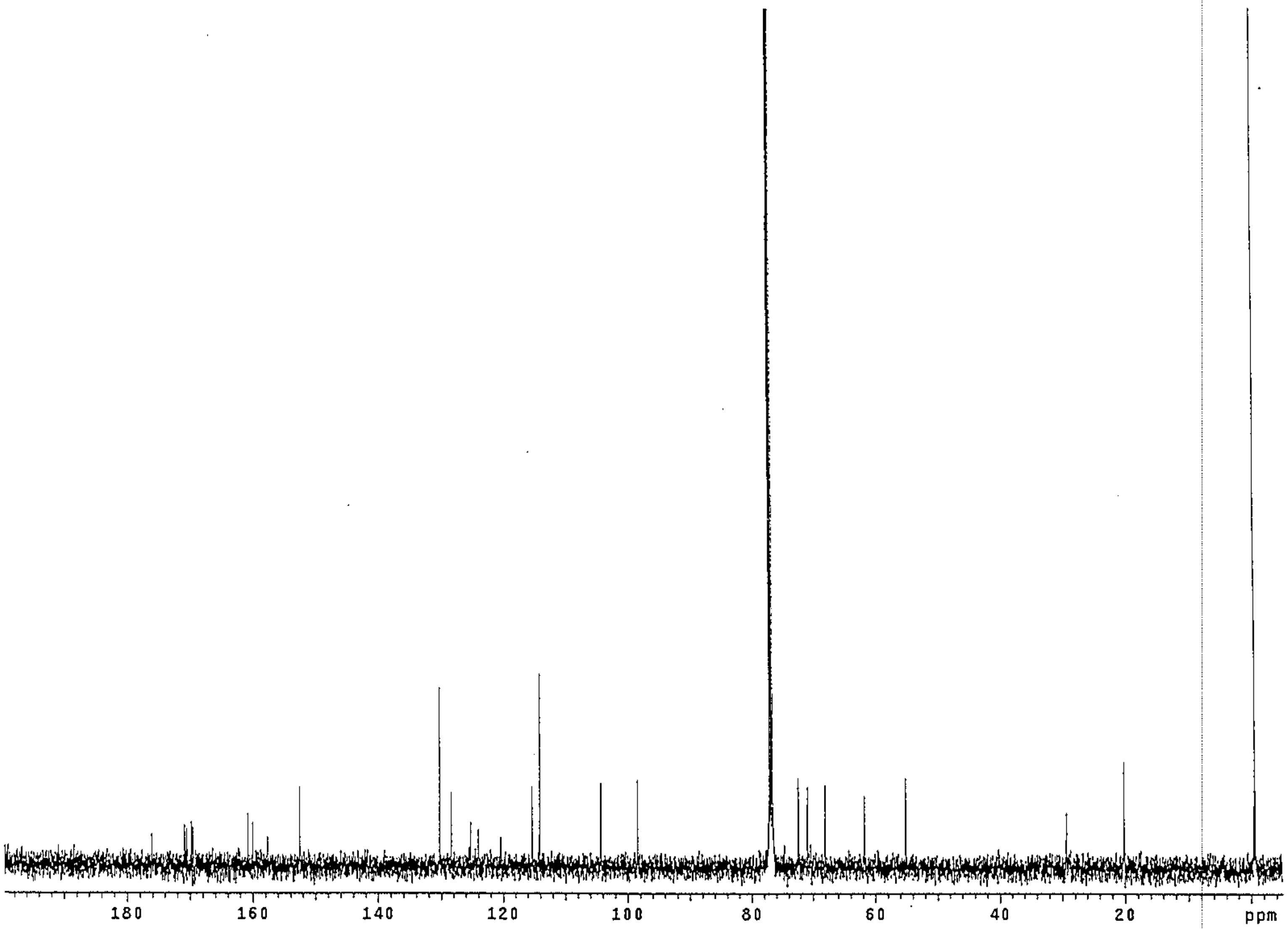


Figure 66 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **AcYS13**

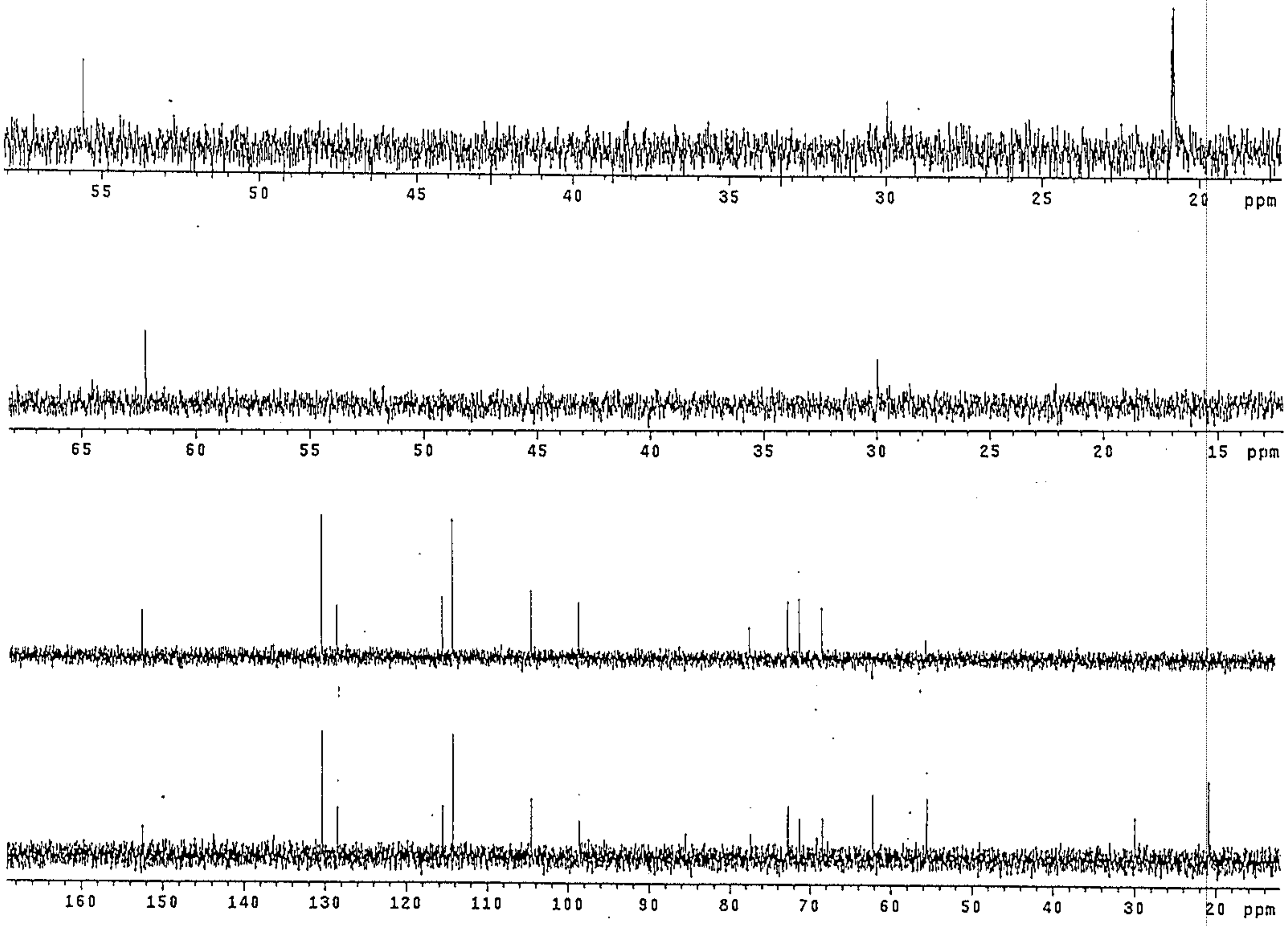


Figure 67 DEPT (135°) ($CDCl_3$) spectrum of **AcYS13**

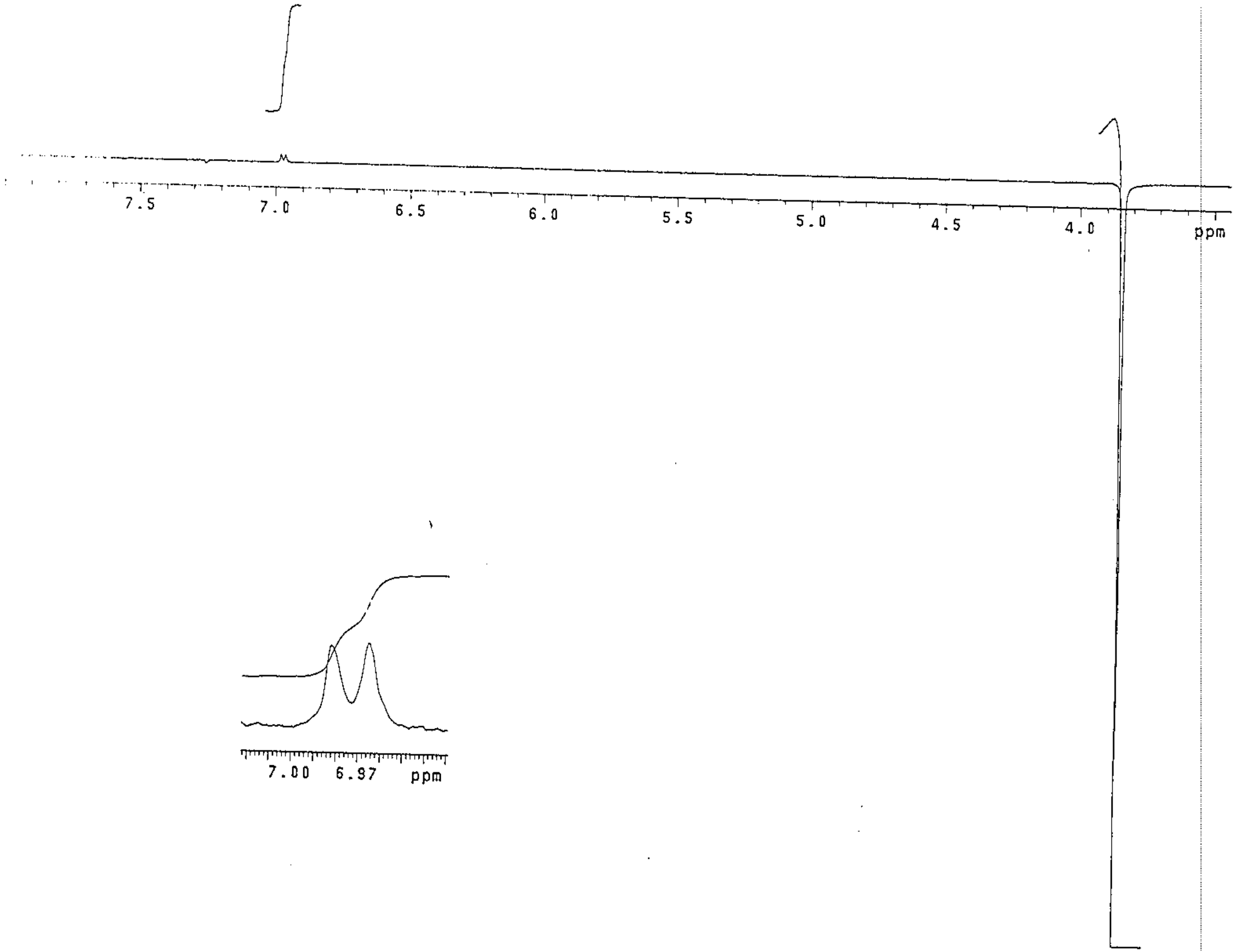


Figure 68 NOEDIFF spectrum of **AcYS13** after irradiation at δ_H 3.85

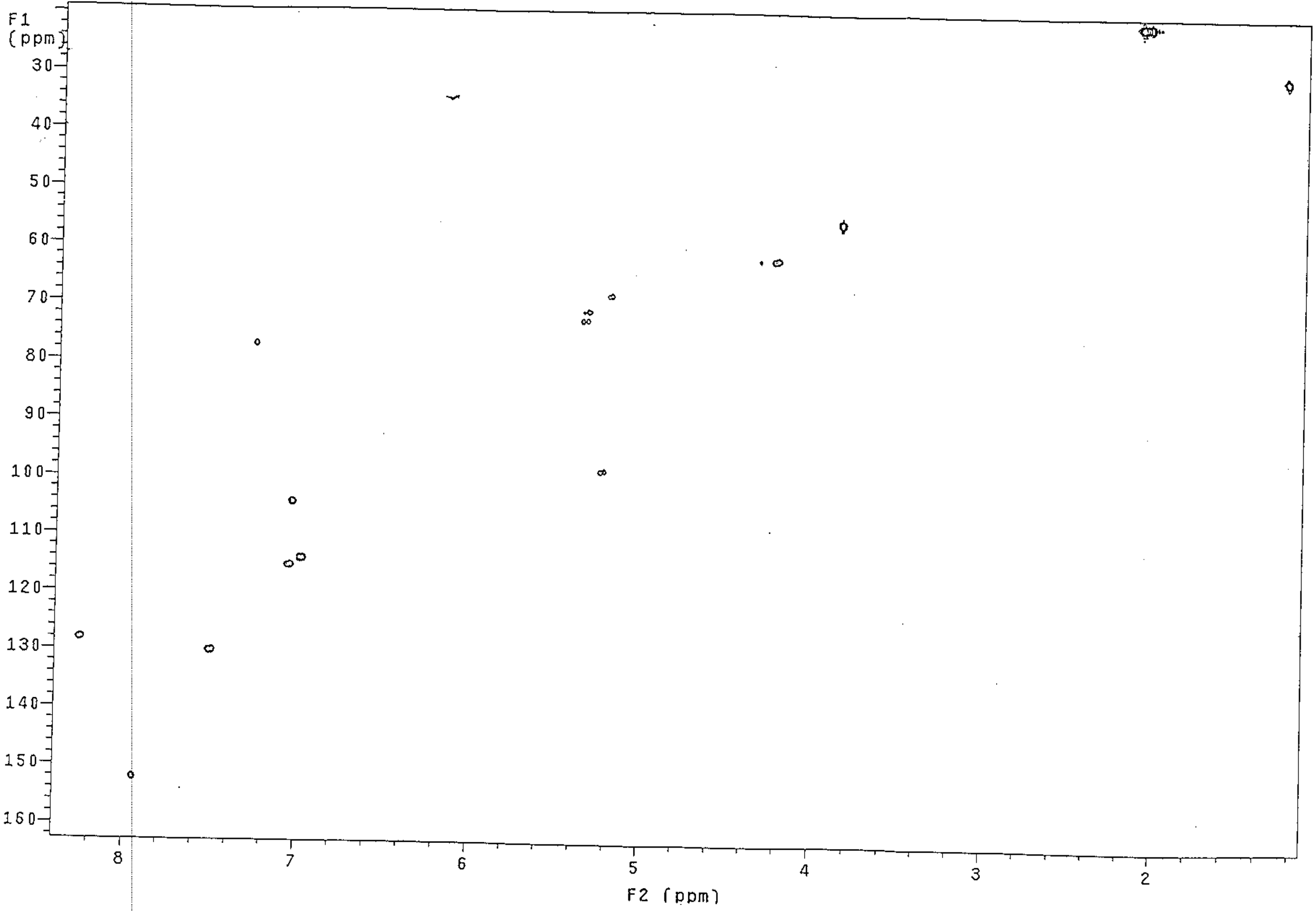


Figure 69 2D HMQC spectrum of AcYS13

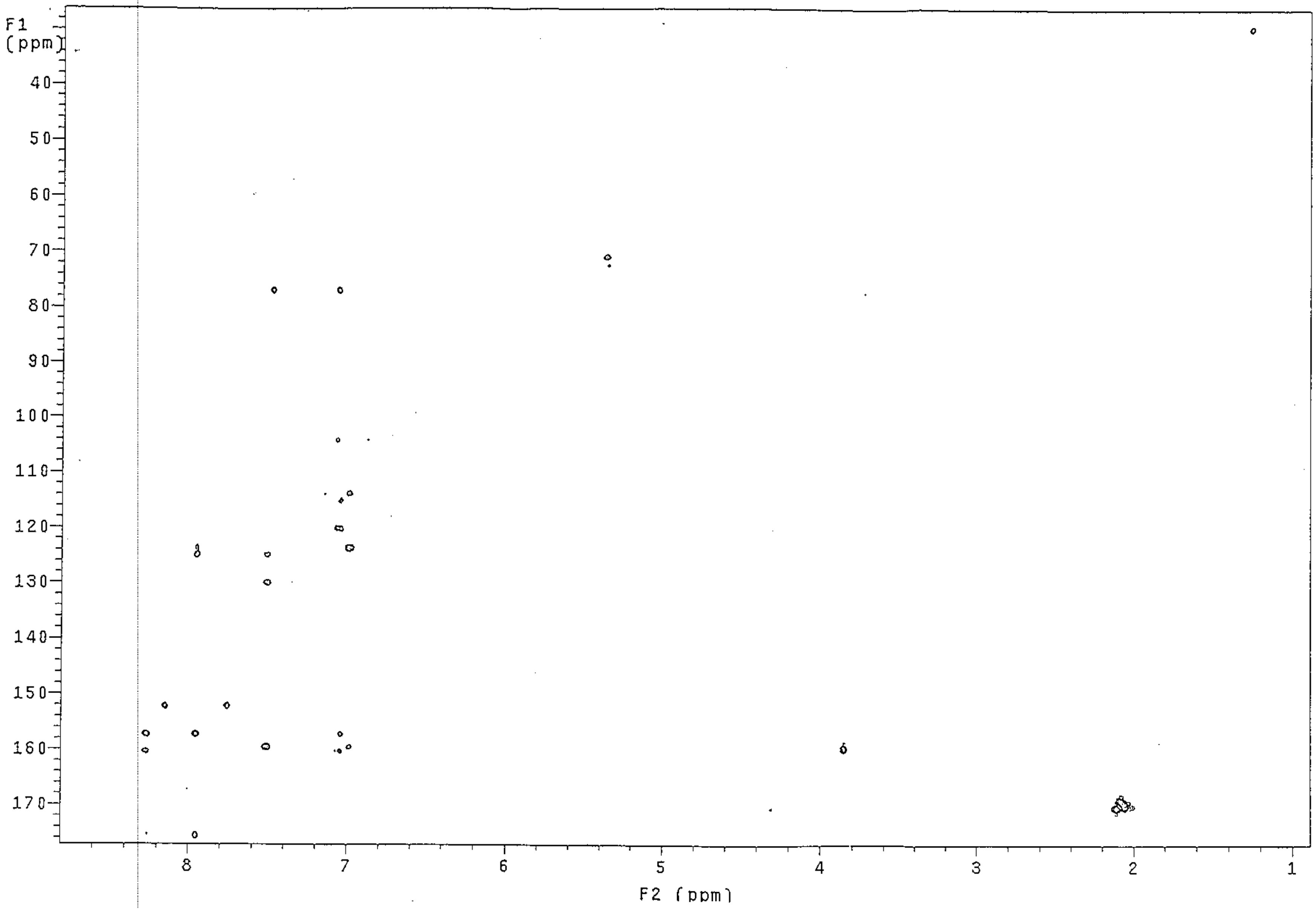


Figure 70 2D HMBC spectrum of AcYS13

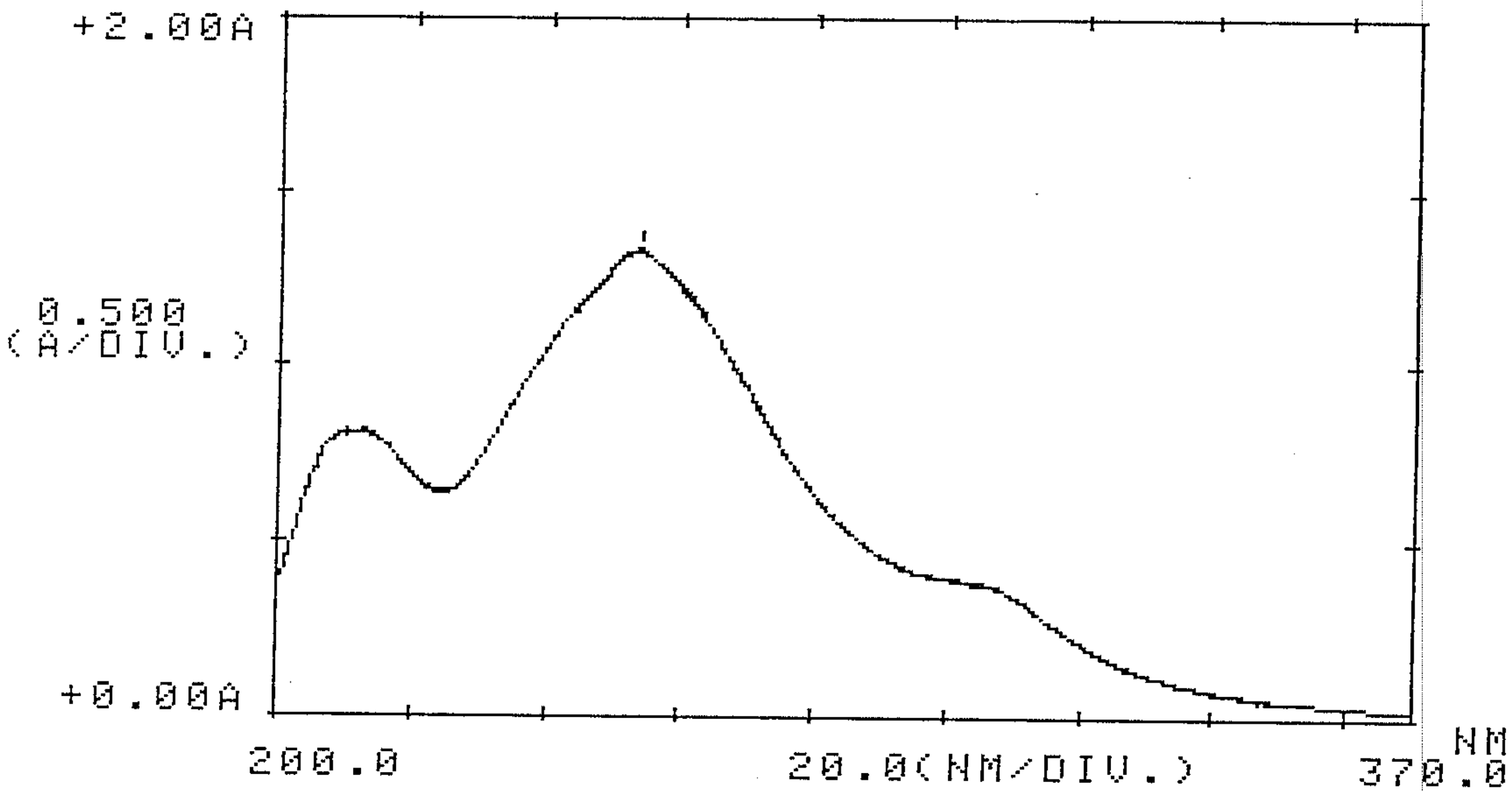


Figure 71 UV ($CHCl_3$) spectrum of **AcYS8**

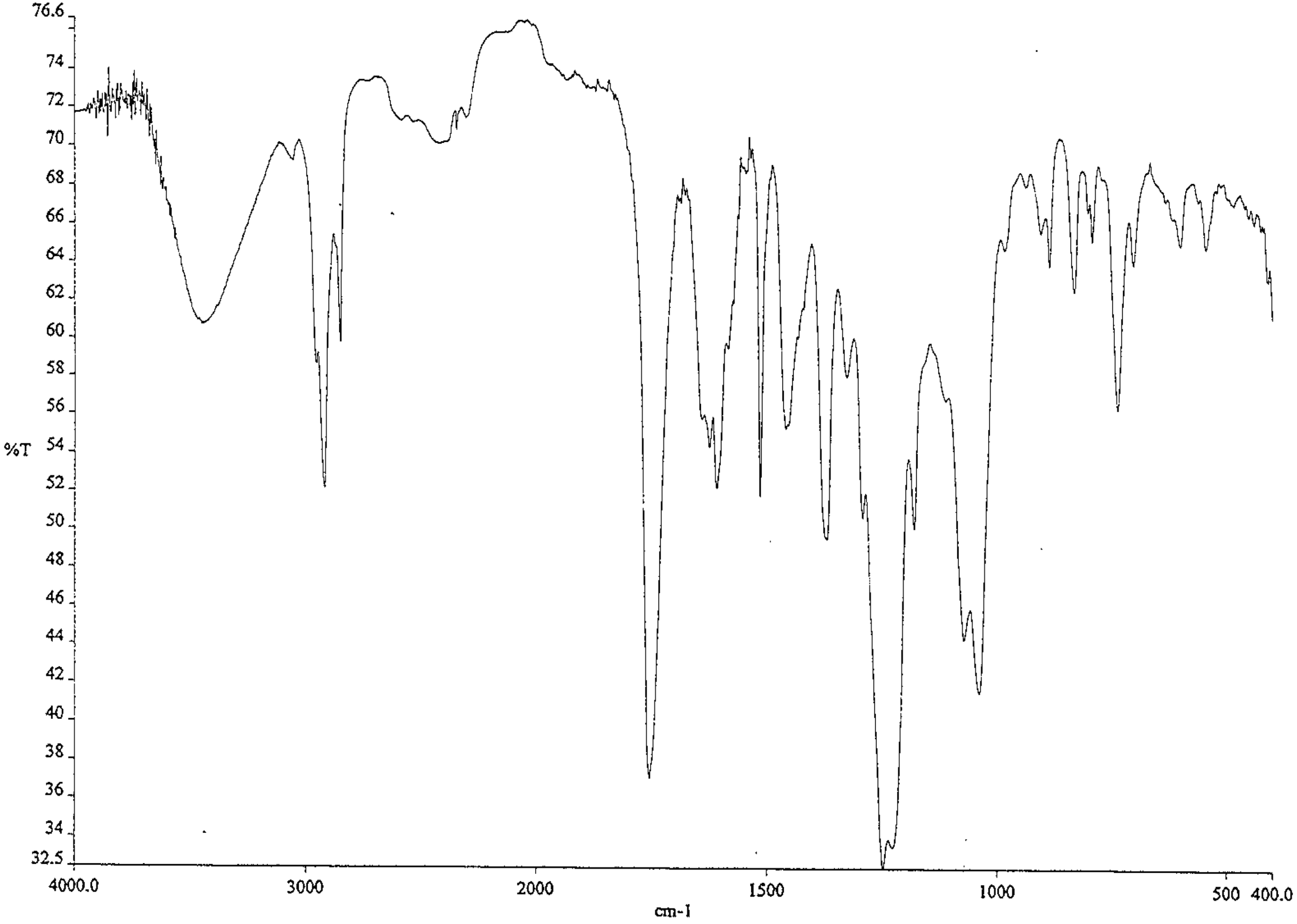


Figure 72 IR (neat) spectrum of **AcYS8**

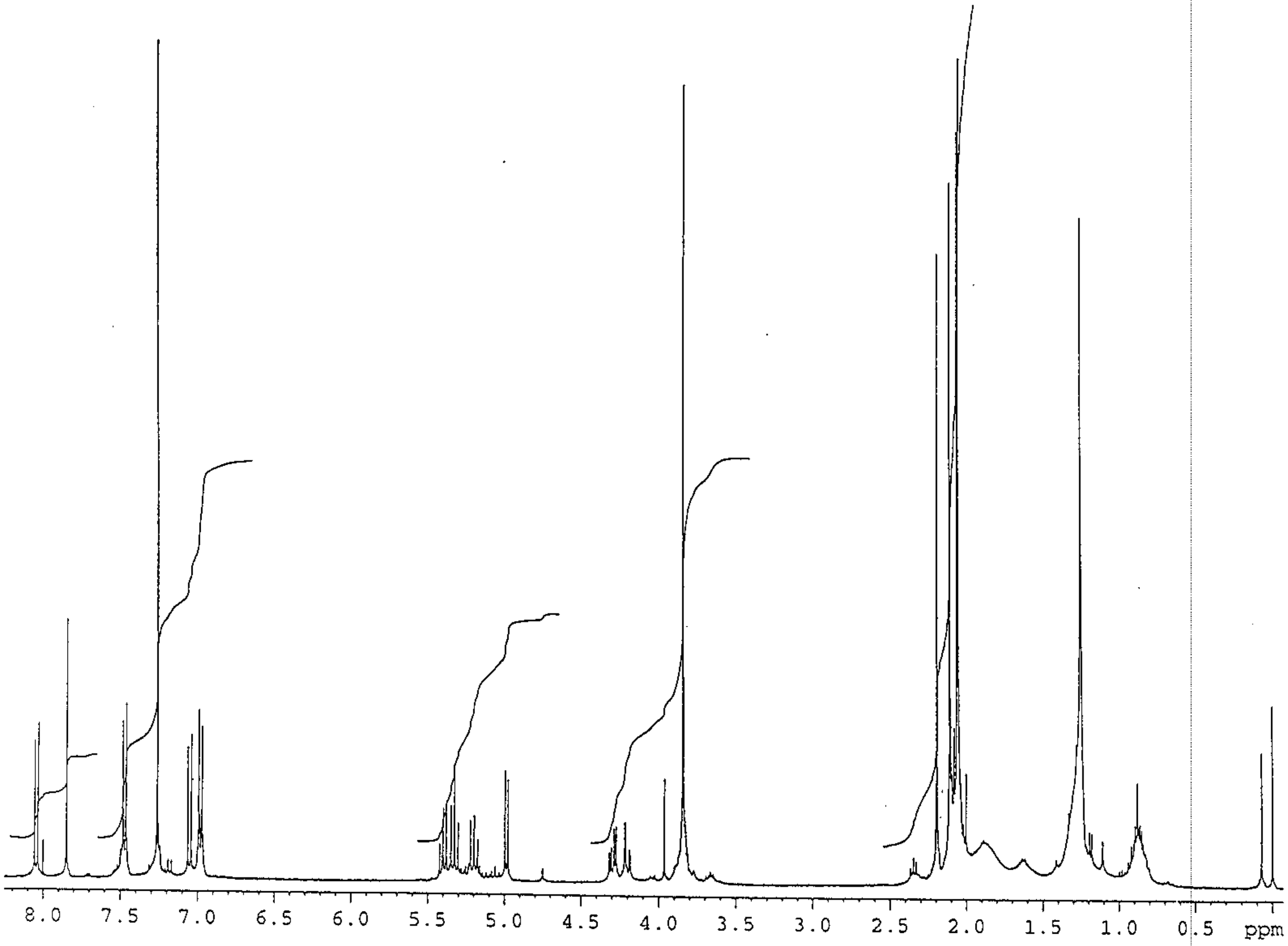


Figure 73 ^{1}H NMR (400 MHz) (CDCl$_3$) spectrum of **AcYS8**

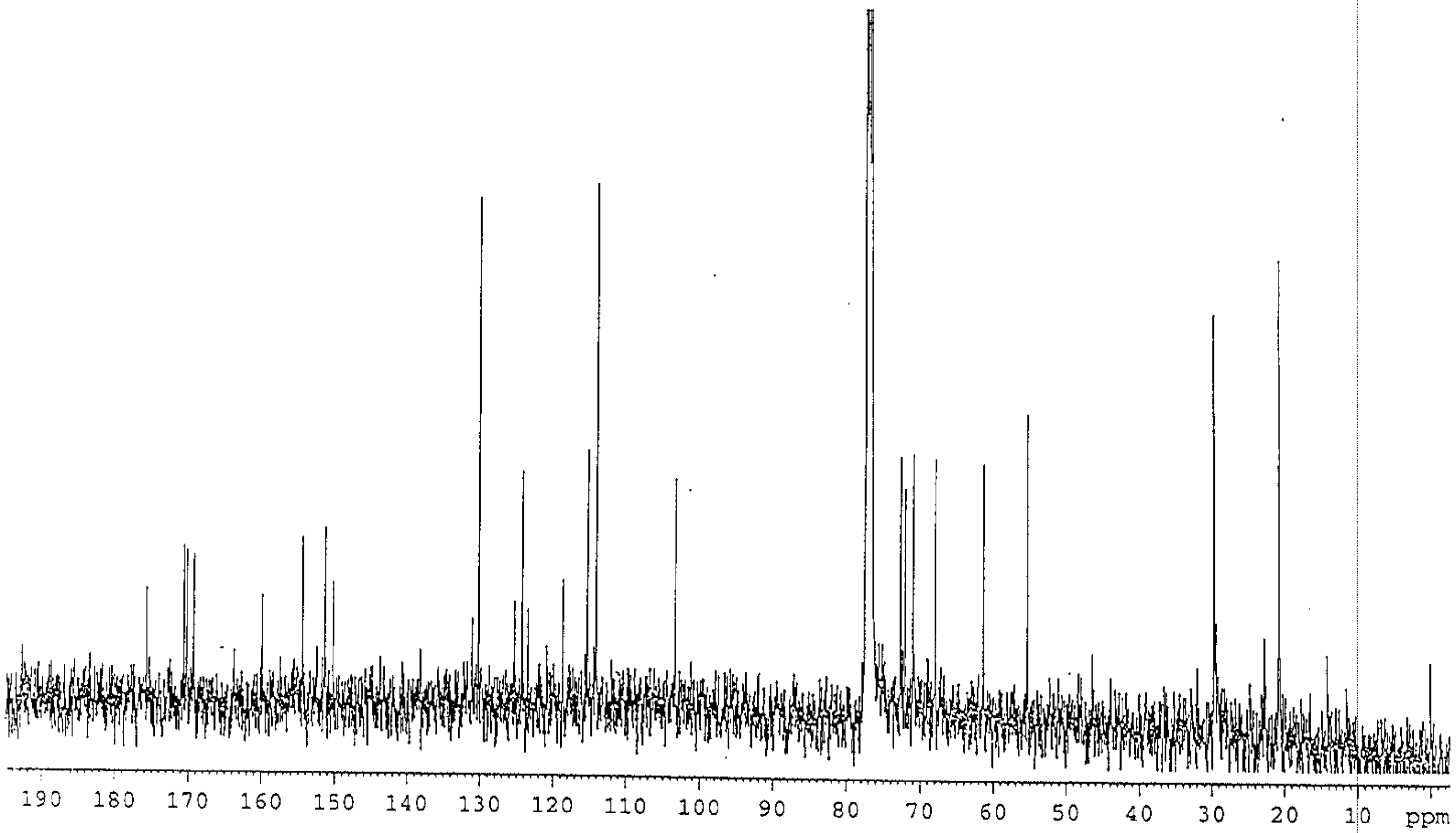


Figure 74 ^{13}C NMR (100 MHz) (CDCl$_3$) spectrum of **AcYS8**

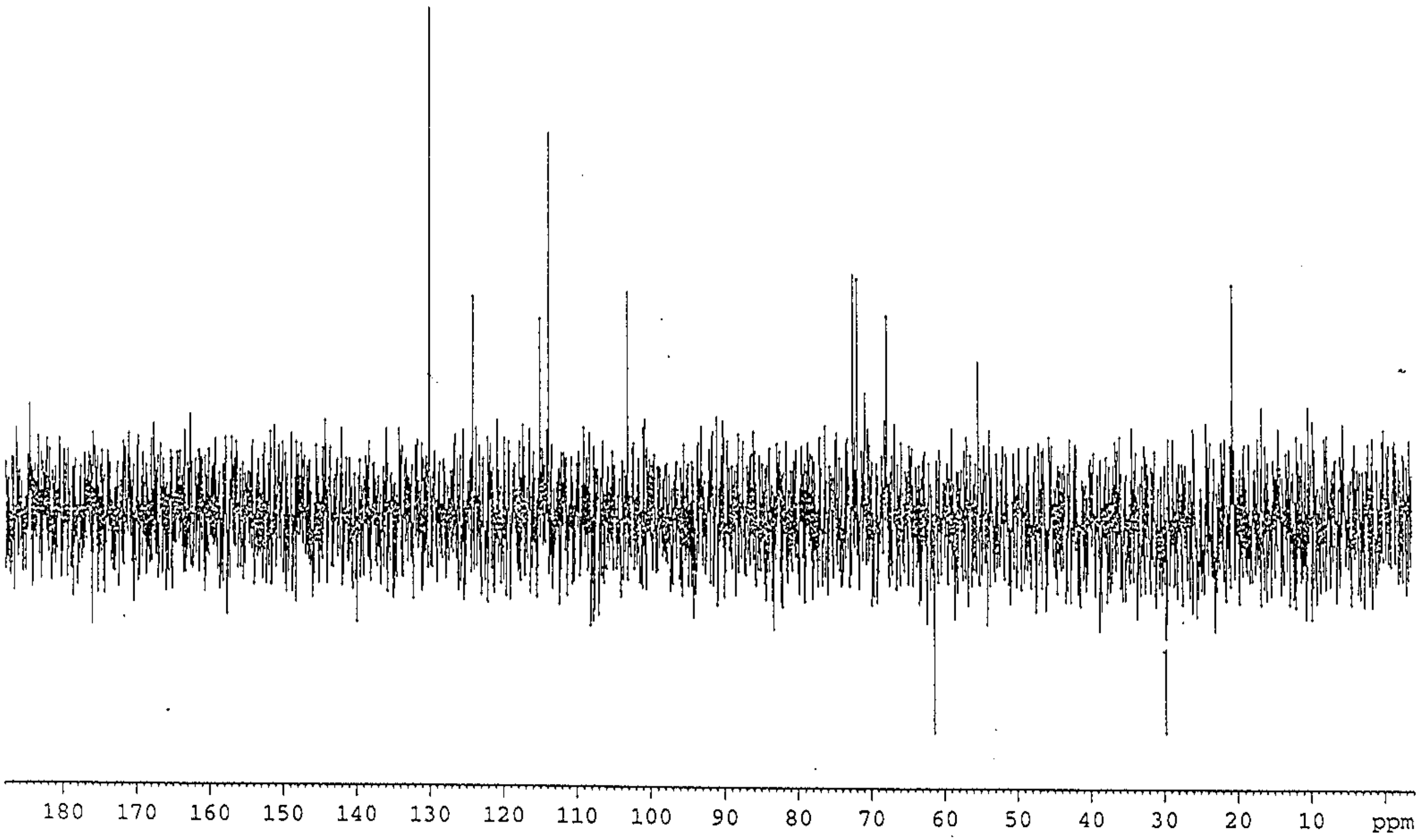


Figure 75 DEPT (135^{O}) (CDCl$_3$) spectrum of **AcYS8**

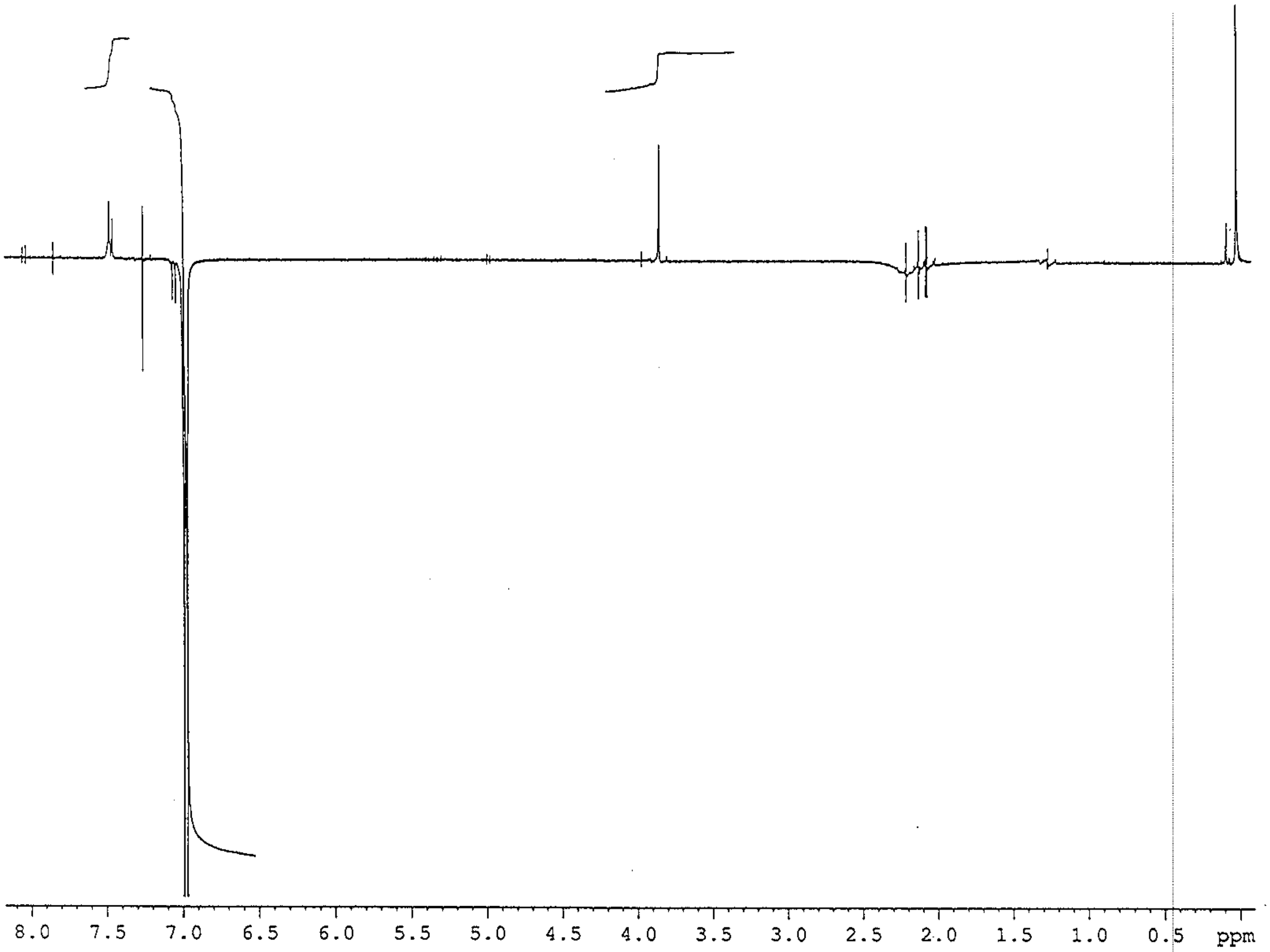


Figure 76 NOEDIFF spectrum of **AcYS8** after irradiation at δ_H 6.98

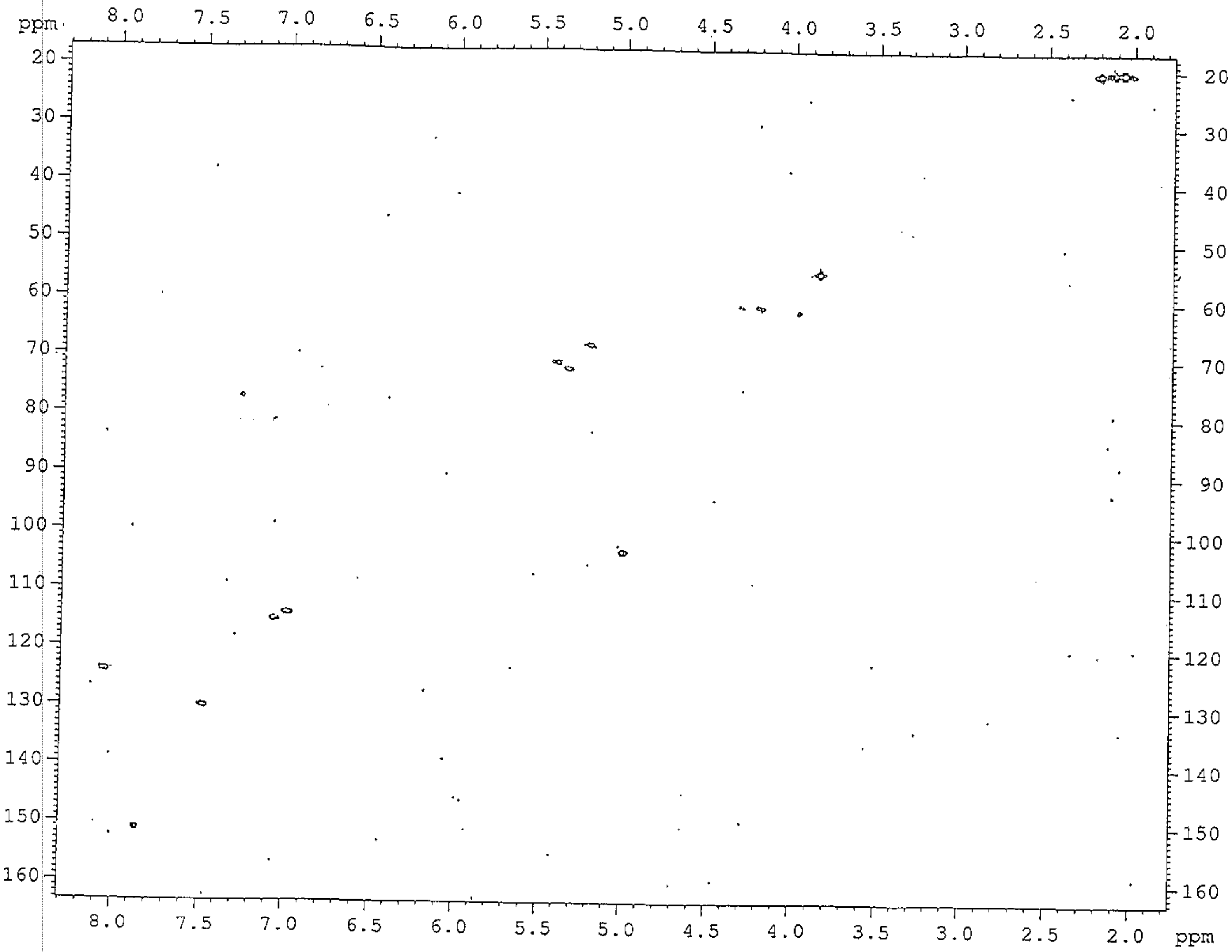


Figure 77 2D HMQC spectrum of **AcYS8**

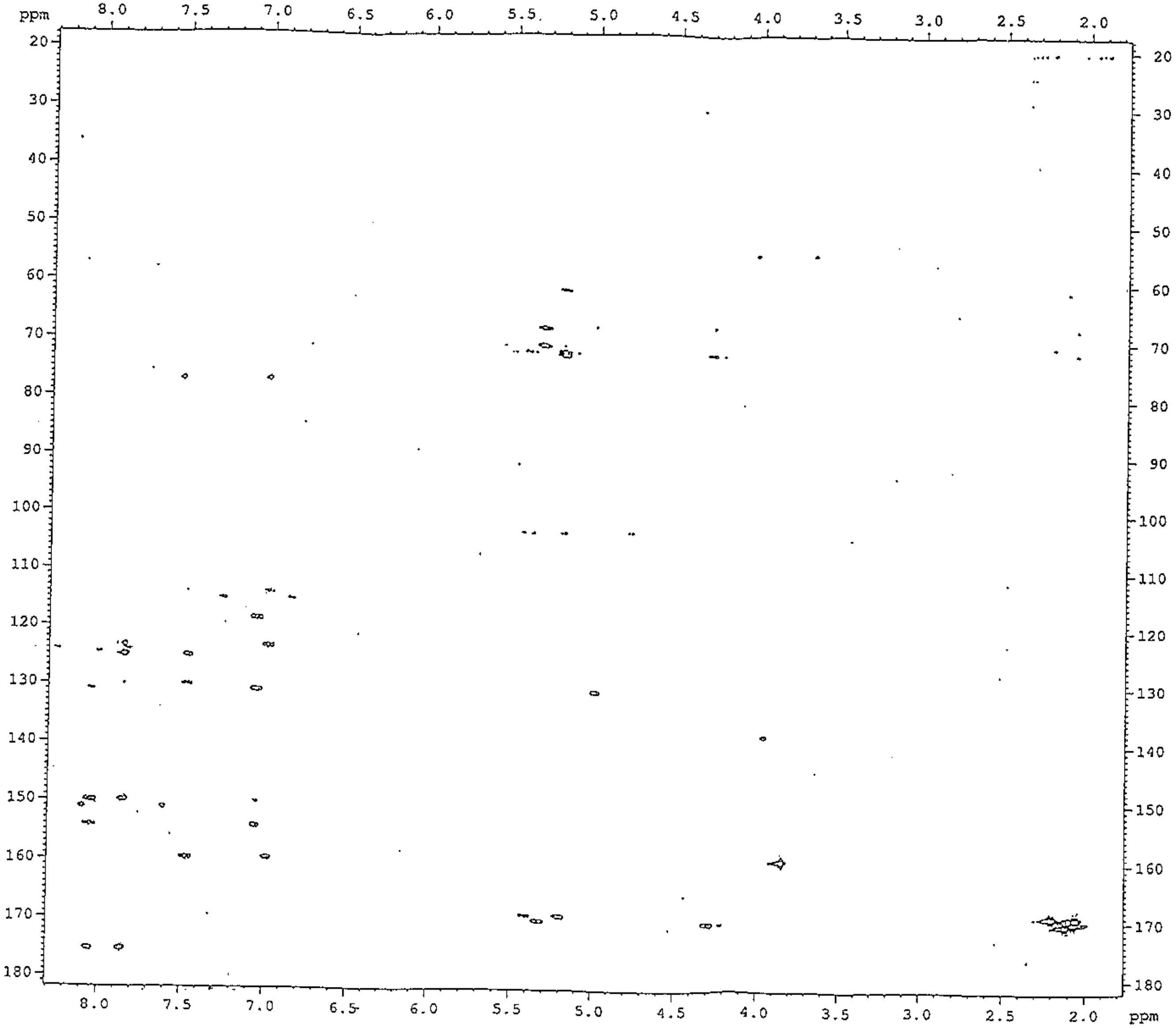


Figure 78 2D HMBC spectrum of **AcYS8**

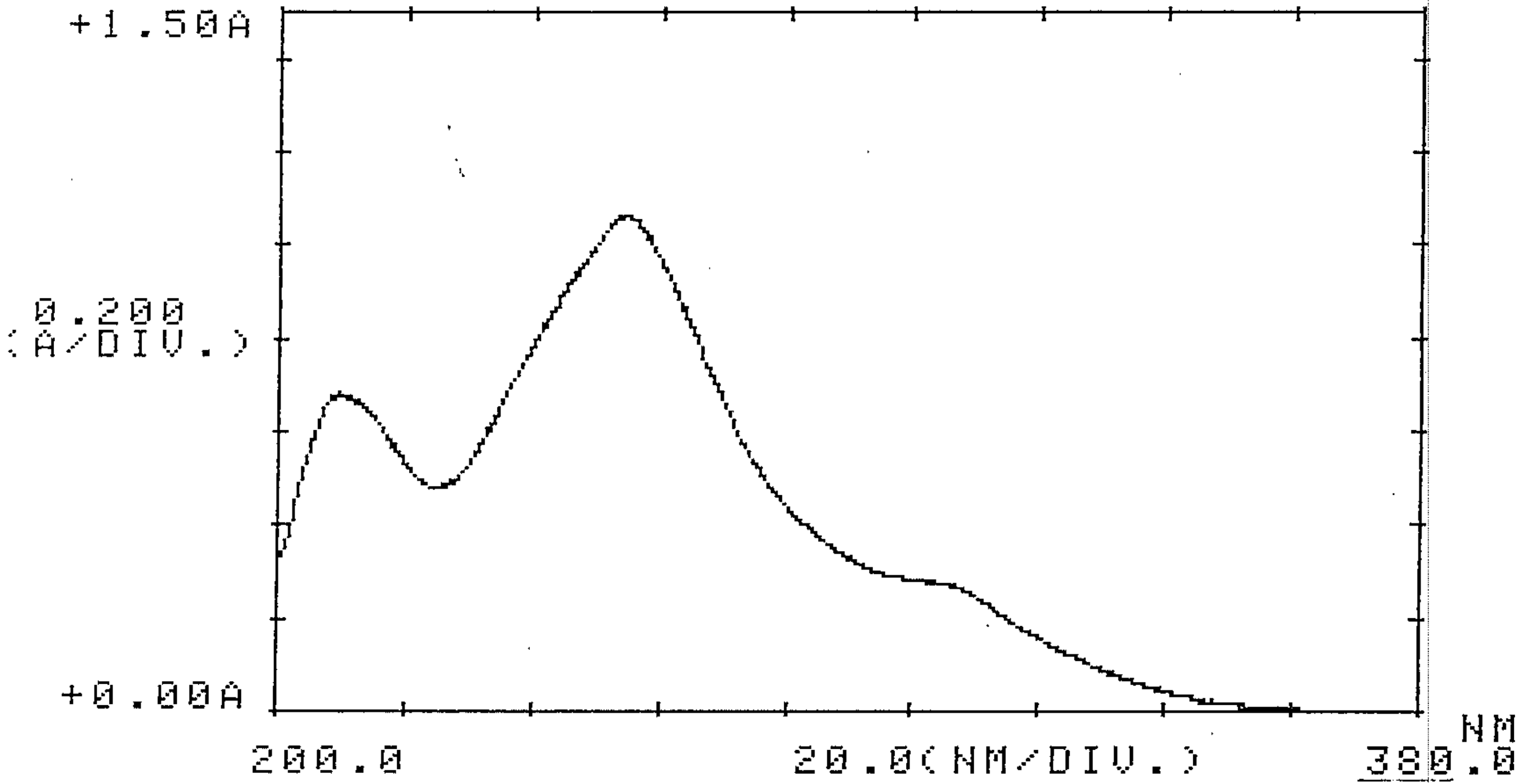


Figure 79 UV (CH_3OH) spectrum of **YS1**

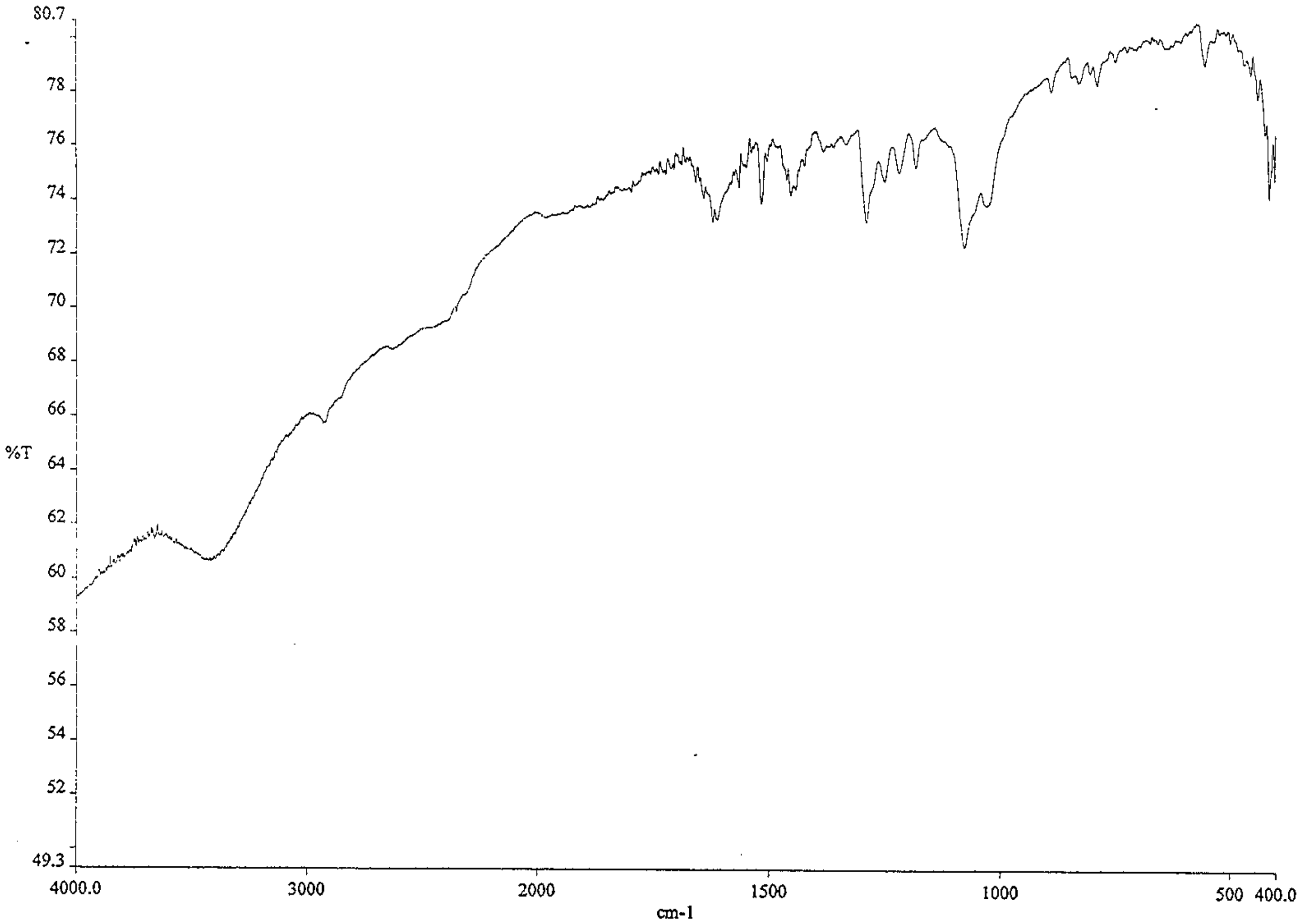


Figure 80 IR (KBr) spectrum of **YS1**

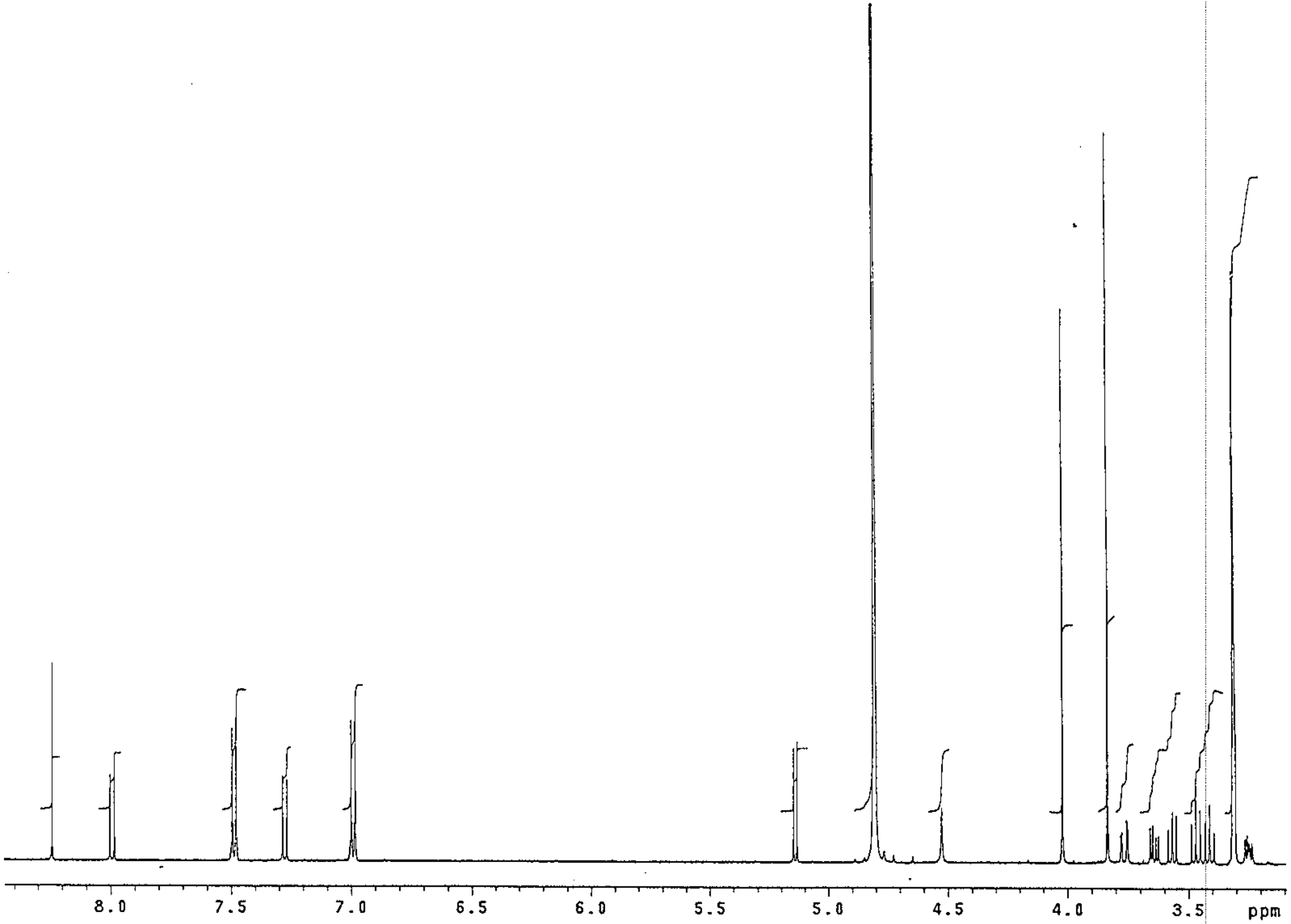


Figure 81 ^{1}H NMR (500 MHz) (CD_3OD) spectrum of **YS1**

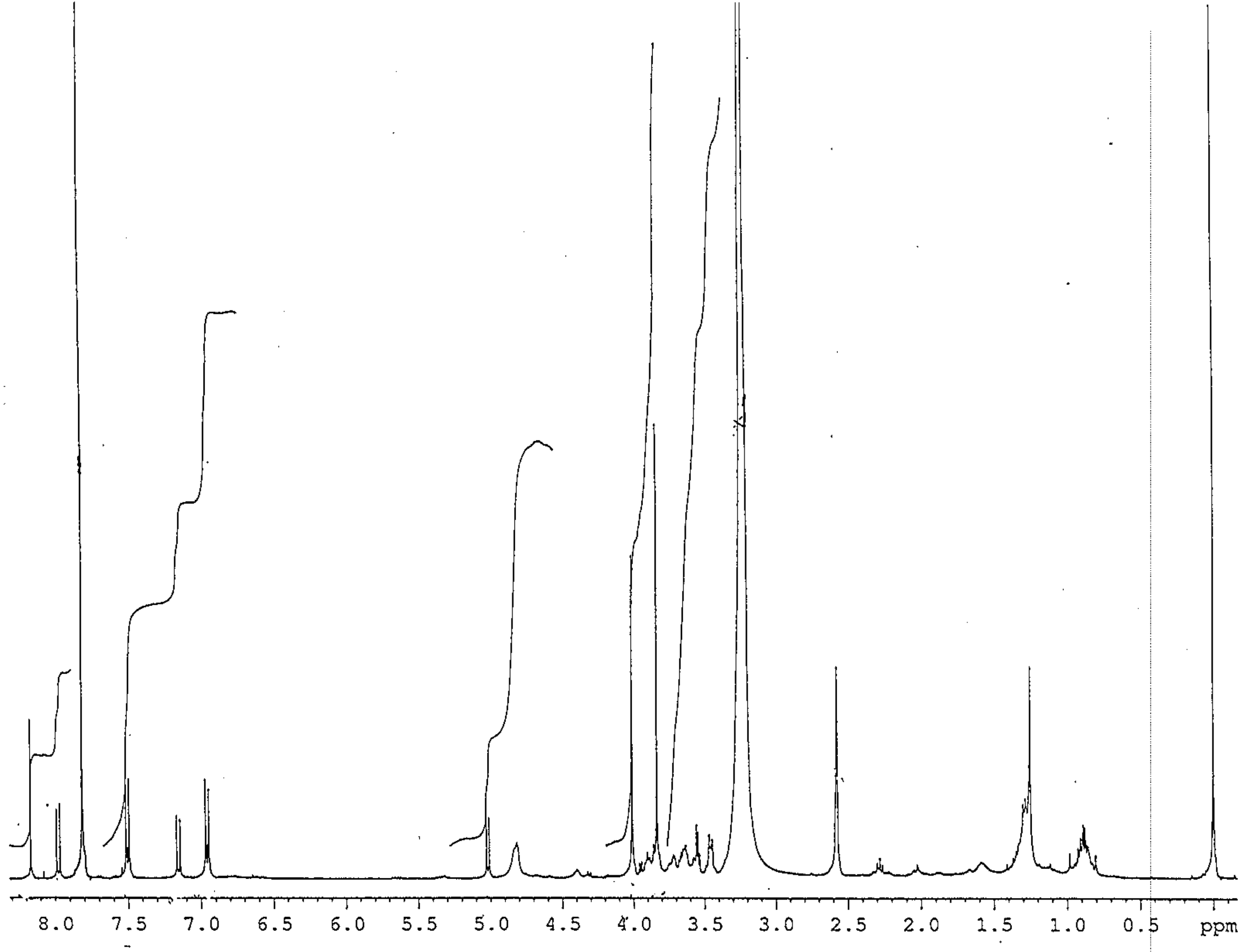


Figure 82 ^{1}H NMR (400 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of **YS1**

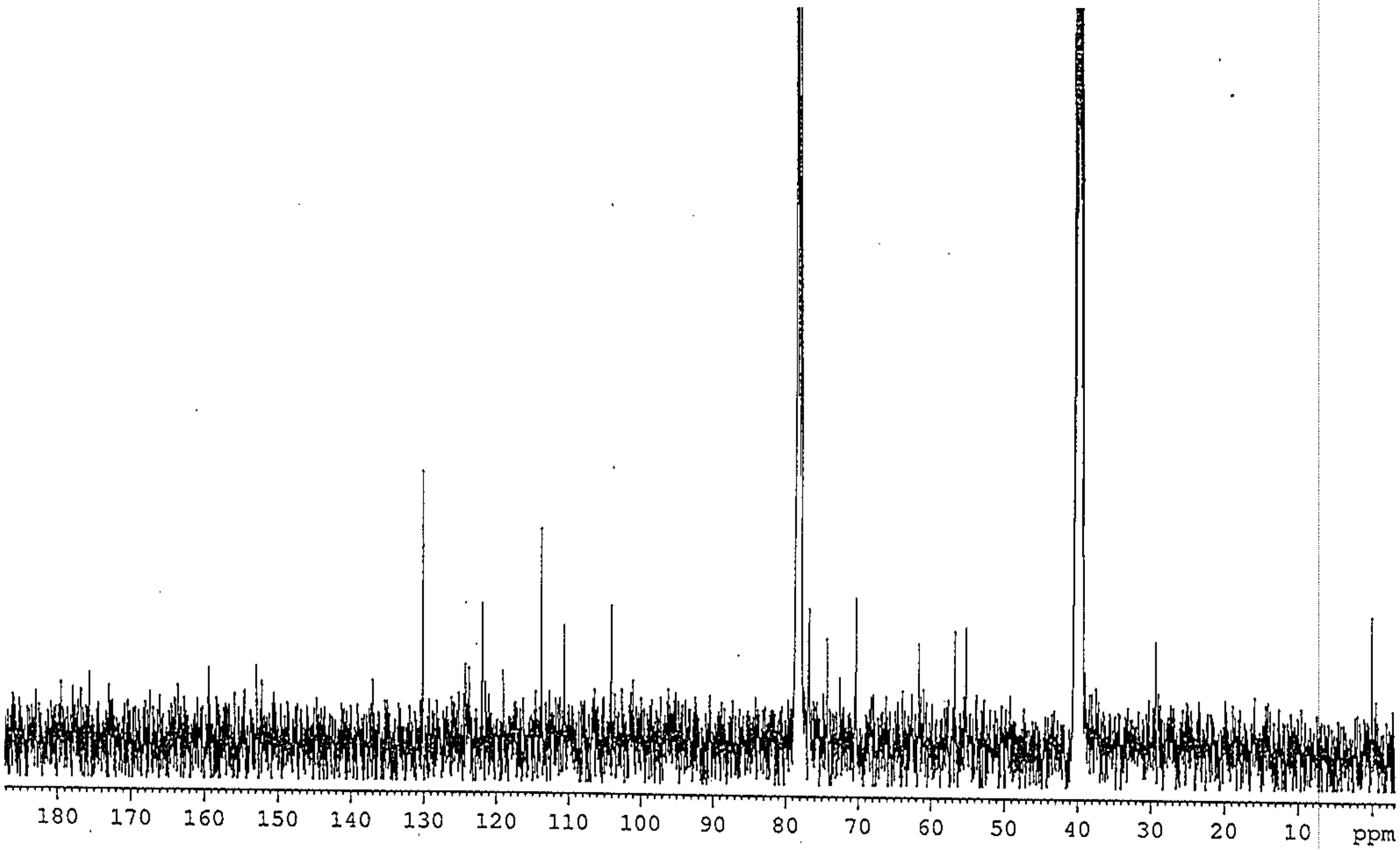


Figure 83 ^{13}C NMR (100 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of **YS1**

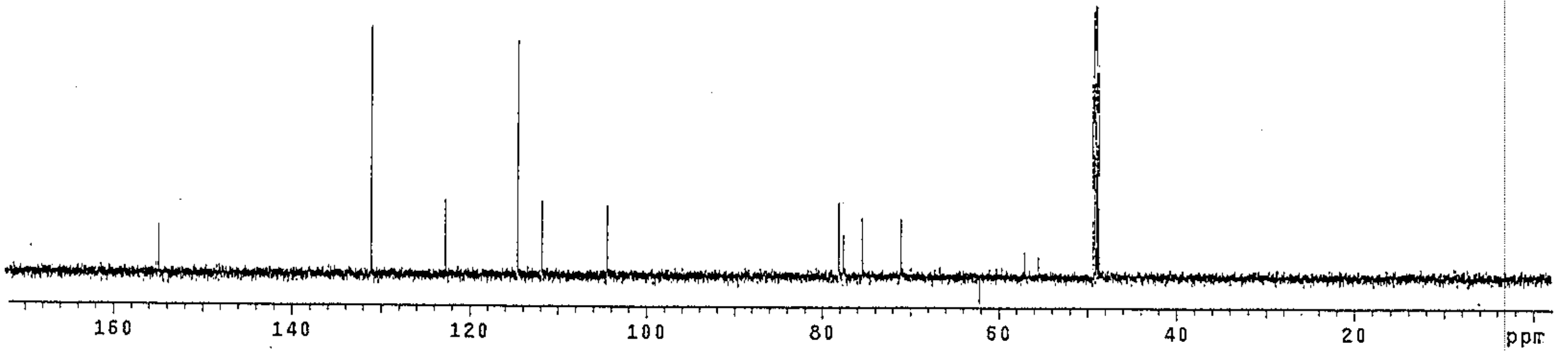


Figure 84 DEPT (135°) (CD_3OD) spectrum of **YS1**

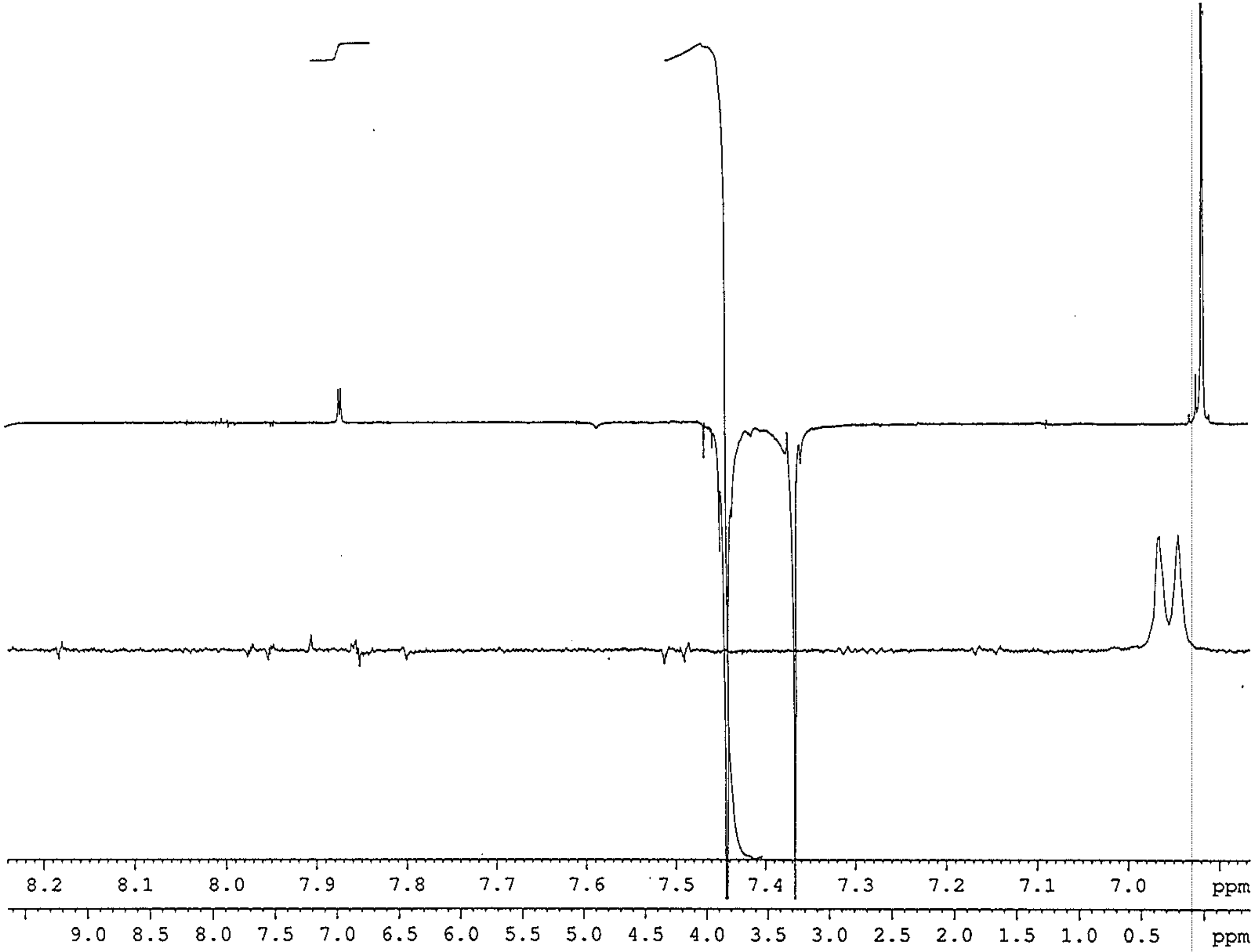


Figure 85 NOEDIFF spectrum of **YS1** after irradiation at δ_H 3.84

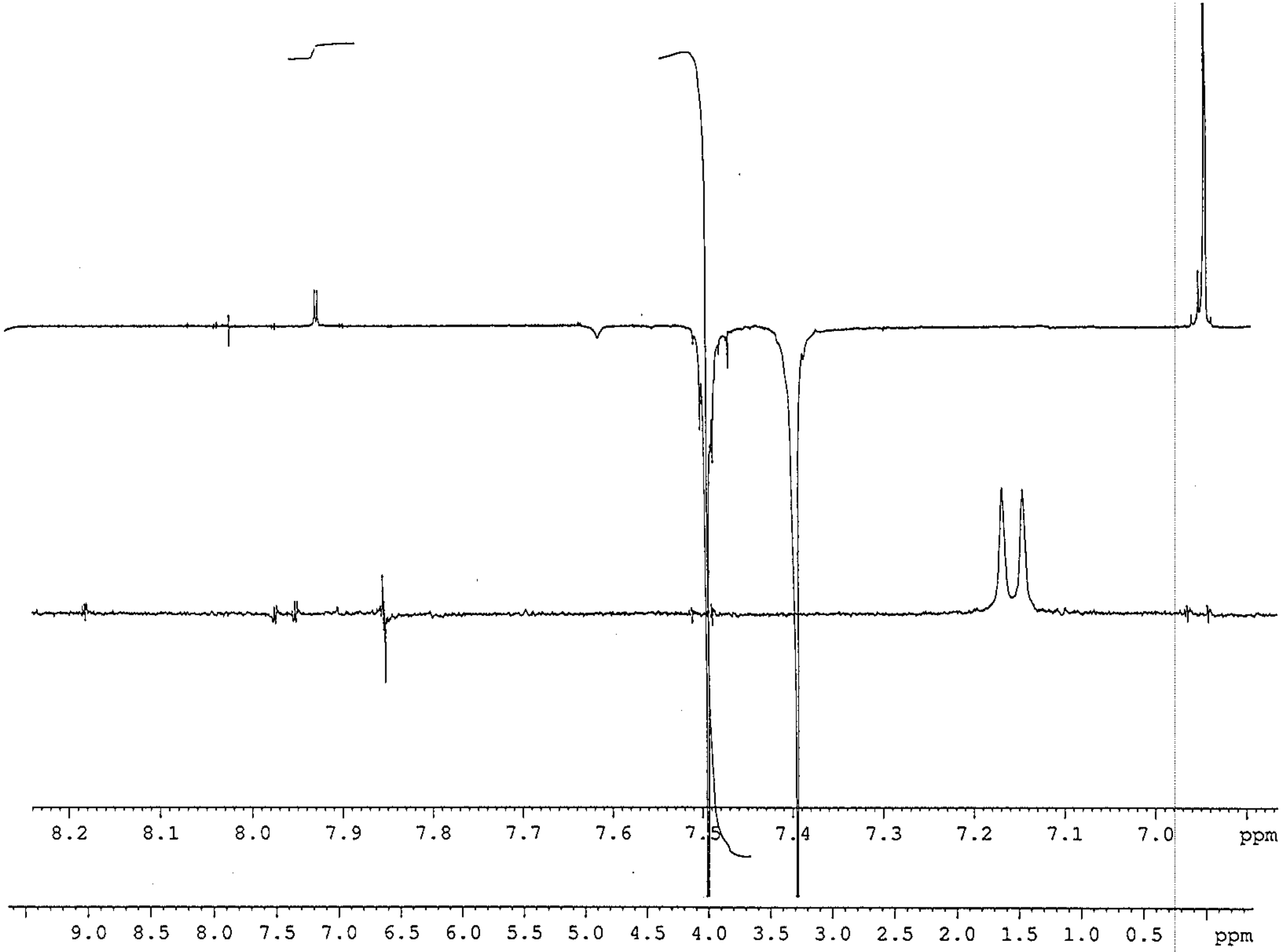


Figure 86 NOEDIFF spectrum of **YS1** after irradiation at δ_H 4.02

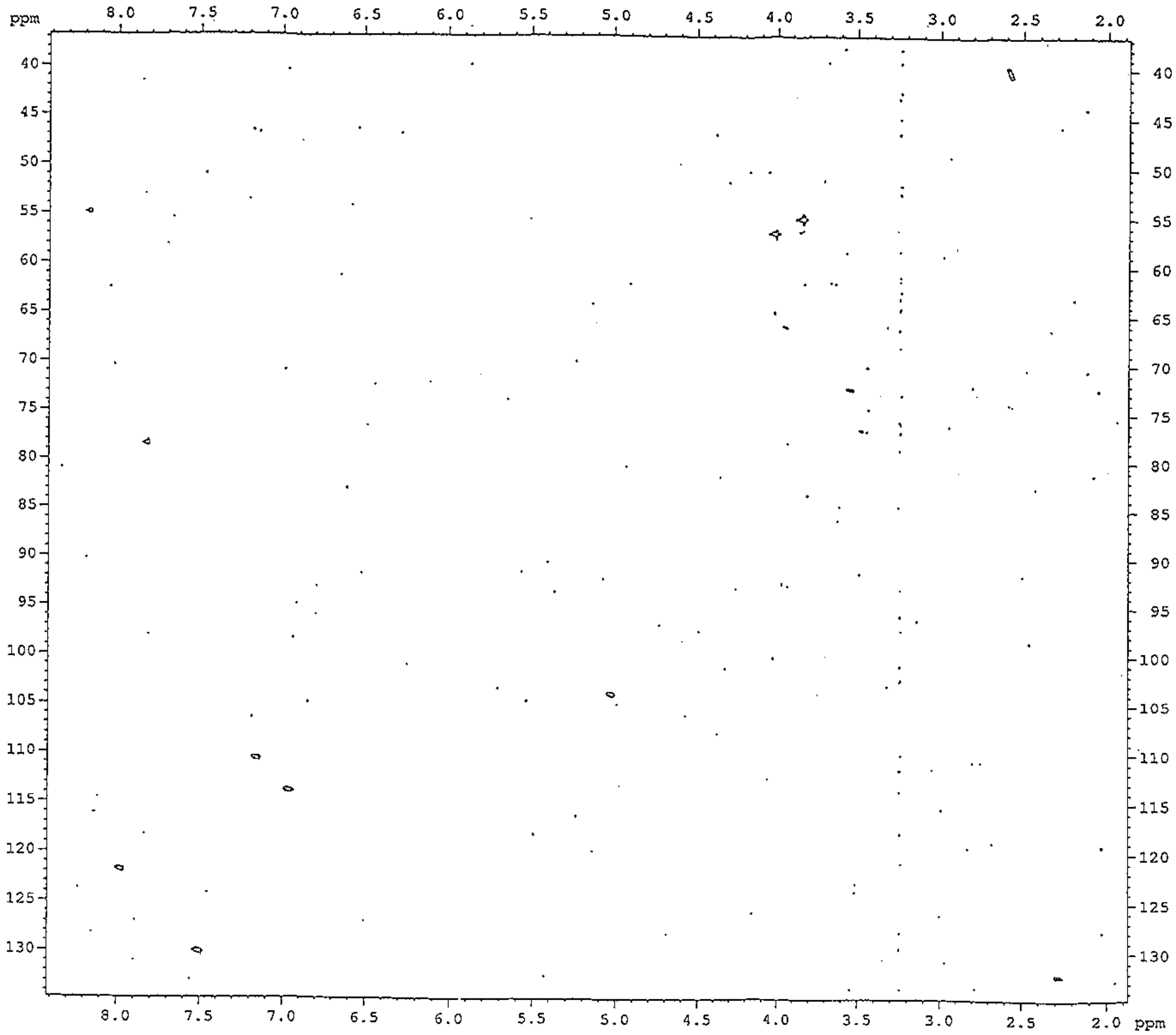


Figure 87 2D HMQC spectrum of **YS1**

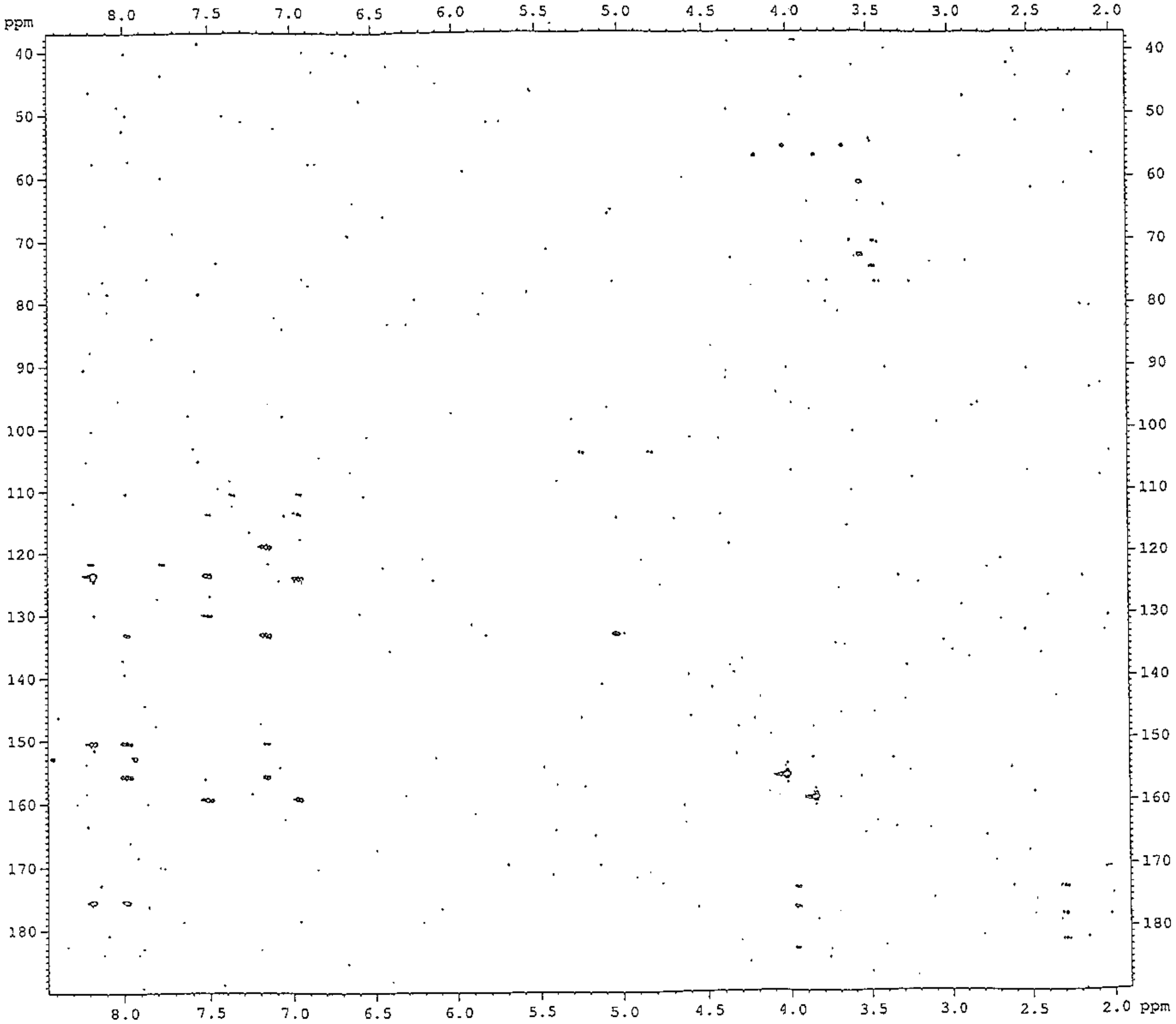


Figure 88 2D HMBC spectrum of YS1

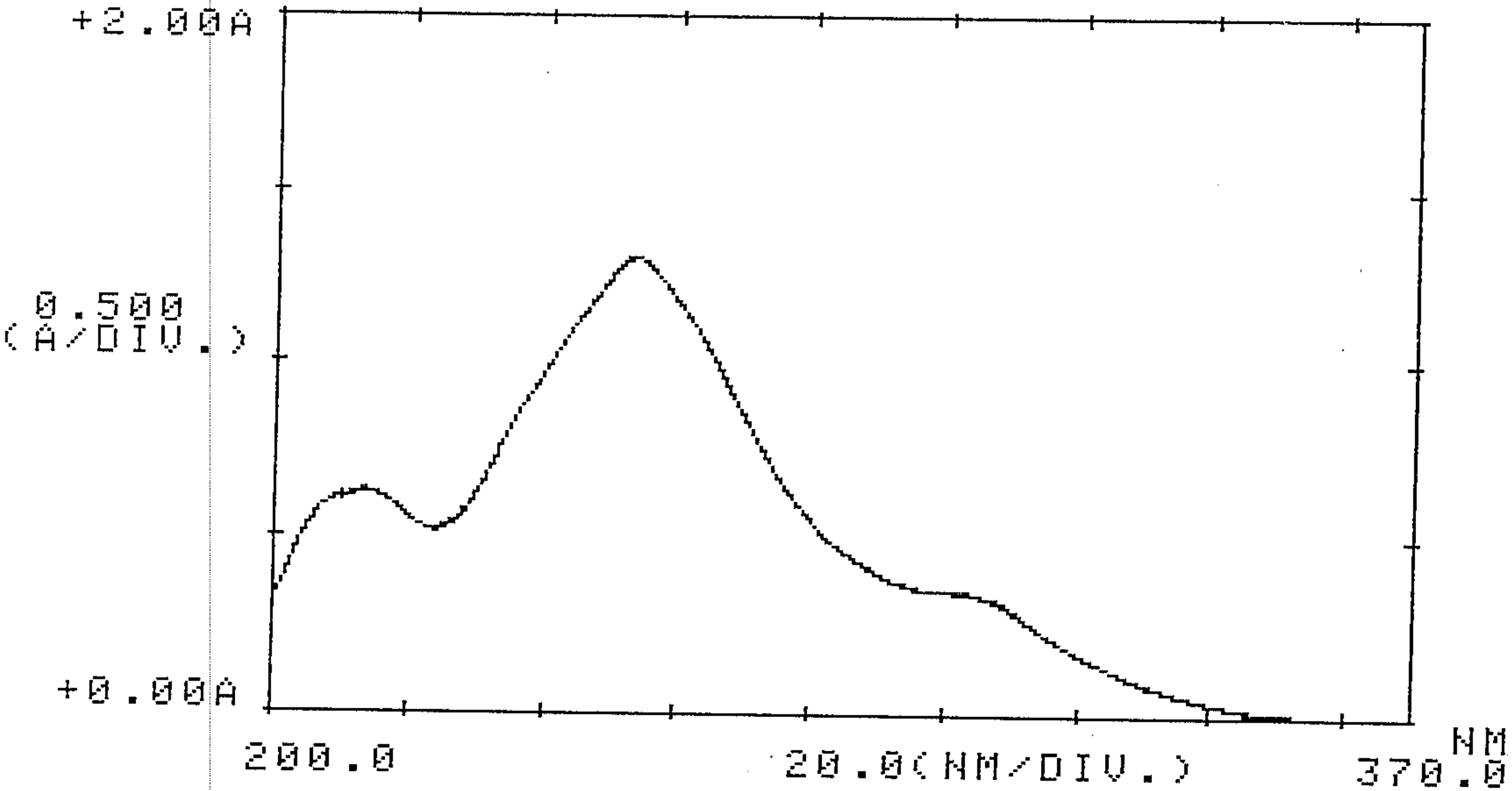


Figure 89 UV ($CHCl_3$) spectrum of **AcYS1**

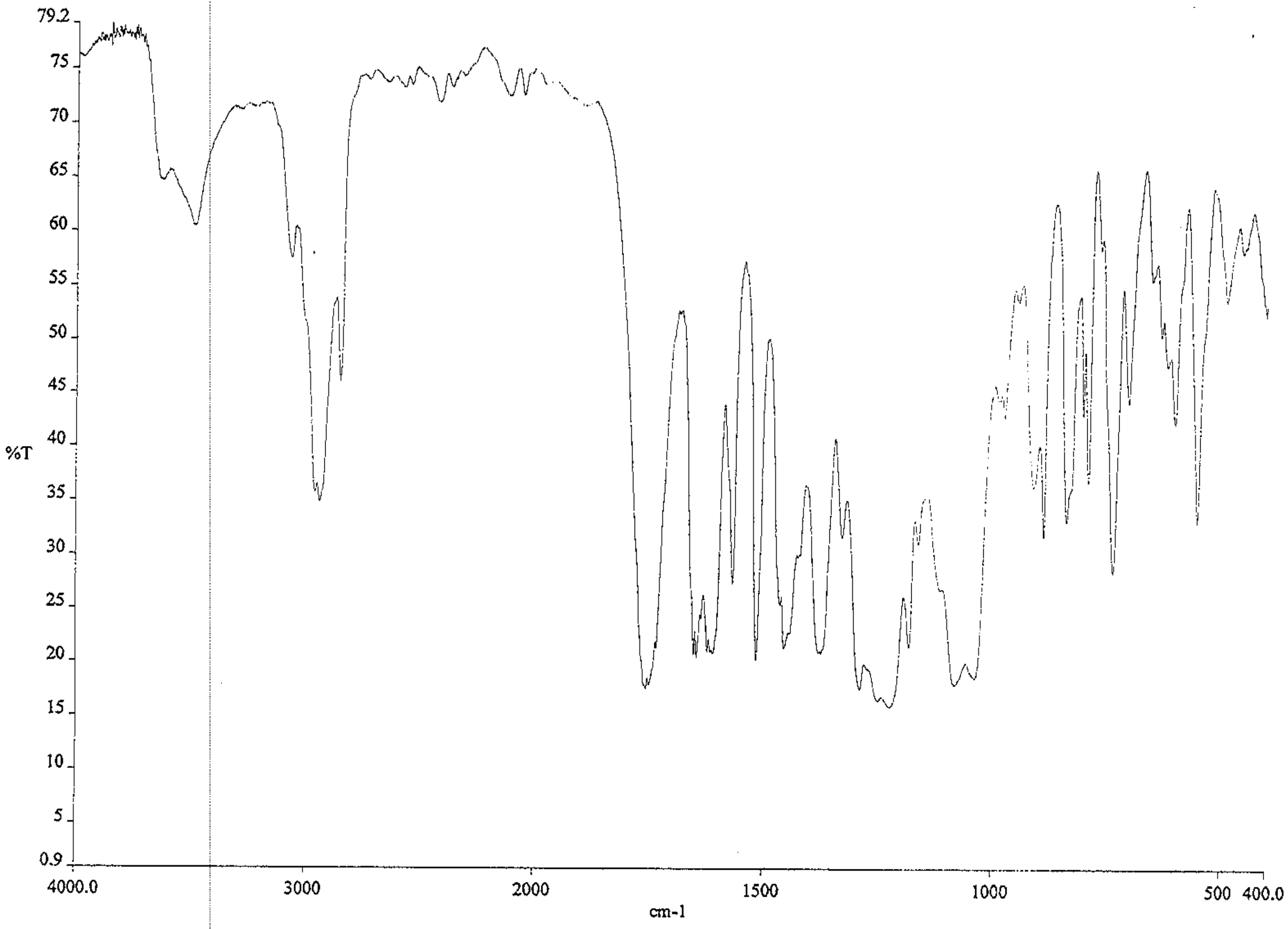


Figure 90 IR (neat) spectrum of **AcYS1**

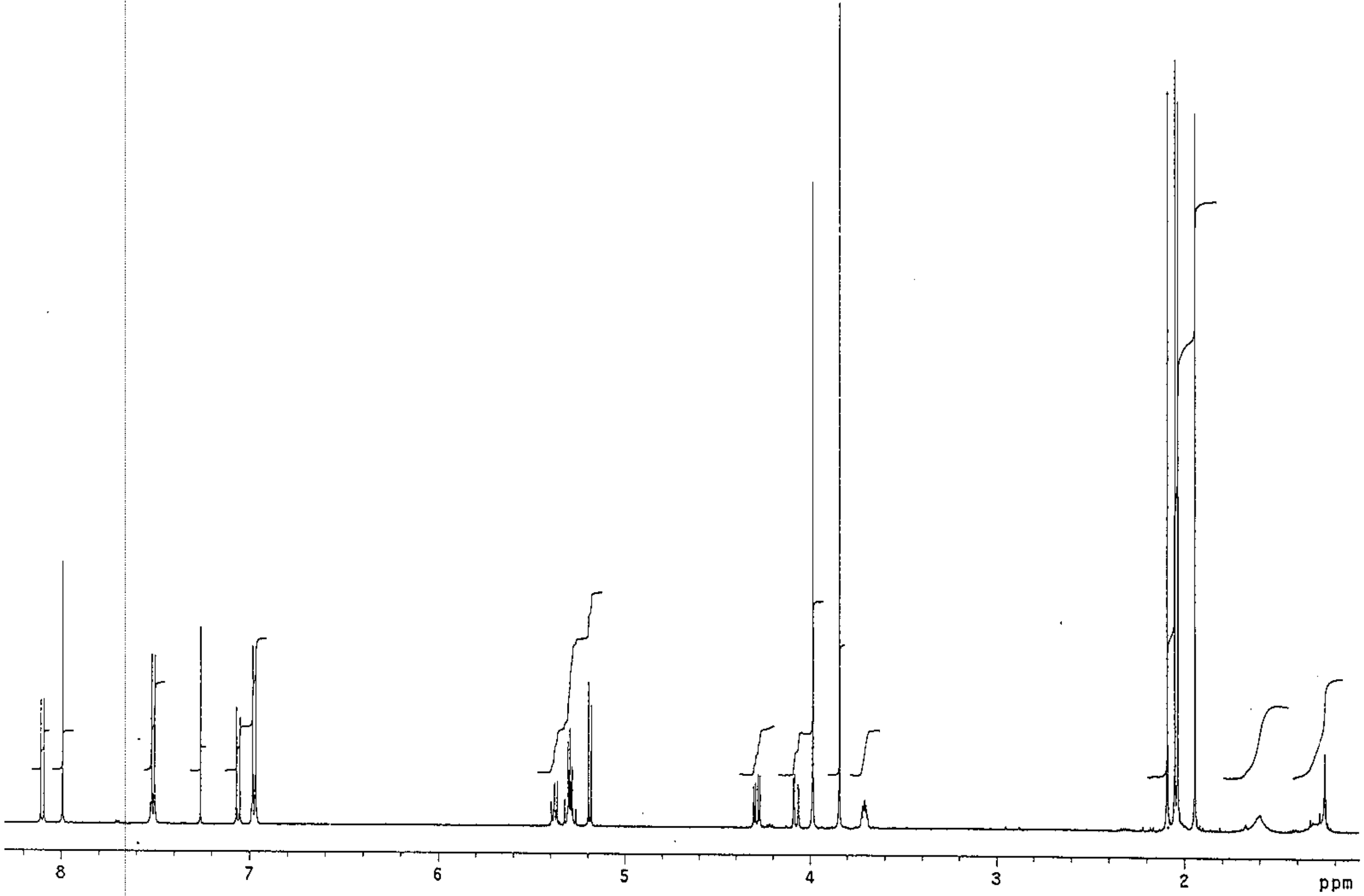


Figure 91 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **AcYS1**

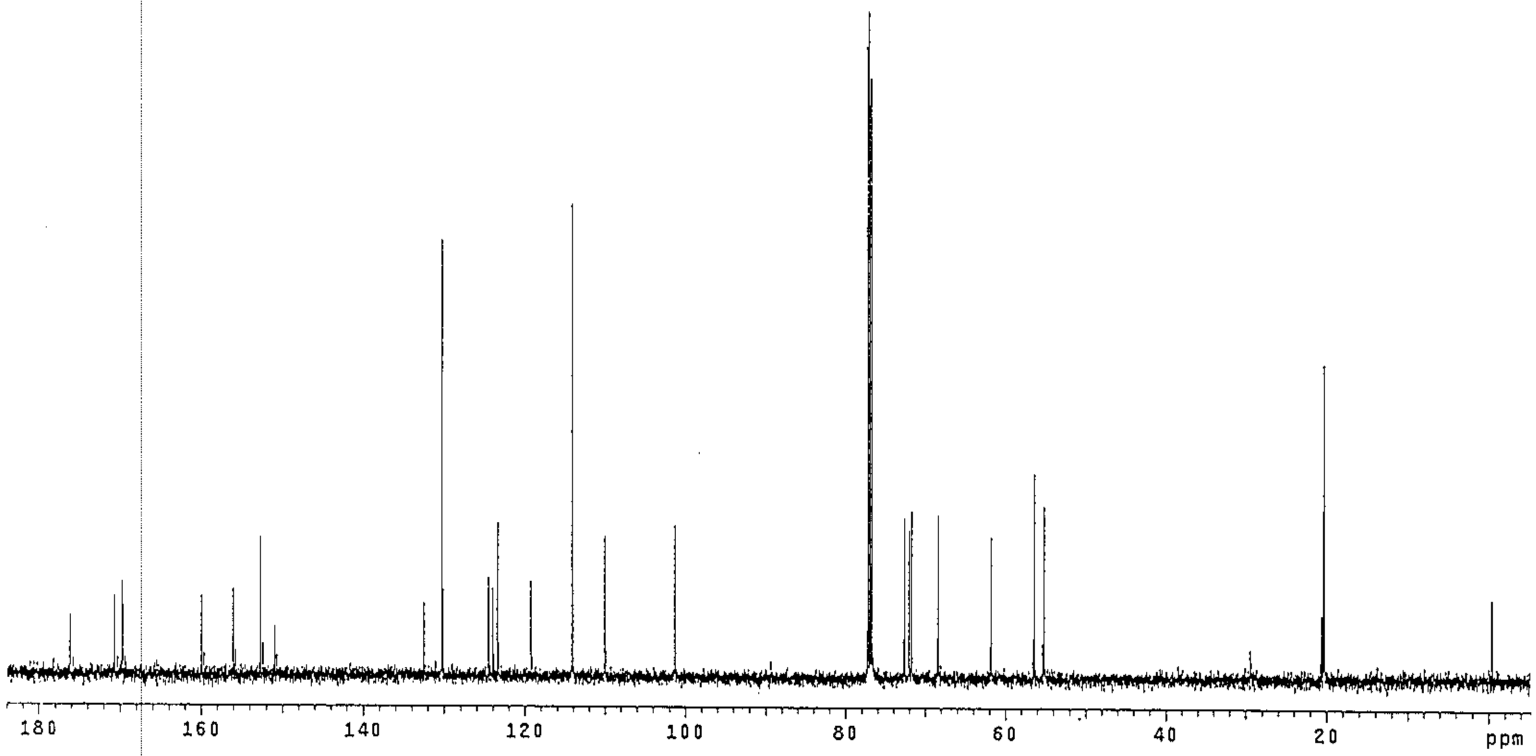


Figure 92 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **AcYS1**

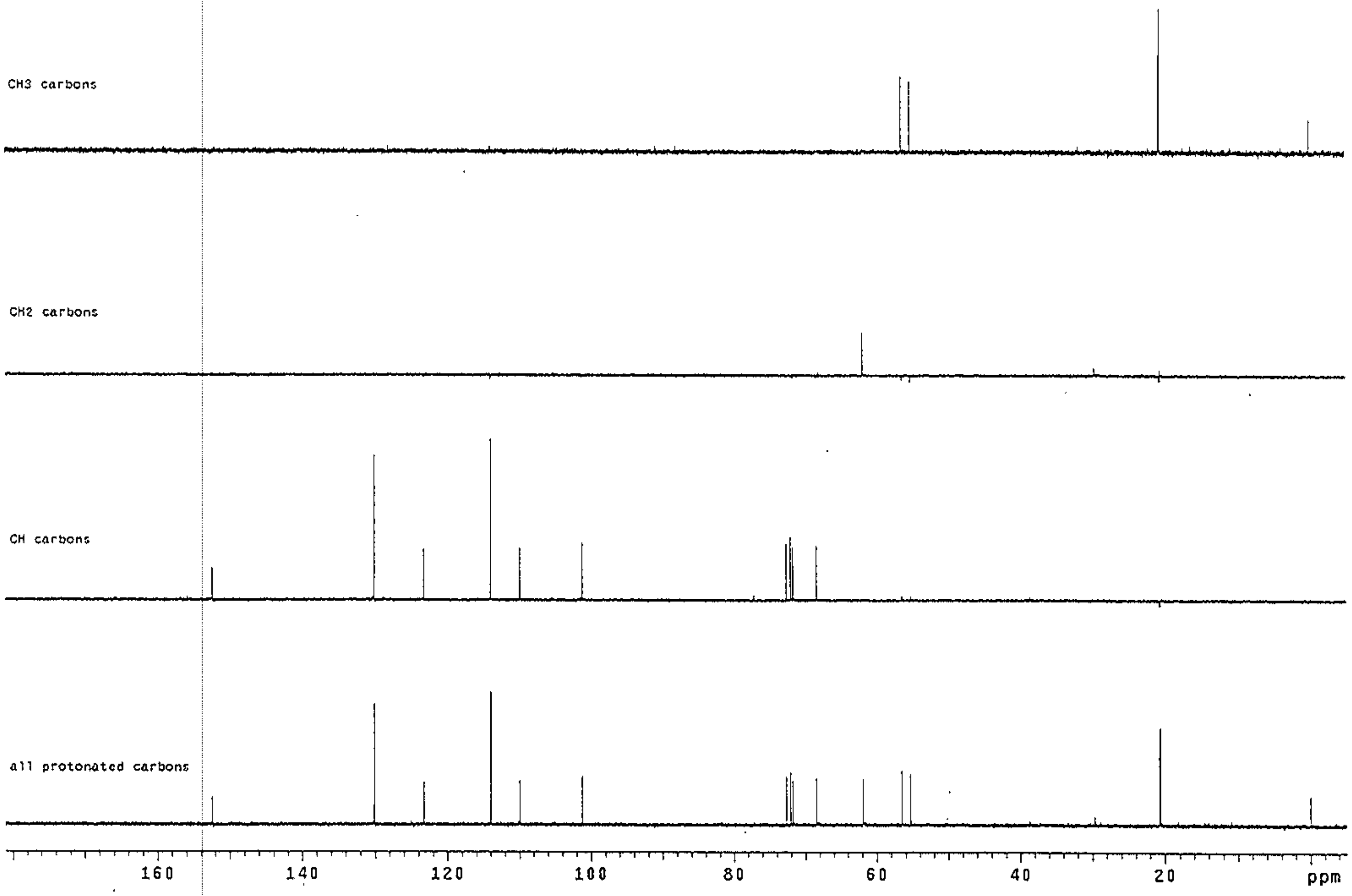


Figure 93 DEPT (135^{O}) ($CDCl_3$) spectrum of **AcYS1**

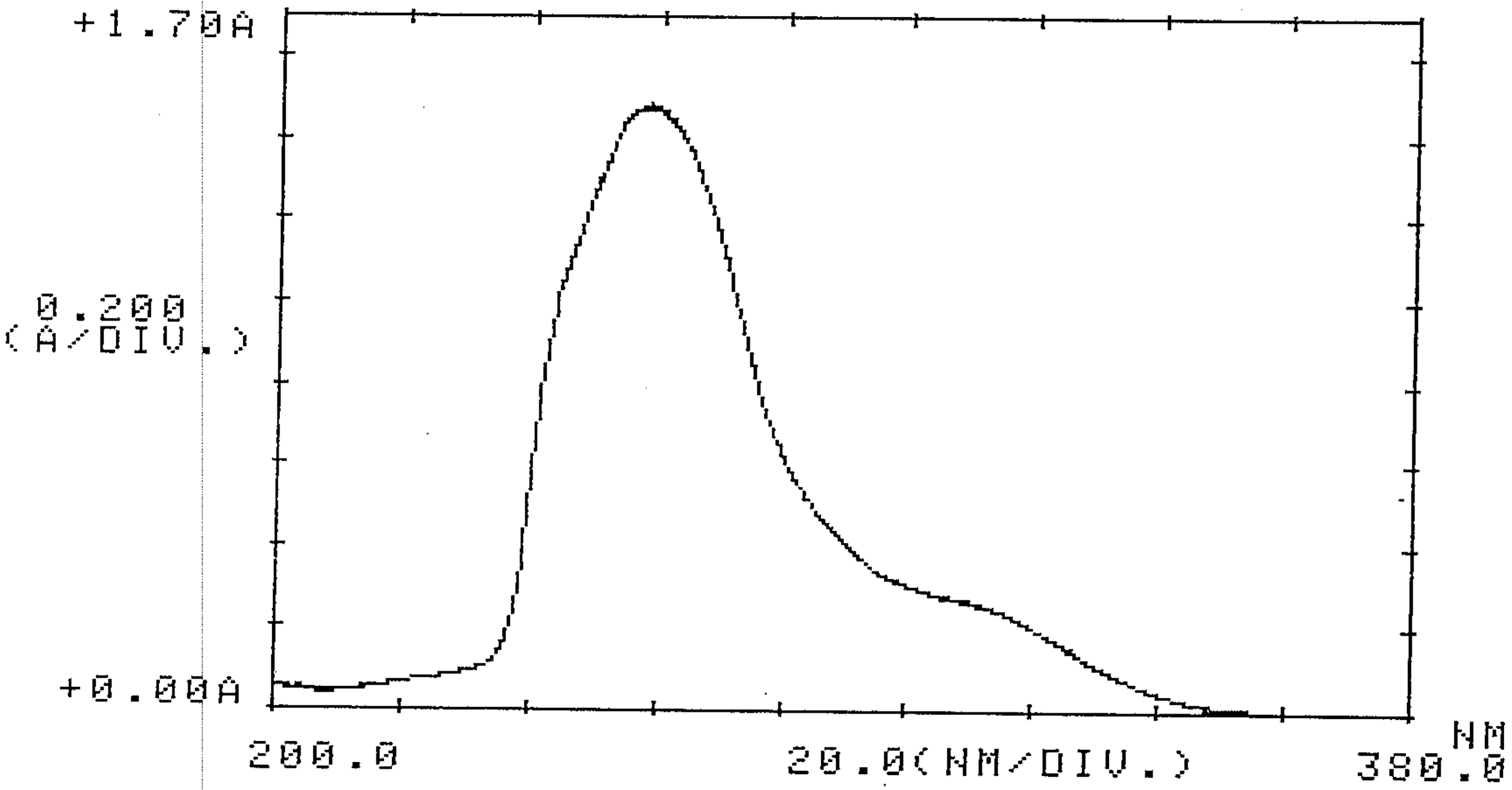


Figure 94 UV ($CHCl_3$) spectrum of **AcYS14**

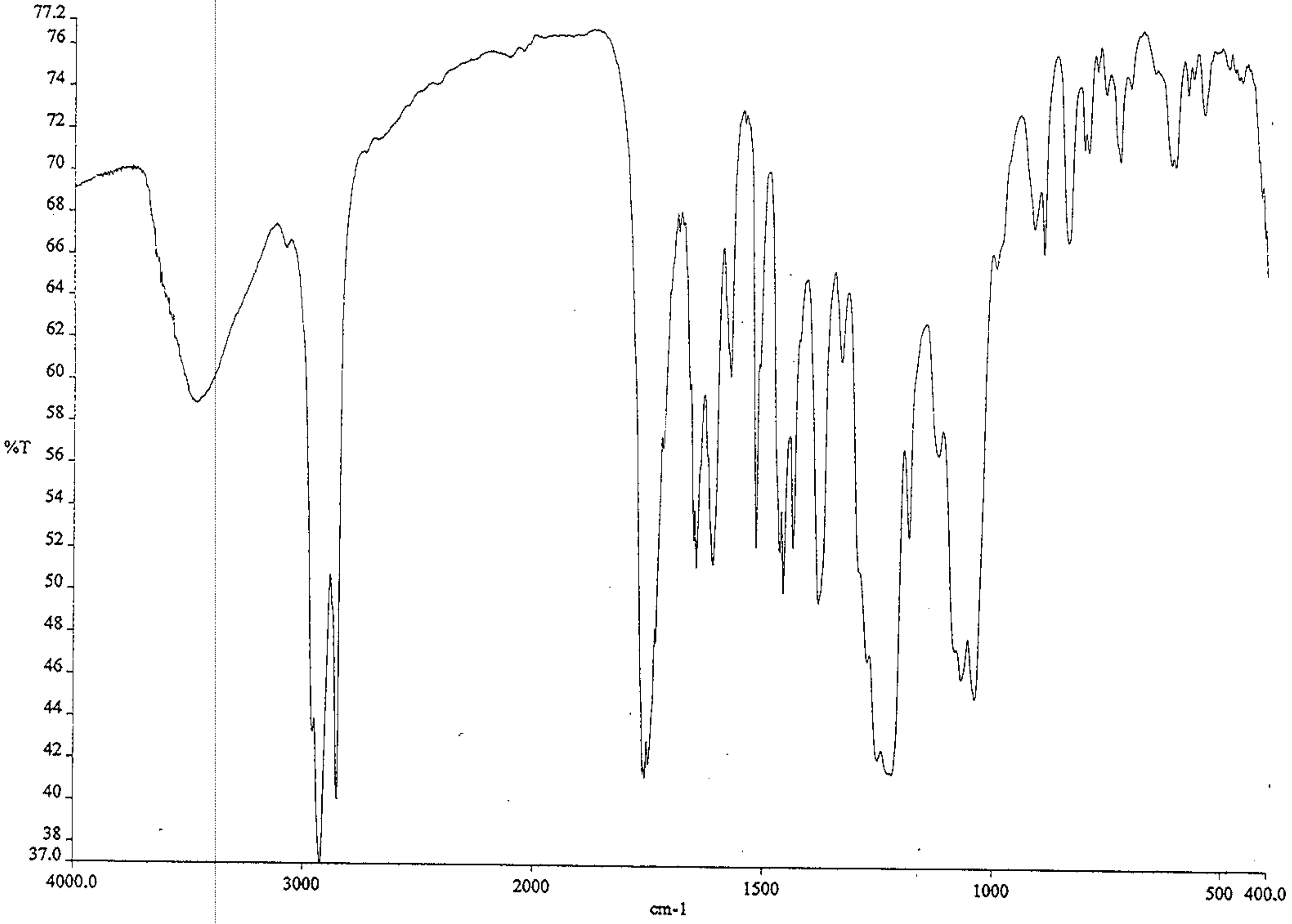


Figure 95 IR (neat) spectrum of **AcYS14**

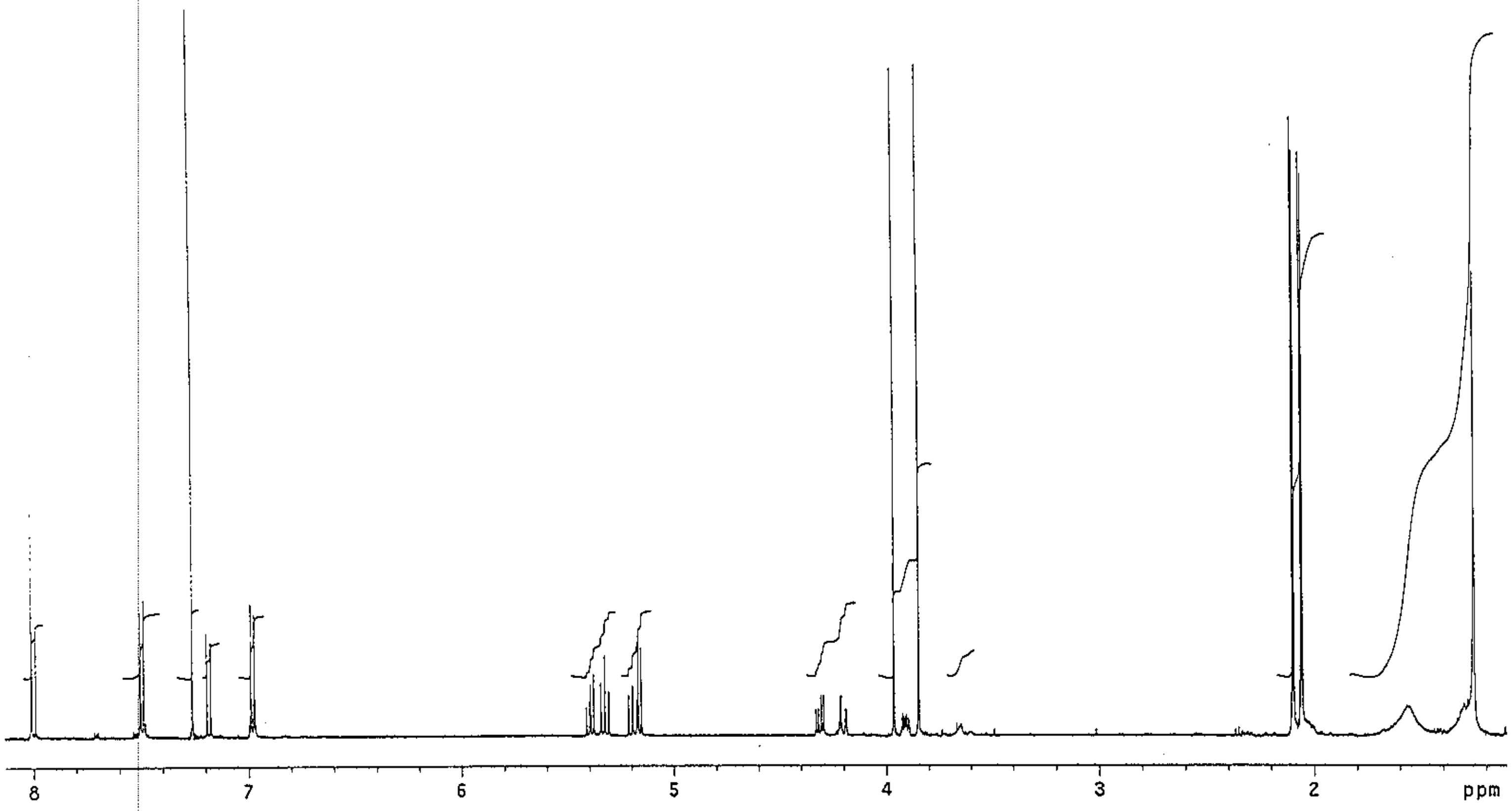


Figure 96 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **AcYS14**

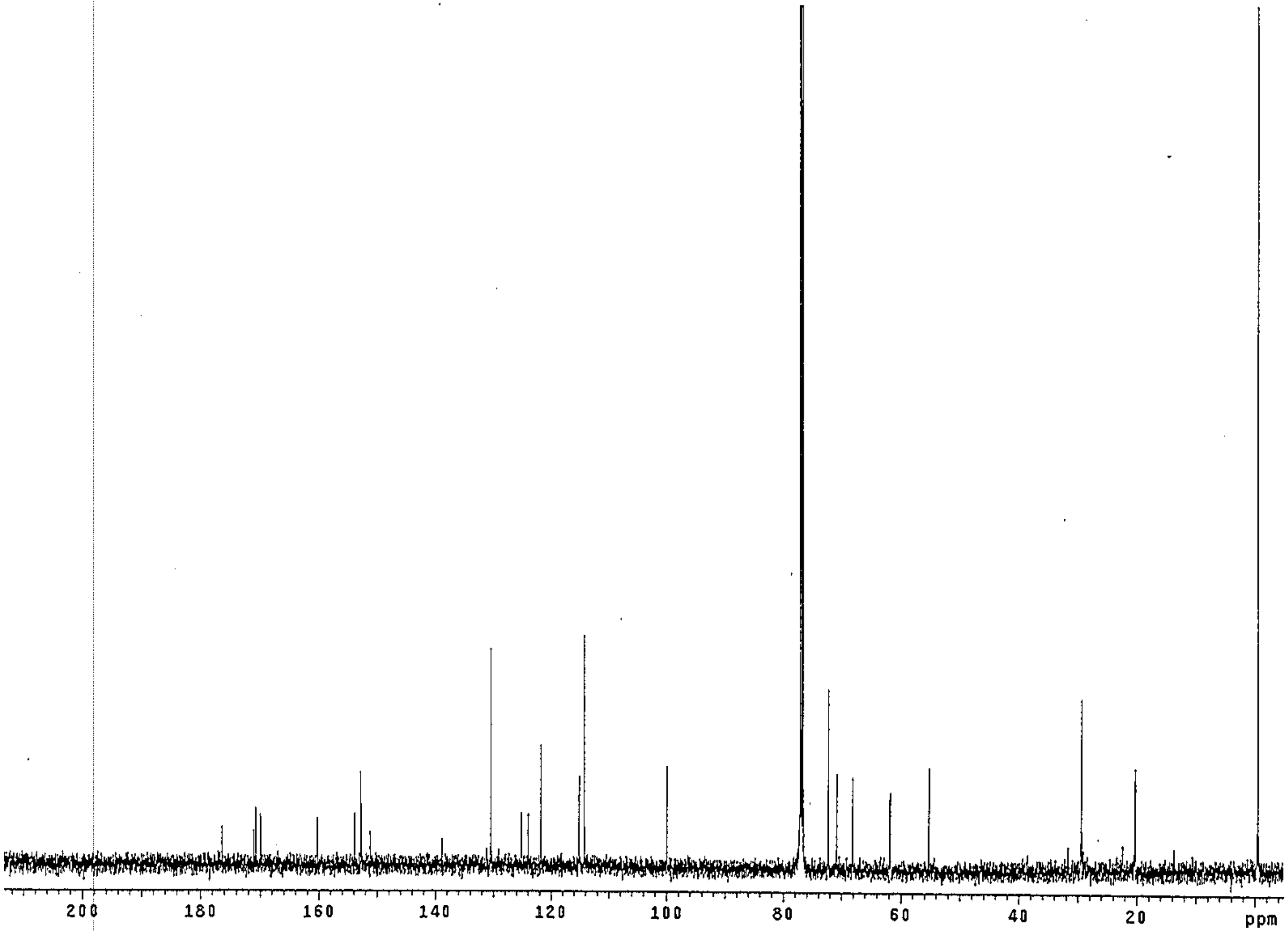


Figure 97 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **AcYS14**

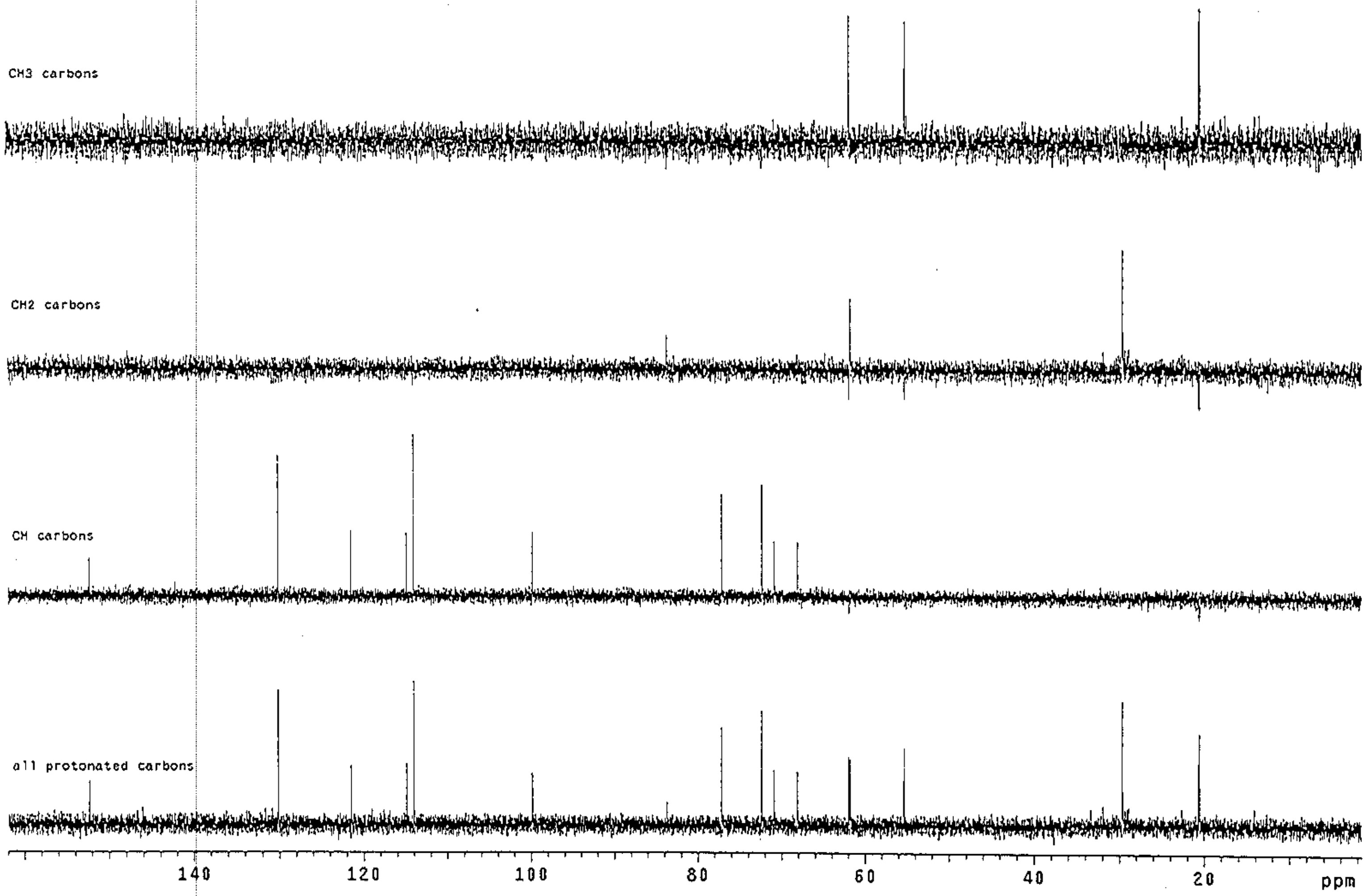


Figure 98 DEPT (135^{O}) ($CDCl_3$) spectrum of **AcYS14**

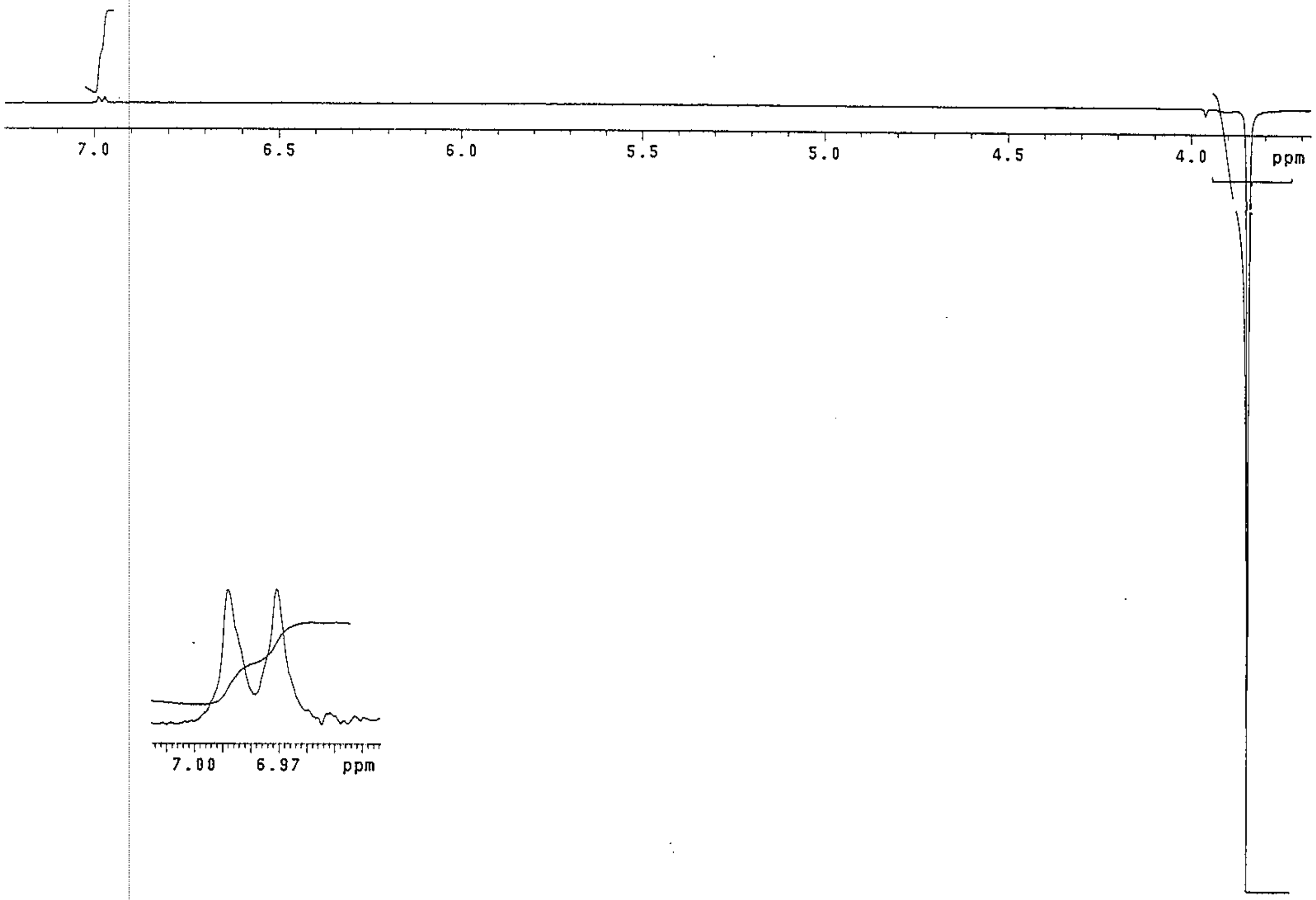


Figure 99 NOEDIFF spectrum of **AcYS14** after irradiation at δ_H 3.85

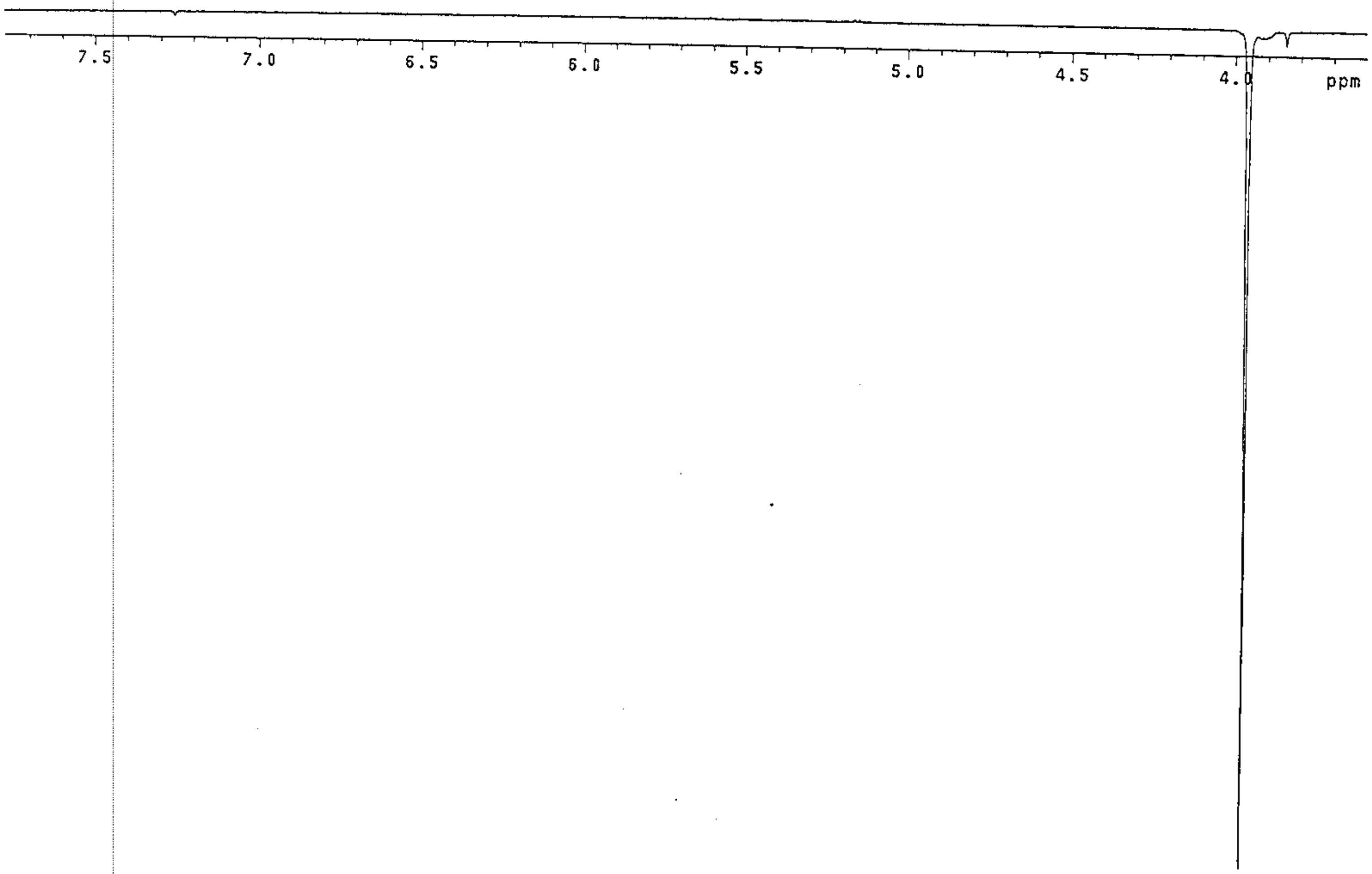


Figure 100 NOEDIFF spectrum of **AcYS14** after irradiation at δ_H 3.96

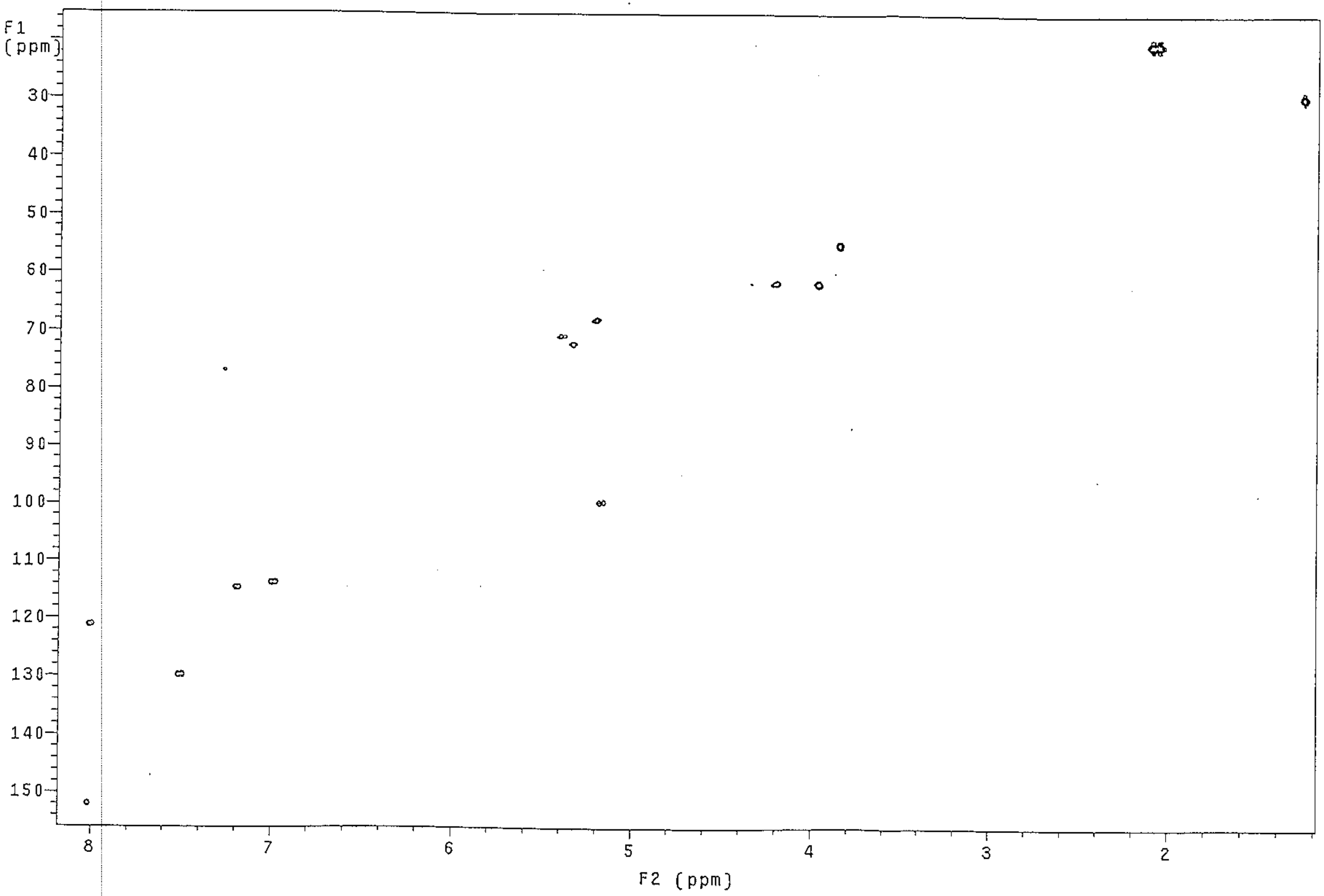


Figure 101 2D HMQC spectrum of **AcYS14**

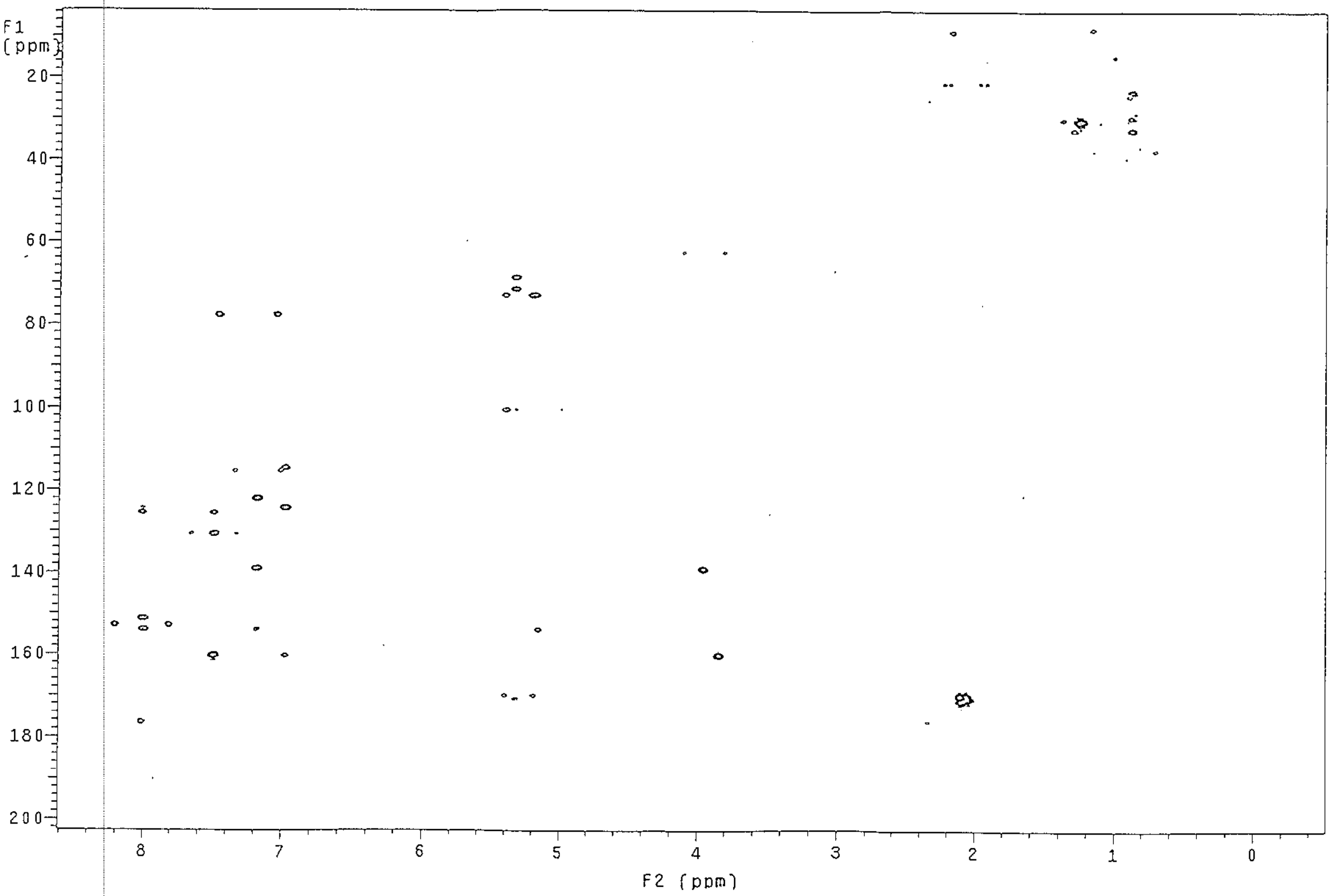


Figure 102 2D HMBC spectrum of **AcYS14**

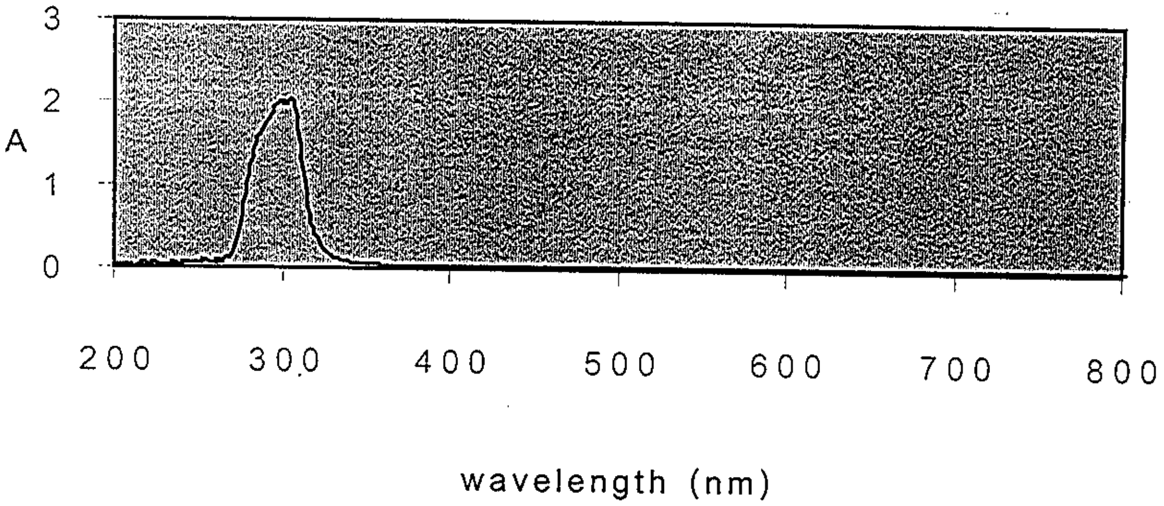


Figure 103 UV ($CHCl_3$) spectrum of **AcYS5**

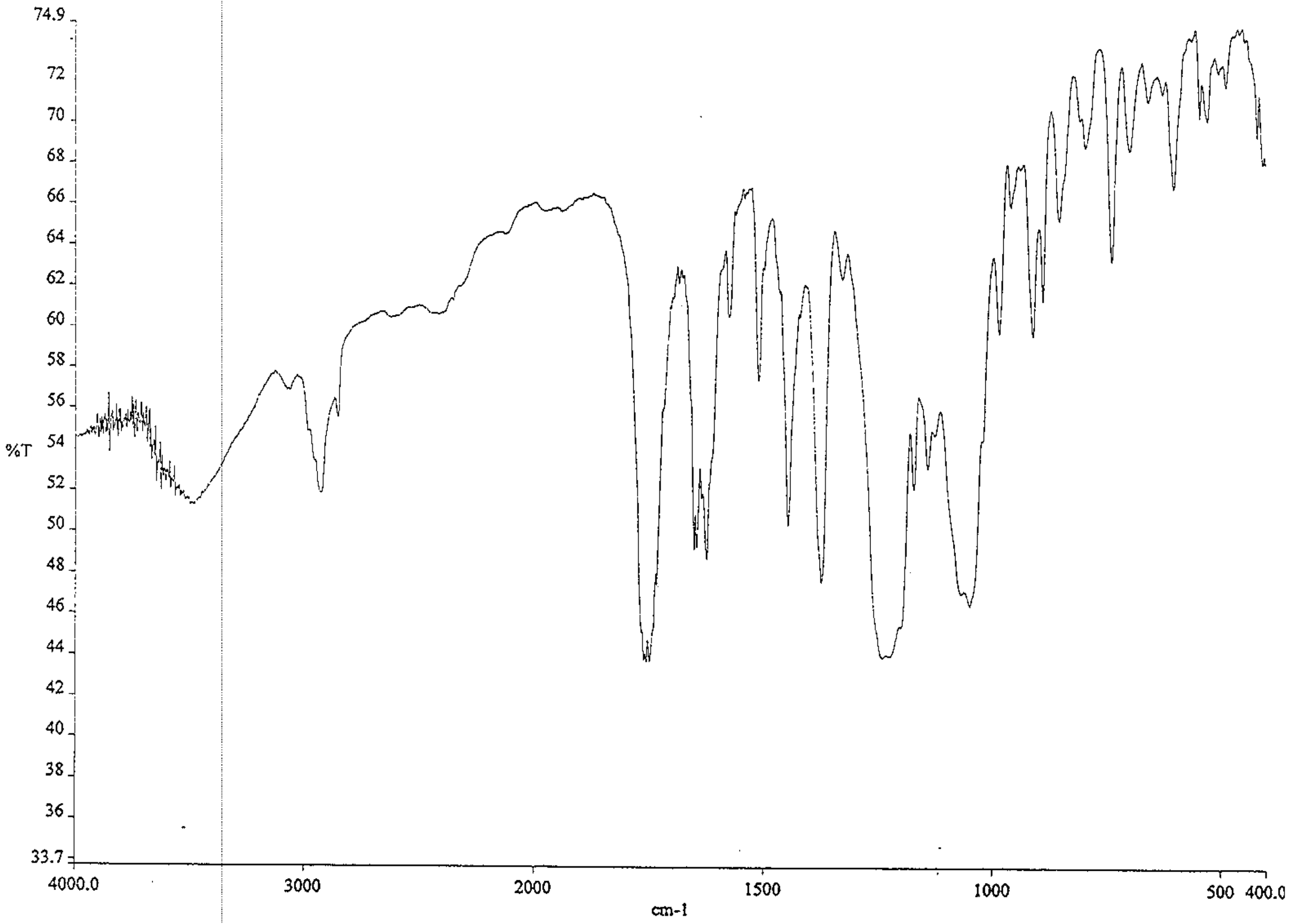


Figure 104 IR (neat) spectrum of **AcYS5**

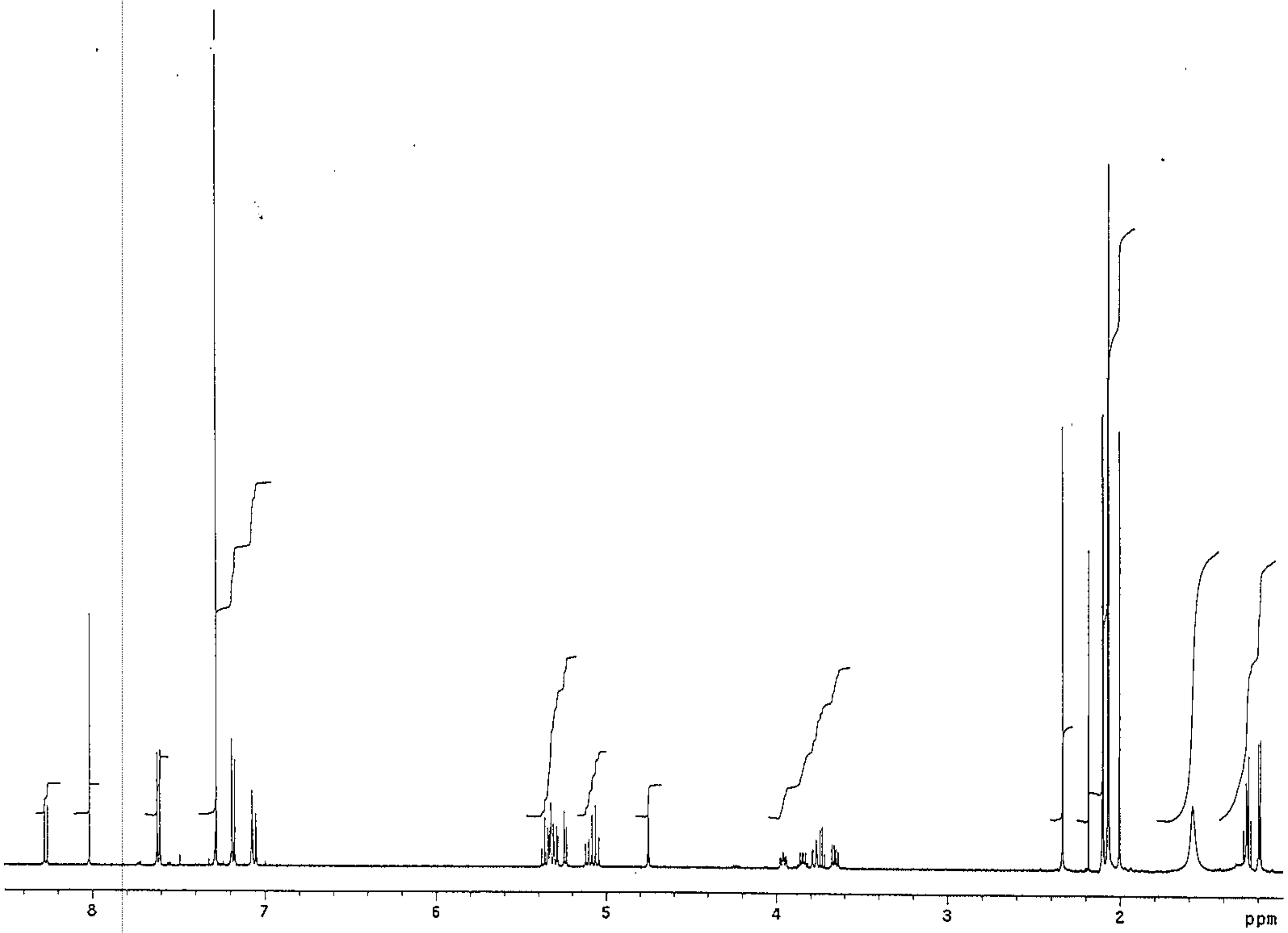


Figure 105 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **AcYS5**

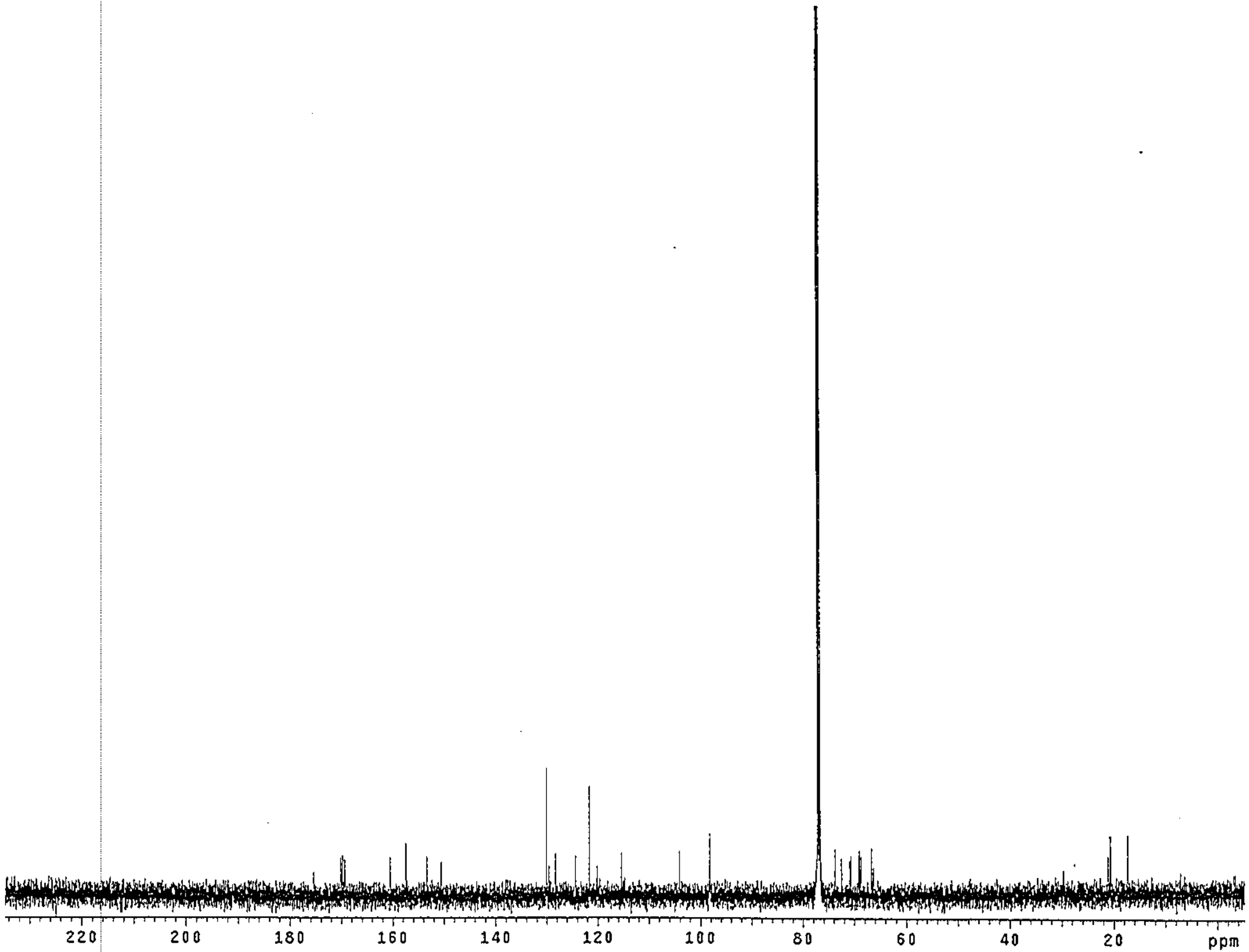


Figure 106 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **AcYS5**

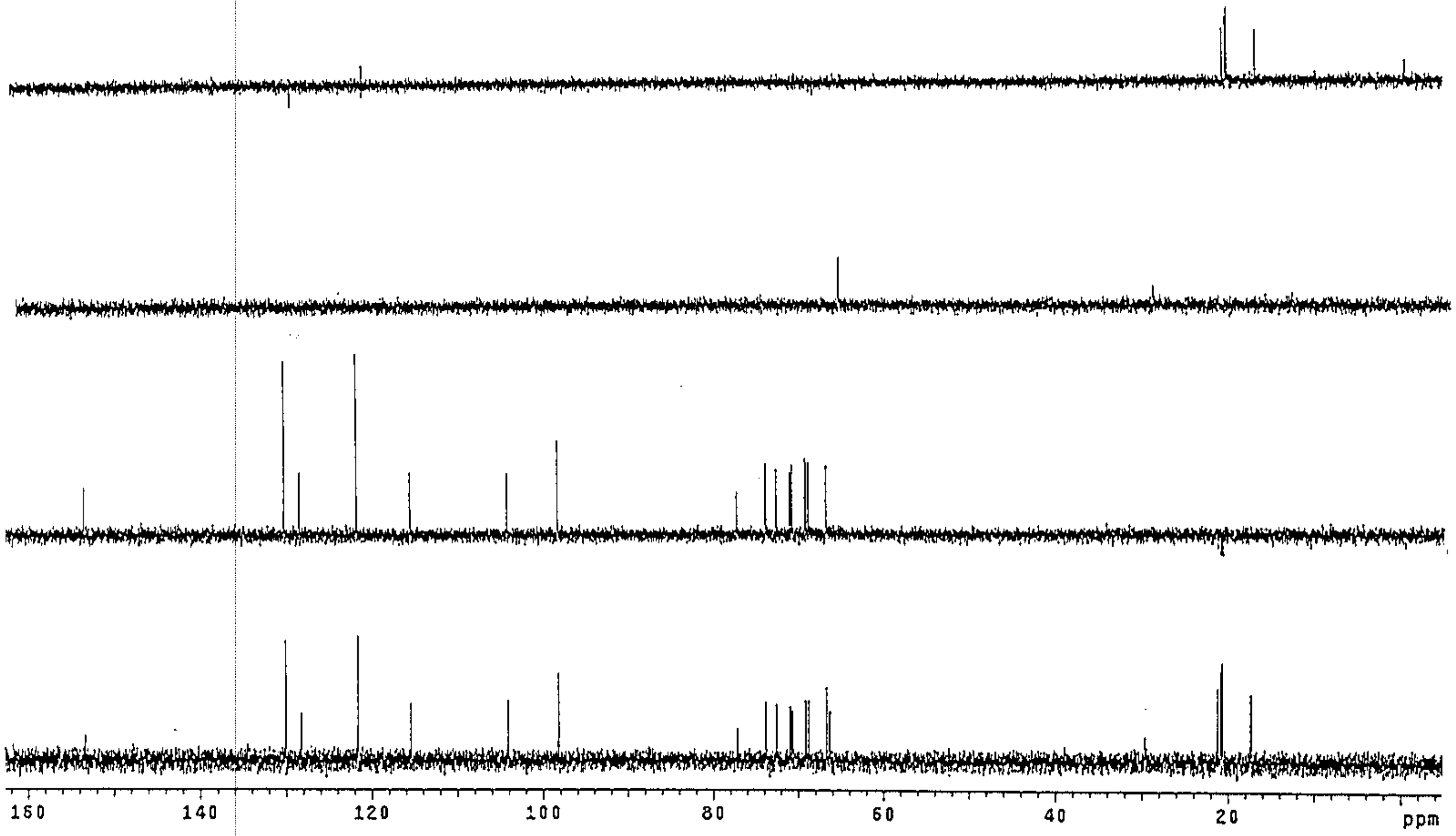


Figure 107 DEPT (135^{O}) (CDCl$_3$) spectrum of **AcYS5**

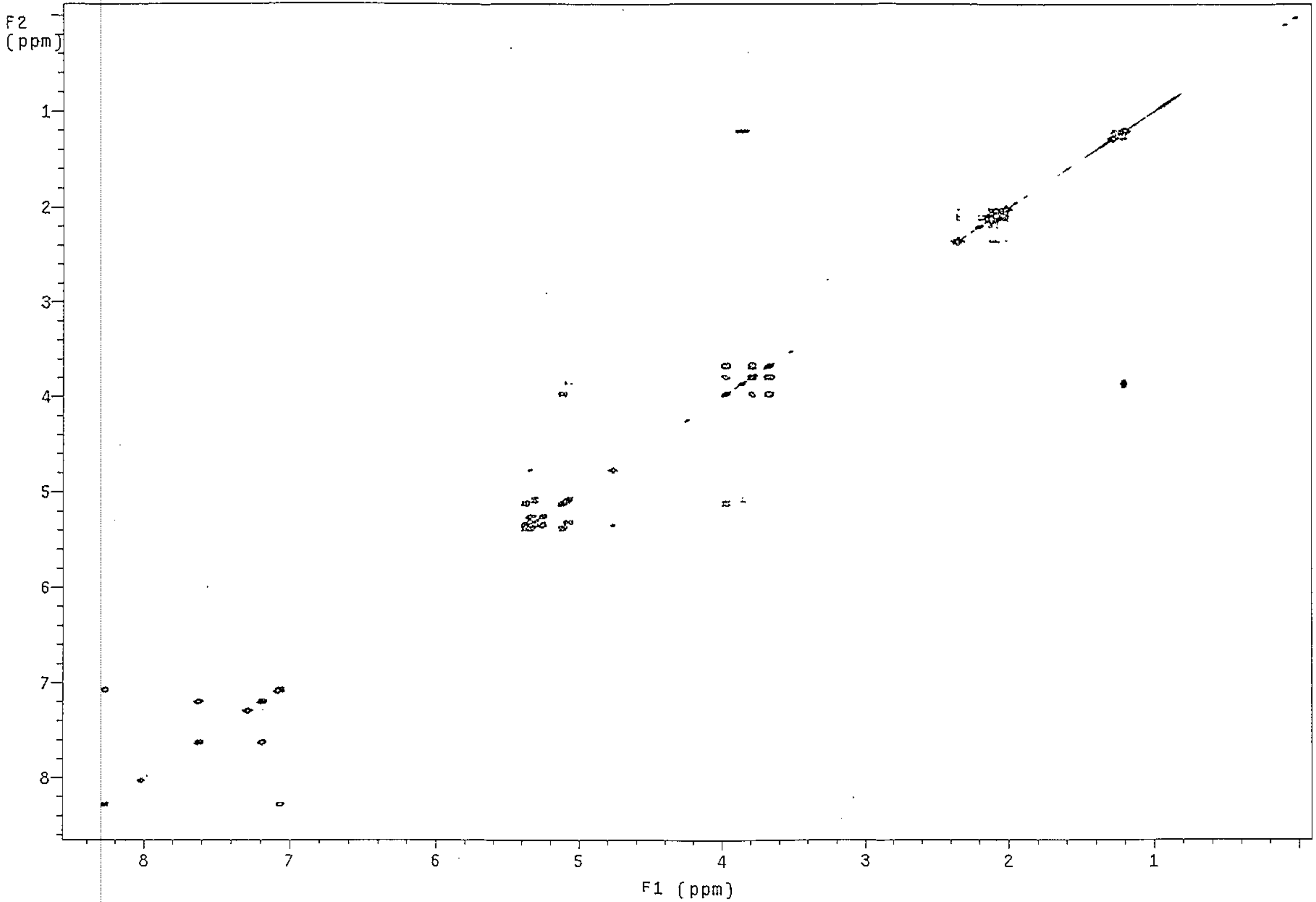


Figure 108 ^{1}H-^{1}H COSY spectrum of AcYS5

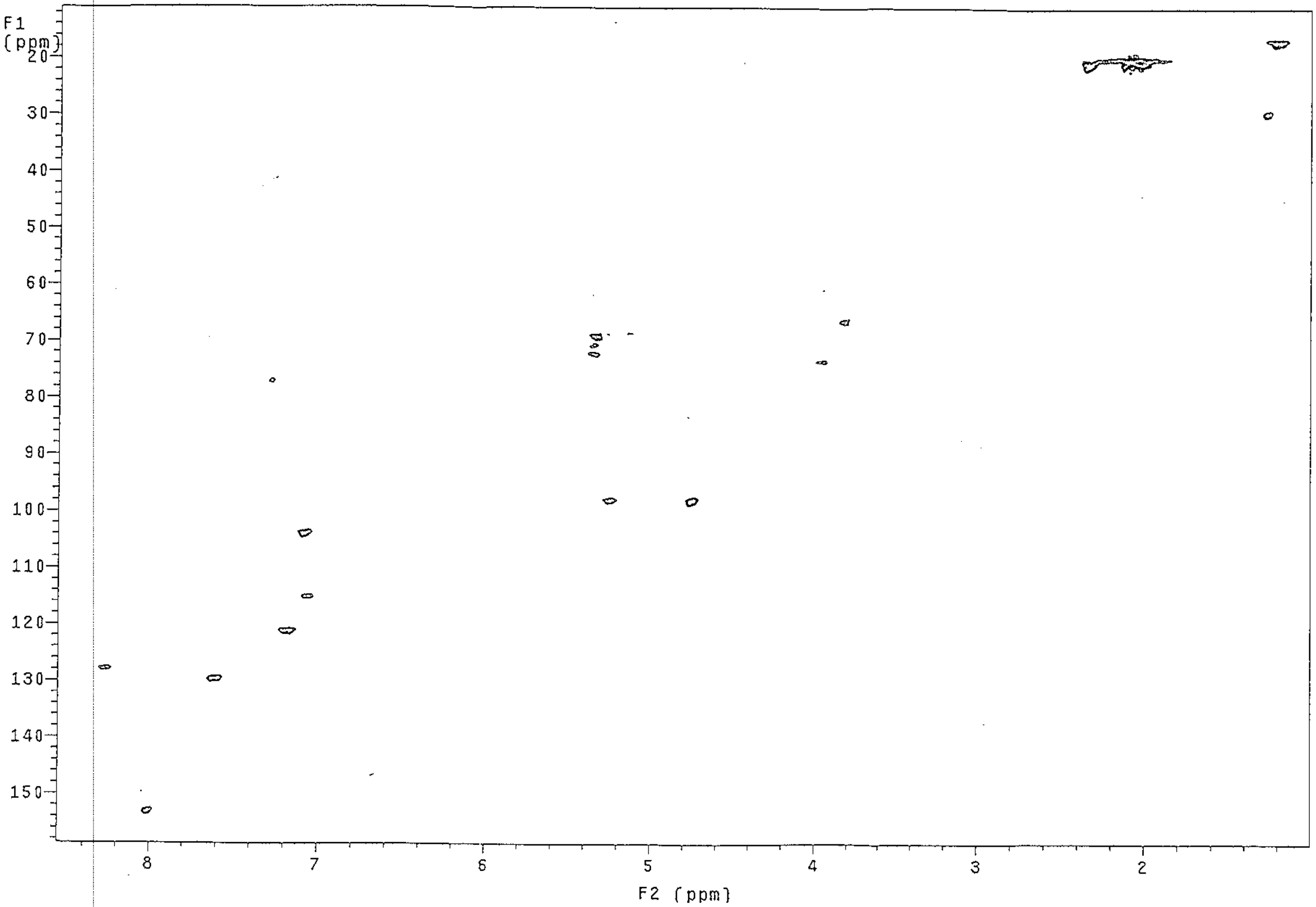


Figure 109 2D HMQC spectrum of **AcYS5**

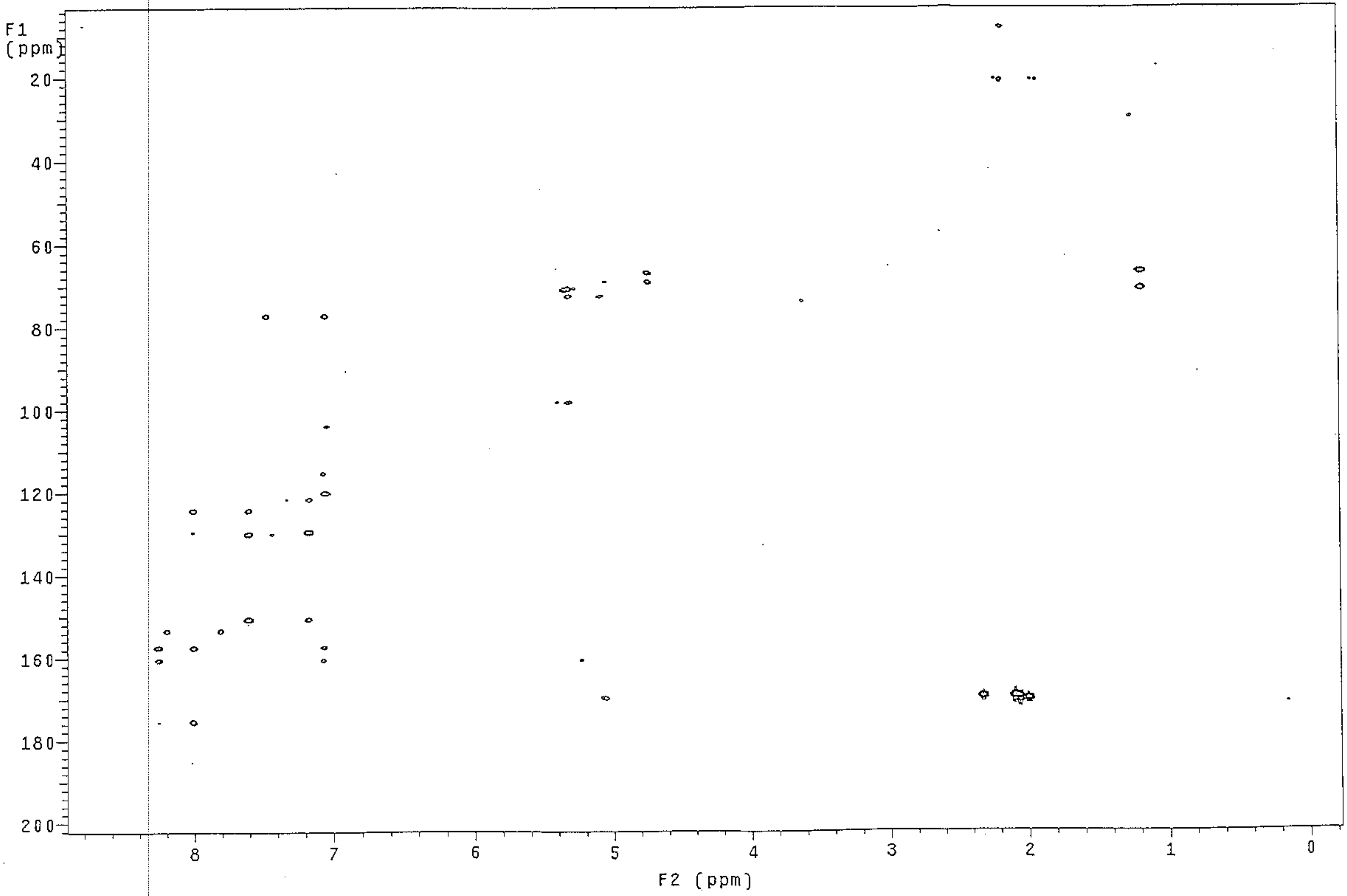


Figure 110 2D HMBC spectrum of AcYS5

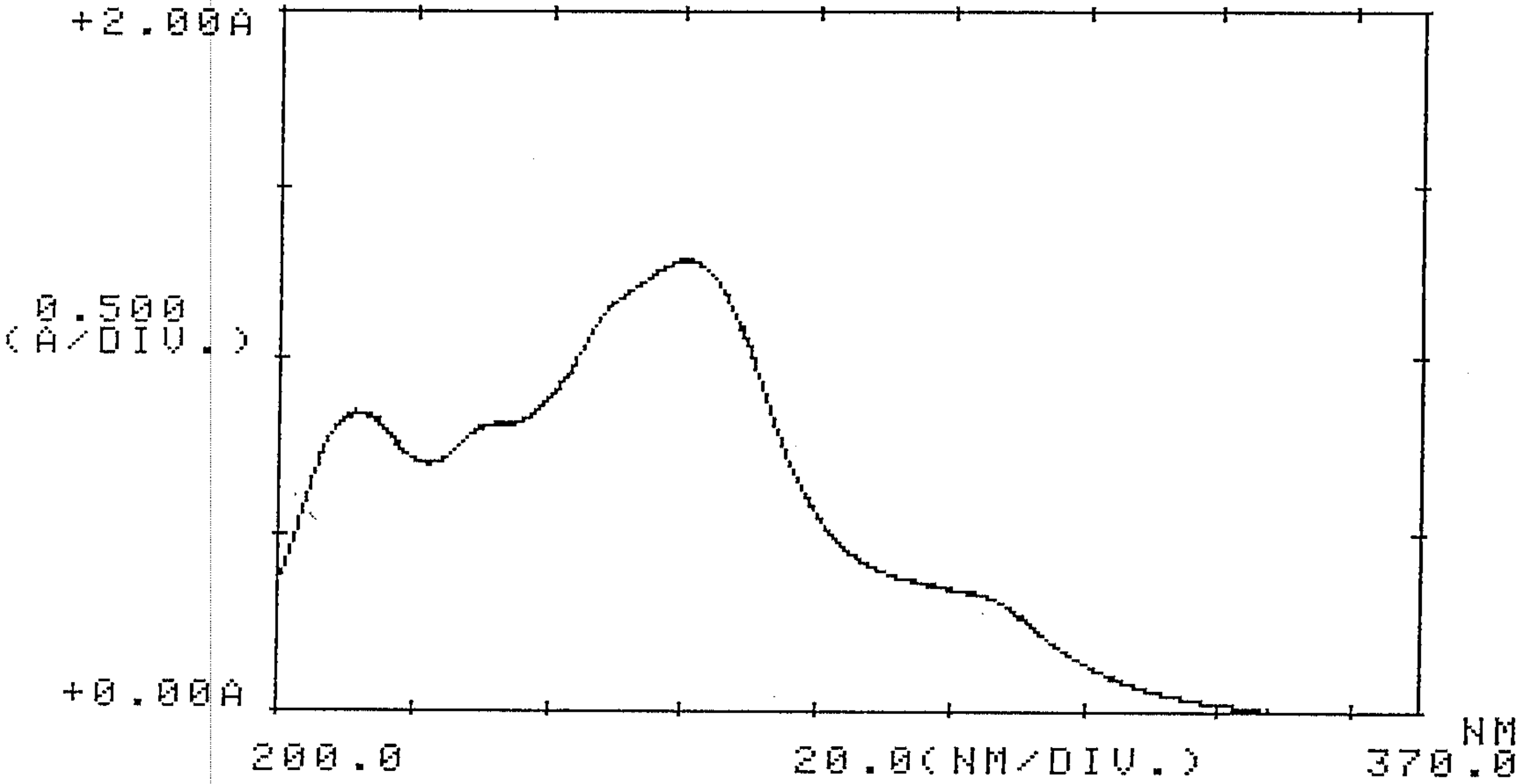


Figure 111 UV ($CHCl_3$) spectrum of **AcYS3**

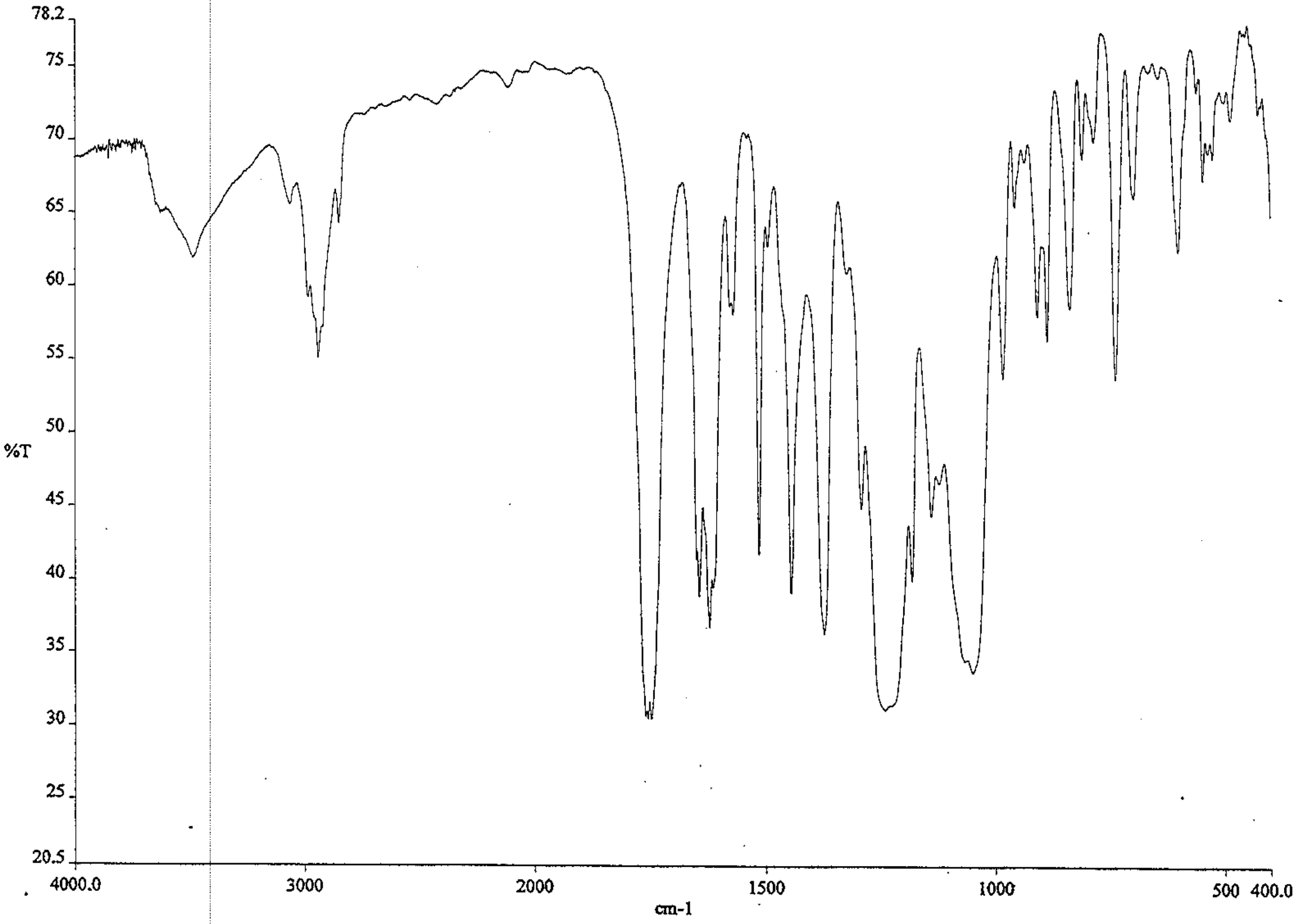


Figure 112 IR (neat) spectrum of **AcYS3**

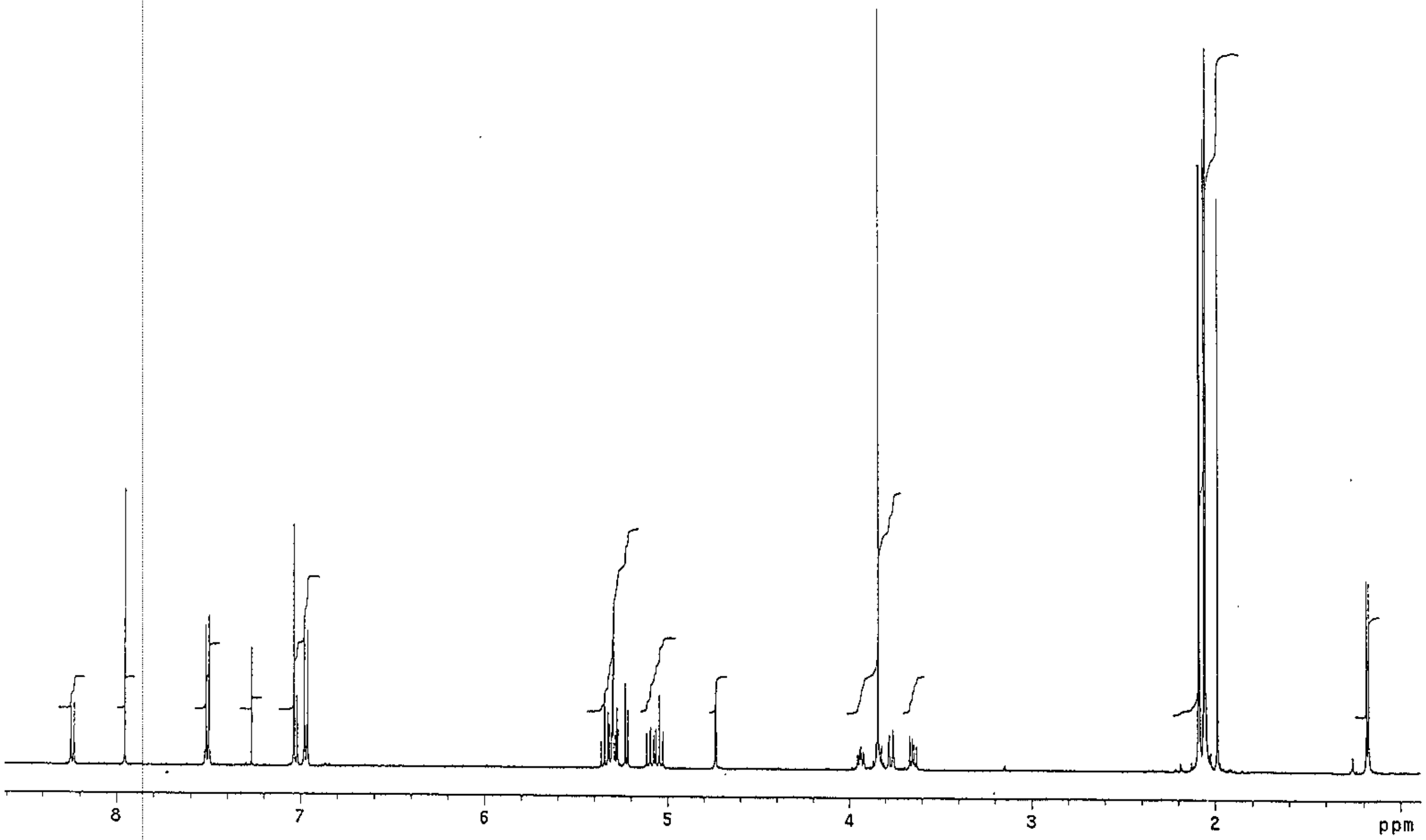


Figure 113 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **AcYS3**

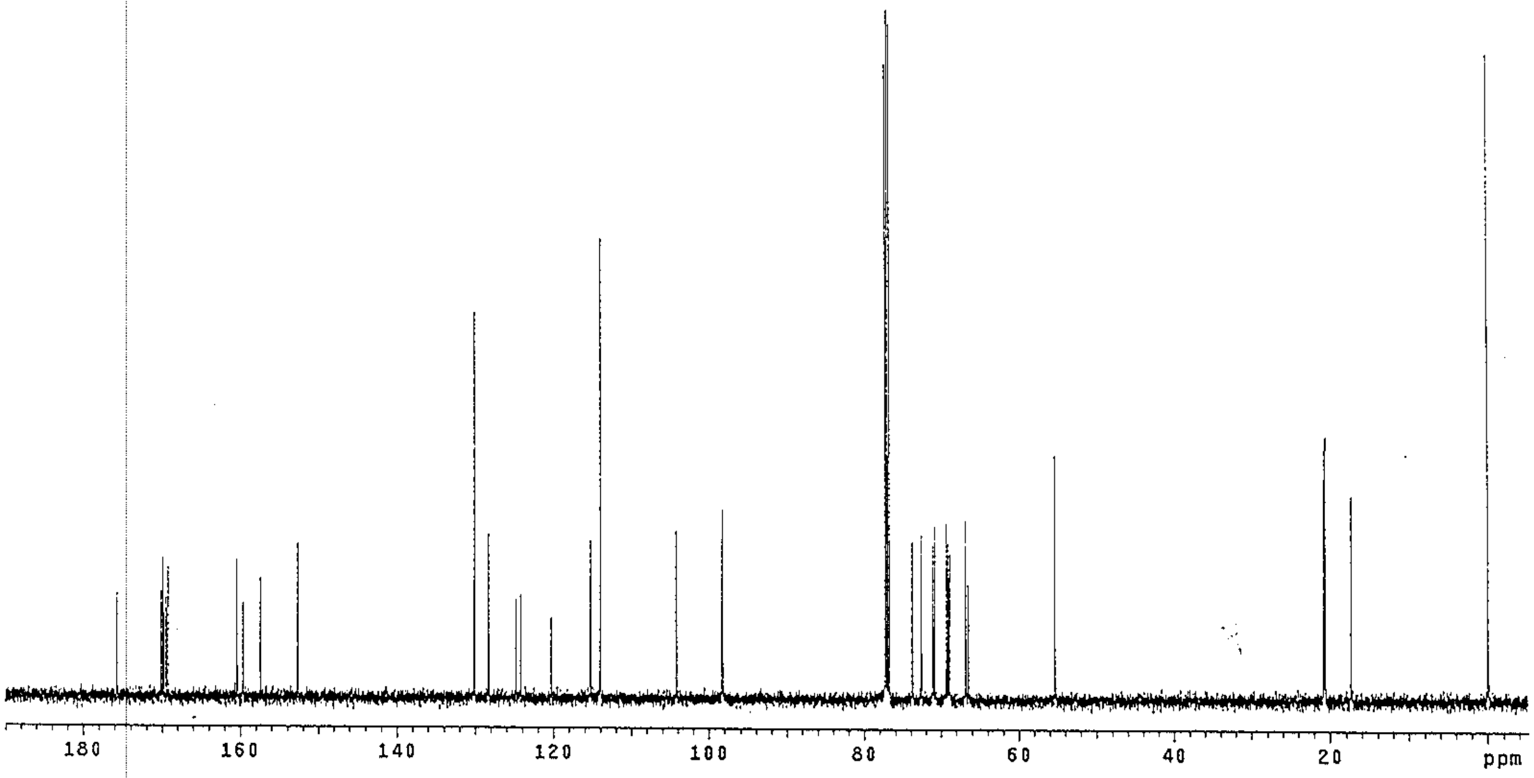


Figure 114 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **AcYS3**

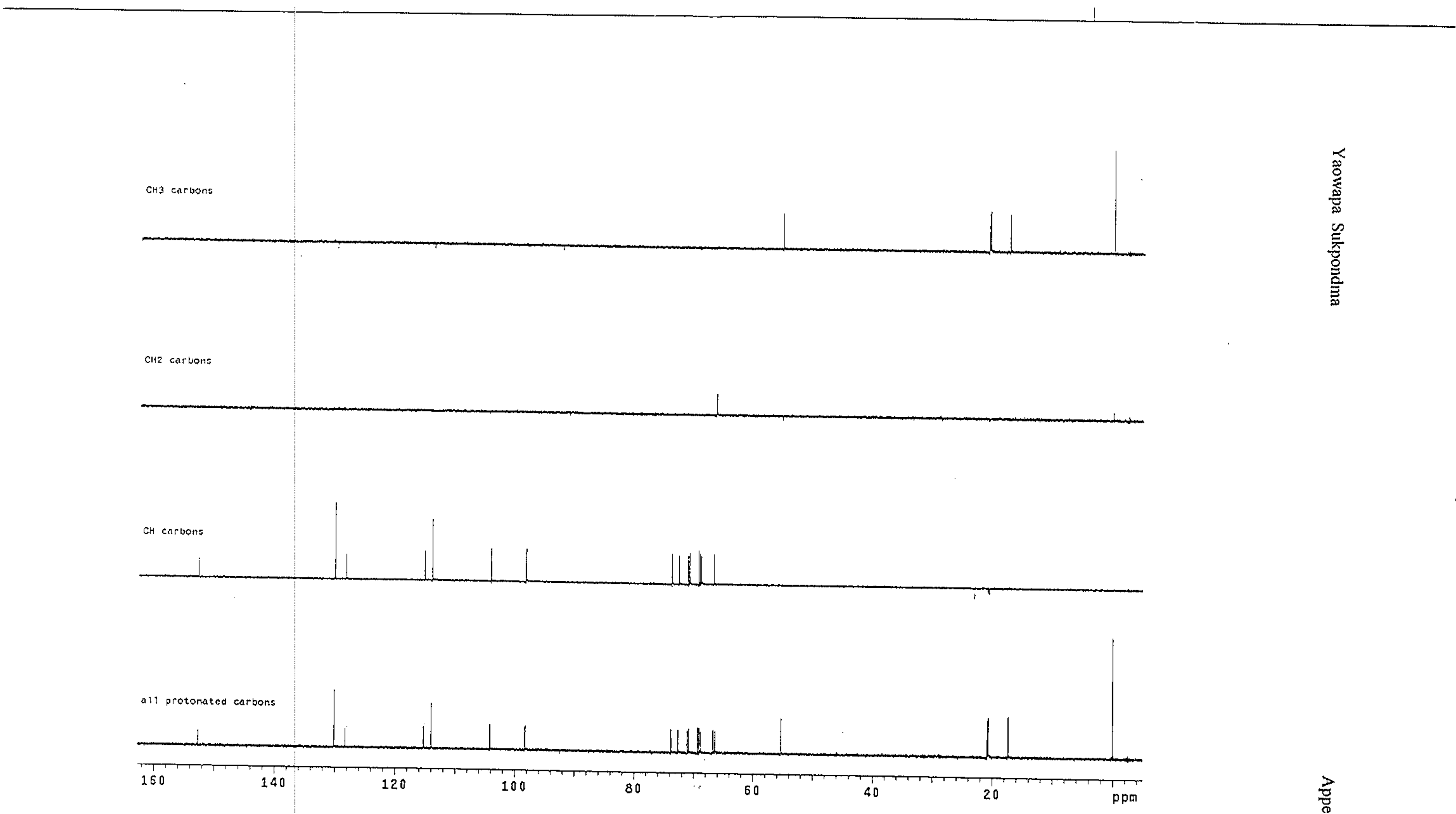


Figure 115 DEPT (135°) ($CDCl_3$) spectrum of **AcYS3**

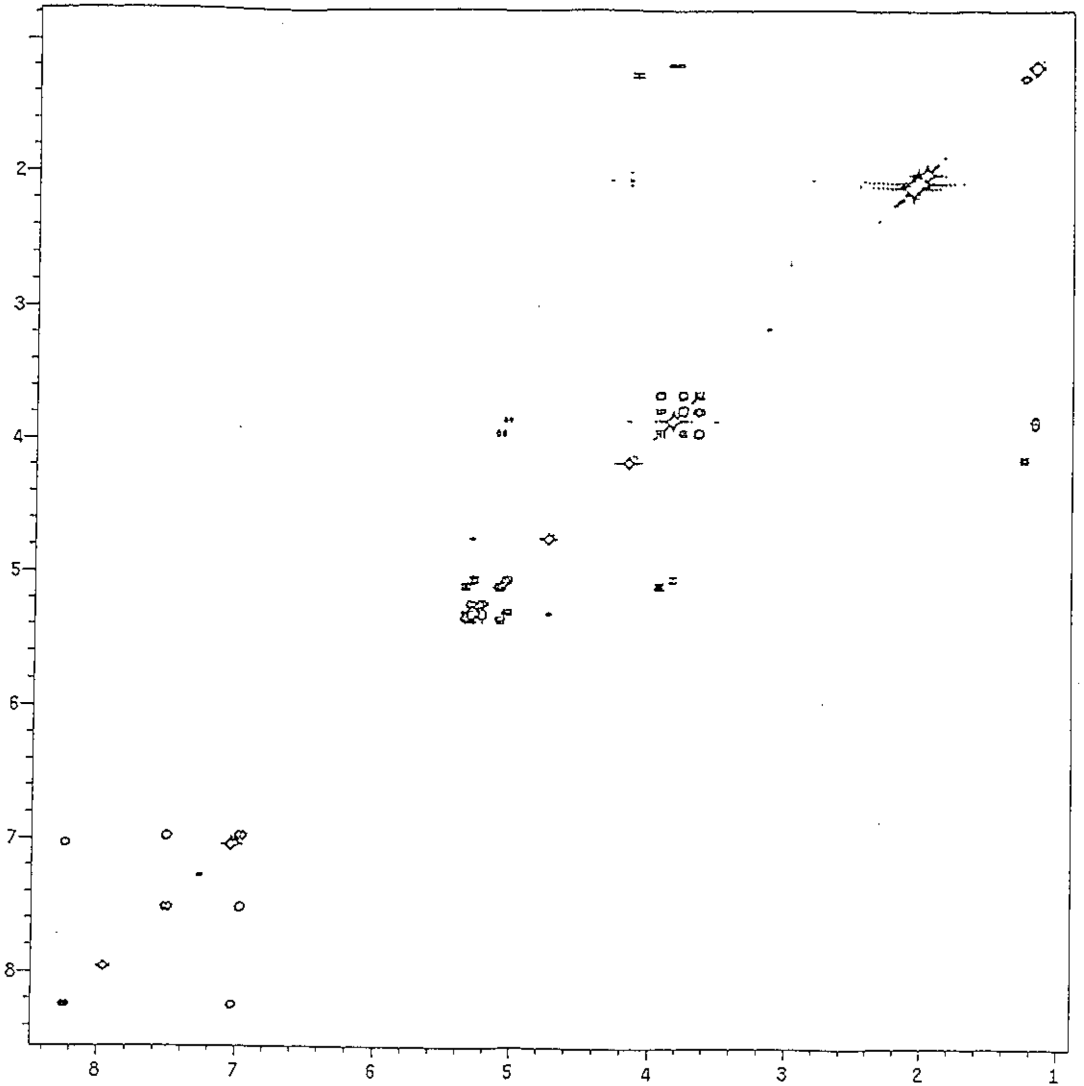


Figure 116 ^{1}H-^{1}H COSY spectrum of AcYS3

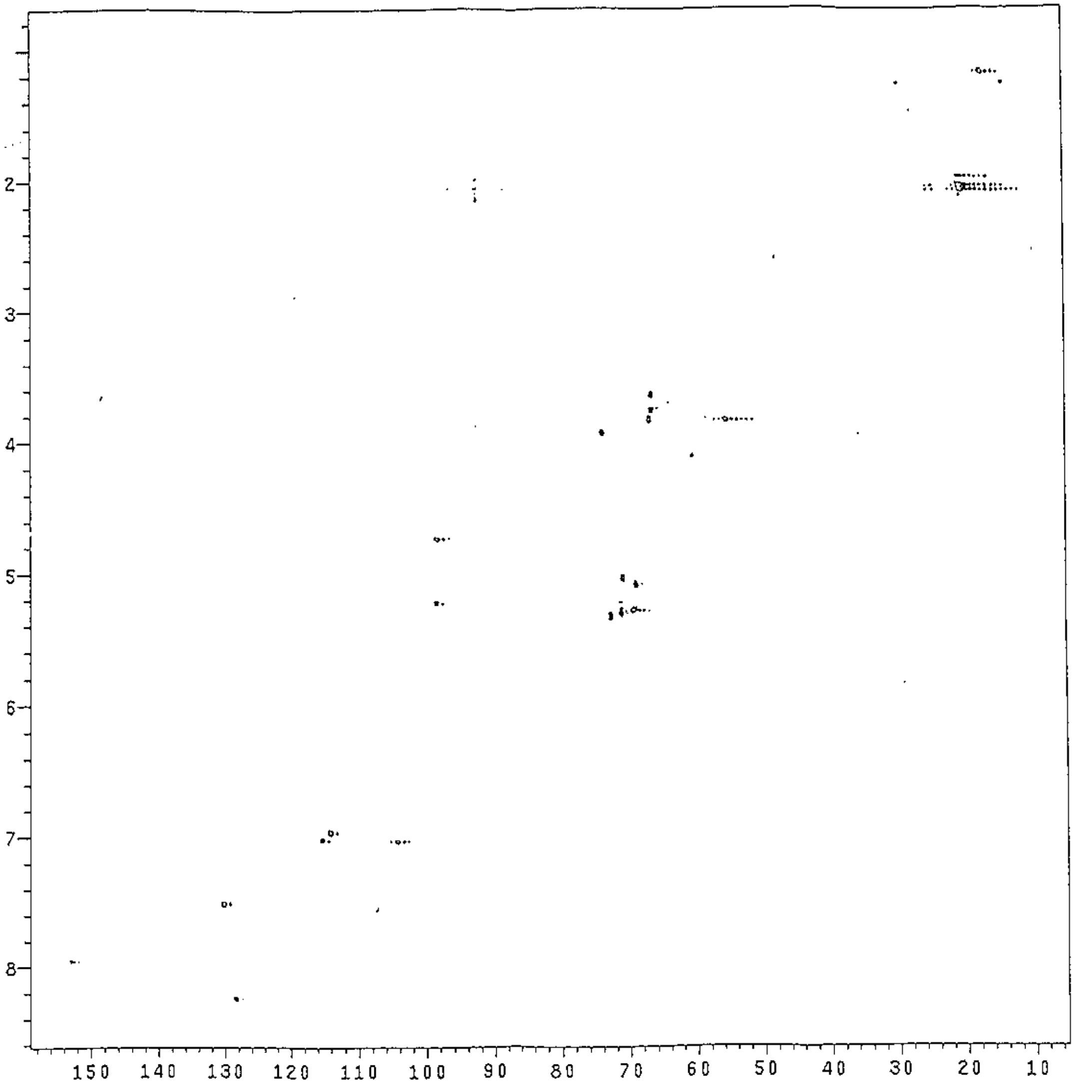


Figure 117 2D HMQC spectrum of AcYS3

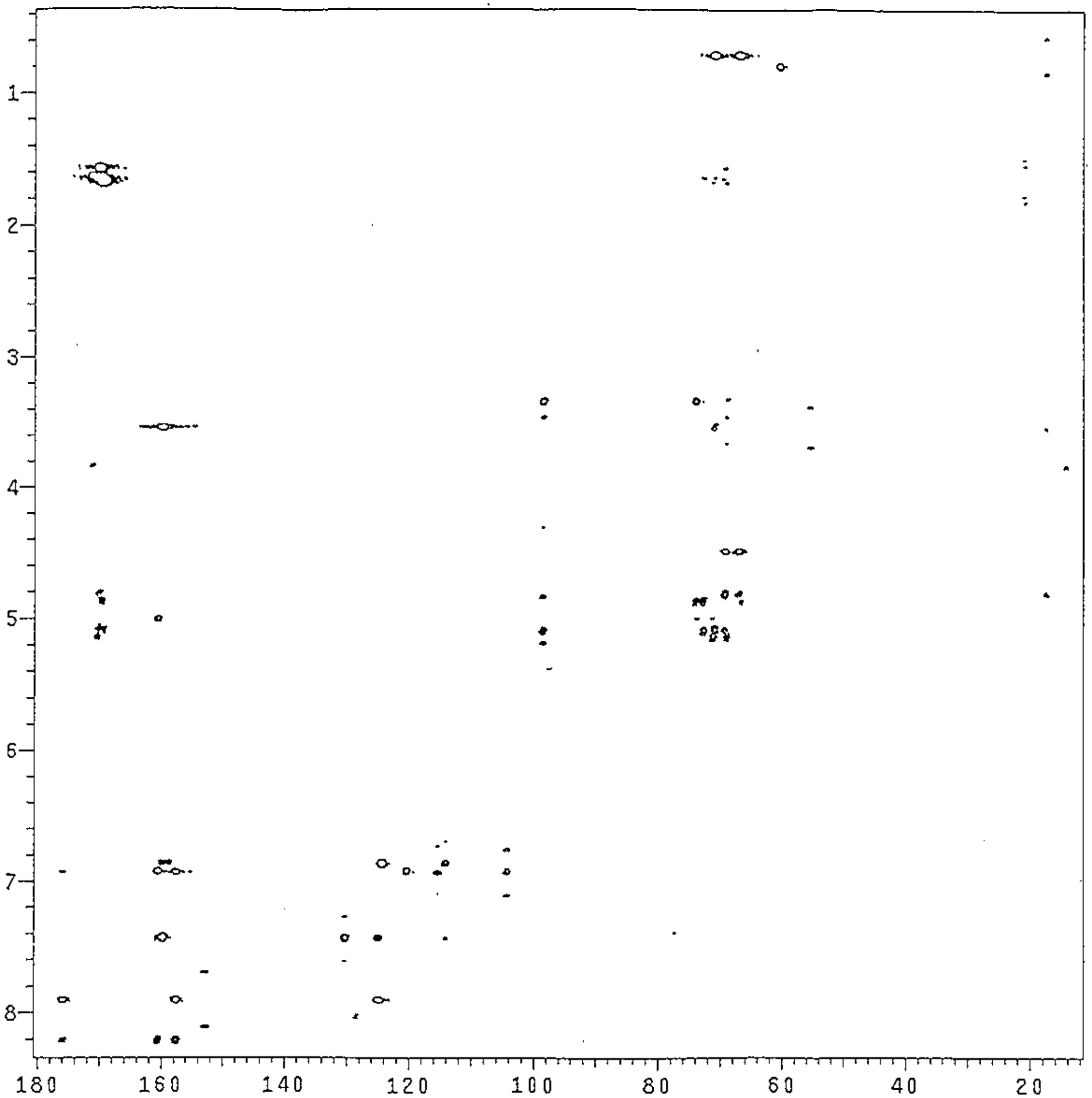


Figure 118 2D HMBC spectrum of **AcYS3**

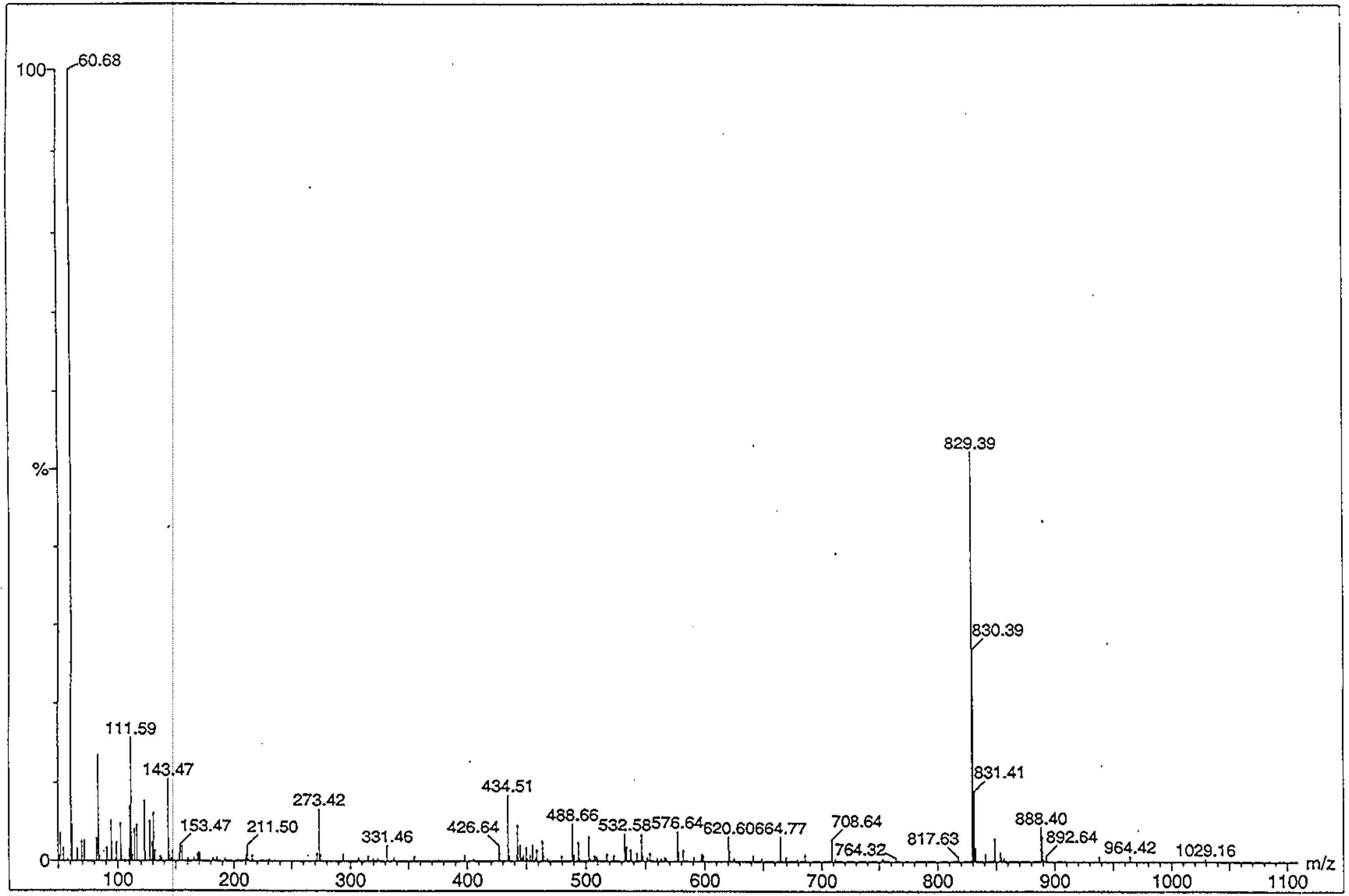


Figure 119 Mass spectrum of **AcYS3**

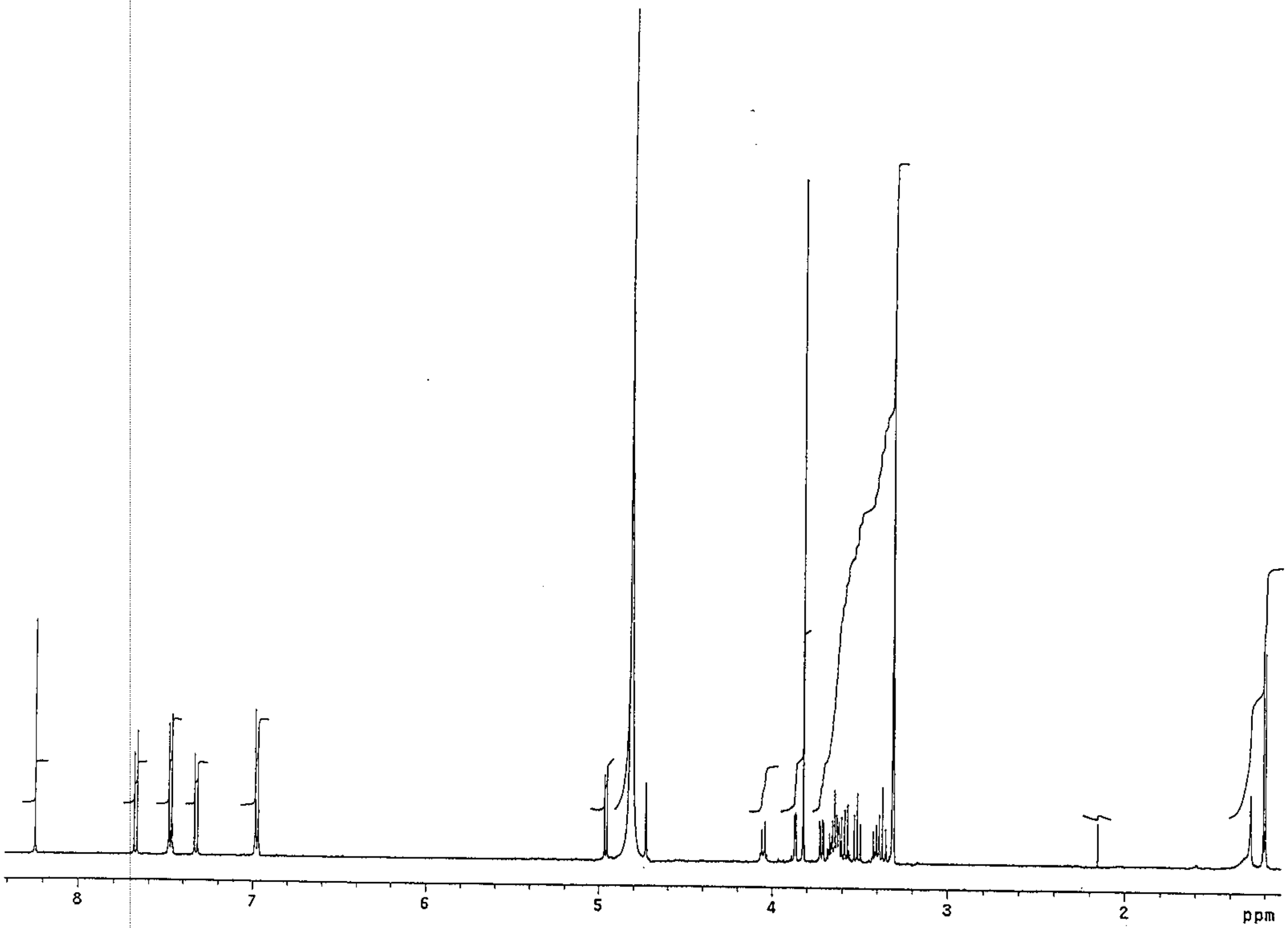


Figure 120 ^{1}H NMR (500 MHz) (CD$_3$OD) spectrum of **YS10**

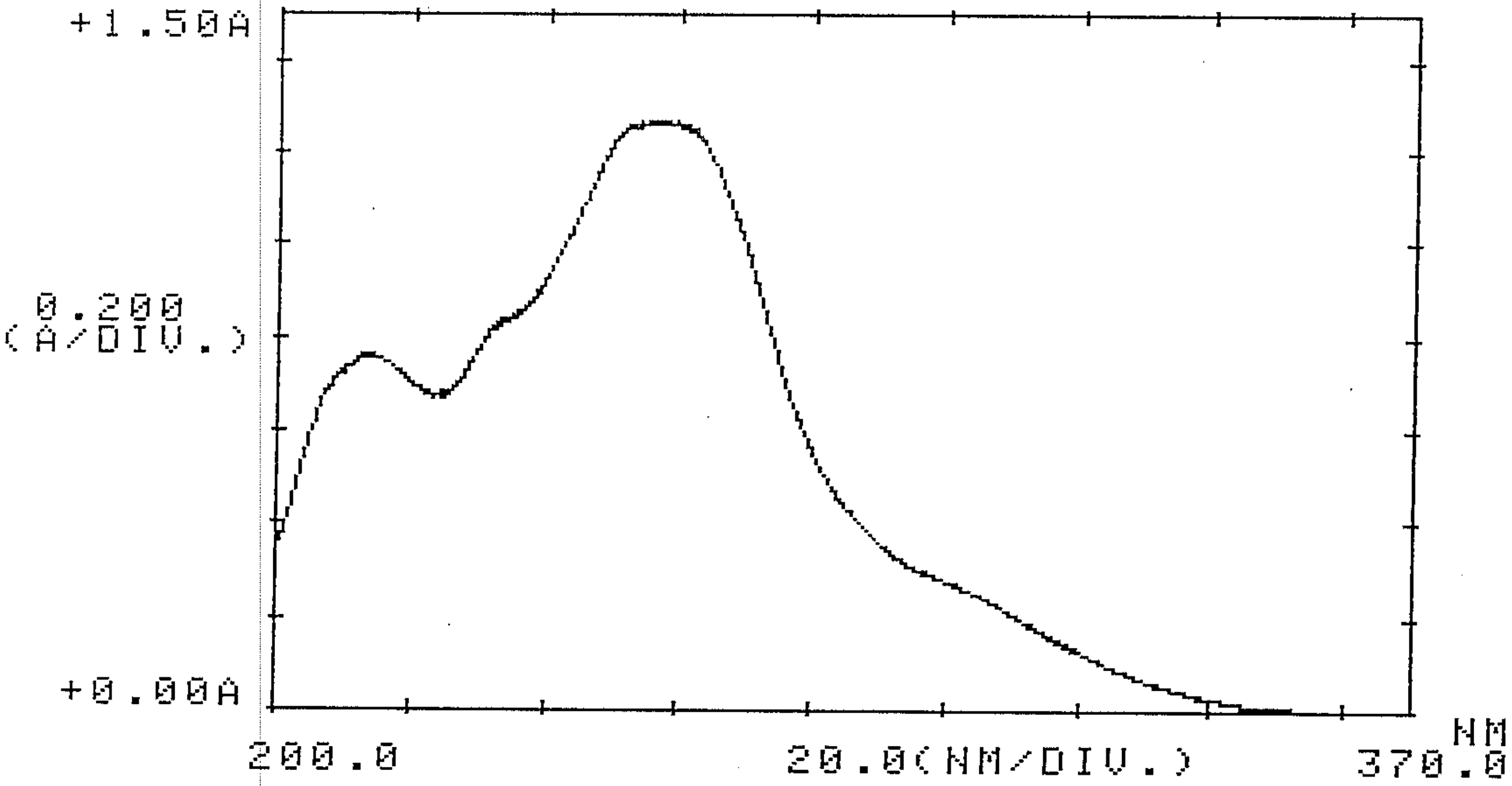


Figure 121 UV ($CHCl_3$) spectrum of **AcYS10**

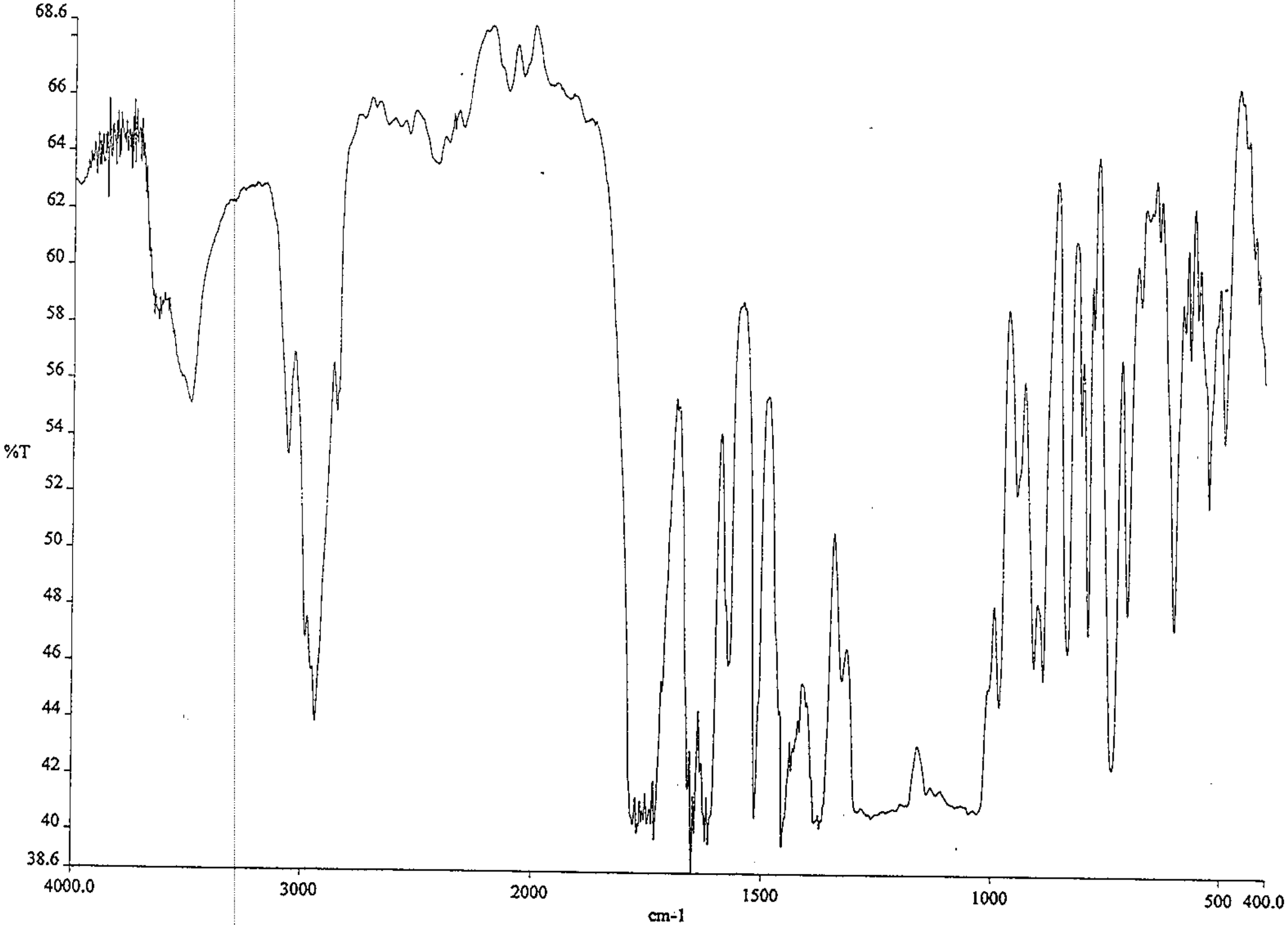


Figure 122 IR (neat) spectrum of **AcYS10**

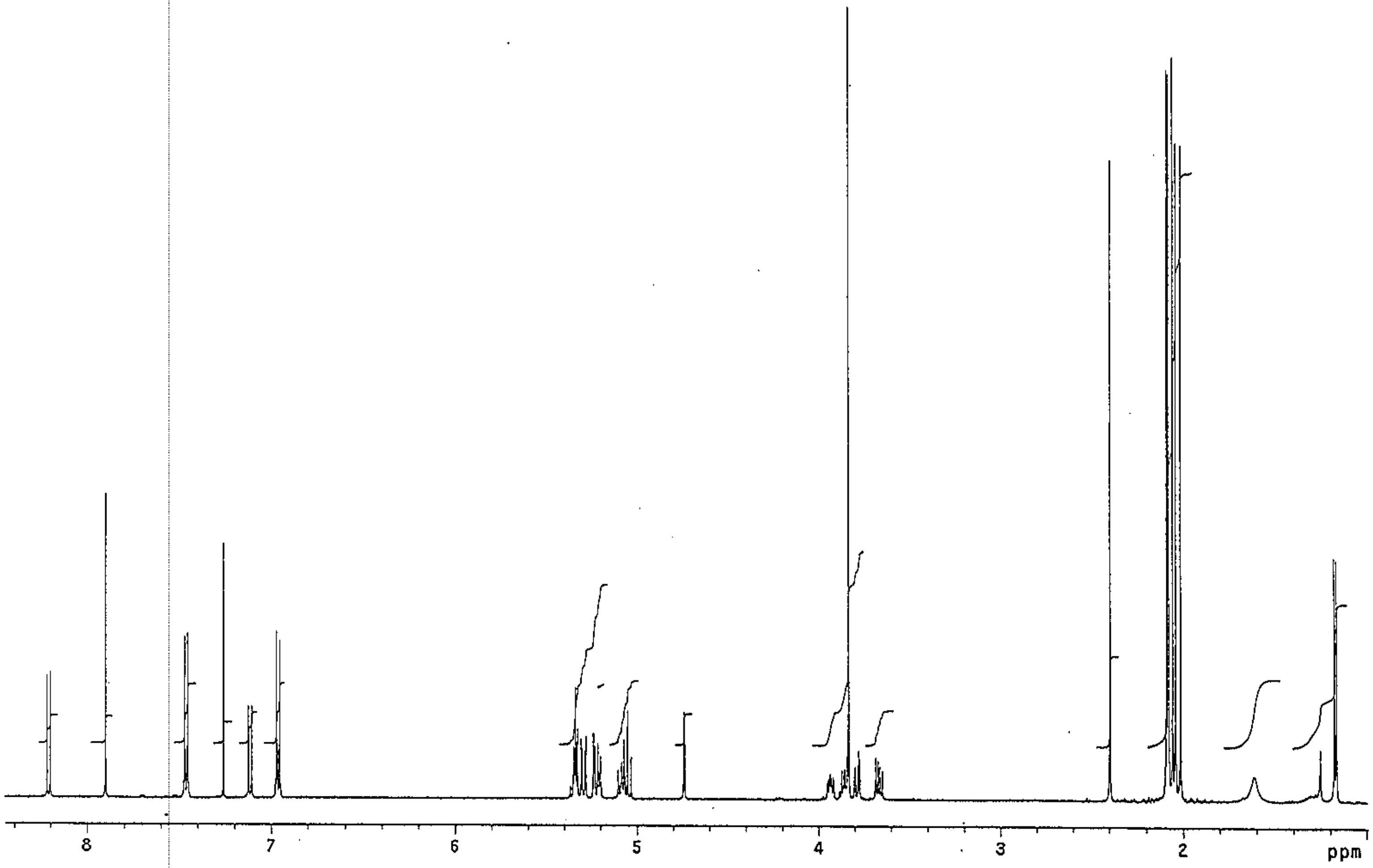


Figure 123 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **AcYS10**

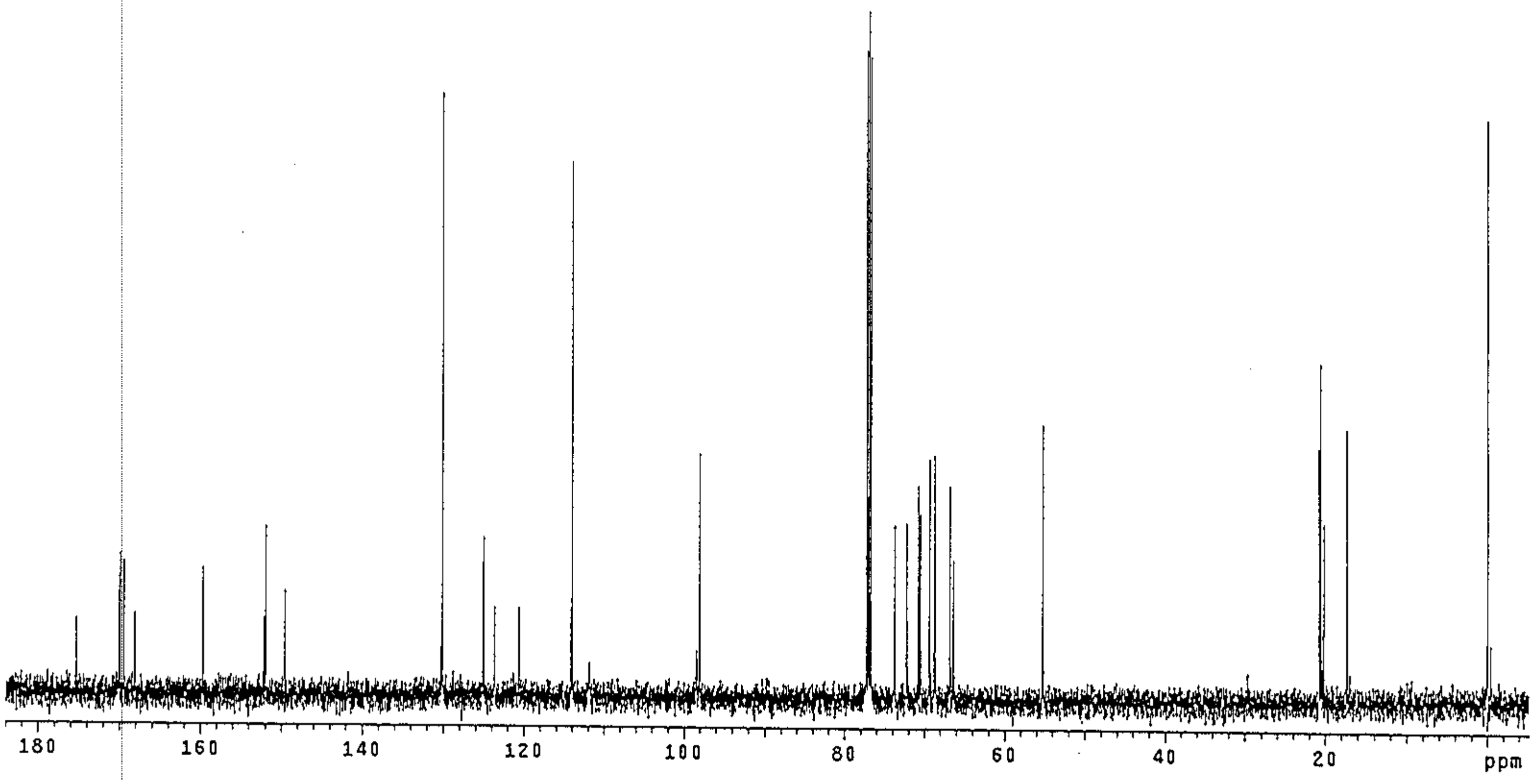


Figure 124 ^{13}C NMR (125 MHz) ($CDCl_3$) spectrum of **AcYS10**

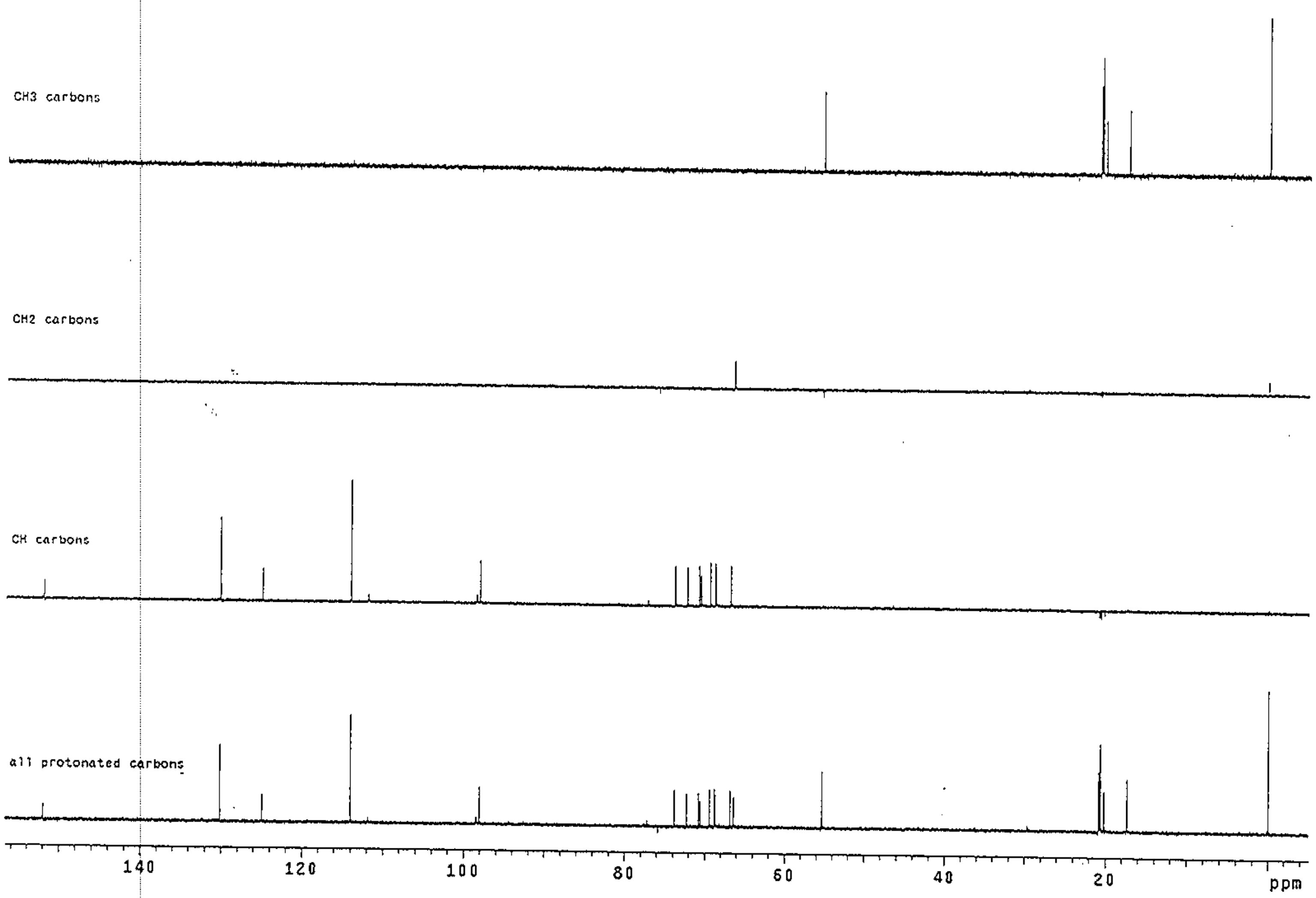


Figure 125 DEPT (135°) ($CDCl_3$) spectrum of **AcYS10**

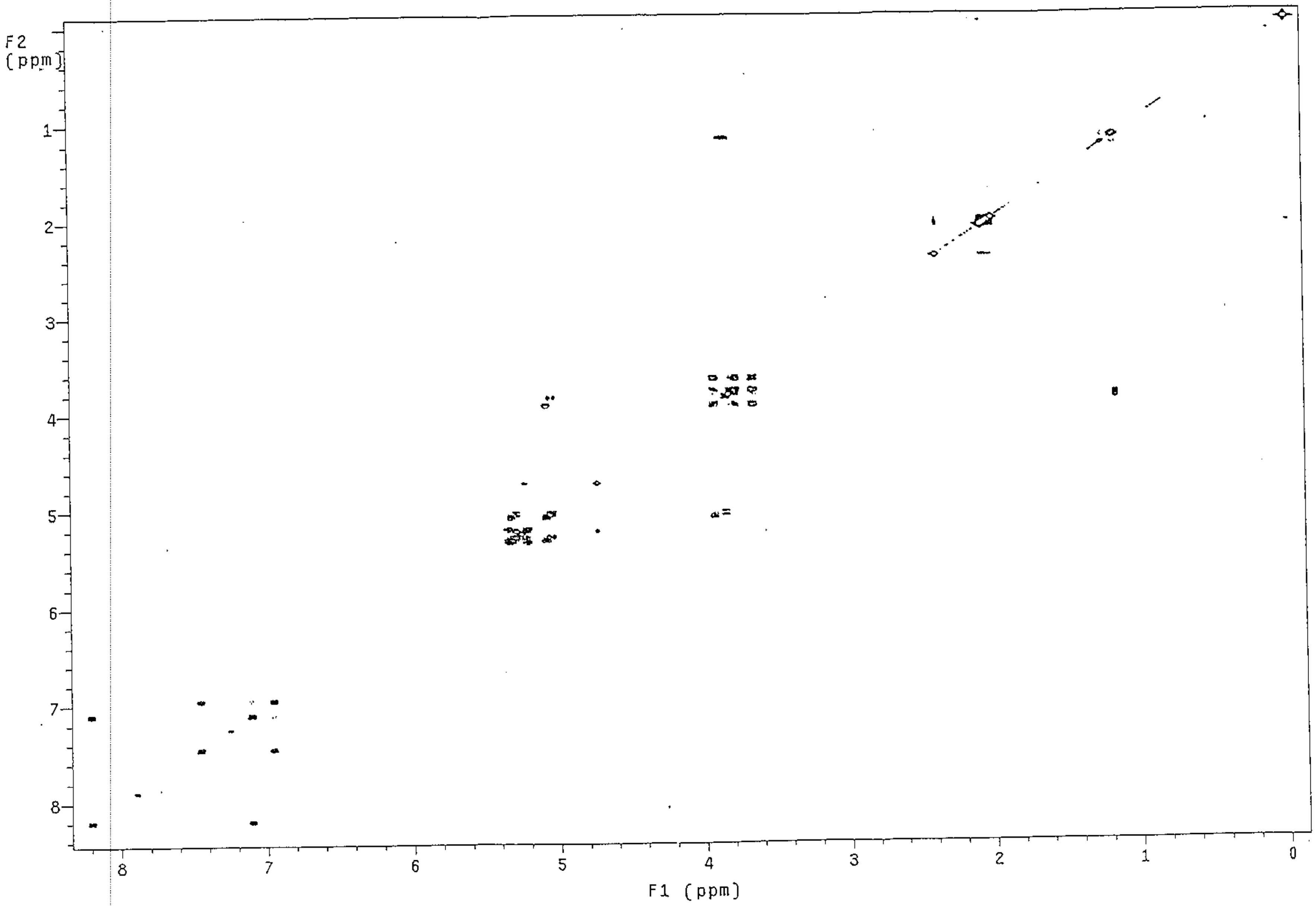


Figure 126 ^{1}H-^{1}H COSY spectrum of **AcYS10**

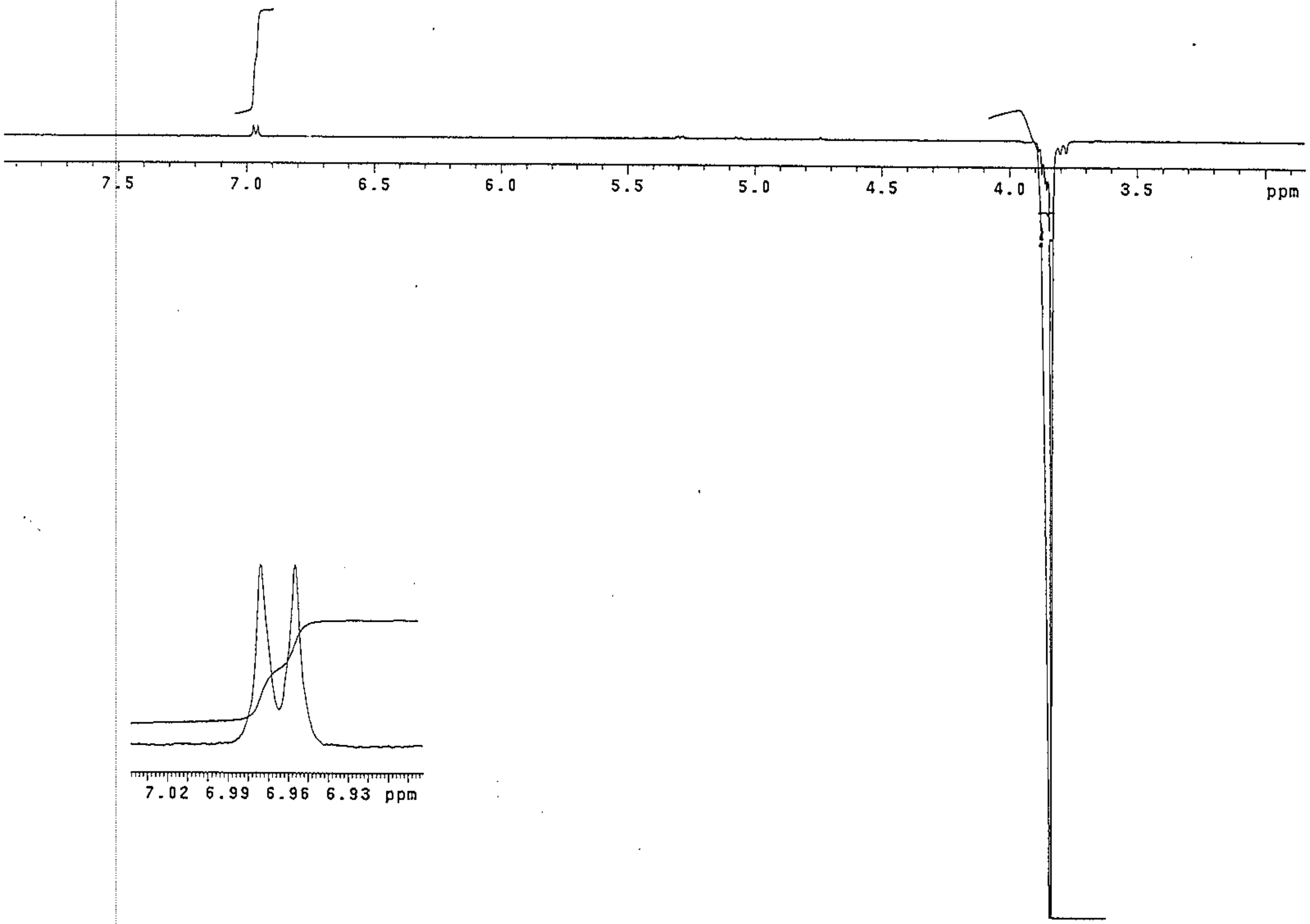


Figure 127 NOEDIFF spectrum of **AcYS10** after irradiation at δ_H 3.84

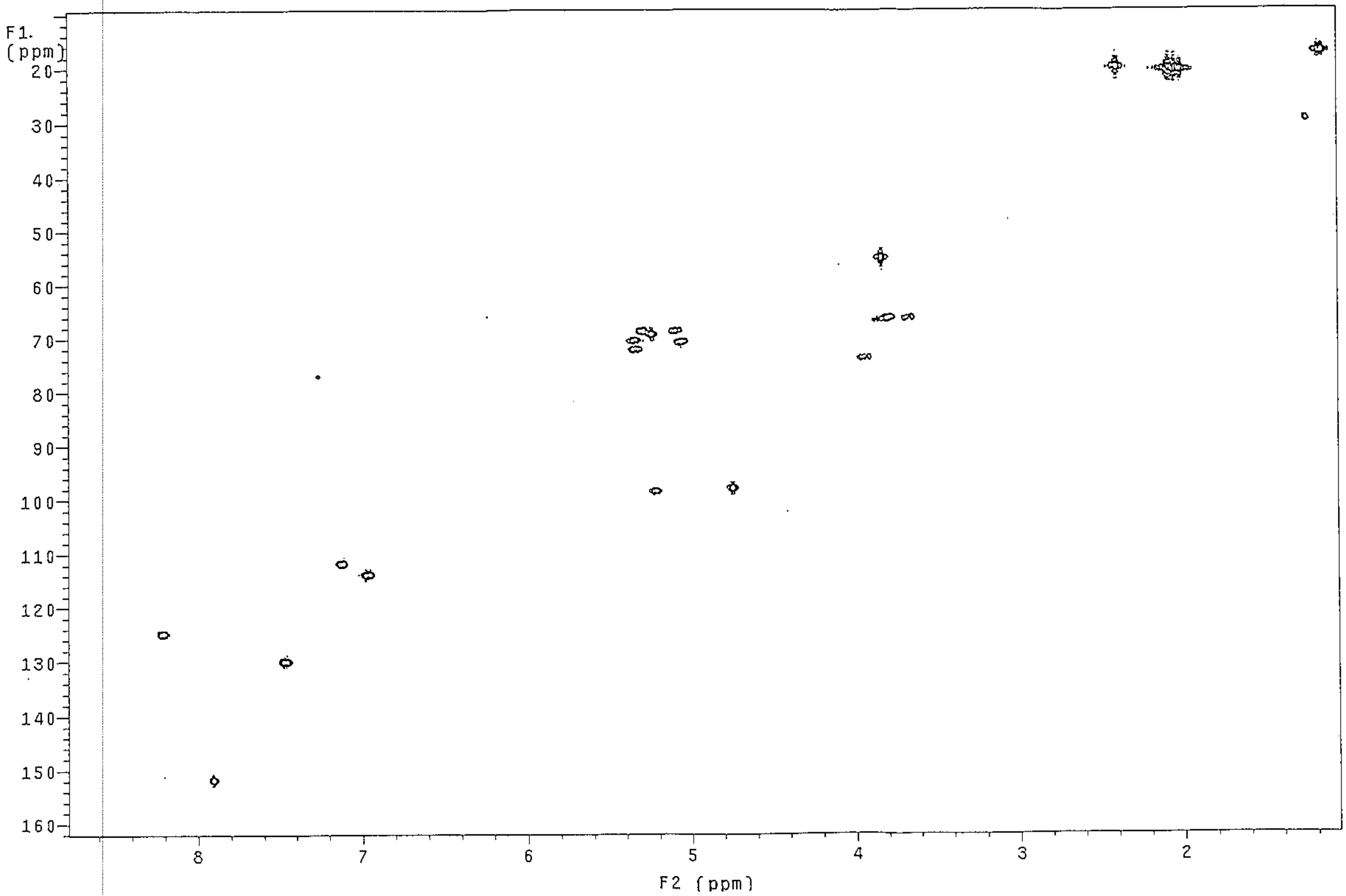


Figure 128 2D HMQC spectrum of AcYS10

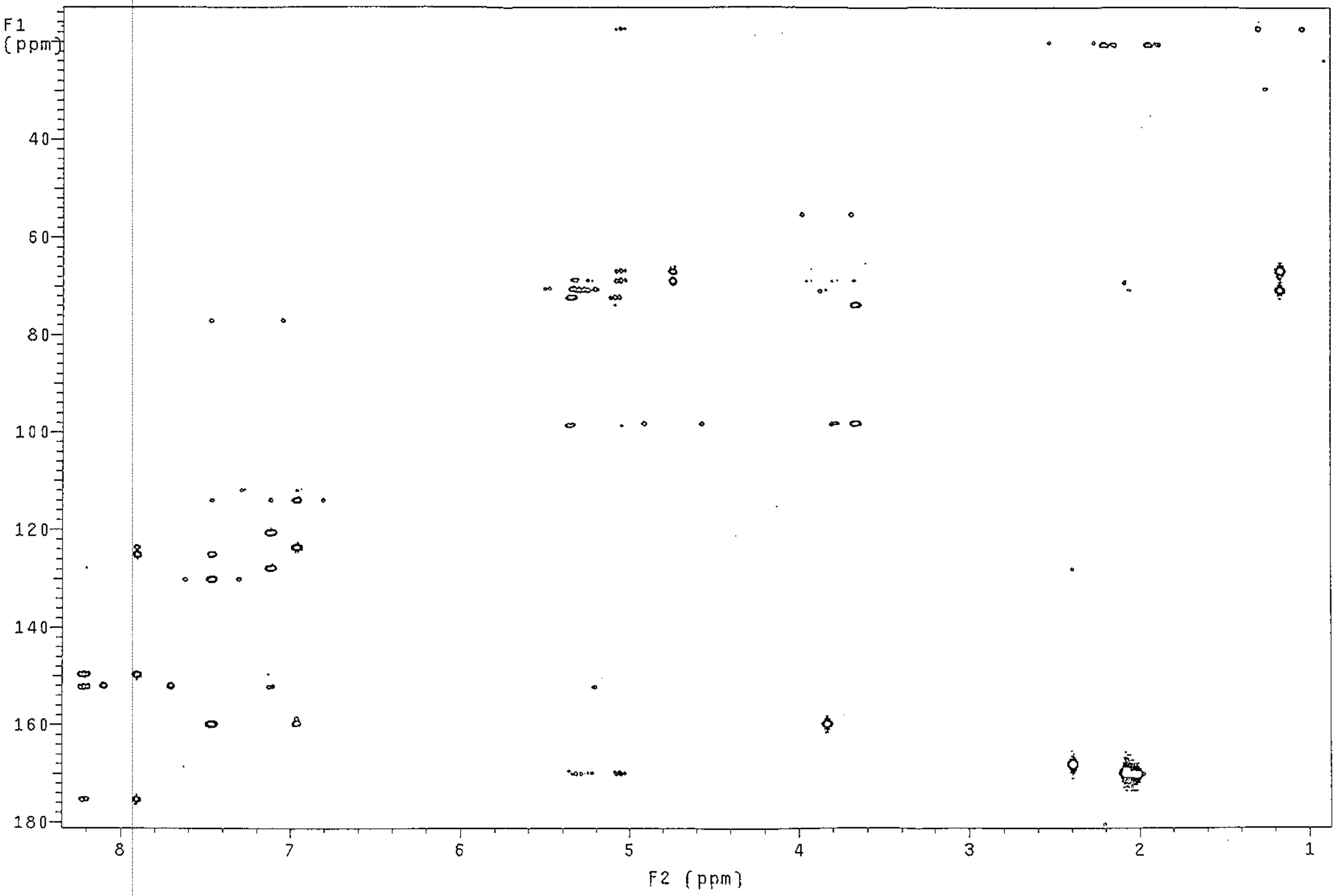


Figure 129 2D HMBC spectrum of **AcYS10**

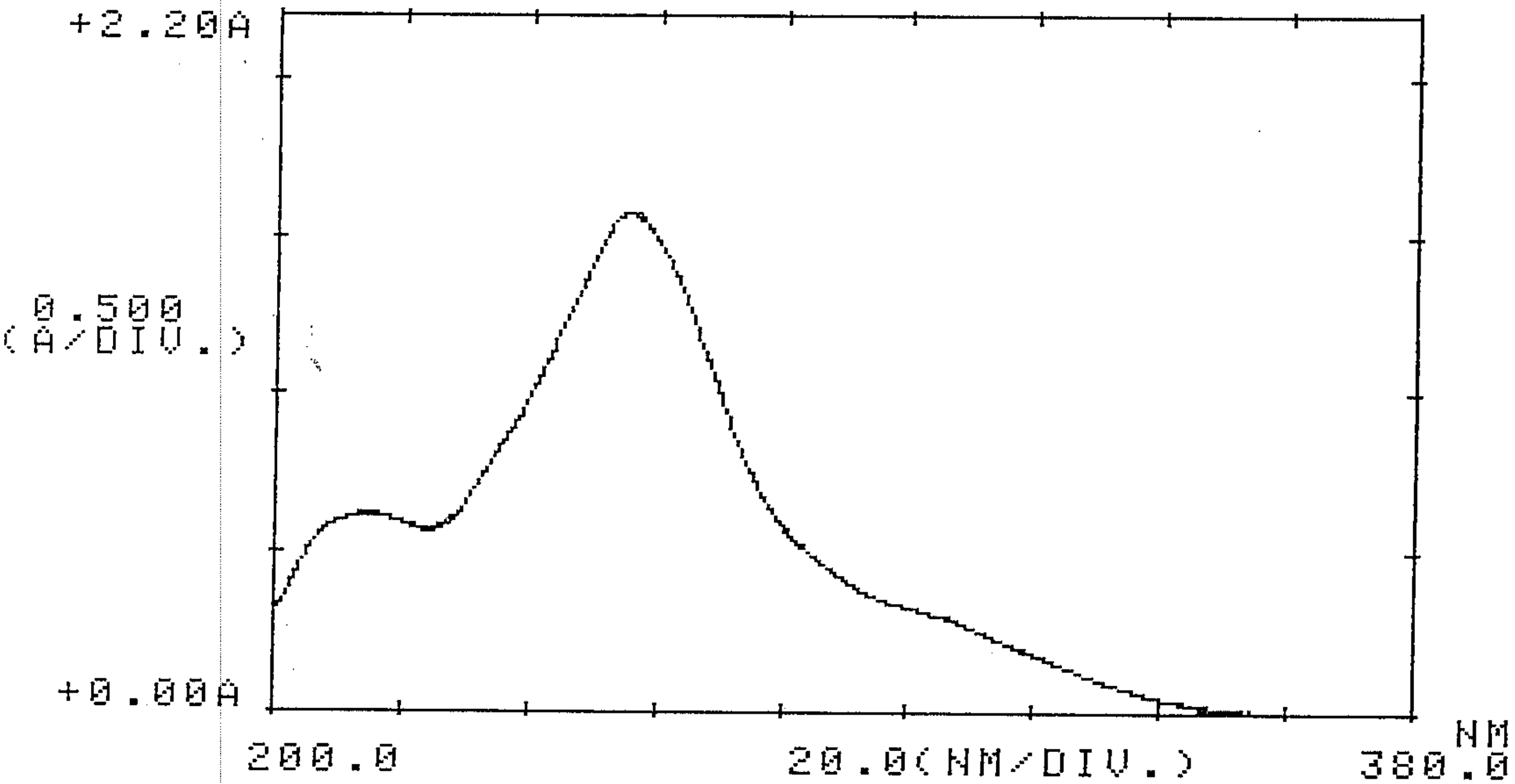


Figure 130 UV (CH_3OH) spectrum of **YS2**

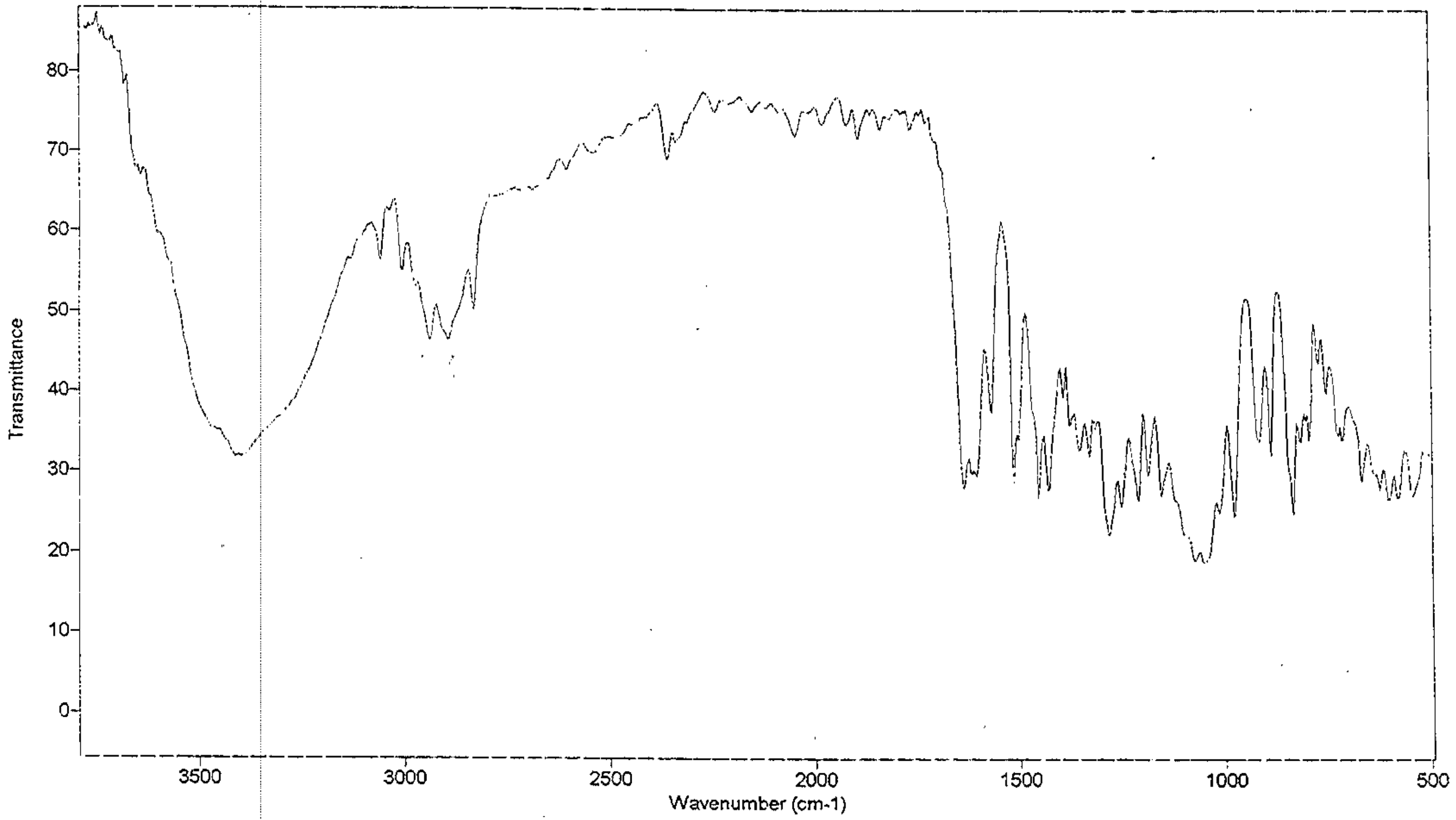


Figure 131 IR (KBr) spectrum of **YS2**

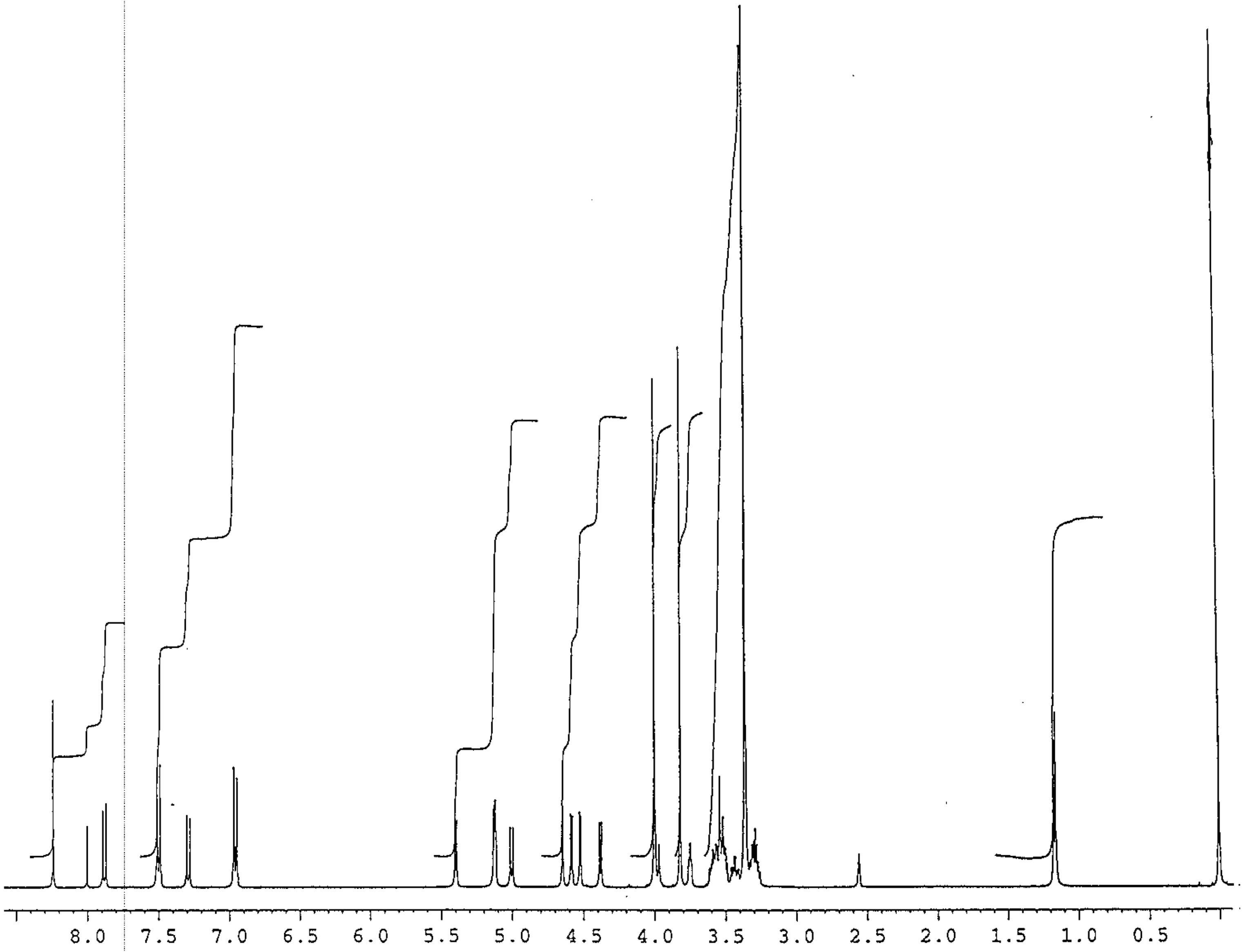


Figure 132 ^{1}H NMR (400 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of **YS2**

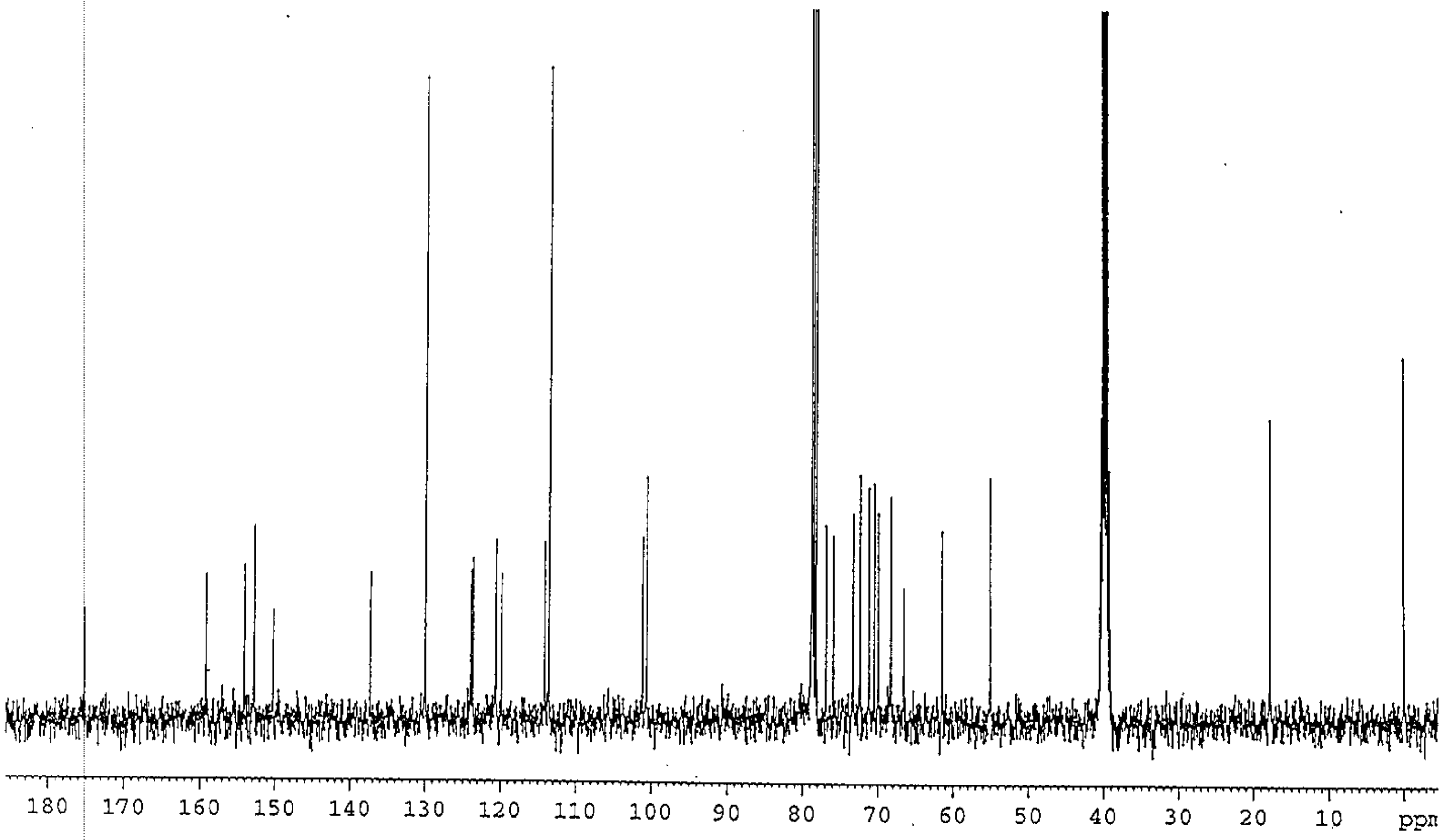


Figure 133 ^{13}C NMR (100 MHz) ($CDCl_3$ +DMSO-d_6) spectrum of **YS2**

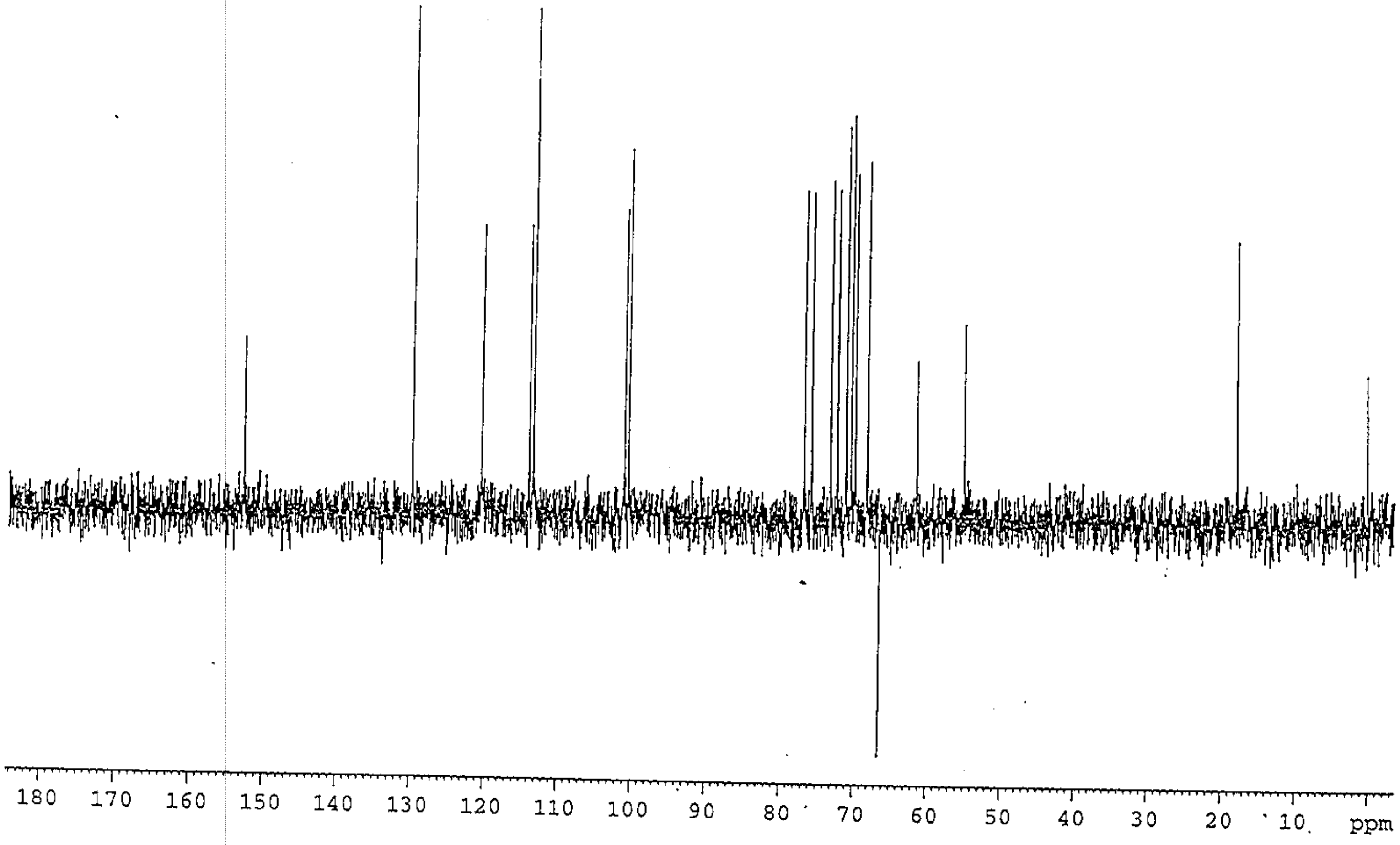


Figure 134 DEPT (135°) ($CDCl_3$ +DMSO-d_6) spectrum of **YS2**

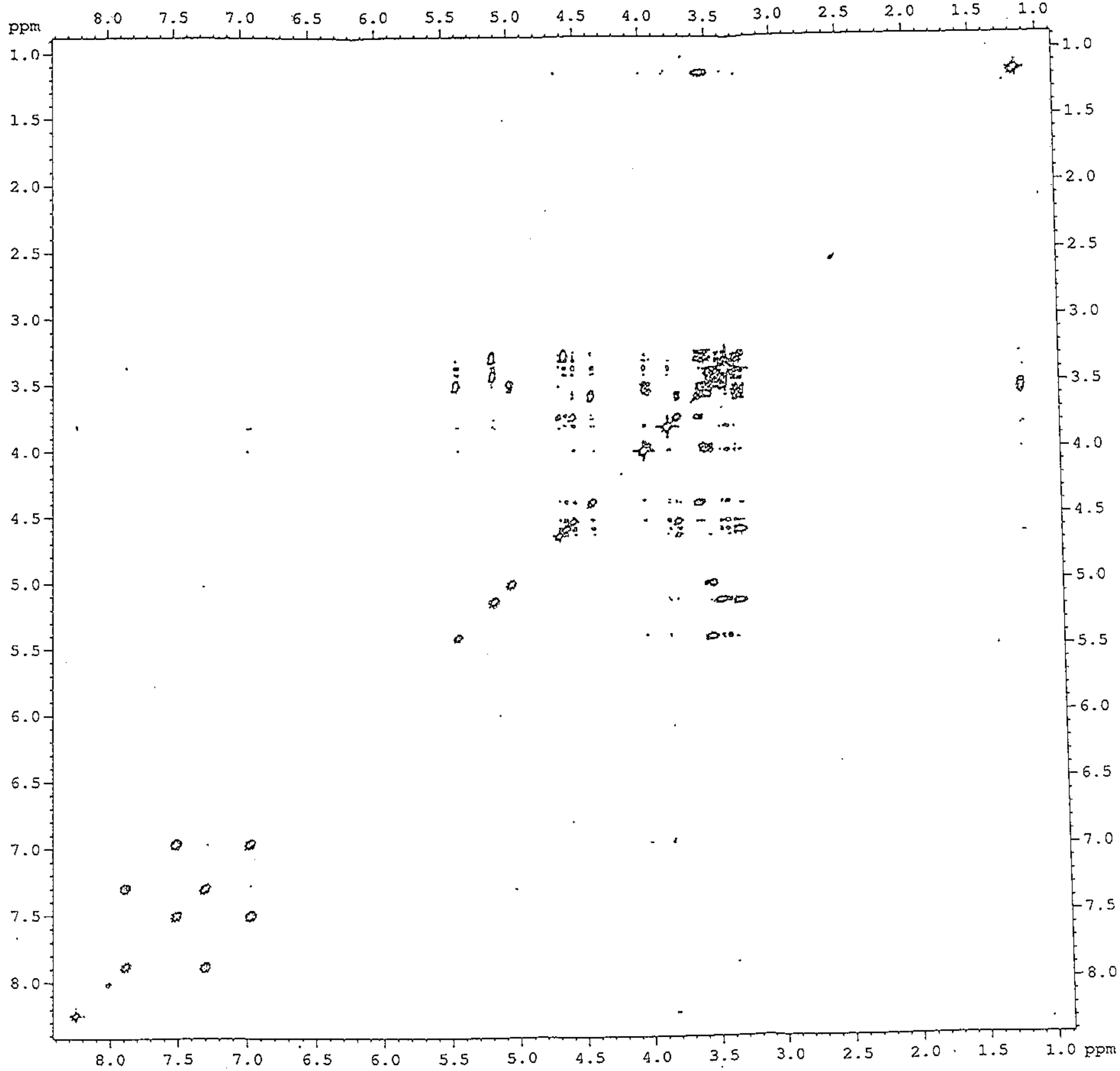


Figure 135 ${}^1H$-${}^1H$ COSY spectrum of **YS2**

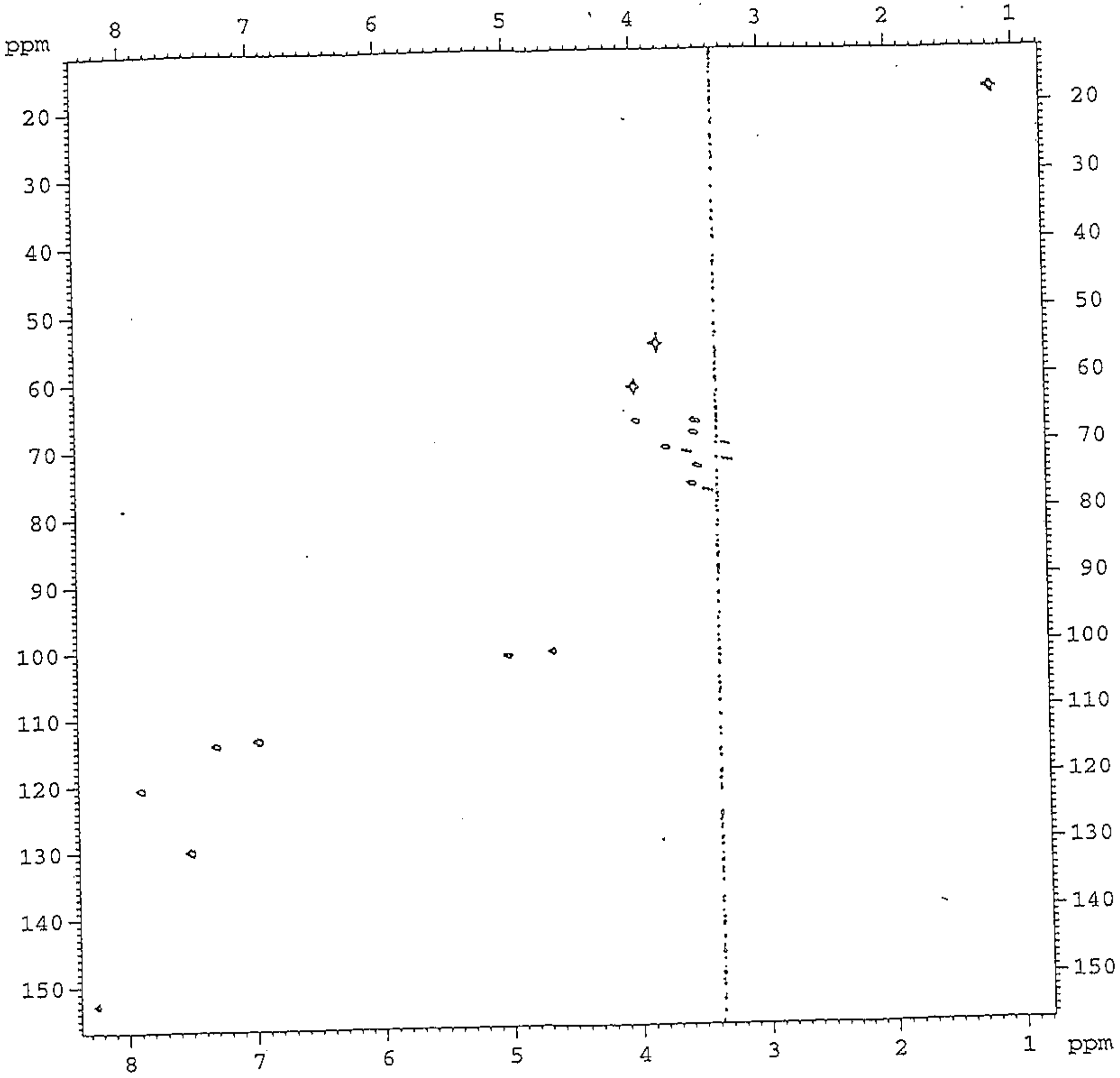


Figure 136 2D HMQC spectrum of YS2

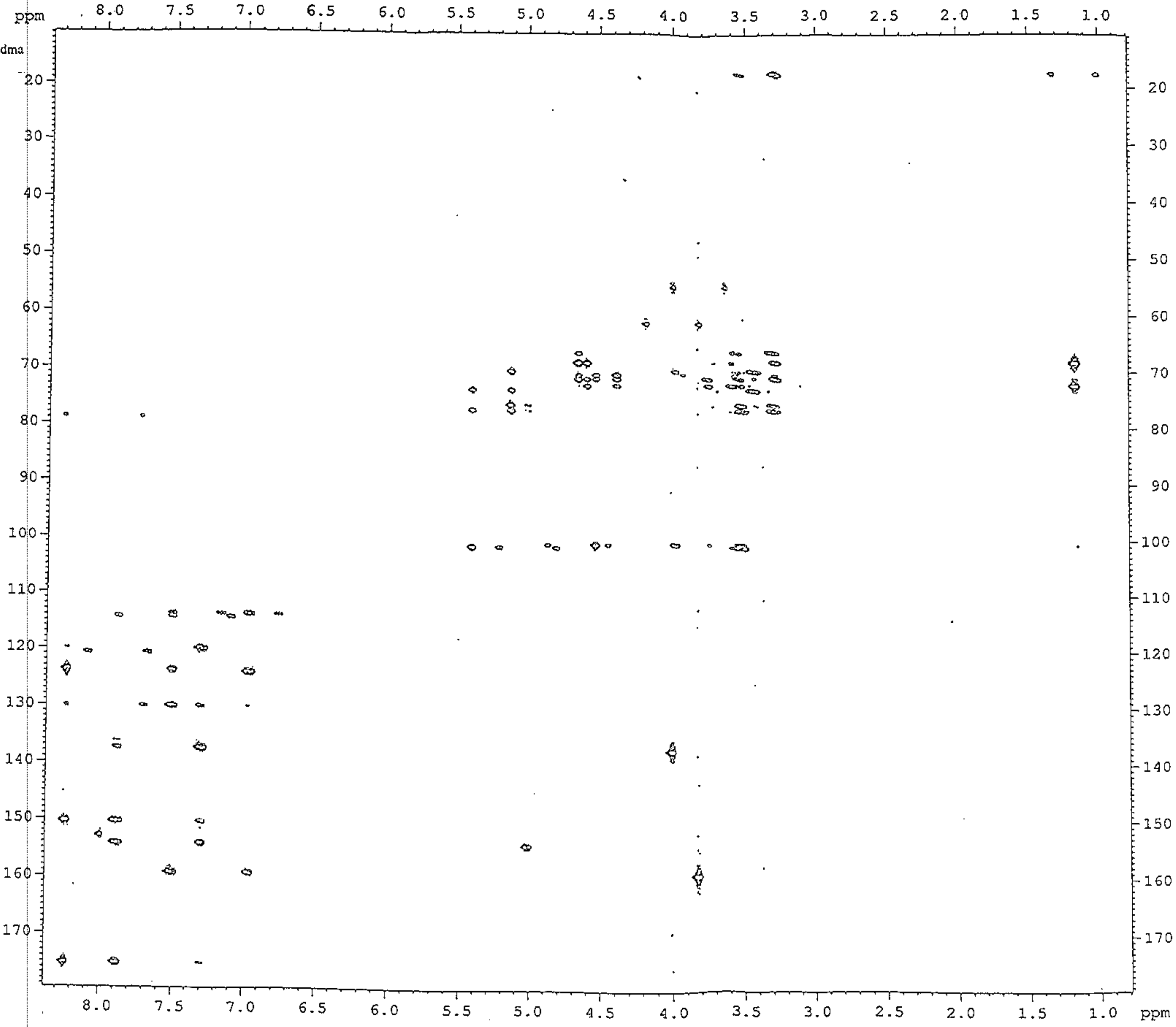


Figure 137 2D HMBC spectrum of YS2

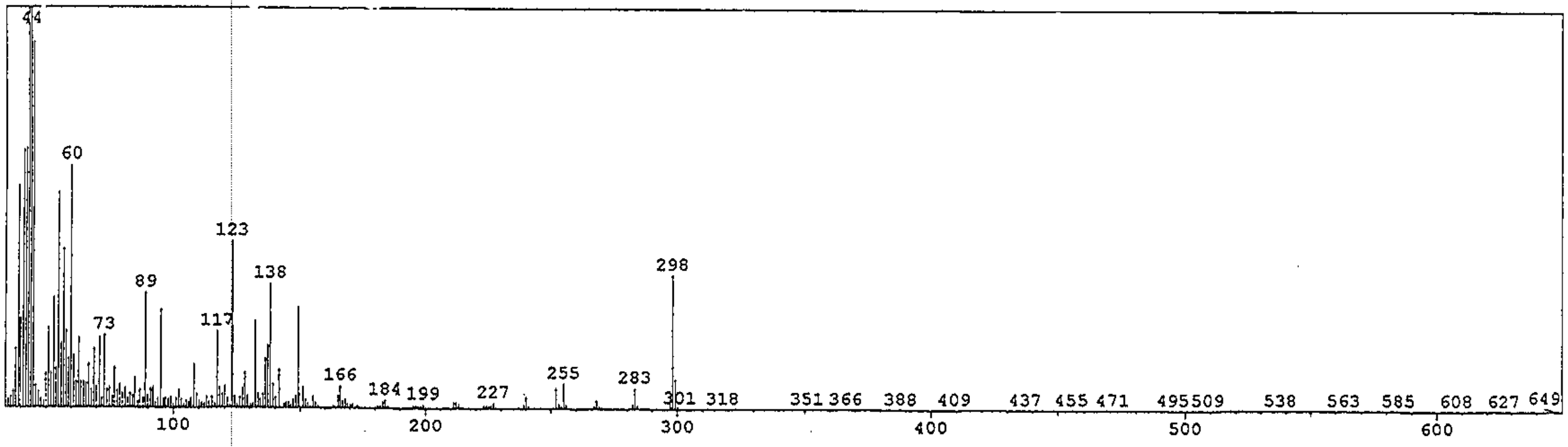


Figure 138 Mass spectrum of **YS2**

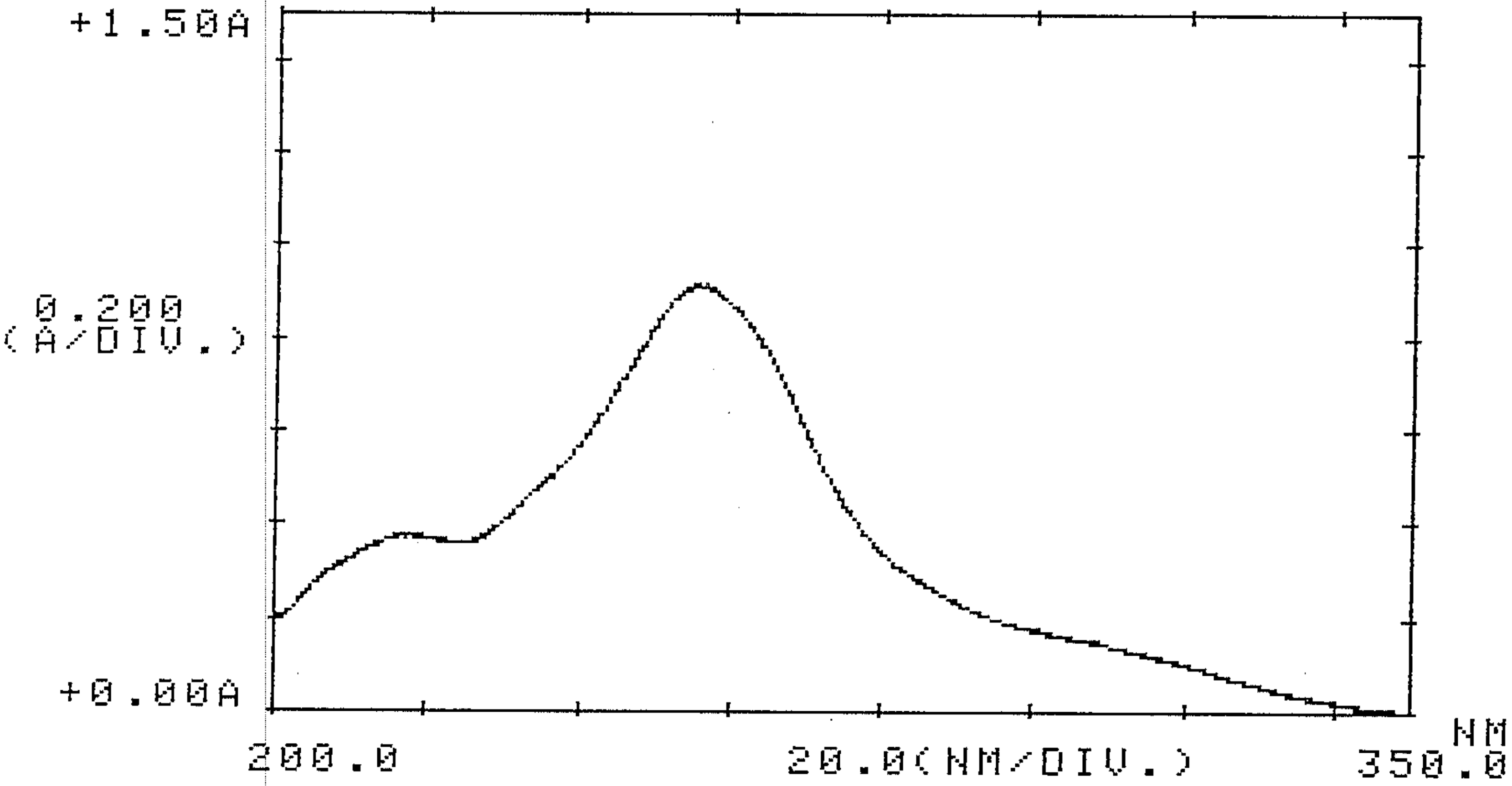


Figure 139 UV ($CHCl_3$) spectrum of **AcYS2**

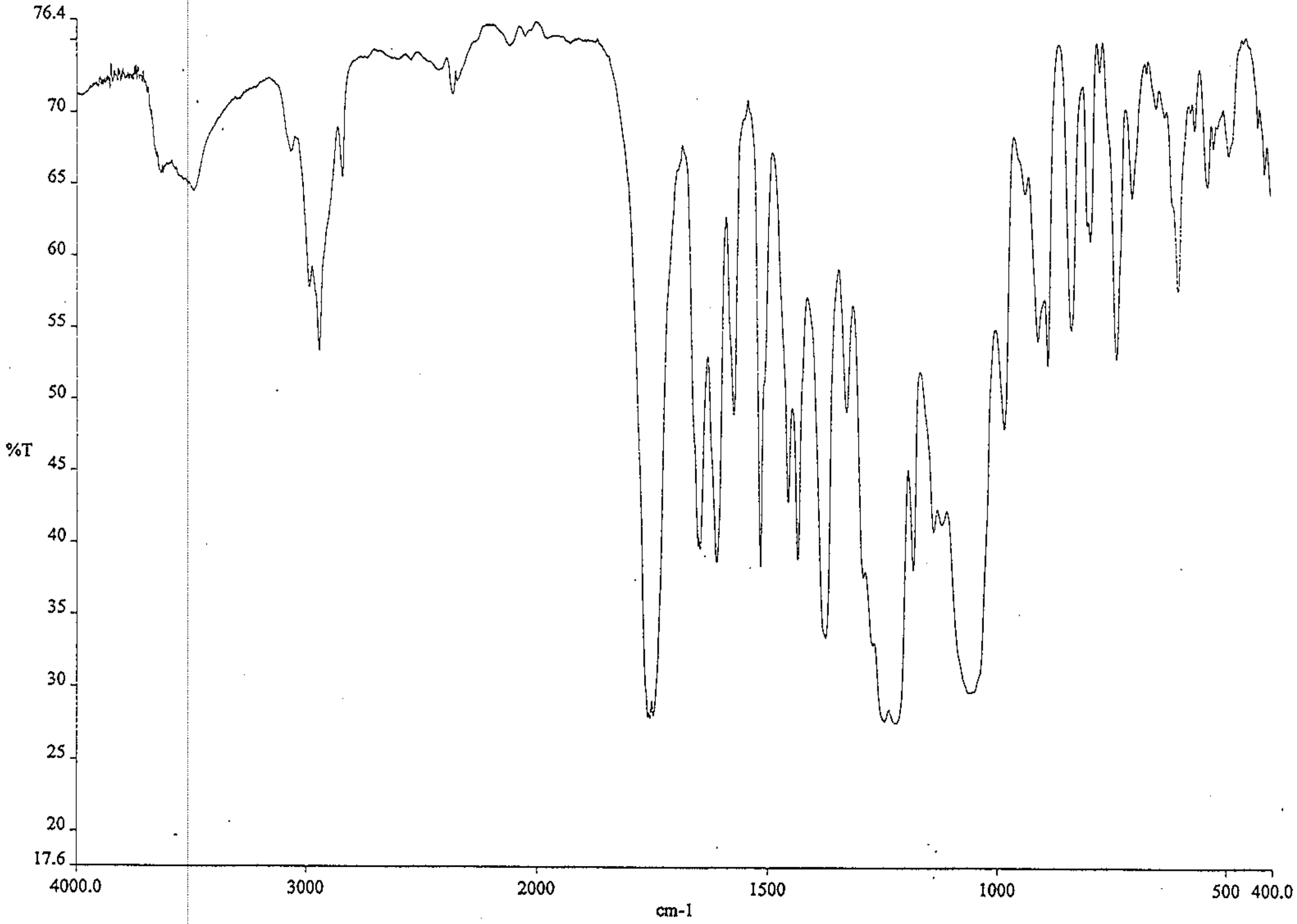


Figure 140 IR (neat) spectrum of **AcYS2**

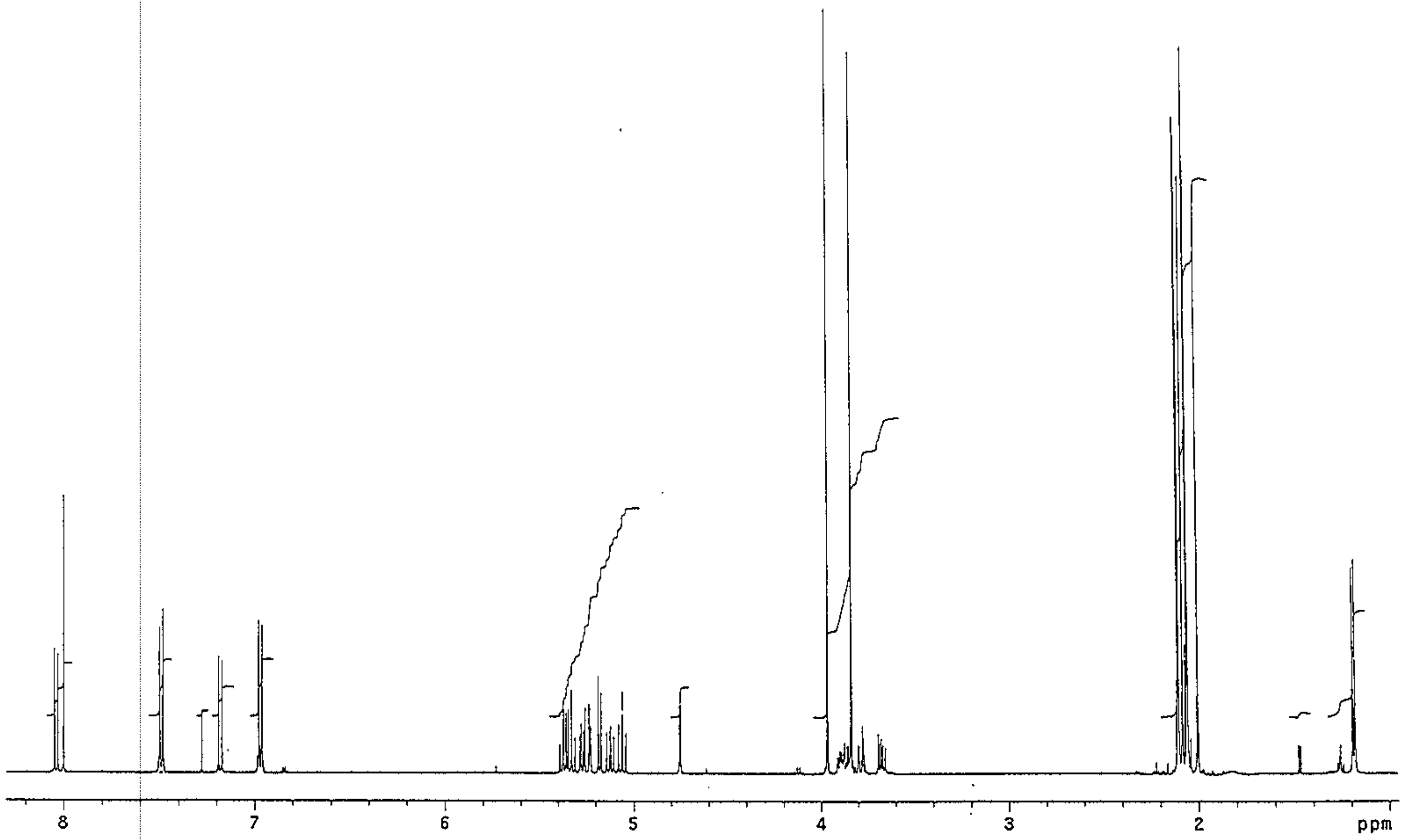


Figure 141 ^1H NMR (500 MHz) ($CDCl_3$) spectrum of **AcYS2**

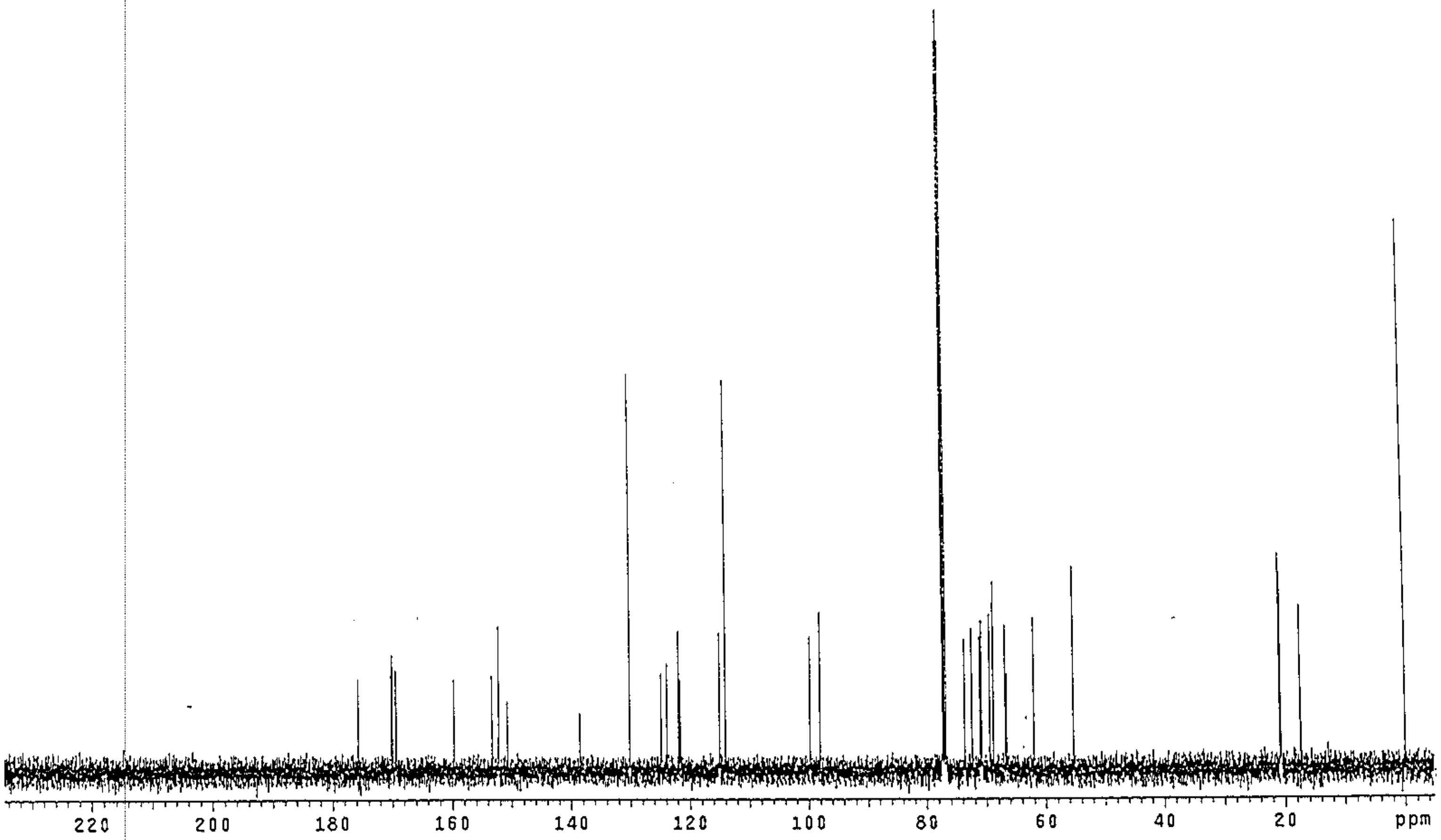


Figure 142 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **AcYS2**

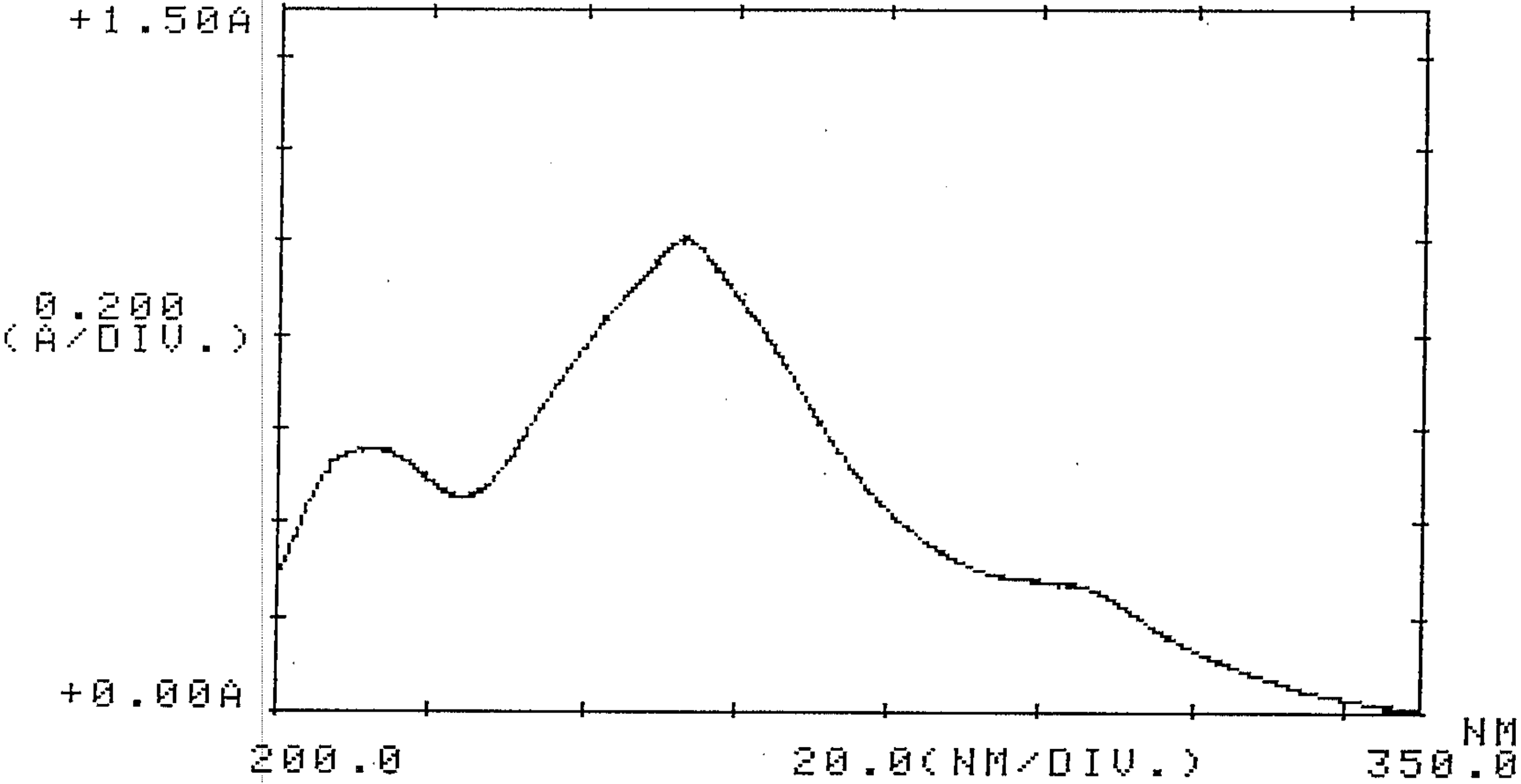


Figure 143 UV ($CHCl_3$) spectrum of **AcYS11**

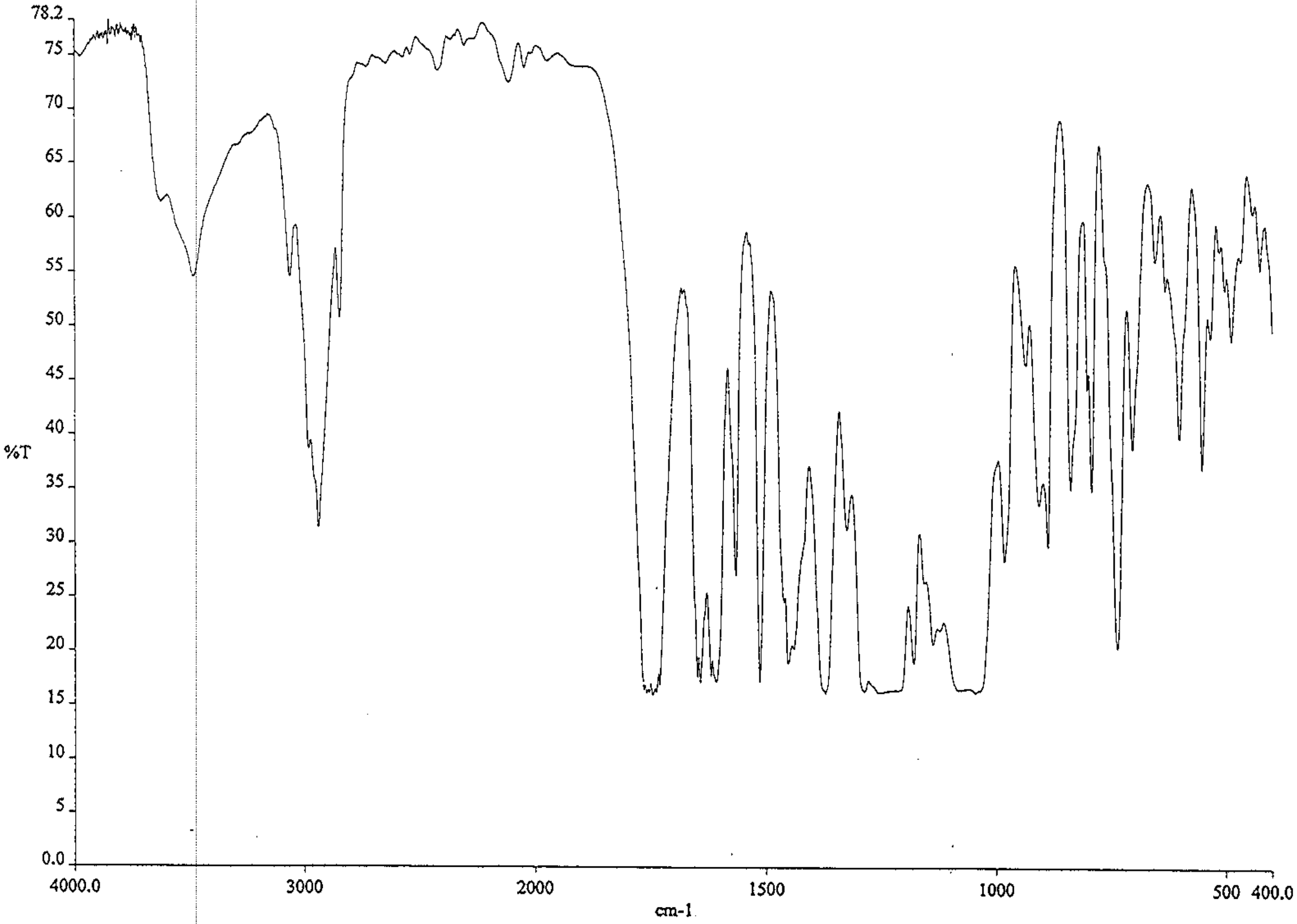


Figure 144 IR (neat) spectrum of **AcYS11**

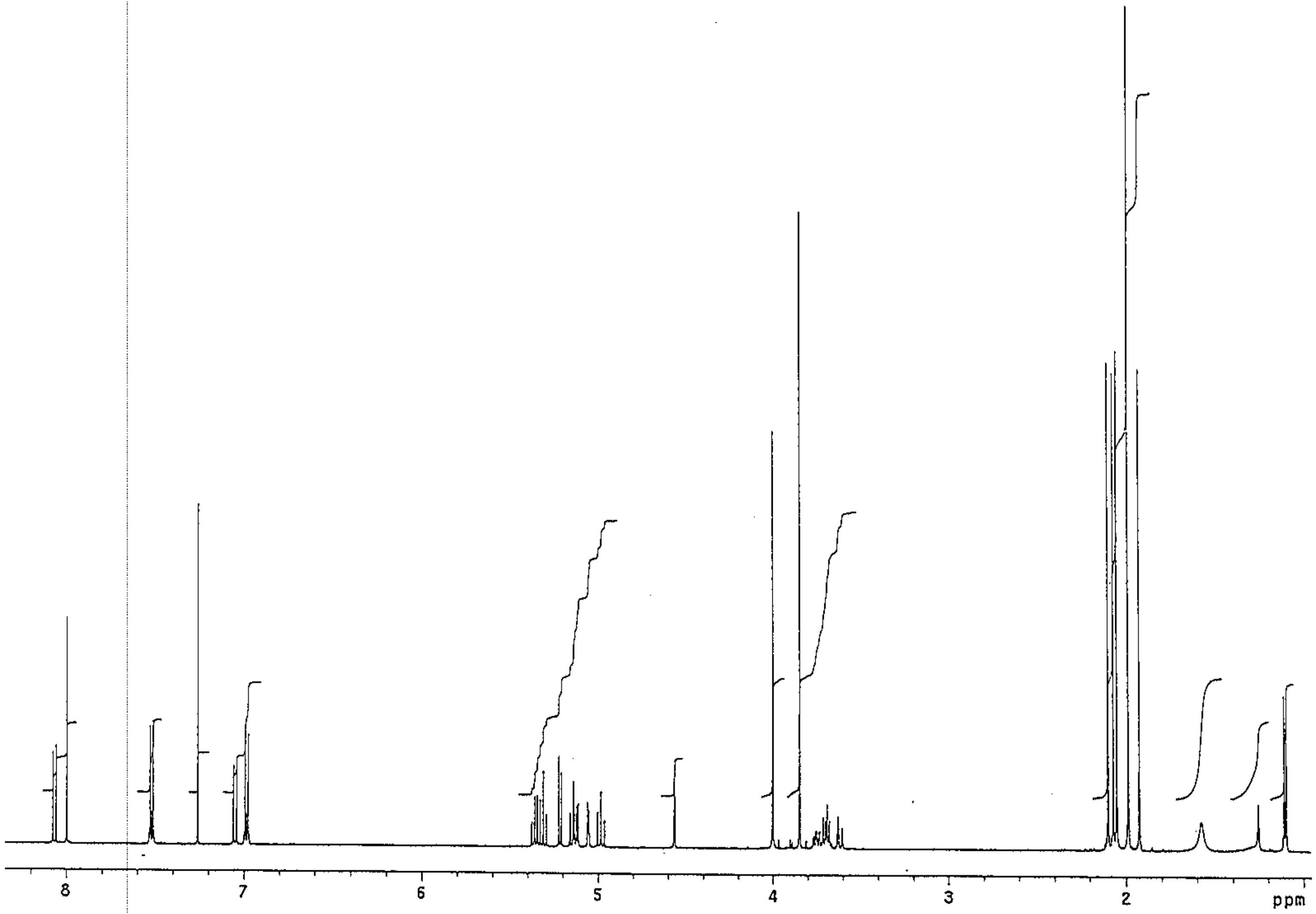


Figure 145 ^{1}H NMR (500 MHz) ($CDCl_3$) spectrum of **AcYS11**

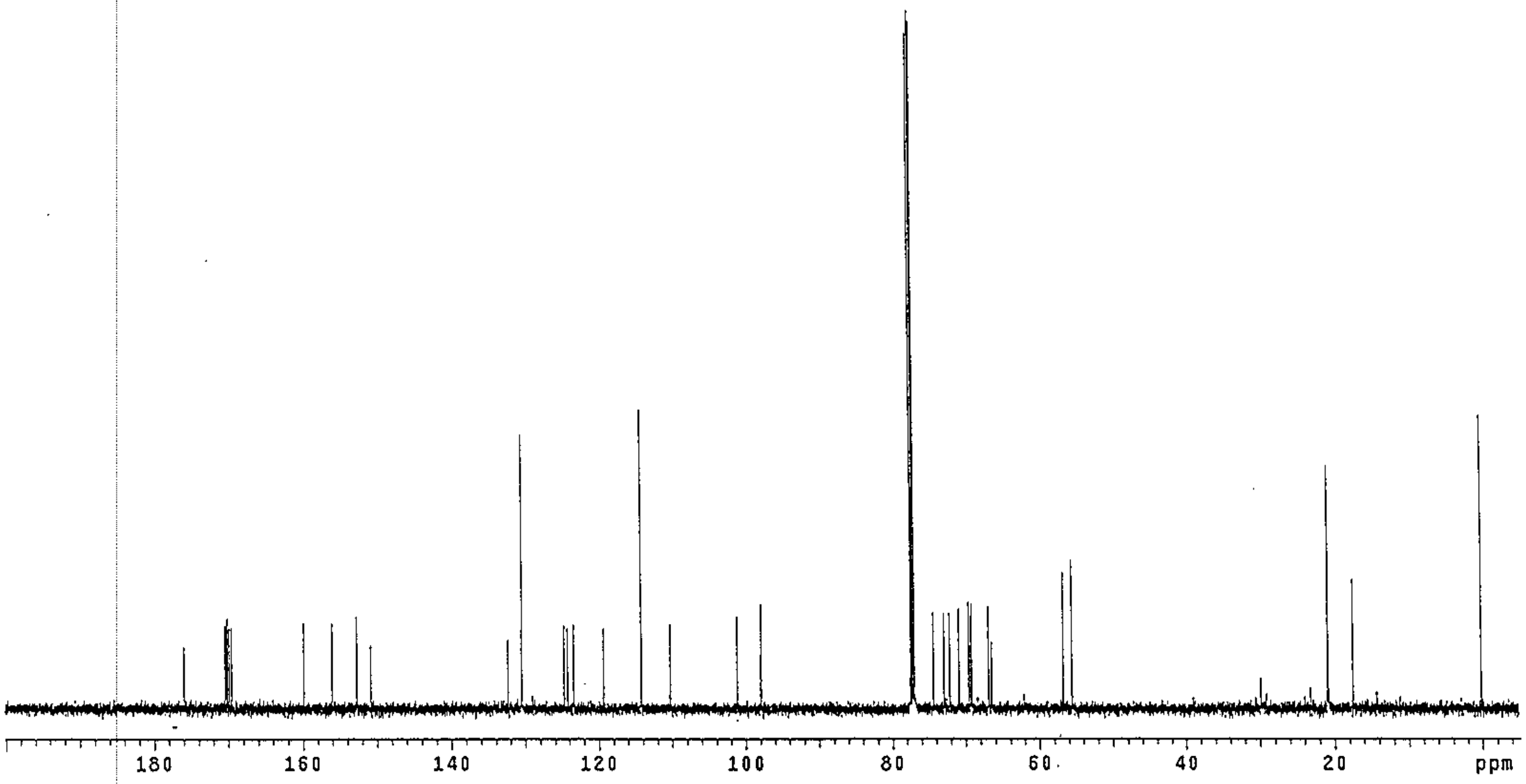


Figure 146 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **AcYS11**

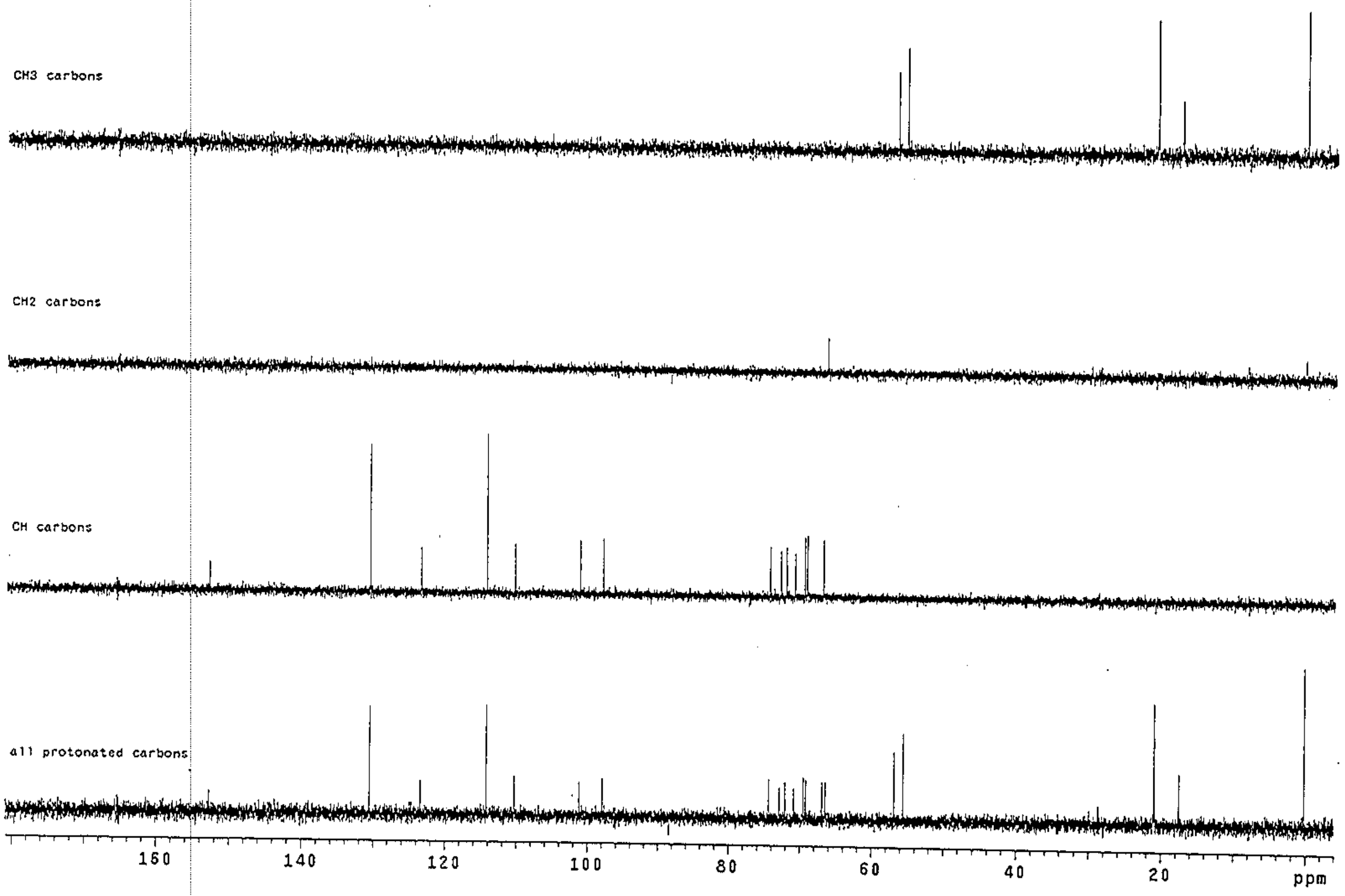


Figure 147 DEPT (135^{O}) ($CDCl_3$) spectrum of **AcYS11**

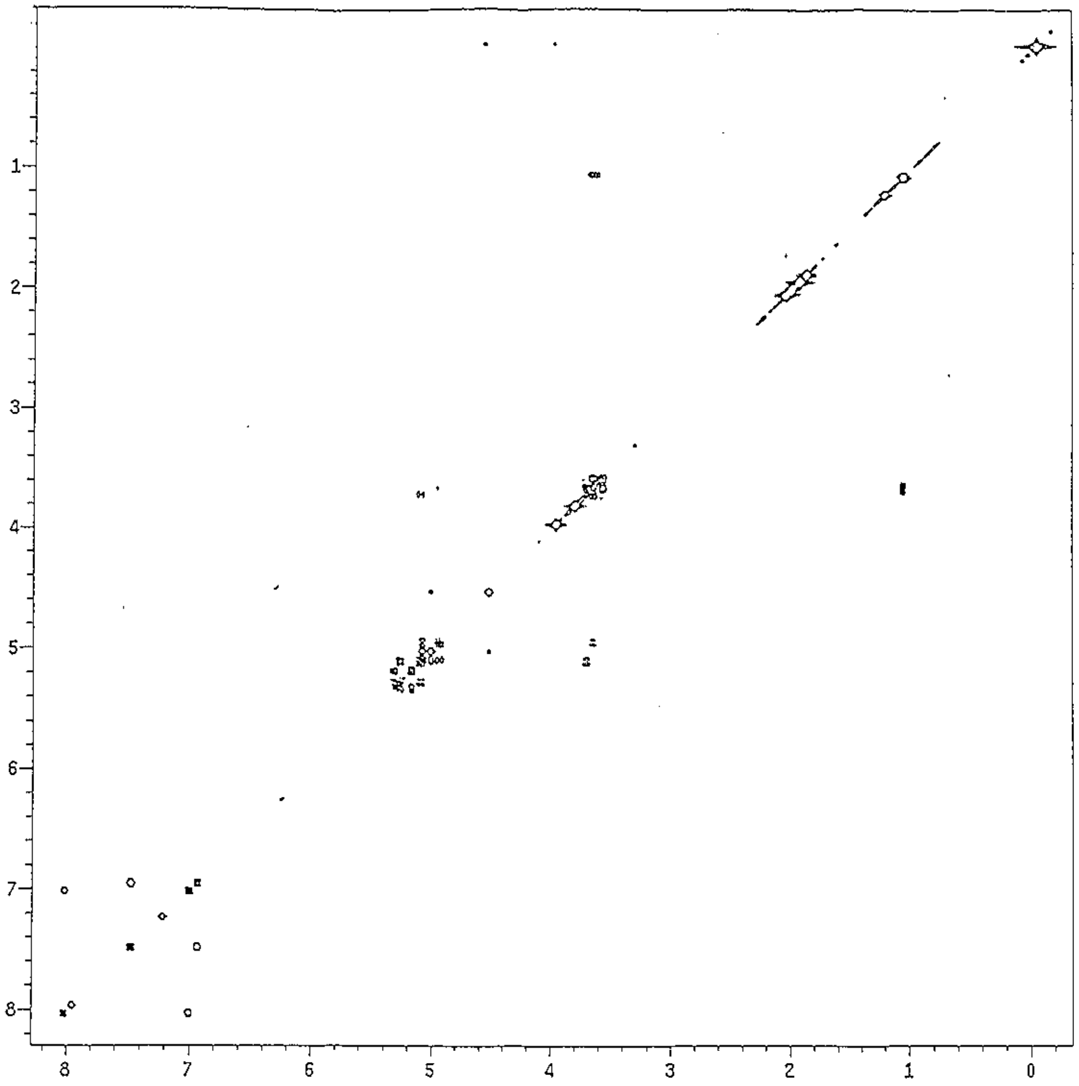


Figure 148 ^{1}H-^{1}H COSY spectrum of **AcYS11**

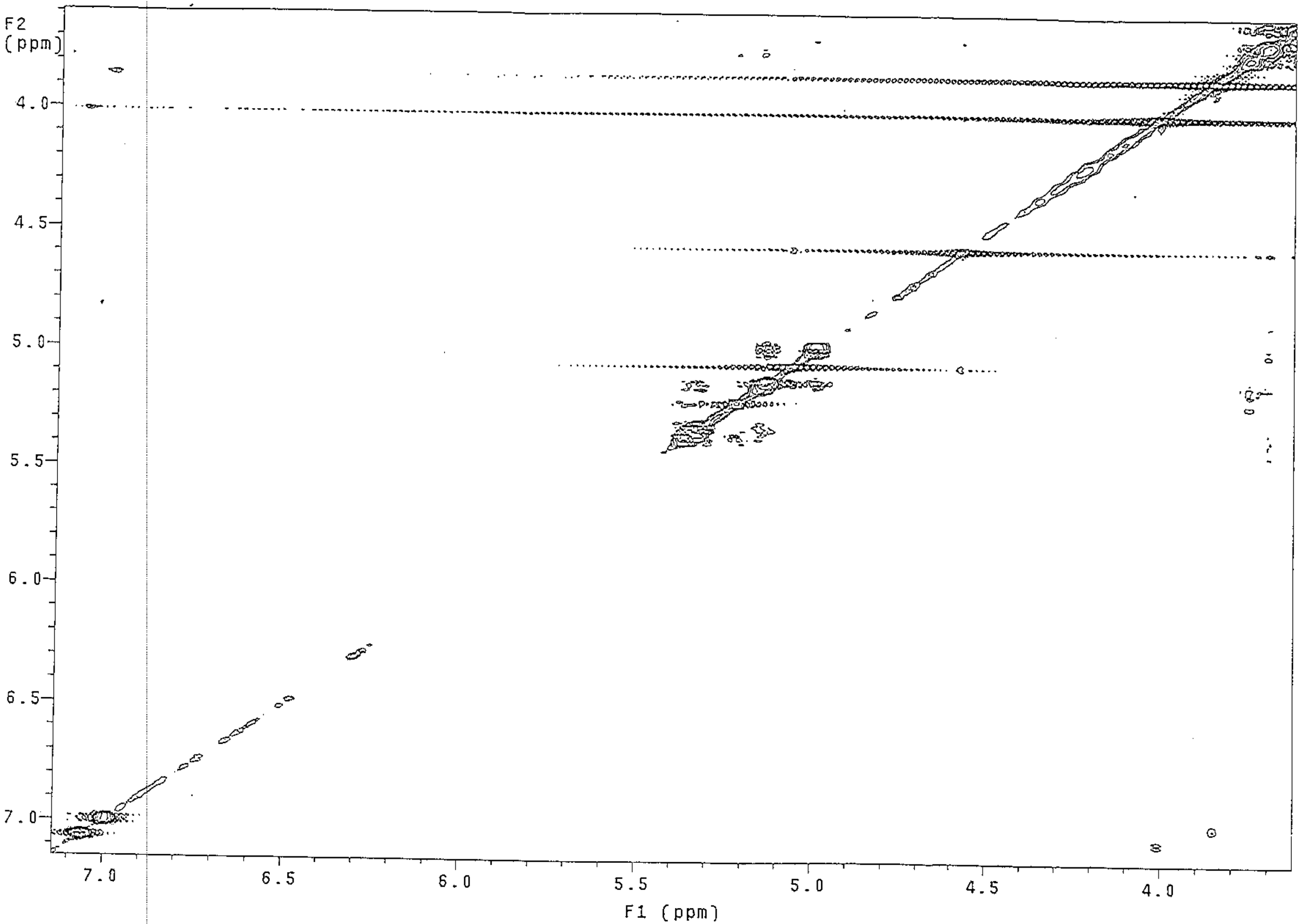


Figure 149 2D NOESY spectrum of **AcYS11**

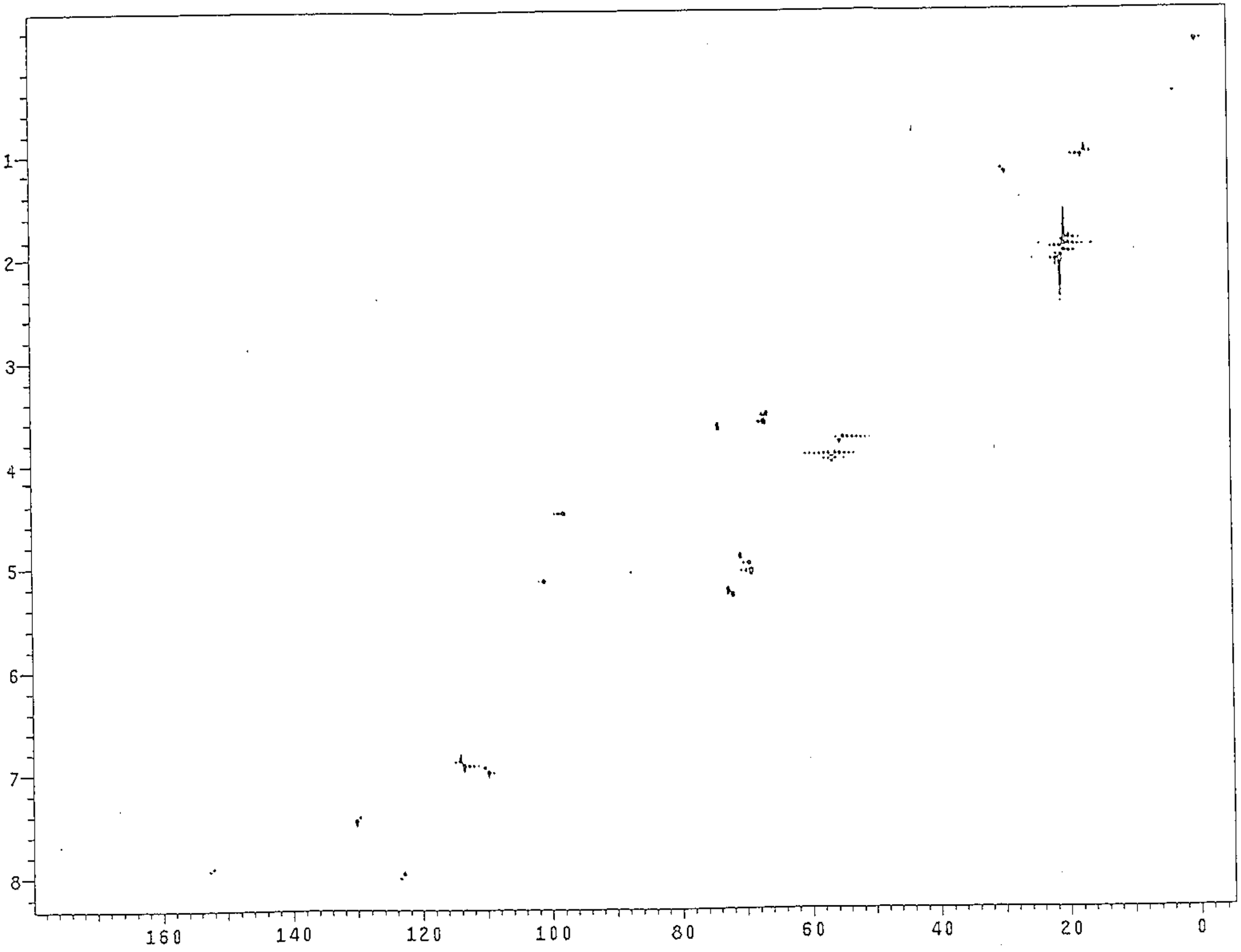


Figure 150 2D HMQC spectrum of **AcYS11**

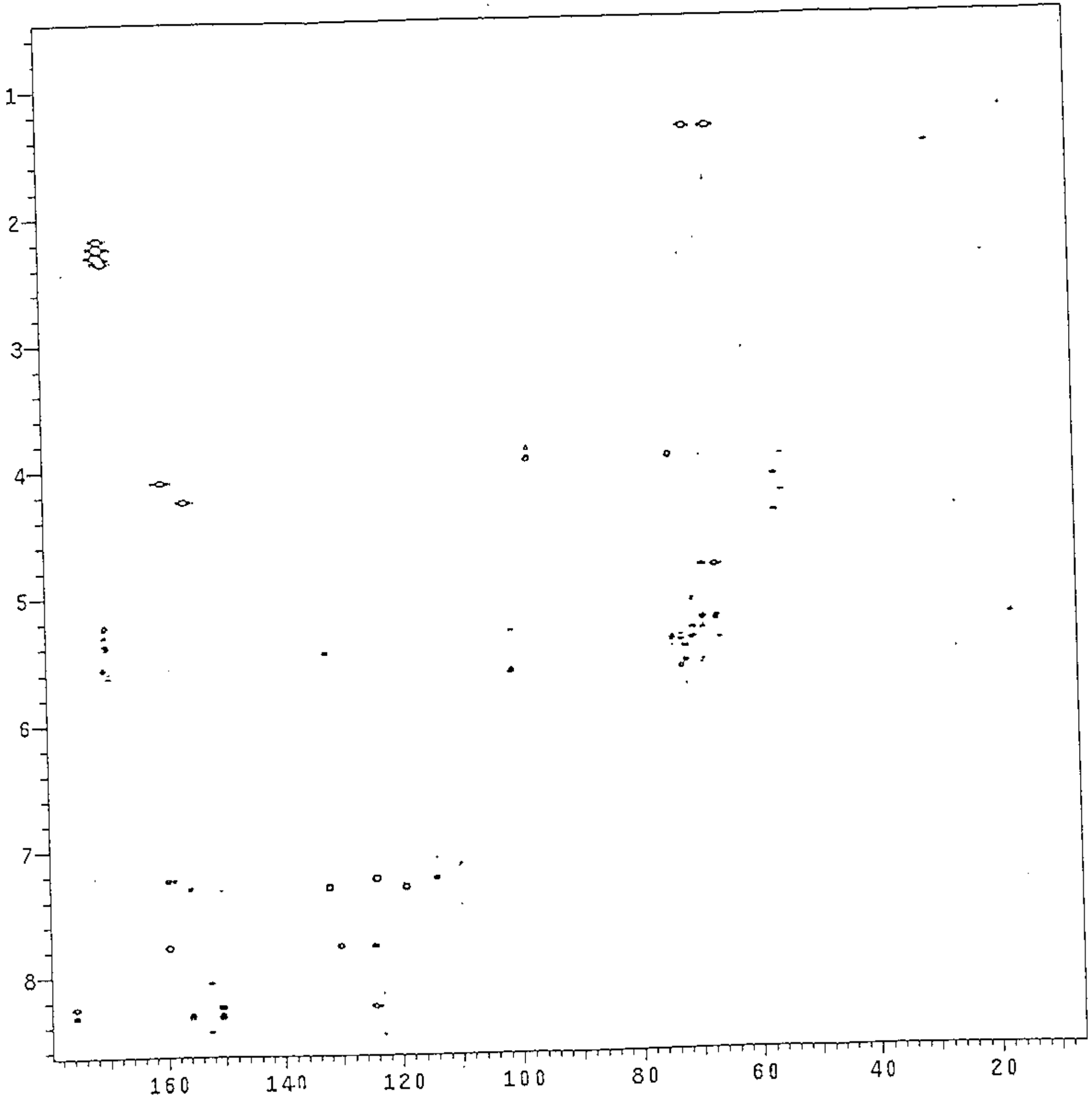


Figure 151 2D HMBC spectrum of AcYS11

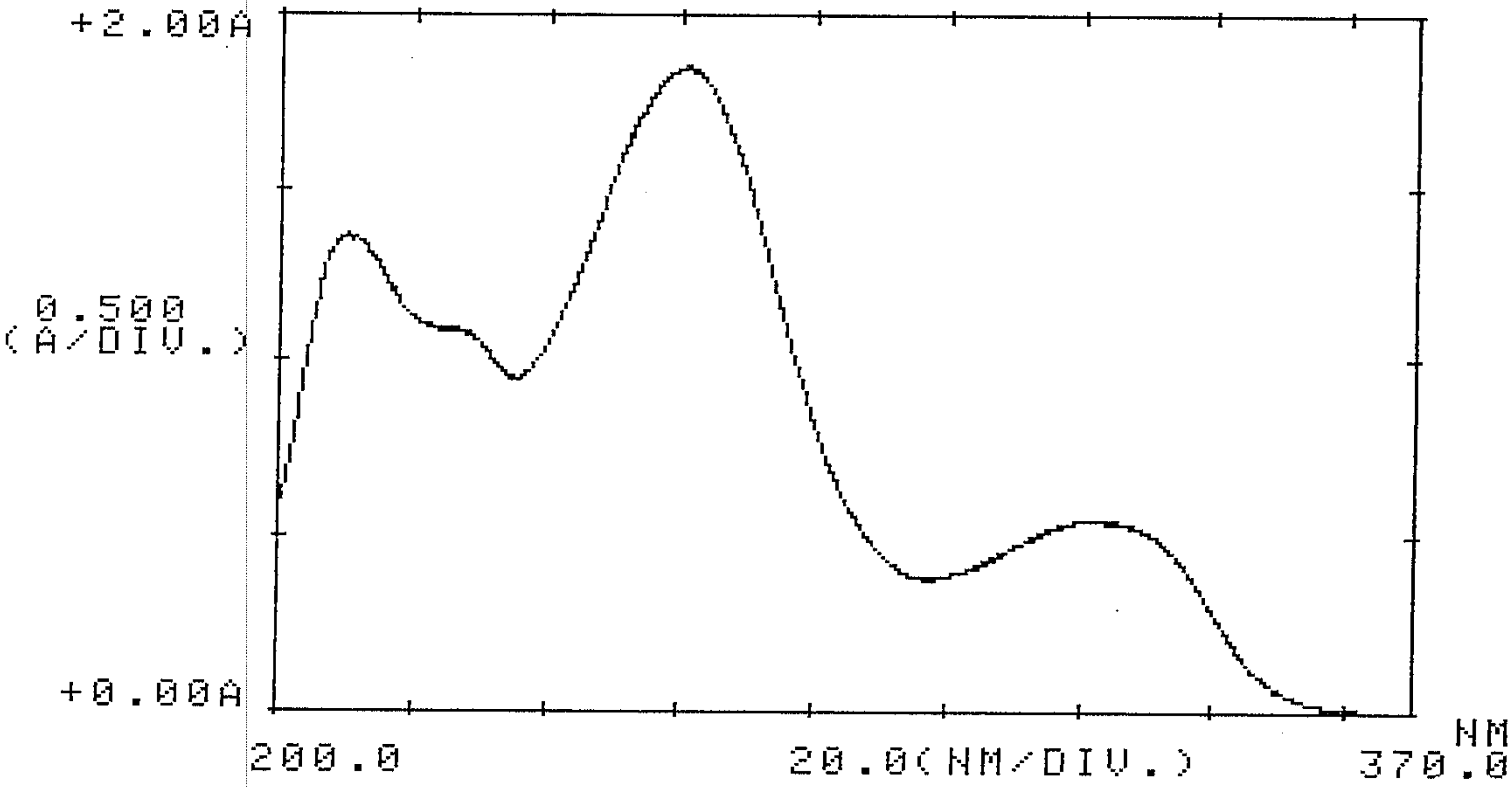


Figure 152 UV ($CHCl_3$) spectrum of **AcYS6**

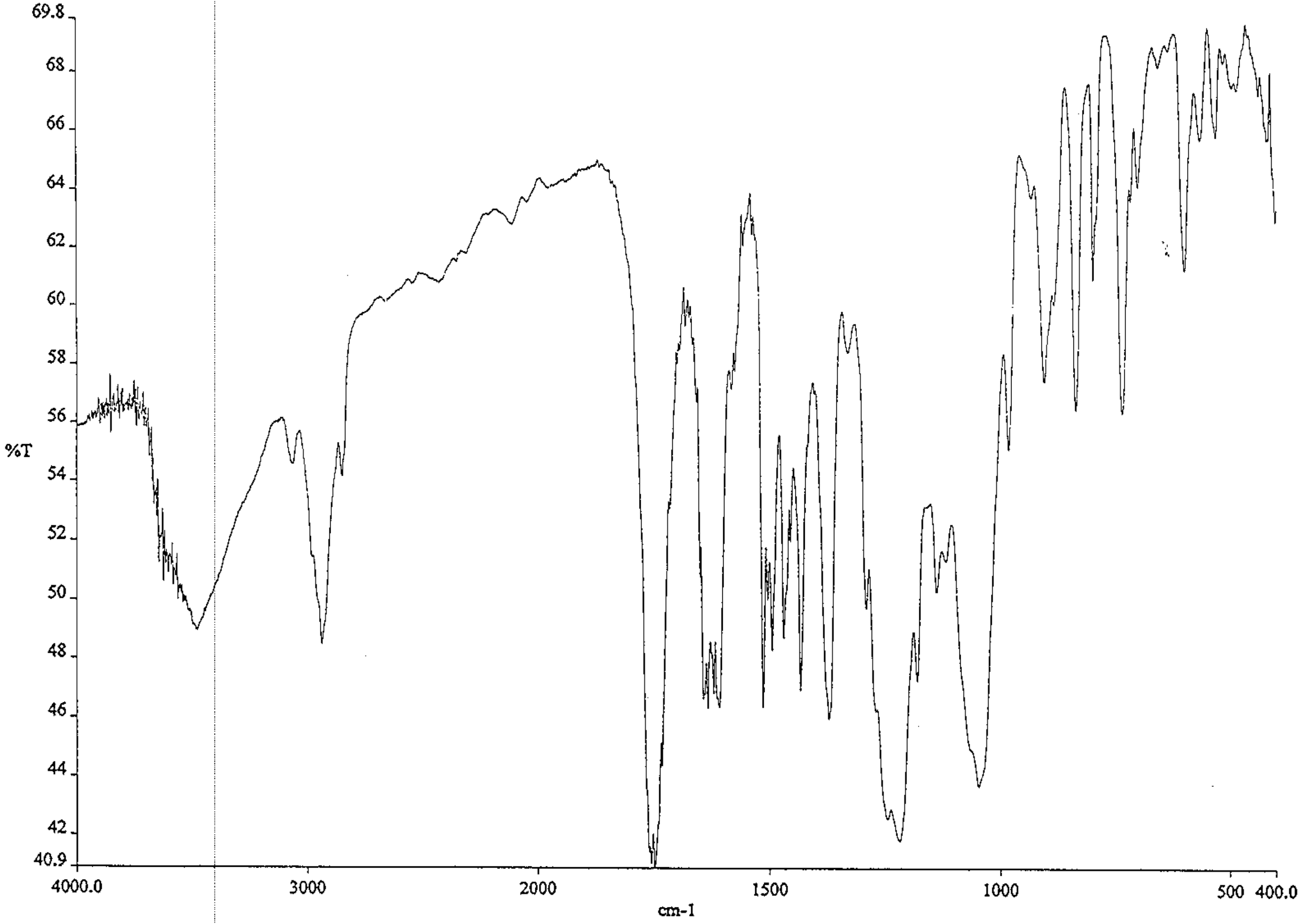


Figure 153 IR (neat) spectrum of **AcYS6**

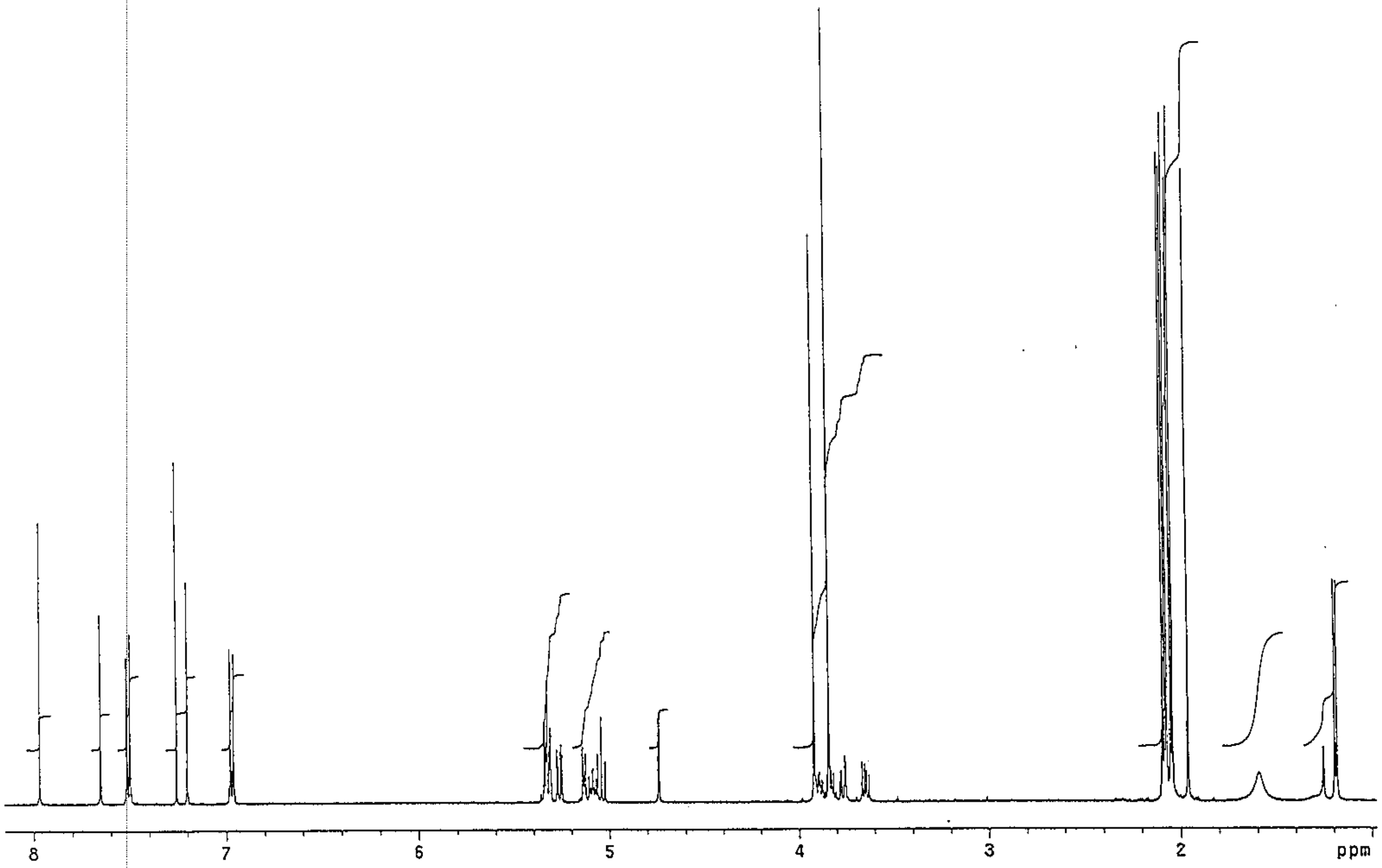


Figure 154 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **AcYS6**

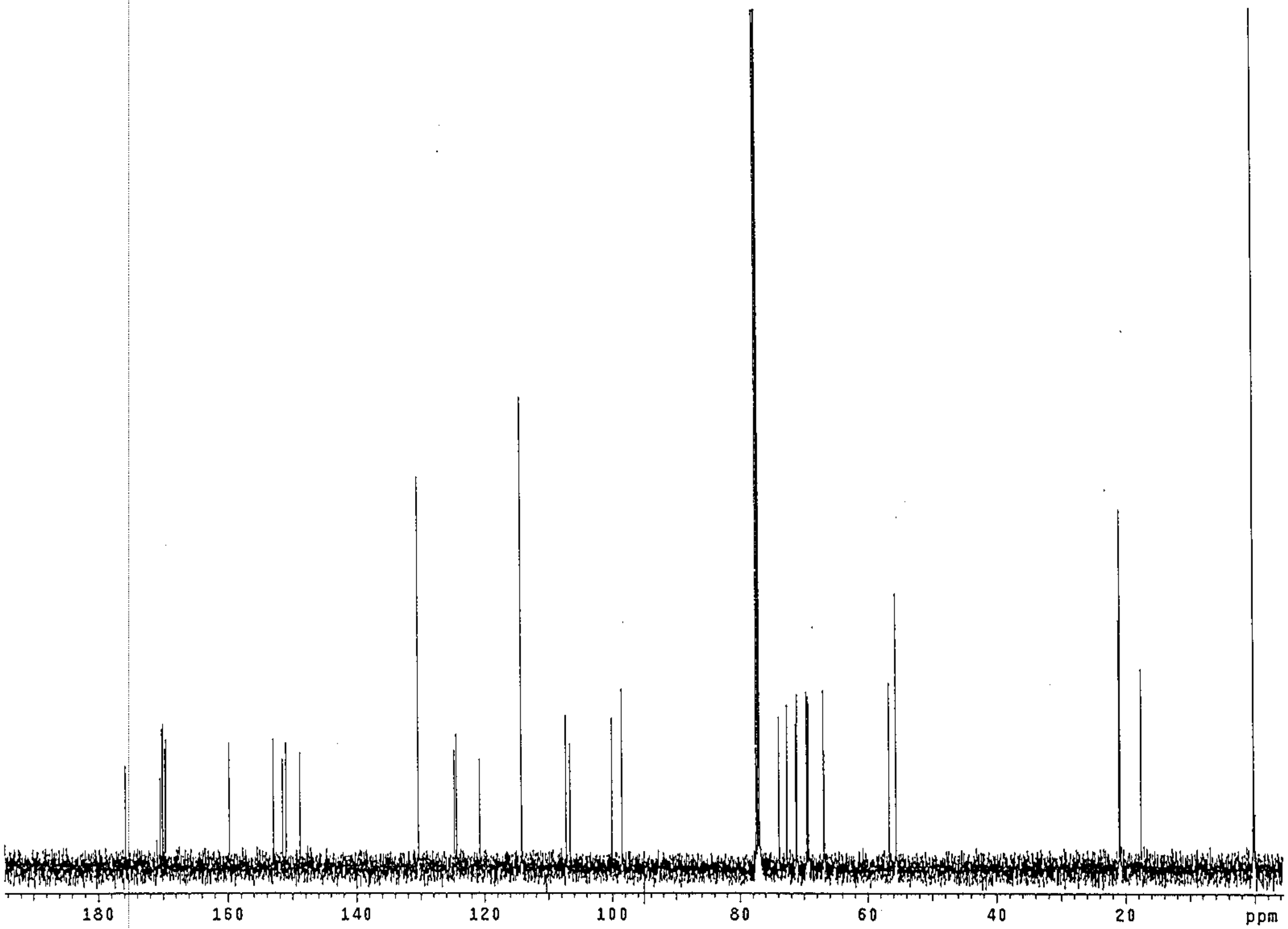


Figure 155 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **AcYS6**

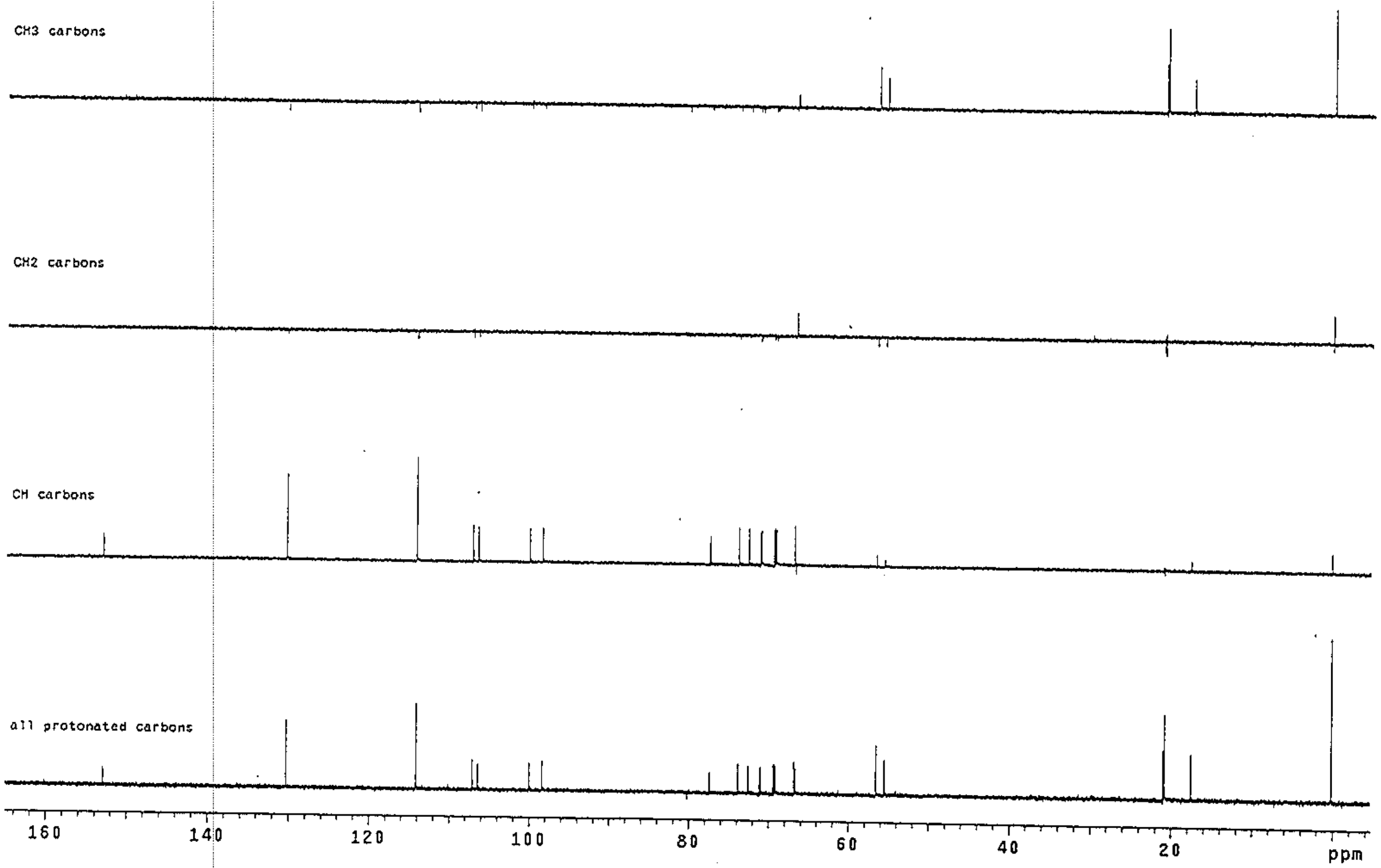


Figure 156 DEPT (135^{O}) (CDCl$_3$) spectrum of **AcYS6**

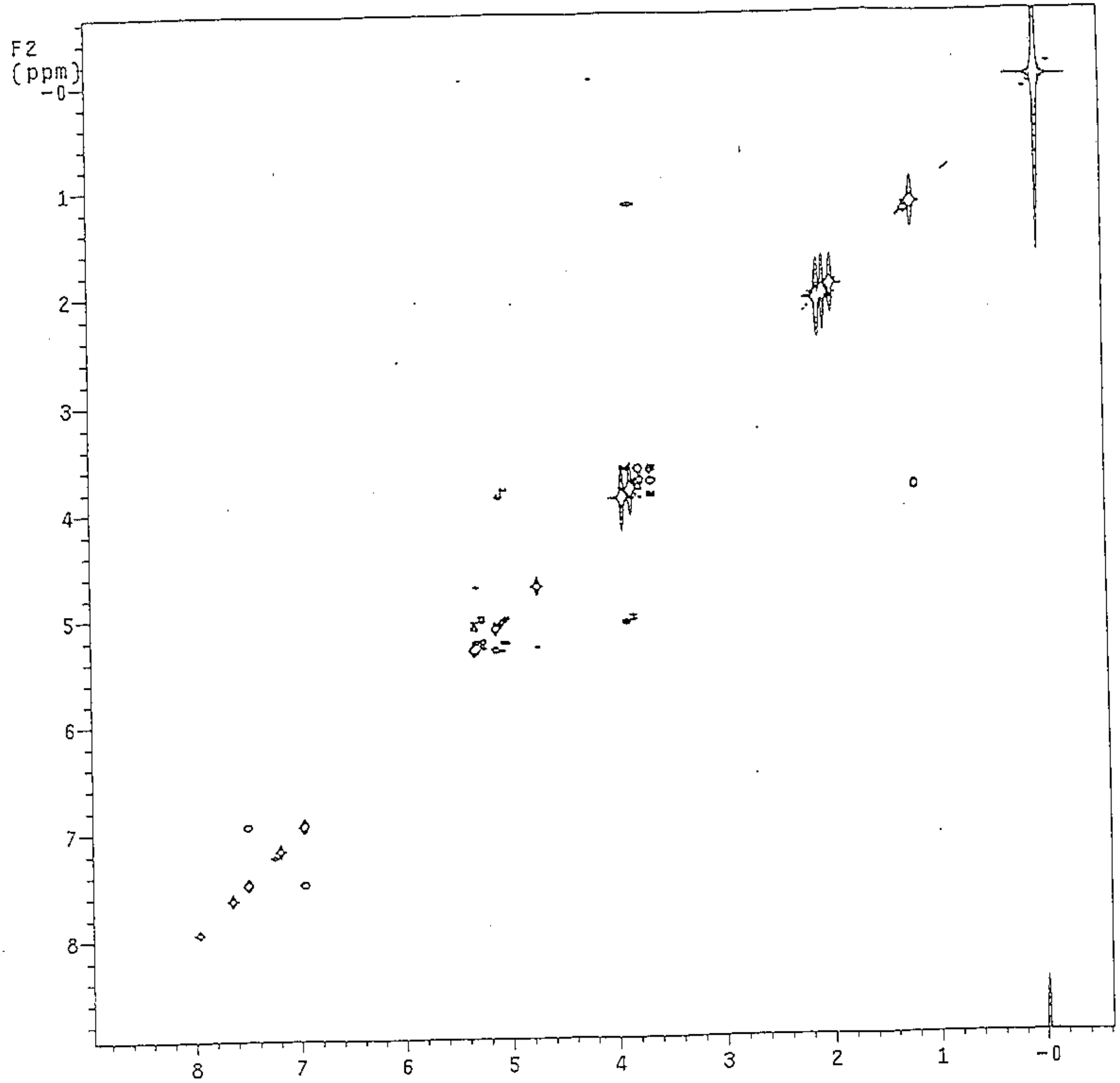


Figure 157 ^{1}H-^{1}H COSY spectrum of **AcYS6**

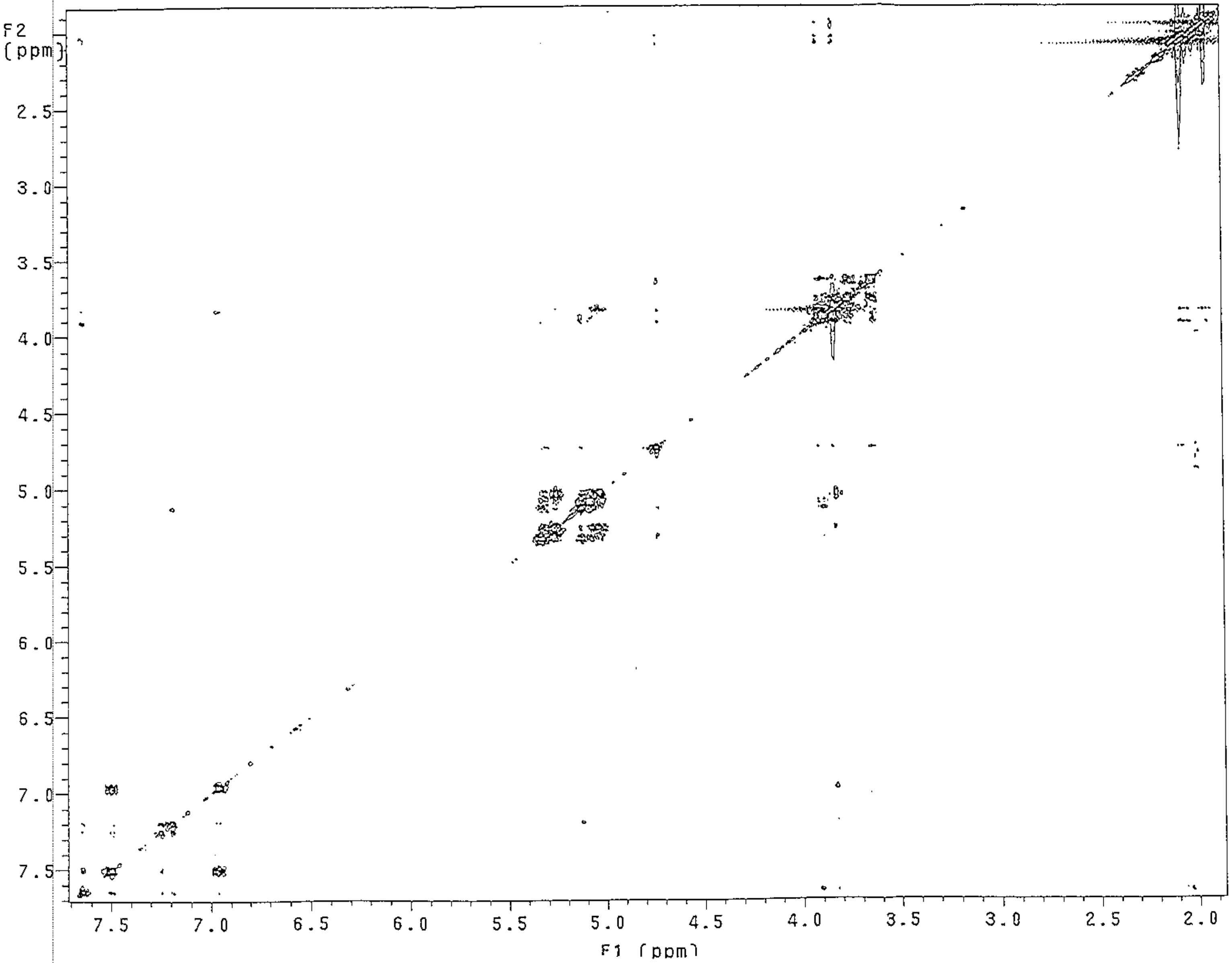


Figure 158 2D NOESY spectrum of AcYS6

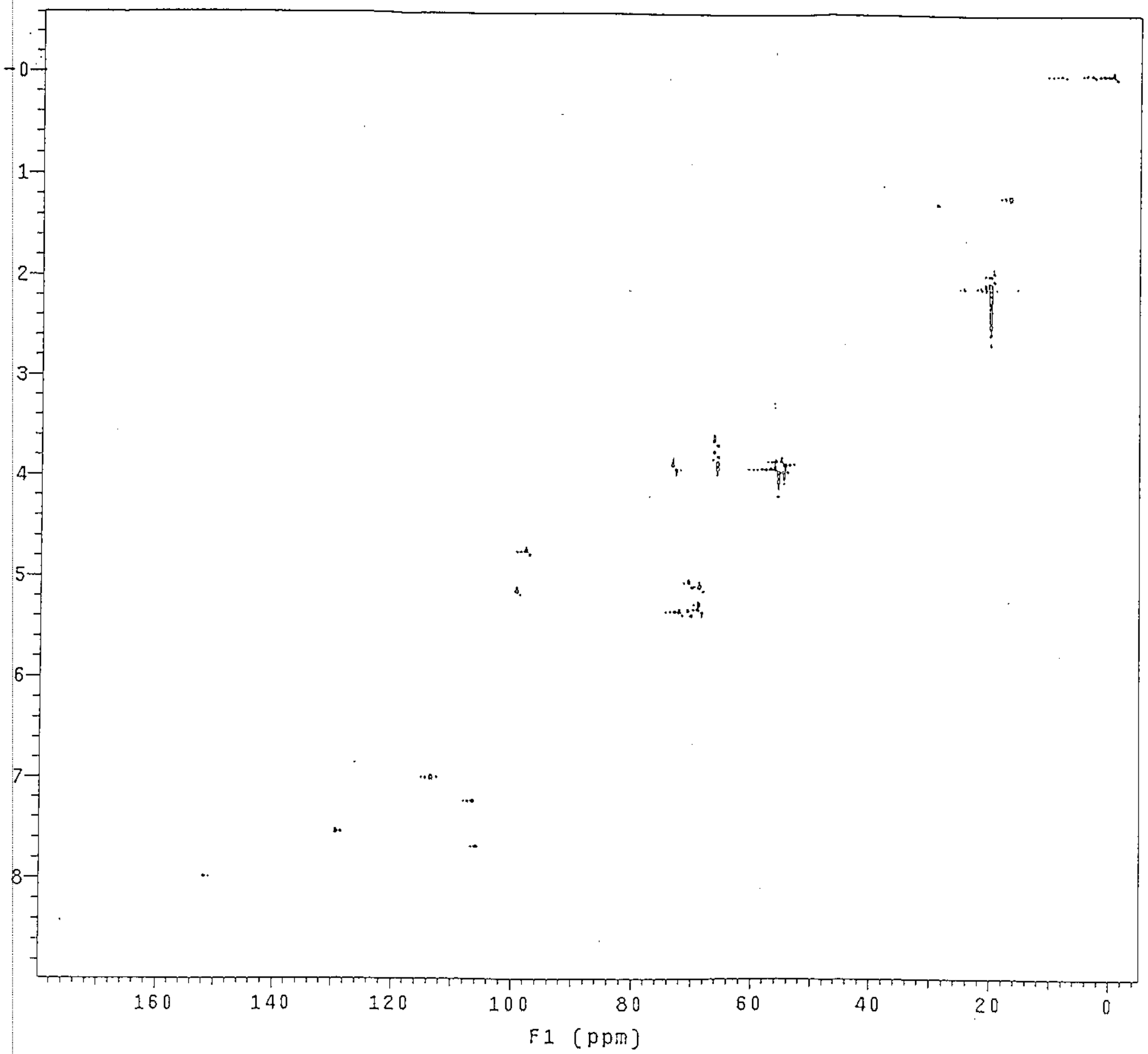


Figure 159 2D HMQC spectrum of AcYS6

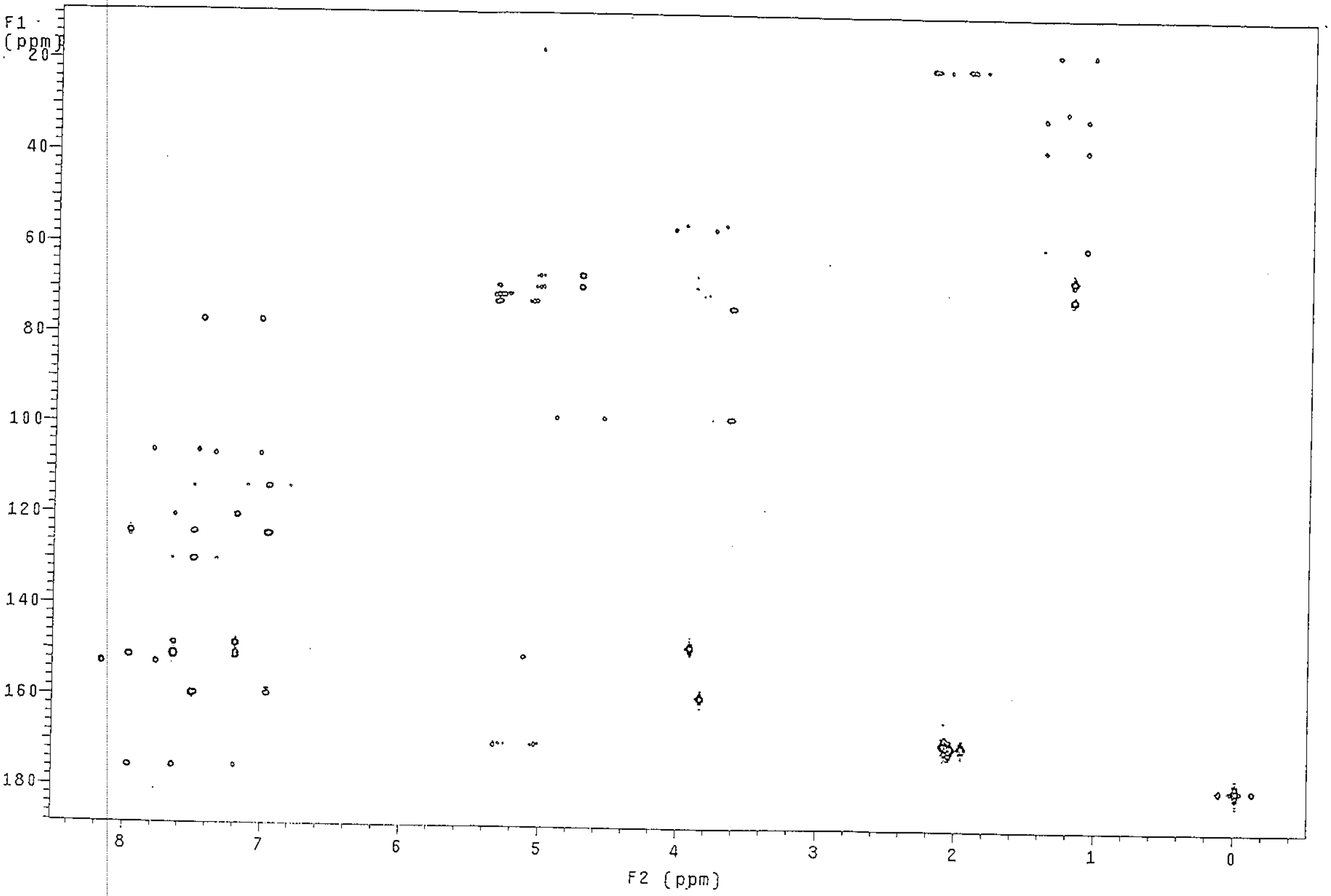


Figure 160 2D HMBC spectrum of **AcYS6**

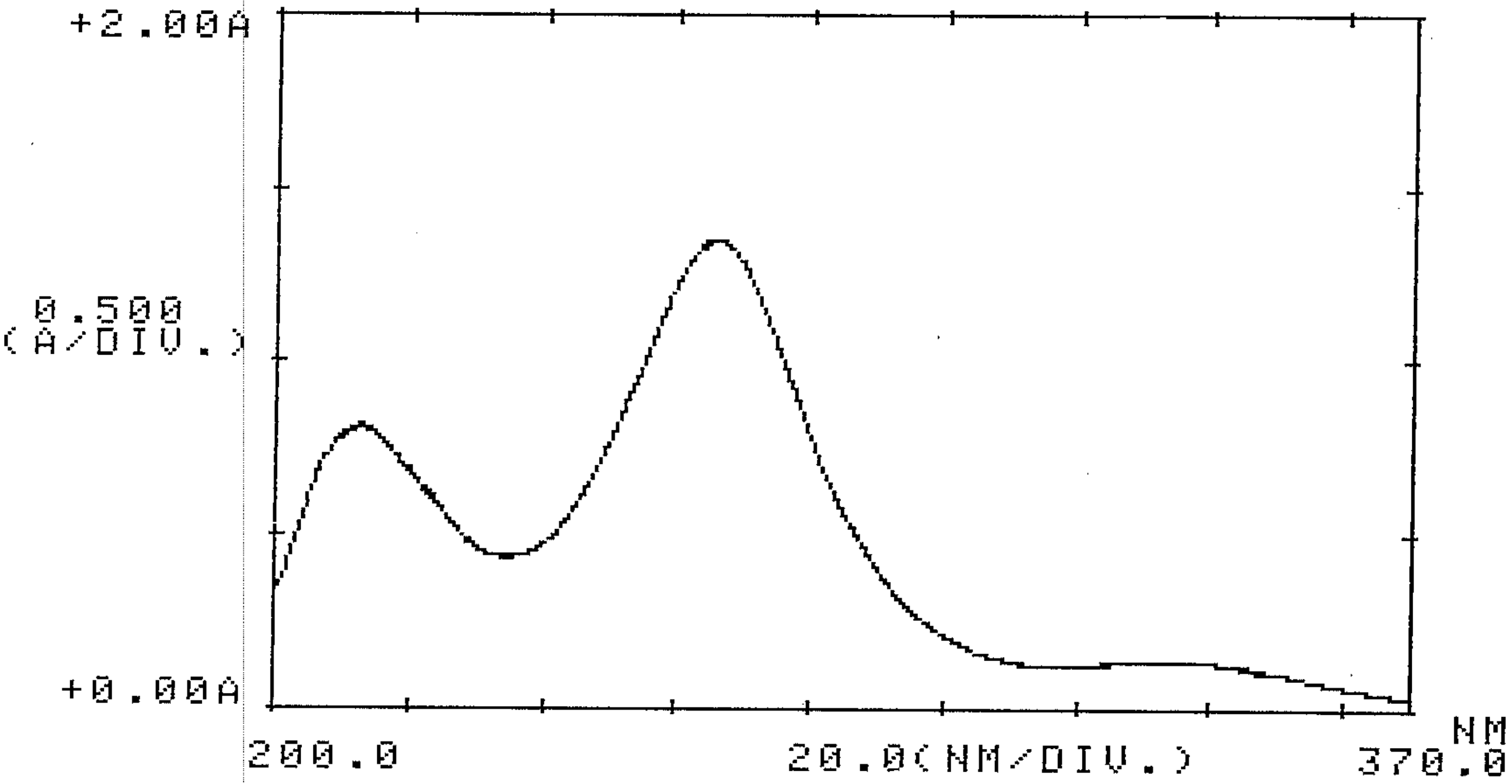


Figure 161 UV ($CHCl_3$) spectrum of **AcYS7**

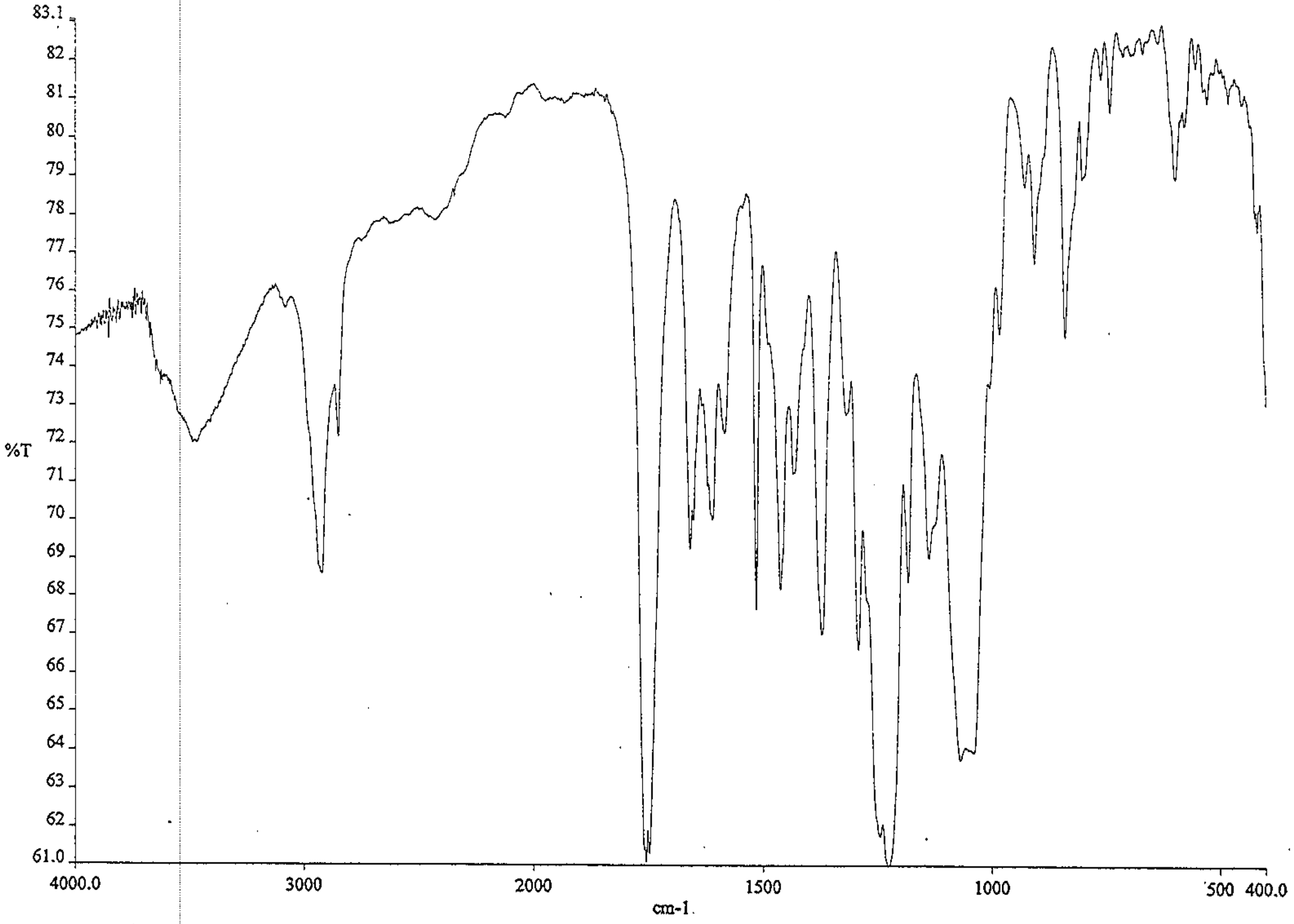


Figure 162 IR (neat) spectrum of **AcYS7**

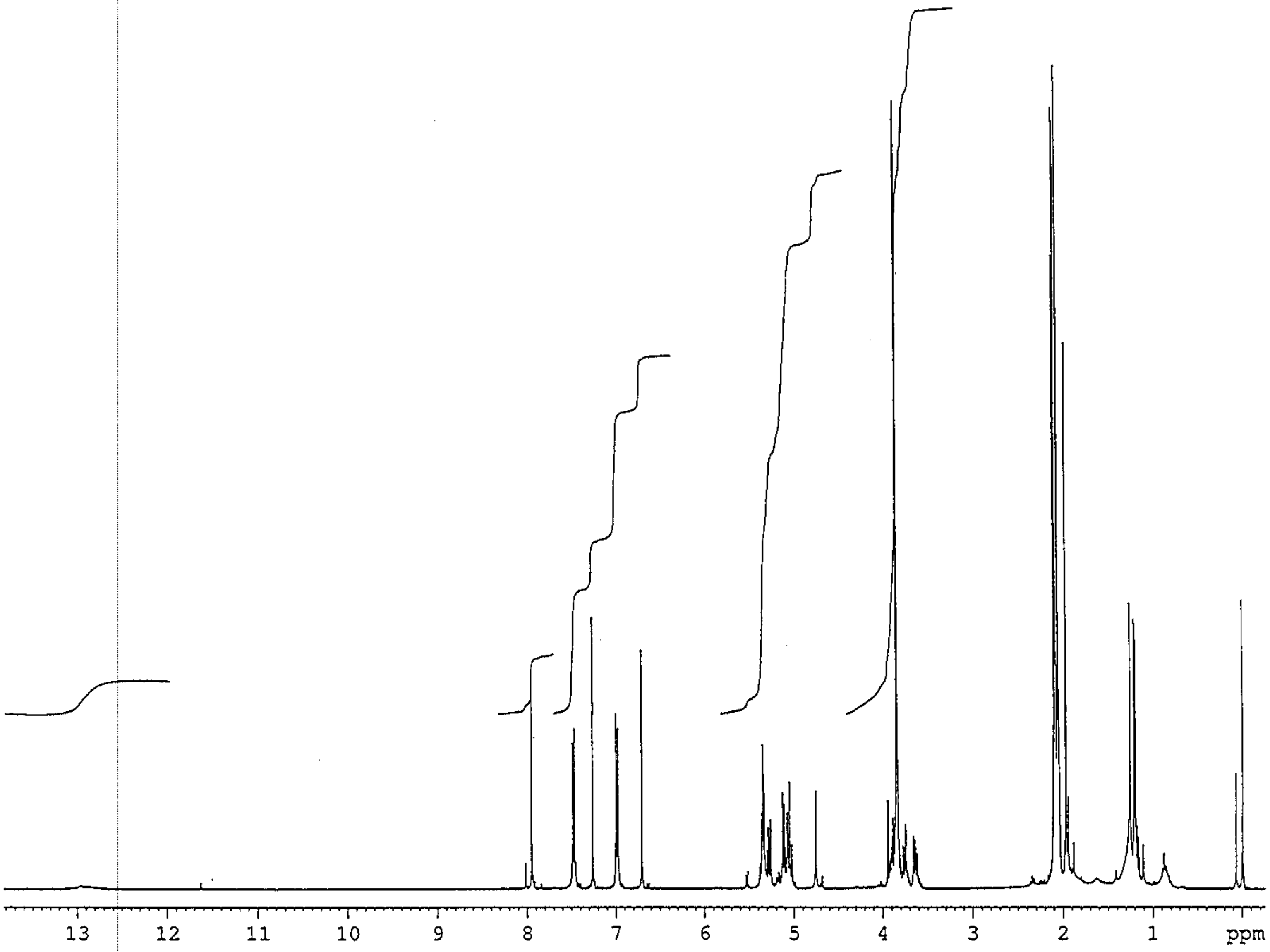


Figure 163 ^{1}H NMR (400 MHz) (CDCl$_3$) spectrum of **AcYS7**

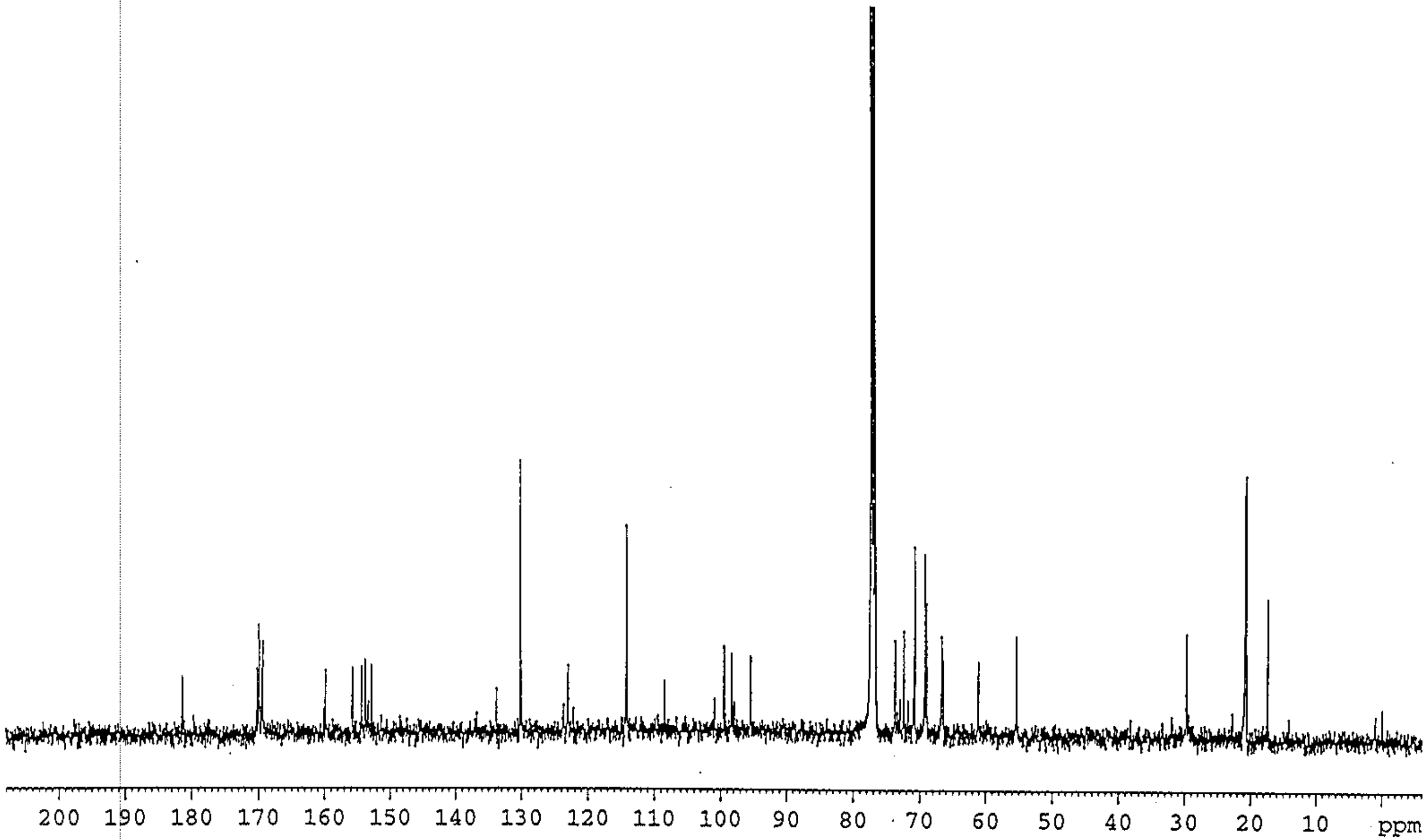


Figure 164 ^{13}C NMR (100 MHz) (CDCl$_3$) spectrum of **AcYS7**

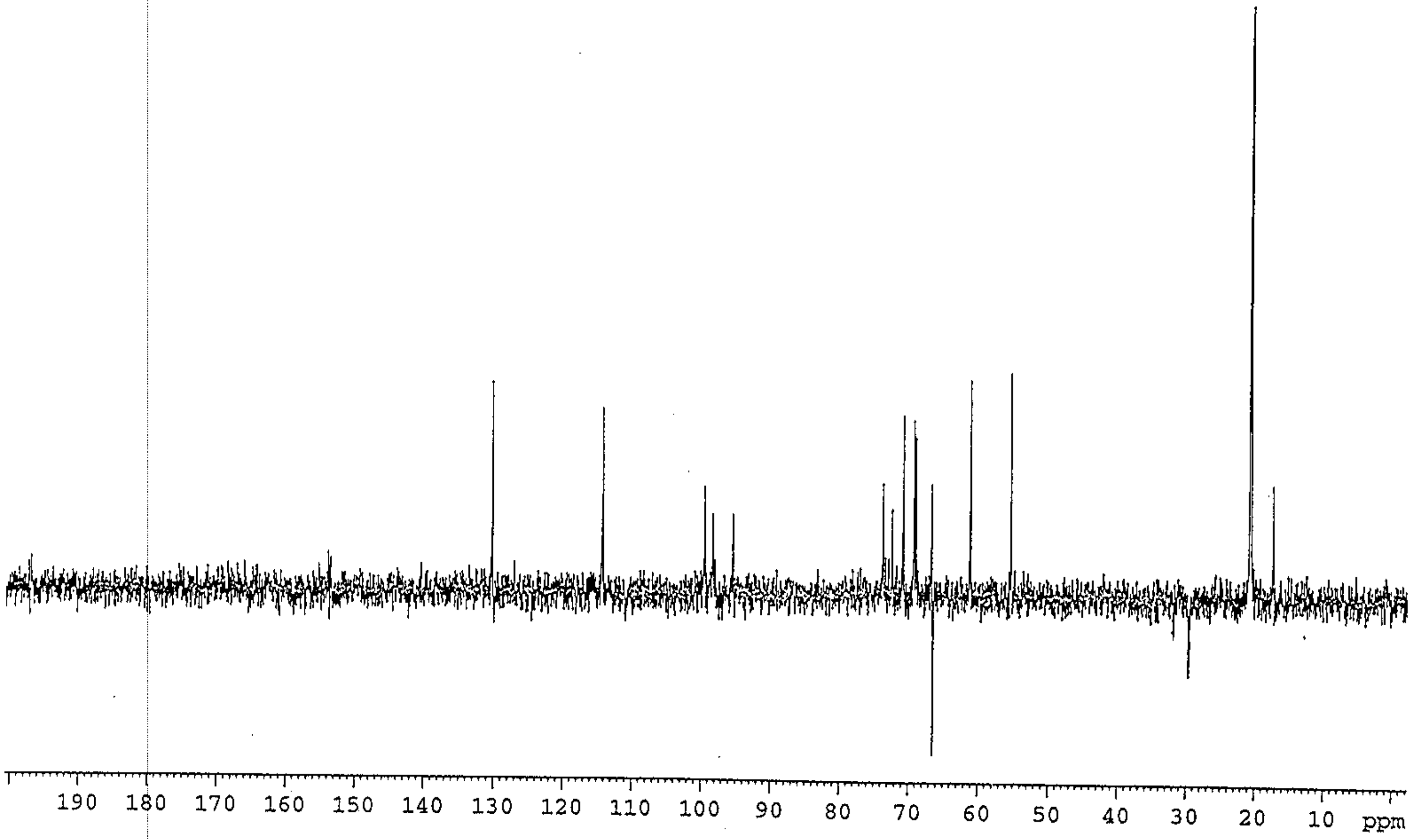


Figure 165 DEPT (135^{O}) (CDCl$_3$) spectrum of **AcYS7**

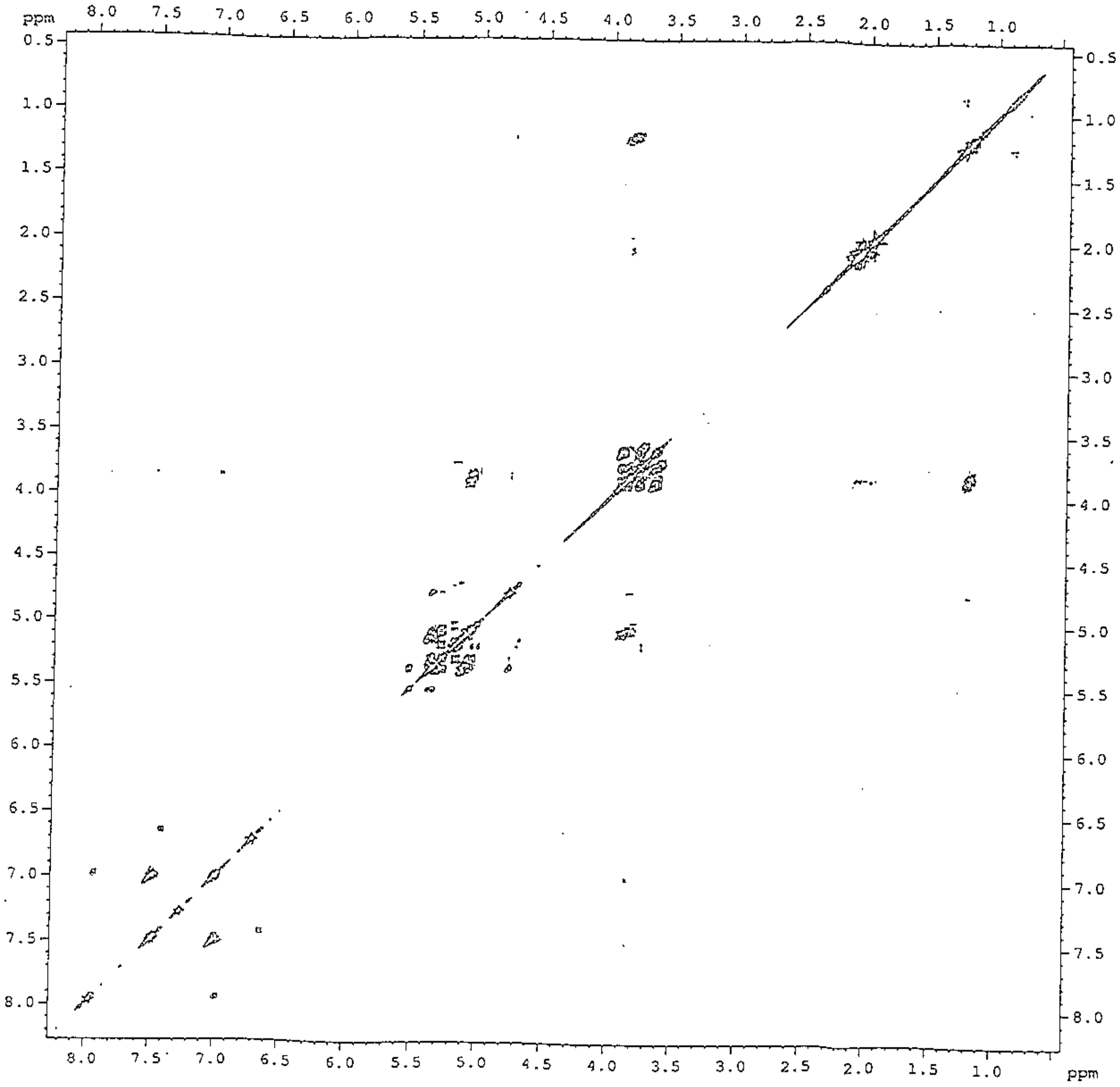


Figure 166 ^{1}H-^{1}H COSY spectrum of AcYS7

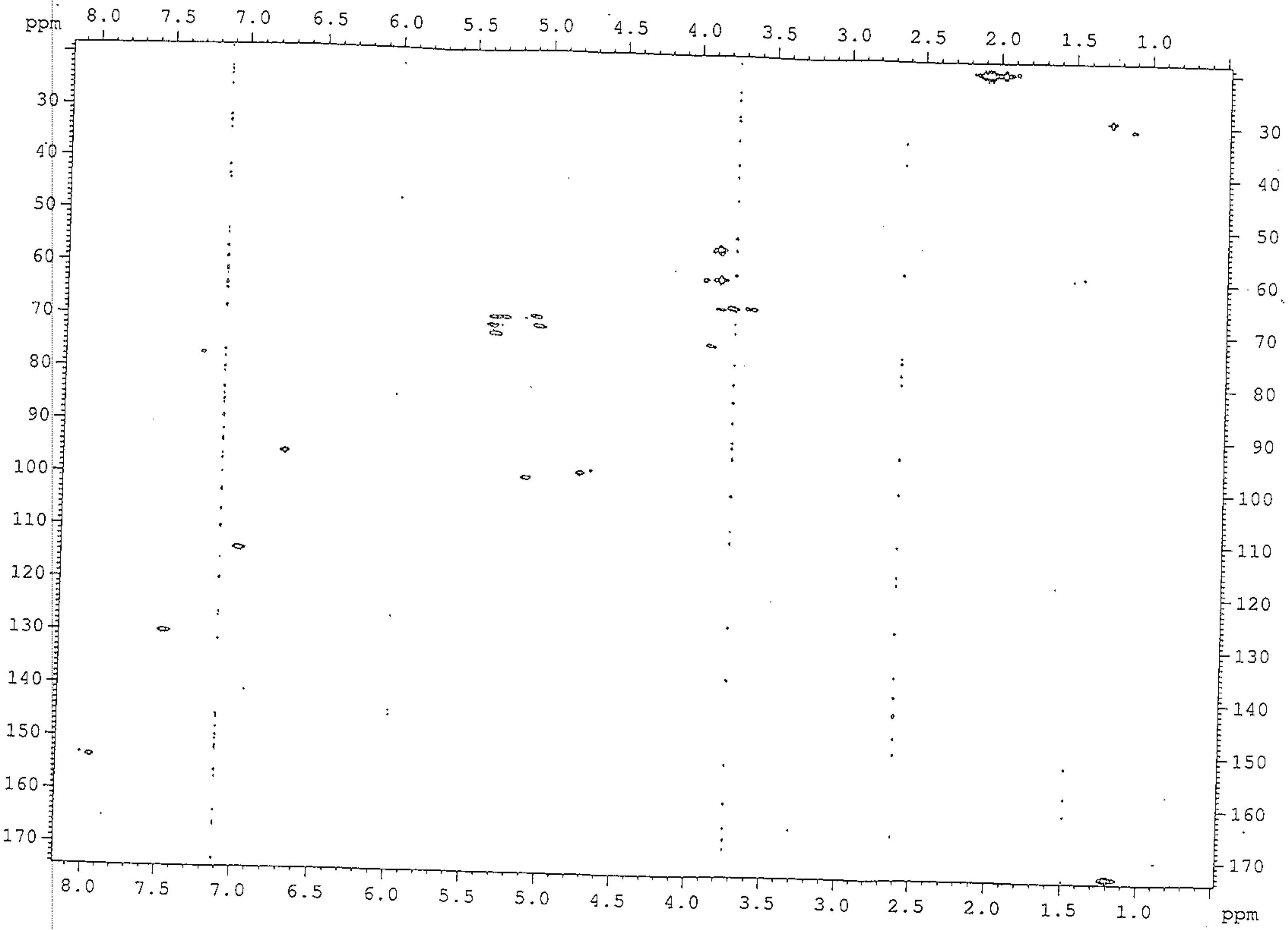


Figure 167 2D HMQC spectrum of **AcYS7**

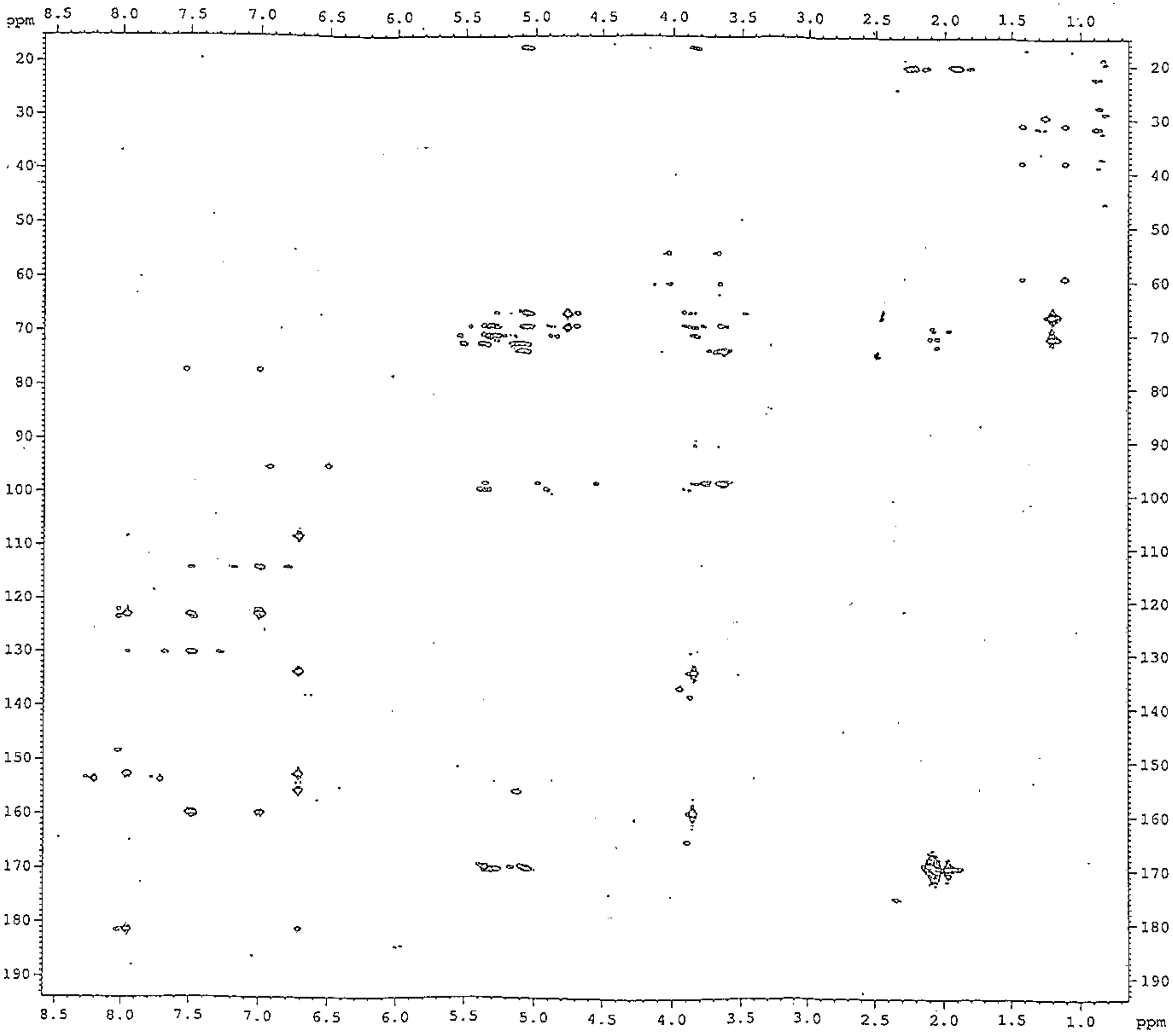


Figure 168 2D HMBC spectrum of AcYS7

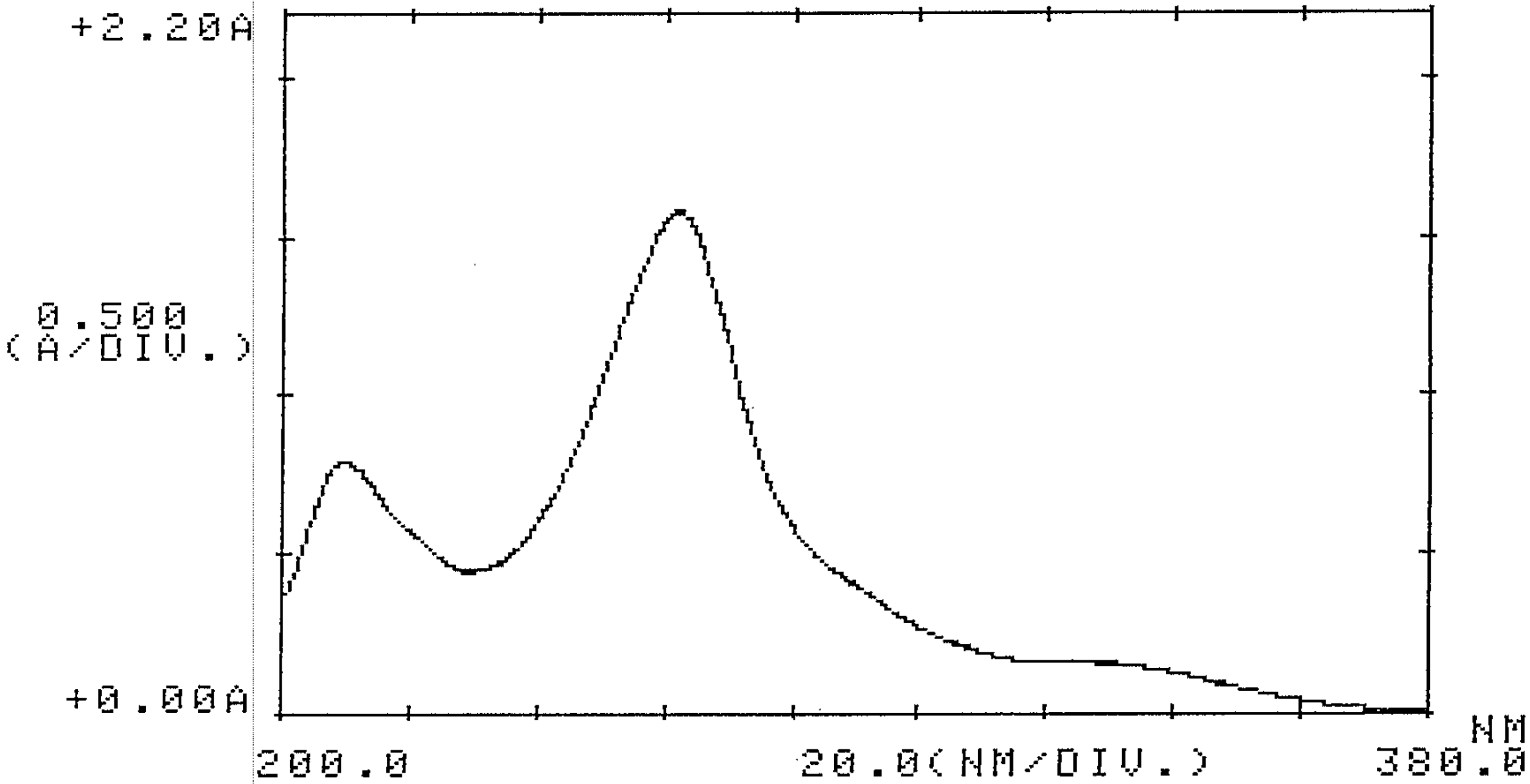


Figure 169 UV (CH_3OH) spectrum of **YS4**

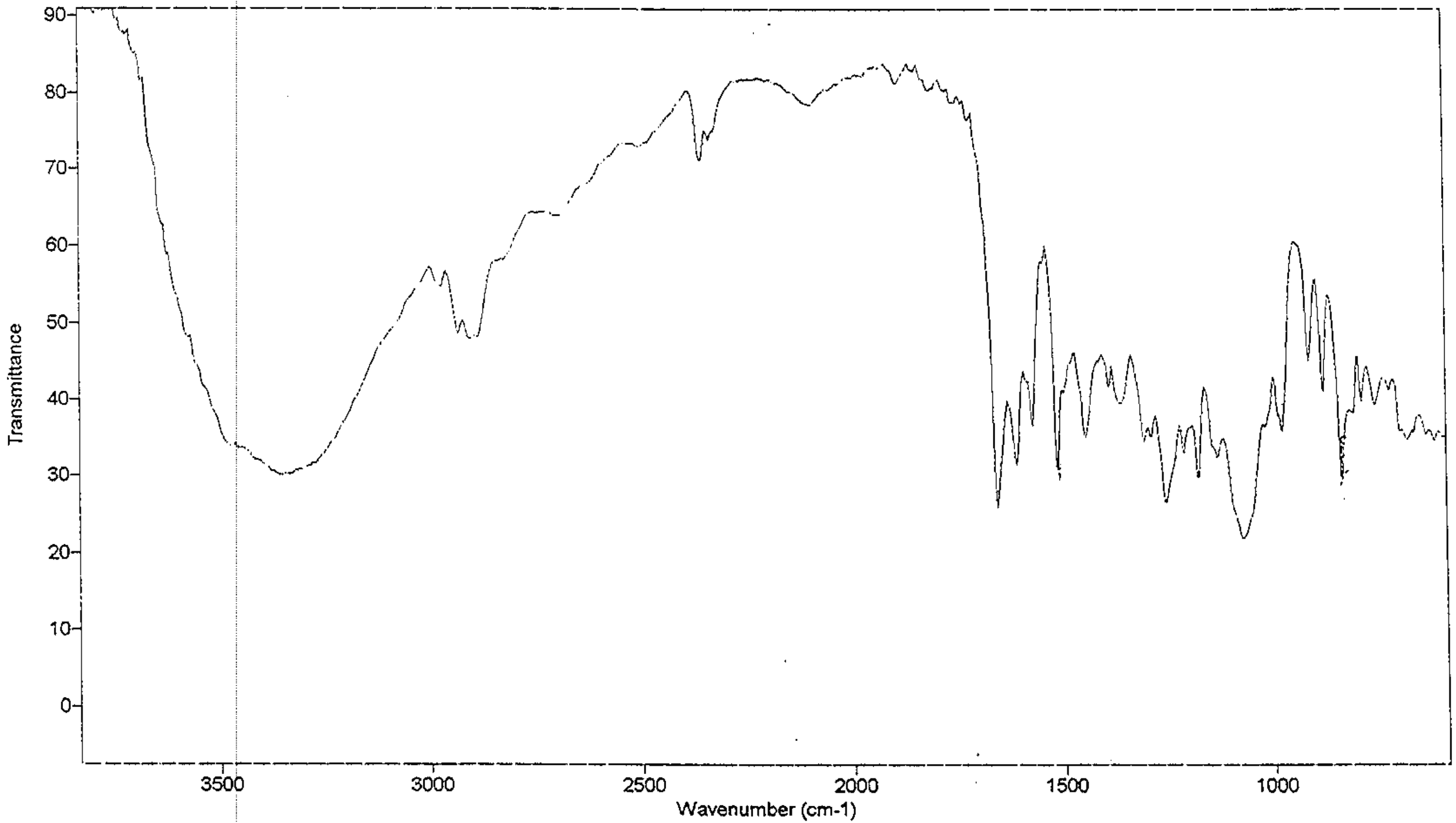


Figure 170 IR (KBr) spectrum of **YS4**

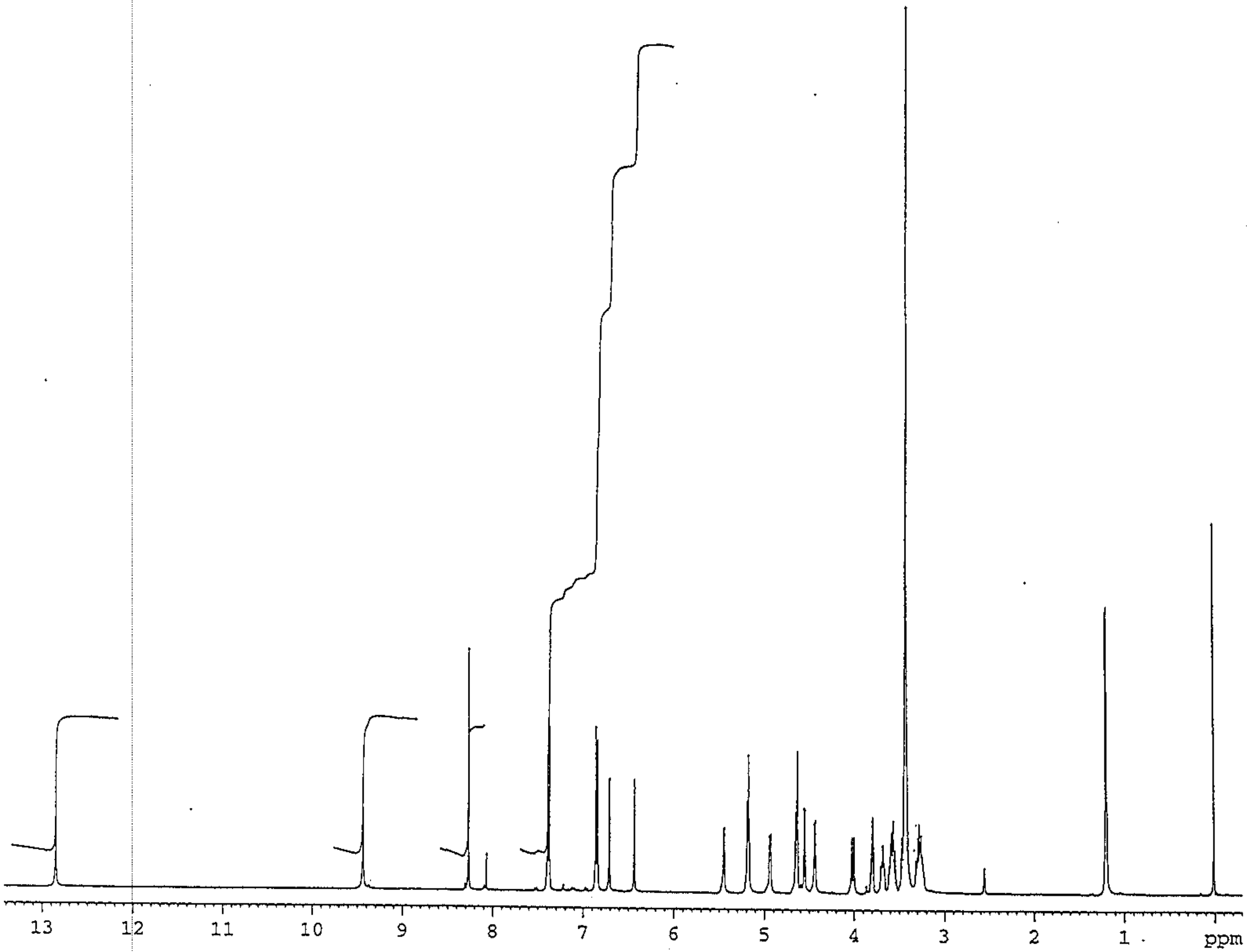


Figure 171 ^{1}H NMR (400 MHz) ($CDCl_3$ +DMSO-d_6) spectrum of **YS4**

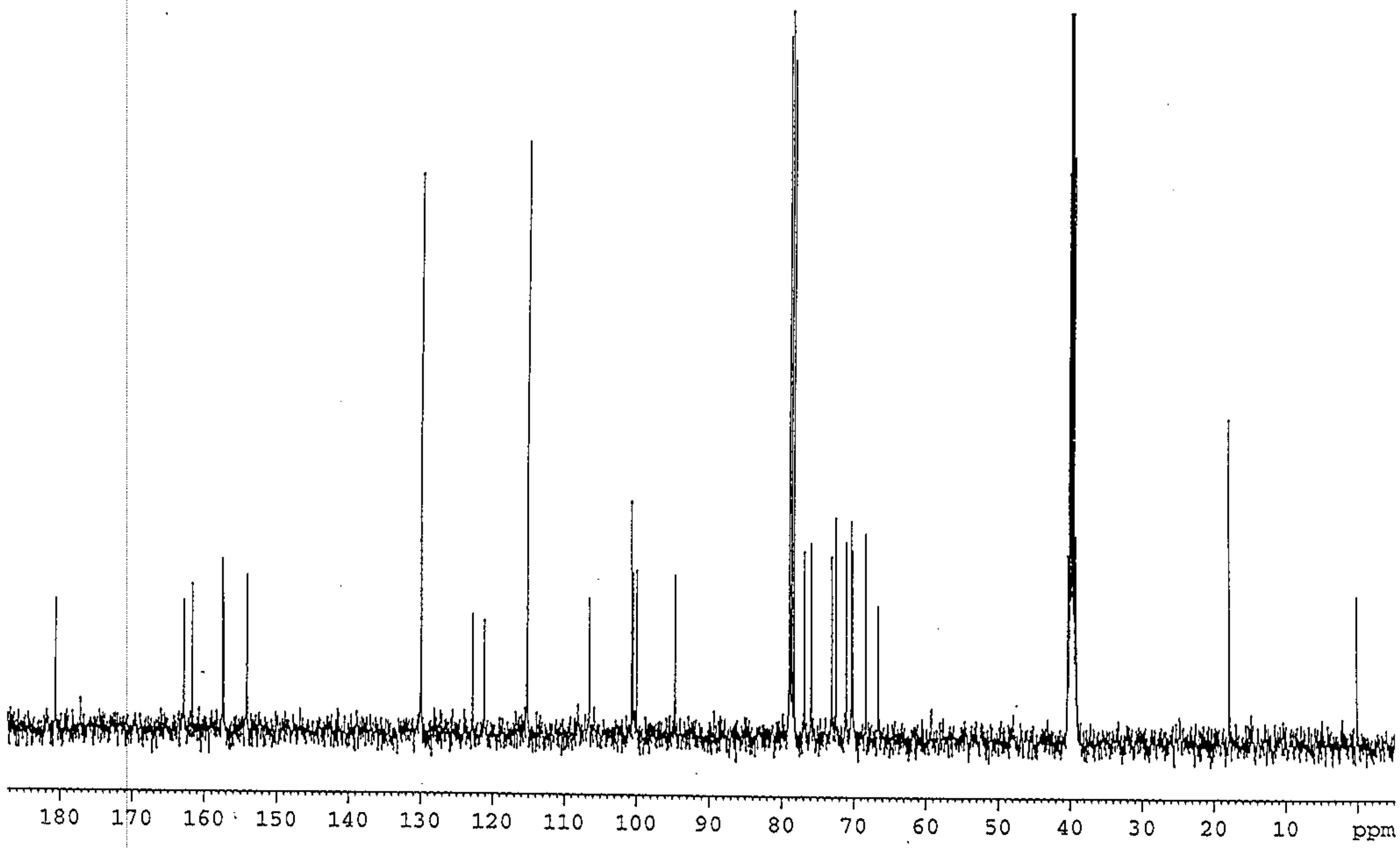


Figure 172 ^{13}C NMR (100 MHz) ($CDCl_3$ + DMSO-d_6) spectrum of **YS4**

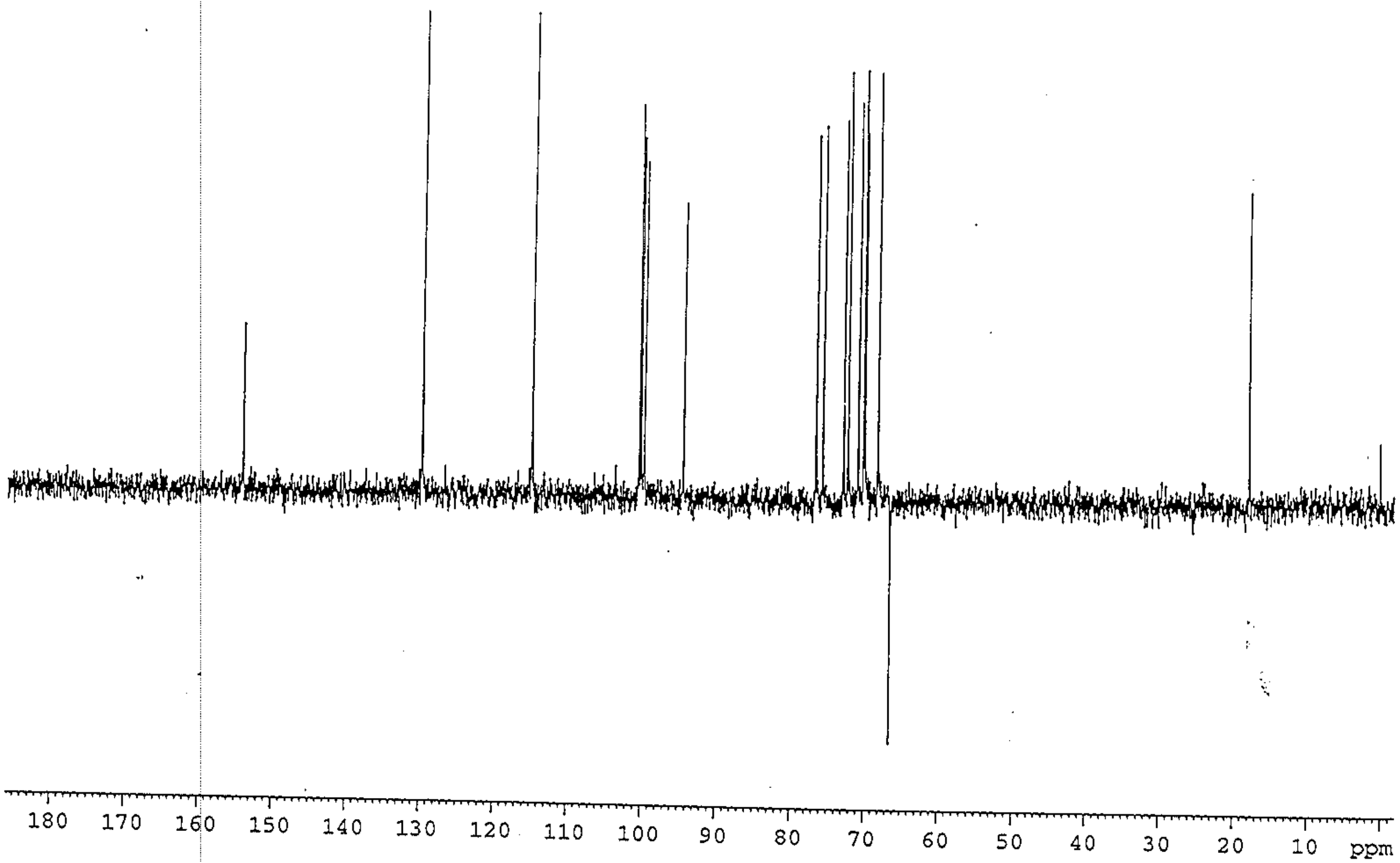


Figure 173 DEPT (135°) ($CDCl_3$ + DMSO-d_6) spectrum of **YS4**

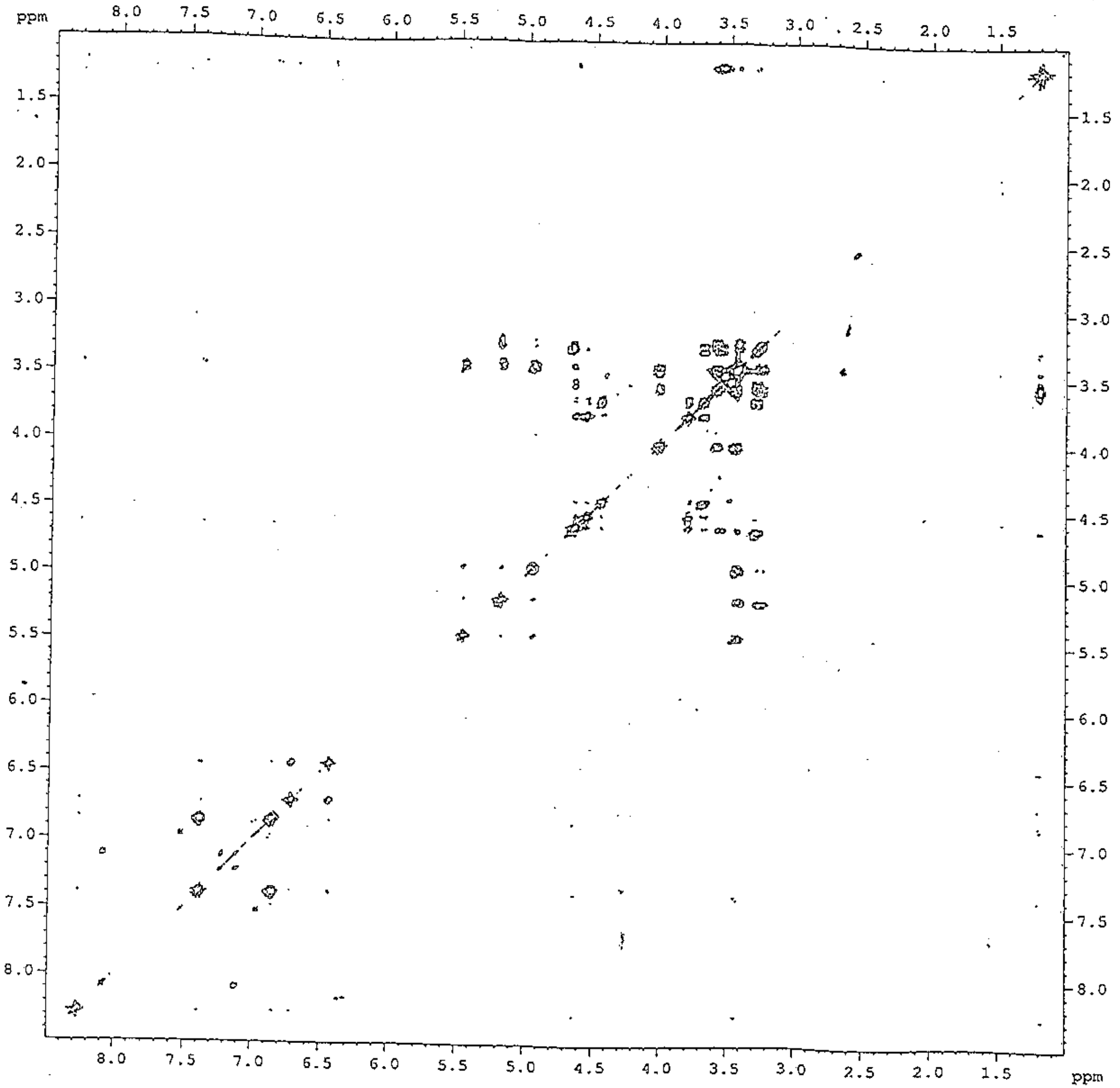


Figure 174 ^{1}H-^{1}H COSY spectrum of YS4

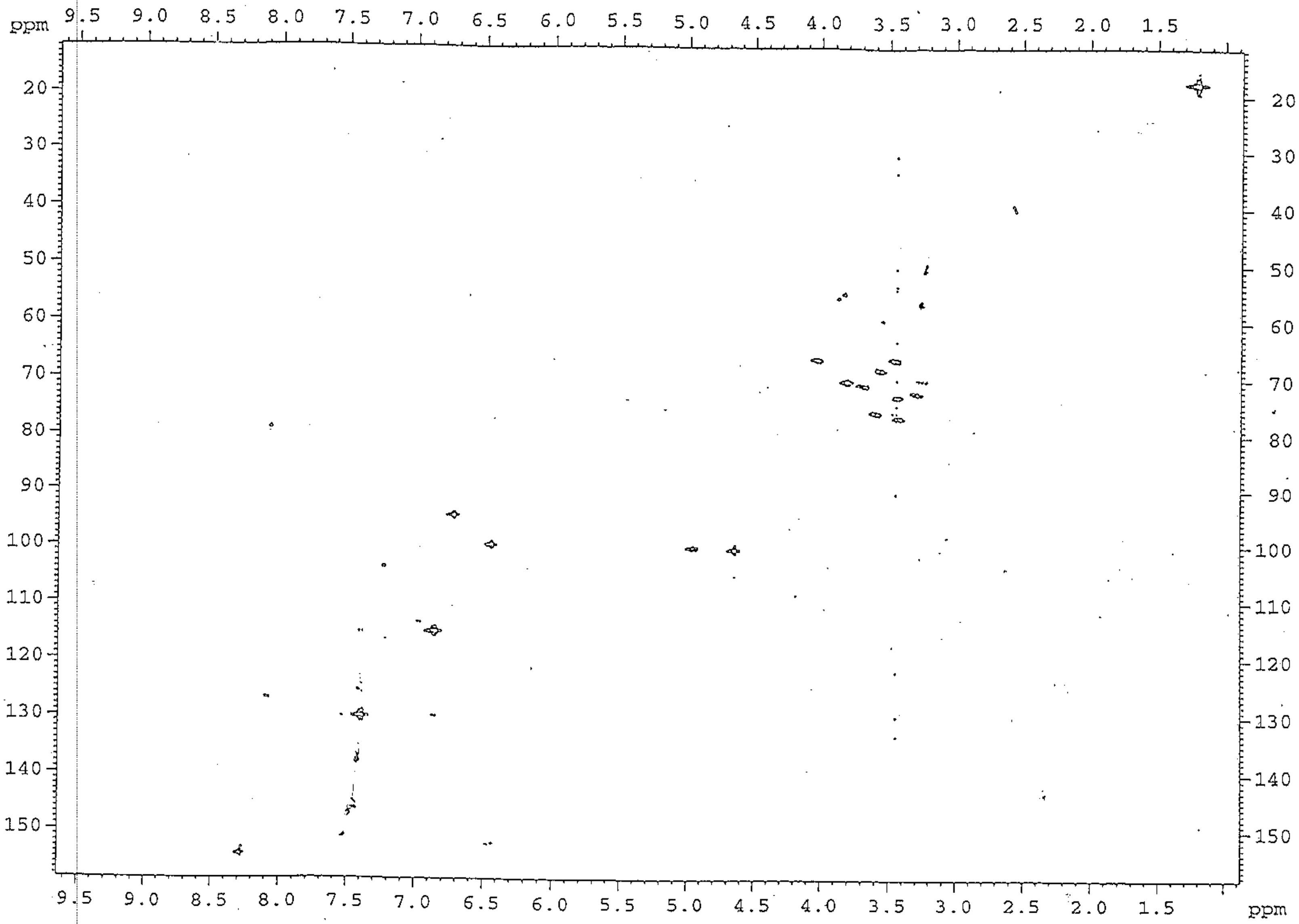


Figure 175 2D HMQC spectrum of YS4

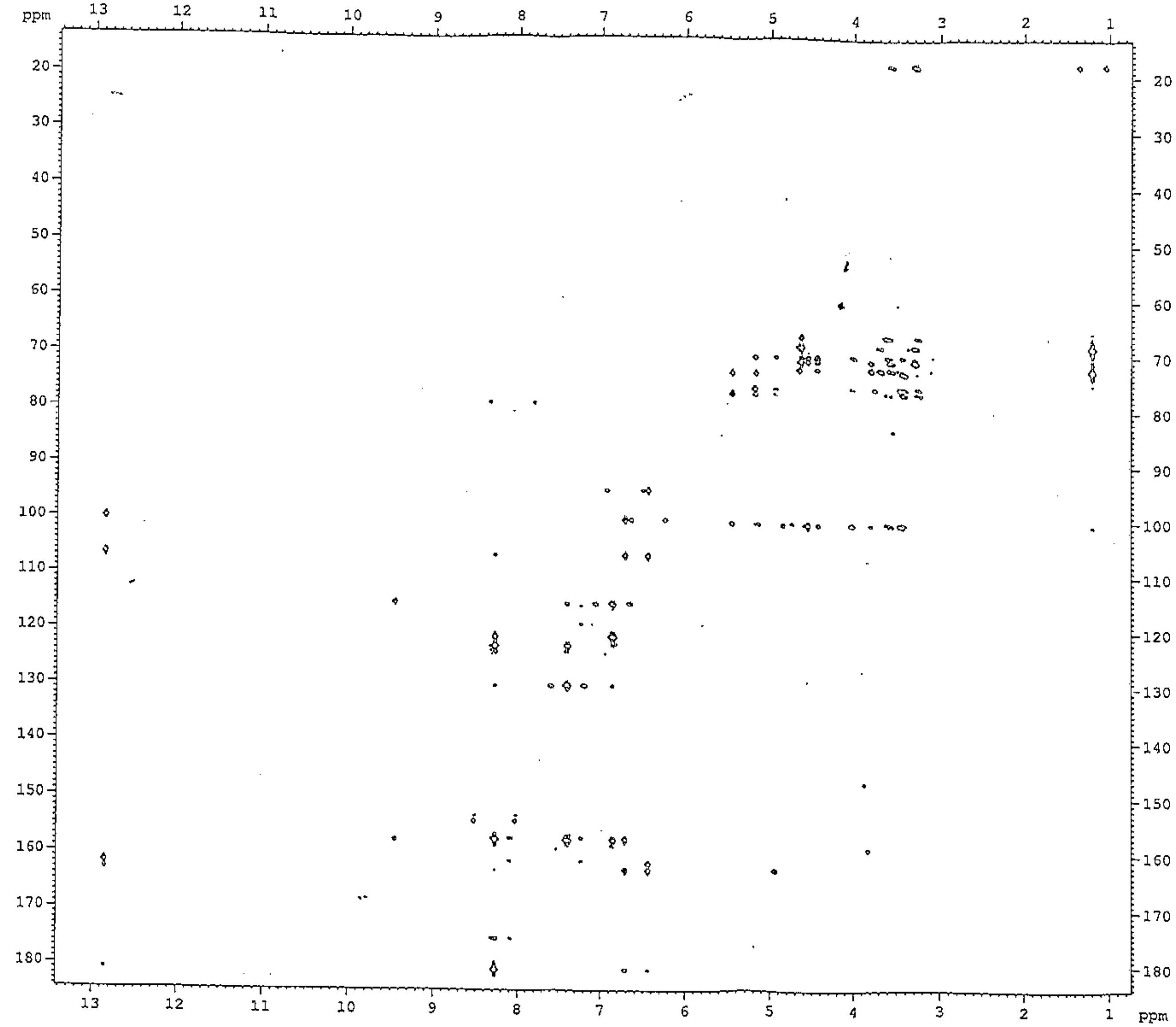


Figure 176 2D HMBC spectrum of YS4

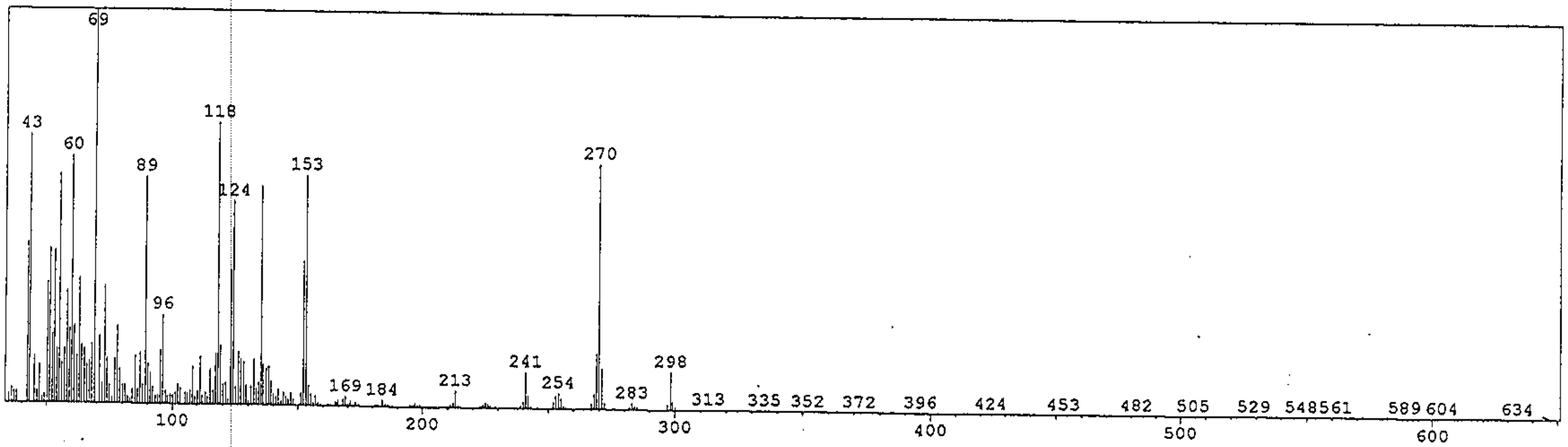


Figure 177 Mass spectrum of **YS4**

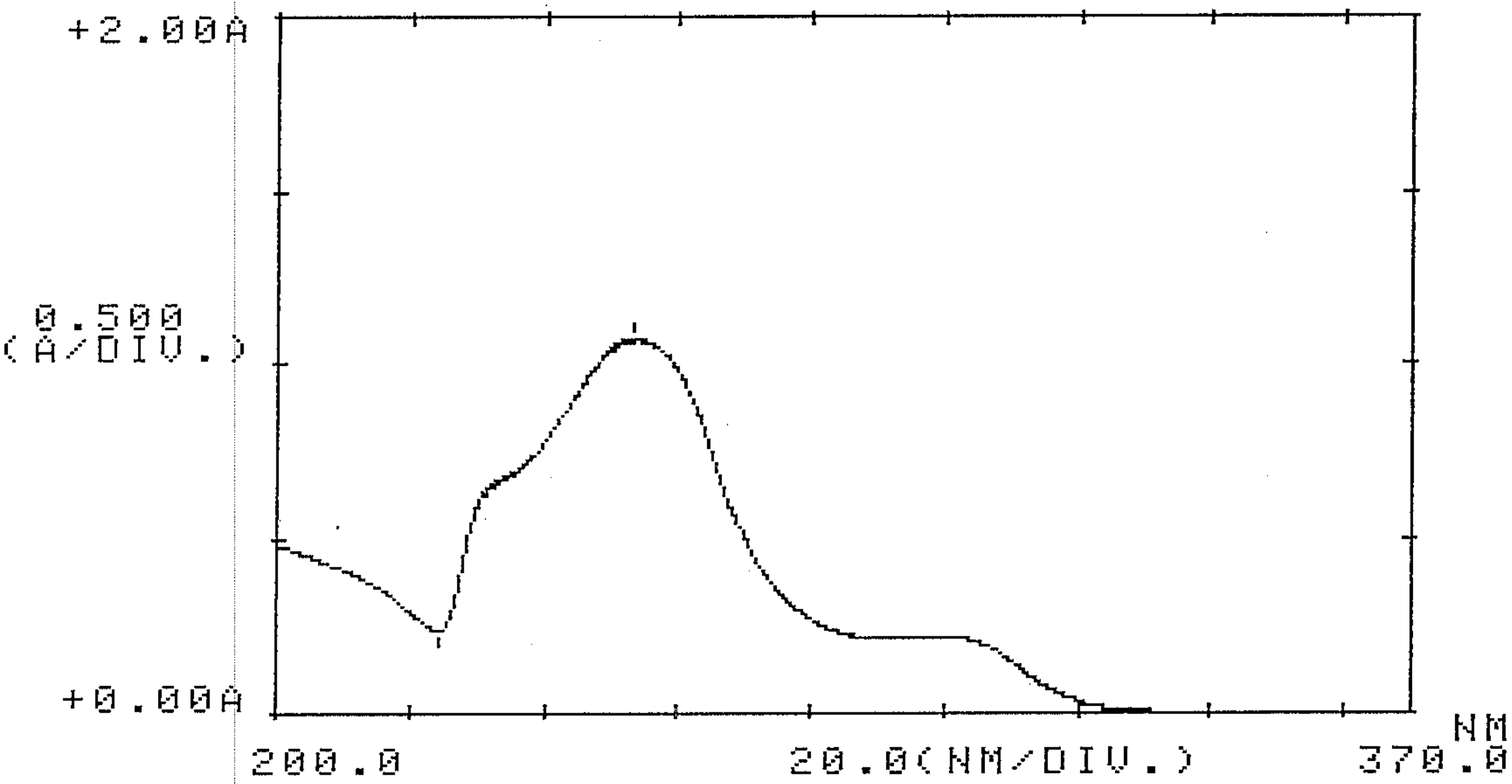


Figure 178 UV (CHCl$_3$) spectrum of **AcYS4**

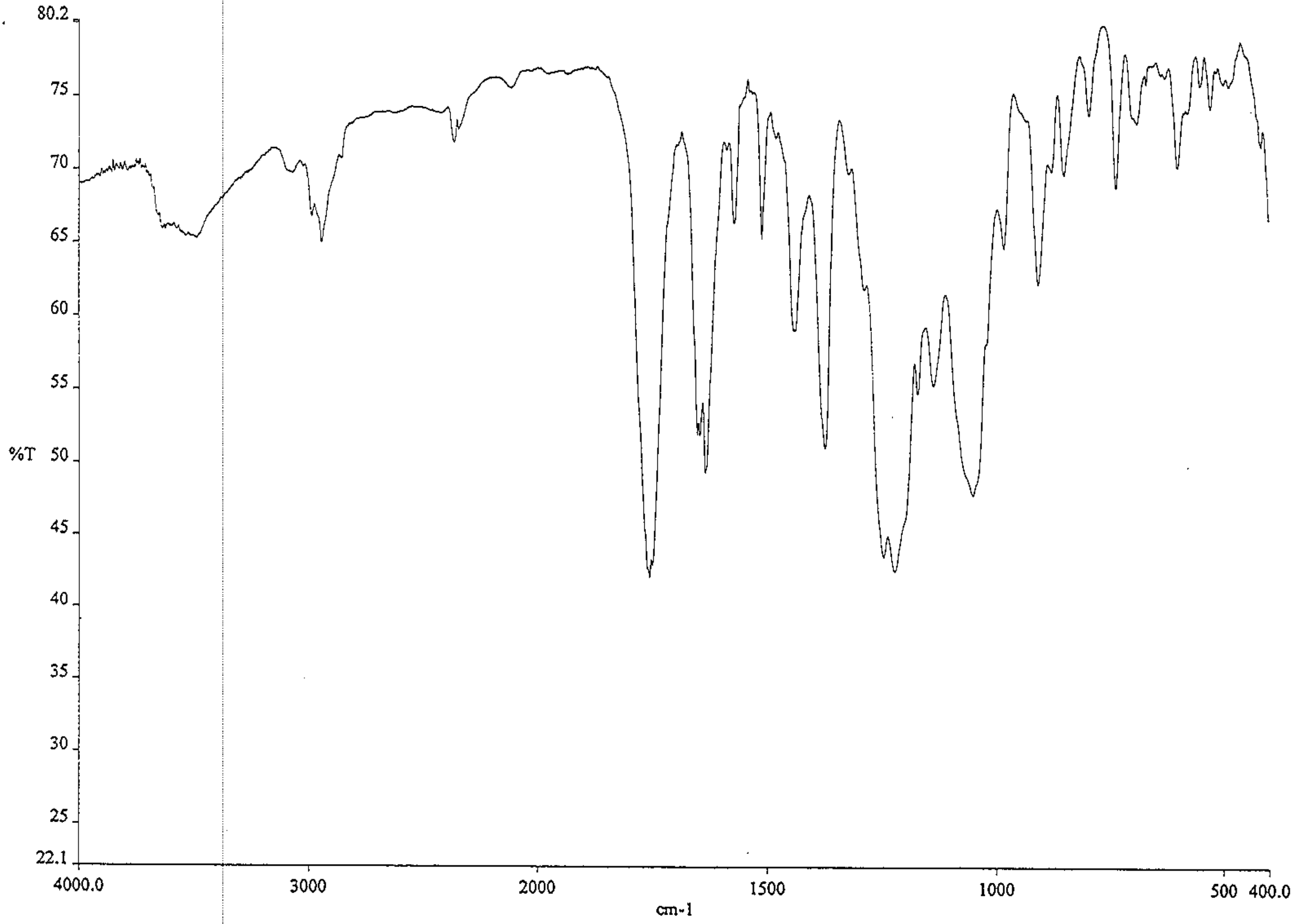


Figure 179 IR (neat) spectrum of **AcYS4**

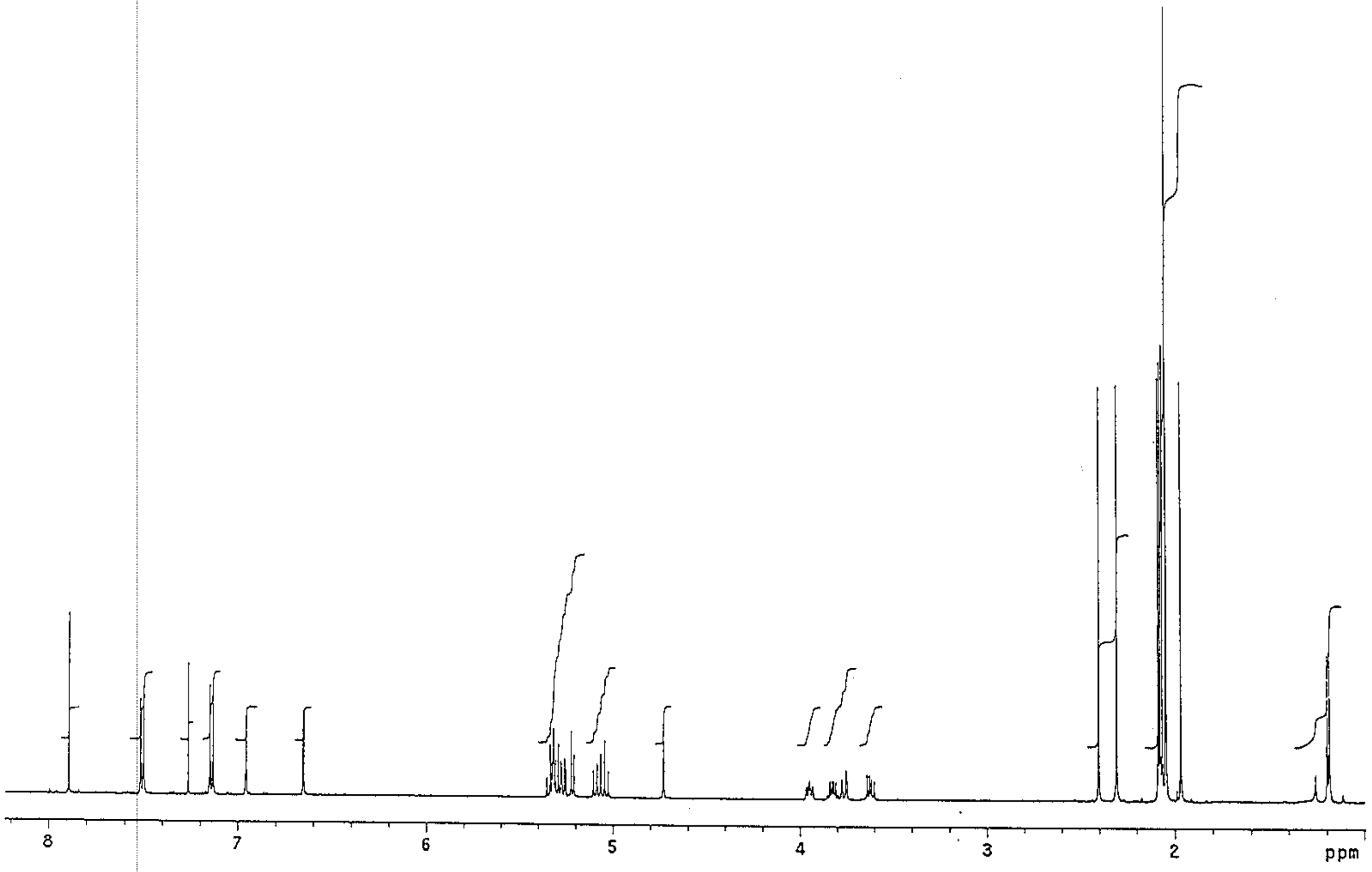


Figure 180 ^{1}H NMR (500 MHz) (CDCl$_3$) spectrum of **AcYS4**

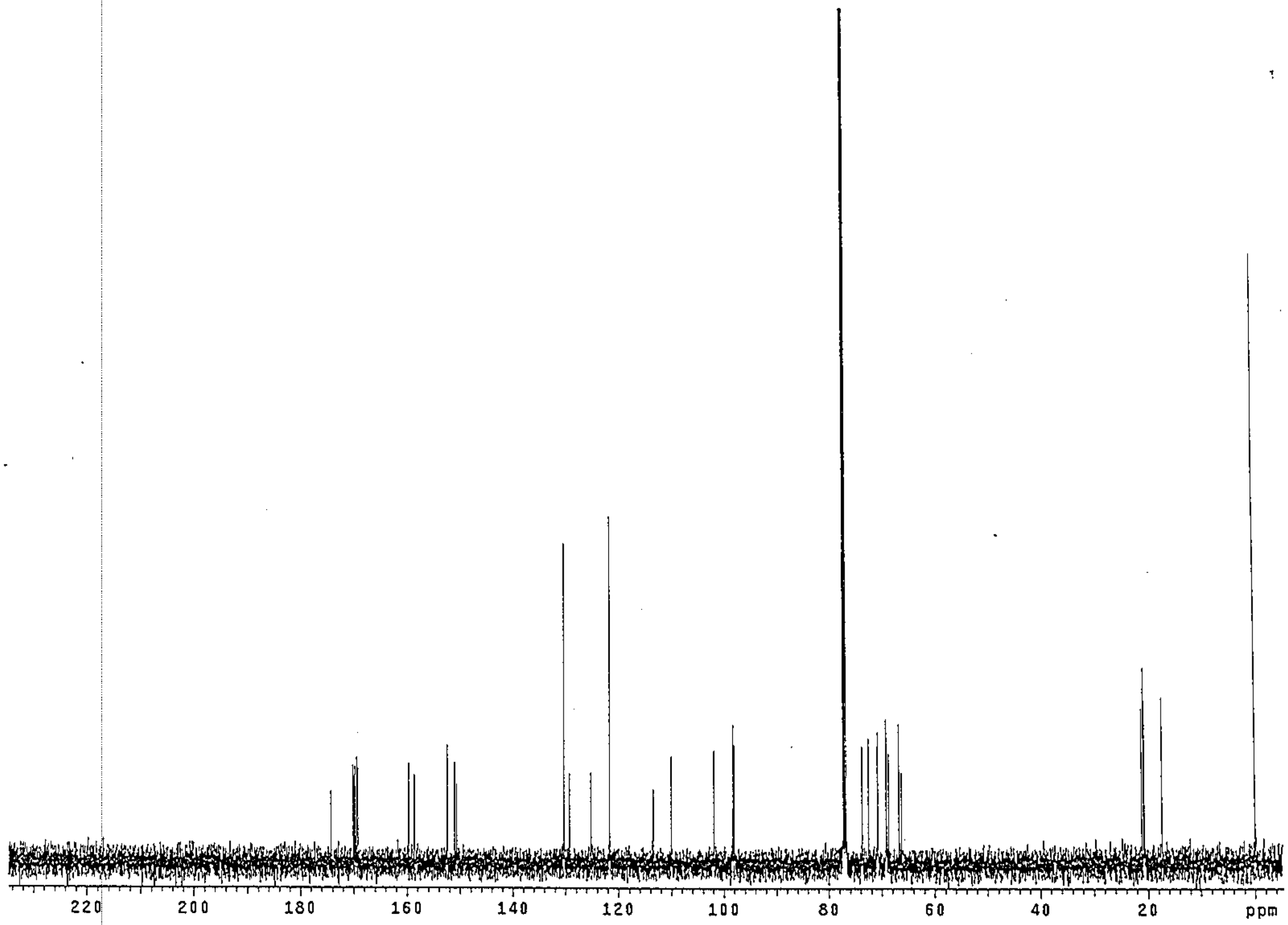


Figure 181 ^{13}C NMR (125 MHz) (CDCl$_3$) spectrum of **AcYS4**

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