Chemical Constituents from Stem Bark of

Garcinia speciosa

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Master of Science Thesis in Organic Chemistry

Prince of Songkla University

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Thesis Title	Chemical Constituents from Stem Bark of *Garcinia speciosa*
Author	Mr. Assdhawut Hiranrat
Major Program	Organic Chemistry
Academic Year	2000

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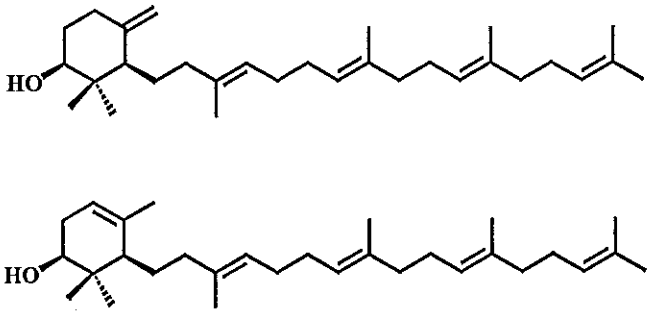
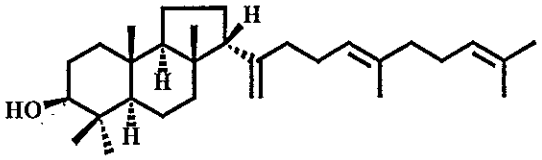
ABSTRACT

The crude methanol extract from stem bark of *Garcinia speciosa*, upon chromatographic separation, yielded five new compounds : four triterpenoids (**AH3**, **AH4**, **AH5** and **AH10**) and one benzophenone derivative (**AH7**) together with eight known compounds : one xanthone [8-deoxygartanin (**AH11**)], one flavonoid derivative [(-)-epicatechin (**AH6**)], one steroid [stigmasterol (**AH8**)], one malabaricane triterpene (**AH2**), a mixture of two steroid glycosides (**AH9**) and a mixture of two triterpenoids (**AH1**).

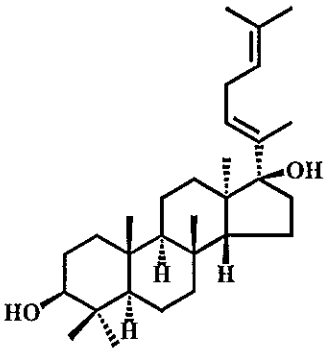
The structures of **AH2**, **AH3**, **AH4**, **AH7**, **AH10** and **AH11** were elucidated by analysis of 1D and 2D NMR spectroscopic data. The structural analysis of **AH5** as its monoacetate derivative (**AH5-Ac**) was accomplished by comparison of its ^{1}H and ^{13}C NMR spectral data with those of **AH4**. The remainders were identified by comparison of their spectroscopic data, melting points and/or optical rotation with those of reported compounds.



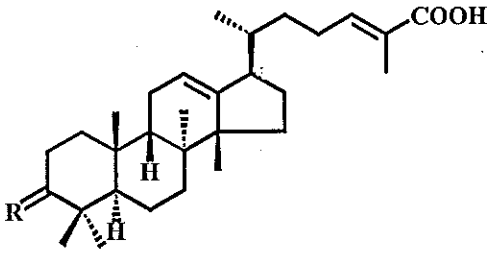
AH1



AH2



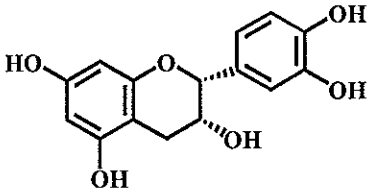
AH3



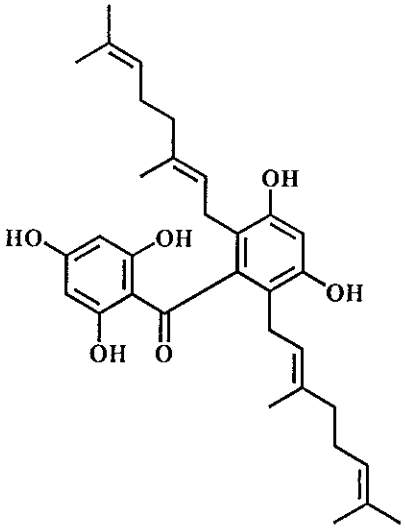
AH4 : R = α-H, β-OAc

AH5 : R = α-H, β-OH

AH10 : R = O



AH6



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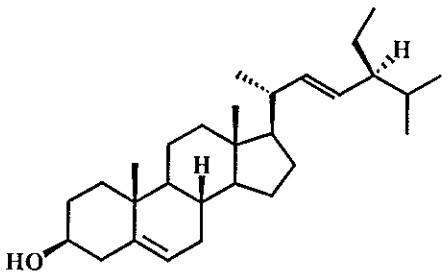
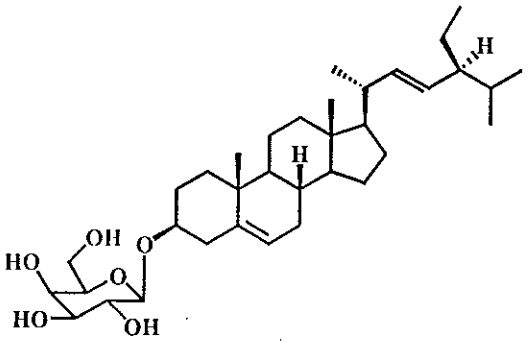
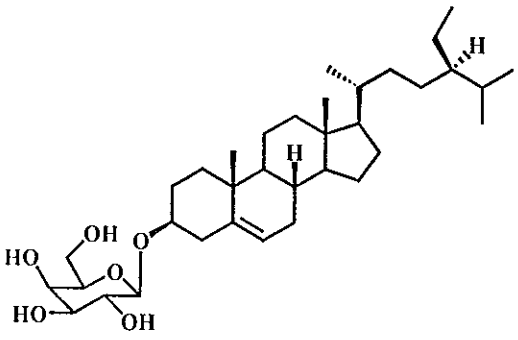
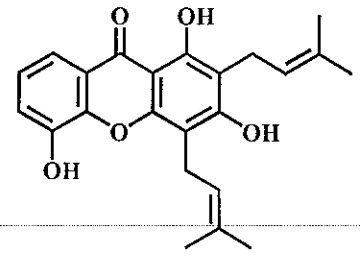


AH8





AH9



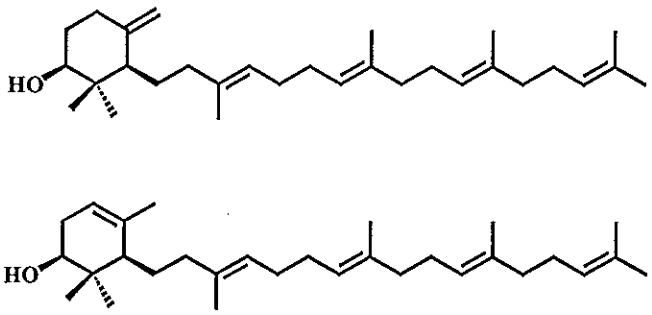
AH11

ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมีจากเปลือกต้นพะวา (*Garcinia speciosa*)
ผู้เขียน	นายอัษฎาวุธ หิรัญรัตน์
สาขาวิชา	เคมีอินทรีย์
ปีการศึกษา	2543

บทคัดย่อ

ส่วนสกัดหยาบเมธานอลของเปลือกต้นพะวา เมื่อนำมาแยกและทำให้บริสุทธิ์ด้วยวิธีทางโครมาโทกราฟี สามารถแยกสารใหม่ได้จำนวน 5 สาร ซึ่งเป็นสารประเภท triterpene จำนวน 4 สาร (**AH3, AH4, AH5** และ **AH10**) และอนุพันธ์ benzophenone จำนวน 1 สาร (**AH7**) และสารที่ทราบโครงสร้างแล้ว 8 สาร เป็นสารบริสุทธิ์จำนวน 4 สาร ได้แก่ สารประเภท xanthone จำนวน 1 สาร [8-deoxygartanin (**AH11**)] สารประเภท flavonoid จำนวน 1 สาร [(-)-epicatechin (**AH6**)] สารประเภท steroid จำนวน 1 สาร [stigmasterol (**AH8**)] สารประเภท malabaricane triterpene จำนวน 1 สาร (**AH2**) และเป็นสารผสมประเภท steroid glycoside จำนวน 2 สาร (**AH9**) และประเภท triterpene จำนวน 2 สาร (**AH1**)

โครงสร้างของ **AH2 AH3 AH4 AH7 AH10** และ **AH11** วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี โดยเฉพาะ 1D และ 2D NMR สเปกโทรสโกปี สำหรับโครงสร้างของ **AH5** วิเคราะห์ในรูปอนุพันธ์อะซิเตท (**AH5-Ac**) โดยการเปรียบเทียบข้อมูลทางสเปกโทรสโกปีกับสาร **AH4** ส่วนโครงสร้างของสารประกอบอื่นๆ ได้จากการเปรียบเทียบข้อมูลทาง ^{1}H และ ^{13}C NMR สเปกตรัม จุดหลอมเหลวและ/หรือค่าการหมุนระนาบแสงกับสารที่ทราบโครงสร้างแล้ว



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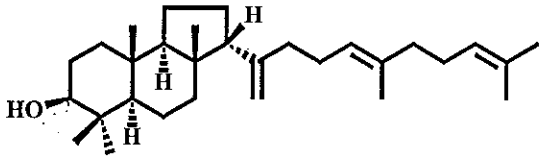

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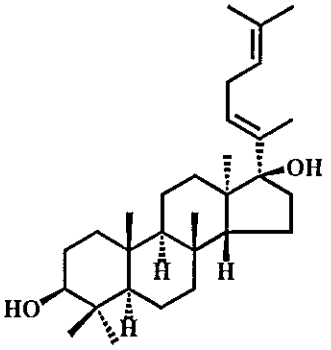

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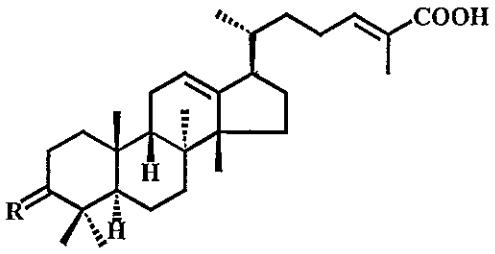

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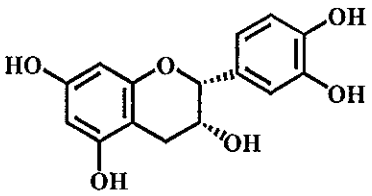

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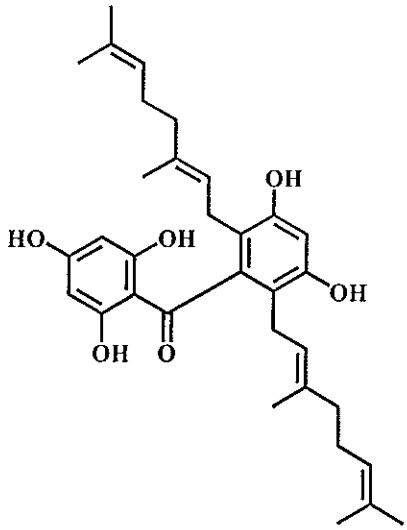

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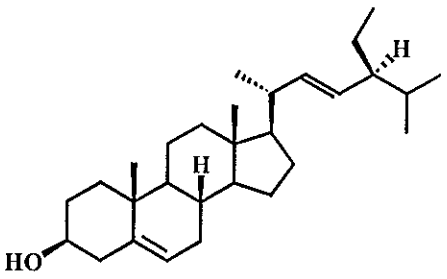

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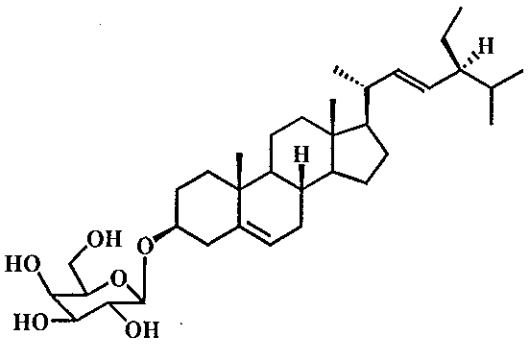

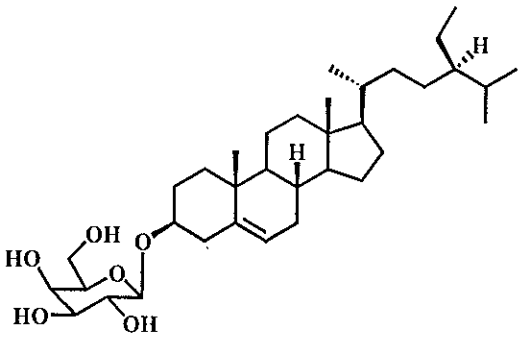

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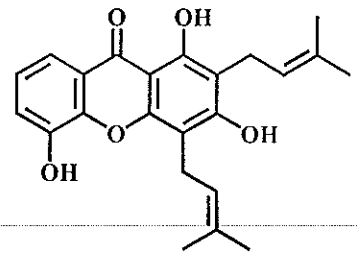

AH11

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I would like to extend my appreciation to the staff of the Department of Chemistry, Faculty of Science, Prince of Songkla University for making this thesis possible and to Ms.Dusanee Langjae, the Scientific Equipment Center, Prince of Songkla University for recording the 500 MHz NMR spectra.

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

Assdhawut Hiranrat

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ABBREVIATIONS AND SYMBOLS

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
q	=	*quartet*
m	=	*multiplet*
br	=	*broad*
brs	=	*broad singlet*
dd	=	*doublet of doublet*
dt	=	*doublet of triplet*
td	=	*triplet of doublet*
qt	=	*quartet of triplet*
qd	=	*quartet of doublet*
ht	=	*heptet of triplet*
sxt	=	*sextet of triplet*
ddd	=	*doublet of doublet of doublet*
δ	=	chemical shift relative to TMS
J	=	coupling constant
m/z	=	a value of mass divided by charge
^{o}C	=	degree celcius
R_f	=	retention factor
g	=	gram
mL	=	milliliter
cm^{-1}	=	reciprocal centimeter (wavenumber)
nm	=	nanometer

ABBREVIATIONS AND SYMBOLS (Continued)

λ_{max}	=	maximum wavelength
ν	=	absorption frequencies
ε	=	Molar extinction coefficient
Hz	=	hertz
MHz	=	megahertz
ppm	=	part per million
rel.int.	=	relative intensity
$[\alpha]_D$	=	specific rotation
c	=	concentration
C_n	=	position of carbons
UV	=	Ultraviolet
IR	=	Infrared
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimentional Nuclear Magnetic Resonance
MS	=	Mass Spectroscopy
HMQC	=	Heteronuclear Multiple Quantum Coherence
HMBC	=	Heteronuclear Multiple Bond Correlation
COSY	=	Correlation Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
NOE	=	Nuclear Overhauser Effect
TLC	=	Thin-Layer Chromatography

ABBREVIATIONS AND SYMBOLS (Continued)

TMS	=	tetramethylsilane
DMSO	=	dimethylsulphoxide
MeOH	=	methanol
$CDCl_3$	=	deuterochloroform
CD_3OD	=	dueteromethanol
ASA	=	anisaldehyde-sulphuric acid in acetic acid solution

CHAPTER 1

INTRODUCTION

1.1 Introduction

Garcinia speciosa, a plant belonging to the Guttiferae family, was found at Tenasserim near Amherst University, Monlmein, Martaban, Andaman Island in Myanmar (Hooker, 1875) and widely distributed in Thailand (ธงชัย, 2525 ; Craib, 1931). The family Guttiferae contains about 40 genera and over 1000 species. Only 6 genera and 60 species are found in Thailand; i.e., *Calophyllum*, *Cratoxylum*, *Garcinia*, *Mesua*, *Kayea* and *Orchrocarpus* (Panthong, 1999). *G. speciosa* is a medium-size tree about 50 feet high, trunk straight, erect, 2 feet diameter; bark thin, greyish-black. Leaves are oblong or elliptic-oblong narrowed at both ends, size (5-12)x(1.75-3) inches, leathery; petiole 0.75 inch thick angled. Male flowers are bright yellow with 1.5 inches diameter as in *G. cornea* but larger and very fragrant; female flowers unknown. Stamens of male flower are in a central shortly-stalked 4-angled or columnar mass; anthers quadrate, dehiscing vertically; no rudimentary ovary. Male flowers are 3 to many flowered terminal and axillary fascicles. Fruits are subglobose or ovoid, tip mamillar. *G. speciosa* is closely allied to *G. cornea* (Hooker, 1875). In Thailand, *G. speciosa* has various local names : "Pha Waa" (พะวา) in Surat Thani, "Cha Muang" (ชะมวง) in Phichit, "Mara Ki Nok" (มะระขี้นก) in Chiang mai, "Khwaat" (ขวาด) in Chiang rai, "Kwak mai or Maak kwak" (กวักไหม หรือ หมากกวัก) in Nong Khai, "Mapong" (มะป่อง) in Northern, "Waa nam" (วาน้ำ) in Trang, "Sarapheepaa" (สาระภีป่า) in Central, Chiang mai (เต็ม, 2523 ; ธงชัย, 2525 ; Craib, 1931).

1.2 Review of Literatures

Plants in the *Garcinia* genus (Guttiferae) are well known to be rich in a variety of compounds : xanthones (Thoison, 2000 ; Kosela, 2000 ; 1999a ; Rukachaisirikul, 2000a ; Nguyen, 2000 ; Cao, 1998a,b ; Iinuma, 1996b,c), benzophenones (Ali, 2000 ; Kosela, 1999a ; Iinuma, 1996a ; Spino, 1995 ; Fukuyama, 1993 ; Gustafson, 1992 ; Nyemba, 1990), biflavonoids (Thoison, 2000 ; Spino, 1995 ; Fukuyama, 1993 ; Goh, 1992 ; Gunatilaka, 1983), benzophenone-xanthone dimers (Kosela, 2000 ; 1999a ; Iinuma, 1996b,c) and triterpenes (Thoison, 2000 ; Nguyen, 2000 ; Rukachaisirikul, 2000b ; Gunatilaka, 1984b). Some of these exhibit a wide range of biological and pharmacological activities, e.g., cytotoxic (Thoison, 2000 ; Kosela, 2000 ; Cao, 1998a,b ; Minami, 1996), antiinflammatory (Minami, 1996 ; Ilyas, 1994 ; Parveen, 1991), antimicrobial (Kosela, 2000 ; Parveen, 1991), antifungal (Kosela, 2000 ; Minami, 1996), antiprotozoal (Parveen, 1991), antibacterial (Rukachaisirikul, 2000a ; Iinuma, 1996a ; Parveen, 1991), antiimmunosuppressive (Ilyas, 1994 ; Parveen, 1991), antimalarial (Kosela, 2000 ; Minami, 1996 ; Ilyas, 1994), anti-HIV (Lin, 1997 ; Gustafson, 1992) activities and healing of skin infections and wounds (Ilyas, 1994 ; Parveen, 1994).

Chemical constituents isolated from 54 species of the genus *Garcinia* were summarized by Wanrudee Kaewnok in 1998. Information from NAPRALERT database developed by University of Illinois at Chicago and Chemical Abstracts of the year 2000 reported additional constituents from nine new species of the *Garcinia* genus (*G. bracteata, G. neglecta, G. pseudoguttifera, G. puat, G. scortechinii, G. sessilis, G. speciosa, G. vilersiana* and *G. vitiensis*). These compounds are presented in **Table 1** together with previously missing information.

Table 1 Compounds from plants of the *Garcinia* genus

Scientific name	Investigated part	Compound	Structure	Bibliography
G. andamanica King.	leaves	sorbifolin 6-galactoside	6a	Sarwar Alam, *et al.*, 1986
		scutellarein 7-digluco-side	-	
G. assigu Lantb.	stem bark	maclurin	1g	Ito, *et al.*, 1998
		pancixanthone A	11.3k	
		toxyloxanthone B	11.4v	
		1,3,5-tri(OH)xanthone	11.3a	
		1,5-di(OH)xanthone	11.2d	
		assiguxanthone A	11.4k	
G. bracteata	leaves	bractatin	11.7s	Thoison, *et al.*, 2000
		isobractatin	11.7u	
		1-O-methylbractatin	11.7t	
		1-O-methylisobractatin	11.7v	
		1-O-(Me)-8-(OMe)-8,8a-dihydrobractatin	11.7w	
		1-O-methylneobractatin	11.7x	
G. cambogia	root bark	garbogiol	11.4s	Iinuma, *et al.*, 1998
		garcinol	1e	
		isogarcinol	1d	
		rheediaxanthone A	11.4r	

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. cowa Roxb.	stem bark	7-O-methylgarcinone E	11.4h	Likhitwitaya-wuid, *et al.*, 1997
G. dulcis	root	garciduol A	2a	Iinuma, *et al.*,
		garciduol B	2b	1996b,c
		garciduol C	2c	Iinuma, *et al.*,
		1,3,6-tri(OH)-7-(OMe) xanthone	11.4c	1996b
		2,5-di(OH)-1-(OMe) xanthone	11.3d	
		1,4,5-tri(OH)xanthone (subelliptenone G)	11.3c	
		1,3,5-tri(OH)xanthone	11.3a	
		1,3,6-tri(OH)-5-(OMe) xanthone	11.4e	
		1,3,6-tri(OH)- 8-iso-prenyl-7-(OMe)-xanthone	11.4d	
	leaves	friedelin	10t	Kosela, *et al.*, 1999a
		dulxanthone E	11.5a	Kosela, *et al.*, 1999a; 2000

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. dulcis	leaves	dulxanthone F	11.5b	Kosela, *et al.*,
		dulxanthone G	11.6b	2000
		dulxanthone H	11.6a	
	stem bark	dulxanthone A	11.4bb	Ito, *et al.*,
		dulxanthone B	11.4cc	1998
		dulxanthone C	11.4dd	
		gentisein	11.3j	
		jacareubin	11.4x	
		toxyloxanthone B	11.4v	
		xanthone VI	11.4y	
		1,3,7-tri(OH)-2-(3-methyl-2-butenyl)-xanthone	11.3i	
	branches	3,8″-biapigenin	3i	Harrison, *et*
		podocarpusflavone A	3k	*al.*, 1994
		1,4,6-tri(OH)-5-(OMe)-7-(3-methylbut-2-enyl)-xanthone	11.4w	
		friedelin	10t	
G. forbesii	branches and stem	forbesione	11.7i	Leong, *et al.*,
		pyranojacareubin	11.4u	1996

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. forbesii	branches and stem	1,3,7-tri(OH)-2-(3-methylbut-2-enyl)-xanthone	11.3i	Leong, *et al.*, 1996
G. gaudicaudii	leaves	gaudichaudione A	11.7a	Cao, *et al.*, 1998a,b
		gaudichaudione B	11.7c	
		gaudichaudione C	11.7d	
		gaudichaudione D	11.7b	
		gaudichaudione E	11.7e	Cao, *et al.*, 1998b
		gaudichaudione F	11.7g	
		gaudichaudione G	11.7k	
		gaudichaudione H	11.7j	
		gaudichaudiic acid A	11.7f	
		gaudichaudiic acid B	11.7h	
		gaudichaudiic acid C	11.7n	
		gaudichaudiic acid D	11.7o	
		gaudichaudiic acid E	11.7l	
		morellic acid	11.7m	
		forbesione	11.7i	
	bark	gaudispirolactone	11.8a	Wu, *et al.*, 2001
		7-isoprenylmorellic acid	11.7y	
		morellic acid	11.7m	
		isomorellic acid	11.7bb	

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. gaudichaudii	bark	isomorellin	11.7aa	Wu, *et al.*,
		isomoreollin	11.7z	2001
		isomorellinol	11.7cc	
		gaudichaudiic acid E	11.7l	
		gaudichaudiic acid F	11.7dd	Xu, *et al.*,
		gaudichaudiic acid G	11.7ee	2000b
		gaudichaudiic acid H	11.7ff	
		gaudichaudiic acid I	11.7gg	
G. hombroniana	pericarp	(24*E*)-3α-(OH)-17,14-friedolanostan-8,14,24-trien-26-oic acid	10a	Rukachaisiri-kul, *et al.*, 2000b
		methyl (24*E*)-3α,23-di(OH)-17,14-friedo-lanostan-8,14,24-trien-26-oat	10b	
		methyl (24*E*)-3α,9,23-tri(OH)-17,14-friedo-lanostan-14,24-dien-26-oat	10c	
		3α-(OH)-23-oxo-9,16-lanostandien-26-oic acid	10e	

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. hombroniana	pericarp	3β-(OH)-23-oxo-9,16-lanostandien-26-oic acid	10d	Rukachaisiri-kul, *et al.*, 2000b
G. kola Heckel	root bark stem	garcinianin	3b	Terashima, *et al.*, 1995
		GB-1	3a	
		(+)-GB-1b	3f	Terashima, *et al.*, 1999a
		(-)-GB-1a	3g	
		(-)-GB-2a	3h	
		3,8$''$-biapigenin	3i	
		amentoflavone	3j	
		garcinianin	3b	
		garcifuran A	7b	Terashima, *et al.*, 1999b
		1,2,8-tri(OMe)xan-thone	11.3g	
		1,2-di(OMe)xanthone	11.2c	
		1,3,5-tri(OH)-2-(OMe) xanthone	11.4t	
		1,5-di(OH)xanthone	11.2d	
		2,5-di(OH)-1-(OMe)-xanthone	11.3d	
		2-(OH)-1,8-di(OMe)-xanthone	11.3h	

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. kola Heckel	stem	2-(OH)-1-(OMe)-xanthone	11.2e	Terashima, *et al.*, 1999b
		3-(OH)-4-(OMe)-xanthone	11.2b	
		2-(OH)xanthone	11.1b	
		4-(OH)xanthone	11.1a	
G. lateriflora Bl.	stem bark	lateriflorone	8a	Kosela, *et al.*, 1999b
G. latissima	stem bark	latisxanthone A	11.4ee	Ito, *et al.*, 1998
		latisxanthone C	11.4aa	
		pyranojacareubin	11.4u	
G. mangostana	fruit hull	α-mangostin	11.4f	Chairungsri-lerd, *et al.*, 1996
		γ-mangostin	11.4g	
	fruit	2,7-di(3-methylbut-2-enyl)-1,3,8-tri(OH)-4-(Me)xanthone	11.3m	Gopalakrish-nan and Bala-ganesan,2000
		2,8-di(3-methylbut-2-enyl)-7-carboxy-1,3-di(OH)xanthone	11.2a	
G. neglecta Vieill.	leaves	garcinisidone A	5e	Ito, *et al.*, 2001
		garcinisidone B	5f	
		garcinisidone C	5g	

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. neglecta Vieill.	leaves	garcinisidone D garcinisidone E	5h 5I	Ito, *et al.*, 2001
G. parvifolia	leaves	garcidepsidone A garcidepsidone B garcidepsidone C garcidepsidone D	5a 5b 5c 5d	Xu, *et al.*, 2000a
G. pseudogutti-fera	heartwood	myrtiaphenone-A myrtiaphenone-B vismiaphenone-C pseudoguttiaphenone-A eupha-8,24-dien-3β-ol	1b 1c 1a 1f 10bb	Ali, *et al.*, 2000
G. puat Guillaumin.	leaves	garcinisidone F 1,3,7-tri(OH)-2-(2-butenyl-3-methyl)-xanthone 5-(OH)methyl-2-fural-dehyde naringenin apigenin	5j 11.3i 7a 6c 6b	Ito, *et al.*, 2001
G. schomburg-kiana	root	3-O-methylgarcinone B	11.4l	Na Pattalung, *et al.*, 1984

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. schomburg-kiana	root	1,3,7-tri(OH)-2,5,8-tris(3-methylbut-2-enyl)-6-(OMe)xanthone	11.4i	Na Pattalung, *et al.*, 1984
	heartwood	volkensiflavone	3c	Häfner and Frahm, 1993
		morelloflavone	3d	
		fukugeside	3e	
G. scortechinii	twigs	scortechinone A	11.7p	Rukachaisiri-kul, *et al.*, 2000a
		scortechinone B	11.7q	
		scortechinone C	11.7r	
		friedelin	10t	
		stigmasterol	-	
G. sessilis	heartwood	*δ*-tocotrienol	4a	Ali, *et al.*, 1999
		5,9-di(OH)-8-(OMe)-2,2-di(Me)-7-(3-methylbut-2-enyl)-2*H*,6*H*-pyrano[3,2-*b*]- xanthen-6-one	11.4z	
G. speciosa	bark	*α*-mangostin	11.4f	Okudaira, *et al.*, 2000
		cowanin	11.4n	
		cowanol	11.4o	

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. speciosa	leaves	5,9,10-tri(OH)-12-[1,1-di(Me)prop-2-enyl]-2,2-di(Me)-2*H*-pyrano(3,2-*b*) xanthen-6-one	11.4j	Mahabusara-kam, 1992
G. subelliptica	seed	garcinielliptin oxide	10f	Lin, *et al.*, 1996; Chung, *et al.*, 1998
		garcinielliptone	10g	Chung, *et al.*, 1998
	root bark	subelliptenone A	11.4p	Iinuma, *et al.*, 1994
		subelliptenone B	11.4q	
		subelliptenone H	11.4b	Iinuma, *et al.*, 1995
		subelliptenone I	11.4m	
	wood	1,4,5-tri(OH)xanthone (subelliptenone G)	11.3c	Fukuyama, *et al.*, 1997
		symphoxanthone	11.4a	
		garciniaxanthone B	11.3l	
		subellinone	9c	
		garsubellin A	9a	
		garsubellin B	9b	
		garsubellin C	9d	
		garsubellin D	9e	
		garsubellin E	9f	

Table 1 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. vilersiana	bark	globuxanthone	11.3e	Nguyen, *et al.*, 2000
		subelliptenone B	11.4q	
		subelliptenone H	11.4b	
		12b-hydroxy-des-D-garcigerrin A	11.3b	
		symphoxanthone	11.4a	
		1-O-methylglobu-xanthone	11.3f	
		olean-12-ene-3β, 11α-diol	10x	
		oleanolic acid	10n	
		lupeol	10s	
		β-amyrin	10w	
G. vitiensis	heartwood	δ-tocotrienol	4a	Ali, *et al.*, 1999
		5,9-di(OH)-8-(OMe)-2,2-di(Me)-7-(3-methylbut-2-enyl)-2*H*,6*H*-pyrano [3,2-*b*]- xanthen-6-one	11.4z	

It is found from our investigation on the stem bark of *G. speciosa* that its major constituents were triterpenoid compounds. Therefore the triterpenes isolated from the *Garcinia* genus were summarized in **Table 2.**

Table 2 Triterpenes from the *Garcinia* genus

Scientific name	Investigated part	Compound	Structure	Bibliography
G. dulcis	leaves	friedelin	10t	Kosela, *et al.*, 1999a
G. indica	seed oil	α-amyrenone β-amyrenone 24-methylenecycloar-tenone cycloartenone	- - 10q 10o	Kolhe, *et al.*, 1982
G. hombroniana	pericarp	(24*E*)-3α-(OH)-17,14-friedolanostan-8,14, 24-trien-26-oic acid methyl (24*E*)-3α,23-di (OH)-17,14-friedo-lanostan-8,14,24-trien-26-oat	10a 10b	Rukachaisiri-kul, *et al.*, 2000b
		methyl (24*E*)-3α,9,23-tri(OH)-17,14-friedo-lanostan-14,24-dien-26-oat	10c	

Table 2 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. hombroniana	pericarp	3α-(OH)-23-oxo-9,16-lanostandien-26-oic acid	10e	Rukachaisiri-kul, *et al.*, 2000b
		3β-(OH)-23-oxo-9,16-lanostandien-26-oic acid	10d	
G. kola Heckel	nut	cycloartenol	10n	Cotterill and Scheinmann, 1978
		24-methylenecyclo-artenol	10o	
	root	cycloartenol	10n	Iwu, *et al.*, 1990
		24-methylenecyclo-artenol	10o	
G. lucida	bark	31-*nor*-9β,19-cyclo-lanost-24-en-3β-ol	10i	Nyemba, *et al.*, 1990
		24,25-epoxy-31-*nor*-9β,19-cyclolanost-24-en-3β-ol	10m	
		9β,19-cyclolanost-24-en-3β,30-diol	10j	
G. mangostana	leaves	3β-(OH)-26-*nor*-9,19-cyclolanost-23-en-25-one	10h	Parveen, *et al.*, 1991

Table 2 (Continued)

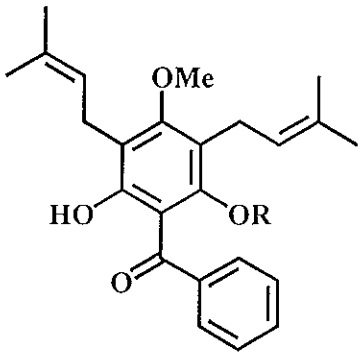
Scientific name	Investigated part	Compound	Structure	Bibliography
G. mangostana	leaves	betulin cycloartenol friedelin mangiferadiol mangiferolic acid 9,19-cyclolanost-25-en-3β,24-diol	10r 10n 10t 10k 10l -	Parveen, *et al.*, 1990
G. myrtifolia	bark	eupha-8,24-dien-3β-ol friedelin	10bb 10t	Spino, *et al.*, 1995
G. opaca	leaves	taraxerol friedelin	10aa 10t	Goh, *et al.*, 1992
G. ovalifolia	stem bark	friedelin	10t	Waterman and Crichton, 1980
G. pseudogutti-fera	heartwood	eupha-8,24-dien-3β-ol	10bb	Ali, *et al.*, 2000
G. pyrifera	stem bark	β-amyrin oleanolic aldehyde	10w -	Ampofo and Waterman, 1986
G. quaesita	bark	3β-(OAc)oleanolic acid	-	Gunatilaka, *et al.*, 1984a

Table 2 (Continued)

Scientific name	Investigated part	Compound	Structure	Bibliography
G. scortechinii	twigs	friedelin	10t	Rukachaisiri-kul, *et al.*, 2000a
G. spicata	leaves	friedelin friedelan-3β-ol	10t 10u	Gunatilaka, *et al.*, 1984b
G. subbelliptica	seed	garcinielliptin oxide garcinielliptone	10f 10g	Lin, *et al.*, 1996;Chung, *et al.*, 1998 Chung, *et al.*, 1998
G. thwaitesii	bark	β-amyrin tirucallol	10w 10cc	Gunatilaka, *et al.*, 1983
G. vilersiana	bark	olean-12-ene-3β,11α-diol oleanolic acid lupeol β-amyrin	10x 10n 10s 10w	Nguyen, *et al.*, 2000
G. xanthochy-mus	leaves	friedelin betulin canophyllol	10t 10r 10v	Baslas and Kumar,1981; Singh, *et al.*, 1991

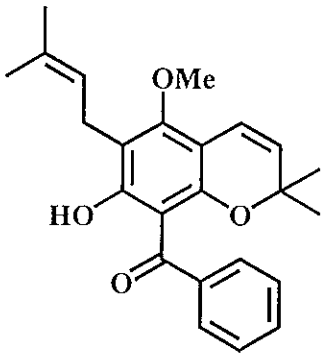
Structures of Compounds Isolated from Plants of the *Garcinia* genus

1. Benzophenone

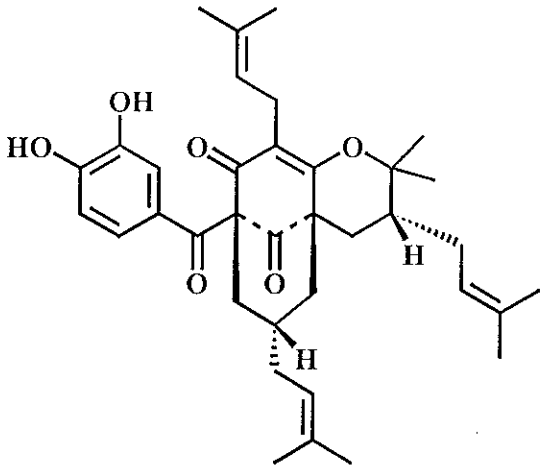


1a: R = H : vismiaphenone-C

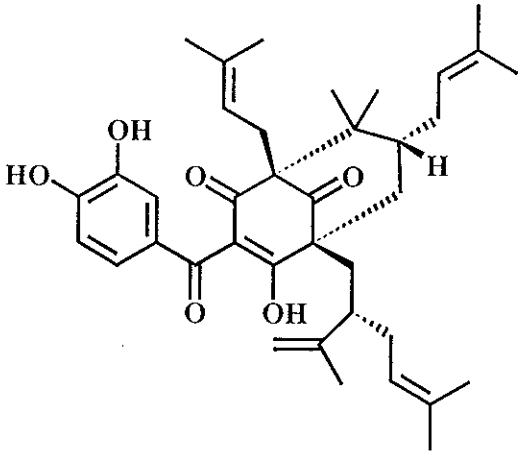
1b: R = Me : myrtiaphenone-A



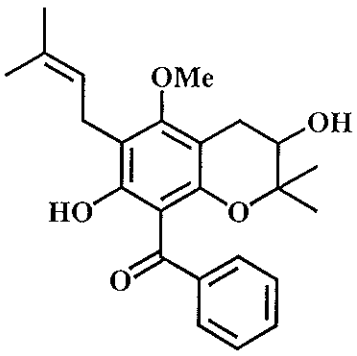
1c: myrtiaphenone-B



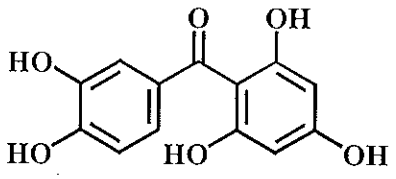
1d: isogarcinol



1e: garcinol

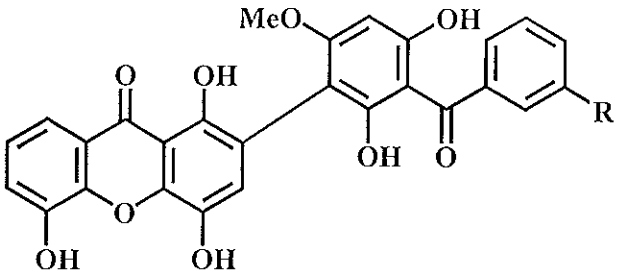


1f: pseudoguttiaphenone-A



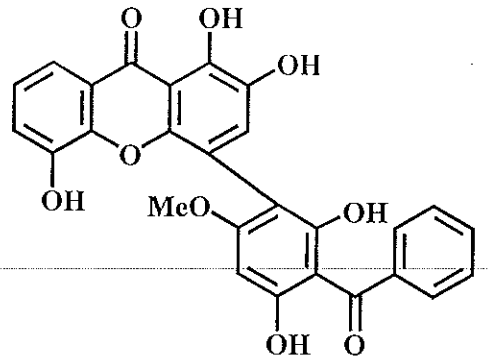
1g: maclurin

2. Benzophenone-xanthone dimer



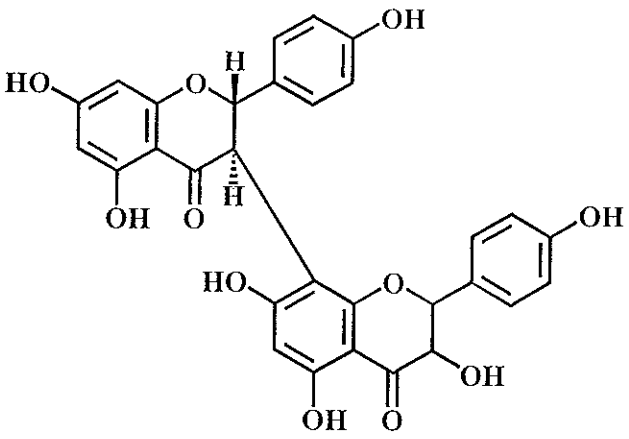
2a: R = H : garciduol A

2b: R = OH : garciduol B

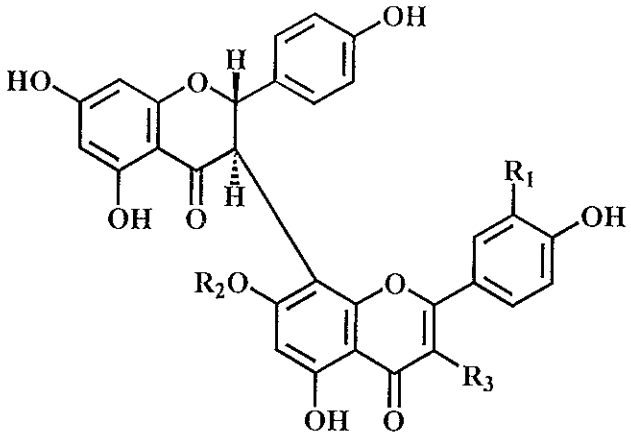


2c: garciduol C

3. Biflavonoid



3a: GB-1

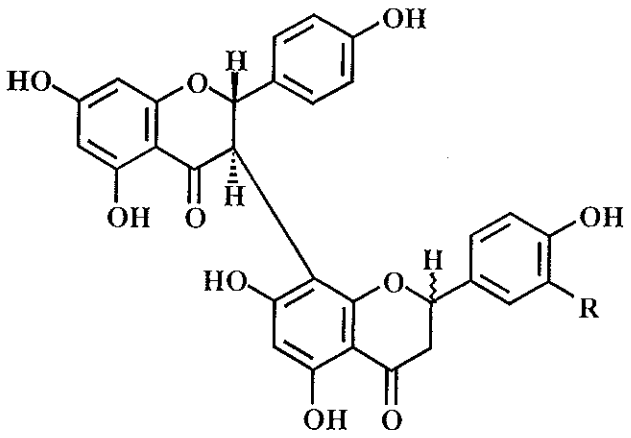


3b: $R_1 = R_2 = H$; $R_3 = OH$: garcinianin

3c: $R_1 = R_2 = R_3 = H$: volkensiflavone

3d: $R_1 = OH$; $R_2 = R_3 = H$: morelloflavone

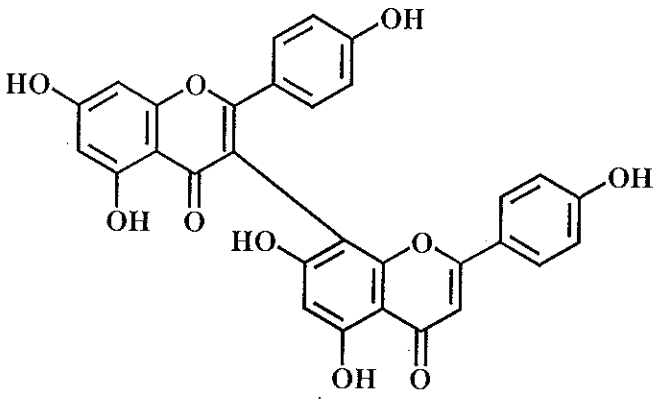
3e: $R_1 = OH$; $R_2 = \beta$-D-glucose ; $R_3 = H$: fukugeside



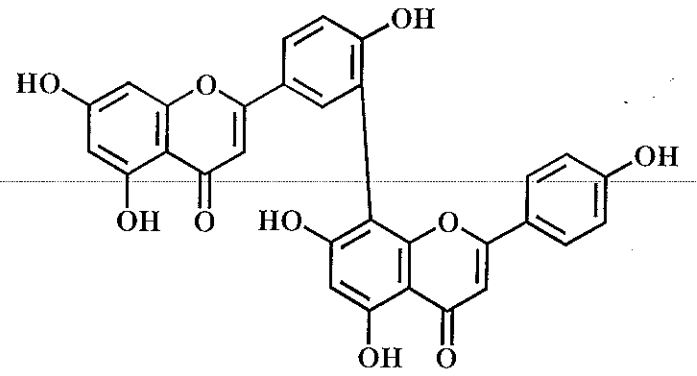
3f: R = H ; H-2″β : (+)-GB-1b

3g: R = H ; H-2″α : (-)-GB-1a

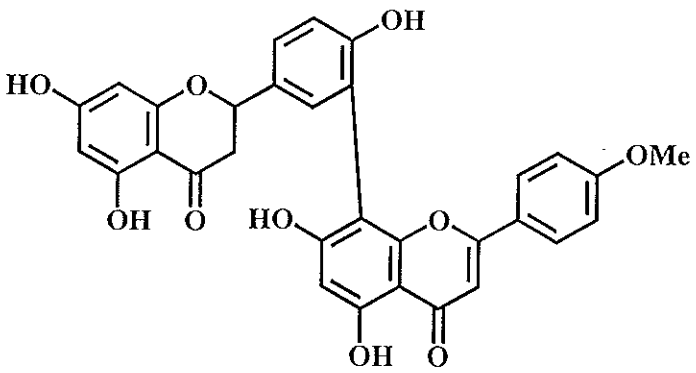
3h: R = OH ; H-2″β : (-)-GB-2a



3i: 3,8″-biapigenin

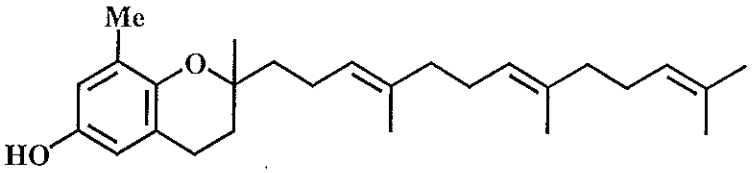


3j: amentoflavone



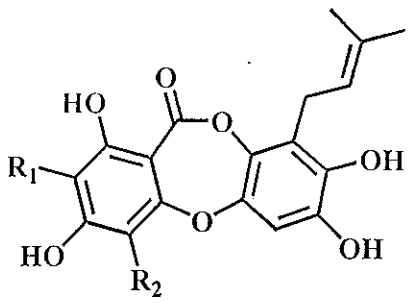
3k: podocarpusflavone A

4. Chromanol



4a: δ-tocotrienol

5. Depsidone



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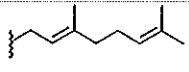
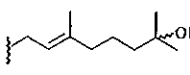
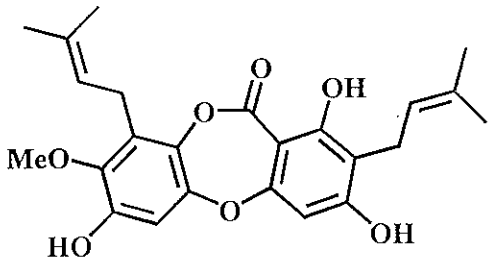
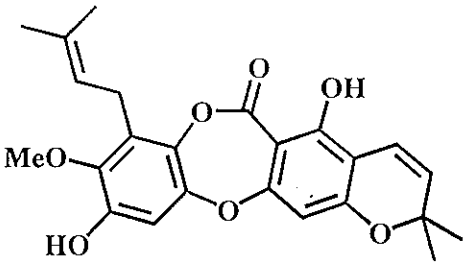
5b: R_1 =  ; R_2 = H : garcidepsidone B

5c: R_1 =  ; R_2 = H : garcidepsidone C

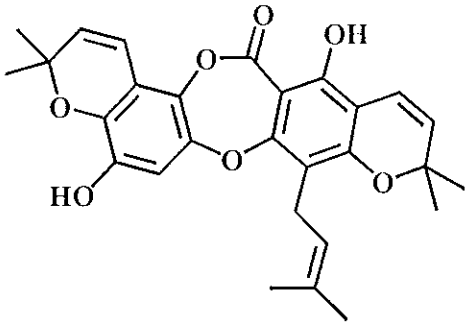
5d: R_1 =  ; R_2 = H : garcidepsidone D



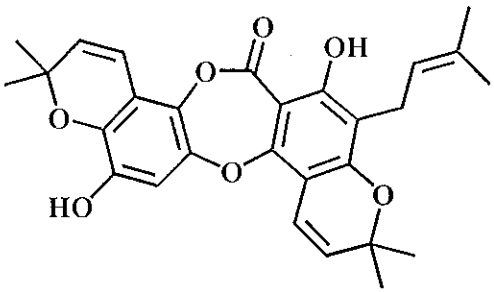
5e: garcinisidone A



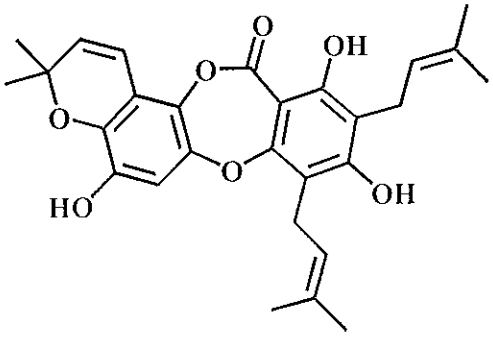
5f: garcinisidone B



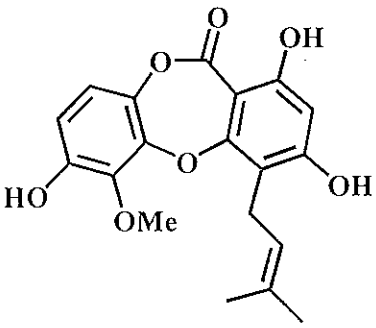
5g: garcinisidone C



5h: garcinisidone D

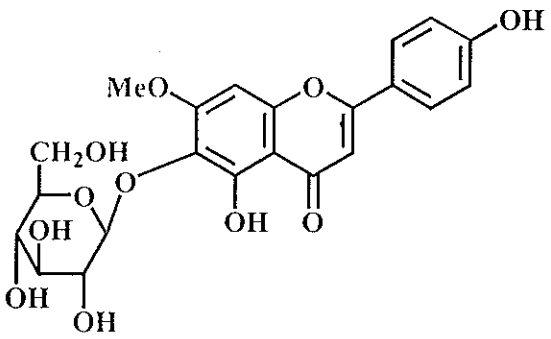


5i: garcinisidone E

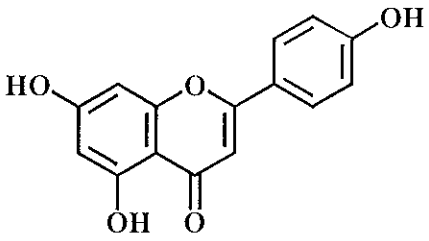


5j: garcinisidone F

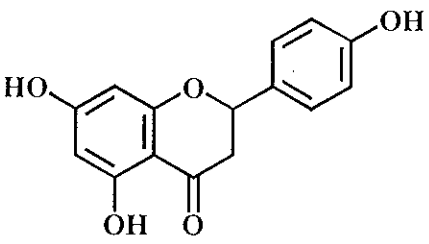
6. Flavonoid



6a: sorbifolin 6-galactoside

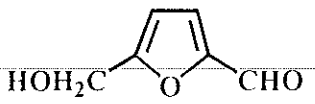


6b: apigenin

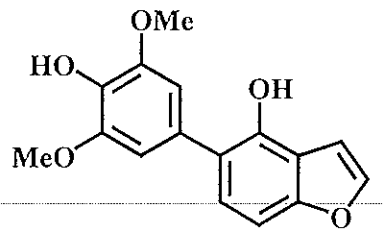


6c: naringenin

7. Furan

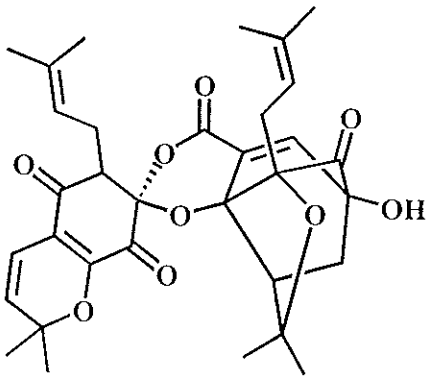


7a: 5-(OH)methyl-2-furaldehyde



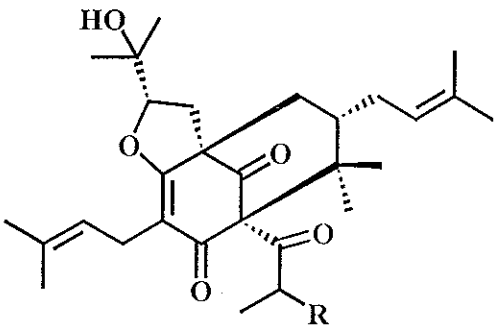
7b: garcifuran A

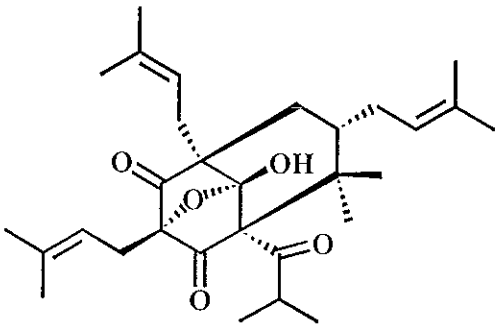
8. Lactone



8a: lateriflorone

9. Phloroglucinol

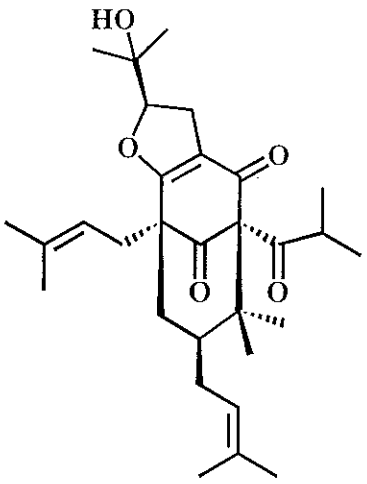




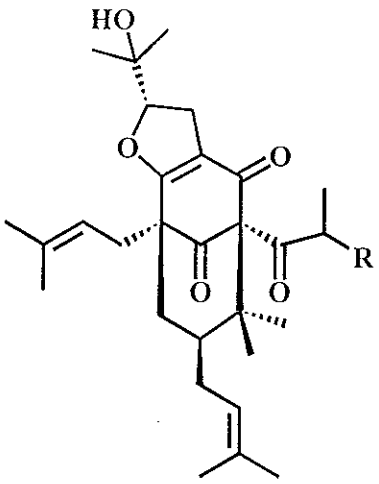
9a: R = Me : garsubellin A

9b: R = CH_2CH_3 : garsubellin B

9c: subellinone



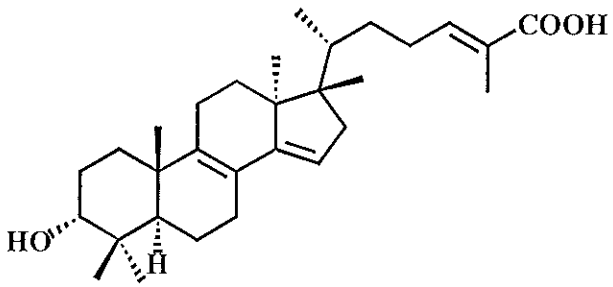
9d: garsubellin C



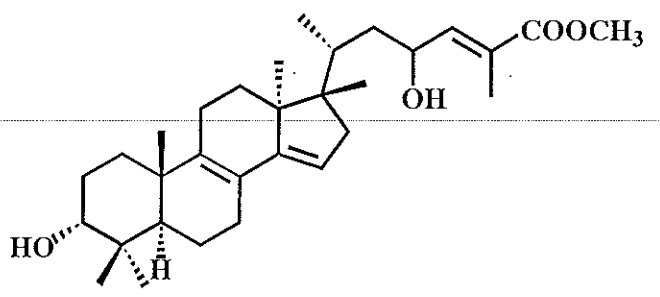
9e: R = Me : garsubellin D

9f: R = CH_2CH_3 : garsubellin E

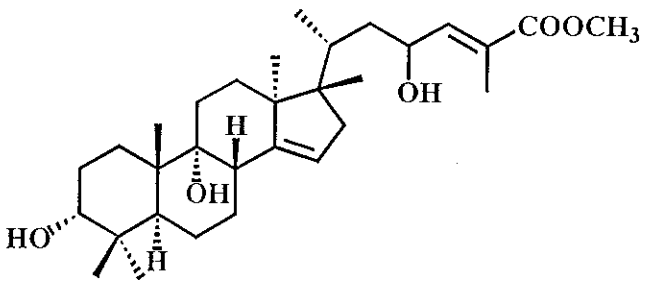
10. Triterpene



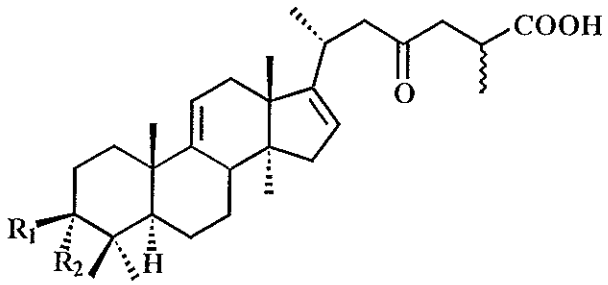
10a: (24*E*)-3α-(OH)-17,14-friedolanostan-8,14,24-trien-26-oic acid



10b: methyl (24*E*)-3α,23-di(OH)-17,14-friedolanostan-8,14,24-trien-26-oat

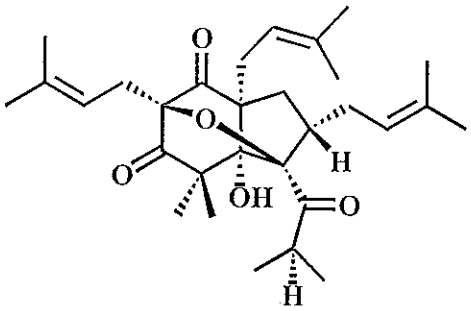


10c: methyl (24*E*)-3α,9,23-tri(OH)-17,14-friedolanostan-14,24-dien-26-oat

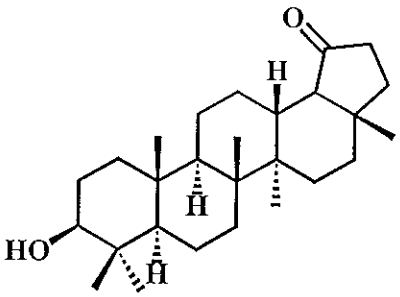


10d: R_1 = OH ; R_2 = H : 3β-(OH)-23-oxo-9,16-lanostadien-26-oic acid

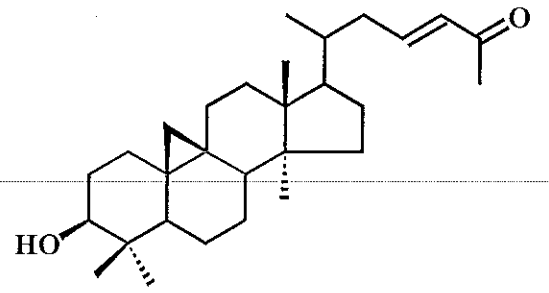
10e: R_1 = H ; R_2 = OH : 3α-(OH)-23-oxo-9,16-lanostadien-26-oic acid



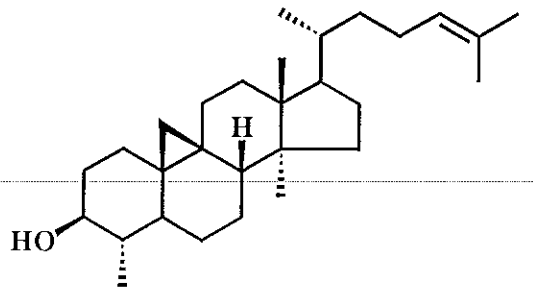
10f: garcinielliptin oxide



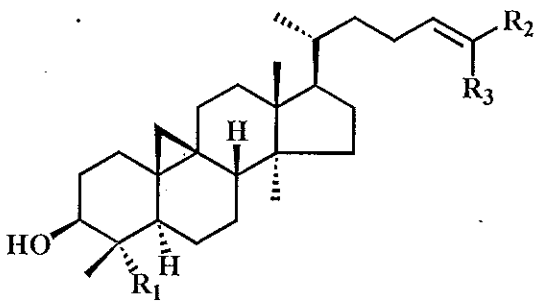
10g: garcinielliptone



10h: 3β-(OH)-26-*nor*-9,19-cyclolanost-23-en-25-one



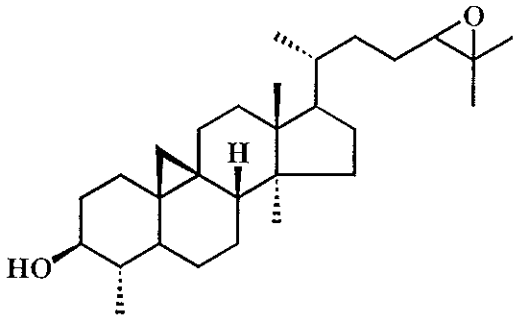
10i: 31-*nor*-9β,19-cyclolanost-24-en-3β-ol



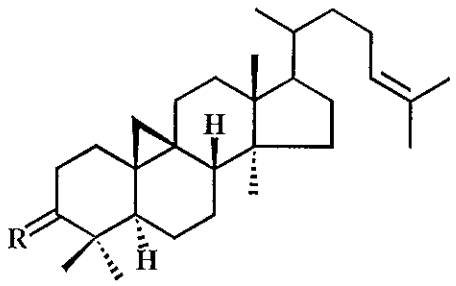
10j: $R_1 = CH_2OH$; $R_2 = R_3 = Me$: 9β,19-cyclolanost-24- ene-3β,30-diol

10k: $R_1 = R_3 = Me$; $R_2 = CH_2OH$: mangiferadiol

10l: $R_1 = R_2 = Me$; $R_3 = COOH$: mangiferolic acid

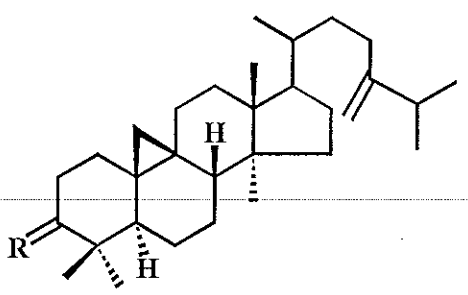


10m: 24,25-epoxy-31-*nor*-9β,19-cyclo-lanostan-3β-ol



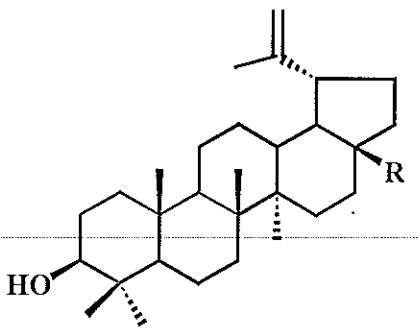
10n: R = α-H; β-OH : cycloartenol

10o: R = O : cycloartenone



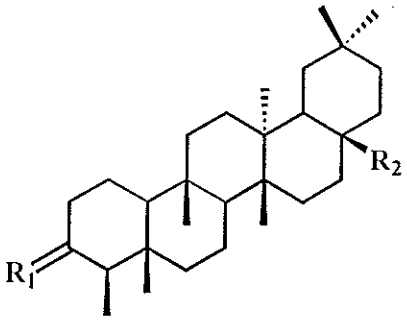
10p: R = α-H; β-OH : 24-methylenecycloartenol

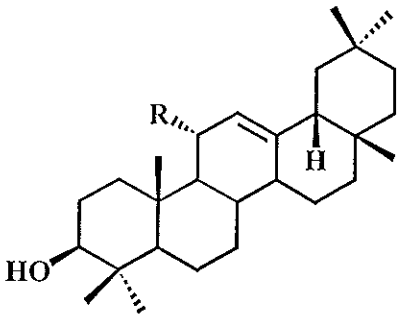
10q: R = O : 24-methylenecycloartenone



10r: R = CH_2OH : betulin

10s: R = Me : lupeol





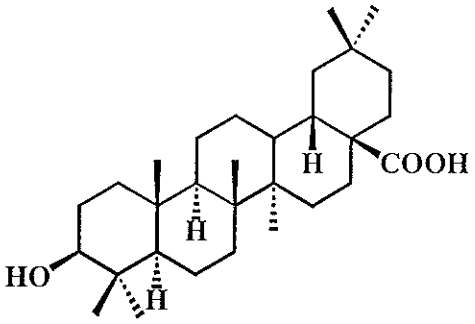
10t: R_1 = O; R_2 = Me : friedelin

10u: R_1 = α-H, β-OH; R_2 = Me : friedelan-3β-ol

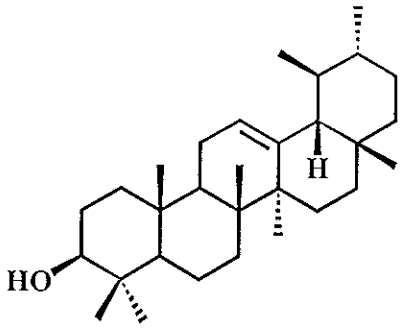
10v: R_1 = O; R_2 = CH_2OH : canophyllol

10w: R_1 = H : β-amyrin

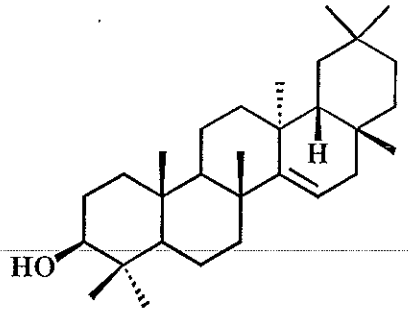
10x: R_1 = OH : olean-12-ene-3β,11α-diol



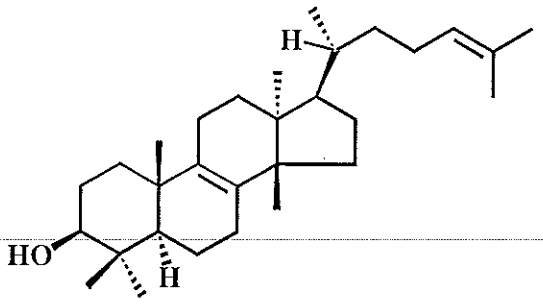
10y: oleanolic acid



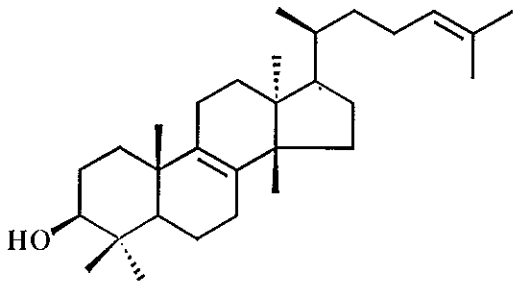
10z: α-amyrin



10aa: taraxerol



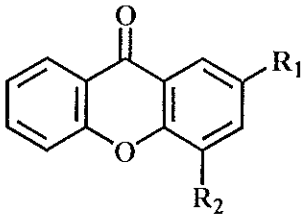
10bb: eupha-8,24-dien-3β-ol



10cc: tirucallol

11. Xanthone

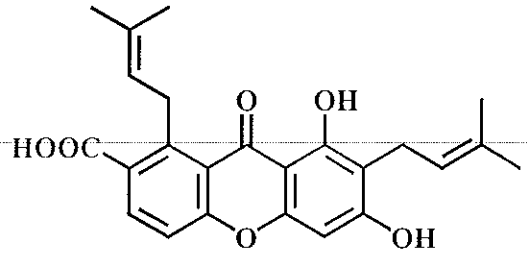
11.1 Monooxyxanthone



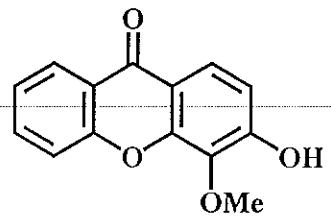
11.1a: R_1 = H ; R_2 = OH : 4-(OH)xanthone

11.1b: R_1 = OH ; R_2 = H : 2-(OH)xanthone

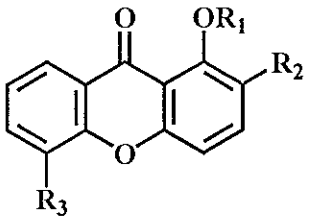
11.2 Dioxyxanthone



11.2a: 2,8-di(3-methylbut-2-enyl)-7-carboxy-1,3-di(OH)xanthone



11.2b: 3-(OH)-4-(OMe)-xanthone

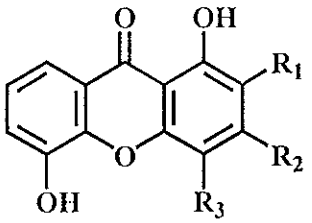


11.2c: R_1 = Me ; R_2 = OMe ; R_3 = H : 1,2-di(OMe)xanthone

11.2d: R_1 = R_2 = H ; R_3 = OH : 1,5-di(OH)xanthone

11.2e: R_1 = Me ; R_2 = OH ; R_3 = H : 1-(OMe)-2-(OH)xanthone

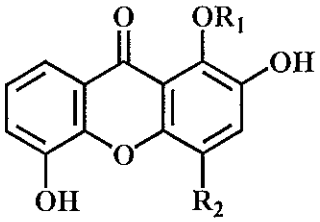
11.3 Trioxyxanthone



11.3a: R_1 = R_3 = H ; R_2 = OH : 1,3,5-tri(OH)xanthone

11.3b: R_1 =  ; R_2 = H ; R_3 = OH : 12b-(OH)-des-D-garcigerrin A

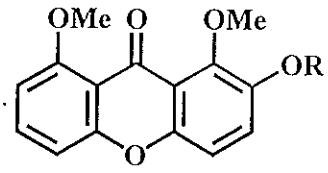
11.3c: R_1 = R_2 = H ; R_3 = OH : 1,4,5-tri(OH)xanthone (subelliptenone G)



11.3d: R_1 = Me; R_2 = H : 2,5-di(OH)-1-(OMe)xanthone

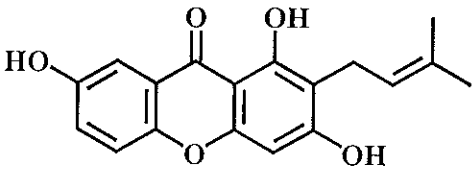
11.3e: R_1 = H; R_2 =  : globuxanthone

11.3f: R_1 = Me ; R_2 =  : 1-O-methylglobuxanthone

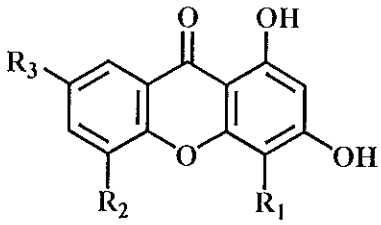


11.3g: R = Me : 1,2,8-tri(OMe)xanthone

11.3h: R = H : 2-(OH)-1,8-di(OMe)xanthone

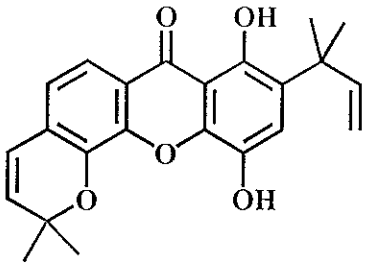


11.3i: 1,3,7-tri(OH)-2-(3-methyl-2-butenyl)xanthone

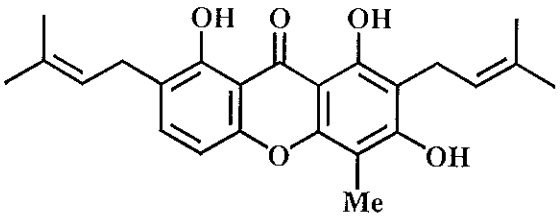


11.3j: $R_1 = R_2 = H$; $R_3 = OH$: gentisein

11.3k: R_1 =  ; $R_2 = OH$; $R_3 = H$: pancixanthone

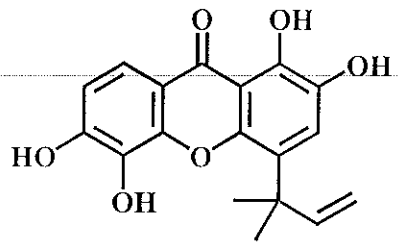


11.3l: garciniaxanthone B

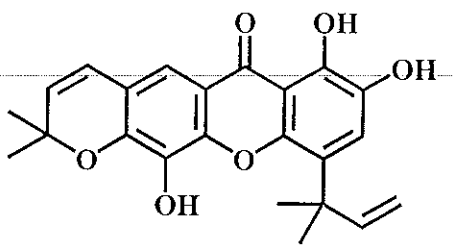


11.3m: 2,7-di(3-methylbut-2-enyl)-1,3,8-tri(OH)-4-(Me)xanthone

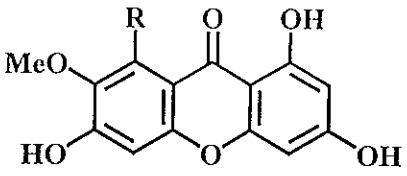
11.4 Tetraoxyxanthone



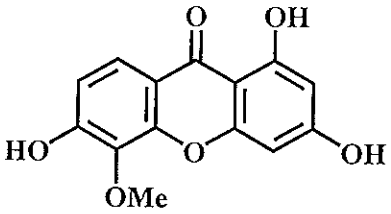
11.4a: symphoxanthone



11.4b: subelliptenone H

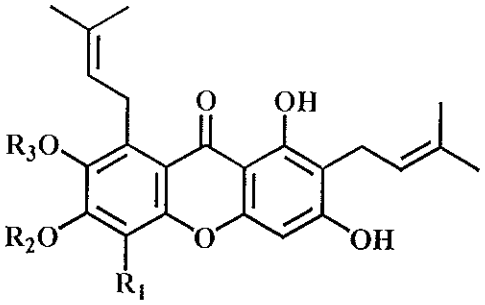


11.4c: R = H : 1,3,6-tri(OH)-7-(OMe) xanthone



11.4e: 1,3,6-tri(OH)-5-(OMe)xanthone

11.4d: R =  : 1,3,6-tri(OH)-8-isoprenyl-7-(OMe)xanthone

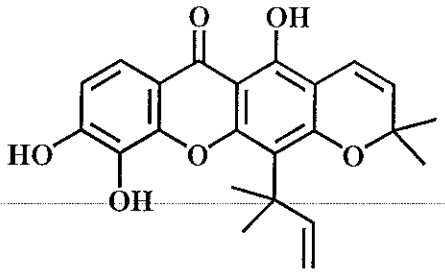


11.4f: $R_1 = R_2 = H$; $R_3 = Me$: α-mangostin

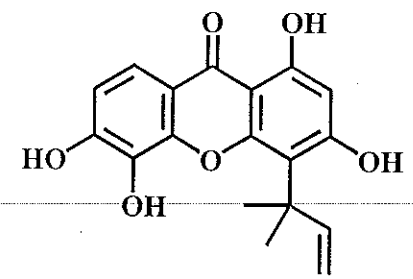
11.4g: $R_1 = R_2 = R_3 = H$: γ-mangostin

11.4h: R_1 =  ; $R_2 = H$; $R_3 = Me$: 7-O-methylgarcinone E

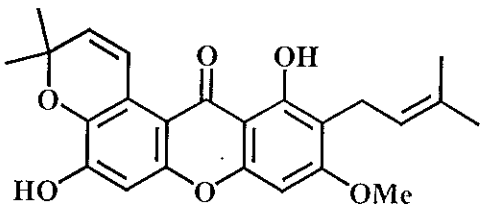
11.4i: R_1 =  ; $R_2 = Me$; $R_3 = H$: 1,3,7-tri(OH)-2,5,8-tris(3-methylbut-2-enyl)-6-(OMe)xanthone



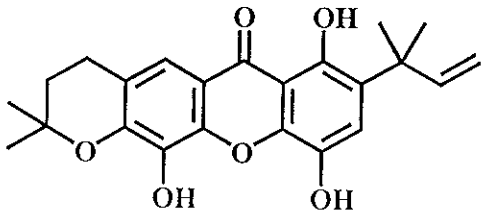
11.4j: 5,9,10-tri(OH)-12-[1,1-di(Me)prop-2-enyl]-2,2-di(Me)-2*H*-pyrano[3,2-*b*] xanthen-6-one



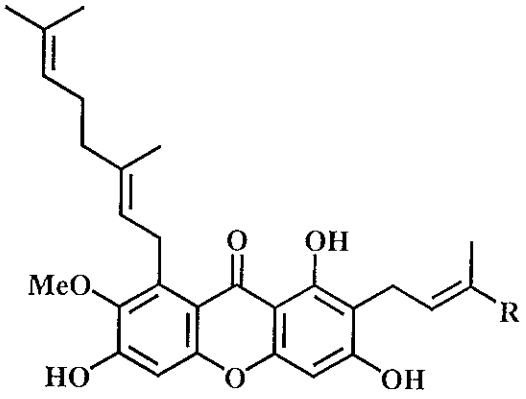
11.4k: assiguxanthone A



11.4l: 3-O-methylgarcinone B

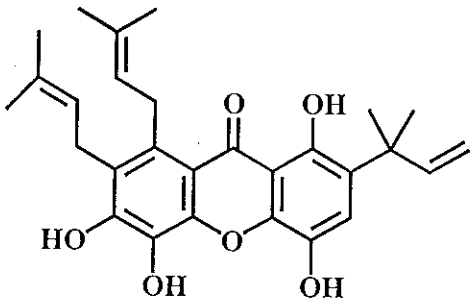


11.4m: subelliptenone I

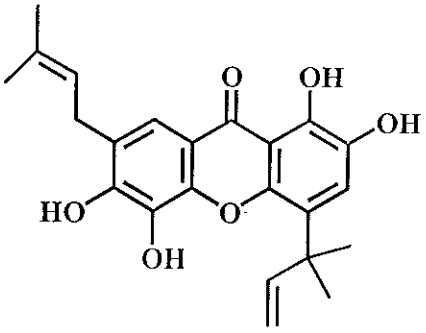


11.4n: R = Me : cowanin

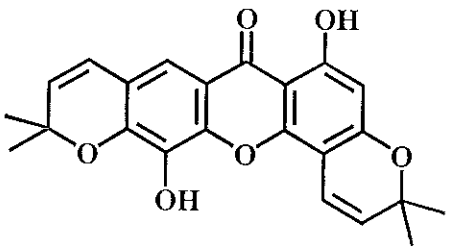
11.4o: R = CH_2OH : cowanol



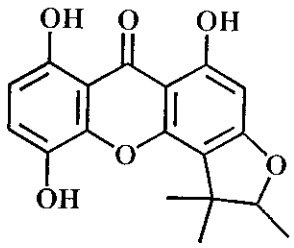
11.4p: subelliptenone A



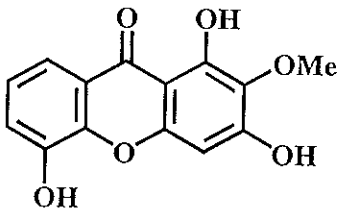
11.4q: subelliptenone B



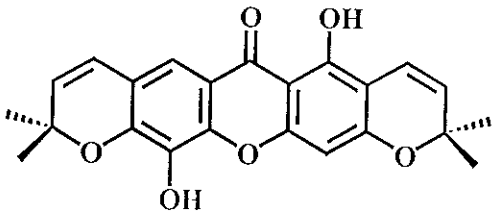
11.4r: rheediaxanthone A



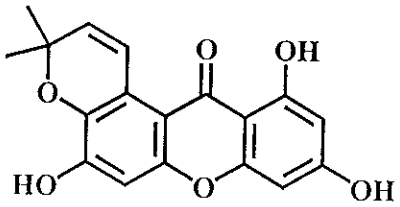
11.4s: garbogiol



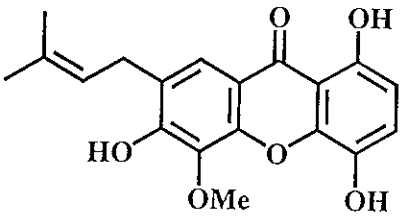
11.4t: 1,3,5-tri(OH)-2-(OMe)xanthone



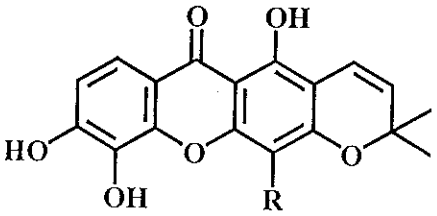
11.4u: pyranojacareubin



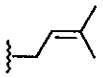
11.4v: toxyloxanthone B

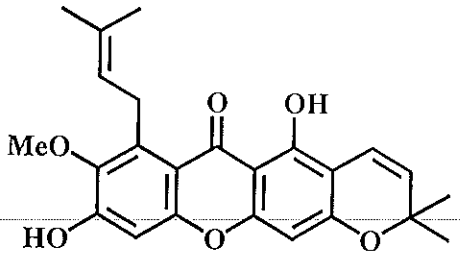


11.4w: 1,4,6-tri(OH)-5-(OMe)-7-(3-methylbut-2-enyl)xanthone

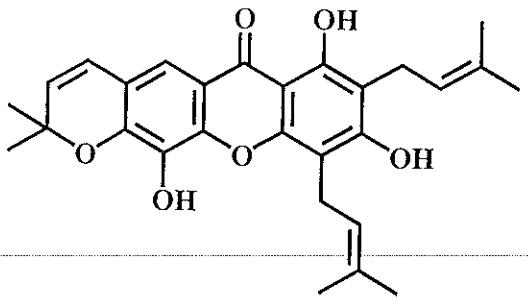


11.4x: R = H : jacareubin

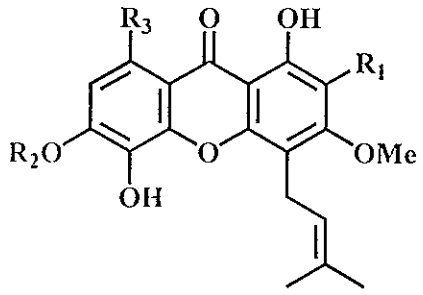
11.4y: R =  : xanthone V1



11.4z: 5,9-di(OH)-8-(OMe)-2,2-di(Me)-7-(3-methylbut-2-enyl)-2*H*,6*H*-pyrano[3,2-*b*]xanthen-6-one



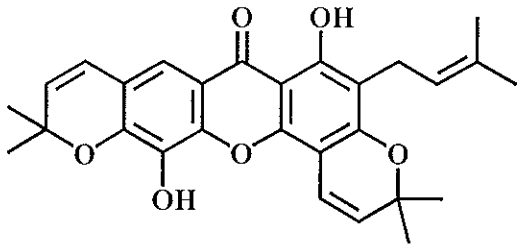
11.4aa: latisxanthone C



11.4bb: $R_1 = R_2 = R_3 = H$: dulxanthone A

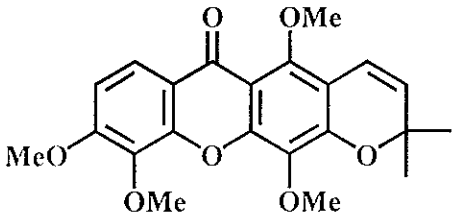
11.4cc: R_1 =  ; $R_2 = R_3 = H$: dulxanthone B

11.4bb: $R_1 = H$; $R_2 = Me$; R_3 =  : dulxanthone C

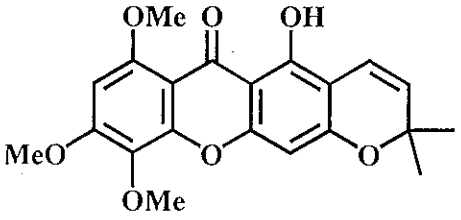


11.4ee: latisxanthone A

11.5 Pentaoxyxanthone

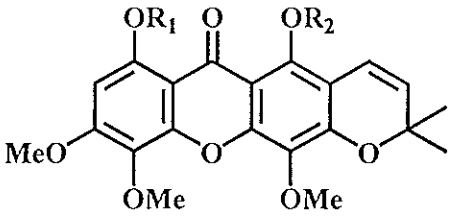


11.5a: dulxanthone E



11.5b: dulxanthone F

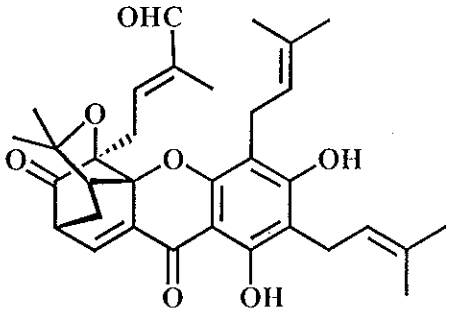
11.6 Hexaoxyxanthone



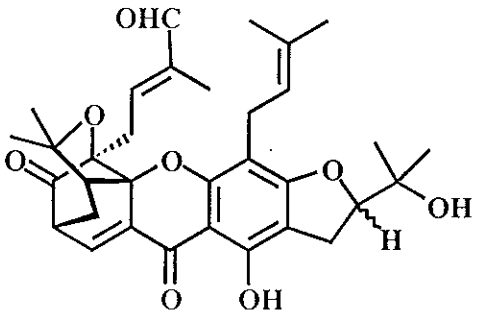
11.6a: R_1 = H ; R_2 = Me : dulxanthone H

11.6b: R_1 = Me ; R_2 = H : dulxanthone G

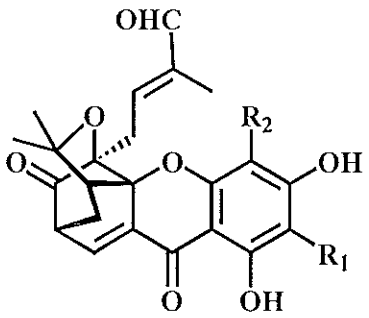
11.7 Caged polyprenylated xanthone

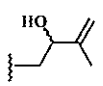
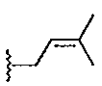


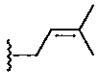
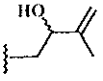
11.7a: gaudichaudione A

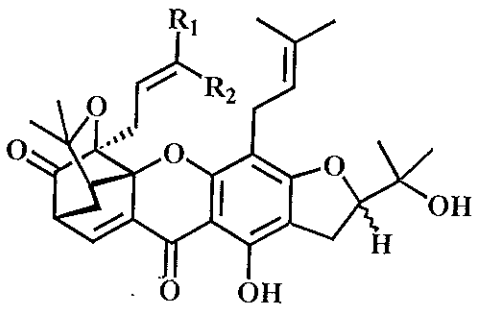


11.7b: gaudichaudione D



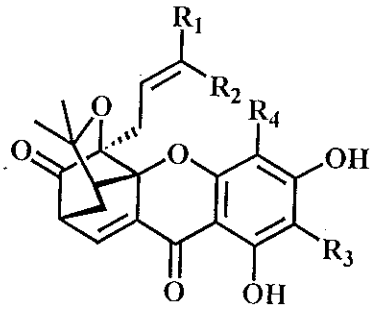
11.7c: R_1 =  ; R_2 =  : gaudichaudione B

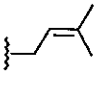
11.7d: R_1 =  ; R_2 =  : gaudichaudione C

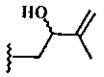


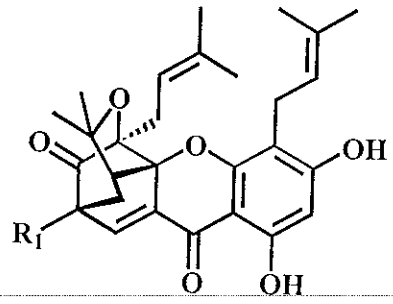
11.7e: R_1 = CHO ; R_2 = Me : gaudichaudione E

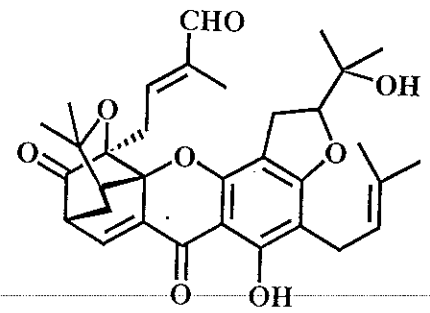
11.7f: R_1 = Me ; R_2 = COOH : gaudichaudiic acid A



11.7g: R_1 = CHO ; R_2 = Me ; R_3 =  ; R_4 =  : gaudichaudione F

11.7h: R_1 = Me ; R_2 = COOH ; R_3 =  ; R_4 =  : gaudichaudiic acid B

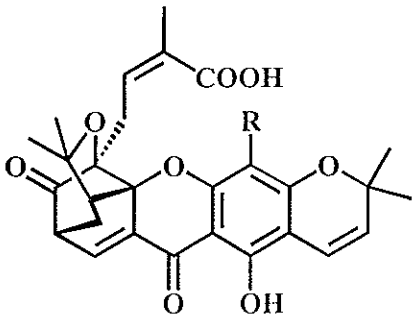


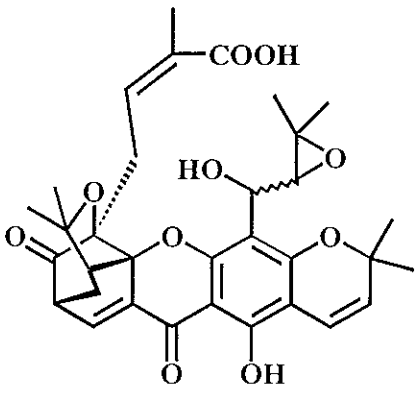


11.7i: R_1 = H : forbesione

11.7j: R_1 = OMe : gaudichaudione H

11.7k: gaudichaudione G



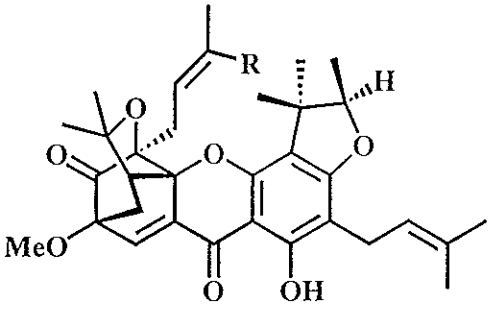


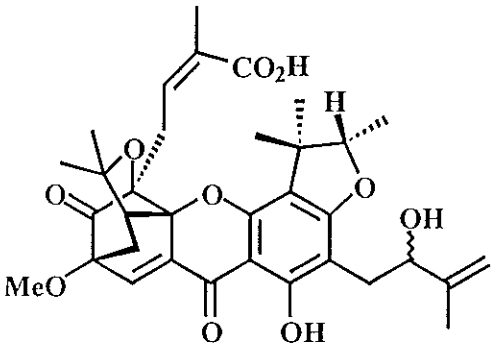
11.7l:  : gaudichaudiic acid E

11.7m:  : morellic acid

11.7n: gaudichaudiic acid C

11.7o: gaudichaudiic acid D

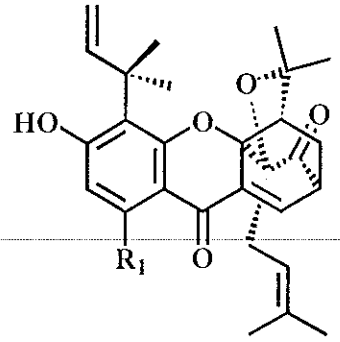


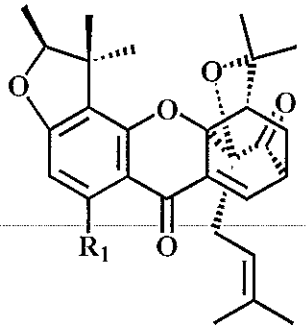


11.7p: R = Me : scortechinone A

11.7q: R = COOH : scortechinone B

11.7r: scortechinone C



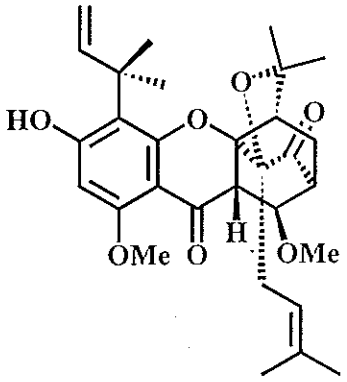


11.7s: R_1 = OH : bractatin

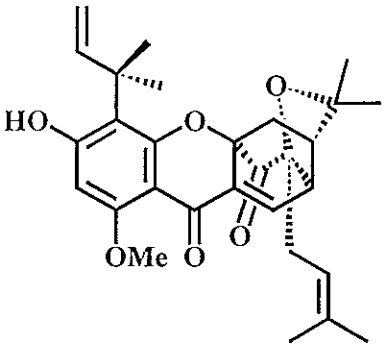
11.7t: R_1 = OMe : 1-O-methylbractatin

11.7u: R_1 = OH : isobractatin

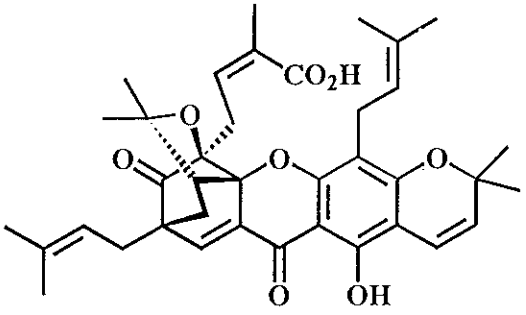
11.7v: R_1 = OMe : 1-O-methylisobractatin



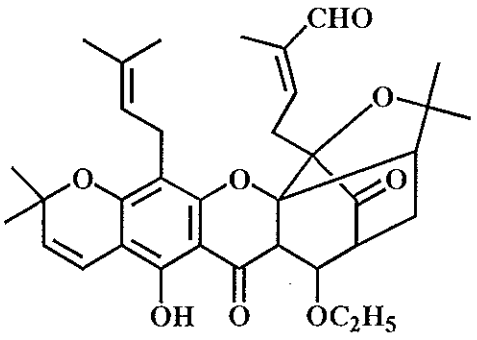
11.7w: 1-O-methyl-8-(OMe)-8,8a-dihydrobractatin



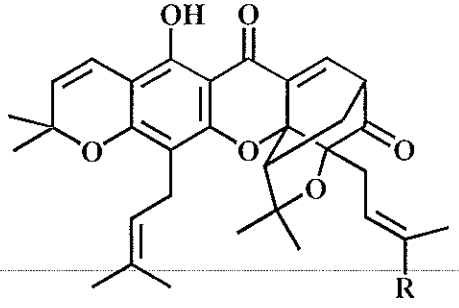
11.7x: 1-O-methylneobractatin



11.7y: 7-isoprenylmorellic acid

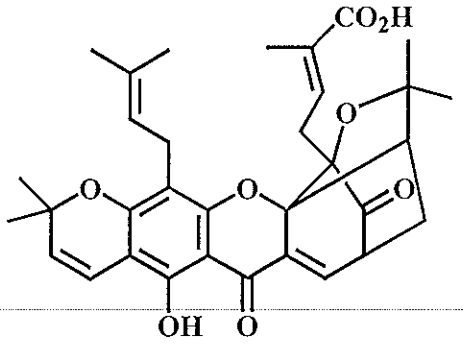


11.7z: isomoreollin

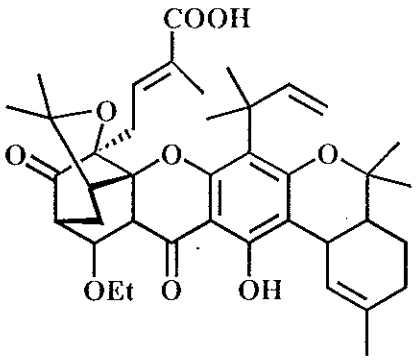


11.7aa: R = CHO : isomorellin

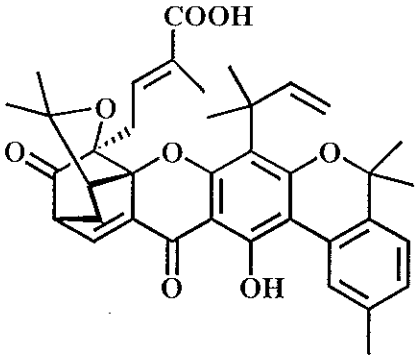
11.7bb: R = COOH : isomorellic acid



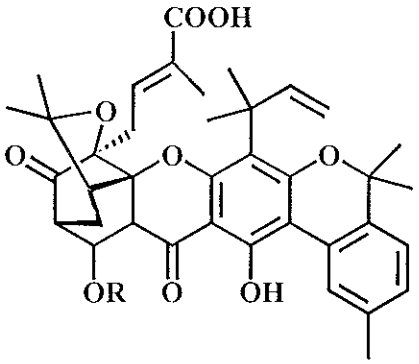
11.7cc: isomorellinol



11.7dd: gaudichaudiic acid F



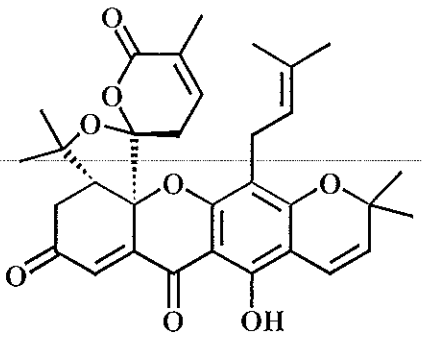
11.7ee: gaudichaudiic acid G



11.7ff: R = Me : gaudichaudiic acid H

11.7gg: R = Et : gaudichaudiic acid I

11.8 Spiroxanthone



11.8a: gaudispirolactone

Furthermore, the spectroscopic data of some triterpenes which had similar structure to the constituents from stem bark of *G. speciosa* were summarized as follow.

(24*E*)-3α-Hydroxy-17,14-friedolanostan-8,14,24-trien-26-oic acid

(Rukachaisirikul, *et al.*, 2000b)

physical appearance : white solid

mp. 231-232^{O}C

$[\alpha]_D^{29}$ -59^{O} (c = 0.84, MeOH)

UV(MeOH) λ_{max} nm : 228 (ε 11,000), 244 (ε 11,615), 250 (ε 12,000), 262 (ε 7,692)

IR(KBr) $\nu_{cm^{-1}}$: 3400, 2960, 1695

^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz) 6.94 (1H, *qt*, *J* = 7.2 and 1.1 Hz, H-24), 5.27 (1H, *s*, H-15), 3.46 (1H, *brs*, H-3), 2.40-2.23 (3H, *m*, 2xH-7, H-16), 2.20-1.90 (6H, *m*, 2xH-11, H-12, H-16, 2xH-22), 1.90-1.85 (1H, *m*, H-20), 1.86 (3H, *d*, *J* = 1.1 Hz, Me-27), 1.70-1.50 (8H, *m*, 2xH-1, 2xH-2, H-5, 2xH-6, H-12), 1.22-1.10 (2H, *m*, 2xH-23), 1.02 (3H, *s*, Me-19), 1.00 (3H, *s*, Me-28), 0.90 (3H, *d*, *J* = 6.6 Hz, Me-21), 0.89 (3H, *s*, Me-29), 0.84 (3H, *s*, Me-30), 0.77 (3H, *s*, Me-18)

^{13}C NMR($CDCl_3$+DMSO-d_6)(δ ppm) (100 MHz) 169.6 (C-26), 148.0 (C-14), 142.0 (C-9), 141.8 (C-24), 127.1 (C-25), 122.1 (C-8), 115.0 (C-15), 74.5 (C-3), 49.8 (C-17), 47.4 (C-13), 44.8 (C-16), 43.7 (C-5), 37.4 (C-20), 37.2 (C-10), 37.0 (C-4), 31.0 (C-23), 29.5 (C-1), 28.7 (C-2), 27.7 (C-28), 26.7 (C-22), 26.1 (C-7), 25.2 (C-12), 22.1 (C-11), 21.7 (C-29), 18.4 (C-19), 17.1 (C-6), 16.1 (C-30), 15.1 (C-18), 14.8 (C-21), 11.7(C-27)

MS(*m/z*)(% rel. int.)	454 ($[M]^+$, 100), 439 (26), 421 (85), 313 (15)
HR-MS(*m/z*)	454.34598 for $C_{30}H_{46}O_3$ (calcd. 454.34470)

Methyl (24*E*)-3α,23-dihydroxy-17,14-friedolanostan-8,14,24-trien-26-oate

(Rukachaisirikul, *et al.*, 2000b)

physical appearance : white powder

mp. 112-113°C

$[\alpha]_D^{29}$ -35° (c = 0.28, MeOH)

UV(MeOH) λ_{max} nm : 228 (ε 13,583), 250 (ε 10,416), 260 (ε 9,583)

IR(KBr) $\nu_{cm^{-1}}$: 3400, 2960, 1705

^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz)	6.72 (1H, *qd*, *J* = 7.2 and 1.1 Hz, H-24), 5.27 (1H, *s*, H-15), 4.60 (1H, *ddd*, *J* = 10.7, 7.2 and 2.5 Hz, H-23), 3.77 (3H, *s*, OMe), 3.45 (1H, *brs*, H-3), 2.40-2.23 (3H, *m*, 2xH-7, H-16), 2.25-2.15 (1H, *m*, H-20), 2.15-2.00 (2H, *m*, 2xH-22), 2.00-1.90 (1H, *m*, H-16), 1.87 (3H, *d*, *J* = 1.1 Hz, Me-27), 1.80-1.40 (9H, *m*, 2xH-1, H-2, H-5, 2xH-6, H-11, 2xH-12), 1.30-1.05 (2H, *m*, H-2, H-11), 1.01 (3H, *s*, Me-30), 0.99 (3H, *s*, Me-28), 0.95 (3H, *d*, *J* = 7.0 Hz, Me-21), 0.91 (3H, *s*, Me-19), 0.89 (3H, *s*, Me-29), 0.76 (3H, *s*, Me-18)
^{13}C NMR($CDCl_3$)(δ ppm) (100 MHz)	168.5 (C-26), 148.8 (C-14), 144.5 (C-9), 142.3 (C-24), 127.0 (C-25),122.8 (C-8), 115.8 (C-15), 75.8 (C-3), 65.8 (C-23), 51.9 (OMe), 50.7 (C-17), 48.0 (C-13), 45.5 (C-16), 44.4 (C-5), 39.2 (C-11), 37.8 (C-10), 37.6 (C-4), 33.4 (C-20), 30.1 (C-1), 29.2 (C-2), 28.0 (C-28), 26.7

	(C-7), 25.6 (C-12), 22.7 (C-22), 22.2 (C-29), 18.9 (C-30), 18.1 (C-6), 17.1 (C-19), 15.6 (C-18), 15.2 (C-21), 12.7(C-27)
MS(*m/z*)(% rel. int.)	484 ($[M]^+$, 42), 469 (18), 451 (34), 313 (35), 24 (58), 41 (100)
HR-MS(*m/z*)	484.35614 for $C_{31}H_{48}O_4$ (calcd. 484.35526)

Methyl (24*E*)-3α,9,23-trihydroxy-17,14-friedolanostan-14,24-dien-26-oate

(Rukachaisirikul, *et al.*, 2000b)

physical appearance : white solid

mp. 128-130°C

$[\alpha]_D^{29}$ -48° (c = 0.42, MeOH)

UV(MeOH) λ_{max} nm : 228 (ε 13,720)

IR(KBr) $\nu_{cm^{-1}}$: 3495, 2965, 1710

^{1}H NMR($CDCl_3$+C_6D_6)(δ ppm) (400 MHz)	6.70 (1H, *qd*, *J* = 8.0 and 1.4 Hz, H-24), 5.33 (1H, *brm*, H-15), 4.56 (1H, *ddd*, *J* = 10.8, 8.0 and 2.2 Hz, H-23),3.75 (3H, *s*, OMe), 3.40 (1H, *brs*, H-3), 2.50-2.40 (1H, *m*, H-8), 2.40-2.30 (1H, *m*, H-16), 2.30-2.20 (1H, *m*, H-20), 2.20-1.92 (2H, *m*, H-5, H-7), 1.92-1.85 (2H, *m*, H-1, H2), 1.86 (3H, *d*, *J* = 1.4 Hz, Me-27), 1.83-1.75 (2H, *m*, H-11, H-16), 1.75-1.65 (1H, *m*, H-22), 1.65-1.55 (3H, *m*, H-2, H-11, H-12), 1.54-1.47 (2H, *m*, H-6, H-12), 1.45-1.35 (2H, *m*, H-6, H-7), 1.23 (3H, *s*, Me-30), 1.15-1.05 (2H, *m*, H-1, H-22), 0.96 (3H, *s*, Me-28), 0.92 (3H, *d*, *J* = 7.0 Hz, Me-21), 0.91 (3H, *s*, Me-19), 0.85 (3H, *s*, Me-29), 0.75 (3H, *s*, Me-18)

^{13}C NMR($CDCl_3$+C_6D_6)(δ ppm) (100 MHz)	168.4 (C-26), 153.6 (C-14), 144.6 (C-24), 142.3 (C-24), 126.9 (C-25),120.3 (C-15), 76.0 (C-3), 75.3 (C-9), 66.7 (C-23), 54.0 (C-17), 51.8 (OMe), 49.1 (C-13), 44.7 (C-16), 42.1 (C-10), 39.2 (C-8), 39.1 (C-22), 39.0 (C-5), 37.5 (C-4), 33.0 (C-20), 29.6 (C-11), 29.0 (C-12), 28.5 (C-28), 25.7 (C-7), 25.1 (C-2), 23.6 (C-1), 22.0 (C-29), 20.8 (C-6), 19.5 (C-30), 16.3 (C-19), 15.3 (C-18), 15.1 (C-21), 12.7(C-27)
EIMS(*m/z*)(% rel. int.)	502 ($[M]^+$, 14), 484 ($[M-H_2O]^+$, 28), 451 (8), 313 (31), 297 (13), 43 (100)
Electrospray MS(*m/z*)(% rel. int.)	1530 ($[3M+Na]^+$, 85), 1027 ($[2M+Na]^+$, 100), 525 ($[M+Na]^+$, 6), 485 ($[MH-H_2O]^+$, 25)
HR-MS(*m/z*)	484.35441 for $C_{31}H_{48}O_4$ (calcd. 484.35526)

3α-Hydroxy-23-oxo-9,16-lanostadien-26-oic acid

(Rukachaisirikul, *et al.*, 2000b)

physical appearance : white powder

mp. 218-220°C

$[\alpha]_D^{29}$ +58° (c = 0.34, MeOH)

IR(KBr) $\nu_{cm^{-1}}$: 3560, 3350, 2940, 1710

^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz)	5.31 (1H, *d*, *J* = 6.4 Hz, H-11), 5.29 (1H, *s*, H-16), 3.20 (1H, *dd*, *J* = 9.6 and 4.0 Hz, H-3), 2.85 (1H, *dd*, *J* = 20.0 and 8.0 Hz, H-24), 2.80-2.75 (1H, *m*, H-25), 2.68 (1H, *dd*, *J* = 18.0 and 6.0 Hz, H-22), 2.49 (1H, *dd*, *J* = 18.0 and 10.0 Hz, H-22), 2.46 (1H, *dd*, *J* = 20.0 and 10.0 Hz, H-24), 2.40-2.28 (2H, *m*, H-8, H-12), 2.07 (1H, *d*, *J* =

	15.2 Hz, H-15), 1.82 (1H, *dd*, *J* = 15.2 and 3.6 Hz, H-15), 1.78-1.57 (5H, *m*, 2xH-2, H-6, H-7, H-12), 1.57-1.28 (4H, *m*, 2xH-1, H-6, H-7), 1.16 (3H, *d*, *J* = 7.0 Hz, Me-27), 1.04 (3H, *s*, Me-19), 1.02 (3H, *d*, *J* = 6.0 Hz, Me-21), 0.99 (3H, *s*, Me-28), 0.82 (3H, *dd*, *J* = 6.0 and 2.0 Hz, H-5), 0.79 (6H, *s*, Me-29, Me-30)
^{13}C NMR($CDCl_3$)(δ ppm) (100 MHz)	207.8 (C-23), 177.1 (C-26), 155.4 (C-17), 149.5 (C-9), 120.1 (C-16), 113.9 (C-11), 77.4 (C-3), 52.4 (C-5), 50.7 (C-13), 49.2 (C-22), 46.4 (C-14), 46.3 (C-24), 40.5 (C-15), 39.7(C-8), 39.4 (C-10), 39.0 (C-4), 36.1 (C-1), 34.3 (C-25), 31.0 (C-12), 28.4 (C-28), 27.8 (C-7), 27.7 (C-20), 27.6 (C-2), 22.1 (C-19), 21.0 (C-6), 20.9 (C-21), 19.8 (C-30), 19.2 (C-18), 17.0 (C-27), 15.9 (C-29)
MS(*m/z*)(% rel. int.)	470 ($[M]^+$, 17), 437 (15), 313 (79), 43 (100)
HR-MS(*m/z*)	470.34061 for $C_{30}H_{46}O_4$ (calcd. 470.33960)

3α-Acetoxy-23-oxo-9,16-lanostadien-26-oic acid

(Rukachaisirikul, *et al.*, 2000b)

physical appearance : white solid

mp. 144-145°C

^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz)	5.29 (1H, *d*, *J* = 6.4 Hz, H-11), 5.21 (1H, *s*, H-16), 4.50 (1H, *dd*, *J* = 9.6 and 4.0 Hz, H-3), 3.01-2.90 (1H, *m*, H-25), 2.85 (1H, *dd*, *J* = 20.0 and 8.0 Hz, H-24), 2.73-2.63 (1H, *m*), 2.65 (1H, *dd*, *J* = 18.0 and 6.0 Hz, H-22), 2.50 (1H, *dd*, *J* = 18.0 and 10.0 Hz, H-22), 2.46 (1H, *dd*, *J* = 18.0 and 6.0 Hz, H-24), 2.39-2.31 (2H, *m*), 2.06 (3H, *s*,

	OCMe), 1.80-1.40 (10H, *m*), 1.40-1.25 (3H, *m*), 1.20 (3H, *d*, *J* = 7.0 Hz, Me-27), 1.08 (3H, *s*, Me-19), 1.04 (3H, *d*, *J* = 8.0 Hz, Me-21), 0.90 (3H, *s*, Me-28), 0.87 (3H, *s*, Me-29), 0.79 (3H, *s*, Me-30), 0.75 (3H, *s*, Me-18)
MS(*m/z*)(% rel. int.)	512 ($[M]^+$, 4), 480 (2), 355 (12), 307 (4), 43 (100)
HR-MS(*m/z*)	512.35090 for $C_{32}H_{48}O_5$ (calcd. 512.35016)

3*β*-Acetoxy-23-oxo-9,16-lanostadien-26-oic acid

(Rukachaisirikul, *et al.*, 2000b)

physical appearance : white solid

mp. 140-141°C

IR(KBr) $\nu_{cm^{-1}}$: 3570, 2780, 1730, 1690

^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz)	5.29 (1H, *d*, *J* = 6.4 Hz, H-11), 5.21 (1H, *s*, H-16), 4.68 (1H, *s*, H-3), 3.01-2.90 (1H, *m*, H-25), 2.85 (1H, *dd*, *J* = 18.0 and 8.0 Hz, H-24), 2.73-2.63 (1H, *m*), 2.65 (1H, *dd*, *J* = 18.0 and 6.0 Hz, H-22), 2.50 (1H, *dd*, *J* = 18.0 and 10.0 Hz, H-22), 2.46 (1H, *dd*, *J* = 18.0 and 6.0 Hz, H-24), 2.41-2.30 (2H, *m*), 2.07 (3H, *s*, OCMe), 2.00-1.40 (10H, *m*), 1.40-1.23 (3H, *m*), 1.20 (3H, *d*, *J* = 7.0 Hz, Me-27), 1.08 (3H, *s*, Me-19), 1.04 (3H, *d*, *J* = 8.0 Hz, Me-21), 0.90 (3H, *s*, Me-28), 0.87 (3H, *s*, Me-29), 0.79 (3H, *s*, Me-30), 0.75 (3H, *s*, Me-18)
MS(*m/z*)(% rel. int.)	512 ($[M]^+$, 4), 480 (2), 355 (12), 43 (100)
HR-MS(*m/z*)	512.35131 for $C_{32}H_{48}O_5$ (calcd. 512.35016)

1.3 The Objective

Up to the present, only two reports revealed the isolation of three xanthones from stem and one xanthone from leaves of *G. speciosa*. We are therefore interested in further investigation of its stem bark in order to separate other additional chemical constituents.

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and Instruments

Melting points were determined on an electrothermal melting point apparatus (Electrothermal 9100) and reported without correction. Infrared spectra (IR) were obtianed on a FTS165 FT-IR spectrometer and Perkin Elmer Spectrum GX FT-IR system and recorded on wavenumber (cm^{-1}). ^{1}H and ^{13}C-Nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded on a Bruker AMX 400 and a FTNMR, Varian UNITY INOVA 500 MHz by using a solution in either deuterochloroform or deuteromethanol with tetramethylsilane (TMS) as an internal standard. Spectra were recorded as chemical shift parameter (δ) value in ppm down field from TMS (δ 0.00). Ultraviolet spectra (UV) were measured with UV-160A spectrophotometer (SHIMADZU). Principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol solution. Optical rotation was measured in methanol solution with sodium D line (590 nm) on an AUTOPOL®II automatic polarimeter. Quick column chromatography, thin-layer chromatography (TLC) and preparative thin-layer chromatography were performed on silica gel 60 GF_{254} (Merck) or reversed-phase C-18. Column chromatography was performed on silica gel (Merck) type 100 (70-230 Mesh ASTM) or reversed-phase C-18. The solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for petroleum ether (bp.40-60°C), diethyl ether and ethyl acetate which were analytical grade reagent.

2.2 Isolation of Compounds from Stem Bark

The crude methanol extract of stem bark of *Garcinia speciosa* was prepared by Professor Dr. Vichai Reutrakul. Chemical investigation of this extract was performed three times. The first investigation was a preliminary study to separate and purify the major components as well as to find out the purification process. The objectives of the second and third were to increase amount of minor components and to purify other minor components which could not be separated in the first investigation.

The First Investigation

The crude extract (20.00 g) was partitioned with water and ethyl acetate to give the ethyl acetate soluble (ET) fraction (7.76 g) and water soluble (AQ) fraction (10.80 g) as red-brown and brown solid, respectively. TLC of ET fraction, using chloroform as a mobile phase, was composed of three purple spots with R_f values of 0.35, 0.29 and 0.09 while AQ fraction showed no definite spot on TLC after dipping in ASA reagent and subsequent heating. According to above information, ET fraction was further tested for its solubility in various solvents at room temperature. The results were demonstrated in **Table 3**.

Table 3 Solubility of ET fraction in various solvents at room temperature

solvent	solubility at room temperature
petroleum ether	-
dichloromethane	+ (pale yellow solution)
diethyl ether	+ (pale yellow solution)

Table 3 (Continued)

solvent	solubility at room temperature
ethyl acetate	+++ (yellow solution)
acetone	+++ (yellow solution)
methanol	+++ (yellow solution)
water	-
10% HCl	-
10% $NaHCO_3$	++ (orange solution)
10% NaOH	+++ (orange solution)

symbol meaning: - insoluble, + slightly soluble, ++ moderately soluble
+++ well soluble

The solubility results indicated that ET fraction contained moderately polar chemical constituents as it was soluble in polar solvents. In addition, the major constituents would be acids according to its solubility in base. All soluble parts in above organic solvents were chromatographed on TLC with chloroform as a mobile phase. They showed similar chromatogram. ET fraction was therefore unseparable into subfractions by any organic solvents. Further separation by quick column chromatography on silica gel was carried out. Elution was conducted initially with petroleum ether gradually enriched with dichloromethane, followed by increasing amount of ethyl acetate in dichloromethane, methanol in ethyl acetate and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford eleven fractions, as shown in **Table 4**.

Table 4 Fractions obtained from ET fraction by quick column chromatography

fraction	weight(g)	physical appearance
E1	0.042	yellow viscous-liquid
E2	0.512	orange-yellow viscous-liquid
E3	0.161	orange-yellow viscous-liquid
E4	1.161	orange-yellow viscous-liquid
E5	0.598	brown-yellow viscous-liquid
E6	0.770	brown viscous-liquid
E7	0.185	red-brown viscous-liquid
E8	0.329	red-brown viscous-liquid
E9	1.604	red-brown solid
E10	0.638	red-brown solid
E11	1.040	red solid

Fraction E1 contained approximately five compounds on TLC with 40% dichloro-methane in petroleum ether as a mobile phase. Further purification was then not performed.

Fraction E2 was characterized by TLC on reversed-phase silica gel with 90% methanol in water as a mobile phase. Only one purple spot (R_f 0.28) was observed in ASA reagent. Further separation by column chromatography over reversed-phase silica gel, eluting with 90% methanol in water was carried out. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford six subfractions, as shown in **Table 5**.

Table 5 Subfractions obtained from fraction E2 by column chromatography on reversed-phase silica gel

subfraction	weight(g)	physical appearance
E2.1	0.004	white solid
E2.2	0.027	yellow viscous-liquid
E2.3	0.254	yellow viscous-liquid
E2.4	0.173	yellow viscous-liquid
E2.5	0.016	yellow viscous-liquid
E2.6	0.020	yellow viscous-liquid

Subfraction E2.1 showed a single purple spot with R_f value of 0.20 on TLC with dichloromethane as a mobile phase in ASA reagent. It was named as **AH8** (melting at 154-156°C).

$[\alpha]_D^{29}$ -55.56° (c = 1.80x10^{-2} g/ 100 cm^3, MeOH)

UV(MeOH) λ_{max} nm(log ε) 208 (3.39)

FT-IR (KBr) $\nu_{cm^{-1}}$ 3340 (O-H stretching) 2958, 2936, 2868 (C-H stretching)

^{1}H NMR ($CDCl_3$)(δ ppm) (500 MHz) 5.37-5.34 (*m*, 1H), 5.15 (*dd*, *J* = 12.3 and 6.7 Hz, 1H), 5.03 (*dd*, *J* = 12.3 and 6.7 Hz, 1H), 3.56-3.48 (*m*, 1H), 2.29 (*ddd*, *J* = 12.3, 6.0 and 2.1 Hz, 1H), 2.24 (*qd*, *J* = 10.8 and 2.1 Hz, 1H), 2.09-1.94 (*m*, 3H), 1.88-1.80 (*m*, 2H), 1.75-1.66 (*m*, 1H), 1.60-1.39 (*m*, 10H), 1.31-1.04 (*m*, 5H), 1.02 (*d*, *J* = 6.7 Hz, 3H), 1.01 (*s*, 3H), 1.00-0.90 (*m*, 2H), 0.85 (*d*, *J* = 6.7 Hz, 3H), 0.81 (*t*, *J* = 7.1 Hz, 3H), 0.80 (*d*, *J* = 6.5 Hz, 3H), 0.70 (*s*, 3H)

^{13}C NMR($CDCl_3$)(δ ppm) (125 MHz)		140.79, 138.33, 129.34, 121.72, 71.83, 56.91, 56.02, 51.27, 50.22, 42.36, 42.26, 40.49, 39.74, 37.31, 36.55, 31.95, 31.94, 31.91, 31.71, 28.92, 25.42, 24.40, 21.24, 21.11, 21.08, 19.42, 19.01, 12.26, 12.08
DEPT 135°	CH_3 :	21.24, 21.08, 19.42, 19.01, 12.26, 12.08
	CH_2 :	39.74, 37.31, 31.95, 31.91, 31.71, 28.92, 25.42, 24.40, 21.11
	CH :	138.33, 129.34, 121.72, 71.83, 56.91, 56.02, 51.27, 50.22, 42.36, 40.49, 31.94
MS(*m/z*)(% rel. int.)		412 (33), 351 (22), 300 (21), 271 (37), 255 (50), 213 (33), 163 (30), 159 (53), 151 (29), 147 (47), 145 (52), 135 (39), 133 (56), 131 (37), 123 (49), 121 (46), 119 (49), 109 (50), 107 (60), 105 (58), 97 (65), 95 (65), 93 (61), 91 (64), 83 (76), 81 (74), 79 (64), 69 (80), 67 (66), 57 (64), 55 (91), 43 (100), 41 (85), 29 (66), 28 (74), 27 (50)

Subfraction E2.2, upon standing at room temperature, afforded a white solid (0.010 g) which was shown to be identical to **AH8** by TLC. Furthermore **AH8** was also a major component of the filtrate according to its TLC.

Subfractions E2.3-E2.4 contained two major spots on TLC with R_f values of 0.45 and 0.32 with dichloromethane as a mobile phase. These spots became purple in ASA reagent. Upon preparative TLC on silica gel plates, using dichloromethane as a mobile phase (twice), two bands were obtained.

Band 1 was a pale yellow viscous-liquid (0.026 g). Its TLC was identical to the original subfraction.

Band 2 was a pale yellow viscous-liquid (0.038 g) with only one spot on TLC with R_f value of 0.32 with dichloromethane as a mobile phase. However, its ^1H NMR spectra in $CDCl_3$ solution revealed the presence of impurities.

Thus, the residues (0.254 g) was therefore acetylated using acetic anhydride (10 mL) and pyridine. The reaction mixture was stirred at room temperature overnight. After working up, a yellow viscous-liquid (0.220 g) was obtained. Further separation was performed by column chromatography on silica gel. Elution was conducted initially with petroleum ether, followed by increasing amount of dichloromethane in petroleum ether and finally with 50% dichloromethane in petroleum ether. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford subfractions I and II.

Subfraction I was a yellow viscous-liquid (0.138 g). It contained one major spot (R_f 0.56) according to its TLC with 30% dichloromethane in petroleum ether as a mobile phase. This compound appeared as a purple spot after dipping TLC in ASA reagent and subsequent heating. Further chromatography by preparative TLC on silica gel plates, using 30% dichloromethane in petroleum ether as a mobile phase gave a pale yellow viscous-liquid (0.017 g). The ^1H NMR spectra was recorded in $CDCl_3$ solution. Base on ^1H NMR spectra data, it still consisted of the same undesired compound as found in the original subfraction. No further investigation was pursued.

Subfraction II was a yellow viscous-liquid (0.025 g). TLC chromatogram was the same as subfraction I. Therefore further purification was not introduced.

Subfractions E2.5-E2.6 contained many spots without major component. No further separation was conducted.

Fraction E3 showed one major compound on TLC, using chloroform as a mobile phase, with R_f value of 0.25 as a purple spot in ASA reagent. Further chromatography

by preparative TLC on silica gel plates with chloroform as a mobile phase gave two isolated bands.

Band 1 was an orange viscous-liquid (0.055 g). It had the same TLC chromatogram as Band 2.

Band 2 was a yellow viscous-liquid (0.075 g). It had two major spots on reversed-phase TLC, using 90% methanol in water as a mobile phase. Further separation by column chromatography on reversed-phase silica gel with 90% methanol in water as a mobile phase afforded six subfractions after combination of subfractions which had similar TLC chromatograms, as shown in **Table 6**.

Table 6 Subfractions obtained from Band 2 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E3.21	0.001	yellow viscous-liquid
E3.22	0.002	yellow viscous-liquid
E3.23	0.003	yellow viscous-liquid
E3.24	0.042	yellow viscous-liquid
E3.25	0.002	yellow viscous-liquid
E3.26	0.024	yellow viscous-liquid

Subfractions E3.21-E3.23 were combined based on their TLC chromatograms which showed only one yellow spot with R_f value of 0.23, using chloroform as a mobile phase. The combined subfraction was rechromatographed by preparative TLC on silica gel with chloroform as a mobile phase (twice) to give a pure compound (**E3.2m-1**) as a yellow viscous-liquid (0.0008 g). It was not investigated further because it was obtained in low quantity.

Subfractions E3.24-E3.26 contained one major purple spot on TLC with dichloromethane as a mobile phase with R_f value of 0.25 in ASA reagent. Their ^1H NMR spectra showed the interesting signals of two olefinic protons at δ_H 5.13 and 5.09 ppm and one oxymethine proton at δ_H 3.20 ppm. However, it was obtained in low quantity. Therefore no attempts were made for further purification.

Fraction E4 was expected to contain **E3.2m-1**. Further separation by column chromatography on silica gel was performed. Elution was conducted initially with chloroform, gradually enriched with methanol and finally with 60% methanol in chloroform. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford seven subfractions, as shown in **Table 7**.

Table 7 Subfractions obtained from fraction E4 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E4.1	0.041	yellow viscous-liquid
E4.2	0.264	yellow viscous-liquid
E4.3	0.013	yellow viscous-liquid
E4.4	0.475	yellow viscous-liquid
E4.5	0.321	yellow viscous-liquid
E4.6	0.132	yellow viscous-liquid
E4.7	0.015	yellow viscous-liquid

All subfractions were not further purified because their TLC revealed the absence of **E3.2m-1**.

Fraction E5 contained two purple spots on TLC with dichloromethane as a mobile phase in ASA reagent with R_f values of 0.23 and 0.12. Further separation by column chromatography on silica gel was performed. Elution was conducted initially with petroleum ether gradually enriched with dichloromethane, followed by increasing amount of ethyl acetate in dichloromethane and methanol in ethyl acetate and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford thirteen subfractions, as shown in **Table 8.**

Table 8 Subfractions obtained from fraction E5 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E5.1	0.007	white solid
E5.2	0.316	yellow viscous-liquid
E5.3	0.056	yellow viscous-liquid
E5.4	0.035	yellow viscous-liquid
E5.5	0.014	yellow viscous-liquid
E5.6	0.028	orange viscous-liquid
E5.7	0.052	red-orange viscous-liquid
E5.8	0.017	yellow viscous-liquid
E5.9	0.009	yellow viscous-liquid
E5.10	0.010	yellow viscous-liquid
E5.11	0.013	yellow viscous-liquid
E5.12	0.027	orange-yellow viscous-liquid
E5.13	0.026	orange-yellow viscous-liquid

Subfraction E5.1 contained many spots, none of which were major components. Therefore it was not investigated further.

Subfraction E5.2-E5.3 contained many spots on TLC. These spots were not well-separated. Further purification was therefore not attempted.

Subfractions E5.4-E5.13 showed no definite spot on TLC. No further investigation was pursued.

Fraction E6 was separated into two parts: a white solid (E6S) and a yellow solution (E6L), upon standing at room temperature.

Subfraction E6S was a white solid (0.005 g). It was chromatographed on TLC with 5% ethyl acetate in dichloromethane as a mobile phase (twice). It showed one major UV-active spot with R_f value of 0.13. Two additional spots were observed in ASA reagent at higher R_f value. The ^{1}H NMR spectra data indicated the presence of two olefinic protons at δ_H 6.90 and 5.31 ppm and one oxymethine proton at δ_H 3.20 ppm.

Subfraction E6L was separated by column chromatography on silica gel. Elution was conducted initially with petroleum ether gradually enriched with dichloromethane, followed by increasing amount of ethyl acetate in dichloromethane and methanol in ethyl acetate and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness *in vacuo* to afford subfractions E6.1-E6.14, as shown in **Table 9**.

Table 9 Subfractions obtained from subfraction E6L by column chromatography on sililca gel

subfraction	weight (g)	physical appearance
E6.1	0.009	yellow viscous-liquid
E6.2	0.001	yellow viscous-liquid

Table 9 (Continued)

subfraction	weight (g)	physical appearance
E6.3	0.003	yellow viscous-liquid
E6.4	0.002	yellow viscous-liquid
E6.5	0.004	yellow viscous-liquid
E6.6	0.007	orange viscous-liquid
E6.7	0.048	orange viscous-liquid
E6.8	0.244	yellow viscous-liquid
E6.9	0.183	yellow viscous-liquid
E6.10	0.015	yellow viscous-liquid
E6.11	0.052	orange viscous-liquid
E6.12	0.118	orange viscous-liquid
E6.13	0.061	yellow viscous-liquid
E6.14	0.013	yellow viscous-liquid

Subfractions E6.1-E6.6 showed no definite spot on TLC, it was then not investigated further.

Subfraction E6.7 contained many spots on TLC which overlaped each other. Therefore purification was not performed.

Subfraction E6.8 contained one major spot on reversed-phase TLC, using methanol as a mobile phase. It appeared as a yellow spot with R_f value of 0.70. Further separation by column chromatogaphy was performed on reversed-phase silica gel, eluting with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford ten subfractions, as shown in **Table 10**.

Table 10 Subfractions obtained from subfraction E6.8 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E6.8.1	0.001	yellow viscous-liquid
E6.8.2	0.001	yellow viscous-liquid
E6.8.3	0.119	yellow viscous-liquid
E6.8.4	0.021	yellow viscous-liquid
E6.8.5	0.008	yellow viscous-liquid
E6.8.6	0.056	yellow viscous-liquid
E6.8.7	0.024	yellow viscous-liquid
E6.8.8	0.003	yellow viscous-liquid
E6.8.9	0.004	yellow viscous-liquid
E6.8.10	0.016	yellow viscous-liquid

Subfractions E6.8.1-E6.8.2 showed no definite spot on TLC, it was then not investigated further.

Subfraction E6.8.3 contained a major yellow spot with R_f value of 0.70 on reversed-phase TLC with methanol as a mobile phase. Therefore it was rechromatographed by column chromatography over reversed-phase silica gel, eluting with methanol to give a yellow compound. Finally purification by preparative TLC on silica gel plates with 5% methanol in chloroform (twice) gave a yellow viscous-liquid (0.015 g). The ^{1}H NMR spectra data revealed the presence of three chelated hydroxyl groups and four aromatic proton signals in different integral ratio, indicating that it was not a pure compound.

Subfractions E6.8.4-E6.8.10 showed no definite spot on TLC. It was then not investigated further.

Subfraction E6.9 contained on TLC many spots which overlaped each other.

Subfractions E6.10-E6.14 showed no definite spot on TLC. It was not investigated further.

Fraction E7 contained the same constituents as fraction E6. So further purification was not carried out.

Fraction E8 contained one yellow major spot with R_f value of 0.44 on reversed-phase TLC with 34% methanol in water as a mobile phase. Further separation by column chromatography on reversed-phase silica gel was performed. Elution was conducted initially with 34% methanol in water, followed by decreasing amount of water and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 11**.

Table 11 Subfractions obtained from fraction E8 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E8.1	0.015	dark yellow viscous- liquid
E8.2	0.103	dark yellow viscous-liquid
E8.3	0.003	dark yellow viscous-liquid
E8.4	0.066	dark yellow viscous-liquid
E8.5	0.022	yellow viscous-liquid

Subfraction E8.1 showed no definite spot on TLC. It was not investigated further.

Subfraction E8.2 was rechromatographed by preparative TLC on silica gel, using ethyl acetate as a mobile phase to give two isolated bands.

The first band was a dark yellow viscous-liquid (0.022 g). It was further purified by preparative TLC on silica gel plate, using 10% methanol in chloroform as a mobile phase to give a yellow viscous-liquid (0.007 g) which gave a yellow spot with R_f value of 0.22. The ^1H NMR spectra data showed five aromatic proton signals together with undesired signals with low intensity.

The second band was a dark yellow viscous-liquid (0.009 g) and had the same major compound as the first band. Further purification was not carried out.

Subfractions E8.3-E8.5 showed no definite spot on TLC. It was then not investigated further.

Fractions E9-E11 showed no definite spot on TLC. It was then not investigated further.

The Second Investigation

The crude methanol extract (100.00 g) was partitioned with ethyl acetate and water to give two isolated fractions. ET1, ethyl acetate soluble fraction, was a red-brown powder (42.63 g) while AQ1, aqueous soluble fraction, was a brown powder (56.10 g). Both fractions, upon chromatographed on TLC with chloroform and 5% methanol in chloroform, showed the same chromatograms as ET and AQ fractions obtained from the first investigation. Further separation of ET1 (20.47 g) was carried out by quick column chromatography on silica gel, eluting with various proportions of dichloromethane in petroleum ether, ethyl acetate in dichloromethane, followed by increasing amount of methanol in ethyl acetate and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford ten fractions, as shown in **Table 12.**

Table 12 Fractions obtained from fraction ET1 by quick column chromatography on silica gel

fraction	weight (g)	physical appearance
E′1	0.084	yellow liquid
E′2	0.677	dark yellow viscous-liquid
E′3	5.731	yellow viscous-liquid
E′4	1.705	yellow viscous-liquid
E′5	0.339	dark yellow viscous-liquid
E′6	0.676	dark yellow viscous-liquid
E′7	0.671	dark yellow viscous-liquid
E′8	3.179	brown powder
E′9	3.961	brown powder
E′10	1.691	brown powder

Fraction E′1 showed no definite spot on TLC. Therefore it was not investigated further.

Fraction E′2 showed two major spots on reversed-phase TLC with methanol. Further separation by column chromatography on reversed-phase silica gel was carried out. Elution was conducted initially with 50% methanol in water, followed by increasing amount of methanol in water and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford seven subfractions, as shown in **Table 13**.

Table 13 Subfractions obtained from fraction E′2 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E′2.1	0.008	pale yellow viscous-liquid
E′2.2	0.010	yellow viscous-liquid
E′2.3	0.024	yellow viscous-liquid
E′2.4	0.093	yellow viscous-liquid
E′2.5	0.436	yellow viscous-liquid
E′2.6	0.048	yellow viscous-liquid
E′2.7	0.011	yellow viscous-liquid

Subfractions E′2.1-E′2.4 showed no definite spot on TLC. Investigation was then not further carried out.

Subfraction E′2.5 showed two major purple spots on TLC with chloroform in ASA reagent with R_f values of 0.26 and 0.20. Further chromatography by column chromatography on silica gel was performed. Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with 5% methanol in chloroform. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 14**.

Table 14 Subfractions obtained from subfraction E′2.5 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E′2.51	0.028	yellow viscous-liquid
E′2.52	0.040	yellow viscous-liquid
E′2.53	0.274	pale yellow viscous-liquid
E′2.54	0.044	pale yellow viscous-liquid
E′2.55	0.019	yellow viscous-liquid

Subfraction E′2.51 showed no definite spot on TLC and was not investigated further.

Subfraction E′2.52 was rechromatographed by preparative TLC on silica gel plates with chloroform to give **AH1** as a pale yellow viscous- liquid (0.008 g). It showed a single purple spot on TLC with chloroform in ASA reagent with R_f value of 0.26. Its ^{1}H NMR spectrum showed two sets of the oxymethine and methyl proton signals in a ratio of three to two, indicating the presence of two components of triterpene.

Subfraction E′2.53 showed two major spots on TLC with chloroform which were visualized as purple spots in ASA reagent with R_f values of 0.26 and 0.20. Further separation by column chromatography on silica gel, using chloroform as eluent was carried out. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford subfractions E′2.531-E′2.533.

Subfraction E′2.531 was a colorless viscous-liquid (0.018 g). It was the unseparable **AH1** by comparison of R_f value and the ^{1}H NMR spectra data.

Subfraction E′2.532 was a colorless viscous-liquid (0.161 g). It showed the same chromatogram as its original. It was separated further by column chromatography on silica gel, using chloroform as eluent to give subfractions ERa-ERc.

Subfraction ERa was a colorless viscous-liquid (0.007 g). It was the unseparable **AH1**.

Subfraction ERb was a colorless viscous-liquid (0.087 g). It was further purified by preparative TLC on silica gel plates with chloroform to give two isolated bands. The first band (0.006 g) was the unseparable **AH1**. The second band was a colorless viscous-liquid (0.028 g). It showed a single spot on TLC with chloroform which was visualized as a purple spot in ASA reagent with R_f value of 0.20. It was named as **AH2**. The presence of two signals of the oxymethine protons at δ_H 3.44 and δ_H 3.39 ppm in a ratio of five to one, apart from the characteristic signals of triterpenoid at high field, revealed that **AH2** was also a mixture of two triterpenes.

Subfraction ERc was the unseparable **AH2** (0.067 g).

Subfraction E′2.533 was combined with subfraction ERc to give a colorless viscous-liquid (0.154 g). Further purification by preparative TLC on silica gel plates using chloroform as eluent was carried out to give two isolated bands. The first band (0.025 g) was the unseparable **AH1** while the second band (0.009 g) was the unseparable **AH2**, according to their ^{1}H NMR spectra.

Subfraction E′2.54 was therefore rechromatographed by preparative TLC on silica gel plates using chloroform as a mobile phase to give the unseparable **AH2** as a pale yellow viscous-liquid (0.026 g).

Subfraction E′2.55 showed no definite spot on TLC and was not investigated further.

Subfractions E′2.6-E′2.7 showed no definite spot on TLC and then investigation was not further carried out.

Fraction E′3 showed a major spot on TLC with dichloromethane with R_f value of 0.13. This fraction (2.892 g) was fractionated by column chromatography on silica gel. Elution was conducted initially with dichloromethane gradually enriched with ethyl acetate, followed by increasing amount of methanol in ethyl acetate and finally with 2% methanol in ethyl acetate. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford seven subfractions, as shown in **Table 15.**

Table 15 Subfractions obtained from fraction E′3 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E′3.1	0.002	pale yellow viscous-liquid
E′3.2	0.267	orange viscous-liquid
E′3.3	0.149	orange viscous-liquid
E′3.4	0.162	orange viscous-liquid
E′3.5	1.585	dark yellow viscous-liquid
E′3.6	0.358	dark yellow viscous-liquid
E′3.7	0.007	pale yellow viscous-liquid

Subfraction E′3.1 showed no definite spot on TLC and was not investigated further.

Subfraction E′3.2-E′3.2 were mixture of the unseparable **AH1** and **AH2**.

Subfraction E′3.4-E′3.5 showed a single major spot on reversed-phase TLC with 85% methanol in water (twice) with R_f value of 0.34 which was visualized as a

purple spot in ASA reagent. Therefore subfraction E′3.5 was separated by column chromatography on reversed-phase silica gel, eluting with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford seven subfractions, as shown in **Table 16**.

Table 16 Subfractions obtained from subfraction E′3.5 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E′3.51	0.003	pale yellow viscous-liquid
E′3.52	0.012	yellow viscous-liquid
E′3.53	0.412	yellow viscous-liquid
E′3.54	1.002	yellow viscous-liquid
E′3.55	0.045	pale green viscous-liquid
E′3.56	0.023	green viscous-liquid
E′3.57	0.010	colorless viscous-liquid

Subfractions E′3.51-E′3.52 showed no definite spot on TLC and was not investigated further.

Subfraction E′3.53 showed a single major spot which was well-separated from baseline on TLC with 50% diethyl ether in petroleum ether with R_f value of 0.34. This subfraction (0.250 g) was further purified by preparative TLC on silica gel plates using 50% diethyl ether in petroleum ether as a mobile phase to give **AH3** (0.064 g) as white needles, melting at 57.9-59.2°C.

$[\alpha]_D^{29}$	$+3.19^o$	(c = 1.56 x 10^{-1} g/ 100 cm^3, MeOH)
UV(MeOH) λ_{max} nm (log ε)		211 (3.26)
FT-IR(KBr) $\nu_{cm^{-1}}$		3408 (O-H stretching) 2929, 2867 (C-H stretching)
^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz)		5.13 (*qt*, *J* = 7.2 and 1.4 Hz, 1H), 5.09 (*ht*, *J* = 6.8 and 1.4 Hz, 1H), 3.21 (*dd*, *J* = 9.8 and 6.4 Hz, 1H), 2.11-2.02 (*m*, 4H), 2.01-1.95(*m*, 2H), 1.89-1.77 (*m*, 3H), 1.69-1.67 (*m*, 1H), 1.68 (*d*, *J* = 1.4 Hz, 3H), 1.67-1.63 (*m*, 2H), 1.62 (*d*, *J* = 1.4 Hz, 3H), 1.62-1.60 (*m*, 1H), 1.60 (*d*, *J* = 1.4 Hz, 3H), 1.59-1.45 (*m*, 4H), 1.38-1.32 (*m*, 1H), 1.22-1.20 (*m*, 1H), 1.21 (*s*, 3H), 1.06 (*dt*, *J* = 11.9 and 6.4 Hz, 1H), 0.98 (*s*, 3H), 0.94 (*s*, 3H), 0.84 (*s*, 3H), 0.78 (*s*, 3H), 0.76 (*dd*, *J* = 12.6 and 2.8 Hz, 1H)
^{13}C NMR($CDCl_3$)(δ ppm) (100 MHz)		135.19, 131.35, 124.52, 124.26, 79.10, 76.01, 60.04, 58.45, 55.56, 44.47, 39.69, 39.12, 38.69, 37.76, 36.88, 28.04, 27.27, 26.64, 26.48, 25.67, 24.27, 22.74, 21.32, 19.52, 17.67, 16.35, 16.01, 15.23
DEPT 90^o	CH :	124.52, 124.26, 79.10, 60.04, 58.45, 55.56
DEPT 135^o	CH_3 :	28.04, 26.48, 25.67, 17.67, 16.35, 16.01, 15.23
	CH_2 :	39.69, 39.12, 37.76, 27.27, 26.64, 24.27, 22.74, 21.32, 19.52
	CH :	124.52, 124.26, 79.10, 60.04, 58.45, 55.56

In addition, this subfraction (0.100 g) was dissolved in acetic anhydride (10 mL) in the presence of a catalytic amount of pyridine. The reaction mixture was stirred at room temperature overnight. Then the reaction mixture was poured into ice-water and the aqueous solution was extracted with ethyl acetate (3x15 mL). The combined ethyl acetate extracts were washed with 10% HCl, 10% $NaHCO_3$ and water,

respectively, and then dried over anhydrous sodium sulphate. After removal of ethyl acetate solvent, the acetate derivative (**AH3-Ac**) was obtained as a colorless viscous-liquid (0.024 g).

$[\alpha]_D^{29}$ +23.81° (c = 4.20x10^{-2} g/ 100 cm^3, MeOH)

UV(MeOH) λ_{max} nm (log ε) 210 (3.25)

FT-IR(neat) $\nu_{cm^{-1}}$ 3475 (O-H stretching) 2966, 2941, 2873 (C-H stretching) 1732 (C=O stretching)

^{1}H NMR($CDCl_3$)(δ ppm) (500 MHz) 5.13 (*qt*, *J* = 7.2 and 1.3 Hz, 1H), 5.09 (*ht*, *J* = 6.7 and 1.3 Hz, 1H), 4.49 (*dd*, *J* = 9.4 and 7.2 Hz, 1H), 2.10-2.03 (*m*, 2H), 2.04 (*s*, 3H), 2.03-1.97 (*m*, 2H), 1.88-1.77 (*m*, 3H), 1.71-1.66 (*m*, 1H), 1.68 (*d*, *J* = 1.3 Hz, 3H), 1.67-1.63 (*m*, 2H), 1.62 (*d*, *J* = 1.3 Hz, 3H), 1.60 (*s*, 3H), 1.57-1.52 (*m*, 4H), 1.52-1.45 (*m*, 3H), 1.38-1.32 (*m*, 1H), 1.27 (*dd*, *J* = 13.0 and 5.3 Hz, 1H), 1.19 (*s*, 3H), 1.18-1.10 (*m*, 1H), 0.95 (*s*, 3H), 0.88 (*dd*, *J* = 12.9 and 2.7 Hz, 1H), 0.87 (*s*, 3H), 0.86 (*s*, 6H)

^{13}C NMR($CDCl_3$)(δ ppm) (125 MHz) 171.20, 135.28, 131.39, 124.57, 124.33, 81.05, 76.05, 59.85, 58.68, 55.70, 44.56, 39.75, 38.83, 37.72, 37.69, 36.87, 28.06, 26.72, 26.48, 25.71, 24.28, 23.63, 22.77, 21.36, 21.29, 19.44, 17.71, 16.44, 16.38, 16.07

DEPT

CH_3 : 28.06, 26.48, 25.71, 21.29, 17.71, 16.44, 16.38, 16.07

CH_2 : 39.75, 38.83, 37.72, 26.72, 24.28, 23.63, 22.77, 21.36, 19.44

CH : 124.57, 124.33, 81.05, 59.85, 58.68, 55.70

Subfraction E′3.54 was crystallized from methanol to give **AH3** as white needles (0.026 g) and the mother liquor (0.976 g) containing major **AH3** which was not further investigated.

Subfraction E′3.55 contained **AH3** as a major component and was not further purified.

Subfractions E′3.56-E′3.57 showed no definite spot on TLC and was not investigated further.

Subfraction E′3.6 was separated by column chromatography on reversed-phase silica gel, eluting with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford eight subfractions, as shown in **Table 17.**

Table 17 Subfractions obtained from subfraction E′3.6 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E′3.61	0.005	white powder
E′3.62	0.057	yellow viscous-liquid
E′3.63	0.017	pale yellow viscous-liquid
E′3.64	0.065	pale yellow viscous-liquid
E′3.65	0.092	yellow viscous-liquid
E′3.66	0.040	white needles
E′3.67	0.009	white powder
E′3.68	0.016	pale yellow viscous-liquid

Subfractions E′3.61-E′3.63 showed no definite spot on TLC and was not investigated further.

Subfraction E′3.64 consisted of **AH4** as major component separated from subfraction E′3.65. Therefore purification was not carried out.

Subfraction E′3.65 was crystallized from methanol to give **AH4** as white needles (0.020 g), melting at 120.1-120.5°C. It showed a single UV-active spot on reversed-phase TLC with 90% methanol in water with R_f value of 0.19.

$[\alpha]_D^{29}$	+11.72° (c = 1.28 x 10^{-1} g/ 100 cm^3, MeOH)
UV(MeOH) λ_{max} nm (log ε)	218 (3.99)
FT-IR(KBr) $\nu_{cm^{-1}}$	3600-2500 (O-H stretching) 2967, 2952, 2871 (C-H stretching) 1735, 1683 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz)	6.90 (*qt*, *J* = 7.4 and 1.4 Hz, 1H), 5.34-5.29 (*m*, 1H), 4.49 (*dd*, *J* = 9.6 and 6.4 Hz, 1H), 2.32-2.20 (*m*, 1H), 2.16-2.07 (*m*, 1H), 2.05 (*s*, 3H), 1.99-1.92 (*m*, 1H), 1.90-1.84 (*m*, 1H), 1.84 (*d*, *J* = 1.4 Hz, 3H), 1.80-1.70 (*m*, 4H), 1.70-1.66 (*m*, 2H), 1.66-1.60 (*m*, 1H), 1.60-1.55 (*m*, 2H), 1.55-1.50 (*m*, 3H), 1.49-1.40 (*m*, 2H), 1.28-1.14 (*m*, 4H), 1.06 (*s*, 3H), 0.98 (*s*, 3H), 0.95 (*J* = 6.6 Hz, 3H), 0.93 (*s*, 3H), 0.90 (*s*, 3H), 0.84 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ ppm) (100 MHz)	173.24, 171.11, 148.52, 145.70, 126.67, 120.05, 81.16, 51.97, 49.30, 45.49, 45.27, 38.51, 37.63, 37.49, 35.99, 34.72, 32.46, 31.56, 30.02, 28.38, 26.88, 26.76, 26.35, 25.67, 24.78, 22.69, 21.29, 19.80, 18.15, 17.98, 16.83, 11.97
DEPT 90°	CH : 145.70, 120.05, 81.16, 49.30, 45.49, 45.27, 37.49

DEPT 135° CH_3 : 28.38, 26.35, 22.69, 21.29, 19.80, 18.15, 16.83, 11.97

CH_2 : 34.72, 32.46, 31.56, 30.02, 26.88, 26.76, 25.67, 24.78, 17.98

CH : 145.70, 120.05, 81.16, 49.30, 45.49, 45.27, 37.49

Subfraction E′3.66 was **AH4**.

Subfractions E′3.67-E′3.68 showed no definite spot on TLC and was not investigated further.

Subfraction E′3.7 showed no definite spot on TLC and was not investigated further.

Fraction E′4 was fractionated by column chromatography on silica gel. Elution was conducted initially with dichloromethane gradually enriched with ethyl acetate, followed by increasing amount of methanol in ethyl acetate and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford ten subfractions, as shown in **Table 18**.

Table 18 Subfractions obtained from fraction E′4 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E′4.1	0.004	pale yellow viscous-liquid
E′4.2	0.006	yellow viscous-liquid
E′4.3	0.094	dark yellow viscous-liquid
E′4.4	0.570	orange viscous-liquid
E′4.5	0.191	yellow viscous-liquid
E′4.6	0.193	yellow viscous-liquid

Table 18 (Continued)

subfraction	weight (g)	physical appearance
E′4.7	0.062	yellow viscous-liquid
E′4.8	0.015	yellow viscous-liquid
E′4.9	0.301	pale yellow powder
E′4.10	0.004	orange powder

Subfractions E′4.1-E′4.2 showed no definite spot on TLC and was not investigated further.

Subfraction E′4.3 was therefore rechromatographed by preparative TLC on silica gel plates, with chloroform to give three isolated bands. The first band was a yellow viscous-liquid (0.005 g) which contained two adjacent spots on TLC with chloroform with R_f values of 0.36 and 0.30. Therefore it was not further purified. The second band was a yellow viscous-liquid (0.041 g) which contained the unseparable **AH2** by comparison of its R_f value and ^{1}H NMR spectra data. The third band was a yellow powder (0.001 g) which contained a mixture of **AH2** and **E3.2m-1**.

Subfraction E′4.4 contained a single major spot on TLC with 50% diethyl ether in petroleum ether with R_f value of 0.32 which was visualized as a purple spot in ASA reagent. Therefore separation by column chromatography on silica gel, eluting with various proportions of methanol-dichloromethane and finally with methanol was carried out. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford seven subfractions, as shown in **Table 19**.

Table 19 Subfractions obtained from subfraction E′4.4 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E′4.41	0.001	pale yellow powder
E′4.42	0.001	yellow powder
E′4.43	0.004	yellow viscous-liquid
E′4.44	0.035	orange viscous-liquid
E′4.45	0.500	orange viscous-liquid
E′4.46	0.004	orange viscous-liquid
E′4.47	0.003	yellow viscous-liquid

Subfractions E′4.41-E′4.43 showed no definite spot on TLC and was not investigated further.

Subfractions E′4.44-E′4.45 showed only one major spot on TLC with 50% diethyl ether in petroleum ether which was visualized as a purple spot in ASA reagent with R_f value of 0.33. It contained **AH3** as a major component. No further purification was carried out.

Subfractions E′4.46-E′4.47 showed no definite spot on TLC and was not investigated further.

Subfractions E′4.5-E′4.6 contained **AH3** as a major spot on TLC. No further purification was performed.

Subfractions E′4.7-E′4.8 contained many spots on TLC, overlaping with others. Therefore investigation was not further carried out.

Subfraction E′4.9 was separated by column chromatography on reversed-phase silica gel using methanol as an eluent. All fractions were examined by TLC and

combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford three subfractions, as shown in **Table 20.**

Table 20 Subfractions obtained from subfraction E′4.9 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E′4.91	0.126	dark yellow viscous- liquid
E′4.92	0.126	yellow viscous-liquid
E′4.93	0.013	yellow viscous-liquid

Subfractions E′4.91-E′4.92 were rechromatographed by reversed-phase TLC with methanol to give two isolated bands. The first band was a yellow viscous-liquid (0.037 g) which contained mixture of two compounds according to its TLC. Further purification was not performed. The second band was a white powder (0.010 g) of which the ^{1}H NMR spectra demonstrated only high field signals. So it was not investigated further.

Subfraction E′4.93 showed no definite spot on TLC and was not investigated further.

Subfraction E′4.10 showed no definite spot on TLC and was not investigated further.

Fraction E′5 was fractionated by column chromatography on reversed-phase silica gel. Elution was conducted initially with 50% methanol in water, followed by increasing amount of methanol in water and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford nine subfractions, as shown in **Table 21.**

Table 21 Subfractions obtained from fraction E′5 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E′5.1	0.005	yellow viscous-liquid
E′5.2	0.004	yellow viscous-liquid
E′5.3	0.015	yellow viscous-liquid
E′5.4	0.093	yellow viscous-liquid
E′5.5	0.031	yellow viscous-liquid
E′5.6	0.118	yellow viscous-liquid
E′5.7	0.034	yellow viscous-liquid
E′5.8	0.021	yellow viscous-liquid
E′5.9	0.017	yellow viscous-liquid

Subfractions E′5.1-E′5.3 contained many spots on TLC. No further separation was carried out.

Subfractions E′5.4-E′5.5 showed many spots, overlaping with others. Therefore investigation was not further carried out.

Subfraction E′5.6 was separated by column chromatography on silica gel. Elution was conducted initially with 3% methanol in chloroform, follwed by increasing amount of methanol in chloroform and finally with 40% methanol in chloroform. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford subfractions E′5.61-E′5.610, as shown in **Table 22**.

Table 22 Subfractions obtained from subfraction E′5.6 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E′5.61	0.001	yellow viscous-liquid
E′5.62	0.004	yellow viscous-liquid
E′5.63	0.034	yellow viscous-liquid
E′5.64	0.008	yellow viscous-liquid
E′5.65	0.008	yellow viscous-liquid
E′5.66	0.023	yellow viscous-liquid
E′5.67	0.014	yellow viscous-liquid
E′5.68	0.002	yellow viscous-liquid
E′5.69	0.002	pale yellow viscous-liquid
E′5.610	0.003	pale yellow viscous-liquid

Subfractions E′5.61-E′5.62 were **AH3** by comparison of R_f value on TLC.

Subfractions E′5.63-E′5.65 were combined to give a yellow viscous-liquid (0.050 g). It was further purified by preparative TLC on silica gel plates with 3% methanol in chloroform to give **AH3** (0.018 g).

Subfractions E′5.66-E′5.610 showed no definite spot on TLC and investigation was not further carried out.

Subfractions E′5.7-E′5.9 showed no definite spot on TLC and was not investigated further.

Fraction E′6 contained three major spots on TLC with 2% methanol in dichloromethane which appeared as yellow spot with R_f value of 0.17 and as two purple spots in ASA reagent with R_f values of 0.30 and 0.24. Further separation by

column chromatography on silica gel was carried out. Elution was conducted initially with dichloromethane gradually enriched with ethyl acetate, followed by increasing amount of methanol in ethyl acetate and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford eight subfractions, as shown in **Table 23**.

Table 23 Subfractions obtained from fraction E′6 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E′6.1	0.004	pale yellow viscous-liquid
E′6.2	0.027	yellow viscous-liquid
E′6.3	0.218	dark yellow viscous-liquid
E′6.4	0.046	dark yellow viscous-liquid
E′6.5	0.077	dark yellow viscous-liquid
E′6.6	0.034	orange viscous-liquid
E′6.7	0.043	brown viscous-liquid
E′6.8	0.064	brown viscous-liquid

Subfractions E′6.1-E′6.2 showed no definite spot on TLC and was not investigated further.

Subfractions E′6.3 was separated by column chromatography on reversed-phase silica gel using 90% methanol in water as an eluent. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford six subfractions, as shown in **Table 24**.

Table 24 Subfractions obtained from subfraction E′6.3 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E′6.31	0.003	yellow viscous-liquid
E′6.32	0.043	yellow viscous-liquid
E′6.33	0.065	yellow viscous-liquid
E′6.34	0.053	yellow viscous-liquid
E′6.35	0.021	yellow viscous-liquid
E′6.36	0.004	yellow viscous-liquid

Subfraction E′6.31 showed no definite spot on TLC and was not investigated further.

Subfractions E′6.32-E′6.35 were further rechromatographed on reversed-phase TLC with 80% methanol in water (4 runs) to give two isolated bands. The first band (M1) was a yellow viscous-liquid (0.074 g). Its ^{1}H NMR spectrum showed three signals of chelated hydroxyl proton in a ratio of 2:2:1, indicating that it was a mixture of at least three compounds. The second band (M2), a yellow viscous-liquid (0.034 g). It showed three spots on reversed-phase TLC with 80% methanol in water (3 runs). No further purification was further carried out.

Subfraction E′6.36 showed no definite spot on TLC and was not investigated further.

Subfraction E′6.4 contained many spots on TLC, overlaping with others. Investigation was not carried out further.

Subfractions E′6.5-E′6.8 showed no definite spot on TLC and was not investigated further.

Fraction E'7 was crystallized from methanol to give **AH5** as white needles (0.032 g), melting at 201.2-202.1°C. It showed a single spot on TLC with 5% methanol in chloroform with R_f value of 0.34 under UV light and as a purple spot in ASA reagent.

$[\alpha]_D^{29}$	-121.21°	(c = 8.25x10^{-3} g/ 100 cm^3, MeOH)
UV(MeOH) λ_{max} nm (log ε)		217 (4.10)
FT-IR(KBr) $\nu_{cm^{-1}}$		3385 (O-H stretching) 2945, 2868 (C-H stretching) 1675 (C=O stretching)
^{1}H NMR($CDCl_3$+CD_3OD)(δ ppm) (500 MHz)		6.78 (*qt* , *J* = 7.5 and 1.4 Hz, 1H), 5.28-5.25 (*m*, 1H), 3.20 (*dd*, *J* = 10.6 and 6.0 Hz, 1H), 2.30-2.20 (*m*, 2H), 2.14-2.06 (*m*, 1H), 1.99-1.93 (*m*, 1H), 1.91-1.84 (*m*, 2H), 1.82 (*d*, *J* = 1.4 Hz, 3H), 1.80-1.69 (*m*, 1H), 1.68-1.63 (*m*, 2H), 1.63-1.55 (*m*, 3H), 1.55-1.50 (*m*, 2H), 1.49-1.40 (*m*, 5H), 1.28-1.14 (*m*, 3H), 1.04 (*s*, 3H), 0.98 (*s*, 3H), 0.96 (*s*, 3H), 0.95 (*d*, *J* = 6.9 Hz, 3H), 0.91 (*s*, 3H), 0.85 (*s*, 3H)
^{13}C NMR($CDCl_3$+CD_3OD)(δ ppm) (125 MHz)		170.80, 148.50, 144.04, 127.03, 119.91, 79.18, 51.90, 49.25, 45.33, 45.20, 39.52, 37.55, 37.48, 36.02, 34.99, 32.51, 31.49, 30.19, 28.36, 28.16, 26.73, 26.70, 26.17, 25.58, 22.60, 19.85, 18.10, 18.07, 15.73, 12.12
DEPT 135°	CH_3 :	28.36, 26.17, 22.60, 19.85, 18.07, 15.73, 12.12
	CH_2 :	34.99, 32.51, 31.49, 30.19, 28.16, 26.73, 26.70, 25.58, 18.10
	CH :	144.04, 119.91, 79.18, 49.25, 45.33, 45.20, 37.48

The ^{1}H NMR spectra of **AH5** was similar to that of **AH4** except for the fact that the oxymethine proton for **AH5** appeared at δ_H 3.20 ppm (*dd*, *J* = 10.6 and 6.0 Hz, 1H) while this proton of **AH4** occurred at lower field (δ_H 4.49, *dd*, *J* = 9.6 and 6.4 Hz, 1H). In addition, only **AH4** showed the acetyl protons signal at δ_H 2.05 ppm (*s*, 3H). Therefore **AH5** (0.021 g) was dissolved in acetic anhydride (3 mL) in the presence of a catalytic amount of pyridine. The reaction mixture was stirred at room temperature overnight and then poured into ice-water. The aqueous solution was extracted with ethyl acetate (3x15 mL). The combined ethyl acetate extracts were washed with 10% HCl, 10% $NaHCO_3$ and water, respectively, and then dried over anhydrous sodium sulphate. After removal of the ethyl acetate solvent, the acetate derivative (**AH5-Ac**) was obtained as white needles (0.024 g), melting at 119-120°C.

$[\alpha]_D^{29}$	+10.58° (c = 9.45x10^{-2} g/ 100 cm^3, MeOH)
UV(MeOH) λ_{max} nm (log ε)	218 (4.07)
FT-IR(KBr) $\nu_{cm^{-1}}$	3600-2500 (O-H stretching) 2965, 2952, 2873 (C-H stretching) 1735, 1684 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz)	6.90 (*qt*, *J* = 7.4 and 1.4 Hz, 1H), 5.34-5.29 (*m*, 1H), 4.49 (*dd*, *J* = 9.6 and 6.4 Hz, 1H), 2.32-2.20 (*m*, 1H), 2.16-2.07 (*m*, 1H), 2.05 (*s*, 3H), 1.99-1.92 (*m*, 1H), 1.90-1.84 (*m*, 1H), 1.84 (*d*, *J* = 1.4 Hz, 3H), 1.80-1.70 (*m*, 4H), 1.70-1.66 (*m*, 2H), 1.66-1.60 (*m*, 1H), 1.60-1.55 (*m*, 2H), 1.55-1.50 (*m*, 3H), 1.49-1.40 (*m*, 2H), 1.28-1.14 (*m*, 4H), 1.06 (*s*, 3H), 0.98 (*s*, 3H), 0.95 (*d*, *J* = 6.6 Hz, 3H), 0.93 (*s*, 3H), 0.90 (*s*, 3H), 0.84 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ ppm) (100 MHz)	173.24, 171.11, 148.52, 145.70, 126.67, 120.05, 81.16, 51.97, 49.30, 45.49, 45.27, 38.51, 37.63, 37.49, 35.99,

		34.72, 32.46, 31.56, 30.02, 28.38, 26.88, 26.76, 26.35, 25.67, 24.78, 22.69, 21.29, 19.80, 18.15, 17.98, 16.83, 11.97
DEPT 90°	CH :	145.70, 120.05, 81.16, 49.30, 45.49, 45.27, 37.49
DEPT 135°	CH_3 :	28.38, 26.35, 22.69, 21.29, 19.80, 18.15, 16.83, 11.97
	CH_2 :	34.72, 32.46, 31.56, 30.02, 26.88, 26.76, 25.67, 24.78, 17.98
	CH :	145.70, 120.05, 81.16, 49.30, 45.49, 45.27, 37.49

Furthermore, **AH5** (0.010 g) was treated with CrO_3 (20 mg) in pyridine (2 mL) and then stirred at room temperature overnight. Upon working-up the reaction mixture in the usual manner and purification by preparative TLC on silica gel plate (2% methanol in chloroform), the oxidized product was obtained as a colorless solid (0.002 g). It showed the same R_f value, melting point and ^1H NMR spectral data as **AH10.**

The mother liquor (0.639 g) was further separated by column chromatography on silica gel. Elution was conducted initially with dichloromethane gradually enriched with ethyl acetate, followed by increasing amount of methanol in ethyl acetate and finally with methanol. All fractions were evaporated to dryness under reduced pressure to afford eight subfractions, as shown in **Table 25**.

Table 25 Subfractions obtained from mother liquor of fraction E′7 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E′7.1	0.005	yellow viscous-liquid
E′7.2	0.004	yellow viscous-liquid

Table 25 (Continued)

subfraction	weight (g)	physical appearance
E′7.3	0.184	orange viscous-liquid
E′7.4	0.028	orange viscous-liquid
E′7.5	0.078	brown viscous-liquid
E′7.6	0.038	brown viscous-liquid
E′7.7	0.039	brown viscous-liquid
E′7.8	0.015	brown viscous-liquid

Subfractions E′7.1-E′7.2 showed two UV-active spots on TLC with 5% methanol in dichloromethane with R_f values of 0.77 and 0.67. It was not further purified because it was obtained in low quantity.

Subfraction E′7.3 was further rechromatographed by column chromatography on reversed-phase silica gel. Elution was conducted initially with 50% methanol in water, followed by increasing amount of methanol in water and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford subfractions A-I.

Subfraction A showed no definite spot on TLC and was not investigated further.

Subfraction B contained four spots on TLC. Because of low quantity, it was not further separated.

Subfraction C (0.020 g) contained three spots on TLC with 15% methanol in chloroform. No further purification was carried out.

Subfractions D-E showed many spots on TLC with 15% methanol in chloroform. Further investigation was not carried out.

Subfractions F-I showed no definite spot on TLC and investigation was not carried out further.

Subfractions E′7.4-E′7.8 showed no definite spot on TLC and was not investigated further.

Fraction E′8 contained only one major yellow spot on TLC with 5% methanol in ethyl acetate with R_f value of 0.52. Therefore it was fractionated by column chromatography on silica gel. Elution was conducted initially with dichloromethane followed by increasing amount of methanol in dichloromethane and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford seven subfractions, as shown in **Table 26**.

Table 26 Subfractions obtained from fraction E′8 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E′8.1	0.024	yellow viscous-liquid
E′8.2	0.207	dark yellow viscous-liquid
E′8.3	0.174	brown viscous-liquid
E′8.4	0.283	brown viscous-liquid
E′8.5	0.582	brown viscous-liquid
E′8.6	0.368	brown viscous-liquid
E′8.7	0.273	brown viscous-liquid

Subfraction E′8.1 contained three spots on TLC with 10% methanol in chloroform with R_f values of 0.81, 0.73 and 0.65. Therefore purification was not carried out.

Subfraction E′8.2-E′8.3 showed no definite spot on TLC and was not investigated further.

Subfraction E′8.4 was separated by column chromatography on reversed-phase silica gel. Elution was conducted initially with 35% methanol in water, followed by increasing amount of methanol in water and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford fifteen subfractions, as shown in **Table 27**.

Table 27 Subfractions obtained from subfraction E′8.4 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E′8.41	0.004	yellow viscous-liquid
E′8.42	0.025	brown powder
E′8.43	0.042	brown powder
E′8.44	0.009	brown powder
E′8.45	0.056	brown powder
E′8.46	0.007	dark yellow viscous-liquid
E′8.47	0.007	dark yellow viscous-liquid
E′8.48	0.004	dark yellow viscous-liquid
E′8.49	0.003	dark yellow viscous-liquid
E′8.410	0.010	yellow viscous-liquid
E′8.411	0.014	yellow viscous-liquid
E′8.412	0.022	yellow viscous-liquid

Table 27 (Continued)

subfraction	weight (g)	physical appearance
E′8.413	0.019	yellow viscous-liquid
E′8.414	0.034	yellow viscous-liquid
E′8.415	0.022	yellow viscous-liquid

Subfraction E′8.41 showed no definite spot on TLC and it was not investigated further.

Subfraction E′8.42 contained **AH6** as a major component.

Subfraction E′8.43-E′8.44 was crystallized from methanol to give **AH6** as a brown powder (0.040 g), melting at 234.6-237.0°C. It showed a single yellow spot on TLC with 20% methanol in chloroform with R_f value of 0.34.

$[\alpha]_D^{29}$ -41.67° (c = 6.00x10^{-2} g/ 100 cm^3, MeOH)

UV(MeOH) λ_{max}nm (log ε) 281 (3.59), 215 (4.34)

FT-IR(KBr) $\nu_{cm^{-1}}$ 3279 (O-H stretching) 2929 (C-H stretching) 1627 (C=C stretching)

^{1}H NMR($CDCl_3$+DMSO-d_6)(δppm) (400 MHz) 8.73 (*s*, 1H), 8.60 (*s*, 1H), 8.35 (*brs*, 1H), 8.17 (*brs*, 1H), 7.00 (*d*, *J* = 1.6 Hz, 1H), 6.79 (*d*, *J* = 8.0 Hz, 1H), 6.77 (*dd*, *J* = 8.0 and 1.6 Hz, 1H), 6.01 (*d*, *J* = 2.4 Hz, 1H), 5.90 (*d*, *J* = 2.4 Hz, 1H), 4.80 (*s*, 1H), 4.15 (*d*, *J* = 3.2 Hz, 1H), 3.71 (*brs*, 1H), 2.81 (*dd*, *J* = 16.8 and 4.4 Hz, 1H), 2.71 (*dd*, *J* = 16.8 and 3.2 Hz, 1H)

^{13}C NMR($CDCl_3$+DMSO-d_6)(δppm) (100 MHz) 156.63, 156.41, 155.74, 144.60, 144.48, 130.60, 118.04, 115.03, 114.45, 98.53, 95.68, 94.66, 78.22, 65.67, 28.14

DEPT 135° CH_2 : 28.14

CH : 118.04, 115.03, 114.45, 95.68, 94.66, 78.22, 65.67

MS(*m/z*)(% rel.int.) 290 ($[M]^+$, 21), 272 (6), 271 (4), 186 (3), 172 (3), 167 (4), 163 (4), 152 (40), 143 (21), 139 (100), 129 (6), 124 (13), 123 (41), 111 (7), 101 (10), 87 (8), 77 (10), 69 (18), 59 (6), 55 (10), 46 (24), 45 (65), 43 (47), 42 (23), 39 (9)

Subfraction E′8.45 contained **AH6** as a major component and was not further purified.

Subfractions E′8.46-E′8.415 showed no definite spot on TLC and investigation was not carried out further.

Subfraction E′8.5 was separated by column chromatography on reversed-phase silica gel. Elution was conducted initially with 50% methanol in water, followed by increasing amount of methanol in water and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford ten subfractions, as shown in **Table 28.**

Table 28 Subfractions obtained from subfraction E′8.5 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
E′8.51	0.016	brown viscous-liquid
E′8.52	0.161	brown viscous-liquid
E′8.53	0.085	brown viscous-liquid
E′8.54	0.087	brown viscous-liquid

Table 28 (Continued)

subfraction	weight (g)	physical appearance
E′8.55	0.018	brown viscous-liquid
E′8.56	0.018	brown viscous-liquid
E′8.57	0.012	brown powder
E′8.58	0.012	brown powder
E′8.59	0.030	brown powder
E′8.510	0.115	brown viscous-liquid

Subfractions E′8.51-E′8.52 showed no definite spot on TLC and investigation was not further carried out.

Subfraction E′8.53 was further rechromatographed by preparative TLC on silica gel plates using 25% methanol in chloroform as eluent (twice) to give **AH6** (0.003 g).

Subfraction E′8.54 contained **AH6** as a major component. No further purification was carried out.

Subfractions E′8.55-E′8.510 showed no definite spot on TLC and investigation was not further carried out.

Subfractions E′8.6-E′8.7 were combined to give a brown powder (0.641 g). Most of constituents of these combined subfractions remained at baseline on TLC with 10% methanol in chloroform. Thus it was tested for their solubility in various solvents at room temperature, as shown in **Table 29**.

Table 29 Solubility of the combined subfractions E′8.6-E′8.7 in various solvents at room temperature

solvent	solubility at room temperature
petroleum ether	-
dichloromethane	-
diethyl ether	+ (pale yellow solution)
ethyl acetate	+ (yellow solution)
acetone	++ (dark yellow solution)
methanol	+++ (red brown solution)
water	+ (pale yellow solution)
10% HCl	+ (pale yellow solution)
10% $NaHCO_3$	++ (red-orange solution)
10% NaOH	++ (red-orange solution)

symbol meaning: - insoluble, +slightly soluble, ++ moderately soluble, +++ well soluble

The above data indicated that most of the constituents were acids with high polarity. In addition, the IR spectra showed strong signal of hydroxyl group. Two following pathways were therefore employed for separation of these combined subfractions.

Pathway A : The combined subfraction (0.300 g) was separated by column chromatography on reversed-phase silica gel. Elution was conducted initially with 40% methanol in water, followed by increasing amount of methanol in water and finally with methanol. All fractions were examined by TLC and combined on the basis

of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford eight subfractions, as shown in **Table 30**.

Table 30 Subfractions obtained from the combined subfractions E′8.6-E′8.7 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
P1	0.004	yellow viscous-liquid
P2	0.028	brown viscous-liquid
P3	0.033	brown viscous-liquid
P4	0.050	brown viscous-liquid
P5	0.020	brown viscous-liquid
P6	0.010	orange viscous-liquid
P7	0.020	orange viscous-liquid
P8	0.010	orange viscous-liquid

All subfractions (P1-P8) showed no definite spot on TLC. No further investigation was not then carried out.

Pathway B : The combined subfraction (0.100 g) was dissolved in acetic anhydride (10 mL) in the presence of a catalytic amount of pyridine. The reaction mixture was stirred at room temperature for four days. The reaction mixture was poured into ice-water and the aqueous solution was extracted with ethyl acetate (3x25 mL). The combined ethyl acetate extract was washed with water and dried over anhydrous sodium sulphate. After removal of the ethyl acetate solvent, the acetate derivative was obtained as an orange viscous-liquid (0.127 g). Further separation by column chromatography on silica gel with various proportions of dichloromethane-petroleum ether, followed by increasing amount of methanol in dichloromethane and

finally with methanol was carried out. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford subfractions N1-N3. All subfractions (N1-N3) showed no definite spot on TLC. No further investigation was carried out.

From the above unsuccessful results, the combined fraction was not investigated further.

Fraction E′9 was fractionated by column chromatography on silica gel. Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with methanol. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness *in vacuo* to afford nine subfractions, as shown in **Table 31.**

Table 31 Subfractions obtained from fraction E′9 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
E′9.1	0.013	yellow viscous-liquid
E′9.2	0.068	yellow viscous-liquid
E′9.3	0.154	yellow viscous-liquid
E′9.4	0.373	brown viscous-liquid
E′9.5	0.335	brown viscous-liquid
E′9.6	0.804	brown viscous-liquid
E′9.7	0.280	brown viscous-liquid
E′9.8	0.557	brown viscous-liquid
E′9.9	0.612	brown viscous-liquid

Subfraction E′9.1 contained three spots on TLC with 5% methanol in chloroform (twice) with R_f values of 0.62, 0.55 and 0.34 which were visualized as purple spots in ASA reagent. Further it was not investigated.

Subfraction E′9.2 showed the same chromatograms as subfraction E′9.3. No separation was further carried out.

Subfraction E′9.3 was crystallized from methanol to give **AH9** as a white powder (0.009 g), melting at 269-270°C. It showed a single spot on TLC with 10% methanol in chloroform with R_f value of 0.20 which was visualized as a purple spot in ASA reagent.

$[\alpha]_D^{29}$	-120.00° (c = 1.20x10^{-2} g/ 100 cm^3, MeOH)
UV(MeOH) λ_{max} nm	206
FT-IR(KBr) $\nu_{cm^{-1}}$	3403 (O-H stretching) 2955, 2934, 2873 (C-H stretching) 1072, 1024 (C-O stretching)
^{1}H NMR($CDCl_3$+CD_3OD)(δ ppm) (500 MHz)	5.39-5.37 (*m*), 5.17 (*dd*, *J* = 15.1 and 8.6 Hz), 5.04 (*dd*, *J* = 15.1 and 8.6 Hz), 4.42 (*d*, *J* = 7.5 Hz), 3.86 (*dd*, *J* = 12.0 and 2.2 Hz), 3.77 (*dd*, *J* = 12.0 and 4.6 Hz), 3.58-3.55 (*m*), 3.47-3.44 (*m*), 3.33-3.28 (*m*), 3.28-3.23 (*m*), 2.41 (*ddd*, *J* = 13.5, 5.0 and 2.5 Hz), 2.32-2.24 (*m*), 2.09-1.96 (*m*), 1.95-1.82 (*m*), 1.72-1.40 (*m*), 1.40-1.06 (*m*), 1.04 (*d*, *J* = 6.5 Hz), 1.02 (*s*), 0.94 (*d*, *J* = 6.5 Hz), 0.72 (*s*), 0.70 (*s*)

Subfractions E′9.4-E′9.9 showed no definite spot on TLC and investigation was not carried out further.

Fraction E′10 showed no definite spot on TLC and was not investigated further.

The Third Investigation

The crude extract (200.00 g) was first fractionated by quick column chromatography on silica gel. Elution was conducted initially with petroleum ether gradually enriched with dichloromethane, followed by increasing amount of ethyl acetate in dichloromethane, methanol in ethyl acetate and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford ten fractions, as shown in **Table 32**.

Table 32 Fractions obtained from the crude extract by quick column chromatography on silica gel

fraction	weight (g)	physical appearance
K1	0.048	yellow viscous-liquid
K2	1.004	orange-yellow viscous-liquid
K3	3.186	orange-yellow viscous-liquid
K4	9.814	orange-yellow viscous-liquid
K5	10.221	brown-yellow viscous-liquid
K6	3.355	brown powder
K7	10.844	brown powder
K8	40.174	red-brown powder
K9	51.065	red-brown powder
K10	28.545	red-brown powder

Fraction K1 contained many spots on TLC without major component. No further separation was conducted.

Fraction K2 showed two major purple spots on TLC with 50% chloroform in petroleum ether as a mobile phase (twice) in ASA reagent with R_f values of 0.41 and 0.27. Further separation by column chromatography on silica gel was then performed. Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with 1% methanol in chloroform. Fractions with the similar TLC chromatograms were combined and evaporated to dryness *in vacuo* to afford subfractions K2.1-K2.5.

Subfraction K2.1 was a yellow viscous-liquid (0.021 g) which contained many spots without major component. Further separation was not carried out.

Subfraction K2.2 was a yellow viscous-liquid (0.073 g). It showed the same chromatogram as subfraction K4.31.

Subfractions K2.3-K2.4 were yellow viscous-liquids (0.125 and 0.629 g, respectively) and showed the same TLC as subfractions K4.32 and K5.2.

Subfraction K2.5 was a yellow viscous-liquid (0.105 g) which contained one major purple spot on TLC with chloroform as a mobile phase in ASA reagent with R_f value of 0.26. It was shown to be identical to **AH2** by TLC chromatogram.

Fraction K3 was separated by column chromatography on silica gel, eluting with proportions of methanol-chloroform and finally with 5% methanol in chloroform. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford seven subfractions, as shown in **Table 33**.

Table 33 Subfractions obtained from fraction K3 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
K3.1	0.017	yellow viscous-liquid
K3.2	0.213	yellow viscous-liquid

Table 33 (Continued)

subfraction	weight (g)	physical appearance
K3.3	0.049	orange-yellow viscous-liquid
K3.4	0.073	orange-yellow viscous-liquid
K3.5	1.082	yellow viscous-liquid
K3.6	1.731	yellow viscous-liquid
K3.7	0.028	yellow viscous-liquid

Subfraction K3.1 contained **AH1** as a major component. It was combined with subfractions K67.16-K67.17 for further investigation.

Subfraction K3.2 was found to contain a mixture of **AH1** and **AH2.** Therefore it was separated by column chromatography on silica gel into four subfractions.

Subfraction K3.21 showed no definite spot on TLC.

Subfraction K3.22 (0.039 g) was the unseparable **AH1** which was combined with subfractions K67.16-K67.17 for further investigation.

Subfraction K3.23 showed the same TLC as the original subfraction.

Subfraction K3.24 (0.090 g) was shown to be identical to **AH2** by TLC.

Subfraction K3.3 contained the unseparable **AH2.** Further purification was not then performed.

Subfraction K3.4 was a mixture of **AH2** and **AH3.** No further purification was attempted.

Subfractions K3.5-K3.6 contained **AH3** as a major components. No further purification was then carried out.

Subfraction K3.7 showed no definite spot on TLC. No further investigation was conducted.

Fraction K4 was separated by column chromatography on silica gel. Elution was conducted initially with petroleum ether gradually enriched with dichloromethane, followed by increasing amount of ethyl acetate in dichloromethane, methanol in ethyl acetate and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford twelve subfractions, as shown in **Table 34**.

Table 34 Subfractions obtained from fraction K4 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
K4.1	0.055	orange viscous-liquid
K4.2	0.093	orange viscous-liquid
K4.3	0.459	orange-yellow viscous-liquid
K4.4	0.512	orange-yellow viscous-liquid
K4.5	0.195	orange-yellow viscous-liquid
K4.6	3.903	orange viscous-liquid
K4.7	1.054	dark yellow viscous-liquid
K4.8	0.934	dark yellow viscous-liquid
K4.9	0.109	dark yellow viscous-liquid
K4.10	0.262	yellow viscous-liquid
K4.11	0.878	yellow viscous-liquid
K4.12	0.620	brown-yellow viscous-liquid

Subfractions K4.1-K4.2 contained many purple spots on TLC in ASA reagent without major component. Further separation was not then carried out.

Subfraction K4.3 contained two major purple spots on TLC with chloroform as a mobile phase in ASA reagent with R_f values of 0.32 and 0.28. Further it was then separated by column chromatography on silica gel, using chloroform as an eluent. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 35.**

Table 35 Subfractions obtained from subfraction K4.3 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
K4.31	0.026	yellow viscous-liquid
K4.32	0.117	yellow viscous-liquid
K4.33	0.194	yellow viscous-liquid
K4.34	0.088	yellow viscous-liquid
K4.35	0.014	yellow viscous-liquid

Subfraction K4.31 was combined with subfraction K2.2 to give a yellow viscous-liquid (0.099 g). Further separation by column chromatography on reversed-phase silica gel using methanol as an eluent was then performed. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford five subfractions (Ka1-Ka5) which showed the same TLC. The ^{1}H NMR spectra of some of these subfractions indicated that none of subfractions contained pure compound. All subfractions were then recombined and converted to the acetate derivative, using acetic anhydride (5 mL) in the presence of a catalytic amount of pyridine. After work up and purification of the acetate derivatives by column chromatography, all fractions were shown to be a mixture of acetate derivatives according to their ^{1}H NMR spectra. No attempted purification were further performed.

Subfraction K4.32 contained two major spots on TLC with chloroform with R_f values of 0.33 and 0.28 which was visualized as purple spots in ASA reagent. It had the same TLC as subfraction K5.2. It was then examined together with subfraction K5.2.

Subfractions K4.33-K4.35 contained one major spot. It was identical to **AH2** according to its TLC chromatogram.

Subfraction K4.4 was separated by column chromatography on silica gel, eluting with a pure dichlorometane. Fractions with the similar TLC chromatograms were combined and evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 36.**

Table 36 Subfractions obtained from subfraction K4.4 by column chlomatography on silica gel

subfraction	weight (g)	physical appearance
K4.41	0.006	yellow viscous-liquid
K4.42	0.018	yellow viscous-liquid
K4.43	0.399	yellow viscous-liquid
K4.44	0.056	dark yellow viscous-liquid

Subfractions K4.41-K4.42 showed no definite spot on TLC. They were then not carried out further.

Subfractions K4.43 were combined because of their similar TLC chromatograms which showed only one major spot with R_f value of 0.30, using a pure chloroform as a mobile phase in ASA reagent. Further these subfractions were therefore combined and then chromatographed by column chromatography on reversed-phase silica gel, eluting with methanol. Fractions with the similar TLC

chromatograms were combined and evaporated to dryness *in vacuo* to afford six subfractions, as shown in **Table 37.**

Table 37 Subfractions obtained from subfraction K4.43 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
KE1	0.009	yellow viscous-liquid
KE2	0.185	colorless viscous-liquid
KE3	0.042	colorless viscous-liquid
KE4	0.024	colorless viscous-liquid
KE5	0.020	colorless viscous-liquid
KE6	0.023	colorless viscous-liquid

Subfraction KE1 showed no definite spot on TLC.

Subfraction KE2 contained one purple spot on reversed-phase TLC with methanol with R_f value of 0.30 in ASA reagent. It was named as **AH2.**

$[\alpha]_D^{29}$ +60.61° (c = 1.65x10^{-2} g/ 100 cm^3, MeOH)

UV(MeOH) λ_{max} nm (log ε) 213 (3.43)

FT-IR (neat) $\nu_{cm^{-1}}$ 3420 (O-H stretching) 2953, 2928, 2868 (C-H stretching) 1640 (C=C stretching)

^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz) 5.13 (*sxt*, *J* = 6.9 and 1.4 Hz, 1H), 5.10 (*ht*, *J* = 6.9 and 1.4 Hz, 1H), 4.85 (*q*, *J* = 1.2 Hz, 1H), 4.62 (*s*, 1H), 3.25 (*dd*, *J* = 11.4 and 5.1 Hz, 1H), 2.20-2.11 (*m*, 1H), 2.10-1.93 (*m*, 8H), 1.91-1.81 (*m*, 1H), 1.79-1.72 (*m*, 1H), 1.68 (*d*, *J* = 1.4 Hz, 3H), 1.68-1.60 (*m*, 2H), 1.61 (*d*, *J* = 1.4

		Hz, 3H), 1.60 (*d*, *J* = 1.4 Hz, 3H), 1.60-1.49 (*m*, 5H), 1.49-1.37 (*m*, 3H), 1.30-1.20 (*m*, 2H), 1.10 (*s*, 3H), 0.97 (*s*, 3H), 0.94 (*s*, 3H), 0.77 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ ppm) (100 MHz)		155.05, 135.09, 131.26, 124.32, 124.09, 109.09, 79.49, 56.37, 52.19, 46.64, 44.74, 39.66, 39.19, 39.00, 35.20, 34.20, 31.86, 29.62, 29.08, 29.05, 28.31, 26.67, 26.46, 25.69, 23.06, 20.69, 18.51, 17.67, 15.98, 15.80
DEPT 90°	CH :	124.32, 124.09, 79.49, 56.37, 52.19, 46.64
DEPT 135°	CH_3 :	29.62, 29.05, 25.69, 23.06, 17.67, 15.98, 15.80
	CH_2 :	109.09, 39.66, 39.19, 34.20, 31.86, 29.08, 28.31, 26.67, 26.46, 20.69, 18.51
	CH :	124.32, 124.09, 79.49, 56.37, 52.19, 46.64

Subfractions KE3-KE6 contained **AH2** as major component. No further purification was then performed.

Subfraction K4.44 showed no definite spot on TLC and it was then not investigated further.

Subfractions K4.5-K4.6 contained one major spot on TLC. It was shown to be identical to **AH3** by TLC chromatograms. Further purification was not then conducted.

Subfraction K4.7 was chromatographed by column chromatography on silica gel. Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with 50% methanol in chloroform. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford eleven subfractions, as shown in **Table 38**.

Table 38 Subfractions obtained from subfraction K4.7 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
K4.71	0.001	yellow viscous-liquid
K4.72	0.161	yellow viscous-liquid
K4.73	0.235	yellow viscous-liquid
K4.74	0.038	yellow viscous-liquid
K4.75	0.073	yellow viscous-liquid
K4.76	0.057	yellow viscous-liquid
K4.77	0.188	yellow viscous-liquid
K4.78	0.042	yellow viscous-liquid
K4.79	0.010	yellow viscous-liquid
K4.710	0.019	yellow viscous-liquid
K4.711	0.022	orange viscous-liquid

Subfraction K4.71 showed no definite spot on TLC.

Subfractions K4.72-K4.73 contained one major spot on TLC, using 1% methanol in chloroform as a mobile phase, which was visualized as a purple spot in ASA reagent with R_f value of 0.49, indicating the presence of **AH3**. Further purifica tion was not then performed.

Subfractions K4.74-K4.76 showed the same TLC as subfraction K4.77. No further investigation was then carried out.

Subfraction K4.77 contained two yellow spots on reversed-phase TLC with 75% methanol in water as a mobile phase (10 runs) with R_f values of 0.40 and 0.32. Further separation by column chromatography on reversed-phase silica gel, using 75% methanol in water as an eluent was performed. Fractions with the similar TLC

chromatograms were combined and evaporated to dryness *in vacuo* to afford fourteen subfractions, as shown in **Table 39**.

Table 39 Subfractions obtained from subfraction K4.77 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
K4.771	0.005	yellow viscous-liquid
K4.772	0.002	yellow viscous-liquid
K4.773	0.003	yellow viscous-liquid
K4.774	0.006	yellow viscous-liquid
K4.775	0.043	yellow solid
K4.776	0.001	yellow viscous-liquid
K4.777	0.001	yellow viscous-liquid
K4.778	0.003	yellow viscous-liquid
K4.779	0.003	yellow viscous-liquid
K4.7710	0.004	yellow viscous-liquid
K4.7711	0.046	yellow solid
K4.7712	0.024	yellow viscous-liquid
K4.7713	0.011	yellow viscous-liquid
K4.7714	0.003	yellow viscous-liquid

Subfractions K4.771-K4.774 contained two spots on TLC which was **AH7** as a major component. No further purification was then conducted.

Subfraction K4.775 showed only one yellow spot on reversed-phase TLC with 75% methanol in water (twice) as a mobile phase with R_f value of

0.16. It was the same compound as E'6.3 from the second investigation. It was named as **AH7**, melting at 159.3-161.0°C.

UV(MeOH) λ_{max} nm (log ε)		300 (4.15), 218 (4.20)
FT-IR(KBr) ν_{cm}-1		3423 (O-H stretching) 2966, 2929 (C-H stretching) 1631 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz)		13.60 (*s*, 1H), 6.85 (*brs*, 1H), 6.46 (*s*, 1H), 6.13 (*s*, 1H), 5.98 (*d*, *J* = 2.6 Hz, 1H), 5.85 (*brs*, 2H), 5.74 (*d*, *J* = 2.6 Hz, 1H), 5.13 (*mt*, *J* = 6.6 Hz, 2H), 5.00 (*mt*, *J* = 6.6 Hz, 2H), 3.19 (*d*, *J* = 6.6 Hz, 4H), 2.06-1.98 (*m*, 4H), 1.97-1.91 (*m*, 4H), 1.66 (*s*, 6H), 1.61 (*s*, 6H), 1.58 (*s*, 6H)
^{13}C NMR($CDCl_3$)(δ ppm) (100 MHz)		198.40, 166.66, 165.10, 161.14, 155.14, 139.60, 139.20, 132.07, 123.72, 120.69, 115.60, 106.48, 105.90, 96.67, 96.13, 39.50, 26.40, 26.18, 25.60, 17.64, 15.98
DEPT 135°	CH_3 :	25.60, 17.64, 15.98
	CH_2 :	39.50, 26.40, 26.18
	CH :	123.72, 120.69, 106.48, 96.67, 96.13

Subfractions K4.776-K4.779 contained two yellow spots on reversed-phase TLC with 75% methanol in water as a mobile phase (twice) with R_f values of 0.16 and 0.08 which were a mixture of **AH7** and compound from subfractions K4.7710-K4.7712.

Subfractions K4.7710-K4.7712 showed only one yellow spot on reversed-phase TLC with 75% methanol in water as a mobile phase with R_f value of 0.08. The ^{1}H NMR spectra indicated that this subfraction was a mixture of two compounds in the ratio of two to one which showed the similar signals as **AH7**.

^{1}H NMR($CDCl_3$)(δ ppm) (500 MHz)	13.84 (*s*, 2H), 13.50 (*s*, 1H), 6.50 (*brs*, 2H), 6.42 (*s*, 3H), 6.08 (*s*, 1H), 6.01 (*s*, 1H), 5.88 (*brs*, 2H), 5.82 (*brs*, 4H), 5.76 (*s*, 2H), 5.29 (*mt*, J = 7.2 Hz, 2H), 5.16-5.09 (*m*, 8H), 5.01 (*mt*, J = 6.8 Hz, 4H), 3.38 (*d*, J = 7.1 Hz, 4H), 3.20 (*d*, J = 6.2 Hz, 16H), 2.05-1.98 (*m*, 8H), 1.98-1.92 (*m*, 8H), 1.83 (*s*, 6H), 1.76 (*d*, J = 1.0 Hz, 6H), 1.73 (*s*, 3H), 1.70 (*d*, J = 1.0 Hz, 3H), 1.66 (*s*, 9H), 1.63 (*s*, 6H), 1.62 (*s*, 12H), 1.60 (*s*, 3H), 1.59 (*s*, 3H), 1.57 (*s*, 6H)

Further separation was performed by column chromatography on gel using 50% methanol in chloroform as an eluent to afford eight subfractions based on their TLC chromatograms. In addition, the TLC chromatogram indicated unsuccessful separation. Thus all (0.019 g) were combined and then acetylated using acetic anhydride (5 mL) in the presence of a catalytic amount of pyridine. After working up and purification by preparative TLC on silica gel plates with 20% chloroform in petroleum ether, the acetate derivative was obtained as a yellow viscous-liquid (0.006 g). But it decomposed during the record of the ^{1}H NMR spectrum.

Subfractions K4.7713-K4.7714 showed no definite spot on TLC. It was then not investigated.

Subfractions K4.78-K4.79 contained two yellow spots which were a mixture of **AH7** and compound in subfractions K4.7710-K4.7712.

Subfraction K4.710 contained two major yellow spots on reversed-phase TLC using 75% methanol in water as a mobile phase (10 runs) with R_f values of 0.55 and 0.47. It was then combined with subfraction K5.7 as they showed similar TLC chromatograms.

Subfractions K4.8-K4.9 contained many purple spots in ASA reagent without the major components according to TLC chromatogram on silica gel with 5% methanol in chloroform. Further separation was then not performed.

Subfraction K4.10 was crystallized from methanol to give **AH4** as white solid (0.080 g) and mother liquor (0.180 g) which contained **AH4** as a major component.

Subfraction K4.11 was crystallized upon standing at room temperature to afford white solid (K4.11S; 0.288 g) and mother liquor (K4.11L; 0.580 g). The solid contained two UV-active spots with R_f values of 0.46 and 0.39 on reversed-phase TLC using methanol as a mobile phase (twice). Further separation by column chromatography on reversed-phase silica gel, eluting with 90% methanol in water was then performed. Fractions with the similar TLC chromatograms were combined and evaporated to dryness *in vacuo* to afford six subfractions, as shown in **Table 40**.

Table 40 Subfractions obtained from K4.11S by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
11SA	0.090	white solid
11SB	0.052	white solid
11SC	0.026	white solid
11SD	0.042	white solid
11SE	0.060	white solid
11SF	0.021	white solid

Subfraction 11SA showed two overlapped UV-active spots on reversed-phase TLC with 80% methanol in water. Further it was then separated by column chromatography on reversed-phase silica gel eluting with 80% methanol in

water. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford subfractions Fr1-Fr3.

Subfraction Fr1 was a white solid (0.018 g) which showed the same TLC as the original subfraction. Therefore purification was performed by reversed-phase TLC with 80% methanol in water (110 runs) to give white solid (0.002 g) which showed the same TLC as the original subfraction.

Subfraction Fr2 was a white solid (0.017 g). It was then purified by reversed-phase TLC using 80% methanol in water as a mobile phase (50 runs) to afford four bands. All bands showed the same ^{1}H NMR spectra data which indicated a mixture of two compounds.

Subfraction Fr3 was a white solid (0.023 g). Its TLC and the ^{1}H NMR spectra data indicated that Fr3 was a mixture of two triterpenoids with ketone functionality at C-3 as found in **AH10**.

Subfractions 11SB-11SD were a mixture of 11SA and **AH4** by comparison of its TLC and the ^{1}H NMR spectra data. **AH4** was shown to be a major component for this mixture.

Subfractions 11SE-11SF showed only one UV-active spot on TLC. It was shown to be identical to **AH4** by its TLC chromatogram.

Subfraction K4.12 showed the similar TLC as subfraction K4.11L. Therefore they were combined and separated by column chromatography on silica gel. Elution was conducted initially with dichloromethane, followed by increasing amount of methanol in dichloromethane and finally with 80% methanol in dichloromethane. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford eight subfractions, as shown in **Table 41**.

Table 41 Subfractions obtained from subfractions K4.11L and K4.12 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
12A	0.021	yellow viscous-liquid
12B	0.289	yellow viscous-liquid
12C	0.202	yellow viscous-liquid
12D	0.057	yellow viscous-liquid
12E	0.072	yellow viscous-liquid
12F	0.072	orange viscous-liquid
12G	0.065	orange viscous-liquid
12H	0.025	orange viscous-liquid

Subfraction 12A contained many spots on TLC without major component. Further investigation was not carried out.

Subfraction 12B was crystallized upon standing at room temperature to afford a white solid (12BS; 0.161 g) and mother liquor (0.120 g) which contained 12BS residue as a major component. The 12BS residue was a mixture according to its reversed-phase TLC. It was then separated further by column chromatography on reversed-phase silica gel using 90% methanol in water as an eluent. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford fifteen subfractions, as shown in **Table 42.**

Table 42 subfractions obtained from 12BS residue by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
12BS1	0.001	colorless viscous-liquid
12BS2	0.002	colorless viscous-liquid
12BS3	0.001	colorless viscous-liquid
12BS4	0.002	colorless viscous-liquid
12BS5	0.010	white solid
12BS6	0.011	white solid
12BS7	0.032	white solid
12BS8	0.024	white solid
12BS9	0.004	white solid
12BS10	0.006	white solid
12BS11	0.016	white solid
12BS12	0.026	white solid
12BS13	0.013	white solid
12BS14	0.006	white solid
12BS15	0.003	white solid

Subfraction 12BS1 showed no definite spot on TLC.

Subfractions 12BS2-12BS4 were combined according to their TLC which showed one UV-active spot on reversed-phase TLC with pure methanol as a mobile phase (twice) with R_f value of 0.51. Its ^{1}H NMR spectrum indicated that it was not a pure compound.

Subfractions 12BS5-12BS6 contained a mixture of subfractions 12BS4 and 12BS7.

Subfractions 12BS7-12BS9 showed one UV-active spot on reversed-phase TLC with methanol (twice) with R_f value of 0.44. It also gave the same ^{1}H NMR spectra data as that of subfraction Fr3.

FT-IR(KBr) $\nu_{cm^{-1}}$	3700-2500 (O-H stretching) 2969, 2946, 2914, 2860 (C-H stretching) 1706, 1675 (C=O stretching)
^{1}H NMR($CDCl_3$)(δ ppm) (500 MHz)	6.90 (*t*, J = 7.5 Hz), 5.28 (*d*, J = 6.5 Hz), 2.71 (*ddd*, J = 15.5, 13.5 and 6.5 Hz), 2.57 (*ddd*, J = 15.5, 11.0 and 7.0 Hz), 2.43-2.36 (*m*), 2.32-2.18 (*m*), 2.16-2.00 (*m*), 2.00-1.86 (*m*), 1.83 (*s*), 1.81-1.66 (*m*), 1.65-1.50 (*m*), 1.50-1.26 (*m*), 1.21 (*s*), 1.21-1.12 (*m*), 1.10 (*s*), 1.08 (*s*), 1.06 (*s*), 1.05 (*s*), 0.93 (*d*, J = 6.0 Hz), 0.91 (*d*, J = 6.5 Hz), 0.88 (*s*), 0.73 (*s*), 0.70 (*s*), 0.66 (*s*)

Subfraction 12BS10 was the same mixture as subfractions 12BS7-12BS9 with an additional **AH4**.

Subfractions 12BS11-12BS15 were shown to be identical to **AH4** by its TLC and ^{1}H NMR spectral data.

Subfraction 12C was separated by column chromatography on reversed-phase silica gel, eluting with 90% methanol in water. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford six subfractions, as shown in **Table 43.**

Table 43 Subfractions obtained from subfraction 12C by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
12C1	0.008	yellow viscous-liquid
12C2	0.003	yellow viscous-liquid
12C3	0.002	yellow viscous-liquid
12C4	0.002	yellow viscous-liquid
12C5	0.008	yellow viscous-liquid
12C6	0.148	yellow viscous-liquid

Subfractions 12C1-12C4 showed no definite spot on TLC. No further separation was then performed.

Subfraction 12C5 showed the same TLC as subfraction 12C6.

Subfraction 12C6 was separated by column chromatography on reversed-phase silica gel, eluting with 90% methanol in water. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford nine subfractions, as shown in **Table 44.**

Table 44 Subfractions obtained from subfraction 12C6 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
12C6.81	0.001	white solid
12C6.82	0.001	white solid
12C6.83	0.002	white solid
12C6.84	0.010	white solid

Table 44 (Continued)

subfraction	weight (g)	physical appearance
12C6.85	0.029	white solid
12C6.86	0.024	white solid
12C6.87	0.016	white solid
12C6.88	0.009	white solid
12C6.89	0.019	white solid

Subfractions 12C6.81-12C6.82 showed no definite spot on TLC.

Subfractions 12C6.83-12C6.84 contained **AH10**, obtained from subfraction 12C6.85, as a major component.

Subfractions 12C6.85-12C6.86 contained one UV-active spot on reversed-phase TLC with 95% methanol in water (twice) with R_f value of 0.38. It was named as **AH10**, melting at 93.0-95.0°C.

$[\alpha]_D^{29}$ +125.00° (c = 8.00x10^{-3} g/ 100 cm^3, MeOH)

UV(MeOH) λ_{max} nm(log ε) 216 (4.07)

FT-IR(KBr) $\nu_{cm^{-1}}$ 3600-2500 (O-H stretching) 2955,2867 (C-H stretching) 1708,1680 (C=O stretching)

^{1}H NMR($CDCl_3$)(δ ppm) (500 MHz) 6.90 (*qt*, *J* = 7.5 and 1.5 Hz, 1H), 5.25-5.23 (*m*, 1H), 2.66 (*ddd*, *J* = 16.0, 11.5 and 5.5 Hz, 1H), 2.36 (*ddd*, *J* = 16.0, 9.5 and 4.0 Hz, 1H), 2.32-2.23 (*m*, 2H), 2.16-2.01 (*m*, 4H), 1.97 (*dd*, *J* = 12.0 and 2.0 Hz, 1H), 1.93 (*td*, *J* = 11.3 and 3.0 Hz, 1H), 1.90-1.86 (*m*, 1H),

		1.83 (*d*, *J* = 1.5 Hz, 3H), 1.71-1.53 (*m*, 5H), 1.49-1.42 (*m*, 2H), 1.33-1.15 (*m*, 4H), 1.08 (*s*, 3H), 1.06 (*s*, 3H), 0.99 (*s*, 3H), 0.96 (*d*, *J* = 7.0 Hz, 3H), 0.94 (*s*, 3H), 0.88 (*s*, 3H)
^{13}C NMR($CDCl_3$)(δ ppm) (125 MHz)		220.05, 173.27, 148.49, 145.63, 126.79, 116.84, 51.56, 49.79, 49.29, 47.07, 41.24, 37.43, 36.18, 36.13, 34.57, 34.06, 33.20, 31.84, 31.50, 29.01, 27.06, 25.61, 24.30, 24.10, 23.32, 22.51, 20.32, 19.80, 18.10, 11.99
DEPT 135°	CH_3 :	29.01, 24.30, 23.32, 22.51, 19.80, 18.10, 11.99
	CH_2 :	34.57, 34.06, 33.20, 31.84, 31.50, 27.06, 25.61, 24.10, 20.32
	CH :	145.63, 116.84, 49.79, 49.29, 41.24, 36.18

Subfractions 12C6.87-12C6.89 were shown to be identical to **AH10** by its TLC and the ^{1}H NMR spectra data.

Subfractions 12D-12F contained one major spot on reversed-phase TLC with methanol as a mobile phase, it was identical to **AH10**. No further purification was then performed.

Subfractions 12G-12H showed no definite spot on TLC. No further separation was then carried out.

Fraction K5 was fractionated by column chromatography on silica gel with various proportions of methanol-chloroform and finally with 80% methanol in chloroform. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford nine subfractions, as shown in **Table 45.**

Table 45 Subfractions obtained from fraction K5 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
K5.1	0.061	yellow viscous-liquid
K5.2	0.935	yellow viscous-liquid
K5.3	0.381	yellow viscous-liquid
K5.4	5.934	orange viscous-liquid
K5.5	2.002	orange viscous-liquid
K5.6	0.406	orange viscous-liquid
K5.7	0.115	orange viscous-liquid
K5.8	0.025	red-brown viscous-liquid
K5.9	0.032	red-brown viscous-liquid

Subfraction K5.1 was combined with subfraction K4.2 to give a yellow viscous-liquid (0.154 g) which contained **AH1** as a major component. Then the combined subfraction was dissolved in acetic anhydride (8 mL) in the presence of a catalytic amount of pyridine. The reaction mixture was stirred at room temperature for one day. The reaction mixture was poured into ice-water and the aqueous solution was extracted with ethyl acetate (3x20 mL). The combined ethyl acetate extracts were washed with 10% HCl, 10% $NaHCO_3$ and water, respectively, and then dried over anhydrous sodium sulphate. After removal of the ethyl acetate solvents, a pale yellow viscous- liquid was obtained in 0.167 g. Further separation was then carried out by column chromatography on silica gel, eluting with 2% ethyl acetate in petroleum ether to give a yellow viscous-liquid (0.119 g). Then it was separated into three bands by preparative TLC with 2% ethyl acetate in petroleum ether (3 runs). The first band (KD1) was a colorless-viscous liquid (0.015 g). Its ^1H NMR spectra data indicated that

KD1 was the impure compound. The second and third bands (KD2 and KD3) were colorless viscous-liquids (0.013 and 0.006 g, respectively) of which ^{1}H NMR spectra data were identical to that of KD1.

Subfraction K5.2 was combined with subfractions K2.3, K2.4 and K4.32 to give a yellow viscous-liquid (1.806 g). Further separation was performed by column chromatography on silica gel using dichloromethane as an eluent to afford three subfractions.

Subfraction KB1 was a pale yellow viscous-liquid (0.135 g) which contained unseparable **AH1**. Then KB1 was acetylated by acetic anhydride (2 mL) in the presence of a catalytic amount of pyridine. After working up, a yellow viscous-liquid was produced in 0.132 g and then was separated by column chromatography to give a colorless viscous-liquid (0.073 g). Further purification by preparative TLC on silica gel plates with 20% chloroform in petroleum ether (3 runs) was then carried out. Subfractions a-g were obtained as a colorless viscous-liquid (0.006, 0.005, 0.007, 0.004, 0.004, 0.005 and 0.014 g, respectively). All subfractions showed single purple spot on TLC with 50% chloroform in petroleum ether with R_f value of 0.42. However, the ^{1}H NMR spectra data indicated that they are a mixture. Thus subfractions a-g were then combined with subfractions KD1-KD3. A portion of these subfractions (0.030 g) was purified by preparative TLC on silica gel plates with 50% chloroform in petroleum ether (50 runs) to give a pure **AH1-1** as a colorless viscous-liquid (0.001 g). Because **AH1-1** was obtained in low quantity which was not enough for structural elucidation, **AH1** was identified as a mixture of triterpenes. In addition, its acetate derivative (**AH1-Ac**) was unstable. Decomposition was detected during TLC examination.

FT-IR(neat) $\nu_{cm^{-1}}$	3400 (O-H stretching) 2961, 2922, 2844 (C-H stretching)
^{1}H NMR($CDCl_3$)(δ ppm) (500 MHz)	5.27-5.23 (*m*, 0.8H), 5.18-5.08 (*m*, 7.2H), 4.89 (*s*, 1H), 4.62 (*s*, 1H), 3.48 (*dd*, *J* = 7.0 and 5.9 Hz, 0.8H), 3.42 (*dd*, *J* = 10.5 and 4.4 Hz, 1H), 2.35 (*td*, *J* = 13.5 and 5.0 Hz, 1H), 2.28-2.21 (*m*, 0.8H), 2.21-1.96 (*m*), 1.89-1.74 (*m*), 1.73 (*brs*, 2.4H), 1.69 (*d*, *J* = 1.0 Hz, 5.4H), 1.68-1.64 (*m*), 1.61 (*s*, 21.6H), 1.60-1.48 (*m*), 1.04 (*s*, 3H), 0.98 (*s*, 2.4H), 0.85 (*s*, 2.4H), 0.73 (*s*, 3H)

Subfraction K5.3 contained one major purple spot on TLC with chloroform with R_f value of 0.30 in ASA reagent. It was shown to be identical to **AH2** by its TLC. No further purification was then carried out.

Subfraction K5.4 contained **AH3** as a major component. Further separation was then not performed.

Subfraction K5.5 showed similar TLC to subfraction K5.6.

Subfraction K5.6 was crystallized upon standing at room temperature to afford **compound AH5** as white needles (0.060 g). The remainder (0.340 g) was separated by column chromatography on reversed-phase silica gel using 75% methanol in water as eluents. Fractions with the similar TLC chromatograms were combined and evaporated to dryness *in vacuo* to afford twelve subfractions, as shown in **Table 46.**

Table 46 Subfractions obtained from the remainder of subfraction K5.6 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
K5.61	0.034	yellow viscous-liquid
K5.62	0.017	yellow viscous-liquid
K5.63	0.021	yellow viscous-liquid
K5.64	0.006	yellow viscous-liquid
K5.65	0.009	yellow viscous-liquid
K5.66	0.007	yellow viscous-liquid
K5.67	0.009	yellow viscous-liquid
K5.68	0.020	yellow viscous-liquid
K5.69	0.011	yellow viscous-liquid
K5.610	0.005	yellow viscous-liquid
K5.611	0.006	yellow viscous-liquid
K5.612	0.009	yellow viscous-liquid

Subfractions K5.61-K5.63 showed no definite spot on TLC. No further investigation was then carried out.

Subfractions K5.64-K5.65 were shown to be identical to **AH7** by its TLC and the ^{1}H NMR spectra data.

Subfraction K5.66 was a mixture of **AH7** and compounds found in subfraction K5.67.

Subfractions K5.67-K5.69 contained one yellow spot on reversed-phase TLC with 80% methanol in water (3 runs) with R_f value of 0.33. Its ^{1}H NMR spectrum indicated that it contained the same mixture as subfractions K4.7710-K4.7712.

Subfractions K5.610-K5.612 showed no definite spot on TLC. Further investigation was then not performed.

Subfraction K5.7 was combined with subfraction K4.710 to give a yellow viscous-liquid (0.134 g). Further separation was then performed by column chromatography on reversed-phase silica gel. Elution was conducted initially with 75% methanol in water, followed by increasing amount of methanol in water and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness *in vacuo* to afford eight subfractions, as shown in **Table 47.**

Table 47 Subfractions obtained from the combined subfractions K5.7 and K4.710 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
K5.71	0.009	yellow viscous-liquid
K5.72	0.006	yellow viscous-liquid
K5.73	0.009	yellow viscous-liquid
K5.74	0.004	yellow viscous-liquid
K5.75	0.010	yellow viscous-liquid
K5.76	0.005	yellow viscous-liquid
K5.77	0.007	yellow viscous-liquid
K5.78	0.072	yellow viscous-liquid

Subfraction K5.71 showed no definite spot on TLC and was not then investigated.

Subfraction K5.72 contained the compound found in subfraction K5.73 as a major component. No further separation was then performed.

Subfraction K5.73 contained one yellow spot on reversed-phase TLC with 75% methanol in water (5 runs) with R_f value of 0.48, indicating the presence of the similar as **AH7**.

Subfractions K5.74-K5.76 were a mixture of compounds found in subfractions K5.73 and K5.75. No further purification was then carried out.

Subfractions K5.77-K5.78 showed no definite spot on TLC. No further investigation was performed.

Subfractions K5.8-K5.9 were not well-separated on TLC. Further investigation was then not carried out.

Fractions K6-K7 were combined to give a brown powder (14.199 g). Further separation by column chromatography on silica gel was performed. Elution was conducted initially with dichloromethane, followed by in creasing amount of methanol in dichloromethane and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford nine subfractions, as shown in **Table 48.**

Table 48 Subfractions obtained from the combined fractions K6-K7 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
K67.1	0.113	yellow viscous-liquid
K67.2	3.463	yellow viscous-liquid
K67.3	3.264	yellow-brown solid
K67.4	1.289	yellow-brown solid
K67.5	0.457	yellow-brown viscous-liquid

Table 48 (Continued)

subfraction	weight (g)	physical appearance
K67.6	0.395	yellow-brown solid
K67.7	0.756	brown solid
K67.8	0.508	brown solid
K67.9	0.659	brown solid

Subfraction K67.1 contained one major purple spot on TLC with 1% methanol in chloroform with R_f value of 0.50 in ASA reagent, indicating the presence of the unseparated mixture **AH1**. Further separation was then carried out by column chromatography on silica gel, eluting with a pure chloroform to afford yellow viscous-liquid (0.039 g), containing the unseparated mixture **AH1**. Then it was combined with subfractions K3.1 and K3.22 to give KG as a yellow viscous-liquid (0.095 g) and reseparated by column chromatography on reversed-phase silica gel, eluting with 95% methanol in water to afford fourteen subfractions, as shown in **Table 49**.

Table 49 Subfractions obtained from KG by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
KG1	0.001	yellow viscous-liquid
KG2	0.002	yellow viscous-liquid
KG3	0.003	yellow viscous-liquid
KG4	0.002	yellow viscous-liquid
KG5	0.002	yellow viscous-liquid
KG6	0.001	yellow viscous-liquid

Table 49 (Continued)

subfraction	weight (g)	physical appearance
KG7	0.001	yellow viscous-liquid
KG8	0.004	yellow viscous-liquid
KG9	0.003	yellow viscous-liquid
KG10	0.001	yellow viscous-liquid
KG11	0.004	yellow viscous-liquid
KG12	0.042	yellow viscous-liquid
KG13	0.040	yellow viscous-liquid
KG14	0.011	yellow viscous-liquid

Subfraction KG1 contained two yellow spots on TLC with chloroform with R_f values of 0.39 and 0.30. No further purification was performed because of a small quantity.

Subfractions KG2-KG3 were combined and then purified by preparative TLC using chloroform as a mobile phase (3 runs) to afford three bands. The first band (KG23A) was a pale yellow viscous-liquid (0.001 g) which showed one yellow spot on TLC with chloroform (twice) with R_f value of 0.49. Its ^{1}H NMR spectrum indicated that KG23A was a mixture. The second band (KG23B) was a colorless viscous-liquid (0.001 g). It showed a single UV-active spot on TLC with chloroform as a mobile phase (twice) with R_f value of 0.45. The ^{1}H NMR spectra data showed the signals of impure compound. The third band (KG23C) was a yellow solid (0.001 g) which contained one yellow spot on TLC using chloroform as a mobile phase (twice) with R_f value of 0.38. It was named as **AH11**, melting at 156.0-159.0°C.

UV(MeOH) λ_{max} nm (log ε)	375 (3.54), 322 (4.15), 260 (4.35), 254 (4.30), 245 (4.45), 209 (4.46)
FT-IR(KBr) $\nu_{cm^{-1}}$	3427 (O-H stretching) 1644 (C=O stretching) 1045, 1025 (C-O stretching)
^{1}H NMR($CDCl_3$)(δ ppm) (400 MHz)	13.20 (*s*, 1H), 7.77 (*dd*, *J* = 8.0 and 1.6 Hz, 1H), 7.30 (*dd*, *J* = 8.0 and 1.6 Hz, 1H), 7.24 (*t*, *J* = 8.0 Hz, 1H), 6.54 (*s*, 1H), 5.72 (*br*, 1H), 5.29 (*mt*, *J* = 6.8 Hz, 1H), 5.27 (*mt*, *J* = 6.6 Hz, 1H), 3.56 (*d*, *J* = 6.6 Hz, 2H), 3.49 (*d*, *J* = 6.8 Hz, 2H), 1.88 (*s*, 3H), 1.86 (*s*, 3H), 1.79 (*d*, *J* = 1.0 Hz, 3H), 1.76 (*d*, *J* = 1.0 Hz, 3H)
^{13}C NMR($CDCl_3$)(δ ppm) (125 MHz)	181.79, 161.45, 159.21, 152.90, 144.82, 144.68, 136.68, 133.86, 124.15, 122.53, 121.45, 121.19, 120.01, 117.21, 109.29, 105.62, 103.52, 25.57, 25.35, 21.76, 21.31, 17.61

Subfraction KG4 showed no definite spot on TLC. No further investigation was then carried out.

Subfractions KG5-KG7 contained one yellow spot and one purple spot on TLC with chloroform as a mobile phase (twice) with R_f values of 0.38 and 0.08, respectively. Because of a low quantity, purification was then not performed further.

Subfractions KG8-KG10 were combined based on their chromatographic data. The combined subfraction was then purified by preparative TLC on silica gel plate with chloroform as a mobile phase (3 runs) to give KG8910P as a pale yellow viscous-liquid (0.002 g). It showed a single UV-active spot on TLC with chloroform (twice) with R_f value of 0.40. Its ^{1}H NMR spectrum indicated that KG8910P was not pure.

Subfractions KG11-KG14 contained one purple spot on TLC with chloroform as a mobile phase with R_f value of 0.42 in ASA reagent. TLC and ^1H NMR spectral data indicated that it was an unseparated mixture **AH1**.

Subfraction K67.2 contained two major purple spots on TLC, indicating the presence of a mixture of **AH2** and **AH3**. No further separation was then performed.

Subfraction K67.3 contained **AH3** as a major component and then was not separated.

Subfraction K67.4 was separated by column chromatography on reversed-phase silica gel using various proportions of water-methanol and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 50**.

Table 50 Subfractions obtained from subfraction K67.4 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
K67.41	0.067	yellow viscous-liquid
K67.42	0.058	yellow viscous-liquid
K67.43	0.181	yellow viscous-liquid
K67.44	0.068	dark yellow viscous-liquid
K67.45	0.322	dark yellow viscous-liquid

Subfractions K67.41-K67.42 contained compound **AH7** as a major component. Further separation was then not carried out.

Subfraction K67.43 was separated by column chromatography on reversed-phase silica gel, eluting with 75% methanol in water. Fractions with the similar TLC chromatograms were combined and evaporated to dryness *in vacuo* to afford eleven subfractions, as shown in **Table 51.**

Table 51 Subfractions obtained from subfraction K67.43 by column chromatography on reversed-phase silica gel

subfraction	weight (g)	physical appearance
KF1	0.003	yellow viscous-liquid
KF2	0.005	yellow viscous-liquid
KF3	0.018	yellow viscous-liquid
KF4	0.030	yellow viscous-liquid
KF5	0.011	yellow viscous-liquid
KF6	0.027	yellow viscous-liquid
KF7	0.026	yellow viscous-liquid
KF8	0.014	yellow viscous-liquid
KF9	0.013	yellow viscous-liquid
KF10	0.013	yellow viscous-liquid
KF11	0.012	yellow viscous-liquid

Subfractions KF1-KF2 showed no definite spot on TLC. Further investigation was then not carried out.

Subfractions KF3-KF4 were shown to be identical to **AH7** based on its TLC and the ^{1}H NMR spectra data.

Subfraction KF5 was found to contain a mixture of **AH7** and compounds from subfraction KF6.

Subfractions KF6-KF9 contained only one yellow spot on reversed-phase TLC with 75% methanol in water with R_f value of 0.27. It was the same compounds as subfractions K4.7710-K4.7712.

Subfraction K67.44 showed similar TLC to subfraction K67.45.

Subfraction K67.45 crystallized upon standing at room temperature to afford **AH5** as white needles (0.022 g). The filtrate contained **AH5** as well as major component according to TLC.

Subfraction K67.5 showed no definite spot on TLC and then was not investigated further.

Subfraction K67.6 contained one major yellow spot on TLC with 20% methanol in chloroform with R_f value of 0.28, indicating the presence of **AH6**. Further separation was then not carried out.

Subfractions K67.7-K67.9 showed no definite spot on TLC. It was then not investigated further.

Fraction K8 contained the polar constituents because its was found near the baseline on TLC with 5% methanol in chloroform as a mobile phase. This fraction (20.065 g) was further fractionated by quick column chromatography on silica gel. Elution was conducted initially with dichloromethane, followed by increasing amount of methanol in dichloromethane and finally with 50% methanol in dichloromethane. Fractions with the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford fourteen subfractions, as shown in **Table 52.**

Table 52 Subfractions obtained from fraction K8 by quick column chromatography on silica gel

subfraction	weight (g)	physical appearance
K8.1	0.082	yellow viscous-liquid
K8.2	0.148	yellow viscous-liquid
K8.3	0.265	dark yellow viscous-liquid
K8.4	0.208	dark yellow viscous-liquid
K8.5	0.753	dark yellow viscous-liquid
K8.6	0.260	brown powder
K8.7	0.189	brown powder
K8.8	0.104	brown viscous-liquid
K8.9	0.052	brown powder
K8.10	0.354	brown powder
K8.11	1.245	brown viscous-liquid
K8.12	3.033	brown powder
K8.13	2.560	brown powder
K8.14	3.616	brown powder

Subfractions K8.1-K8.2 contained two major purple spots on TLC. It was a mixture of the unseparable **AH1** and **AH2** by comparison of their TLC chromatograms. Then separation was not carried out further.

Subfractions K8.3-K8.4 contained a mixture of **AH2** and **AH3** by comparison of TLC chromatograms.

Subfraction K8.5 was separated by column chromatography on silica gel. Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with 20% methanol in chloroform. Fractions with

the similar TLC chromatograms were combined and evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 53.**

Table 53 Subfractions obtained from subfraction K8.5 by column chromatography on silica gel

subfraction	weight (g)	physical appearance
K8.51	0.010	pale yellow viscous-liquid
K8.52	0.315	dark yellow viscous-liquid
K8.53	0.194	dark yellow viscous-liquid
K8.54	0.052	yellow viscous-liquid
K8.55	0.017	yellow viscous-liquid

Subfraction K8.51 contained an **AH2** as a major component according to its characteristic chromatogram.

Subfraction K8.52 showed two major purple spots on TLC in ASA reagent which were a mixture of **AH3** and **AH4** by TLC.

Subfraction K8.53 was found to contain the compound **AH4** as a major component by comparison of the TLC chromatogram.

Subfraction K8.54 was subjected to preparative TLC on silica gel plates using a pure chloroform as an eluent (6 runs) to give **55P** as white solid (0.005 g). It showed only one UV-active spot on TLC with 2% methanol in chloroform (2 runs) with R_f value of 0.24 which appeared as a purple spot in ASA reagent. Its ^1H NMR spectrum indicated that the compound **55P** was a mixture of two triterpenoids which showed the similar signals as **AH10** but it had the β-hydroxyl group at C-3. Because of low quantity, purification was then not attempted.

Subfraction K8.55 showed no definite spot on TLC. No further purification was conducted.

Subfractions K8.6-K8.10 were not well separated on TLC in various mobile phase systems. Further investigation was then not carried out.

Subfractions K8.11-K8.14 showed no definite spot on TLC. No further investigation was conducted.

Fraction K9 (25.09 g) was fractionated by quick column chromatography on silica gel. Elution was conducted initially with chloroform, followed by increasing amount of methanol in chloroform and finally with methanol. Fractions with the similar TLC chromatograms were combined and evaporated to dryness *in vacuo* to afford nine subfractions, as shown in **Table 54**.

Table 54 Subfractions obtained from fraction K9 by quick column chromatography on silica gel

subfraction	weight (g)	physical appearance
K9.1	0.042	pale yellow viscous-liquid
K9.2	0.178	yellow viscous-liquid
K9.3	0.037	yellow viscous-liquid
K9.4	0.037	yellow viscous-liquid
K9.5	0.061	orange-yellow viscous-liquid
K9.6	0.511	orange-yellow viscous-liquid
K9.7	0.094	brown powder
K9.8	2.443	brown powder
K9.9	3.122	brown powder

Subfraction K9.1 contained a mixture of the unseparable **AH1** and **AH2** by comparison of its TLC with **AH1** and **AH2**.

Subfraction K9.2 showed two major purple spots on TLC which were **AH2** and **AH3**. No further separation was then carried out.

Subfractions K9.3-K9.5 were not well separated on TLC. Further investigation was then not performed.

Subfractions K9.6-K9.9 showed no definite spot on TLC and then were not investigated further.

Fraction K10 showed no definite spot on TLC. Further investigation was then not carried out.

CHAPTER 3

RESULTS AND DISCUSSION

The methanol extract of the stem bark of *G. speciosa* was separated by chromatographic methods to yield five new compounds ; four triterpenoids (**AH3, AH4, AH5** and **AH10**) and one benzophenone derivative (**AH7**) together with eight known compounds ; one xanthone [8-deoxygartanin (**AH11**)], one flavonoid [(-)-epi-catechin (**AH6**)], one steroid [stigmasterol (**AH8**)], one malabaricane triterpene (**AH2**), a mixture of two triterpenoids (**AH1**) and a mixture of two steroid glucosides (**AH9**). The structures of **AH2, AH3, AH4, AH7, AH10** and **AH11** were elucidated using 1D and 2D NMR spectroscopic data. **AH5** was transformed to its monoacetate derivative (**AH5-Ac**) and then identified by comparison of its spectroscopic data with those of **AH4**. The structure elucidation of remainders was accomplished by comparison of their spectroscopic data, especially its ^{1}H NMR spectra data with known compounds. For structural elucidation, the ^{13}C NMR signals were assigned from DEPT, HMQC and HMBC spectra.

3.1 Compound AH3

Compound AH3 was obtained as white needles, melting at 57.9-59.2°C. The IR spectrum (**Figure 3.2**) exhibited an absorption band for a hydroxyl group at 3408 cm^{-1}. It also gave a positive Leibermann-Burchard test for triterpene compound. The ^{1}H NMR spectrum (**Table 55**) (**Figure 3.3**) of **AH3** indicated the presence of five tertiary methyls (δ_H 1.21, *s* ; δ_H 0.98, *s* ; δ_H 0.94, *s* ; δ_H 0.84, *s* and δ_H 0.78, *s*), three

vinylic methyls (δ_H 1.68, *d*, *J* = 1.4 Hz ; δ_H 1.62, *d*, *J* = 1.4 Hz and δ_H 1.60, *d*, *J* = 1.4 Hz) and one oxymethine proton (δ_H 3.21, *dd*, *J* = 9.8 and 6.4 Hz). These signals were regarded as being due to a tetracyclic triterpene having a 3β-hydroxyl group (Rukachaisirikul, 2000b). Furthermore, **AH3** showed the signals of two olefinic protons (δ_H 5.13, *qt*, *J* = 7.2 and 1.4 Hz and δ_H 5.09, *ht*, *J* = 6.8 and 1.4 Hz). The DEPT experiments (**Figures 3.5** and **3.6**) demonstrated that both olefinic protons belonged to two trisubstituted double bonds which were shown by the UV spectrum (**Figure 3.1**) (λ_{max} 211 nm) to be unconjugated. The ^{13}C NMR spectrum (**Table 55**) (**Figure 3.4**) exhibited seven quaternary carbons [sp^3C : δ_C 76.01, 44.47 (2xC), 38.69 and 36.88 ; sp^2C : δ_C 135.19 and 131.35], six methine carbons (sp^3C : δ_C 79.10, 60.04, 58.45 and 55.56 ; sp^2C : δ_C 124.52 and 124.26), nine methylene carbons (δ_C 39.69, 39.12, 37.76, 27.27, 26.64, 24.27, 22.74, 21.32 and 19.52) and eight methyl carbons [δ_C 28.04, 26.48, 25.67 (2xC), 17.67, 16.35, 16.01 and 15.23].

Table 55 The ^{1}H and ^{13}C NMR spectral data of compound AH3

Position	δ_H, *mult.*, *J* (Hz)	Type of Carbon	δ_C
1	1.59-1.45 (*m*, 1H) ; 1.06 (*dt*, *J* = 11.9 and 6.4 Hz, 1H)	CH_2	39.12
2	1.67-1.63 (*m*, 1H) ; 1.62-1.60 (*m*, 1H)	CH_2	27.27
3	3.21 (*dd*, *J* = 9.8 and 6.4 Hz, 1H)	CH	79.10
4	-	C	38.69
5	0.76 (*dd*, *J* = 12.6 and 2.8 Hz, 1H)	CH	55.56
6	1.67-1.63 (*m*, 1H) ; 1.59-1.45 (*m*, 1H)	CH_2	19.52
7	1.89-1.77 (*m*, 2H)	CH_2	37.76
8	-	C	44.47
9	1.22-1.20 (*m*, 1H)	CH	60.04

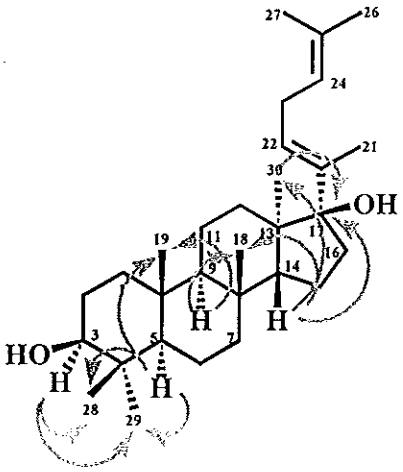
Table 55 (Continued)

Position	δ_H, *mult.*, *J* (Hz)	Type of Carbon	δ_C
10	-	C	36.88
11	1.59-1.45 (*m*, 1H) ; 1.38-1.32 (*m*, 1H)	CH_2	21.32
12	1.89-1.77 (*m*, 1H) ; 1.59-1.45 (*m*, 1H)	CH_2	24.27
13	-	C	44.47
14	1.69-1.67 (*m*, 1H)	CH	58.45
15	2.11-2.02 (*m*, 1H) ; 2.01-1.95 (*m*, 1H)	CH_2	26.64
16	2.11-2.02 (*m*, 2H)	CH_2	22.74
17	-	C	76.01
18	0.94 (*s*, 3H)	CH_3	26.48
19	0.84 (*s*, 3H)	CH_3	16.35
20	-	C	135.19
21	1.62 (*d*, *J* = 1.4 Hz, 3H)	CH_3	16.01
22	5.13 (*qt*, *J* = 7.2 and 1.4 Hz, 1H)	CH	124.52
23	2.11-2.02 (*m*, 1H) ; 2.01-1.95 (*m*, 1H)	CH_2	39.69
24	5.09 (*ht*, *J* = 6.8 and 1.4 Hz, 1H)	CH	124.26
25	-	C	131.35
26	1.68 (*d*, *J* = 1.4 Hz, 3H)	CH_3	25.67
27	1.60 (*d*, *J* = 1.4 Hz, 3H)	CH_3	17.67
28	0.78 (*s*, 3H)	CH_3	15.23
29	0.98 (*s*, 3H)	CH_3	28.04
30	1.21 (*s*, 3H)	CH_3	25.67

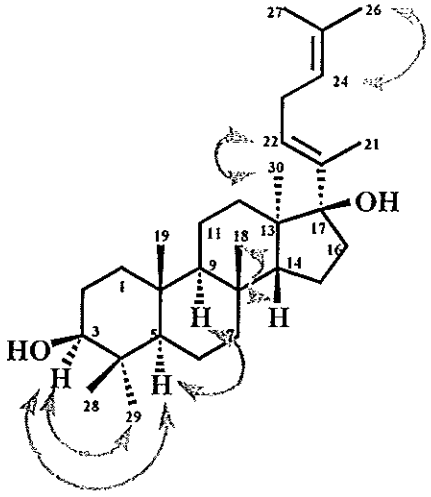
The decoupling experiments demonstrated that the irradiation of the methylene protons at δ_H 2.11-1.95 changed the splitting pattern of both olefinic protons at δ_H 5.13

from *quartet of triplet* (J = 7.2 and 1.4 Hz) to *quartet* (J = 1.4 Hz) and δ_H 5.09 from *heptet of triplet* (J = 6.8 and1.4 Hz) to *heptet* (J = 1.4 Hz). These data suggested that both olefinic protons coupled to these methylene protons with the vicinal coupling (J = 7.2 and 6.8 Hz). The structure of the side chain was then suggested to be [MeC=CHCH$_2$CH=C(Me)$_2$]. Apart from the signals of 3-oxymethine carbon (C-3, δ_C 79.10), the ^{13}C NMR and DEPT spectra displayed a signal for an oxyquaternary carbon at δ_C 76.01, indicating that **AH3** contained an additional hydroxyl group of a tertiary alcohol in the molecule. When **AH3** was subjected to an acetylation reaction, the acetylated product (**AH3-Ac**) obtained showed only one acetoxyl group (δ_H 2.04, *s*) in the ^{1}H NMR spectrum (**Figure 3.18**) and a hydroxyl group at 3475 cm^{-1} in the IR spectrum (**Figure 3.17**) together with two signals of oxycarbon atoms ; one acetoxy-methine carbon (δ_C 81.05) and one hydroxyquaternary carbon (δ_C 76.05) in the ^{13}C NMR spectrum (**Figure 3.19**). The presence of the intact hydroxyl group in the IR spectrum (**Figure 3.17**) together with the unshifted signal of oxyquaternary carbon (**Figure 3.19**) of its monoacetate derivative (**AH3-Ac**) confirmed that **AH3** carried two hydroxy groups : one for a secondary alcohol and the other for a tertiary alcohol.

The location of all tertiary methyls were established using the HMBC chain correlation data (**Table 56**) (**Figure 3.9**) starting from the observed correlations between H-3 (δ_H 3.21) and the Me-28 (δ_C 15.23) and the Me-29 (δ_C 28.04). In addition, the HMBC spectrum suggested that the hydroxyl group of the tertiary alcohol was located at the C-17 (δ_C 76.01) due to the cross peaks with the Me-30 (δ_H 1.21) and the H-14 (δ_H 1.69-1.67) as shown.



HMBC correlations



NOE correlations

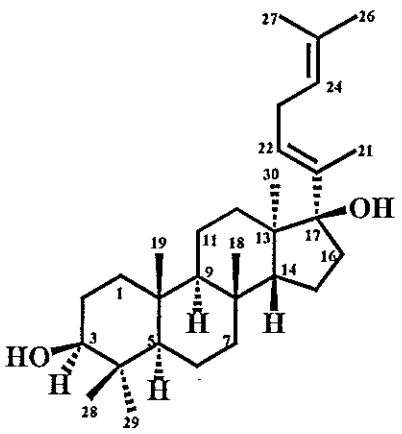
Table 56 Major HMBC correlations of compound AH3

Proton	HMBC correlation ; $\delta_C(C_n)$
H-3	38.69 (4), 28.04 (Me-29), 27.27 (2), 15.23 (Me-28)
H-5	79.10 (3), 60.04 (9), 38.69 (4), 39.12 (1), 37.76 (7), 36.88 (10), 28.04 (Me-29), 19.52 (6), 16.35 (Me-19), 15.23 (Me-28)
H-9	55.56 (5), 44.47 (8), 39.12 (1), 37.76 (7), 36.88 (10), 26.48 (Me-18), 24.27 (12), 21.32 (11), 16.35 (Me-19)
H-14	76.01 (17), 60.04 (9), 44.47 (8,13), 26.64 (15), 25.67 (Me-18, Me-30)
H-22	39.69 (23), 22.74 (16), 16.01 (Me-21)
H-24	124.52 (22), 25.67 (Me-26), 17.67 (Me-27)
Me-18	60.04 (9), 58.45 (14), 44.47 (8), 37.76 (7)

Table 56 (Continued)

Proton	HMBC correlation ; $\delta_C(C_n)$
Me-19	60.04 (9), 55.56 (5), 39.12 (1), 36.88 (10), 15.23 (Me-28)
Me-21	135.19 (20), 124.52 (22), 39.69 (23), 22.74 (16)
Me-26	131.35 (25), 124.26 (24), 17.67 (Me-27)
Me-27	131.35 (25), 124.26 (24), 25.67 (Me-26)
Me-28	79.10 (3), 55.56 (5), 38.69 (4), 28.04 (Me-29)
Me-29	79.10 (3), 55.56 (5), 38.69 (4), 15.23 (Me-28)
Me-30	76.01 (17), 58.45 (14), 24.27 (12), 22.74 (16)

The relative stereochemistry of **AH3** was established by the NOE difference results as shown above. The enhancement of the H-5 (δ_H 0.76) and Me-29 (δ_H 0.98) signals by the irradiation at δ_H 3.21 (H-3) (**Figure 3.13**) indicated that the H-5 and Me-29 were *cis* to the H-3. Additionally, only the signal of the H-9 (δ_H 1.22-1.20) was enhanced when the H-5 was irradiated (**Figure 3.10**), indicating that the H-9 and H-5 were also *cis*. The Me-19 (δ_H 0.84) was therefore *trans* to the H-5 and H-9. The irradiation at the Me-18 (δ_H 0.94) (**Figure 3.11**) enhanced the H-14 signal (δ_H 1.69-1.67) but not the H-9 indicated that the Me-18 was *cis* to H-14 but *trans* to H-9. The Me-30 was finally found to be *cis* to the side chain but *trans* to H-14 as the irradiation at Me-30 (δ_H 1.21) (**Figure 3.12**) affected the olefinic proton H-22 (δ_H 5.13) of the side chain but did not show any effect to the H-14. Furthermore, the Me-26 (δ_H 1.68) was enhanced by the irradiation at the olefinic H-24 (δ_H 5.09) (**Figure 3.14**), indicating that they were *cis*. The Me-21 (δ_H 1.62) was *trans* to the H-22 because it was not enhanced by irradiation at the H-22 (**Figure 3.15**). **AH3** was then elucidated as 3β,17β-dihydroxy-20*E*,24-apotirucalladiene (**1**), a new apotirucallane-type triterpene.



(1)

3.2 Compound AH2

Compound AH2 was obtained as a colorless viscous-liquid. It exhibited an IR absorption band at 3420 cm^{-1} (**Figure 3.22**) for a hydroxyl group while the UV spectra (**Figure 3.21**) revealed the presence of an unconjugated chromophore with a maximum absorption band at 213 nm (log ε 3.43). Additionally, it gave a positive Leibermann-Burchard test for triterpenoid compounds. The ^{1}H NMR spectrum (**Table 57**) (**Figure 3.23**) of **AH2** indicated the presence of four tertiary methyls (δ_H 1.10, *s* ; δ_H 0.97, *s* ; δ_H 0.94, *s* and δ_H 0.77, *s*), three vinylic methyls (δ_H 1.68, *d*, J = 1.4 Hz ; δ_H 1.61, *d*, J = 1.4 Hz and δ_H 1.60, *d*, J = 1.4 Hz), one oxymethine proton (δ_H 3.25, *dd*, J = 11.4 and 5.1 Hz) and four olefinic protons (δ_H 5.13, *sxt*, J = 6.9 and 1.4 Hz ; δ_H 5.10, *ht*, J = 6.9 and 1.4 Hz ; δ_H 4.85, *q*, J = 1.2 Hz and δ_H 4.62, *s*). The DEPT experiments (**Figures 3.25** and **3.26**) demonstrated that these olefinic protons belonged to two trisubstitued double bonds and one terminal disubstituted double bond which were shown by the UV spectrum to be unconjugated. The ^{13}C NMR spectrum (**Table 57**) (**Figure 3.24**) together with DEPT and HMQC spectra (**Figures 3.25, 3.26** and **3.28**) exhibited 30 resonances for 30 carbon atoms ; six quaternary carbons (δ_C 155.05, 135.09, 131.26, 44.74, 39.00 and 35.20), six methine carbons (δ_C 124.32, 124.09, 79.49, 56.37,

and 46.64), eleven methylene carbons (δ_C 109.09, 39.66, 39.19, 34.20, 31.86, 29.08, 28.31, 26.67, 26.46, 20.69 and 18.51) and seven methyl carbons (δ_C 29.62, 29.05, 25.69, 23.06, 17.67, 15.98 and 15.80).

Table 57 The NMR spectral data of compound AH2 and 13βH-malabaricatriene

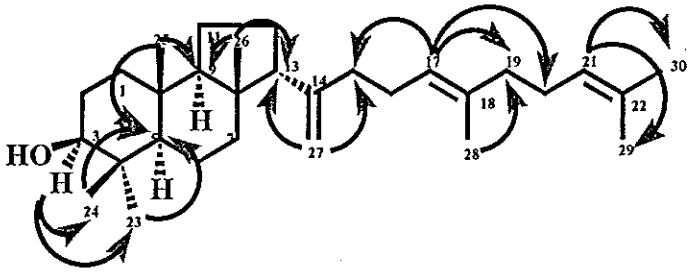
Position	AH2			13βH-malabaricatriene
	δ_H, *mult.*, *J* (Hz)	Type of Carbon	δ_C	δ_C
1	1.49-1.37 (*m*, 2H)	CH_2	34.20	40.5
2	1.79-1.72 (*m*, 1H) ; 1.68-1.60 (*m*, 1H)	CH_2	29.08	18.5
3	3.25 (*dd*, *J* = 11.4 and 5.1 Hz)	CH	79.49	-
		CH_2	-	42.5
4	-	C	39.00	33.0
5	1.60-1.49 (*m*, 1H)	CH	46.64	57.0
6	1.60-1.49 (*m*, 1H) ; 1.30-1.20 (*m*, 1H)	CH_2	18.51	19.3
7	1.68-1.60 (*m*, 1H) ; 1.30-1.20 (*m*, 1H)	CH_2	31.86	39.3
8	-	C	44.74	45.5
9	1.60-1.49 (*m*, 1H)	CH	52.19	55.6
10	-	C	35.20	36.8
11	1.60-1.49 (*m*, 1H) ; 1.49-1.37 (*m*, 1H)	CH_2	20.69	20.7
12	2.10-1.93 (*m*, 2H)	CH_2	26.67	24.9
13	2.10-1.93 (*m*, 1H)	CH	56.37	56.6
14	-	C	155.05	154.5

Table 57 (Continued)

Position	AH2			13βH-malabaricatriene
	δ_H, *mult.*, *J* (Hz)	Type of Carbon	δ_C	δ_C
15	2.10-1.93 (*m*, 1H) ; 1.91-1.81 (*m*, 1H)	CH_2	39.19	36.7
16	2.10-1.93 (*m*, 1H) ; 1.60-1.49 (*m*, 1H)	CH_2	28.31	27.8
17	5.13 (*sxt*, *J* = 6.9 and 1.4 Hz, 1H)	CH	124.09	124.3
18	-	C	135.09	135.0
19	2.10-1.93 (*m*, 2H)	CH_2	39.66	39.7
20	2.20-2.11 (*m*, 1H) ; 2.10-1.93 (*m*, 1H)	CH_2	26.46	26.9
21	5.10 (*sxt*, *J* = 6.9 and 1.4 Hz, 1H)	CH	124.32	124.4
22	-	C	131.26	131.2
23	0.97 (*s*, 3H)	CH_3	29.05	33.5
24	0.77 (*s*, 3H)	CH_3	15.80	21.4
25	0.94 (*s*, 3H)	CH_3	23.06	15.6
26	1.10 (*s*, 3H)	CH_3	29.62	26.8
27	4.85 (*q*, *J* = 1.2 Hz, 1H) ; 4.62 (*s*, 1H)	CH_2	109.09	108.7
28	1.60 (*d*, *J* = 1.4 Hz, 1H)	CH_3	15.98	16.0
29	1.61 (*d*, *J* = 1.4 Hz, 1H)	CH_3	17.67	17.7
30	1.68 (*d*, *J* = 1.4 Hz, 3H)	CH_3	25.69	25.7

Comparison of the ^{13}C NMR spectral data of **AH2** with those of 13βH-malabaricatriene obtained from *Lemmaphyllum microphyllum* var. *obovatum* showed

that their carbon signals were similar except that **AH2** contained one oxymethine carbon (δ_C 79.49). In addition, the chemical shifts of olefinic and methyl protons of **AH2** were similar to those of 13βH-malabaricatriene. These results together with the HMBC correlation data (**Table 58**) (**Figure 3.29**), as shown below, indicated that **AH2** had a malabaricatriene skeleton which is tricyclic triterpene with two six and one five member rings having one exomethylene and two trisubstituted double bonds at the side chain (Masuda, 1989).

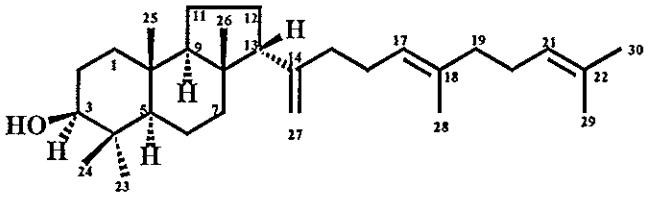


HMBC Correlations

Table 58 Major HMBC correlations of compound AH2

Proton	HMBC correlation ; δ_C (C_n)
H-3	39.00 (4), 34.20 (1), 29.05 (Me-23), 15.80 (Me-24)
H-17	39.66 (19), 39.12 (15), 26.46 (20), 15.98 (Me-28)
H-21	25.69 (Me-26), 17.67 (Me-29)
H-27	155.05 (14), 56.37 (13), 39.19 (15)
Me-23	79.49 (3), 46.64 (5), 39.00 (4), 15.80 (Me-24)
Me-24	79.49 (3), 46.64 (5), 39.00 (4), 29.06 (Me-23)
Me-25	52.19 (9), 46.64 (5), 35.20 (10), 34.20 (1)
Me-26	155.05 (14), 56.37 (13), 52.19 (9), 44.74 (8), 31.86 (7)
Me-28	135.09 (18), 124.09 (17), 39.66 (19)
Me-29	131.26 (22), 124.32 (21), 25.69 (Me-30)
Me-30	131.26 (22), 124.32 (21), 17.67 (Me-29)

The location of the hydroxyl group was also established by the HMBC correlations data to be at the C-3 since the oxymethine proton (H-3) (δ_H 3.25, *dd*, J = 11.4 and 5.1 Hz) showed cross peaks with the Me-23 (δ_C 29.05) and the Me-24 (δ_C 15.80). The splitting pattern of H-3 as *doublet of doublet* with coupling constants of 11.4 and 5.1 Hz implied that the H-3 was in the axial α position (Rukachaisirikul, 2000b ; Mata, 1991). Thus **AH2** was assigned as 3-hydroxymalabarica-14(27),17,21-triene (**2**). Although the relative stereochemistry was not yet established, **AH2** was expected to have the same stereochemistry as 13βH-malabaricatriene.



(2)

3.3 Compound AH4

Compound AH4 was obtained as white needles, melting at 120.1-120.5°C. The IR spectrum (**Figure 3.31**) showed the presence of a hydroxyl (3600-2500 cm^{-1}) and carbonyl groups of an α,β-unsaturated carboxylic acid (1708 cm^{-1}) and a saturated ester (1727 cm^{-1}). In the UV spectrum (**Figure 3.30**), a strong absorption at λ_{max} 218 nm indicated that **AH4** possessed the α,β-unsaturated carboxylic acid chromophore. The carbonyl functionalities were therefore confirmed by the carbon signals at δ_C 173.24 and 171.11 in the ^{13}C NMR data (**Table 60**) (**Figure 3.33**). **AH4** also gave a positive Leibermann-Burchard test for triterpenoid compounds. The ^1H NMR spectrum (**Table 59**) (**Figure 3.32**) demonstrated the presence of five angular methyls (δ_H 1.06, *s* ; δ_H 0.98, *s* ; δ_H 0.93, *s* ; δ_H 0.90, *s* and δ_H 0.84, *s*), one secondary methyl (δ_H 0.95, *d*, J = 6.6 Hz) and one oxymethine proton (δ_H 4.49, *dd*, J = 9.6 and 6.4 Hz). These signals

were regarded as being due to a tetracyclic triterpene (Rukachaisirikul, 2000b). The oxymethine proton appeared at lower field than that of H-3 of 3-hydroxytriterpene (Mata, 1991). The presence of the acetyl methyl protons at δ_H 2.05 together with above information indicated that **AH4** carried an acetoxy group rather than a hydroxyl group at C-3 (Mata, 1991). The splitting pattern of H-3 as *doublet of doublet* with coupling constants of 9.6 and 6.4 Hz implied that the H-3 was in the axail α position (Rukachaisirikul, 2000b). The signals of the vinylic and methyl protons at δ_H 6.90 (*qt*, J = 7.4 and 1.4 Hz) and δ_H 1.84 (*d*, J = 1.4 Hz), respectively, suggested the structure of the side chain to be [-CH(Me)CH_2CH_2CH=C(Me)COOH]. It was in agreement with the ^{1}H-^{1}H COSY (**Figure 3.36**) and HMBC correlation data (**Table 61**) (**Figure 3.38**). Therefore, the other vinylic proton at δ_H 5.34-5.29 (*m*) was expected to be in the tetracyclic system. The signals in the ^{13}C NMR spectrum (**Table 60**) (**Figure 3.33**) and DEPT experiments (**Figures 3.34** and **3.35**) indicated the presence of two carbonyl carbons (δ_C 173.24 and 171.11), six quaternary carbons (δ_C 148.52, 126.67, 51.97, 38.51, 37.63 and 35.99), seven methine carbons (δ_C 145.70, 120.05, 81.16, 49.30, 45.49, 45.27 and 37.49), nine methylene carbons (δ_C 34.72, 32.46, 31.56, 30.02, 26.88, 26.76, 25.67, 24.78 and 17.98) and eight methyl carbons (δ_C 28.38, 26.35, 22.69, 21.29, 19.80, 18.15, 16.83 and 11.97).

Table 59 The ^{1}H NMR spectral data of compounds AH4, AH5 and AH10

Position	AH4 ; δ_H, *mult.*, J(Hz)	AH5 ; δ_H, *mult.*, J(Hz)	AH10 ; δ_H, *mult.*, J(Hz)
1	1.55-1.50 (*m*, 1H) ;	1.49-1.40 (*m*, 2H)	2.16-2.01 (*m*, 1H) ;
2	1.49-1.40 (*m*, 1H) 1.70-1.66 (*m*, 2H)	1.68-1.63 (*m*, 1H) ; 1.49-1.40 (*m*, 1H)	1.71-1.53 (*m*, 1H) β : 2.66 (*ddd*, J = 16.0, 11.5 and 5.5 Hz, 1H) α : 2.36 (*ddd*, J = 16.0, 9.5 and 4.0 Hz, 1H)

Table 59 (Continued)

Position	AH4 ; δ_H, *mult.*, *J*(Hz)	AH5 ; δ_H, *mult.*, *J*(Hz)	AH10 ; δ_H, *mult.*, *J*(Hz)
3	4.49 (*dd*, *J* = 9.6 and 6.4 Hz, 1H)	3.20 (*dd*, *J* = 10.6 and 6.0 Hz, 1H)	-
5	1.66-1.60 (*m*, 1H)	1.49-1.40 (*m*, 1H)	1.97 (*dd*, *J* = 12.0 and 2.0 Hz, 1H)
6	1.60-1.55 (*m*, 2H)	1.63-1.55 (*m*, 1H) ; 1.49-1.40 (*m*, 1H)	1.49-1.42 (*m*, 1H) ; 1.33-1.15 (*m*, 1H)
7	1.90-1.84 (*m*, 1H) ; 1.28-1.14 (*m*, 1H)	1.91-1.84 (*m*, 1H) ; 1.28-1.14 (*m*, 1H)	2.16-2.01 (*m*, 1H) ; 1.33-1.15 (*m*, 1H)
9	1.80-1.70 (*m*, 1H)	1.80-1.69 (*m*, 1H)	1.93 (*td*, *J* = 11.3 and 3.0 Hz, 1H)
11	1.99-1.92 (*m*, 1H) ; 1.80-1.70 (*m*, 1H)	1.99-1.93 (*m*, 1H) ; 1.91-1.84 (*m*, 1H)	2.16-2.01 (*m*, 1H) ; 1.90-1.86 (*m*, 1H)
12	5.34-5.29 (*m*, 1H)	5.28-5.25 (*m*, 1H)	5.25-5.23 (*m*, 1H)
15	1.55-1.50 (*m*, 1H) ; 1.28-1.14 (*m*, 1H)	1.55-1.50 (*m*, 1H) ; 1.28-1.14 (*m*, 1H)	1.71-1.53 (*m*, 1H) ; 1.33-1.15 (*m*, 1H)
16	1.80-1.70 (*m*, 1H) ; 1.49-1.40 (*m*, 1H)	1.68-1.63 (*m*, 1H) ; 1.63-1.55 (*m*, 1H)	1.71-1.53 (*m*, 1H) ; 1.49-1.42 (*m*, 1H)
17	2.32-2.20 (*m*, 1H)	2.30-2.20 (*m*, 1H)	2.32-2.23 (*m*, 1H)
18	0.90 (*s*, 3H)	0.91 (*s*, 3H)	0.94 (*s*, 3H)
19	1.06 (*s*, 3H)	1.04 (*s*, 3H)	0.88 (*s*, 3H)
20	1.55-1.50 (*m*, 1H)	1.55-1.50 (*m*, 1H)	1.71-1.53 (*m*, 1H)
21	0.95 (*d*, *J* = 6.6 Hz, 3H)	0.95 (*d*, *J* = 6.9 Hz, 3H)	0.96 (*d*, *J* = 7.0 Hz, 3H)

Table 59 (Continued)

Position	AH4 ; δ_H, *mult.*, *J*(Hz)	AH5 ; δ_H, *mult.*, *J*(Hz)	AH10 ; δ_H, *mult.*, *J*(Hz)
22	1.28-1.14 (*m*, 2H)	1.63-1.55 (*m*, 1H) ; 1.28-1.14 (*m*, 1H)	1.71-1.53 (*m*, 1H) ; 1.33-1.14 (*m*, 1H)
23	2.16-2.07 (*m*, 1H) ; 1.80-1.70 (*m*, 1H)	2.30-2.20 (*m*, 1H) ; 2.14-2.06 (*m*, 1H)	2.32-2.23 (*m*, 1H) ; 2.16-2.01 (*m*, 1H)
24	6.90 (*qt*, *J* = 7.4 and 1.4 Hz, 1H)	6.78 (*qt*, *J* = 7.5 and 1.4 Hz, 1H)	6.90 (*qt*, *J* = 7.5 and 1.5 Hz, 1H)
27	1.84 (*d*, *J* = 1.4 Hz, 3H)	1.82 (*d*, *J* = 1.4 Hz, 3H)	1.83 (*d*, *J* = 1.5 Hz, 3H)
28	0.93 (*s*, 3H)	0.85 (*s*, 3H)	1.06 (*s*, 3H)
29	0.84 (*s*, 3H)	0.96 (*s*, 3H)	1.08 (*s*, 3H)
30	0.98 (*s*, 3H)	0.98 (*s*, 3H)	0.99 (*s*, 3H)
32	2.05 (*s*, 3H)	-	-

Table 60 Chemical shifts and types of carbon of compounds AH4, AH5 and AH10

Position	Type of Carbon	AH4 ; δ_C	AH5 ; δ_C	AH10 ; δ_C
1	CH_2	34.72	34.99	33.20
2	CH_2	24.78	26.70	34.06
3	CH	81.16	79.18	-
	C	-	-	220.05
4	C	38.51	39.52	47.07
5	CH	45.27	45.20	49.29
6	CH_2	17.98	18.10	20.32
7	CH_2	30.02	30.19	34.57
8	C	37.63	37.55	37.43

Table 60 (Continued)

Position	Type of Carbon	AH4 ; δ_C	AH5 ; δ_C	AH10 ; δ_C
9	CH	45.49	45.33	41.24
10	C	35.99	36.02	36.13
11	CH_2	25.67	25.58	24.10
12	CH	120.05	119.91	116.84
13	C	148.52	148.50	148.49
14	C	51.97	51.90	51.56
15	CH_2	31.56	31.49	31.50
16	CH_2	26.76	28.16	25.61
17	CH	49.30	49.25	49.79
18	CH_3	19.80	19.85	22.51
19	CH_3	26.35	26.17	24.30
20	CH	37.49	37.48	36.18
21	CH_3	18.15	18.07	18.10
22	CH_2	32.46	32.51	31.84
23	CH_2	26.88	26.73	27.06
24	CH	145.70	144.04	145.63
25	C	126.67	127.03	126.79
26	C=O	173.24	170.80	173.27
27	CH_3	11.97	12.12	11.99
28	CH_3	16.83	15.73	19.80
29	CH_3	28.38	28.36	29.01
30	CH_3	22.69	22.60	23.32
31	C=O	171.11	-	-
32	CH_3	21.29	-	-

In the HMBC correlation spectrum (**Table 61**) (**Figure 3.38**), the secondary Me-21 (δ_H 0.95, *d*, *J* = 6.6 Hz) of the side chain exhibited a correlation peak with the methine C-17 (δ_C 49.30), indicating that the side chain was located at C-17. The vinylic proton at δ_H 5.34-5.29 (*m*, H-12) correlated with the C-17, C-9 (δ_C 45.49), C-11 (δ_C 25.67) and C-14 (δ_C 51.97), suggesting that the trisubstituted double bond was located at C-12/C-13. Long-range polarization transfer of the α-methine proton signal (H-3) at δ_H 4.49 to the carbonyl carbon (δ_C 171.11, C-31) of the acetate group confirmed the attachment of acetoxyl group at C-3. The location of all tertiary methyl groups was established using the data from the HMBC spectra (**Table 61**) (**Figure 3.38**).

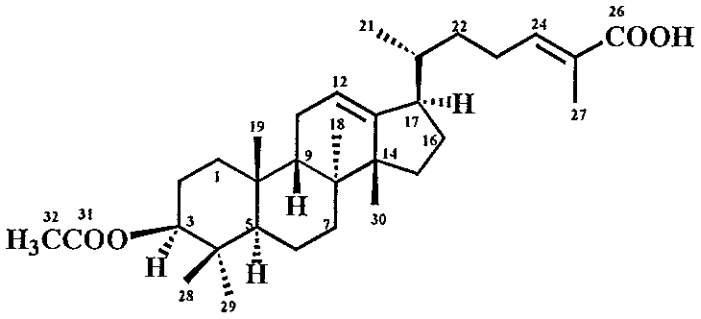
Table 61 Major HMBC correlations of compound AH4

Proton	$\delta_C(C_n)$
H-3	171.11 (31), 38.51 (4), 34.72 (1), 28.38 (Me-29), 24.78 (2), 16.83 (Me-28)
H-5	38.51 (4), 35.99 (10), 34.72 (1), 30.02 (7), 28.38 (Me-29), 26.35 (Me-19), 17.98 (6), 16.83 (Me-28)
H-9	120.05 (12), 51.97 (14), 45.27 (5), 37.63 (8), 34.72 (1), 26.35 (Me-19), 25.67 (11), 19.80 (Me-18)
H-12	51.97 (14), 49.30 (17), 45.49 (9), 25.67 (11)
H-24	173.24 (26), 126.67 (25), 32.46 (22), 26.88 (23), 11.97 (Me-27)
Me-18	51.97 (14), 45.49 (9), 37.63 (8), 30.02 (7)
Me-19	45.49 (9), 45.27 (5), 35.99 (10), 34.72 (1)
Me-21	49.30 (17), 37.49 (20), 32.46 (22)
Me-27	173.24 (26), 145.70 (24), 126.67 (25)

Table 61 (Continued)

Proton	$\delta_C (C_n)$
Me-28	81.16 (3), 45.27 (5), 38.51 (4), 28.38 (Me-29)
Me-29	81.16 (3), 45.27 (5), 38.51 (4), 16.83 (Me-28)
Me-30	148.52 (13), 51.97 (14), 37.63 (8), 31.56 (15)
Me-32	171.11 (31), 81.16 (3)

The enhancement of the allylic Me-27 was not observed by the irradiation at the olefinic proton H-24 (δ_H 6.90) (**Figure 3.43**) in the NOE difference spectrum, indicating that the configuration on the side chain double bond was *E*. Irradiation at the tertiary methyl Me-29 (δ_H 0.84) (**Figure 3.39**) enhanced the signal of axail H-3 (δ_H 4.49) and the H-5 (δ_H 1.66-1.60) while the signals of H-9 and Me-28 were enhanced by the irradiation at the Me-19 (δ_H 1.06) (**Figure 3.42**). These indicated that the H-3 and H-5 were *cis* to the Me-29 and *trans* to the Me-28 which was therefore *cis* to both the H-9 and the Me-19. Moreover, irradiation at the Me-30 (δ_H 0.98) (**Figure 3.41**) resulted in the enhancement of only the methine proton H-9 not the methine proton H-17 (δ_H 2.32-2.20) and the Me-18 (δ_H 0.90), suggesting the Me-30 was *cis* to the H-9 and *trans* to the H-17 and the Me-18. The relative stereochemistry between the H-17 and the Me-21 was shown to be *cis* due to the enhancement of the H-17 by irradiation at the Me-21 (δ_H 0.95) (**Figure 3.40**). On the basis of these spectral studies, the structure of **AH4** was then identified as the new 3-acetylprotostane (**3**).



(3)

3.4 Compound AH10

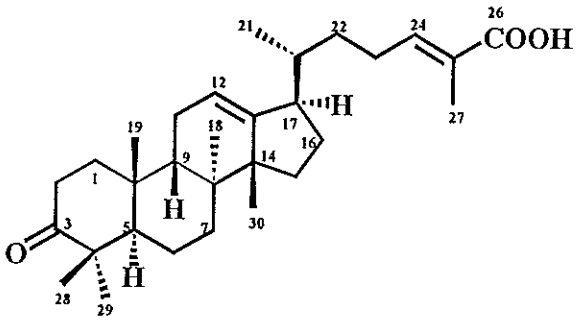
Compound AH10 was obtained as a white solid, melting at 93.0-95.0°C. The IR spectrum (**Figure 3.45**) showed three absorption bands at 3600-2500, 1708 and 1680 cm^{-1}, corresponding to a hydroxyl group of a carboxylic acid and carbonyl groups of a saturated ketone and an α,β-unsaturated carboxylic acid, respectively. The presence of the carbonyl carbon signals at δ_C 173.27 and 220.05 supported the above conclusion. An absorption band at λ_{max} 216 nm in the UV spectrum (**Figure 3.44**) was similar to that of **AH4**, suggesting that **AH10** possessed the same chromophore as **AH4**. The ^{13}C NMR spectrum (**Table 60**) (**Figure 3.47**) together with data from DEPT experiments (**Figure 3.48**) showed the presence of two carbonyl carbons (δ_C 220.05 and 173.27), four sp^2 carbon signals (δ_C 148.49, 145.63, 126.79 and 116.84) of two trisubstituted double bonds, four sp^3 quaternary carbon signals (δ_C 51.56, 47.07, 37.43 and 36.13), four sp^3 methine carbons (δ_C 49.79, 49.29, 41.24 and 36.18), nine sp^3 methylene carbons (δ_C 34.57, 34.06, 33.20, 31.84, 31.50, 27.06, 25.61, 24.10 and 20.32) and seven methyl carbons (δ_C 29.01, 24.30, 23.32, 22.51, 19.80, 18.10 and 11.99). The ^1H NMR spectrum (**Table 59**) (**Figure 3.46**) revealed signals of two trisubstituted olefinic protons (δ_H 6.90, *qt*, J = 7.5 and 1.5 Hz and δ_H 5.25-5.23, *m*) and seven methyl groups ascribed to five tertiary methyls (δ_H1.08, *s* ; δ_H1.06, *s* ; δ_H 0.99, *s* ;

δ_H 0.94, *s* and δ_H 0.88, *s*), one secondary methyl (δ_H 0.96, *d*, *J* = 7.0 Hz) and one vinylic methyl (δ_H 1.83, *d*, *J* = 1.5 Hz). These proton signals were similar to those of **AH4**. **AH10** was initially assigned to have a similar core structure to **AH4** with one trisubstituted double bond at C-12/C-13 and the same side chain as that of **AH4**. The position of this trisubstituted double bond was confirmed by analysis of HMBC spectra (**Table 62**) (**Figure 3.51**), which exhibited cross peaks between the olefinic proton, H-12 (δ_H 5.25-5.23) and the C-9 (δ_C 41.24), C-14 (δ_C 51.56) and C-17 (δ_C 49.79).

Table 62 Major HMBC correlations of compound AH10

Proton	δ_C (C_n)
H-2	220.05 (3), 47.07 (4), 36.13 (10), 33.20 (1)
H-5	47.07 (4), 41.24 (9), 24.30 (Me-19), 20.32 (6), 19.80 (Me-28)
H-9	36.13 (10), 34.57 (7), 33.20 (1)
H-11	148.49 (13), 116.84 (12), 51.56 (14), 37.43 (8), 36.13 (10), 24.30 (Me-19), 22.51 (Me-18)
H-12	51.56 (14), 49.79 (17), 41.24 (9)
H-24	173.27 (26), 126.79 (25), 31.84 (22), 27.06 (23), 11.99 (Me-27)
Me-18	51.56 (14), 41.24 (9), 37.43 (8), 34.57 (7)
Me-19	49.29 (5), 41.24 (9), 36.13 (10), 33.20 (1)
Me-21	49.79 (17), 36.18 (20), 31.84 (22)
Me-27	173.27 (26), 145.63 (24), 126.79 (25)
Me-28	220.05 (3), 49.29 (5), 47.07 (4), 29.01 (Me-29)
Me-29	220.05 (3), 49.29 (5), 47.07 (4), 19.80 (Me-28)
Me-30	148.49 (13), 51.56 (14), 37.43 (8), 31.50 (15)

The presence of the methylene protons (H-2) at δ_H 2.66 (*ddd*, J = 16.0, 11.5 and 5.5 Hz) and δ_H 2.36 (*ddd*, J = 16.0, 9.5 and 4.0 Hz) which were observed at lower field than those (δ_H 1.70-1.66) of **AH4**, the presence of the carbonyl carbon at δ_C 220.05 and the absence of the oxymethine proton in **AH10** indicated that the C-3 was a carbonyl carbon, not an oxymethine carbon. This was confirmed by the HMBC correlation data (**Table 62**) (**Figure 3.51**) ; the Me-28 (δ_H 1.06) and Me-29 (δ_H 1.08) showed correlation peaks with the carbonyl carbon at δ_C 220.05 (C-3). The relative stereochemistry was deduced by NOE difference spectral data to be the same as that of **AH4**. **AH10** was therefore assigned as the new 3-oxoprotostane (**4**).



(4)

3.5 Compound AH5

Compound AH5 was obtained as white needles, melting at 201.2-202.1°C. In the UV spectrum (**Figure 3.52**), an strong absorption at λ_{max} 217 nm indicated that **AH5** possessed the same chromophore as **AH4**. The IR spectrum (**Figure 3.53**) showed the presence of a hydroxyl group (3385 cm^{-1}) and a carbonyl group of an α,β-unsaturated carboxylic acid (1675 cm^{-1}). The ^{13}C NMR spectral data (**Table 60**) (**Figure 3.55**) together with DEPT data (**Figure 3.56**) and HMQC spectrum (**Figure**

3.57) showed 30 resonances for 30 carbon atoms : one carbonyl carbon (δ_C 170.80), six quaternary carbons (δ_C 148.50, 127.03, 51.90, 39.52, 37.55 and 36.02), seven methine carbons (δ_C 144.04, 119.91, 79.18, 49.25, 45.33, 45.20 and 37.48), nine methylene carbons (δ_C 34.99, 32.51, 31.49, 30.19, 28.16, 26.73, 26.70, 25.58 and 18.10) and seven methyl carbons (δ_C 28.36, 26.17, 22.60, 19.85, 18.07, 15.73 and 12.12). The presence of two quaternary sp^2 carbon atoms (δ_C 148.50 and 127.03) and two sp^2 methine carbons (δ_C 144.04 and 119.91) suggested that **AH5** consisted of two trisubstituted double bonds. The ^{1}H NMR spectrum (**Table 59**) (**Figure 3.54**) demonstrated the presence of five tertiary methyls (δ_H 1.04, *s* ; δ_H 0.98, *s* ; δ_H 0.96, *s* ; δ_H 0.91, *s* and δ_H 0.85, *s*), one secondary methyl (δ_H 0.95, *d*, *J* = 6.9 Hz), one vinylic methyl (δ_H 1.82, *d*, *J* = 1.4 Hz) and two olefinic protons (δ_H 6.78, *qt*, *J* = 7.5 and 1.4 Hz ; δ_H 5.28-5.25, *m*). These data were similar to those of **AH4** except for the fact that the oxymethine proton of **AH5** appeared at δ_H 3.20 (*dd*, *J* = 10.6 and 6.0 Hz) while this proton of **AH4** occurred at much lower field (δ_H 4.49, *dd*, *J* = 9.6 and 6.0 Hz). In addition, **AH4** showed the acetyl-proton signal at δ_H 2.05 (*s*, 3H), suggesting that **AH4** might be the acetate derivative of **AH5**. The important signals in the ^{1}H and ^{13}C NMR spectra of **AH4** and **AH5** were compared as shown in **Table 63**.

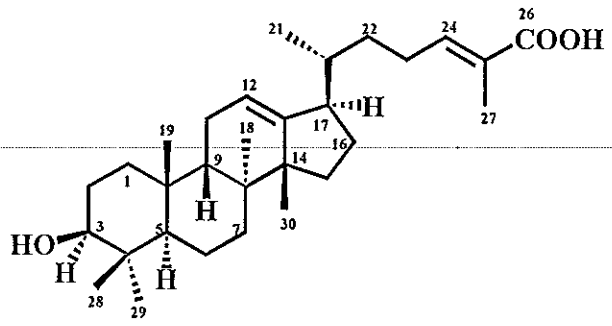
Table 63 The important signals in the ^{1}H and ^{13}C NMR spectra of compounds AH4 and AH5

AH4		AH5	
δ_H ; *mult*, *J* (Hz)	δ_C	δ_H ; *mult*, *J* (Hz)	δ_C
4.49 (*dd*, *J* = 9.6 and 6.4 Hz, 1H)	81.16	3.20 (*dd*, *J* = 10.6 and 6.0 Hz,1 H)	79.18
5.34-5.29 (*m*, 1H)	120.05	5.28-5.25 (*m*, 1H)	119.91
0.95 (*d*, *J* = 6.6 Hz, 3H)	18.15	0.95 (*d*, *J* = 6.9 Hz, 3H)	18.07

Table 63 (Continued)

AH4		AH5	
δ_H ; *mult*, *J* (Hz)	δ_C	δ_H ; *mult*, *J* (Hz)	δ_C
6.90 (*qt*, *J* = 7.4 and 1.4 Hz, 1H)	145.70	6.78 (*qt*, *J* = 7.5 and 1.4 Hz, 1H)	144.04
1.84 (*d*, *J* = 1.4 Hz, 3H)	11.97	1.82 (*d*, *J* = 1.4 Hz, 3H)	12.12
0.84, 0.90, 0.93, 0.98, 1.06	28.38,	0.85, 0.91, 0.96, 0.98, 1.04	15.73,
(*s*, 3H each)	19.80,	(*s*, 3H each)	19.85,
	16.83,		28.36,
	22.69,		22.60,
	26.35		26.17
2.05 (*s*, 3H)	21.29	-	-

AH5 was then converted to the acetate derivative (**AH5-Ac**), using acetic anhydride in the presence of a catalytic amount of pyridine. It was found that **AH5-Ac** exhibited the identical spectroscopic data as **AH4**. Furthermore, oxidation of **AH5** with CrO_3-pyridine (Kumar, 1985 ; Siddiqui, 1986) afforded **AH10**. These supported that **AH5** was an alcohol form of the ester **AH4** and the ketone **AH10**. **AH5** was therefore identified as the new 3-hydroxyprotostane (**5**).



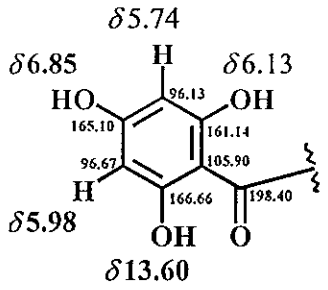
(5)

3.6 Compound AH7

Compound AH7 was obtained as a yellow viscous-liquid. The IR spectrum (**Figure 3.65**) exhibited two absorption bands at 3423 and 1631 cm^{-1}, corresponding to a hydroxyl group and a chelated *ortho*-hydroxyl carbonyl group (Hou, 2001), respectively. The presence of a carbonyl carbon signal at δ_C 198.40 (**Table 64**) supported this conclusion. The UV spectrum (**Figure 3.64**) exhibited typical absorption bands of a benzophenone at λ_{max} 300 and 218 nm (Hou, 2001), indicating that **AH7** might be a benzophenone derivative.

The ^{1}H NMR spectra (**Table 64**) (**Figure 3.66**) together with the data from ^{1}H-^{1}H COSY spectra (**Figure 3.69**) revealed the presence of one chelated hydroxyl proton (δ_H 13.60, *s*, 1-OH), four phenolic hydroxyl groups (δ_H 6.85, *brs*, 3-OH ; δ_H 6.13, *s*, 5-OH and δ_H 5.85, *brs*, 10- and 12-OH), three aromatic protons (δ_H 6.46, *s*, H-11 ; δ_H 5.98, *d*, J = 2.6 Hz, H-2 and δ_H 5.74, *d*, J = 2.6 Hz, H-4), four olefinic protons (δ_H 5.13, *mt*, J = 6.6 Hz, H-15, H-25 and δ_H 5.00, *mt*, J = 6.6 Hz, H-20, H-30), twelve methylene protons (δ_H 3.19, *d*, J = 6.6 Hz, H-14, H-24 ; δ_H 2.06-1.98, *m*, H-19, H-29 and δ_H 1.97-1.91, *m*, H-18, H-28) and six vinylic methyls (δ_H 1.66, *s*, Me-22, Me-32 ; δ_H 1.61, *s*, Me-17, Me-27 and δ_H 1.58, *s*, Me-23, Me-33). The ^{13}C NMR (**Table 64**) (**Figure 3.67**), DEPT (**Figure 3.68**) and HMQC (**Figure 3.70**) spectra displayed resonances for fourteen quaternary carbons [δ_C 198.40, 166.66, 165.10, 161.14, 155.14 (2xC), 139.60, 139.20 (2xC), 132.07 (2xC), 115.60 (2xC) and 105.90], seven methine carbons [δ_C 123.72 (2xC), 120.69 (2xC), 106.48, 96.67 and 96.13], six methylene carbons [δ_C 39.50 (2xC), 26.40 (2xC) and 26.18 (2xC)] and six methyl carbons carbons [δ_C 25.60 (2xC), 17.64 (2xC) and 15.98 (2xC)]. Two sets of *doublet* signals at δ_H 5.98 (H-2) and δ_H 5.74 (H-4) with a coupling constant of 2.6 Hz indicated the *meta* relationship. In addition, the singlet signal of the *peri*-hydroxyl (δ_H 13.60, *s*, 1-OH) showed correlations with the aromatic methine carbon (δ_C 96.67 ; δ_H 5.98) and carbonyl carbon

(δ_C 198.40), suggesting that **AH7** was a benzophenone derivative with a tetrasubstituted benzene ring of the type A. This partail structure was confirmed by the HMBC correlation data **(Table 65)** **(Figure 3.71)**.



structural unit A

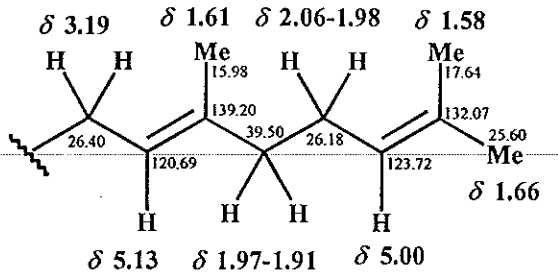
Table 64 The ^{1}H and ^{13}C NMR spectral data of compound AH7

Position	Type of Carbon	δ_C	δ_H, *mult.*, *J* (Hz)
1-OH	C	166.66	13.60 (*s*, 1H)
2	CH	96.67	5.98 (*d*, *J* = 2.6 Hz, 1H)
3-OH	C	165.10	6.85 (*brs*, 1H)
4	CH	96.13	5.74 (*d*, *J* = 2.6 Hz, 1H)
5-OH	C	161.14	6.13 (*s*, 1H)
6	C	105.90	-
7	C	198.40	-
8	C	139.60	-
9,13	C	115.60	-
10,12-OH	C	155.14	5.85 (*brs*, 2H)
11	CH	106.48	6.46 (*s*, 1H)
14,24	CH_2	26.40	3.19 (*d*, *J* = 6.6 Hz, 4H)
15,25	CH	120.69	5.13 (*mt*, *J* = 6.6 Hz, 2H)

Table 64 (Continued)

Position	Type of Carbon	δ_C	δ_H, *mult.*, *J* (Hz)
16,26	C	139.20	-
17,27	CH_3	15.98	1.61 (*s*, 6H)
18,28	CH_2	39.50	1.97-1.91 (*m*, 4H)
19,29	CH_2	26.18	2.06-1.98 (*m*, 4H)
20,30	CH	123.72	5.00 (*mt*, *J* = 6.6 Hz, 2H)
21,31	C	132.07	-
22,32	CH_3	25.60	1.66 (*s*, 6H)
23,33	CH_3	17.64	1.58 (*s*, 6H)

The presence of two types of olefinic protons (δ_H 5.13 and δ_H 5.00, *mt*, *J* = 6.6 Hz, 2H each), three types of methylene groups (δ_H 3.19, *d*, *J* = 6.6 Hz, 4H ; δ_H 2.06-1.98 and δ_H 1.97-1.91, *m*, 4H each) and three types of vinylic methyls (δ_H 1.66 ; δ_H 1.61 and δ_H 1.58, *s*, 6H each) together with the number of protons for each signal in the ^{1}H NMR spectrum further revealed the presence of two identical geranyl chains as shown in structural unit B. This substructure was confirmed by ^{1}H-^{1}H COSY (**Figure 3.69**) and HMBC (**Table 65**) spectra.

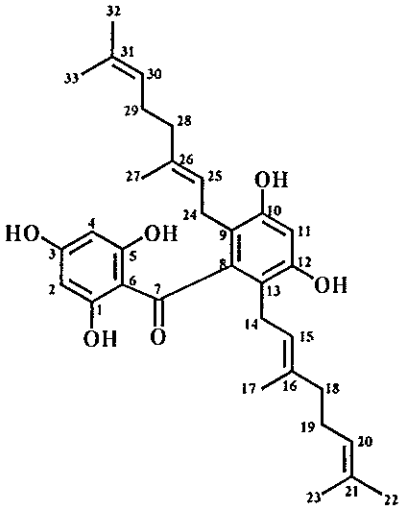


Structural unit B

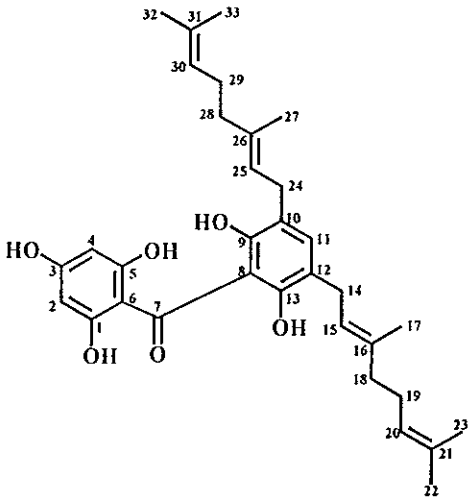
Table 65 Major HMBC correlations of compound AH7

Proton	δ_C (C_n)
1-OH	198.40 (7), 166.66 (1), 165.10 (3), 105.90 (6), 96.67 (2)
H-2	166.66 (1), 165.10 (3), 105.90 (6), 96.13 (4)
H-4	165.10 (3), 161.14 (5), 105.90 (6), 96.67 (2)
5-OH	165.10 (3), 161.14 (5), 105.90 (6), 96.13 (4)
H-11	155.14 (10, 12), 115.60 (9, 13), 26.40 (14, 24)
H-14,24	155.14 (10, 12), 139.60 (8), 139.20 (16, 26), 120.69 (15, 25), 115.60 (9, 13), 39.50 (18, 28), 15.98 (Me-17, Me-27)
H-15,25	115.60 (9, 13), 39.50 (18, 28), 26.40 (14, 24), 15.98 (Me-17, Me-27)
H-18,28	139.20 (16, 26), 123.72 (20, 30), 120.69 (15, 25), 26.18 (19, 29), 15.98 (Me-17, Me-27)
H-19,29	139.20 (16, 26), 132.07 (21, 31), 123.72 (20, 30), 39.50 (18, 28)
H-20,30	39.50 (18, 28), 25.60 (Me-22, Me-32), 17.64 (Me-23, Me-33)
Me-17,27	139.20 (16, 26), 120.69 (15, 25), 39.50 (18, 28)
Me-22,32	132.07 (21, 31), 123.72 (20, 30), 17.64 (Me-23, Me-33)
Me-23,33	132.07 (21, 31), 123.72 (20, 30), 25.60 (Me-22, Me-32)

The remaining proton signals, the aromatic proton (δ_H 6.46, *s*, H-11) and two phenolic hydroxyl protons (δ_H 5.85, *brs*, 10-OH and 12-OH), suggested that the other aromatic ring was a pentasubstituted benzene moiety, containing two geranyl side chains. Two possible structures (**6** and **7**) were then proposed for **AH7**.



(6)



(7)

In the HMBC spectral data (**Table 65**), the methylene protons of the geranyl chain (H-14, H-24 ; δ_C 26.40) showed correlation peaks with three quaternary aromatic carbons at δ_C 155.14 (C-10, C-12), 139.60 (C-8) and 115.60 (C-9, C-13) of the pentasubstituted benzene moiety. These indicated that geranyl chains were located at the C-9 and C-13, rather than at C-10 and C-12. **AH7** was therefore assigned to have the structure (**6**), a new benzophenone derivative.

3.7 Compound AH1

Compound AH1 was a colorless viscous-liquid. The IR spectrum (**Figure 3.72**) showed bands related with an O-H stretching hydroxyl group (3400 cm^{-1}) and a C-H stretching (2961, 2922 and 2844 cm^{-1}). The ^1H NMR signals (**Table 66**) (**Figure 3.73**) at δ_H 3.48 (*dd*, J = 7.0 and 5.9 Hz, 0.8H) and δ_H 3.42 (*dd*, J = 10.5 and 4.4 Hz, 1H) displayed the secondary nature and equatorial disposition on a cyclohexane ring of the hydroxyl group (Barrero, 1989). The signals at δ_H 4.89 (*s*, 1H) and δ_H 4.62 (*s*, 1H) in its ^1H NMR spectrum were also characteristic of a methylene cyclohexane moiety.

In addition, the signals of methyl groups were also observed at δ_H 1.04 (*s*, 3H), δ_H 0.98 (*s*, 2.4H), δ_H 0.85 (*s*, 2.4H) and δ_H 0.73 (*s*, 3H) for tertiary methyls and δ_H 1.73 (*brs*, 2.4H), δ_H 1.69 (*d*, J = 1.0 Hz, 3H) and δ_H 1.61 (*s*, 21.6H) for vinylic methyls. These ^{1}H NMR data indicated that **AH1** was a mixture of two triterpenes having a monocyclic moiety in the molecule in the ratio of 0.8 to 1. Comparison of its data with the prevoiusly reported data (Barrero, 1989 ; Akihisa, 1999) suggested that **AH1** displayed the same signals as **achilleol A** and **camelliol C**. The important data were shonw in **Table 66**.

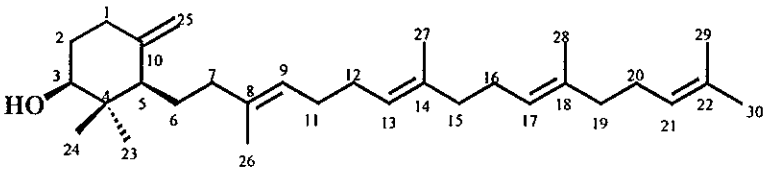
Table 66 The important signals in the ^{1}H NMR spectra of Compounds AH1, Achilleol A and Camelliol C

Position H	Compound AH1; δ_H	Achilleol A; δ_H	Camelliol C; δ_H
1	5.27-5.23 (*m*, 0.8H)	-	5.24 (*m*, 1H)
3α	3.48 (*dd*, J = 7.0 and 5.9 Hz, 0.8H); 3.42 (*dd*, J = 10.5 and 4.4 Hz, 1H)	3.42 (*dd*, J = 10.0 and 4.5 Hz, 1H)	3.46 (*brt*, J = 6.9 Hz, 1H)
9	5.18-5.08 (*m*)	5.12 (*m*)	5.15 (*m*)
13	5.18-5.08 (*m*)	5.12 (*m*)	5.15 (*m*)
17	5.18-5.08 (*m*)	5.12 (*m*)	5.12 (*m*)
21	5.18-5.08 (*m*)	5.12 (*m*)	5.10 (*m*)
23	1.04 (*s*, 3H); 0.98 (*s*, 2.4H)	1.02 (*s*, 3H)	0.97 (*s*, 3H)
24	0.73 (*s*, 3H); 0.85 (*s*, 2.4H)	0.72 (*s*, 3H)	0.83 (*s*, 3H)
25	4.62, 4.89 (*s*, 1H each); 1.73 (*brs*, 2.4H)	4.62, 4.88 (1H each)	1.72 (*brs*, 3H)

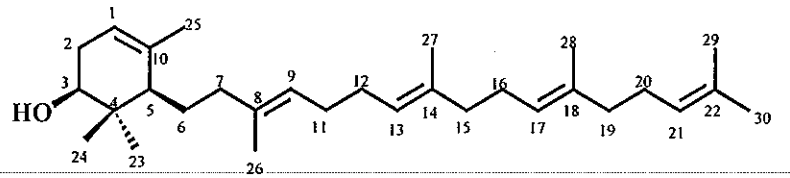
Table 66 (Continued)

Position H	Compound AH1; δ_H	Achilleol A; δ_H	Camelliol C; δ_H
26,27,28, 29	1.61 (*s*, 21.6H)	1.60 (*s*, 12H)	1.60 (*s*, 12H)
30	1.69 (*d*, *J* = 1.0 Hz, 5.4H)	1.69 (*s*, 3H)	1.68 (*s*, 3H)

Furthermore, **AH1** was then transformed to its acetate derivative (**AH1-Ac**). Again, the ^{1}H NMR data of **AH1-Ac** (**Figure 3.74**) were the same as found in **achilleol A** and **camelliol C acetates** (**Table 67**). **AH1** was then identified as a mixture of **achilleol A** (**8**) and **camelliol C** (**9**).



(8)



(9)

Table 67 The important signals in the ^{1}H NMR spectra of Compounds AH1, Achilleol A and Camelliol C monoacetate

Position H	Compound AH1-Ac δ_H	Achilleol A acetate δ_H	Camelliol C acetate δ_H
1	5.27-5.23 (*m*)	-	5.21 (*m*)
3α	4.71 (*dd*, *J* = 7.0 and 5.5 Hz); 4.67 (*dd*, *J* = 10.5 and 4.4 Hz)	4.68 (*dd*, *J* = 10.0 and 4.5 Hz)	4.70 (*dd*, *J* = 7.0 and 5.8 Hz)
9	5.18-5.08 (*m*)	5.12 (*m*)	5.15 (*m*)
13	5.18-5.08 (*m*)	5.12 (*m*)	5.15 (*m*)
17	5.18-5.08 (*m*)	5.12 (*m*)	5.12 (*m*)
21	5.18-5.08 (*m*)	5.12 (*m*)	5.10 (*m*)
23	0.95 (*s*); 0.92 (*s*)	0.95 (*s*)	0.91 (*s*)
24	0.80 (*s*); 0.89 (*s*)	0.80 (*s*)	0.89 (*s*)
25	4.62 (s), 4.89 (*d*, *J* = 1.0 Hz); 1.72 (*brs*)	4.65, 4.90 (*m*)	1.72 (*brs*)
26	1.62 (*s*); 1.60 (*s*)	1.60 (*s*)	1.62 (*s*)
27,28,29	1.60 (*s*)	1.60 (*s*)	1.60 (*s*)
30	1.68 (*d*, *J* = 1.0 Hz)	1.69 (*s*)	1.68 (*s*)
3-OAc	2.05 (*s*); 2.04 (*s*)	2.05 (*s*)	2.04 (*s*)

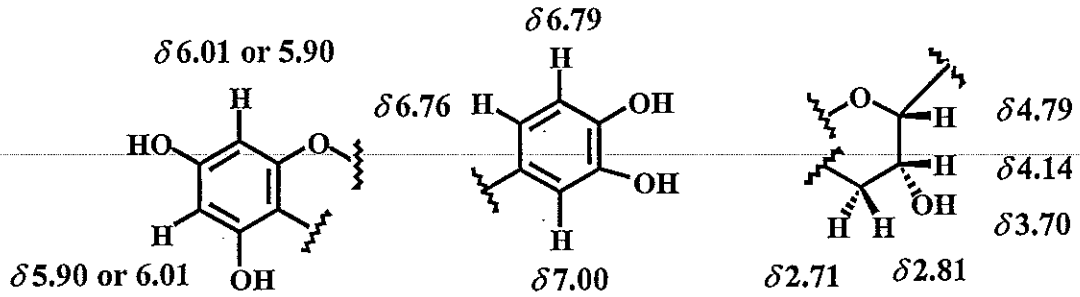
3.8 Compound AH6

Compound AH6 was crystallized from methanol as brown powder, melting at 234.6-237.0^{o}C. The EI mass spectrum (**Figure 3.75**) showed the molecular ion peak at

m/z 290 for molecular formular $C_{15}H_{14}O_6$. The IR spectrum (**Figure 3.77**) showed an absorption band corresponding to a hydroxy group at 3280 cm^{-1} while the UV spectrum (**Figure 3.76**) showed absorption bands of an aromatic moiety at 215 and 281 nm.

The ^{13}C NMR spectrum (**Figure 3.79**) of **AH6** in $CDCl_3$ + DMSO-d_6 showed fiftheen signals for fifteen carbon atoms. Analysis of DEPT spectra (**Figure 3.80**) suggested the presence of seven methine carbon atoms (δ_C 118.04, 115.03, 114.45, 95.68, 94.66, 78.22 and 65.67), one methylene carbon (δ_C 28.14) and seven signals for quaternary carbon atoms (δ_C 156.63, 156.41, 155.74, 144.60, 144.48, 130.60 and 98.53). The ^1H NMR spectrum (**Figure 3.78**) showed the presence of five hydroxyl groups [δ_H 8.73, δ_H 8.59, (*s*, 1H each), δ_H 8.35, δ_H 8.17 and δ_H 3.70 (*brs*, 1H each)]. Two *meta*-coupled aromatic protons at δ_H 6.01 and δ_H 5.90 (*d*, J = 2.4 Hz, 1H each) indicated that **AH6** consisted of the structural unit C in the molecular structure.

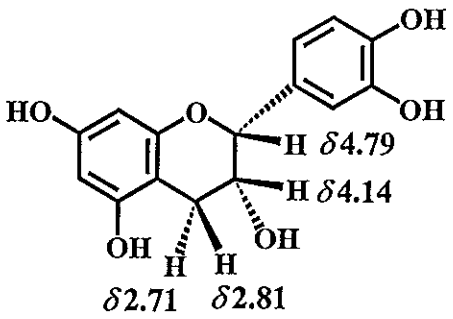
The multiplicity and chemical shift values at δ_H 7.00 (*d*, J = 1.6 Hz, 1H), δ_H 6.79 (*d*, J = 8.0 Hz, 1H) and δ_H 6.76 (*dd*, J = 8.0 and 1.6 Hz, 1H) suggested that **AH6** had the 1,3,4-trisubstituted benzene ring as shown in a structural unit D. Additionally, **AH6** contained a structural unit E due to the presence of two methine protons [δ_H 4.79 (*s*, 1H) and δ_H 4.14 (*d*, J = 3.2 Hz, 1H)] attached to oxygenated carbon and methylene protons [δ_H 2.81 (*dd*, J = 16.8 and 4.4 Hz, 1H) and δ_H 2.71 (*dd*, J = 16.8 and 3.2 Hz, 1H)].



structural unit C structural unit D structural unit E

The relative stereochemistry of the oxymethine protons of the structural unit E was determined by the NOE difference data. The signals of the oxymethine proton (δ_H 4.79) and one of the methylene protons (δ_H 2.81) were enhanced when the oxymethine proton (δ_H 4.14) was irradiated (**Figure 3.81**), indicating that both oxymethine protons were *cis* to that methylene proton.

AH6 was therefore identified as **(-)-epicatechin (10)**, not ***ent*-epicatechin** due to the negative value of $[\alpha]_D$ (Buckingham, 1994). The structure was confirmed by comparision of its ^1H and ^{13}C NMR, mass spectroscopic data, $[\alpha]_D$ and melting point with the previously data (Panthong, 1999; Markham, 1976).

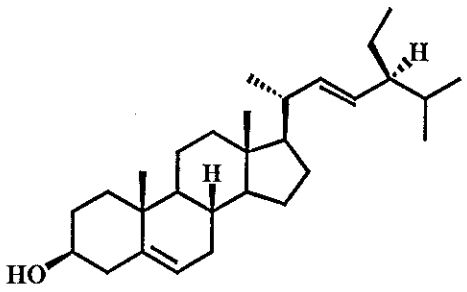


(10)

3.9 Compound AH8

Compound AH8 was obtained as a white solid, melting at 154-156°C. The mass spectrum (**Figure 3.82**) showed the molecular ion peak at *m/z* 412 for molecular formular $C_{29}H_{48}O$ while the IR spectrum (**Figure 3.84**) exhibited an absorbtion band at 3340 cm^{-1} for a hydroxy group. The signals in the ^{13}C NMR spectrum (**Figure 3.86**) and DEPT experiments (**Figure 3.87**) indicated that the presence of six methyl carbons (δ_C 21.24, 21.08, 19.42, 19.01, 12.26 and 12.08), nine methylene carbons (δ_C 39.74, 37.31, 31.95, 31.91, 31.71, 28.92, 25.42, 24.40 and 21.11), eleven methine carbons

(δ_C 138.33, 129.34, 121.72, 71.83, 56.91, 56.02, 51.27, 50.22, 42.36, 40.49 and 31.94) and three quaternary carbons (δ_C 140.79, 42.26 and 36.55). The ^{1}H NMR spectrum (**Figure 3.85**) indicated the presence of one primary methyl (δ_H 0.81, *t*, J = 7.1 Hz), three secondary methyls (δ_H 1.02, *d*, J = 6.7 Hz ; δ_H 0.85, *d*, J = 6.7 Hz and δ_H 0.80, *d*, J = 6.5 Hz), two tertiary methyls (δ_H 1.01, *s* and δ_H 0.70, *s*) and one oxymethine proton (δ_H 3.56-3.48, *m*). Additionally, **AH8** showed three olefinic protons (δ_H 5.37-5.34, *m* ; δ_H 5.15, *dd*, J = 12.3 and 6.7 Hz and δ_H 5.03, *dd*, J = 12.3 and 6.7 Hz) indicating that **AH8** contained one *trans*-disubstituted double bond and one trisubstituted double bond. The characteristic ^{1}H signals were the same as those of **stigmasterol**. Therefore its ^{1}H and ^{13}C NMR, mass spectral data, optical rotation value $[\alpha]_D$ and melting point were compared with the previously reported data of **stigmasterol**. It was found that **AH8** gave identical spectral data as **stigmasterol**. It was therefore identified as **stigmasterol (11)**.

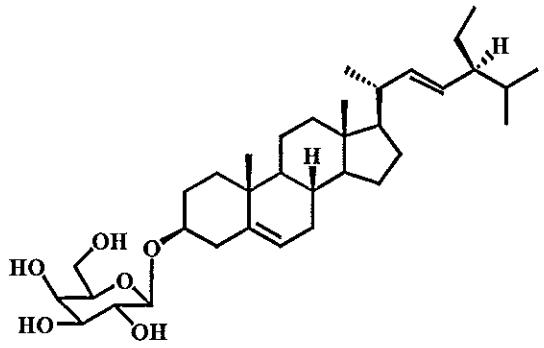


(11)

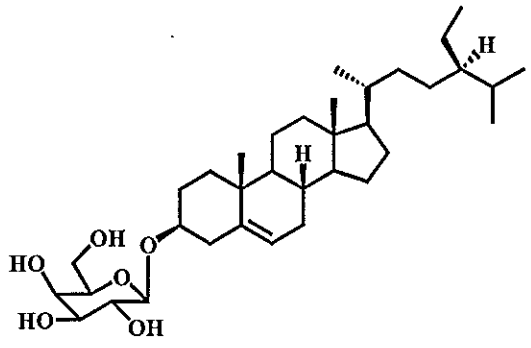
3.10 Compound AH9

Compound AH9 was white powder, melting at 269-270°C. The IR spectrum (**Figure 3.89**) showed the presence of a hydroxyl group at 3403 cm^{-1} and C-O signals at 1072 and 1024 cm^{-1}. It had the similar spectral data as **AH8** (**stigmasterol**) except

for the additional signals at δ_H 4.42-3.23 of a sugar moiety. Moreover, the ^{1}H NMR spectrum (**Figure 3.90**) showed a low-intensity characteristic signals of olefinic protons of stigmasterol [δ_H 5.17 (*dd*, J = 15.1 and 8.6 Hz) ; δ_H 5.04 (*dd*, J = 15.1 and 8.6 Hz)]. These suggested that **AH9** was a mixture of stigmasterol and sitosterol glycoside. Comparison of its ^{1}H NMR spectrum with those of stigmasterol and sitosterol glucosides (**Figure 3.91**) obtained from Professor Dr. Waltor C. Taylor of The University of Sydney confirmed that **AH9** contained both **stigmasterol** and **sitosterol glucosides** (**12** and **13**). The ratio of these compound was judged to be 1:1 by the relative integral of their olefinic protons (δ_H 5.39-5.37, *m* ; δ_H 5.17, *dd*, J = 15.1 and 8.6 Hz and δ_H 5.04, *dd*, J = 15.1 and 8.6 Hz).



(12)



(13)

3.11 Compound AH11

Compound AH11 was a yellow solid, melting at 156-159°C. The IR spectrum (**Figure 3.93**) exhibited two absorption bands at 3427 (a hydroxyl group) and 1644 cm^{-1} (a chelated *ortho*-hydroxyl carbonyl group) (Rukachaisirikul, 2000a ; Hou, 2001). The presence of this carbonyl functionality was confirmed by the signals at δ_C 181.79

in the ^{13}C NMR spectrum (**Table 68**) (**Figure 3.95**). The absorption bands at λ_{max} 375, 322, 260, 254, 245 and 209 nm in the UV spectrum (**Figure 3.92**) suggested that **AH11** was a xanthone derivative. The ^{1}H NMR spectra (**Table 68**) (**Figure 3.94**) revealed the presence of one chelated hydroxyl proton (δ_H 13.20, *s*, 1-OH), two phenolic hydroxyl groups (δ_H 6.54, *s* ; δ_H 5.72, *brs*), three aromatic protons (δ_H 7.77, *dd*, J = 8.0 and 1.6 Hz, H-8 ; δ_H 7.30, *dd*, J = 8.0 and 1.6 Hz, H-6 ; δ_H 7.24, *t*, J = 8.0 Hz, H-7), two olefinic protons (δ_H 5.29, *mt*, J = 6.8 Hz, H-12 ; δ_H 5.27, *mt*, J = 6.6 Hz, H-17), two methylene groups (δ_H 3.56, *d*, J = 6.6 Hz, H-16 ; δ_H 3.49, *d*, J = 6.8 Hz, H-11) and four vinylic methyl groups (δ_H 1.88, *s*, Me-20; δ_H 1.86, *s*, Me-15; δ_H 1.79, *d*, J = 1.0 Hz, Me-14 ; δ_H 1.76, *d*, J = 1.0 Hz, Me-19). The ^{13}C NMR spectra data (**Table 68**) (**Figure 3.95**) deduced from HMQC (**Figure 3.96**) and HMBC (**Table 69**) (**Figure 3.97**) spectra, showed 22 resonances for 23 carbon atoms : twevle quaternary carbons (δ_C 181.79, 161.45, 159.21, 152.90, 144.82, 144.68, 136.68, 133.85, 121.19, 109.29, 105.62 and 103.52), five methine carbons (δ_C 124.15, 122.53, 121.45, 120.01 and 117.21), two methylene carbons (δ_C 21.76 and 21.31) and four methyl carbons [δ_C 25.57, 25.35 and 17.61 (2xC)].

Table 68 The ^{1}H and ^{13}C NMR spectral data of compound AH11

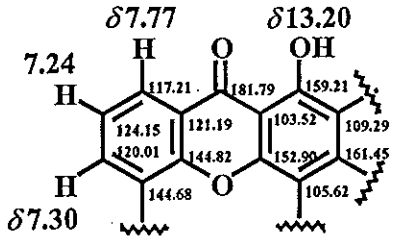
Position	Type of Carbon	δ_C	δ_H (*mult.*, J_{Hz})
1-OH	C	159.21	13.20 (*s*, 1H)
2	C	109.29	-
3-OH	C	161.45	6.54 (*s*, 1H)
4	C	105.62	-
4a	C	152.90	-

Table 68 (Continued)

Position	Type of Carbon	δ_C	δ_H (*mult.*, J_{Hz})
5-OH	C	144.68	5.72 (*brs*, 1H)
6	CH	120.01	7.30 (*dd*, J = 8.0 and 1.6 Hz, 1H)
7	CH	124.15	7.24 (*t*, J = 8.0 Hz)
8	CH	117.21	7.77 (*dd*, J = 8.0 and 1.6 Hz, 1H)
8a	C	121.19	-
9	C	181.79	-
9a	C	103.52	-
10a	C	144.82	-
11	CH_2	21.31	3.49 (*d*, J = 6.8 Hz, 2H)
12	CH	121.45	5.29 (*mt*, J = 6.8 Hz, 1H)
13	C	136.68	-
14	CH_3	25.57	1.79 (*d*, J = 1.0 Hz, 3H)
15	CH_3	17.61	1.86 (*s*, 3H)
16	CH_2	21.76	3.56 (*d*, J = 6.6 Hz, 2H)
17	CH	122.53	5.27 (*mt*, J = 6.6 Hz, 1H)
18	C	133.85	-
19	CH_3	25.35	1.76 (*d*, J = 1.0 Hz, 3H)
20	CH_3	17.61	1.88 (*s*, 3H)

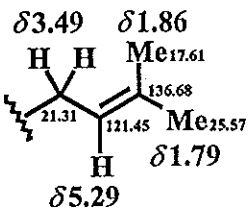
The multiplicity, chemical shift and coupling constant value (J) of the aromatic protons at δ_H 7.77 (*dd*, J = 8.0 and 1.6 Hz, H-8) exhibited *ortho*- and *meta*-coupling with the aromatic protons at δ_H 7.24 (*t*, J = 8.0 Hz, H-7) and δ_H 7.30 (*dd*, J = 8.0 and 1.6 Hz, H-6), respectively. These data indicated that **AH11** contained the 1,2,3-trisubstituted benzene ring. One of the substituents was a carbonyl functionality which

was located at the *ortho* position to the aromatic proton δ_H 7.77. In addition, the singlet signal of hydroxyl group at δ_H 13.20 suggested an intramolecular hydrogen bond of the hydroxyl group to the carbonyl functionality, as shown in the structural unit F. The HMBC correlation data (**Table 69**) (**Figure 3.97**) confirmed the presence of this unit.

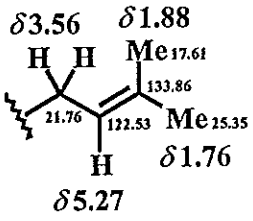


Structural unit F

The olefinic protons δ_H 5.29 (*mt*, J = 6.8 Hz, H-12) and δ_H 5.27 (*mt*, J = 6.6 Hz, H-17) displayed the vicinal coupling to methylene protons at δ_H 3.49 (*d*, J = 6.8 Hz, H-11) and δ_H 3.56 (*d*, J = 6.6 Hz, H-16), respectively. Additionally, this protons showed the allylic coupling to methyl protons, suggesting that **AH11** contained two isoprenyl groups of the structural units G and H.



structural unit G



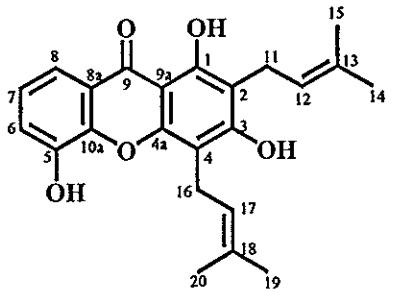
structural unit H

Table 69 Major HMBC correlations of compound AH11

Proton	HMBC correlation ; δ_C (C_n)
1-OH	181.79 (9), 161.45 (3), 159.21 (1), 109.29 (2), 103.52 (9a)
3-OH	161.45(3), 152.90 (4a), 109.29 (2), 105.62 (4)
H-6	144.68 (5), 124.15 (7), 121.19 (8a), 117.21 (8)
H-7	144.68 (5), 121.19 (8a), 120.01 (6), 117.21 (8)
H-8	181.79 (9), 144.82 (10a), 120.01 (6)
H-11	161.45 (3), 159.21 (1), 136.68 (13), 121.45 (12), 109.29 (2), 25.57 (14)
H-12	25.57 (14), 21.31 (11), 17.61 (15)
H-16	161.45 (3), 152.90 (4a), 133.85 (18), 122.53 (17), 105.62 (4), 25.35 (19)
H-17	25.35 (19), 21.76 (16), 17.61 (20)
Me-14	136.68 (13), 121.45 (12), 17.61 (15)
Me-15	136.68 (13), 121.45 (12), 25.57 (14)
Me-19	133.85 (18), 122.53 (17), 17.61 (20)
Me-20	133.85 (18), 122.53 (17), 25.35 (19)

The attachment of structural units G and H was determined by the HMBC spectrum (**Table 69**) (**Figure 3.97**) as follows. The methylene protons (δ_H 3.49, H-11) caused cross peaks to 3 aromatic carbons [δ_C 161.45 (C-3) ; δ_C 159.21 (C-1) and δ_C 109.29 (C-2)] while the other methylene protons (δ_H 3.56, H-16) showed correlations with aromatic carbons at δ_C 161.45 (C-3), 152.90 (C-4a) and 105.62 (C-4). Both methylene groups showed ^3J correlation with the aromatic carbon (δ_C 161.45, C-3), indicating that both isoprenyl units were placed at the *ortho*-position of these carbon. In addition, the chelated hydroxyl group showed a correlation to the aromatic carbon (δ_C 109.29, C-2), suggesting the attachment of the structural unit G at C-2.

Subsequently, the structural unit H was definitely located at C-4. **AH11** was then identified as **8-deoxygartanin (14)**. It was previously reported the isolation of this compound from *Garcinia mangostana* (Govindachari, 1971) and *Rheedia gardneriana* (Monache, 1984). Its physical and spectral data were in agreement with those reported in the literatures.



(14)

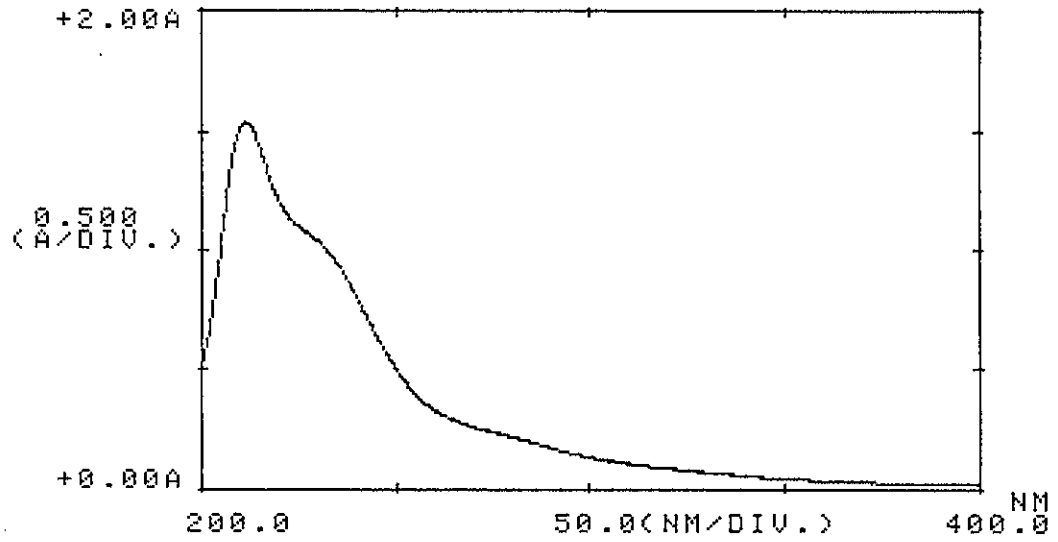


Figure 3.1 UV (MeOH) spectrum of compound AH3

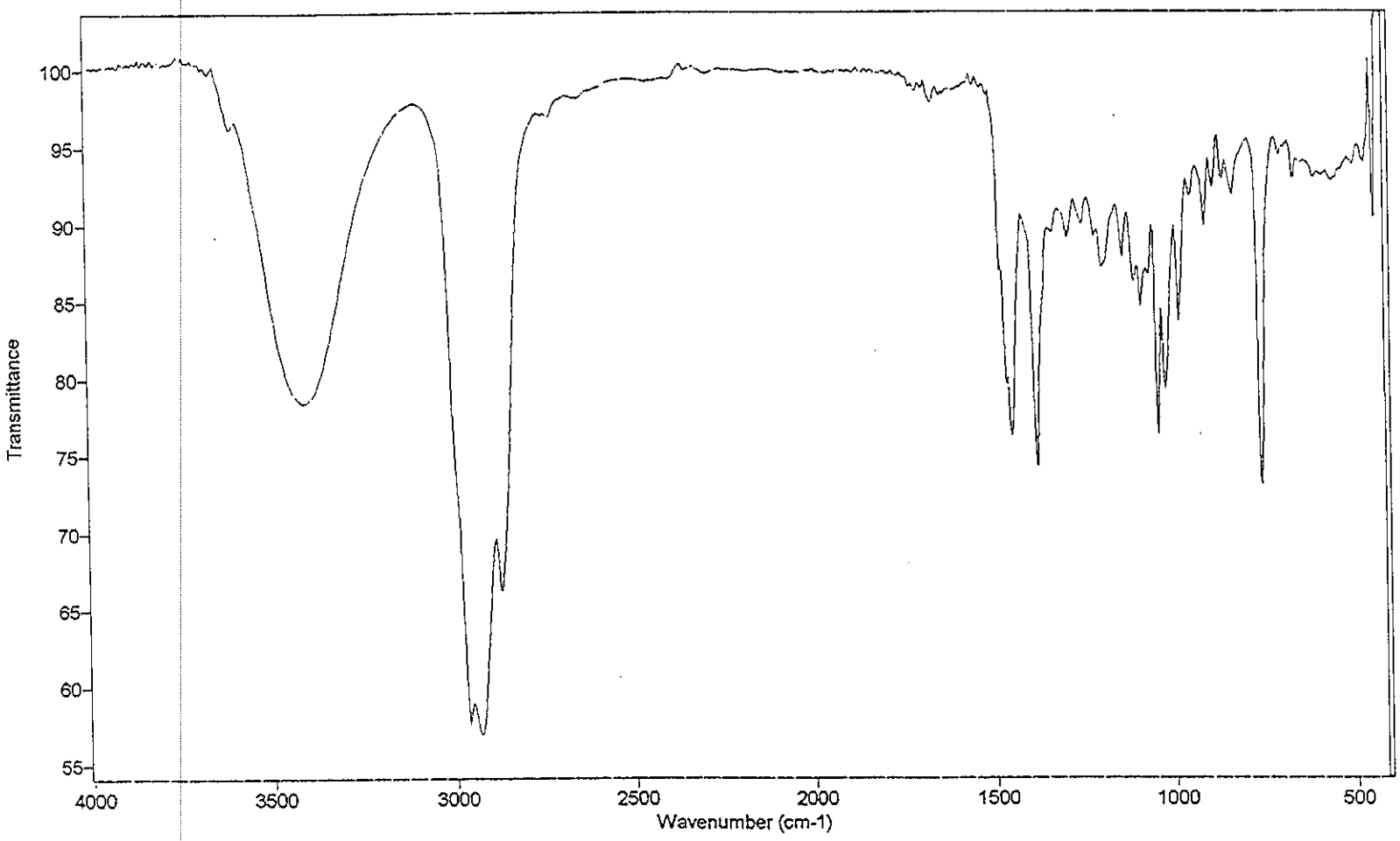


Figure 3.2 FT-IR (KBr) spectrum of compound AH3

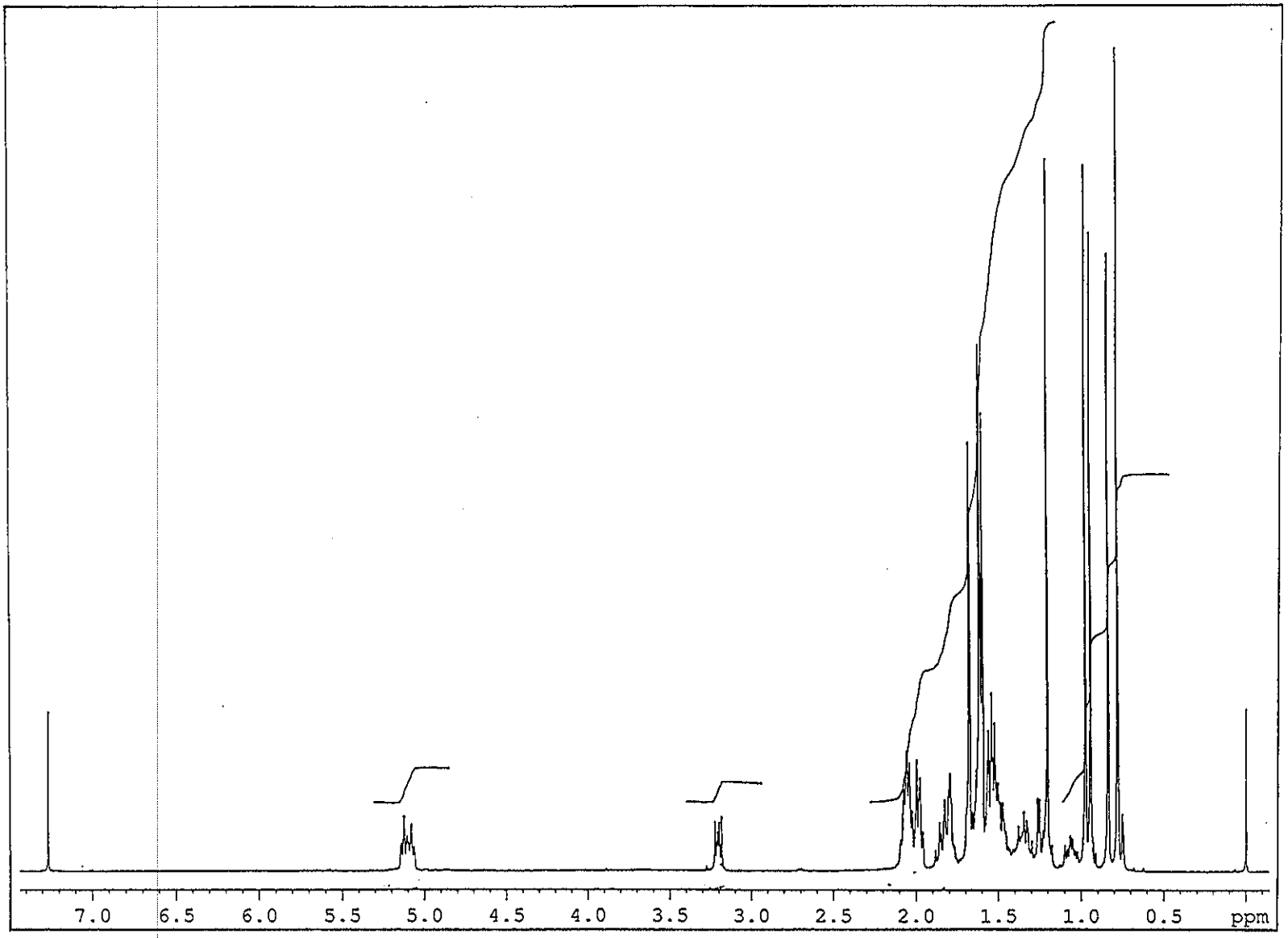


Figure 3.3 ^{1}H NMR (400 MHz)($CDCl_3$) spectrum of compound AH3

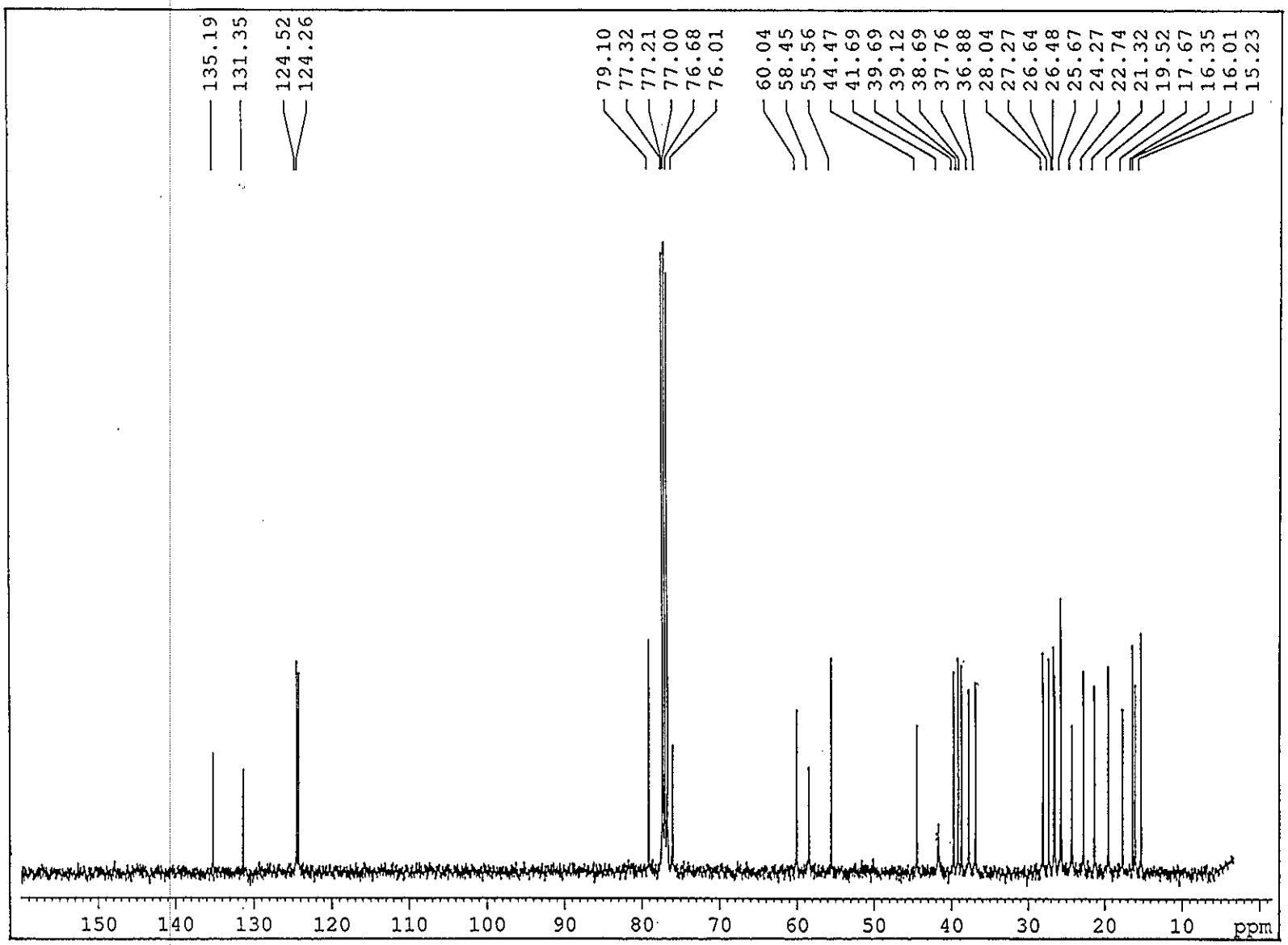


Figure 3.4 ^{13}C NMR (100 MHz)($CDCl_3$) spectrum of compound AH3

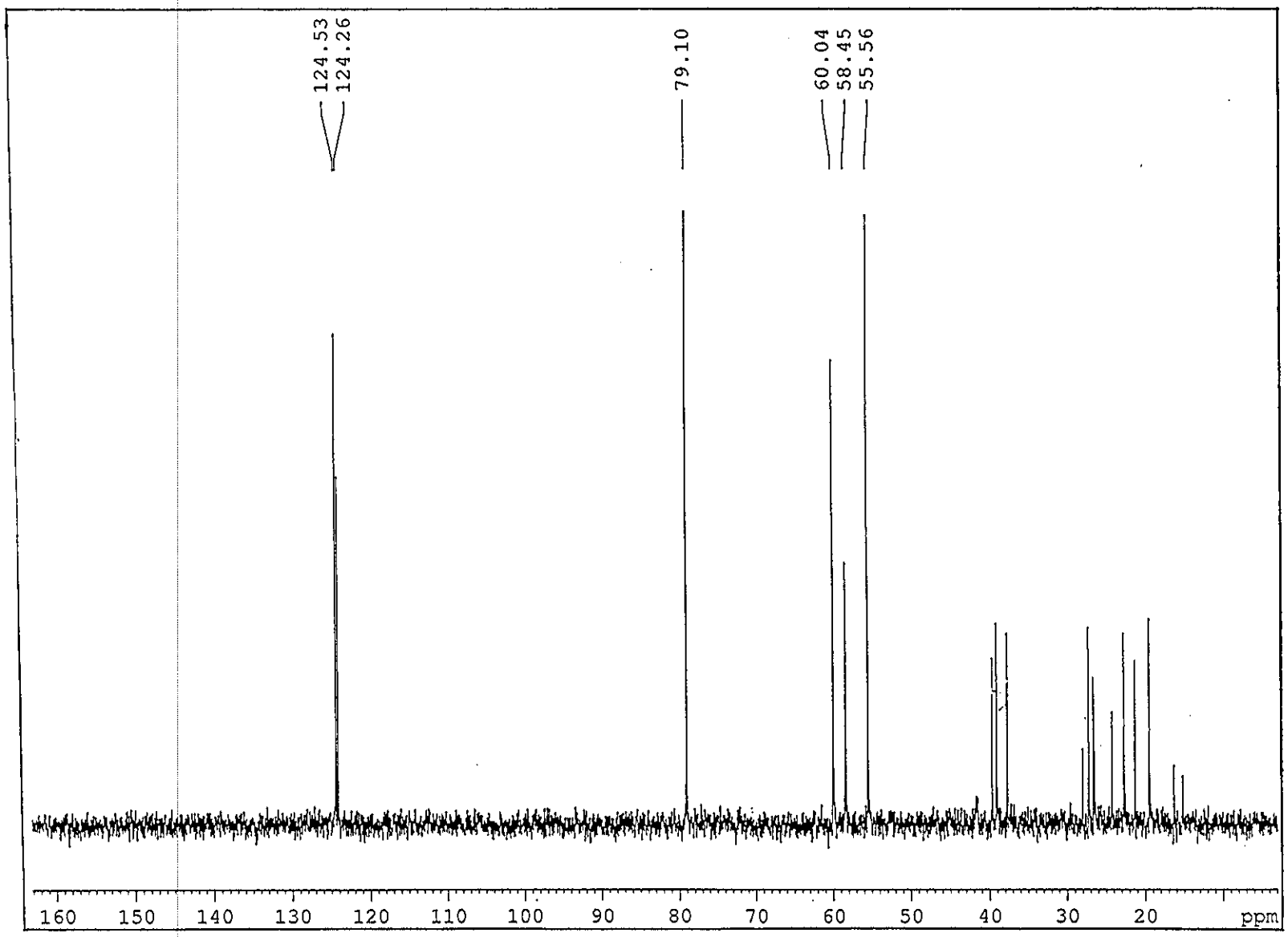


Figure 3.5 DEPT 90° spectrum of compound AH3

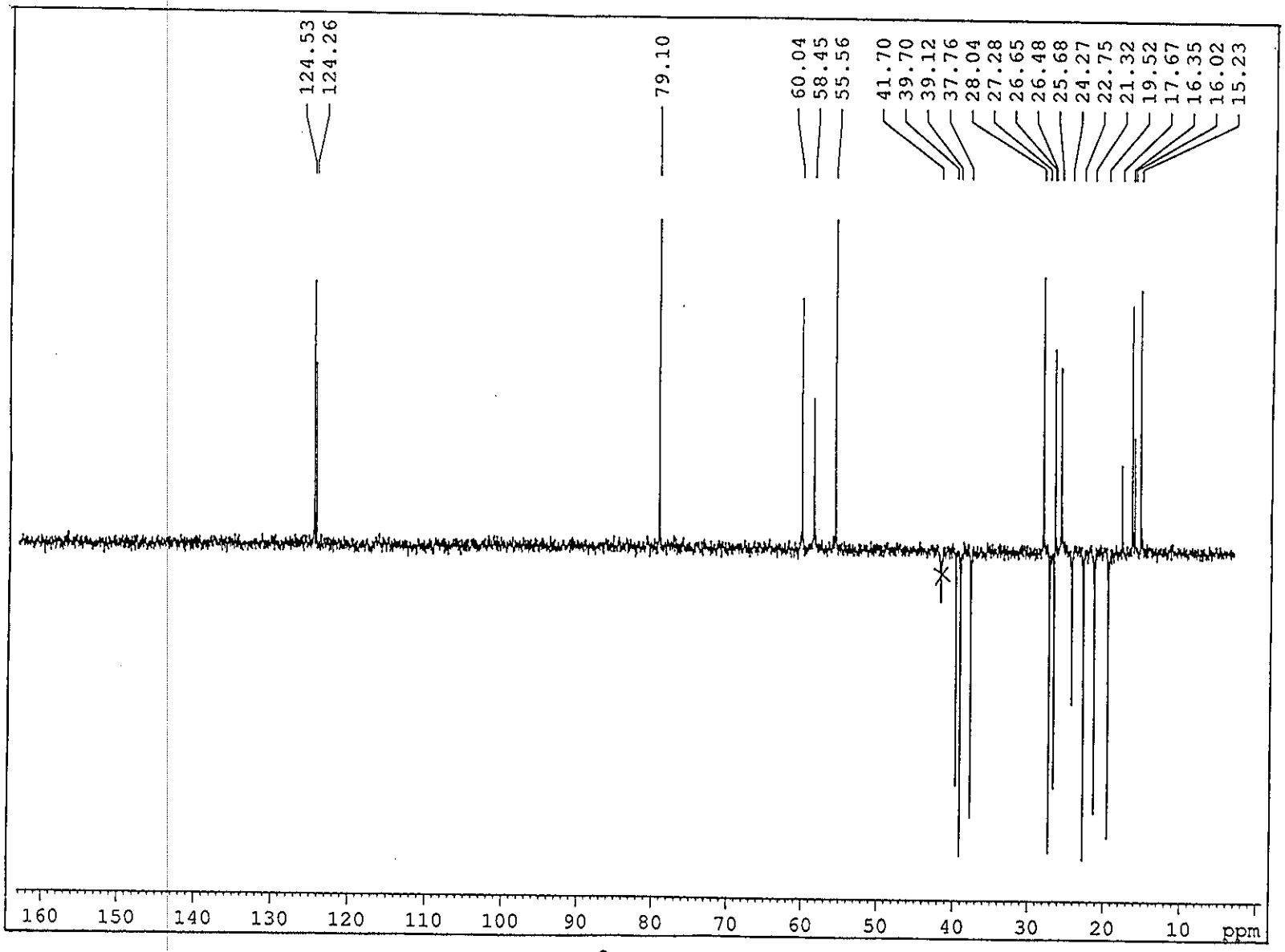


Figure 3.6 DEPT 135° spectrum of compound AH3

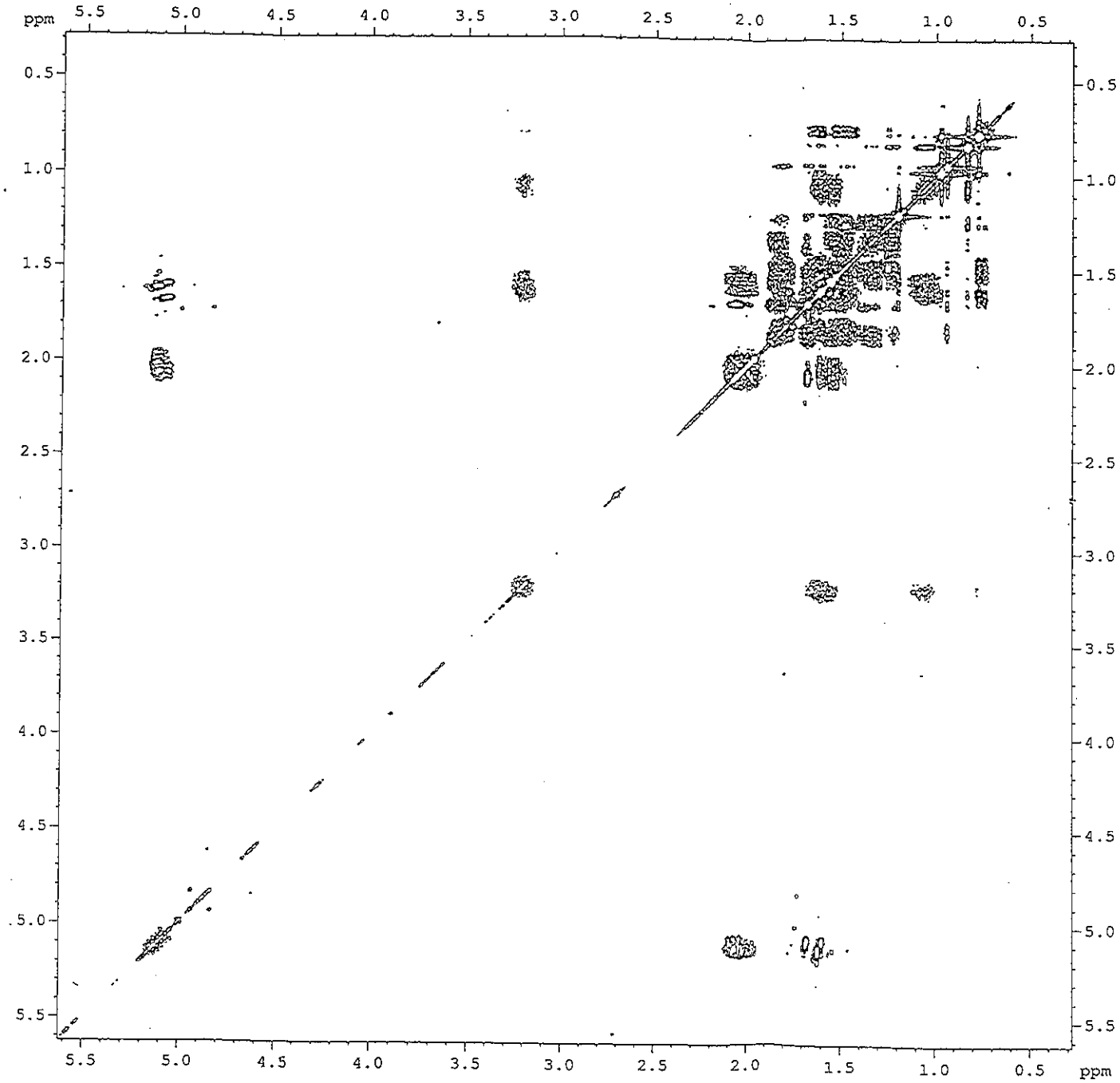


Figure 3.7 ^{1}H-^{1}H COSY spectrum of compound AH3

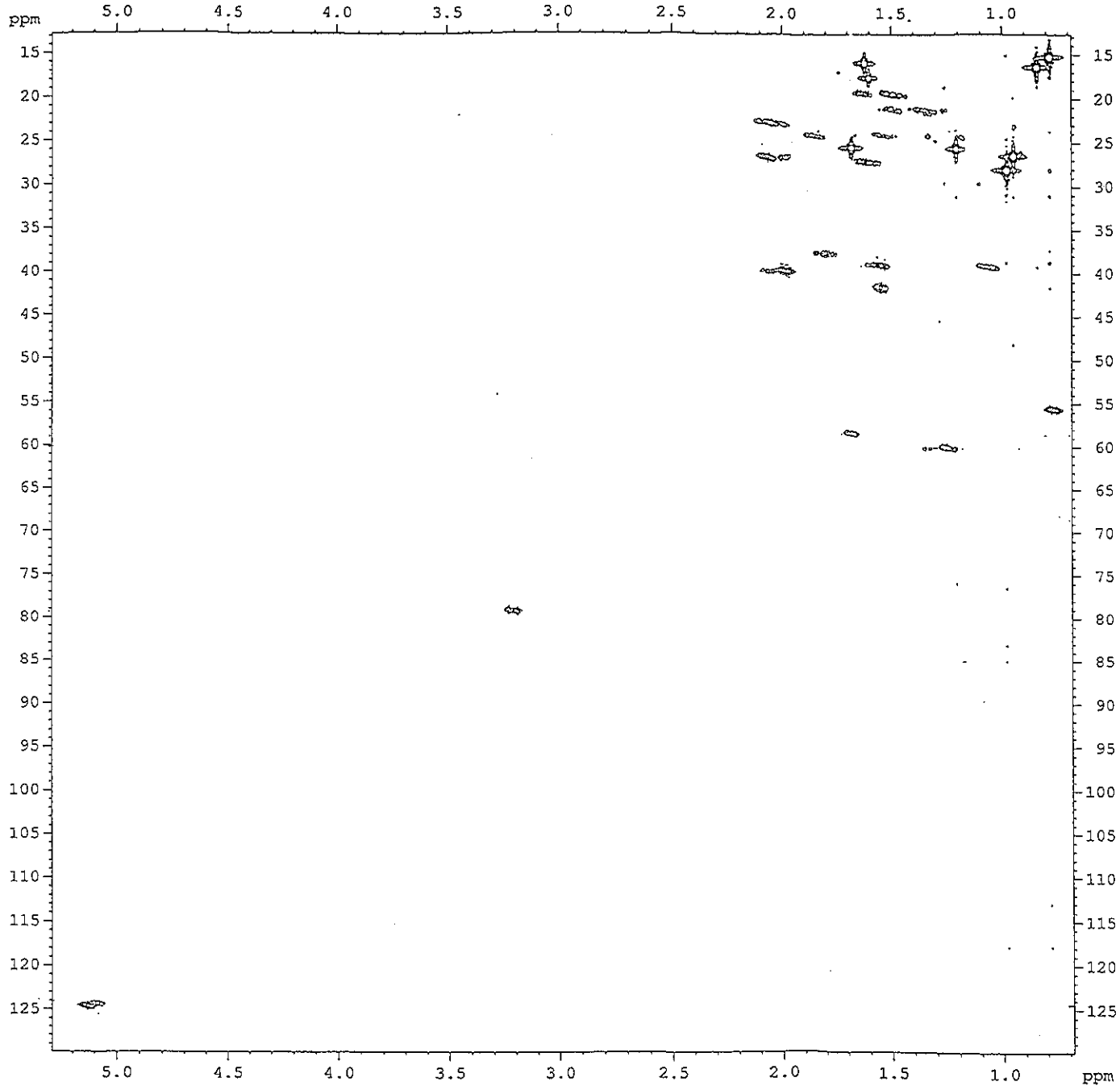


Figure 3.8 2D HMQC spectrum of compound AH3

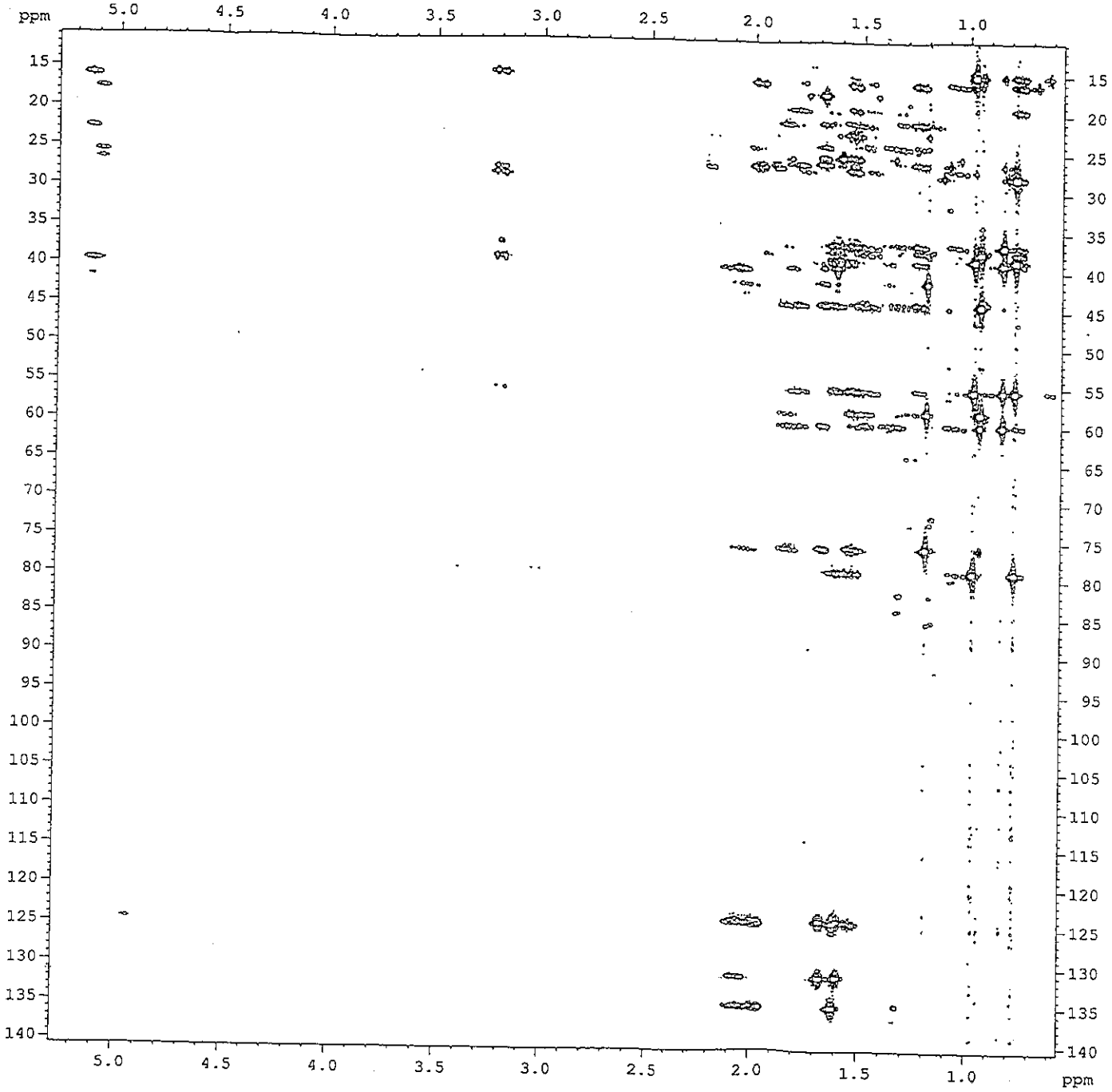


Figure 3.9 2D HMBC spectrum of compound AH3

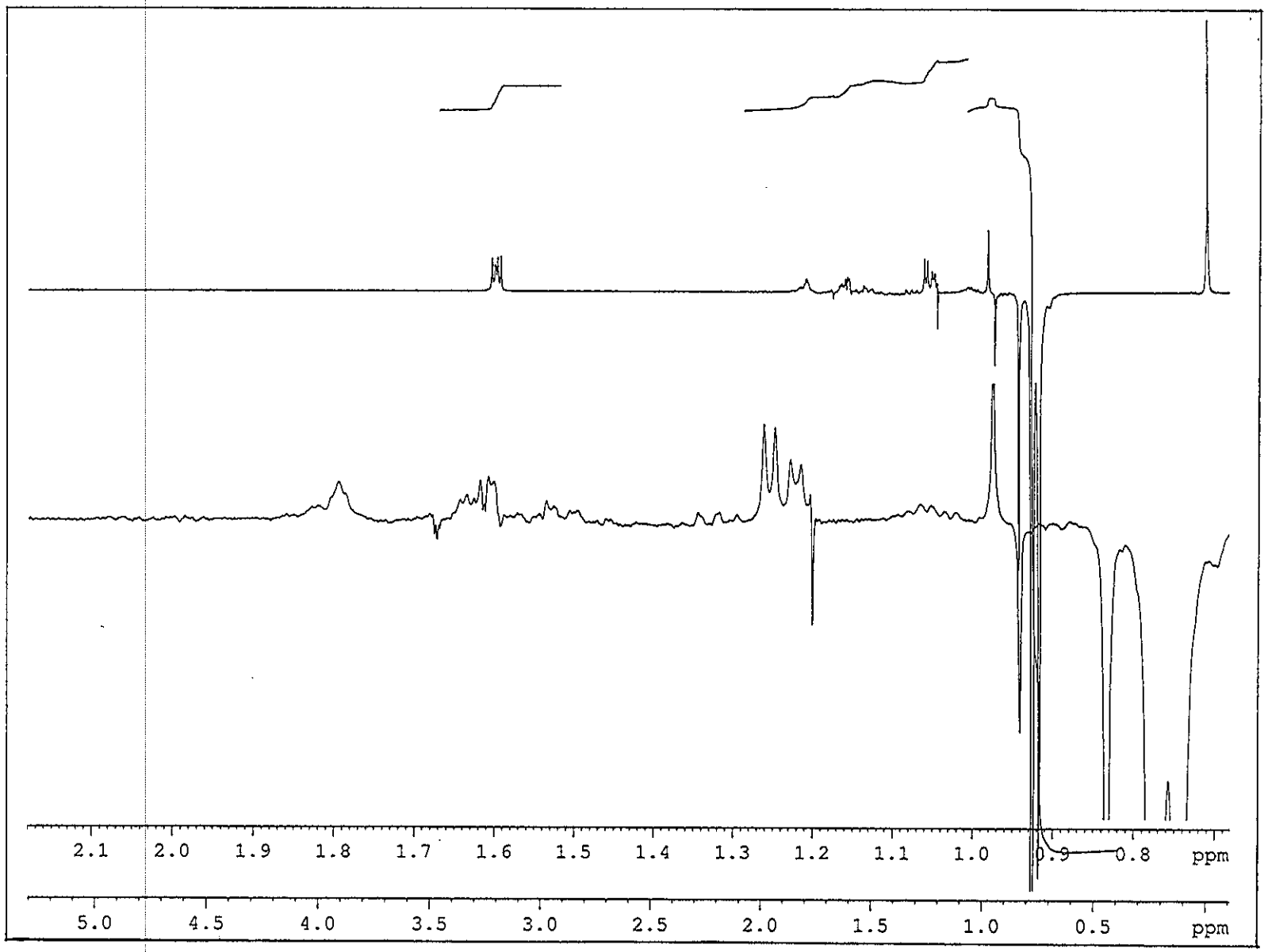


Figure 3.10 NOE difference spectrum of compound AH3 after irradiation at δ_H 0.76 ppm (H-5)

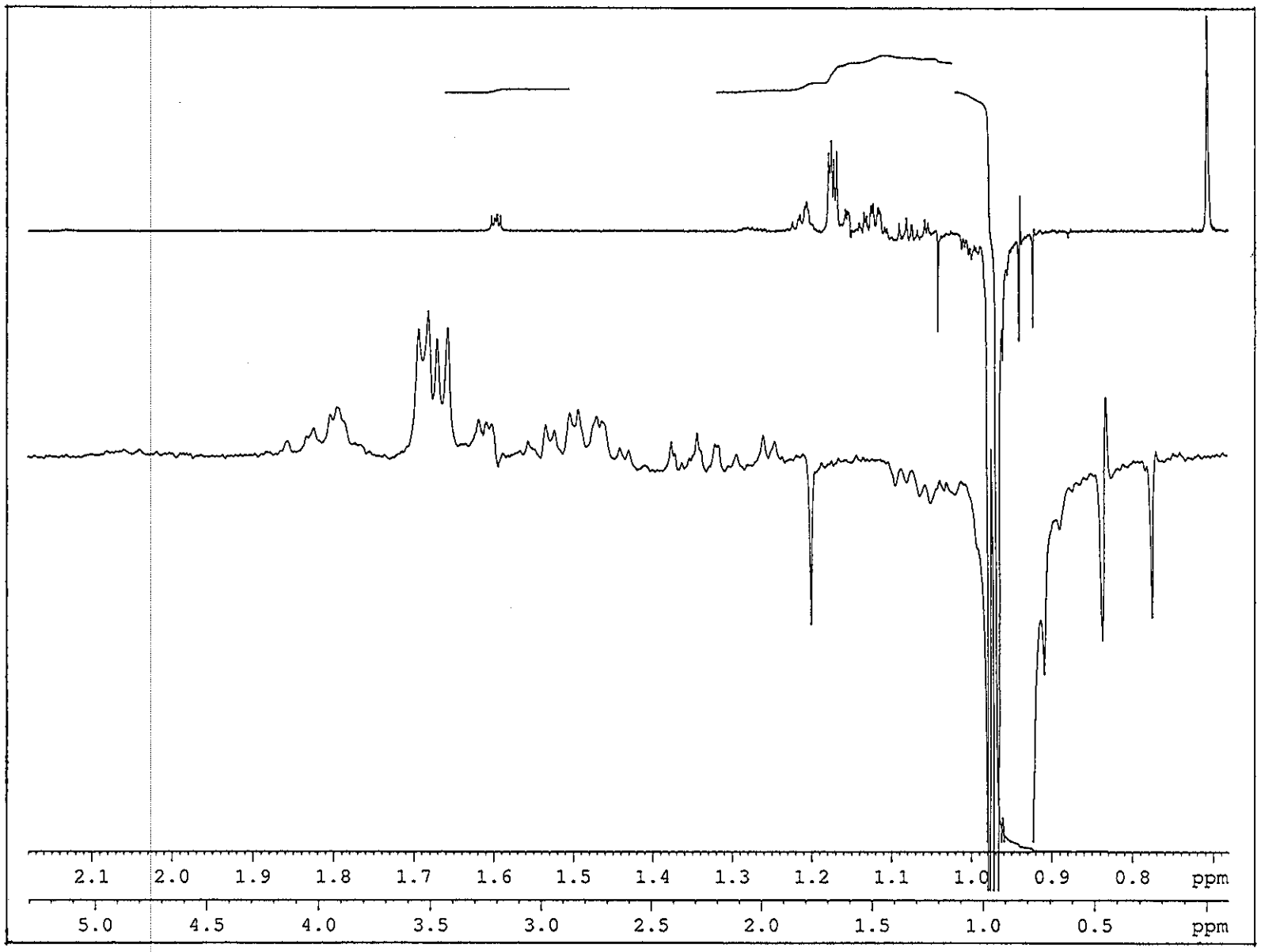


Figure 3.11 NOE difference spectrum of compound AH3 after irradiation at δ_H 0.94 ppm (Me-18)

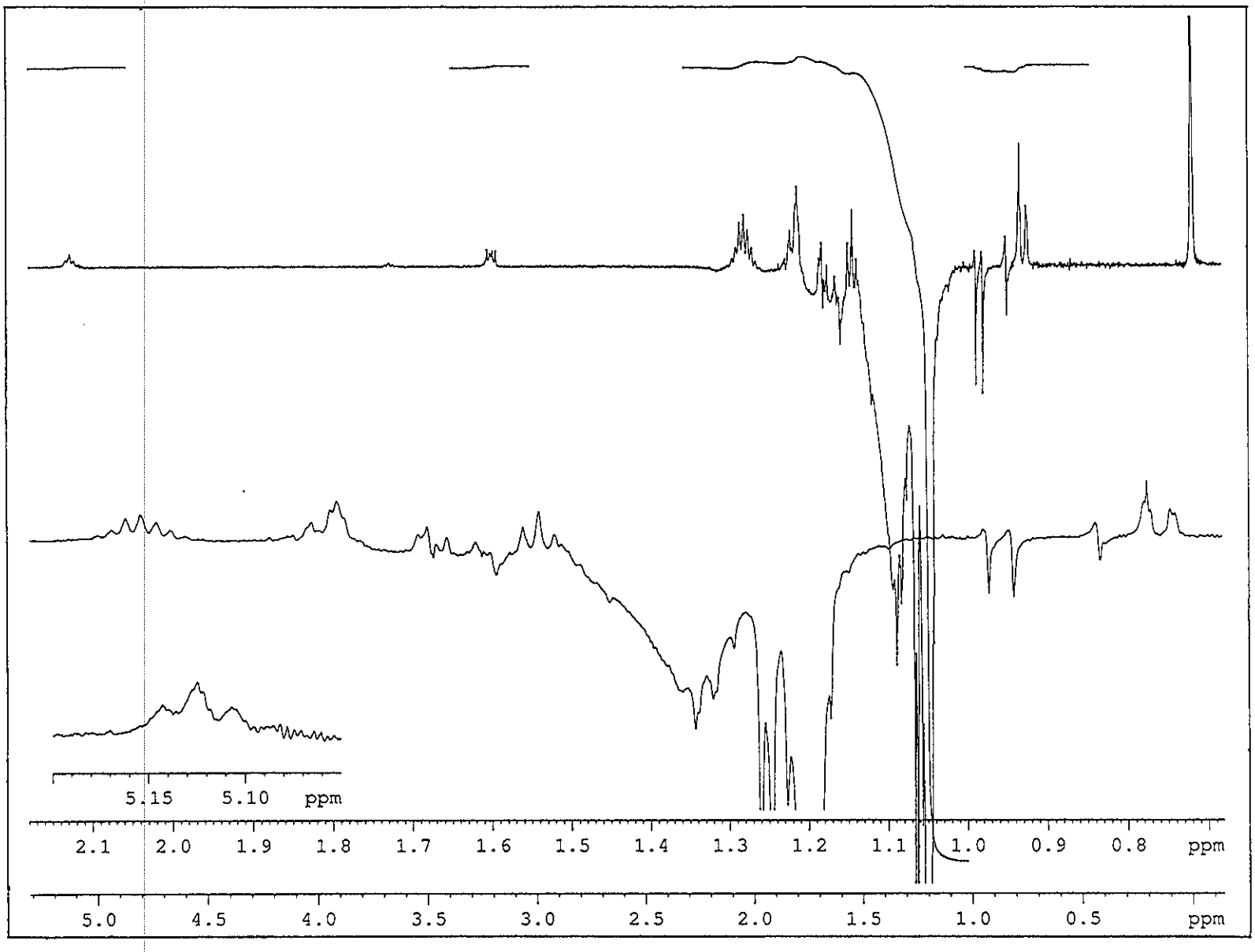


Figure 3.12 NOE difference spectrum of compound AH3 after irradiation at δ_H 1.21 ppm (Me-30)

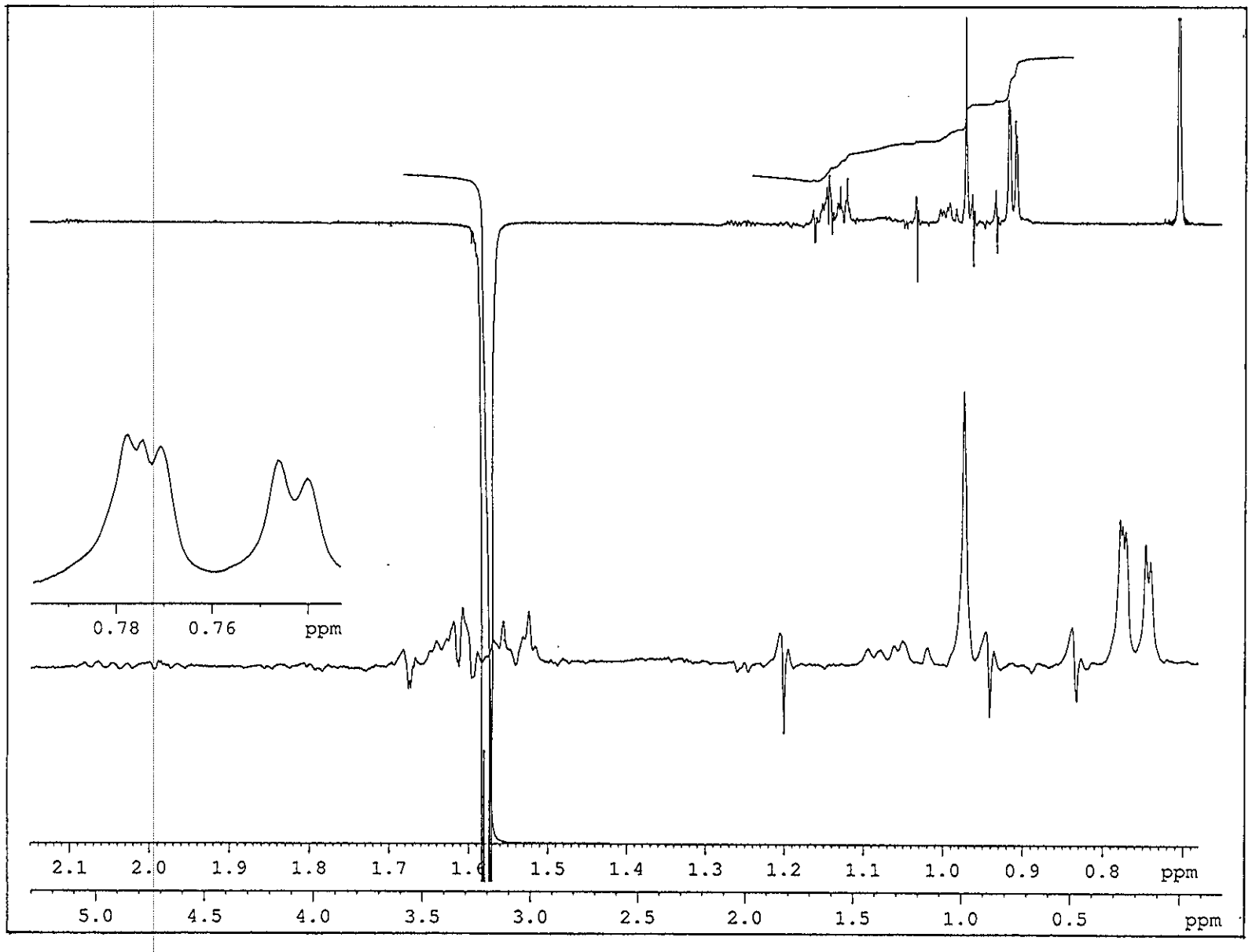


Figure 3.13 NOE difference spectrum of compound AH3 after irradiation at δ_H 3.21 ppm (H-3)

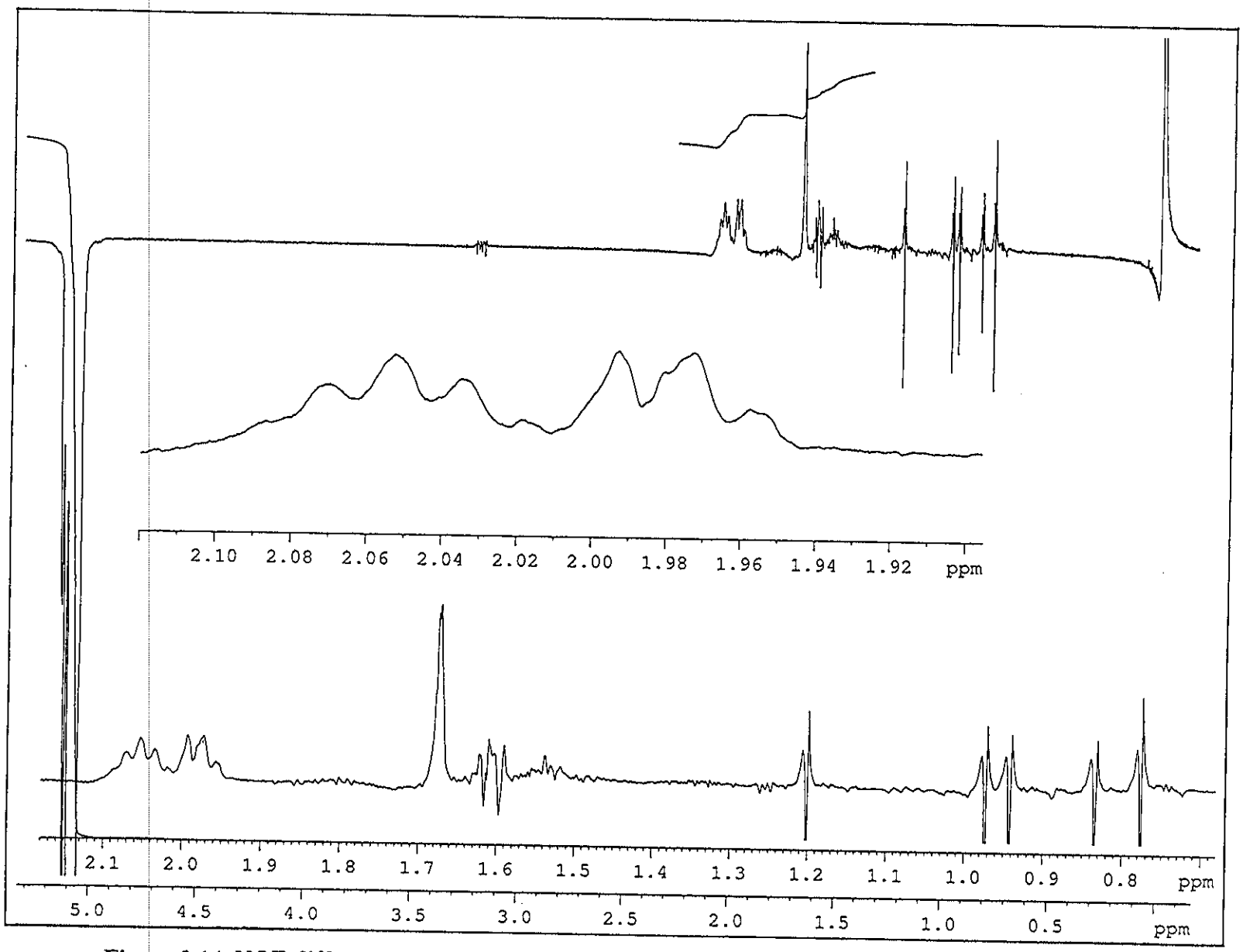


Figure 3.14 NOE difference spectrum of compound AH3 after irradiation at δ_H 5.09 ppm (H-24)

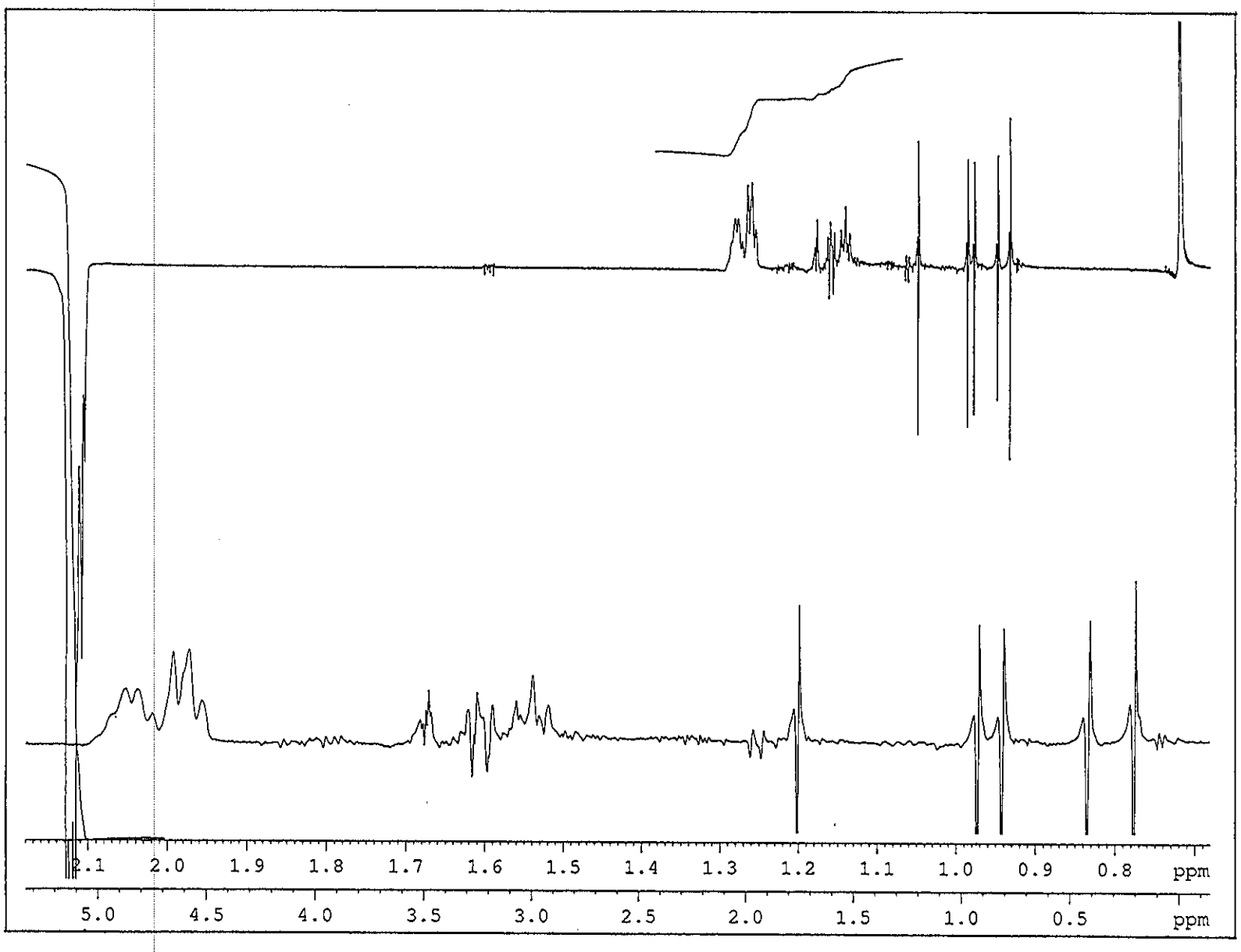


Figure 3.15 NOE difference spectrum of compound AH3 after irradiation at δ_H 5.13 ppm (H-22)

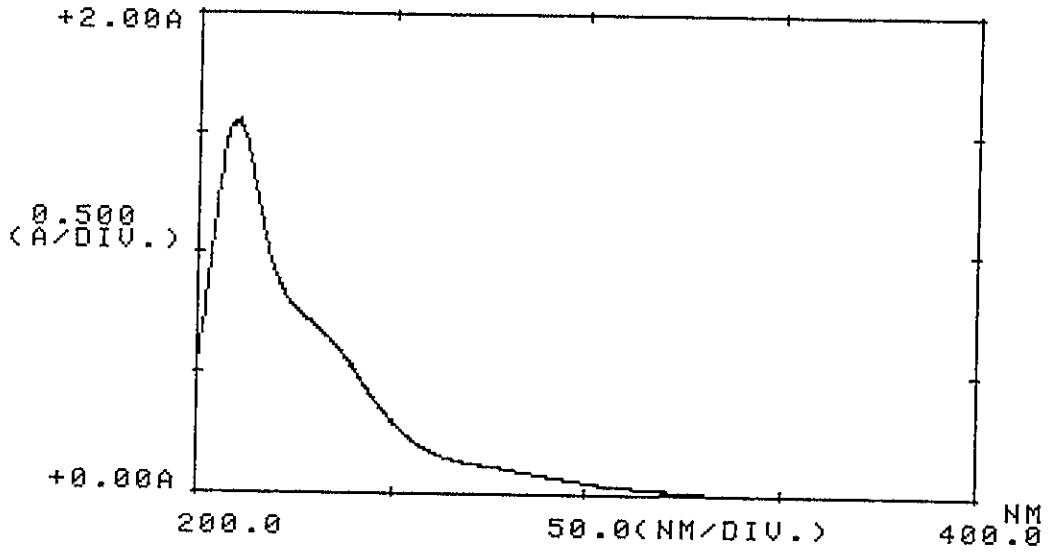


Figure 3.16 UV (MeOH) spectrum of compound AH3-Ac

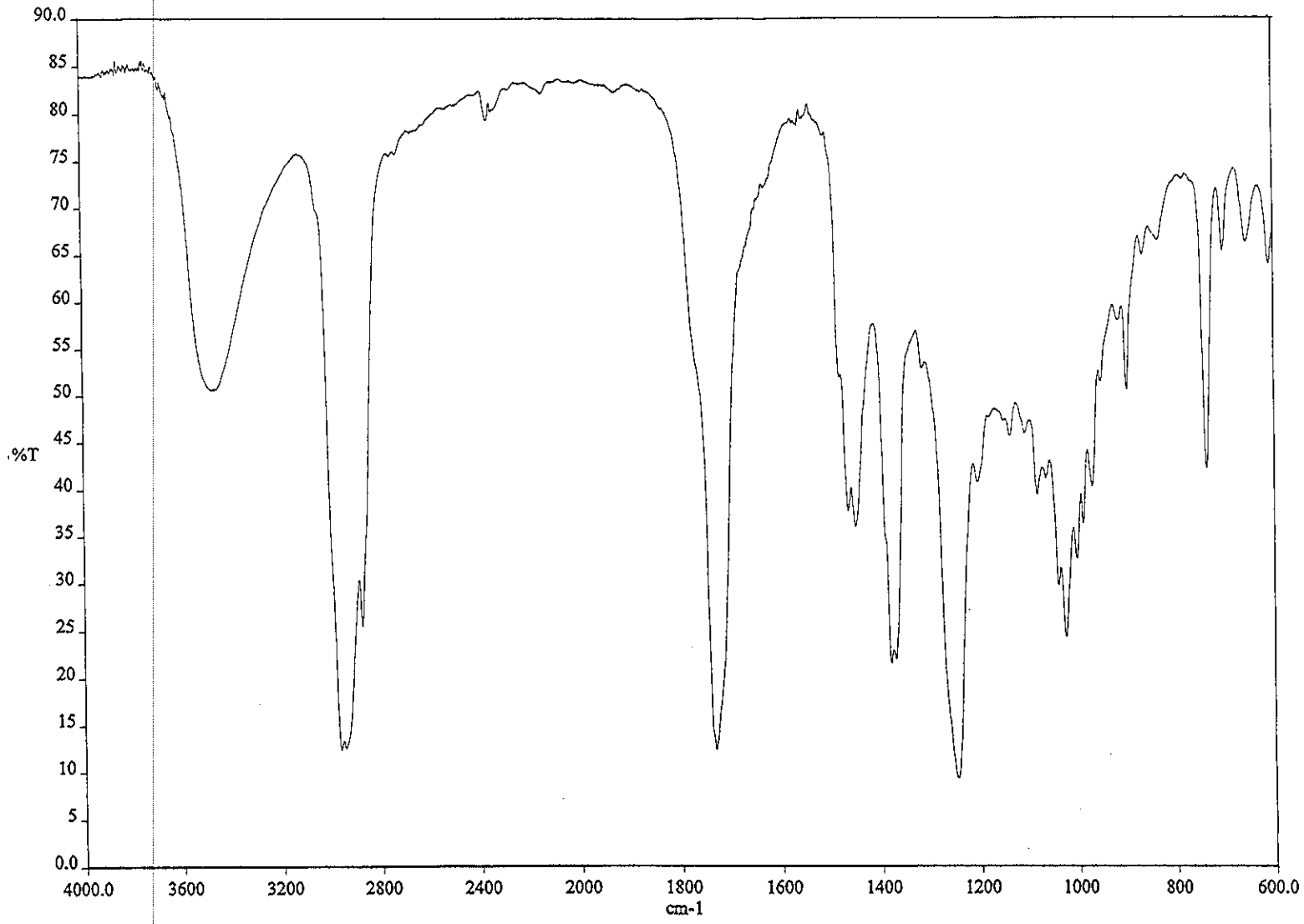


Figure 3.17 FT-IR (neat) spectrum of compound AH3-Ac

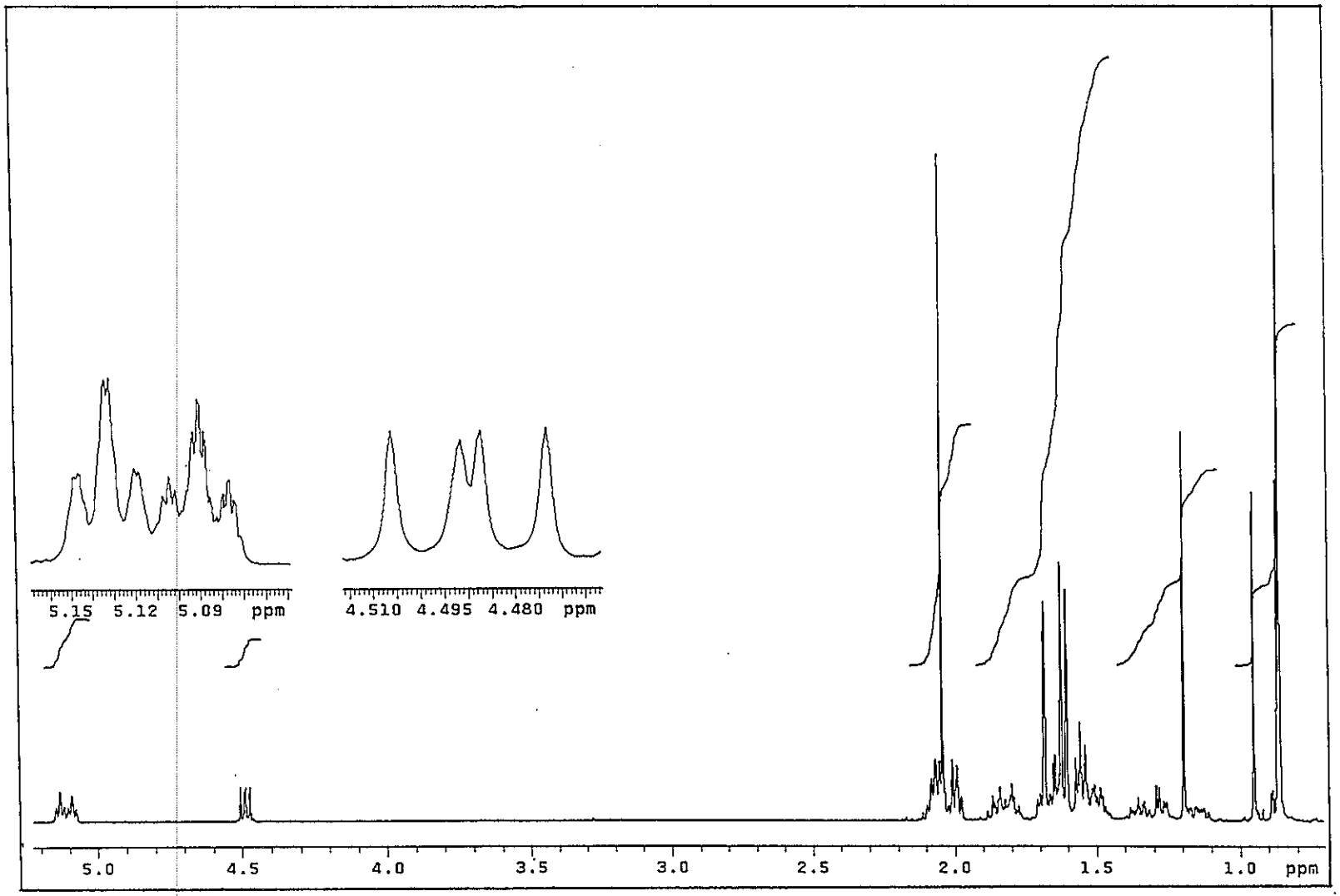


Figure 3.18 ^{1}H NMR (500 MHz)($CDCl_3$) spectrum of compound AH3-Ac

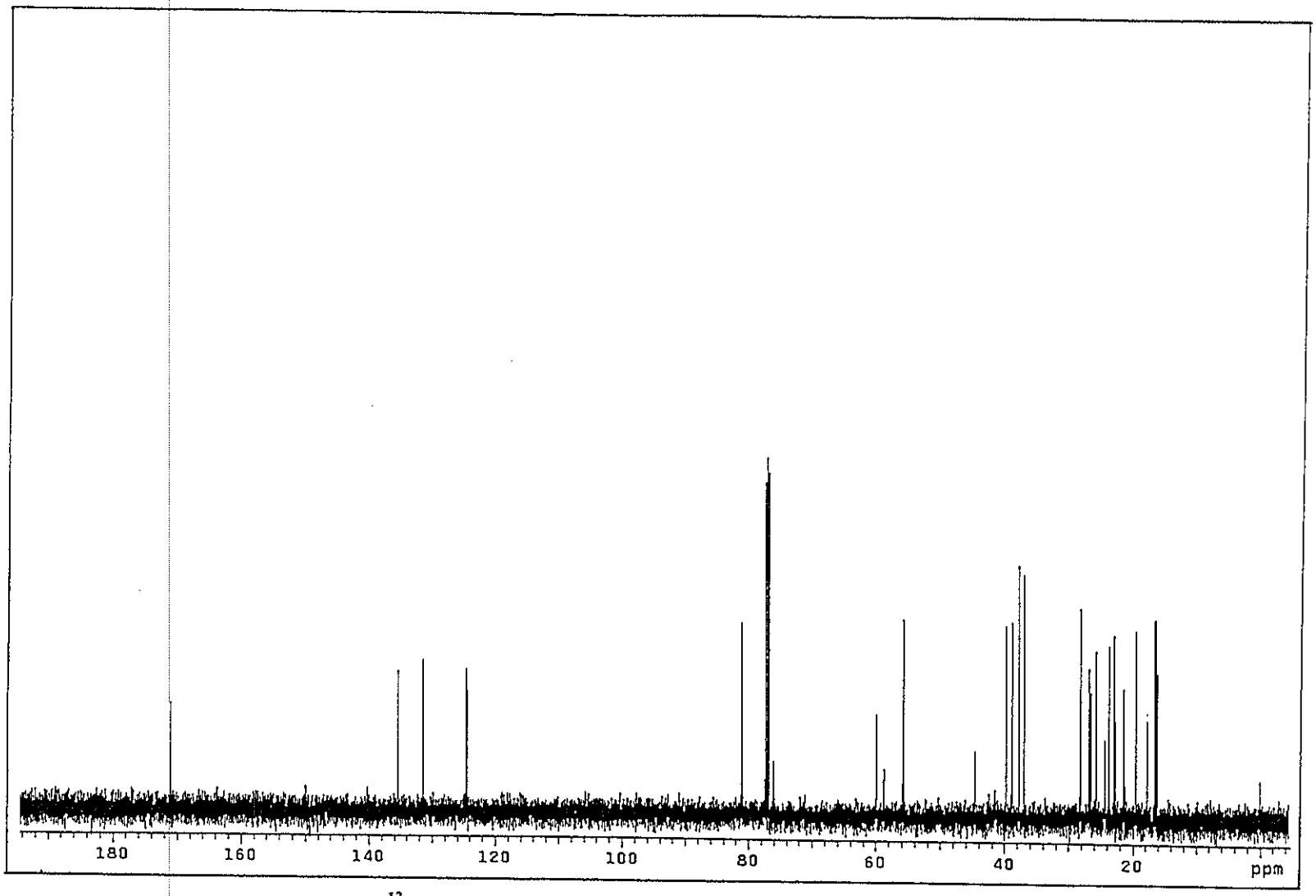


Figure 3.19 ^{13}C NMR (125 MHz)($CDCl_3$) spectrum of compound AH3-Ac

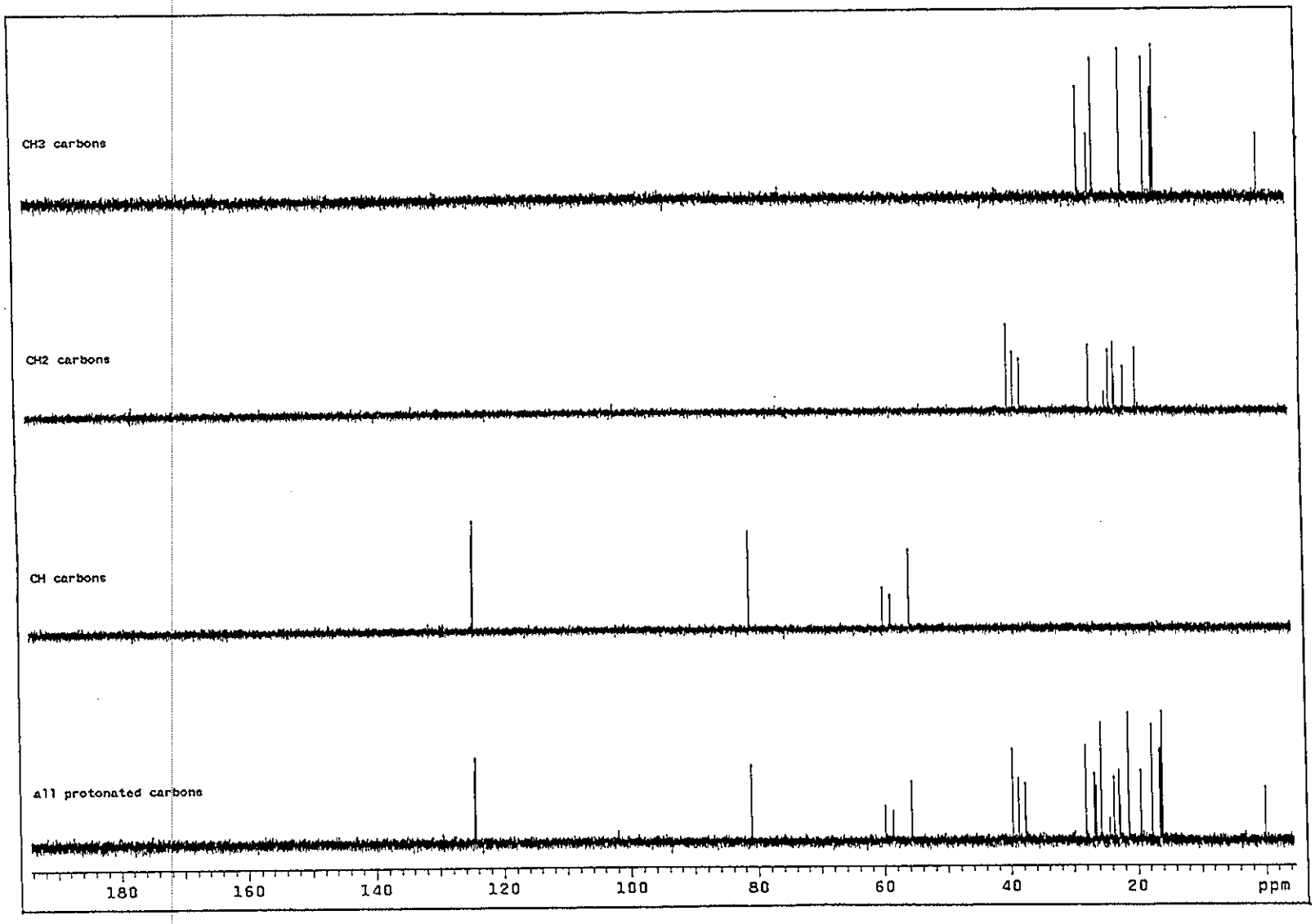


Figure 3.20 DEPT spectrum of compound AH3-Ac

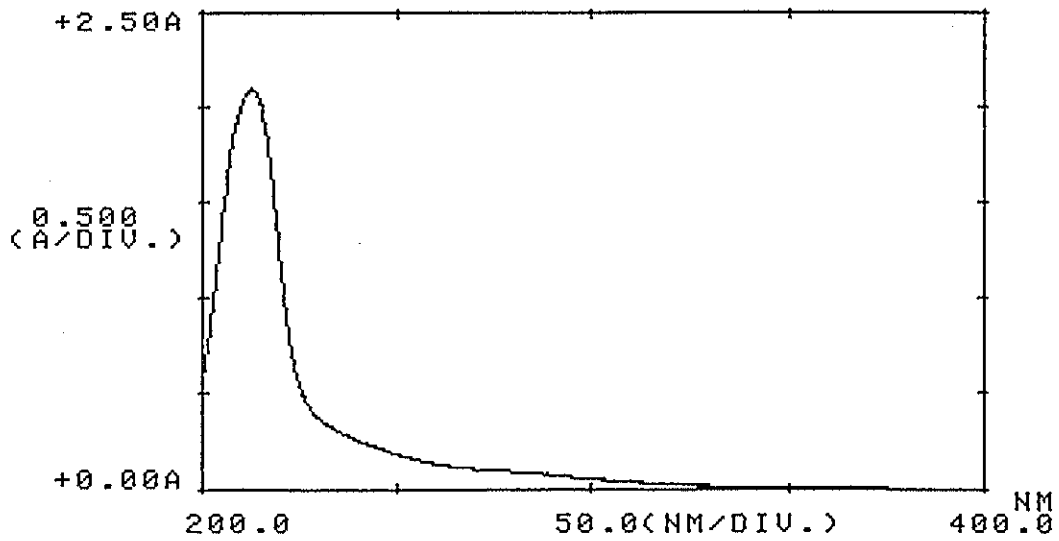


Figure 3.21 UV (MeOH) spectrum of compound AH2

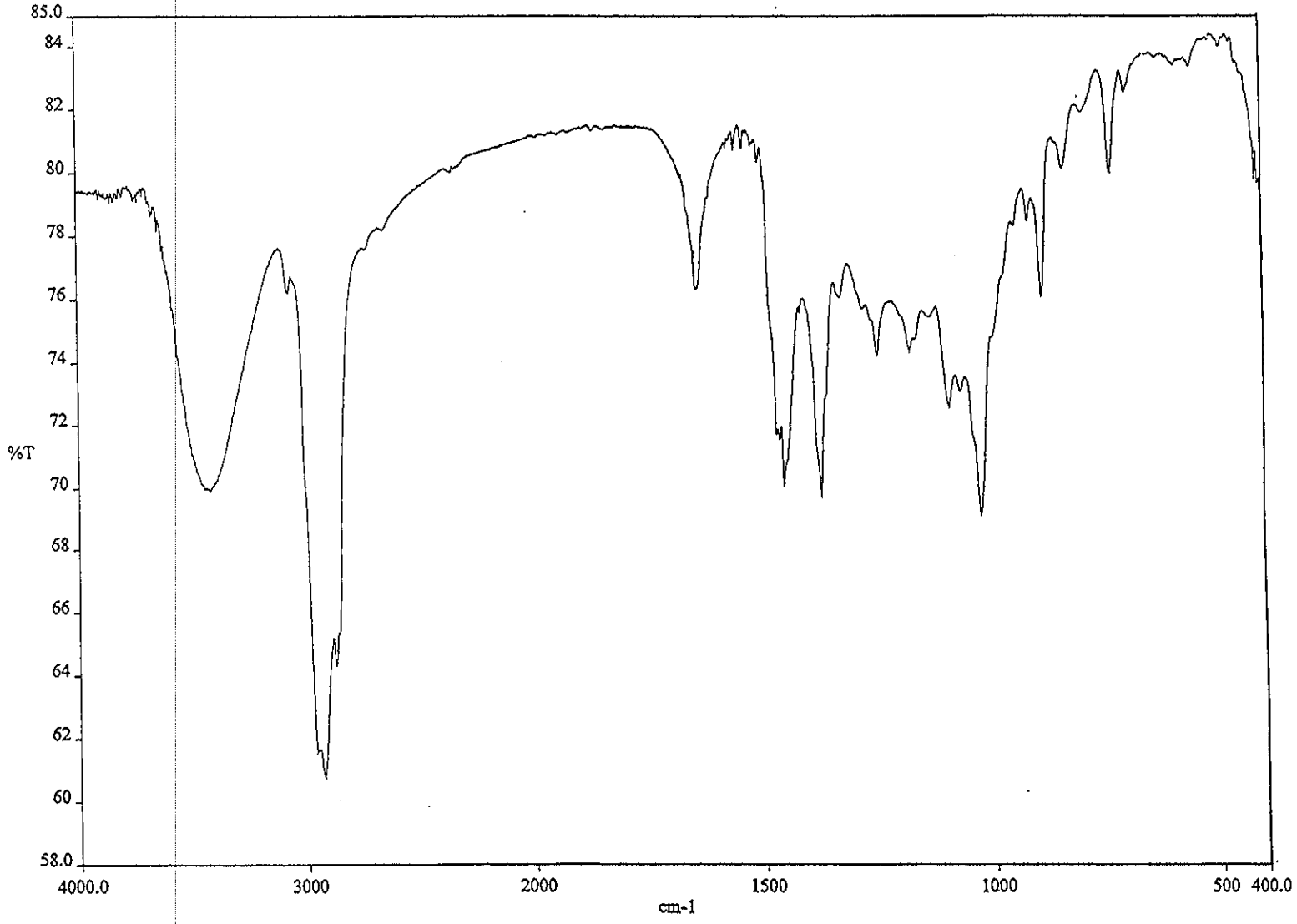


Figure 3.22 FT-IR (neat) spectrum of compound AH2

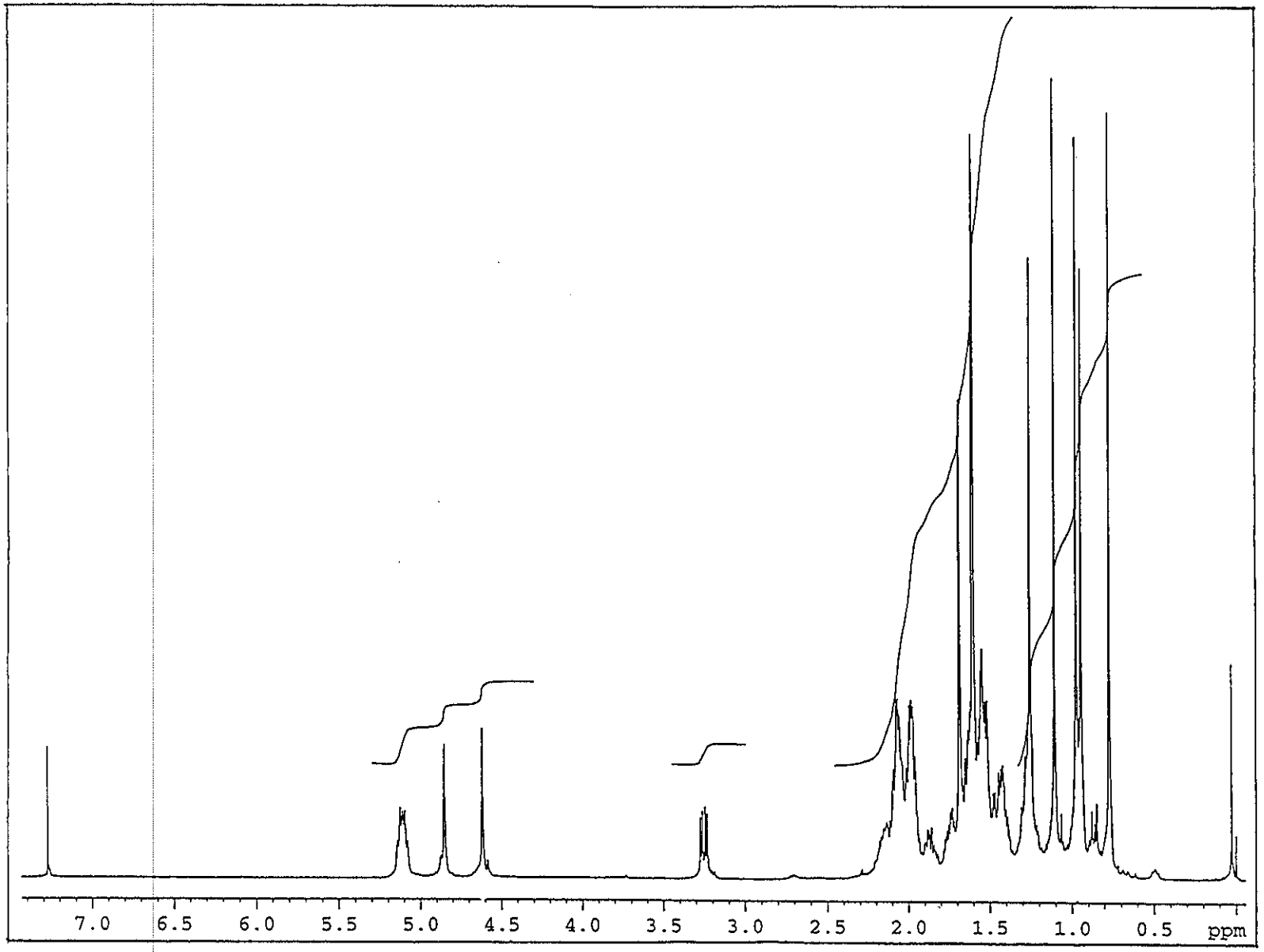


Figure 3.23 ^{1}H NMR (400 MHz)($CDCl_3$) spectrum of compound AH2

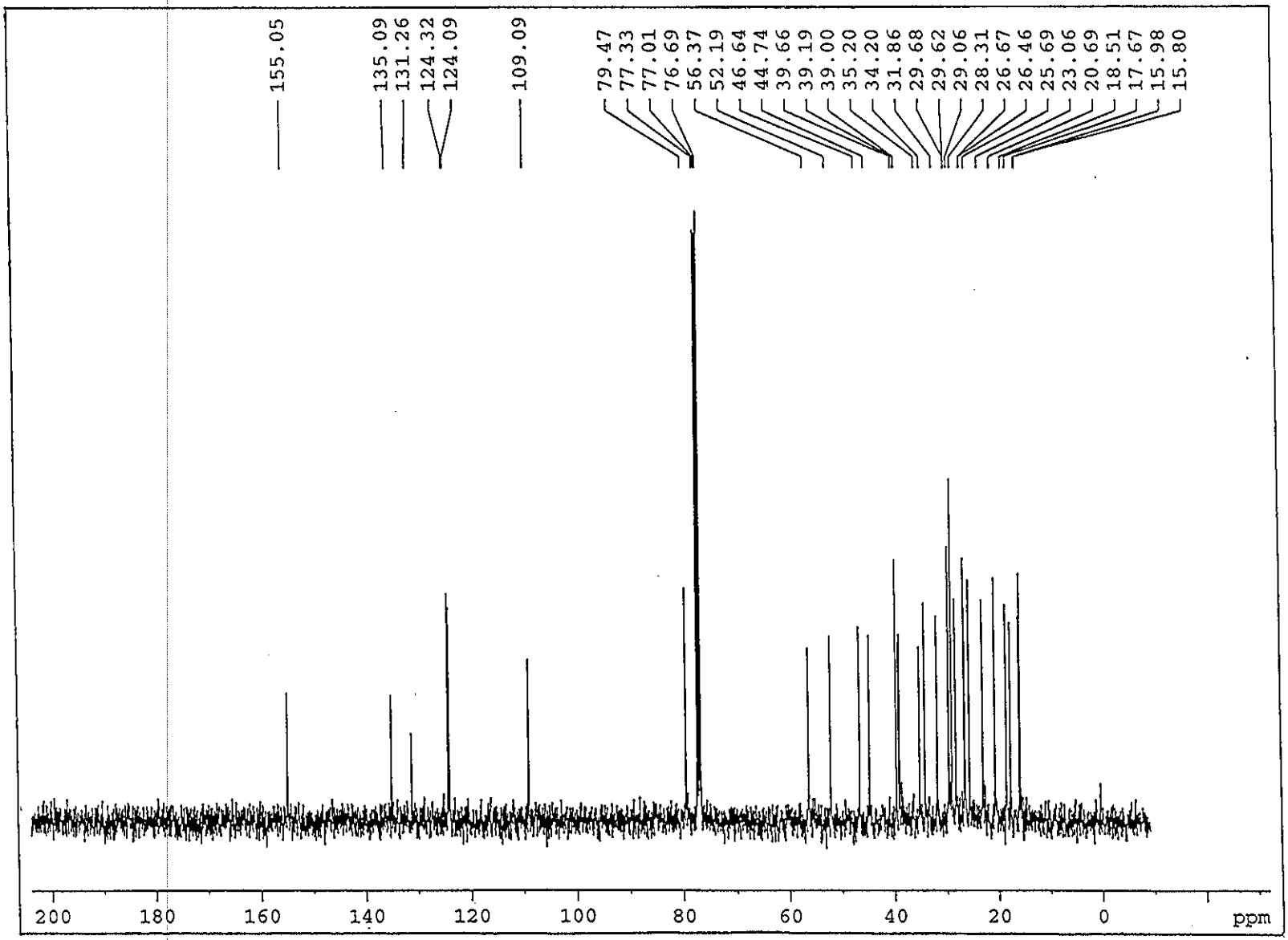


Figure 3.24 ^{13}C NMR (100 MHz)($CDCl_3$) spectrum of compound AH2

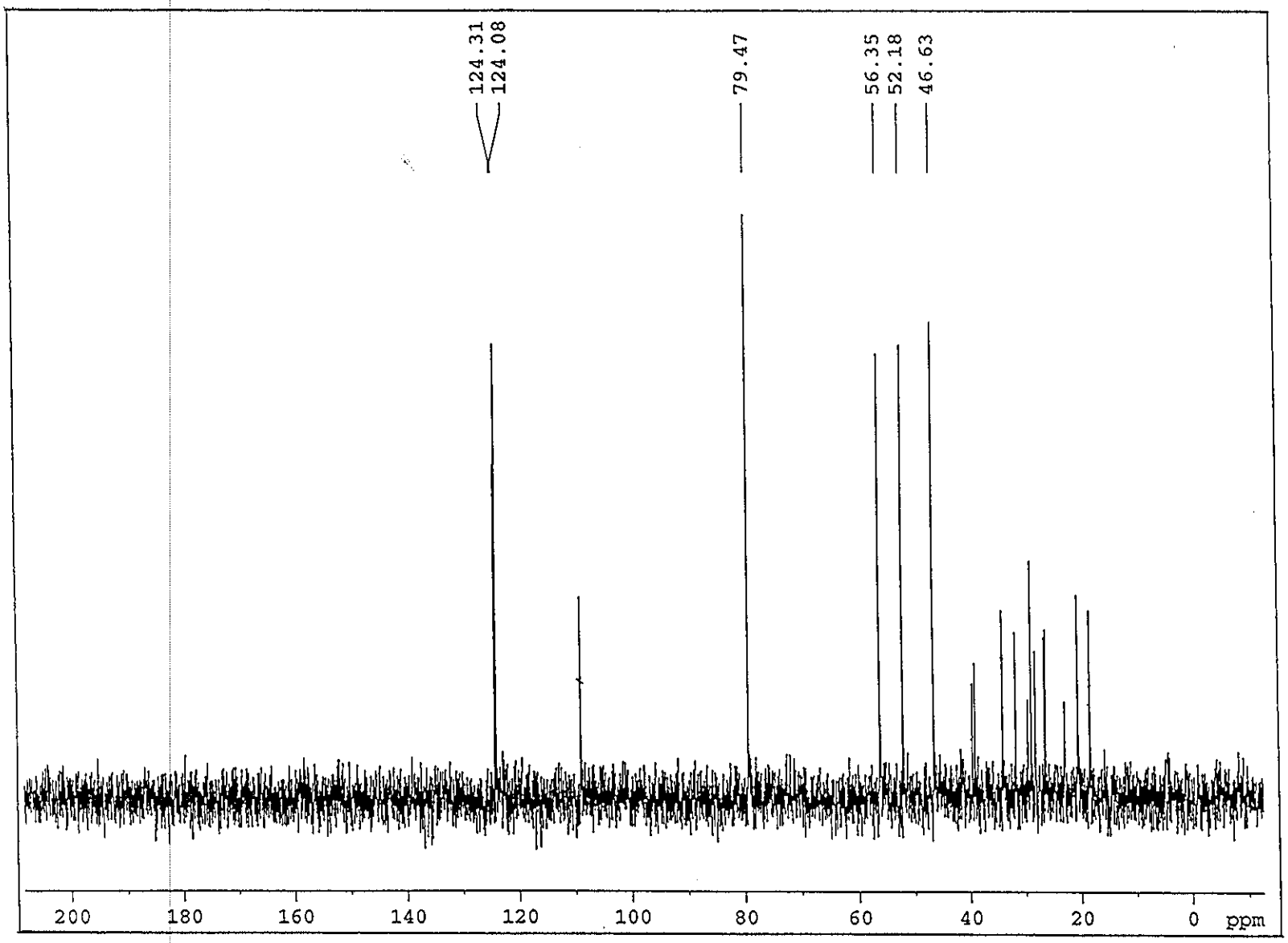


Figure 3.25 DEPT 90° spectrum of compound AH2

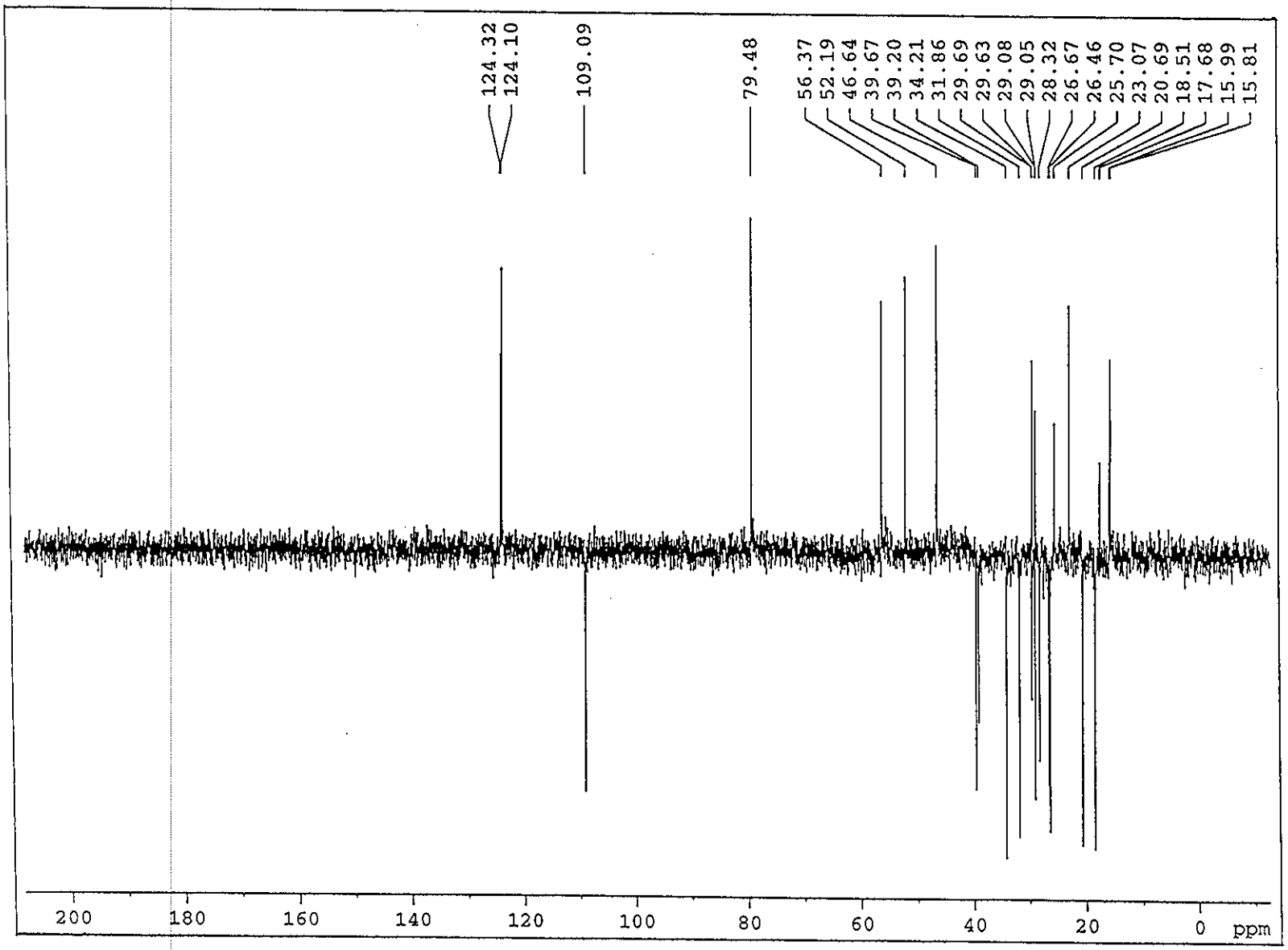


Figure 3.26 DEPT 135° spectrum of compound AH2

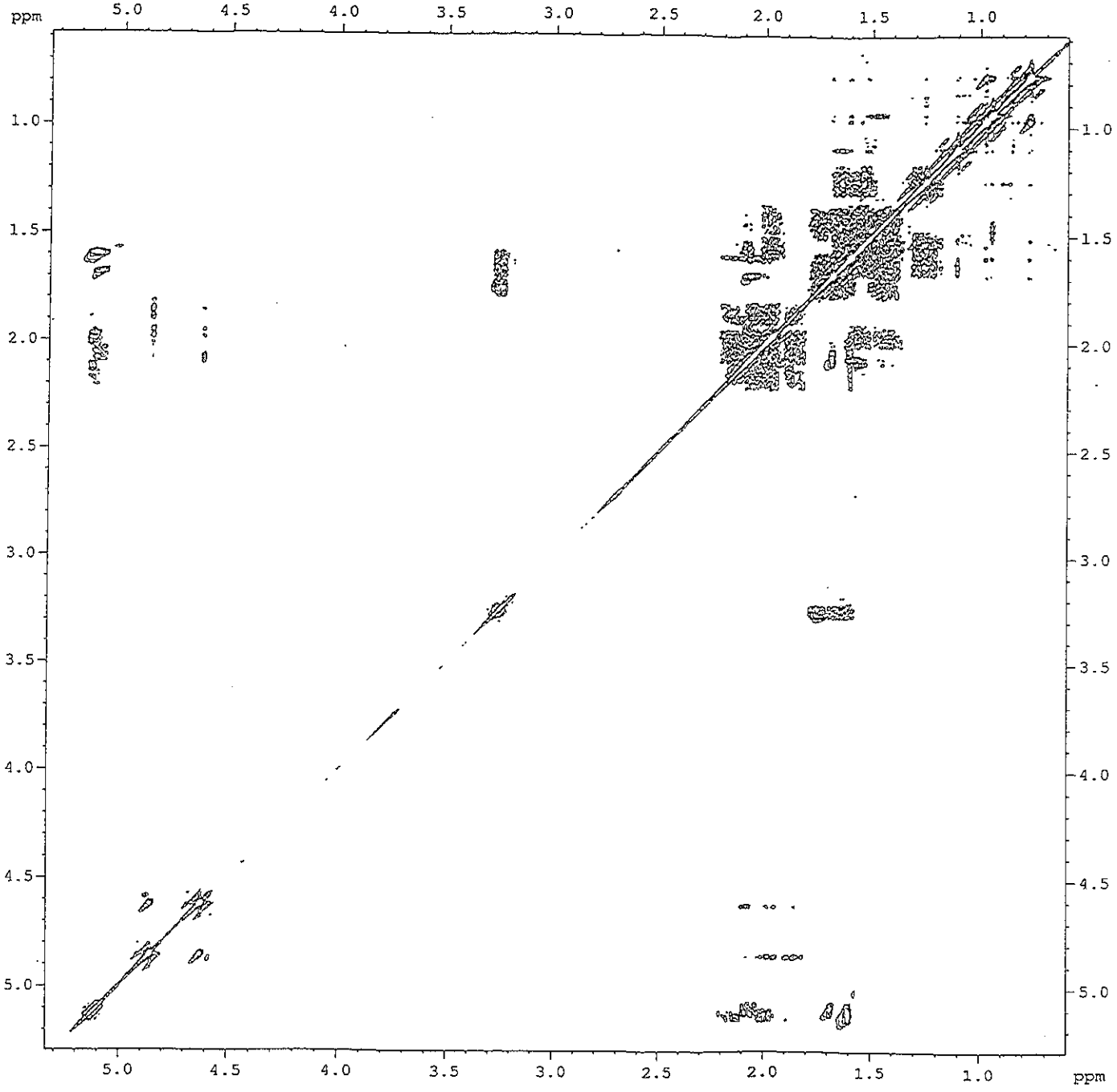


Figure 3.27 ^{1}H-^{1}H COSY spectrum of compound AH2

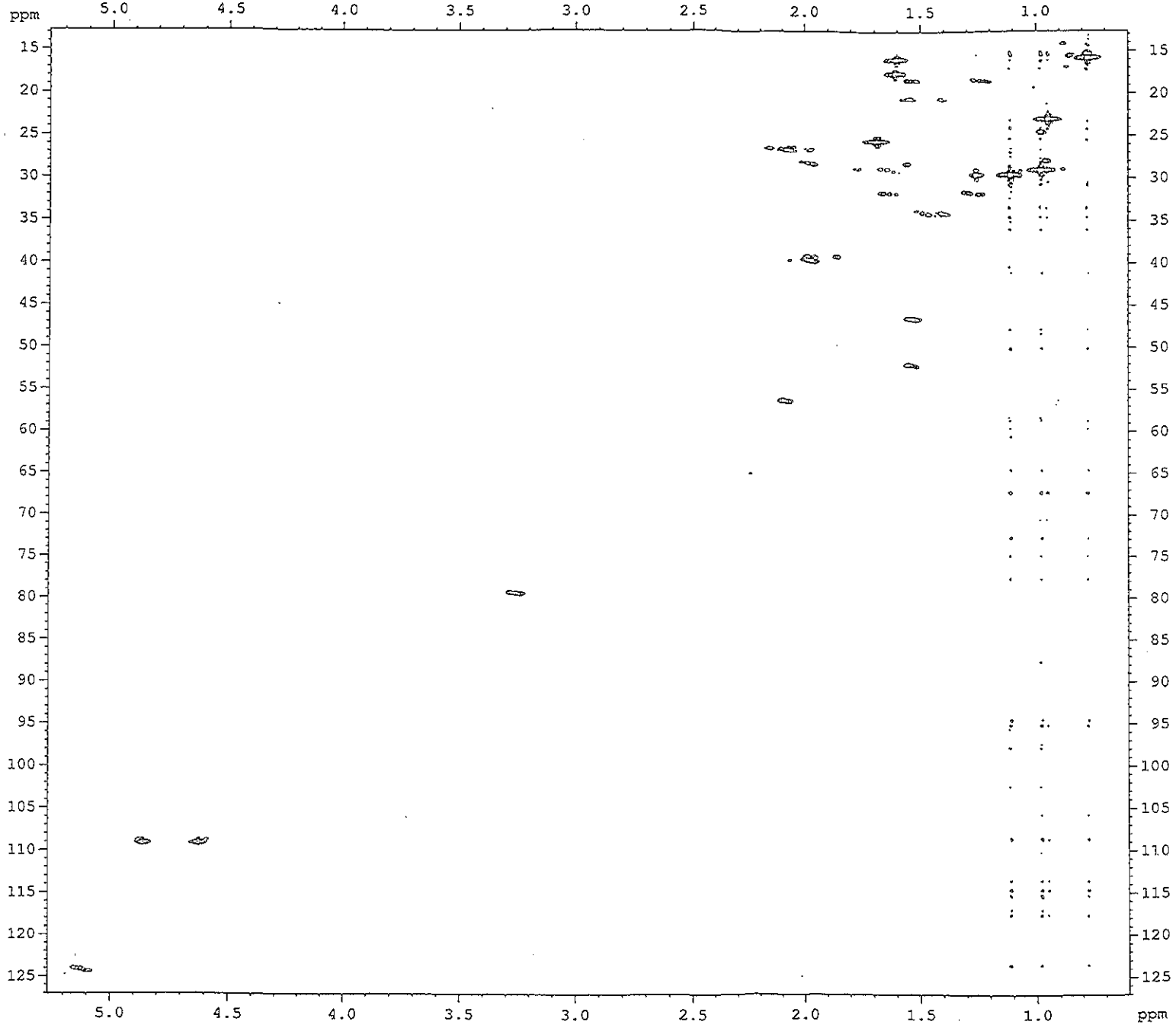


Figure 3.28 2D HMQC spectrum of compound AH2

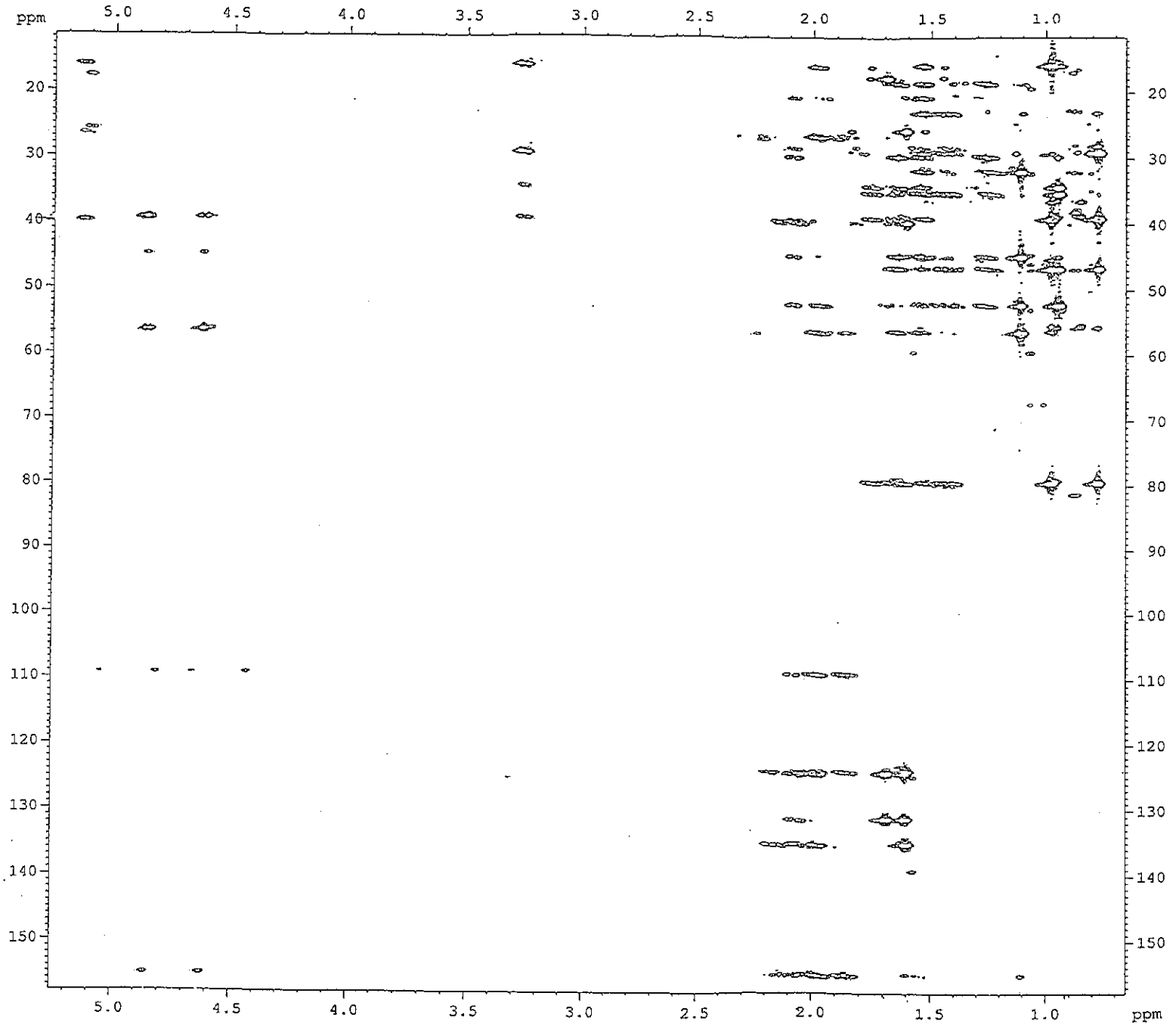


Figure 3.29 2D HMBC spectrum of compound AH2

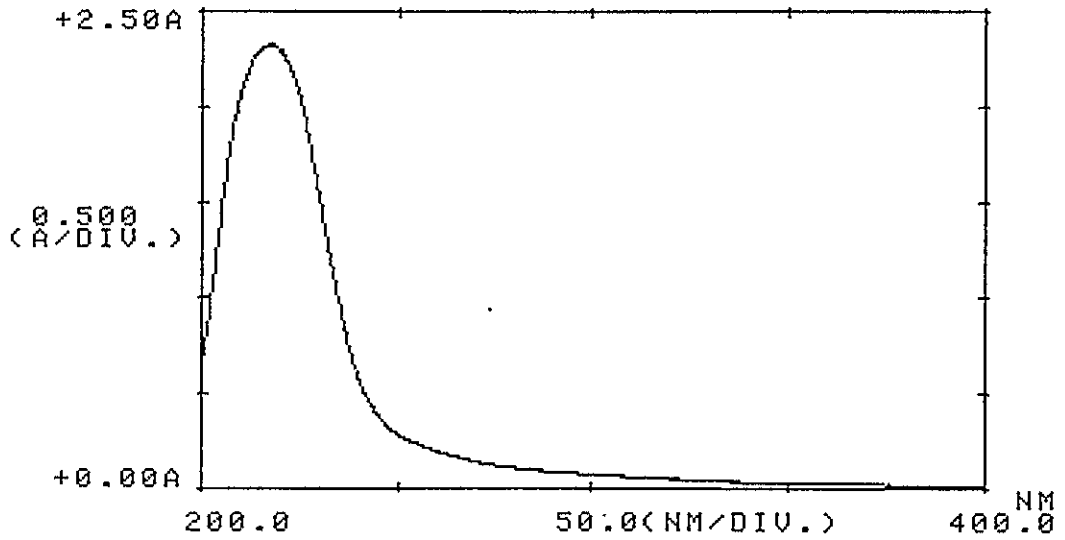


Figure 3.30 UV (MeOH) spectrum of compound AH4

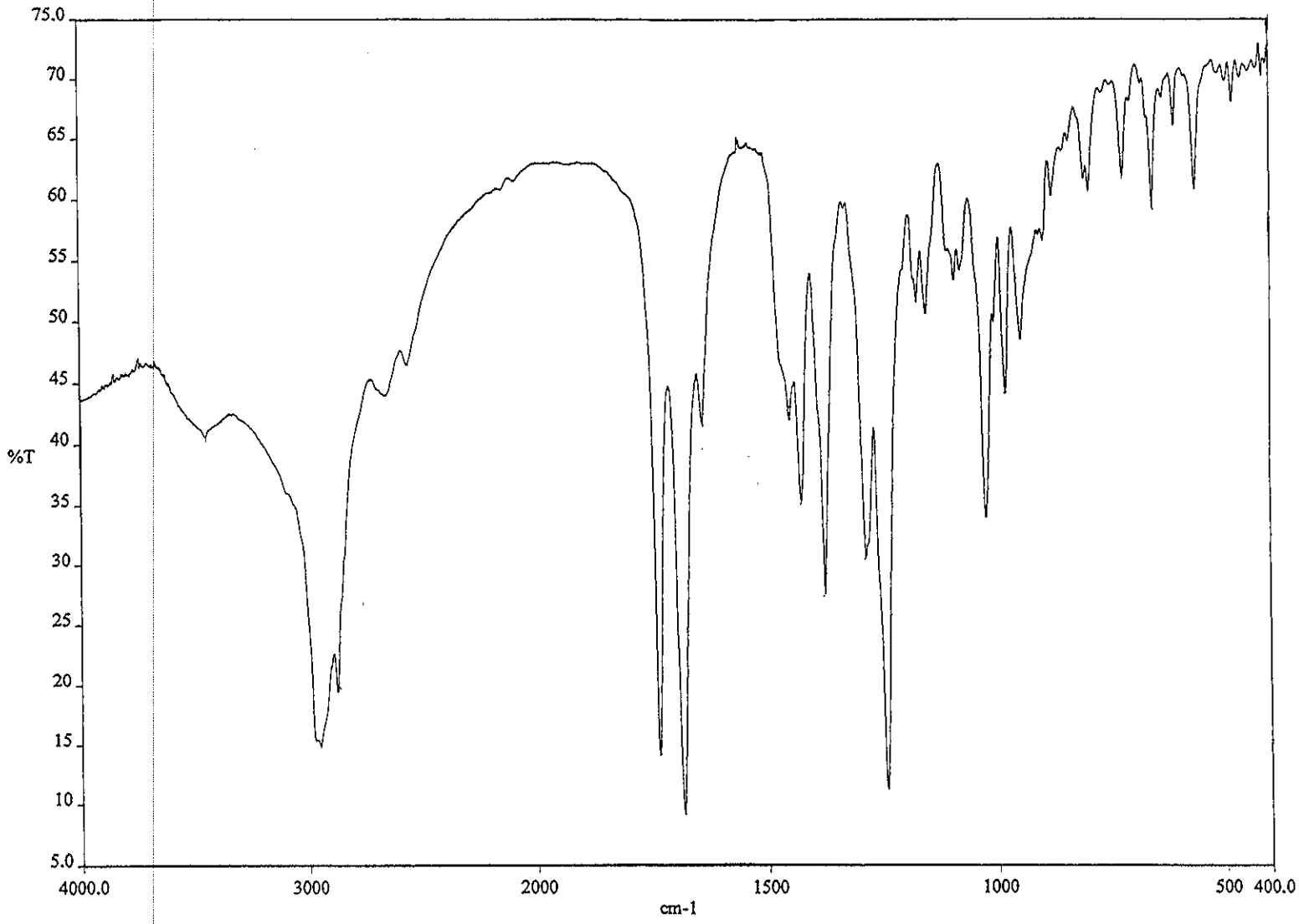


Figure 3.31 FT-IR (KBr) spectrum of compound AH4

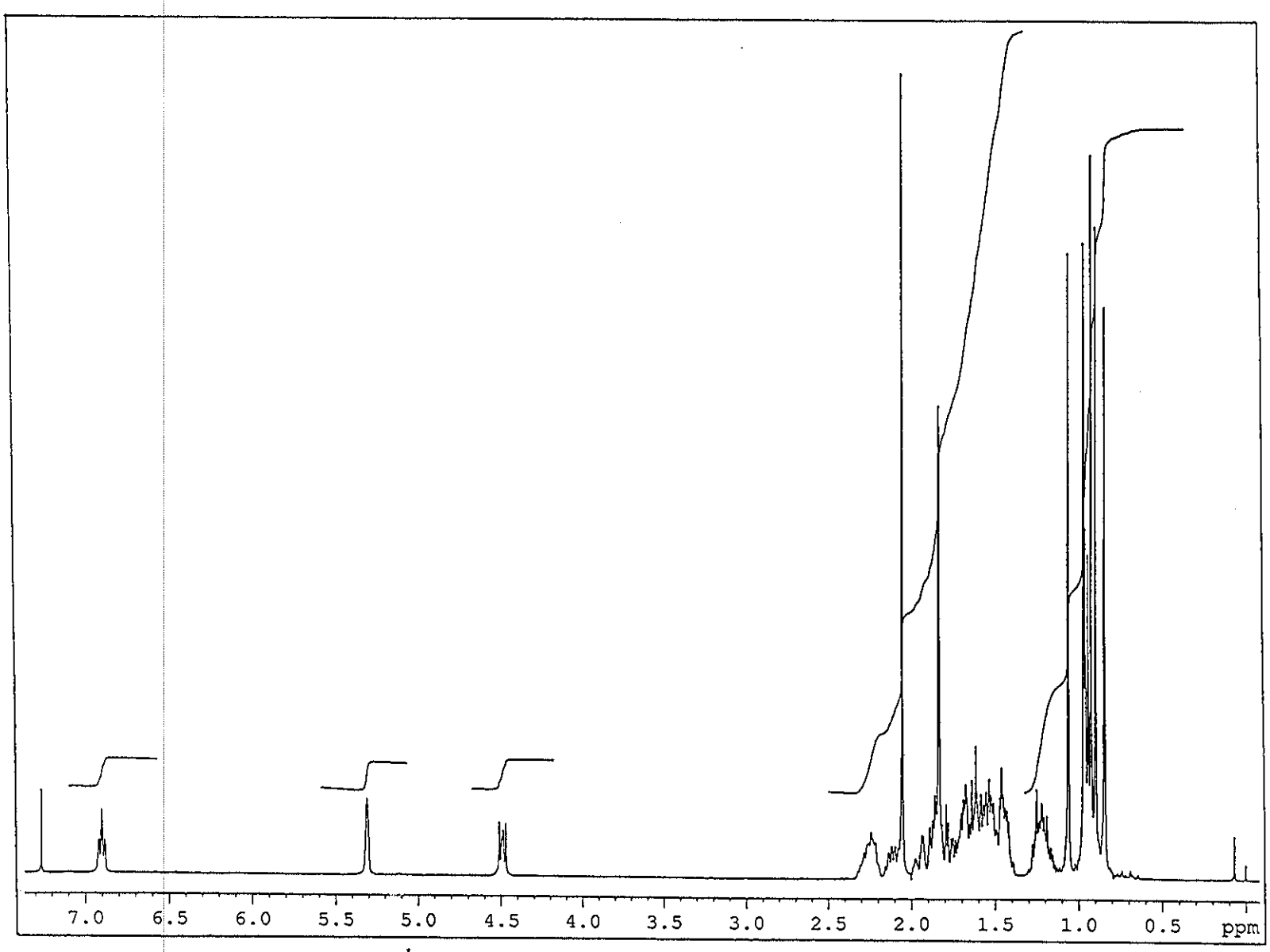


Figure 3.32 ^{1}H NMR (400 MHz)($CDCl_3$) spectrum of compound AH4

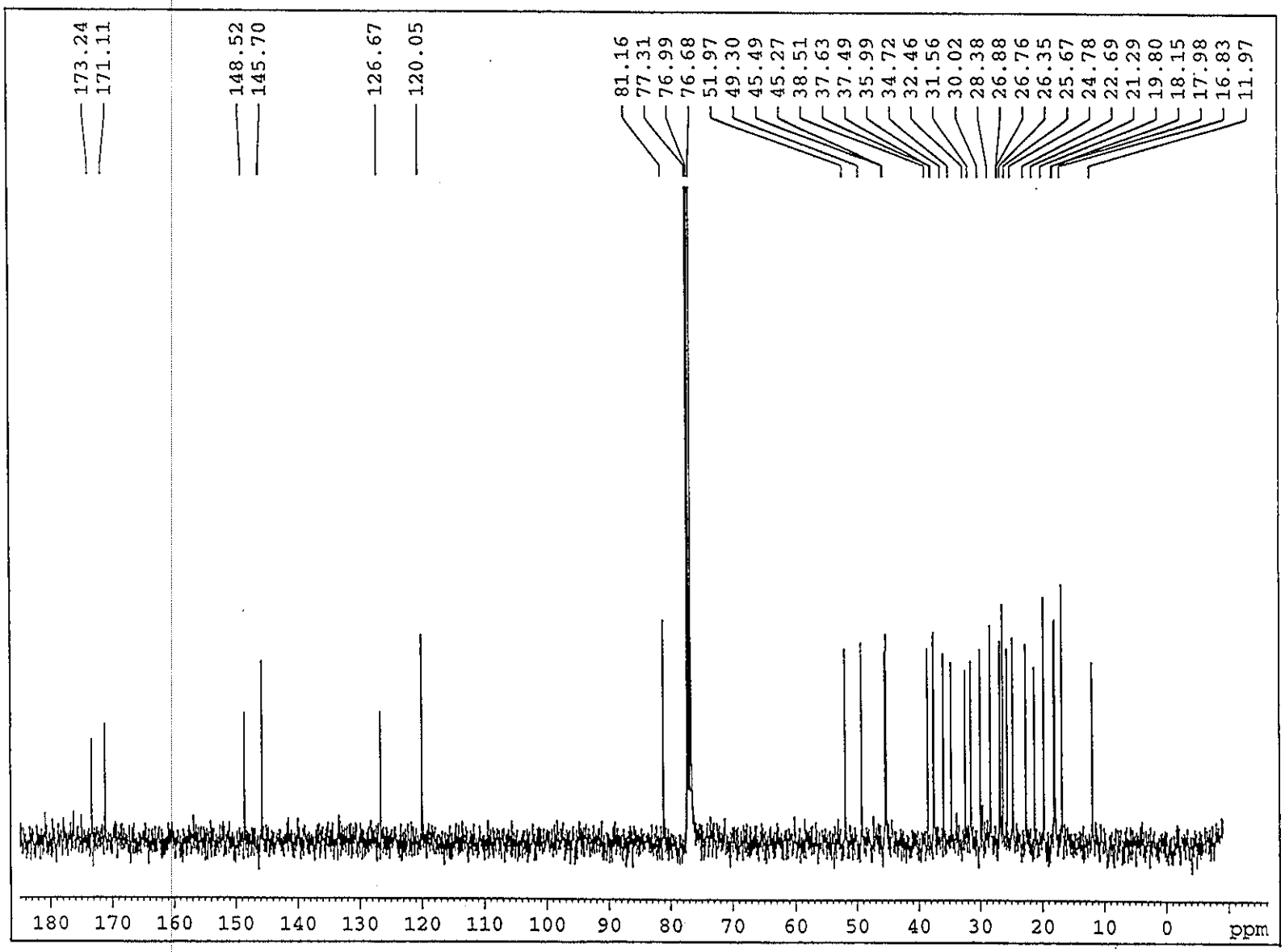


Figure 3.33 ^{13}C NMR (100 MHz)($CDCl_3$) spectrum of compound AH4

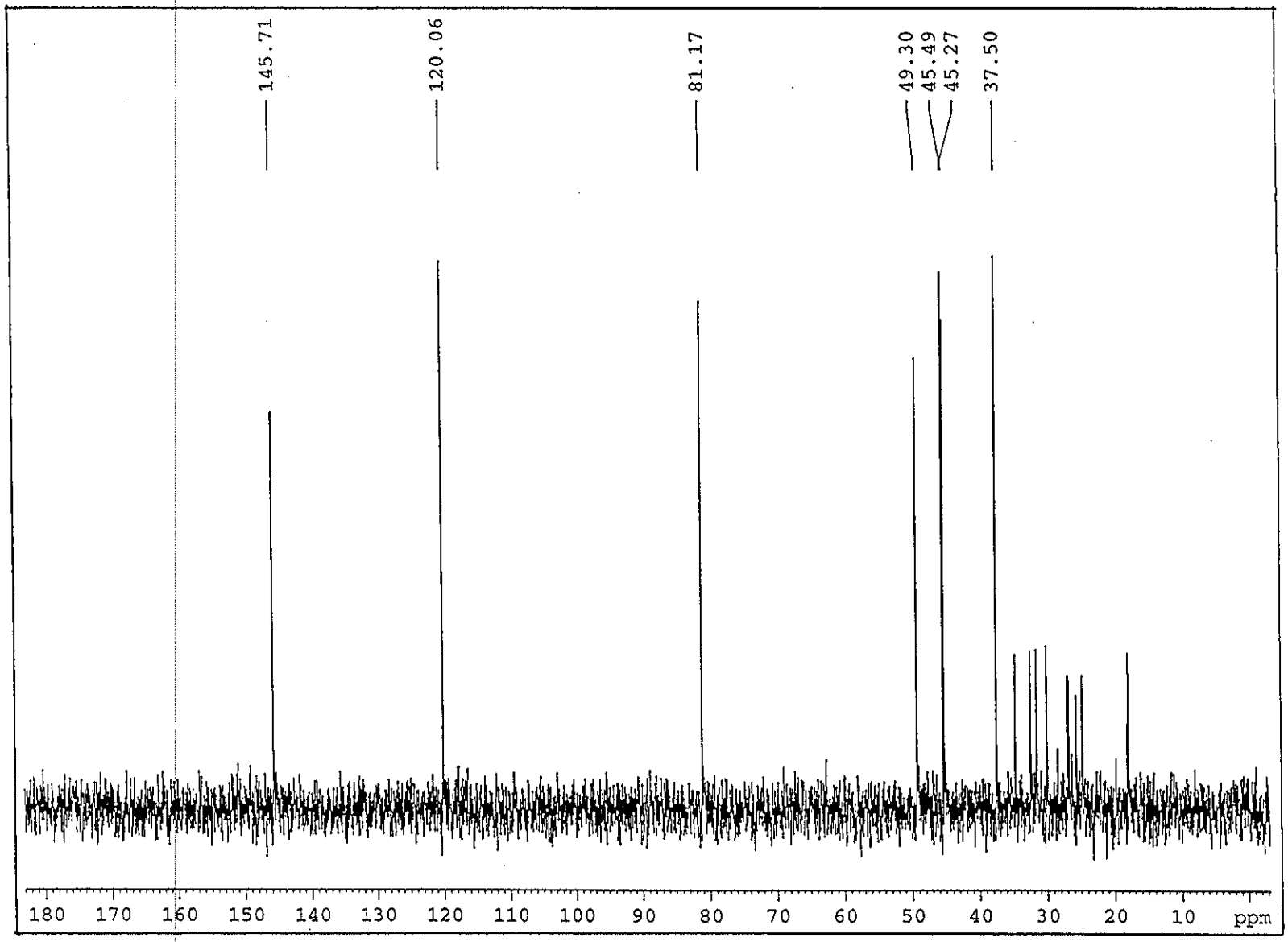


Figure 3.34 DEPT 90° spectrum of compound AH4

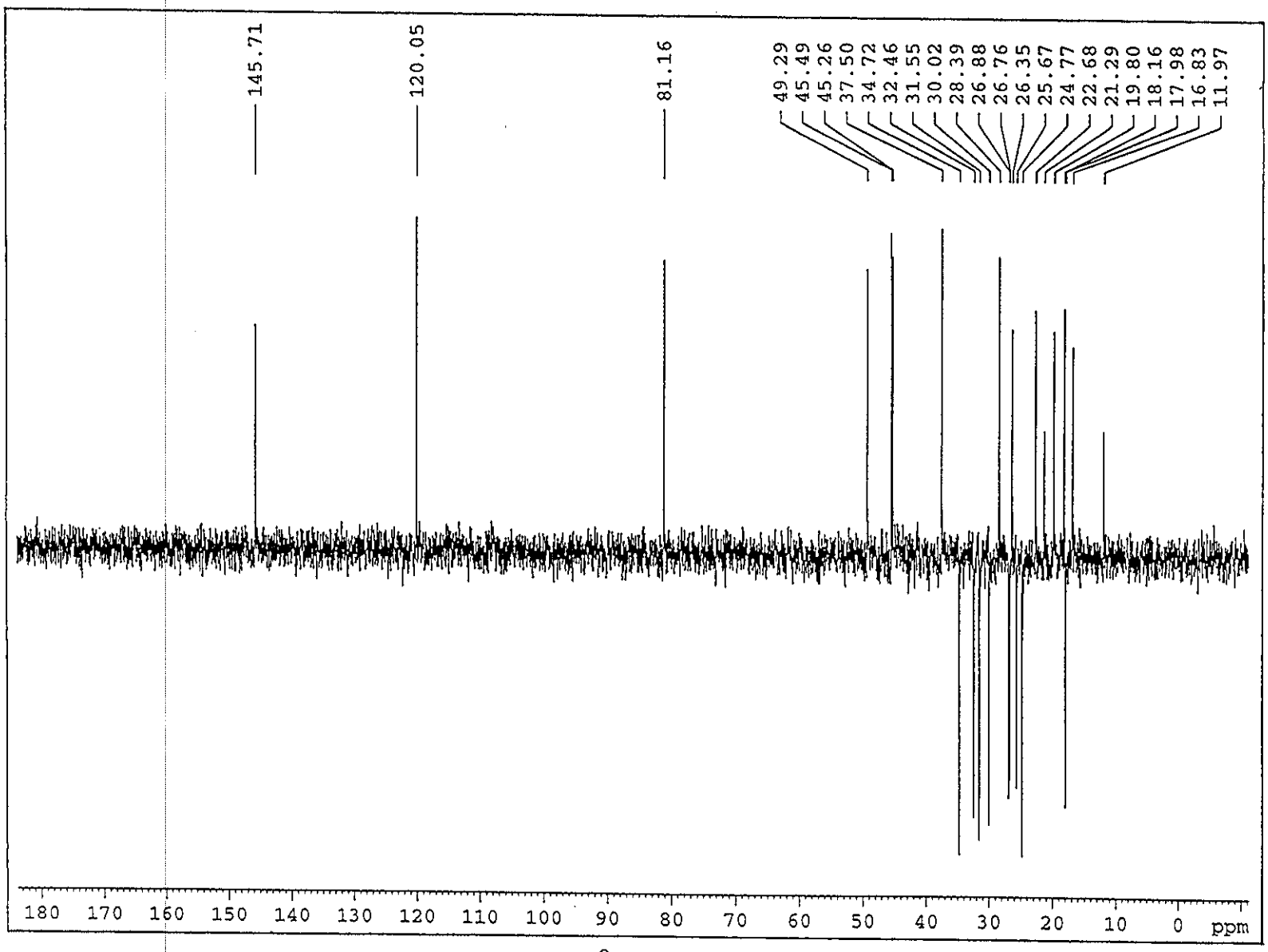


Figure 3.35 DEPT 135° spectrum of compound AH4

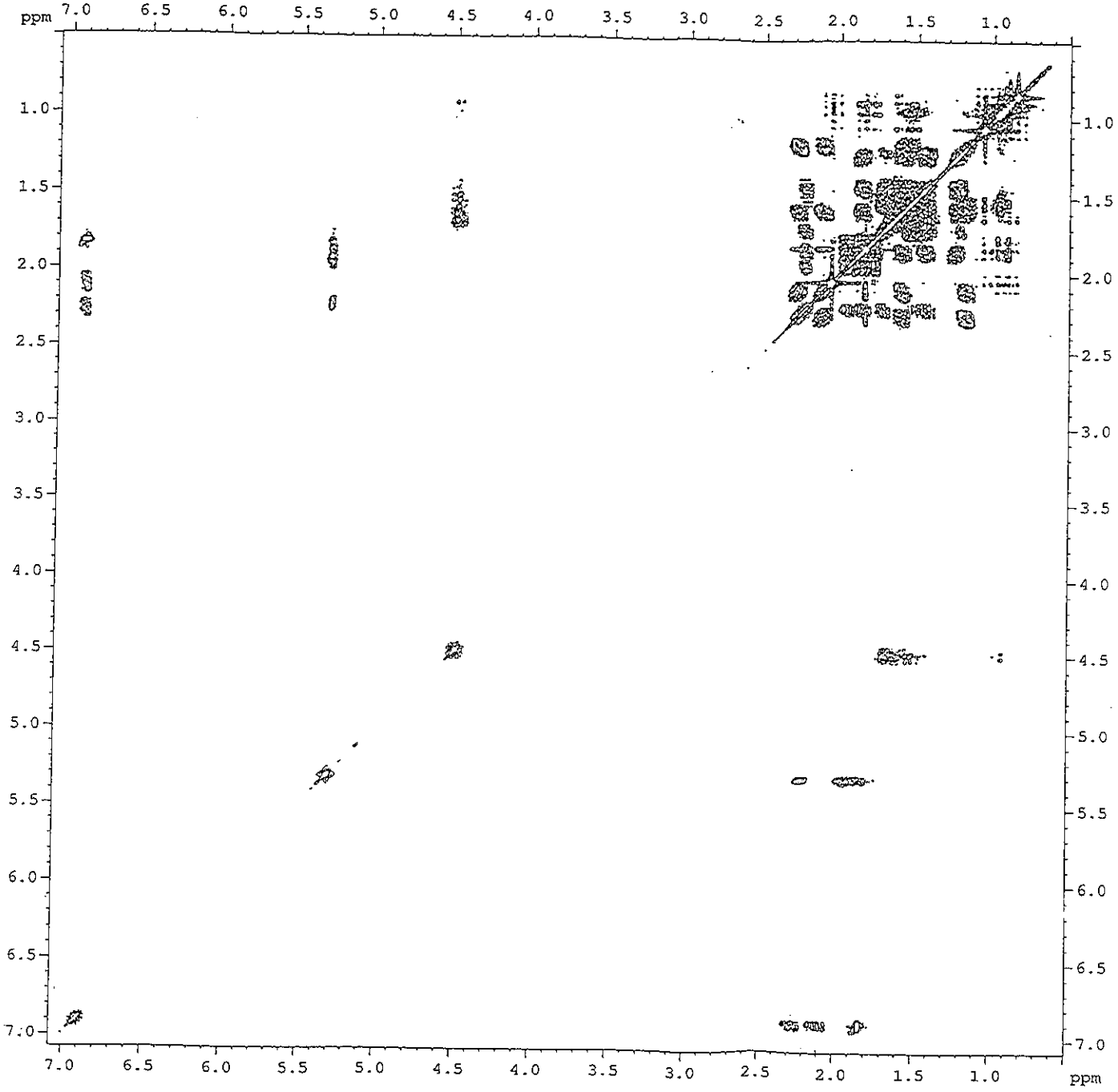


Figure 3.36 ^{1}H-^{1}H COSY spectrum of compound AH4

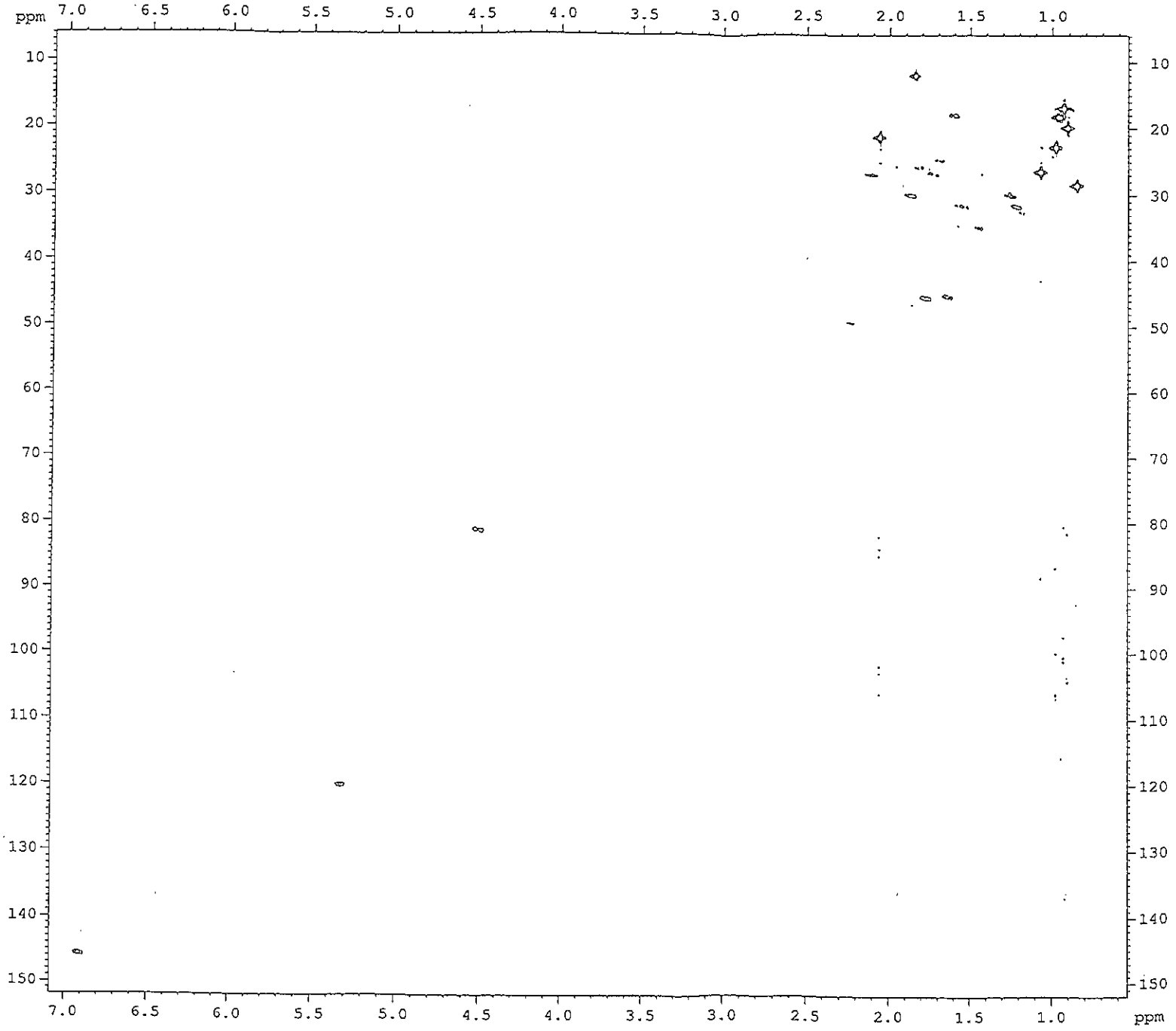


Figure 3.37 2D HMQC spectrum of compound AH4

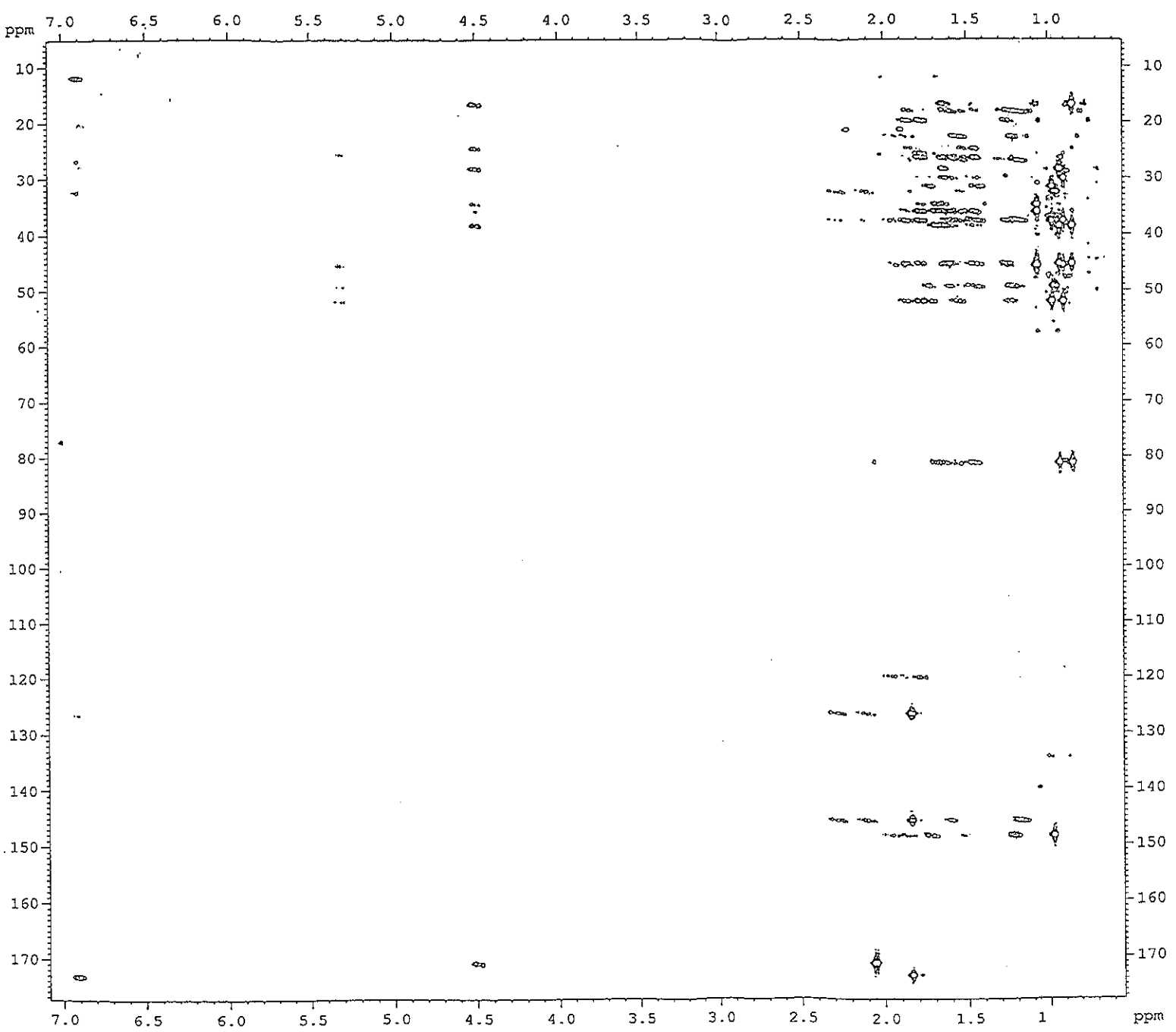


Figure 3.38 2D HMBC spectrum of compound AH4

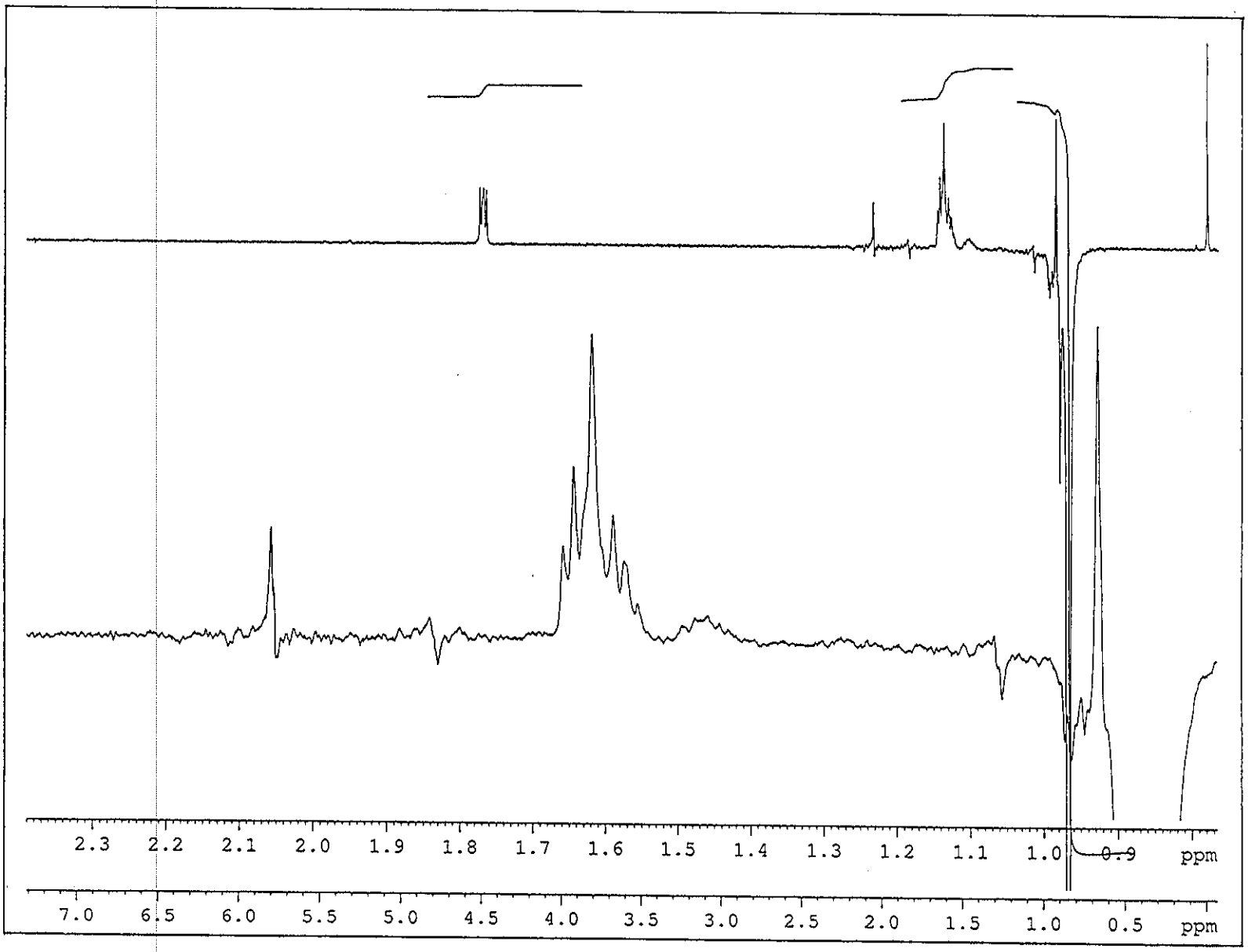


Figure 3.39 NOE difference spectrum of compound AH4 after irradiation at δ_H 0.84 ppm (Me-29)

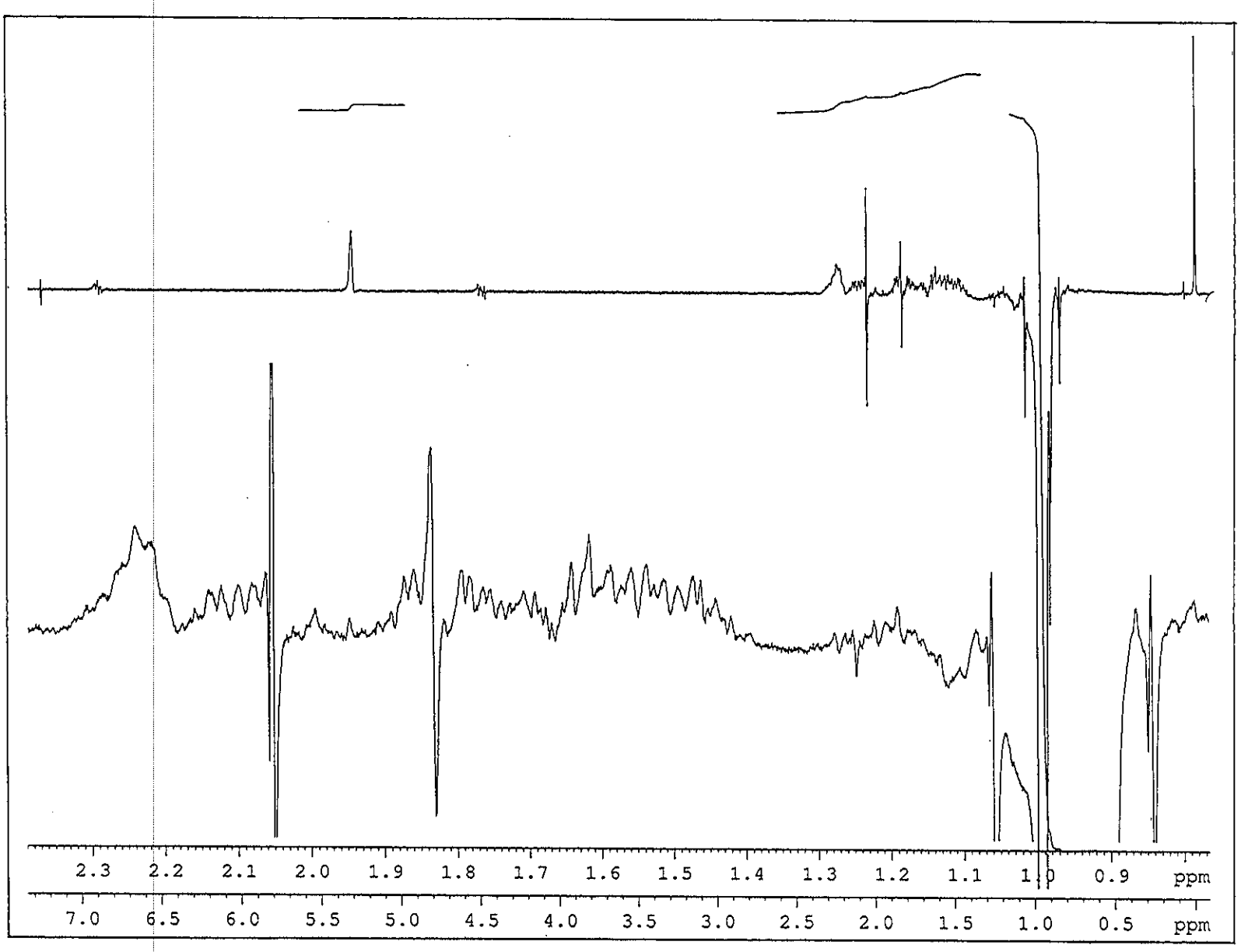


Figure 3.40 NOE difference spectrum of compound AH4 after irradiation at δ_H 0.95 ppm (Me-21)

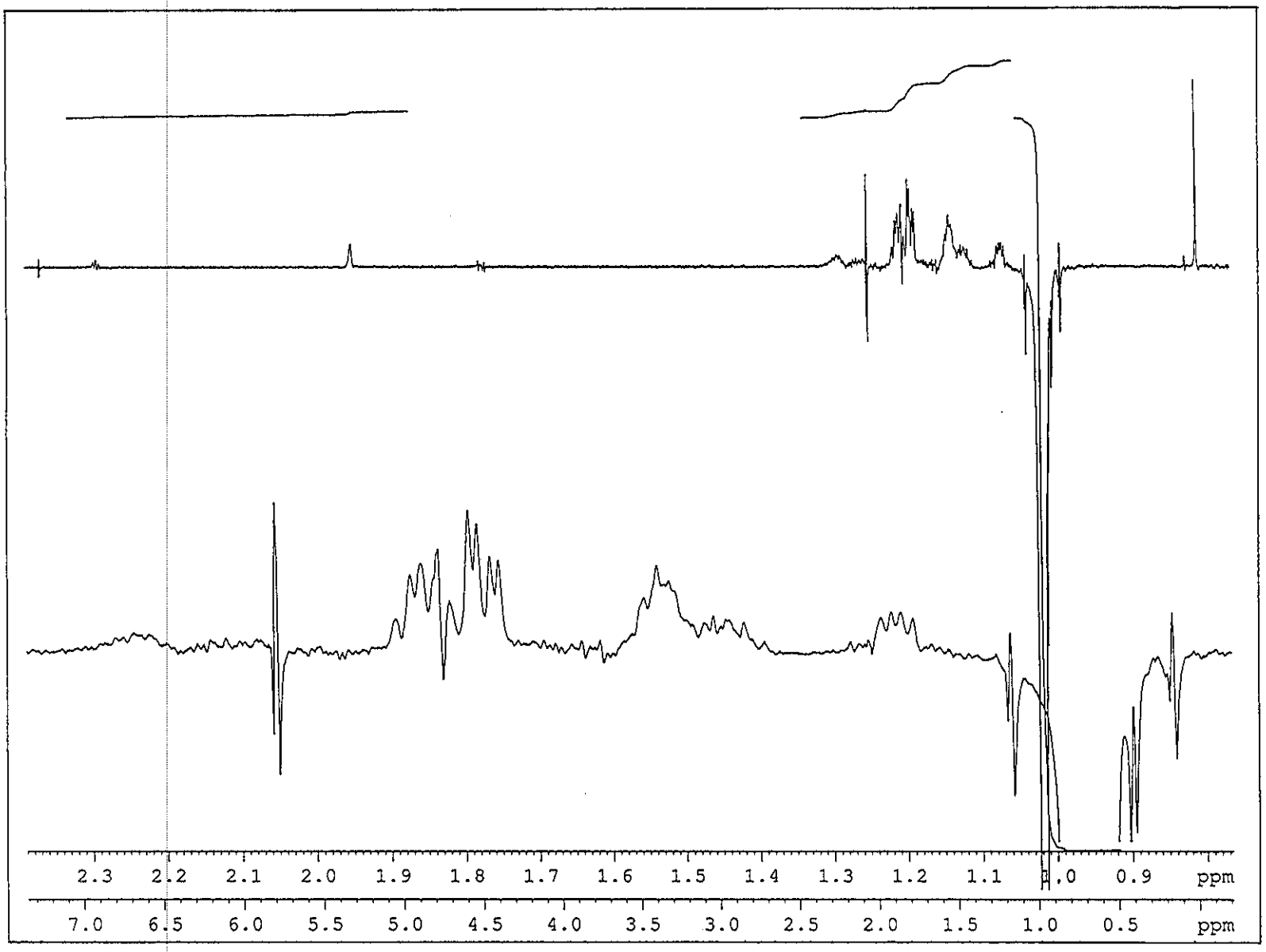


Figure 3.41 NOE difference spectrum of compound AH4 after irradiation at δ_H 0.98 ppm (Me-30)

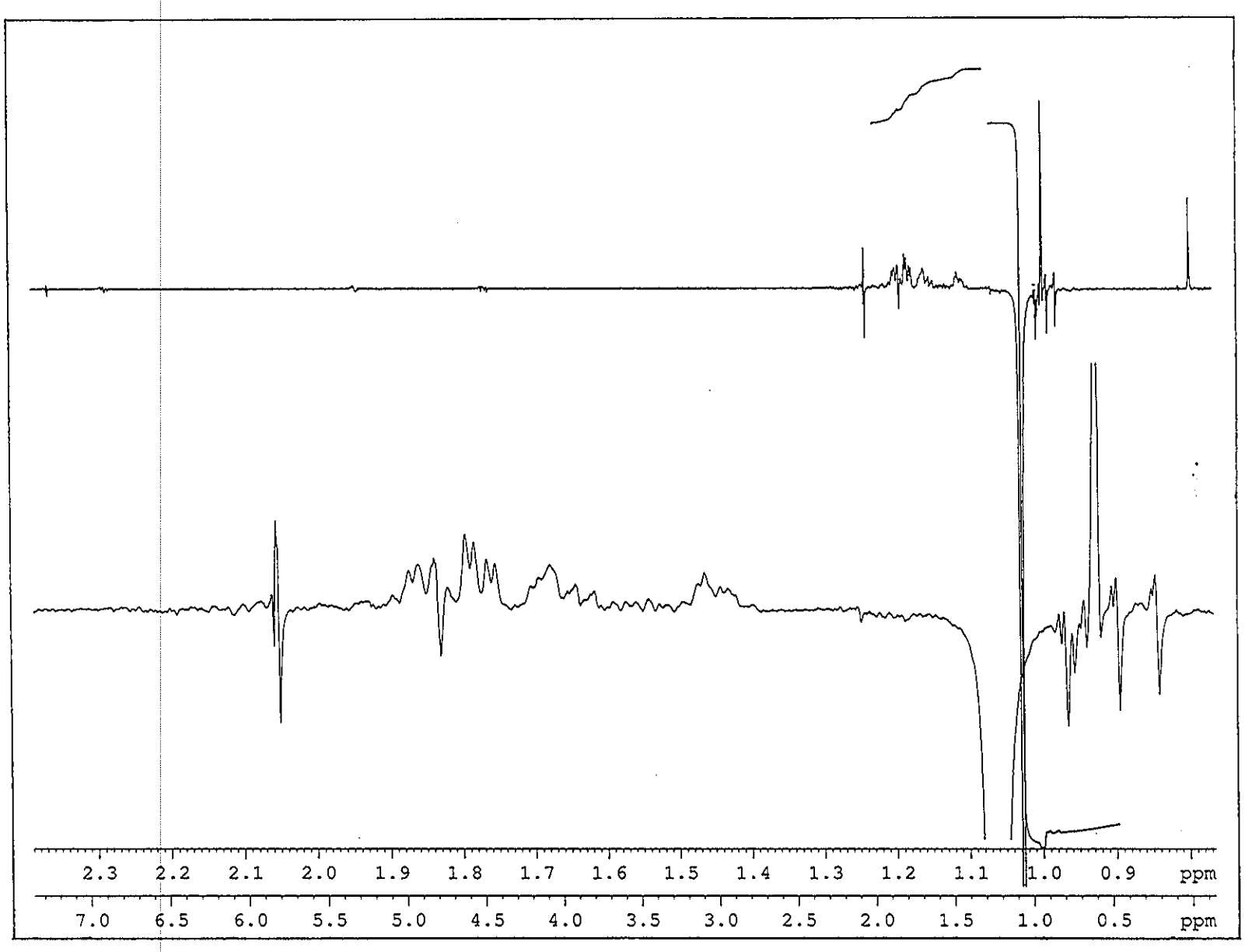


Figure 3.42 NOE difference spectrum of compound AH4 after irradiation at δ_H 1.06 ppm (Me-19)

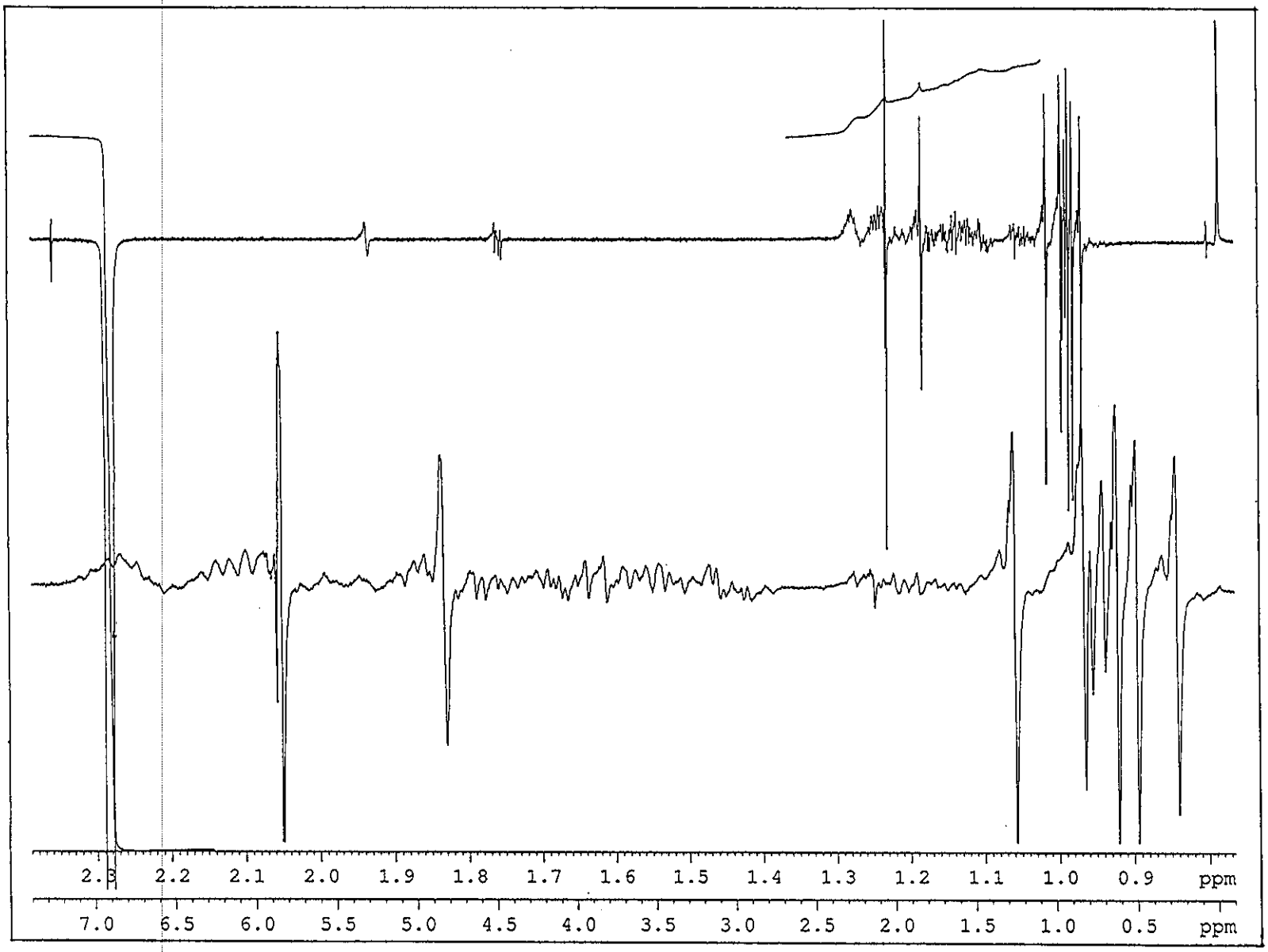


Figure 3.43 NOE difference spectrum of compound AH4 after irradiation at δ_H 6.90 ppm (H-24)

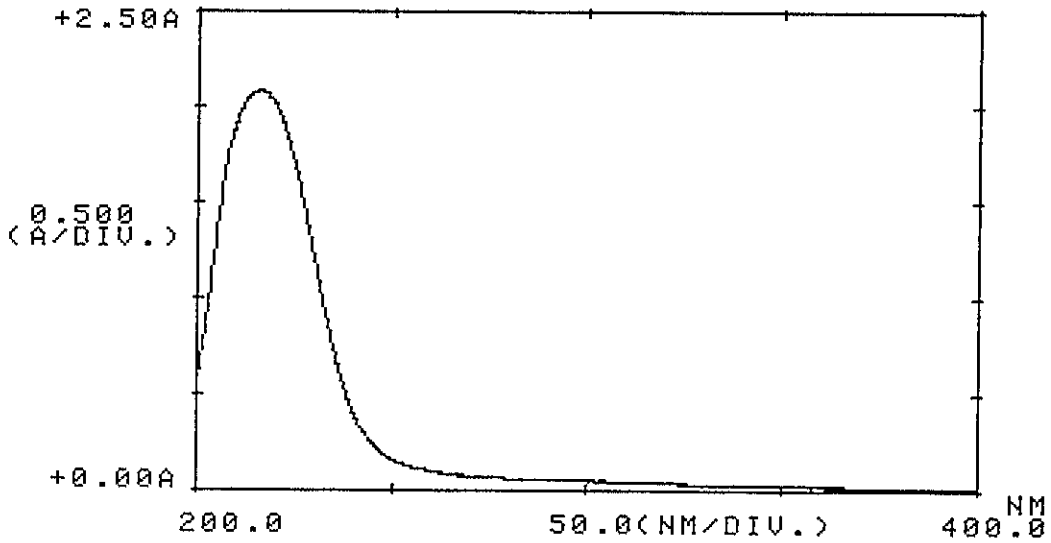


Figure 3.44 UV (MeOH) spectrum of compound AH10

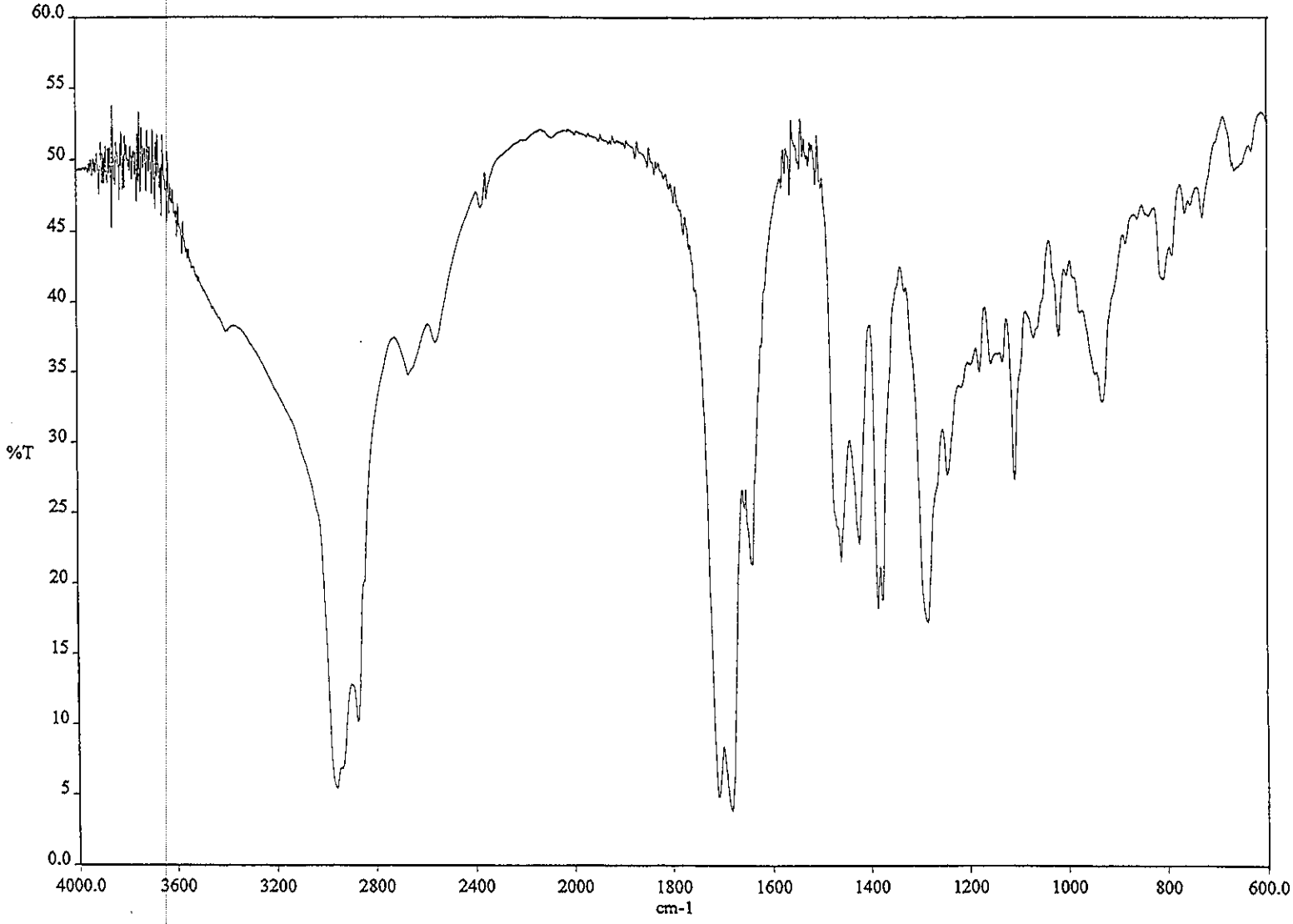


Figure 3.45 FT-IR (KBr) spectrum of compound AH10

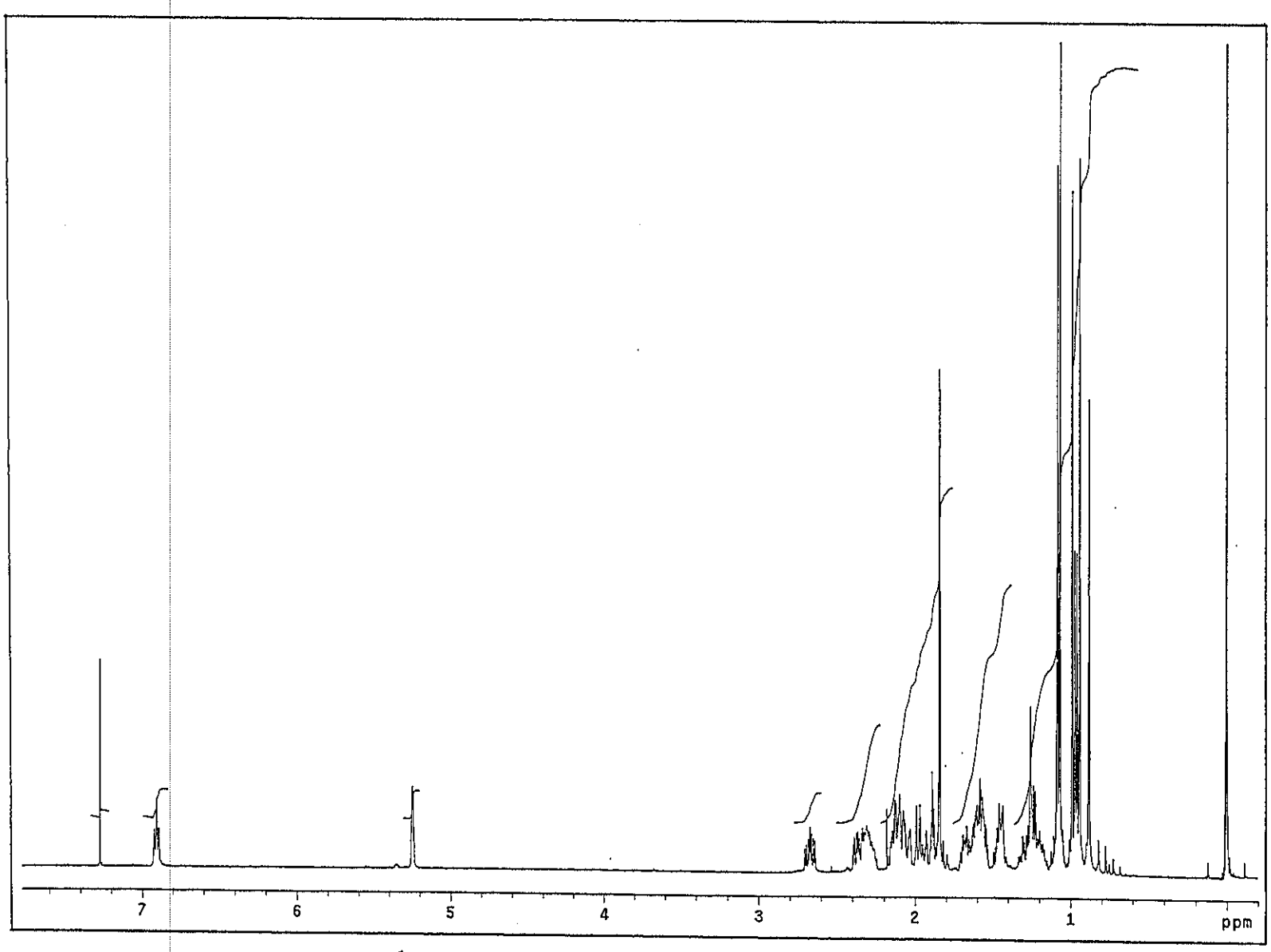


Figure 3.46 ^{1}H NMR (500 MHz)($CDCl_3$) spectrum of compound AH10

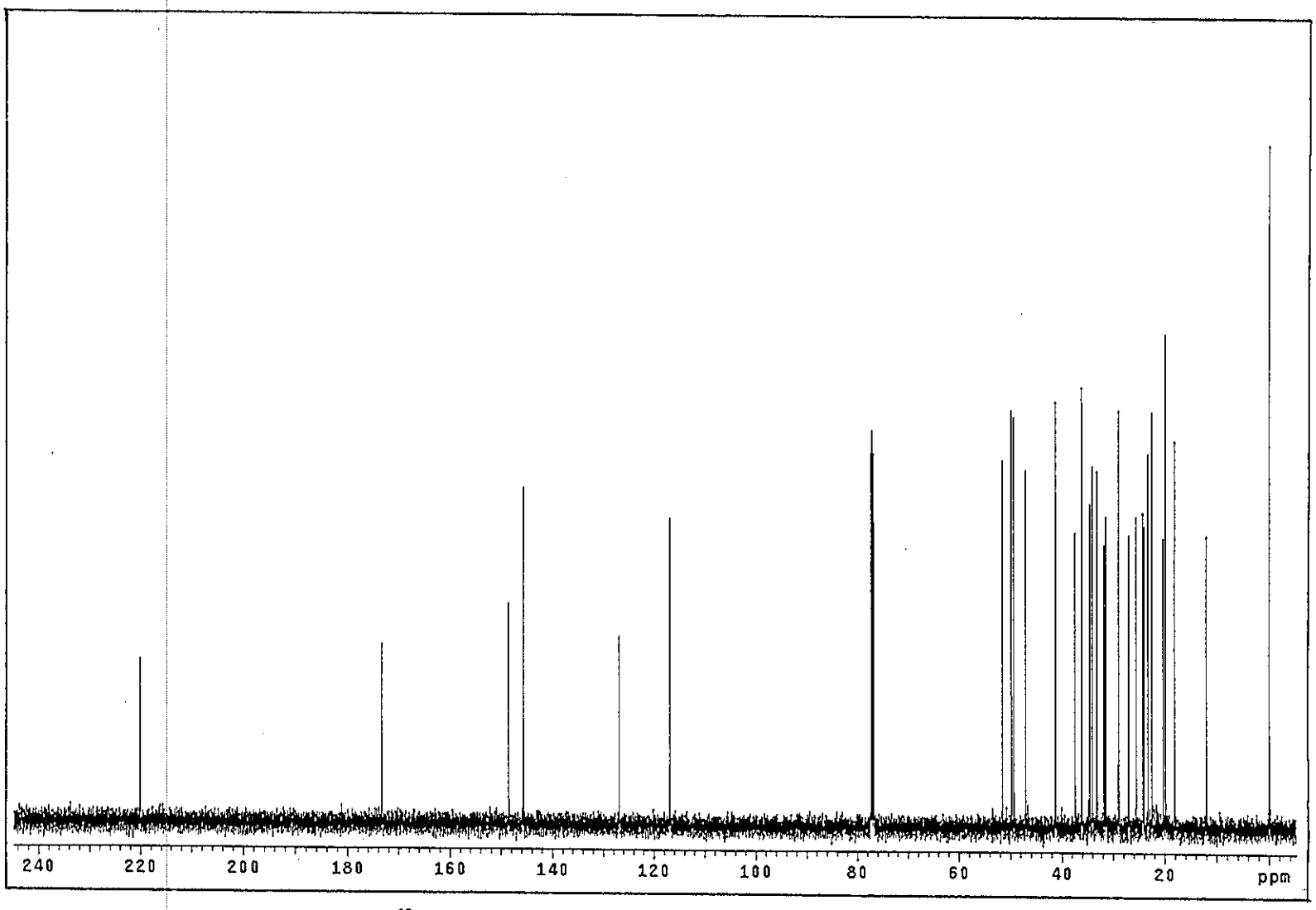


Figure 3.47 ^{13}C NMR (125 MHz)($CDCl_3$) spectrum of compound AH10

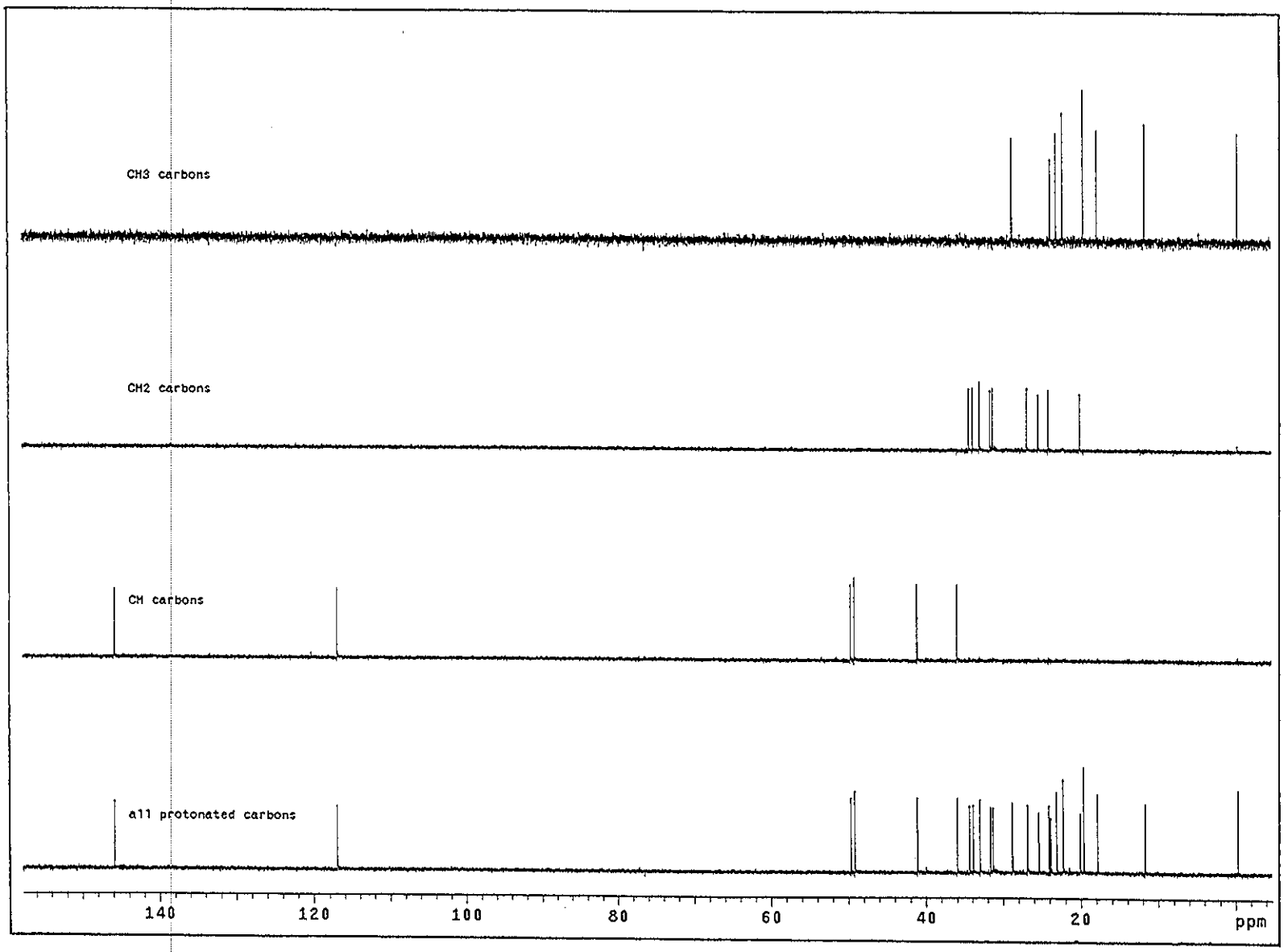


Figure 3.48 DEPT spectrum of compound AH10

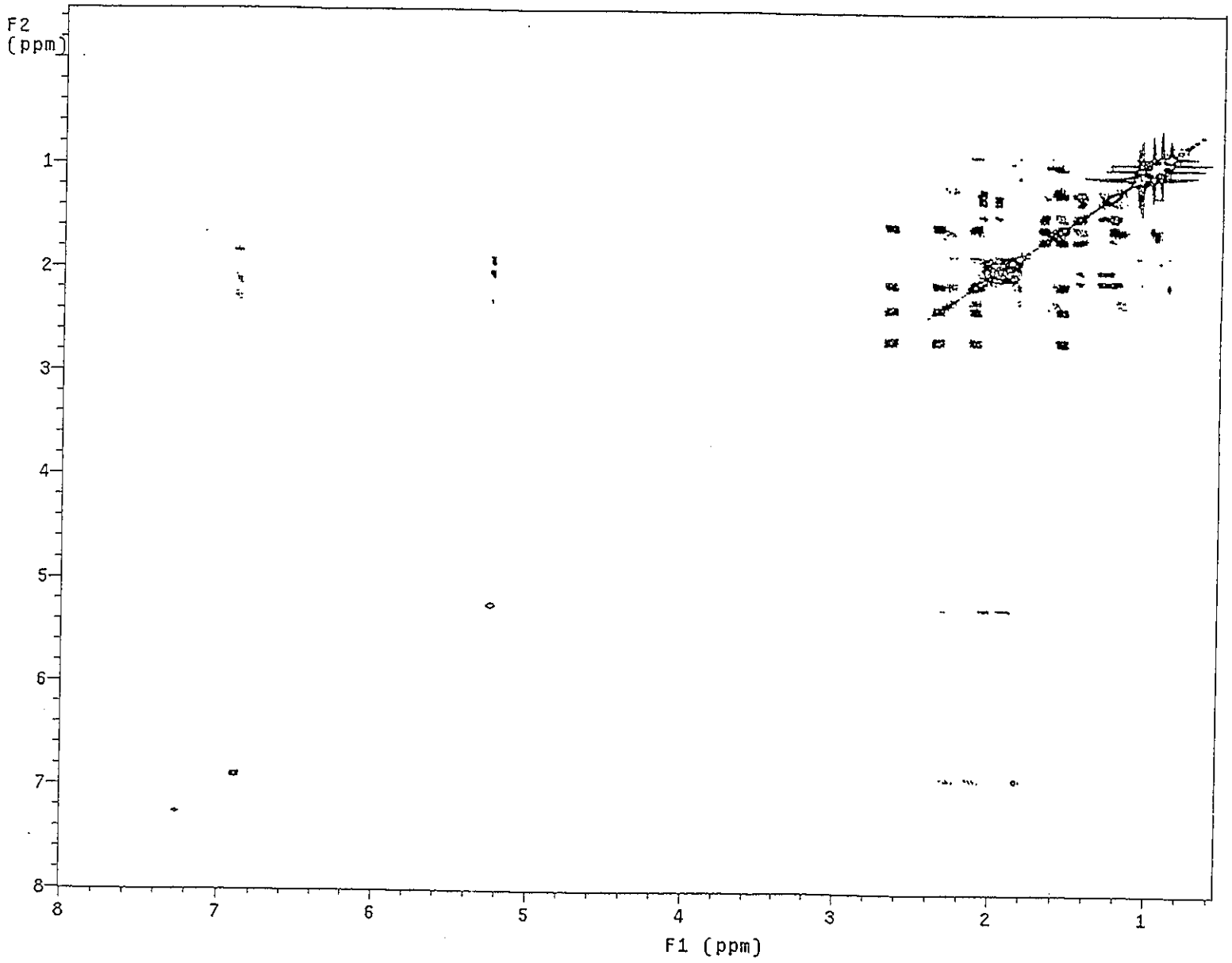


Figure 3.49 ^{1}H-^{1}H COSY spectrum of compound AH10

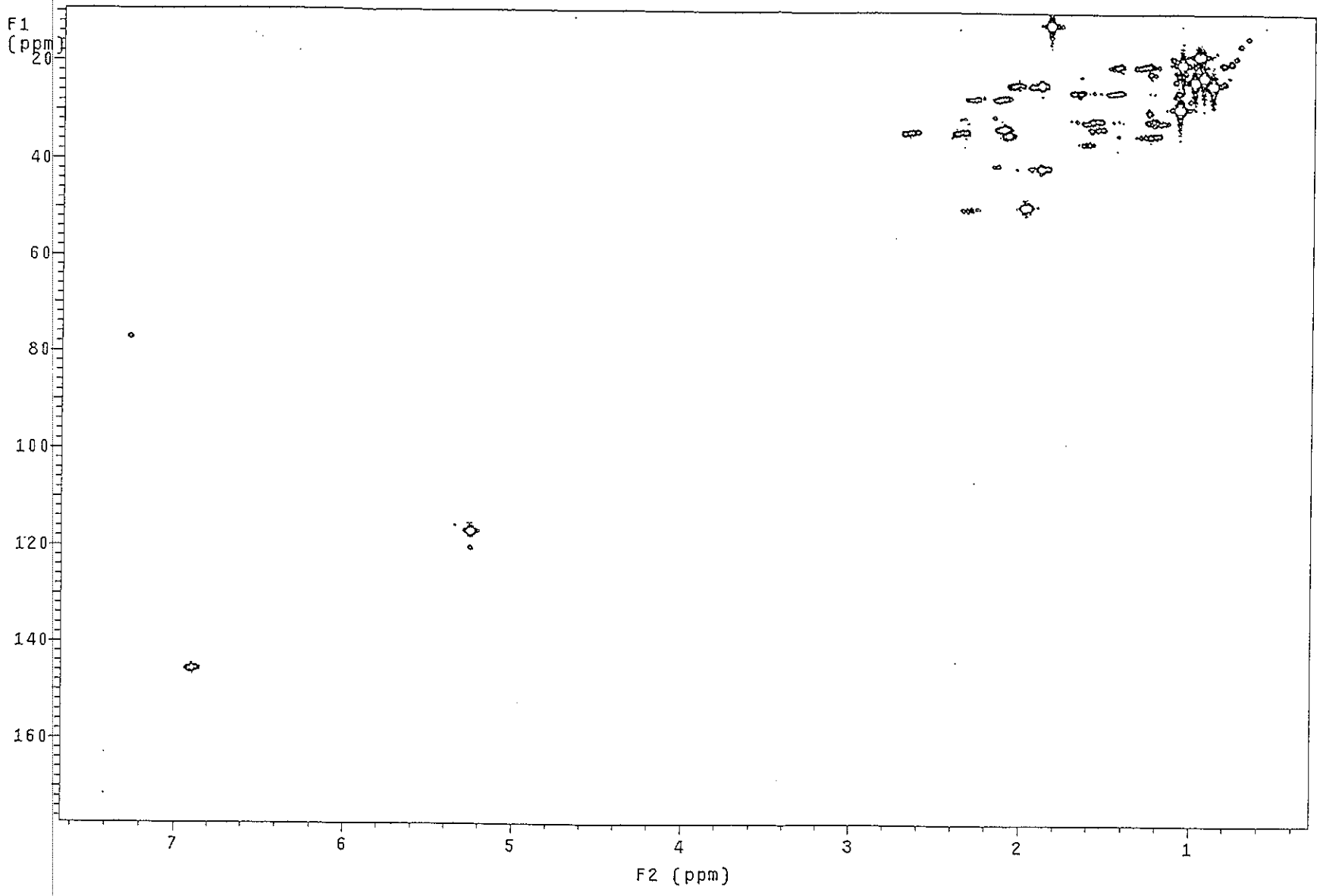


Figure 3.50 2D HMQC spectrum of compound AH10

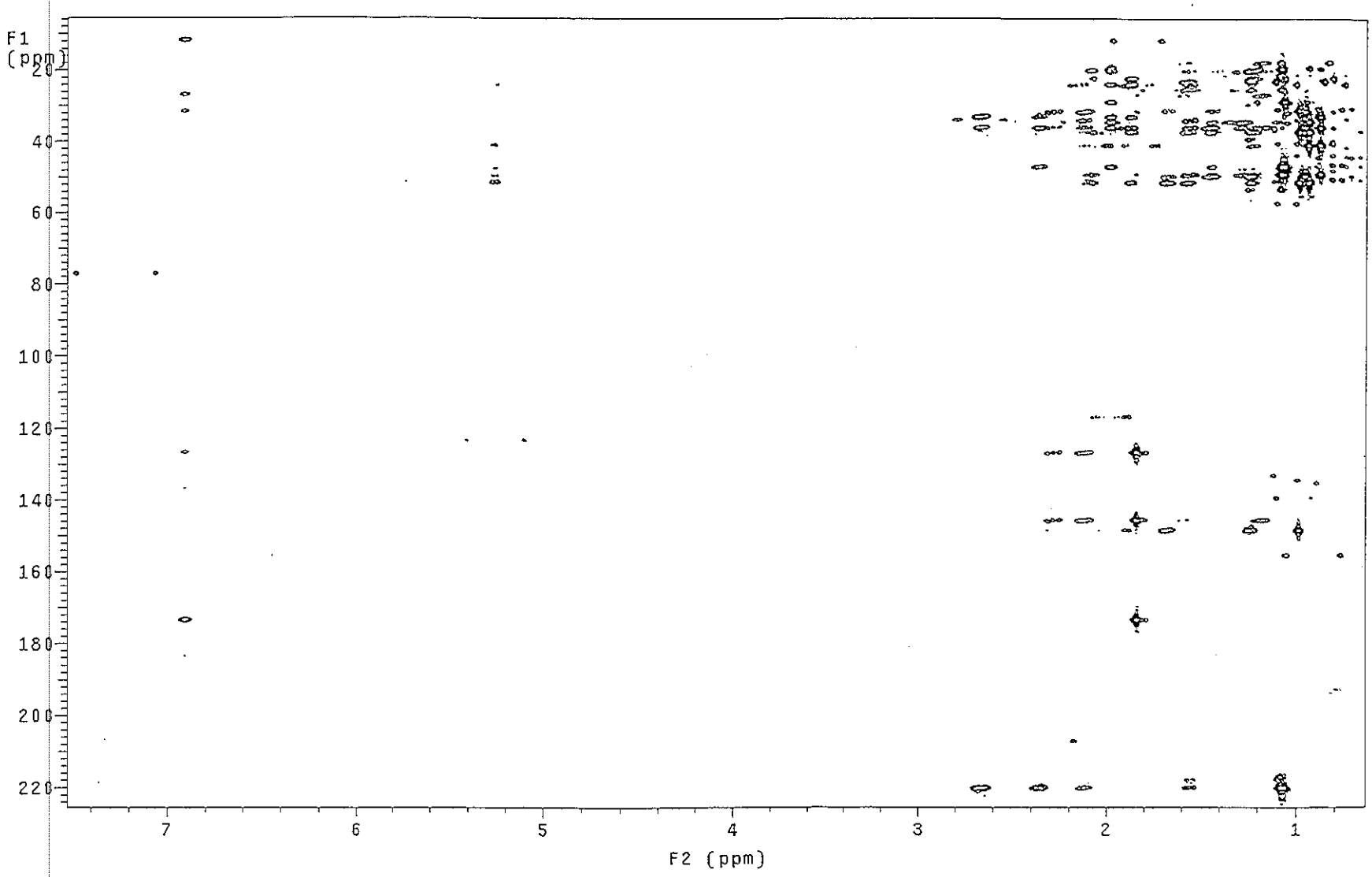


Figure 3.51 2D HMBC spectrum of compound AH10

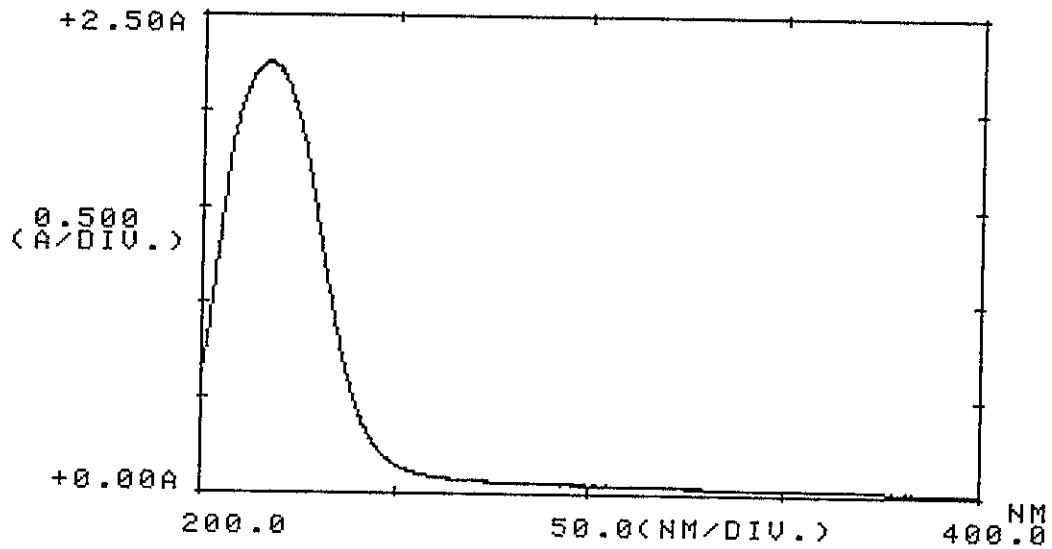


Figure 3.52 UV (MeOH) spectrum of compound AH5

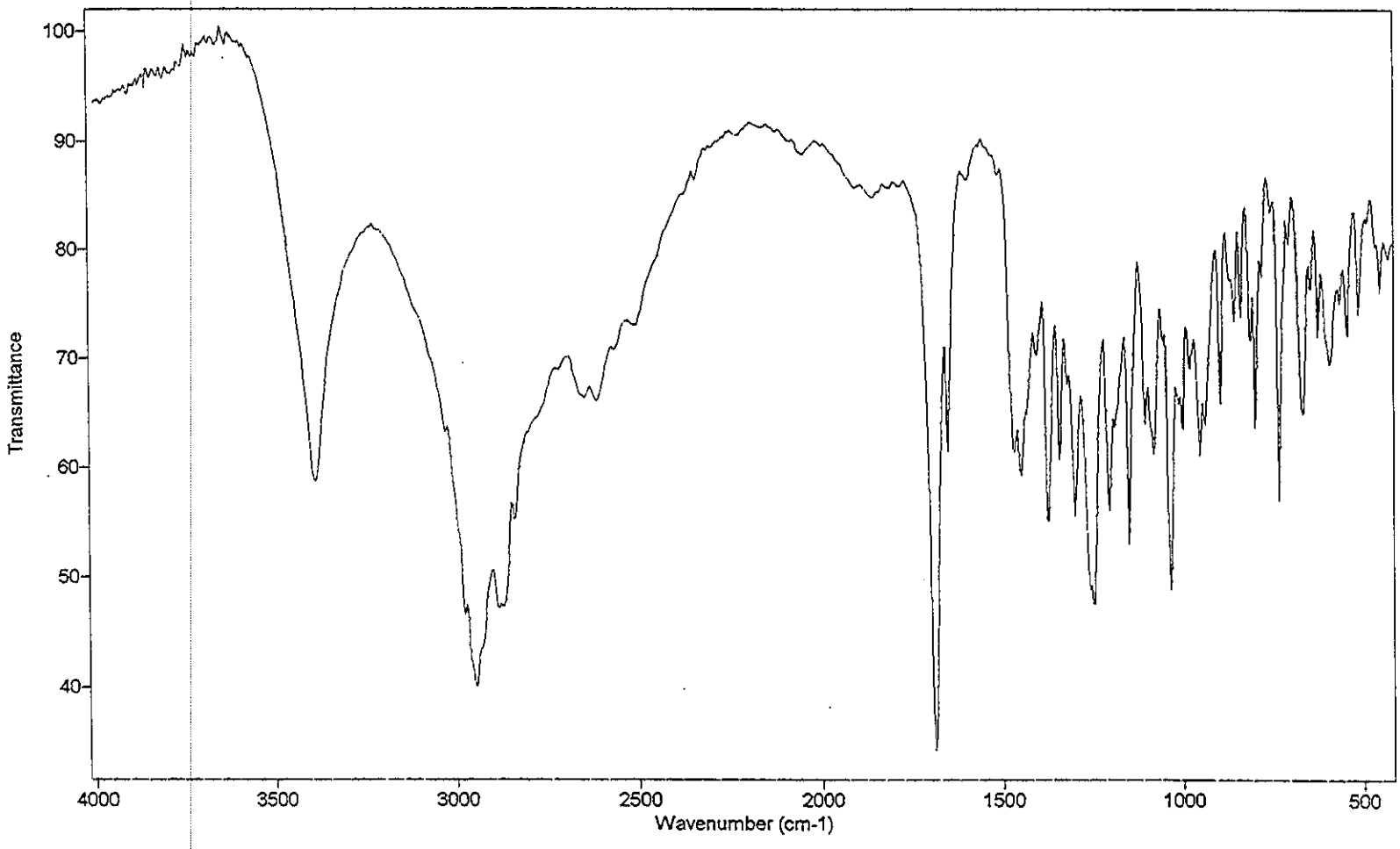


Figure 3.53 FT-IR (KBr) spectrum of compound AH5

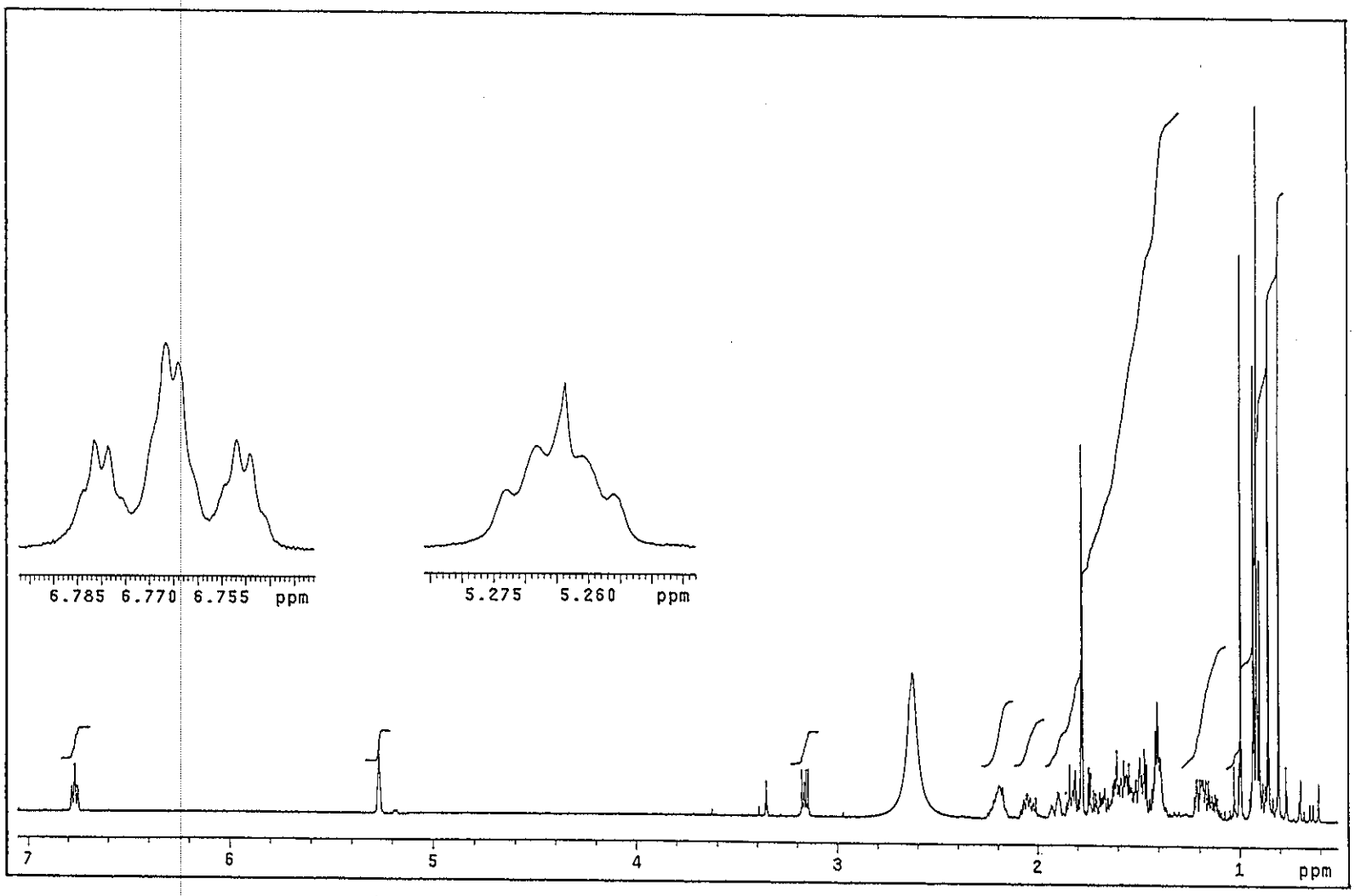


Figure 3.54 ^{1}H NMR (500 MHz)($CDCl_3$+CD_3OD) spectrum of compound AH5

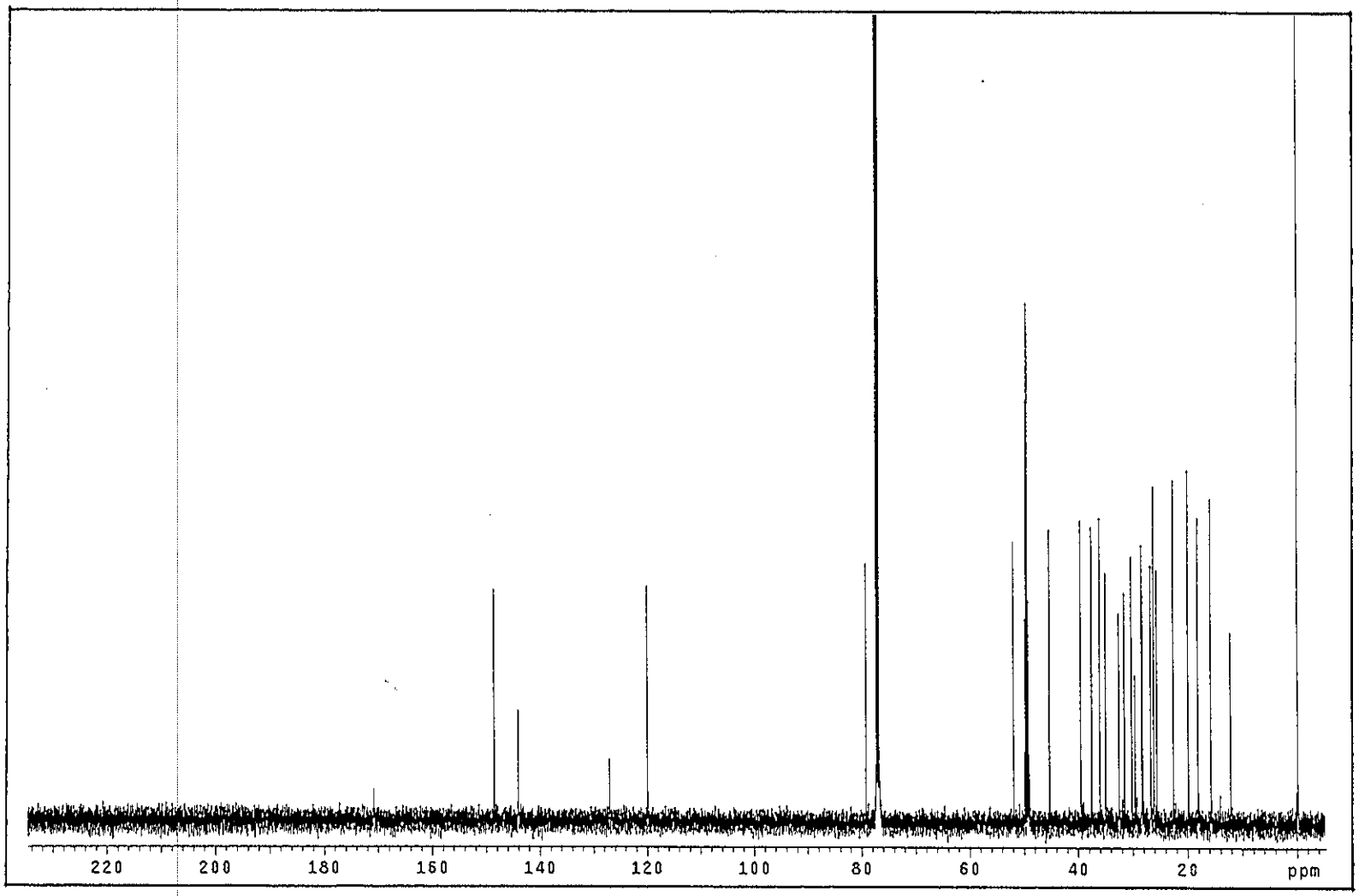


Figure 3.55 ^{13}C NMR (125 MHz)($CDCl_3$+CD_3OD) spectrum of compound AH5

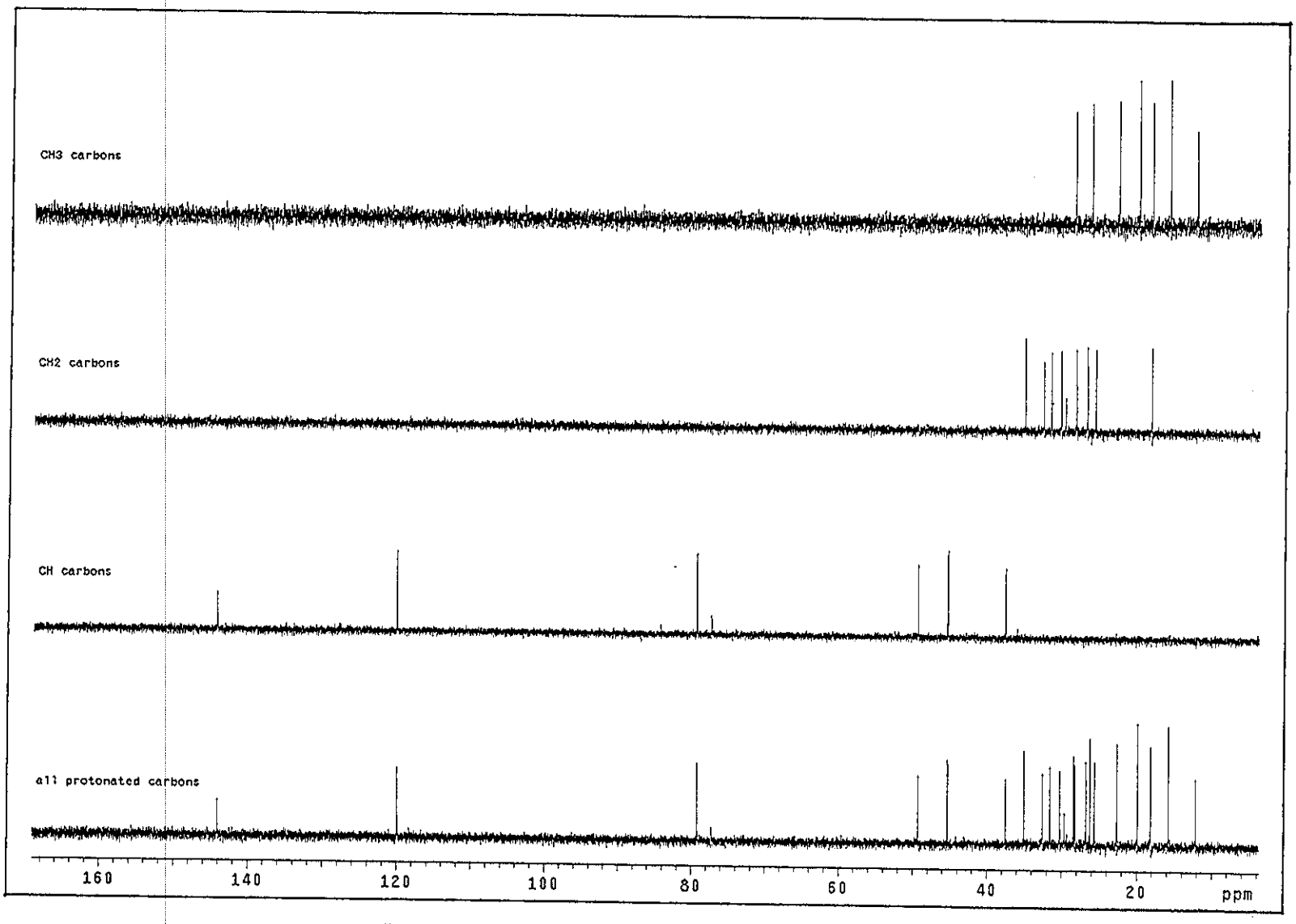


Figure 3.56 DEPT spectrum of compound AH5

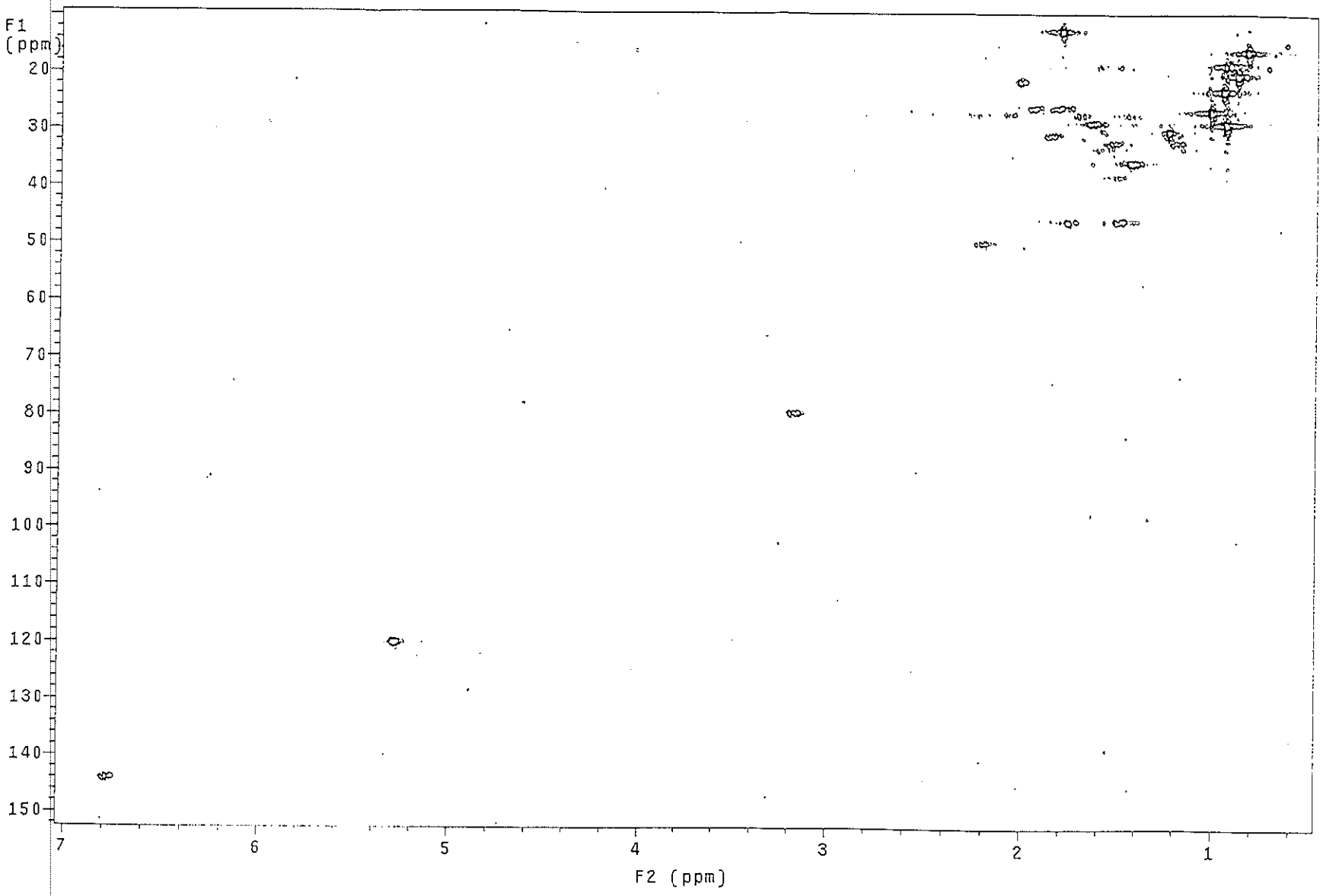


Figure 3.57 2D HMQC spectrum of compound AH5

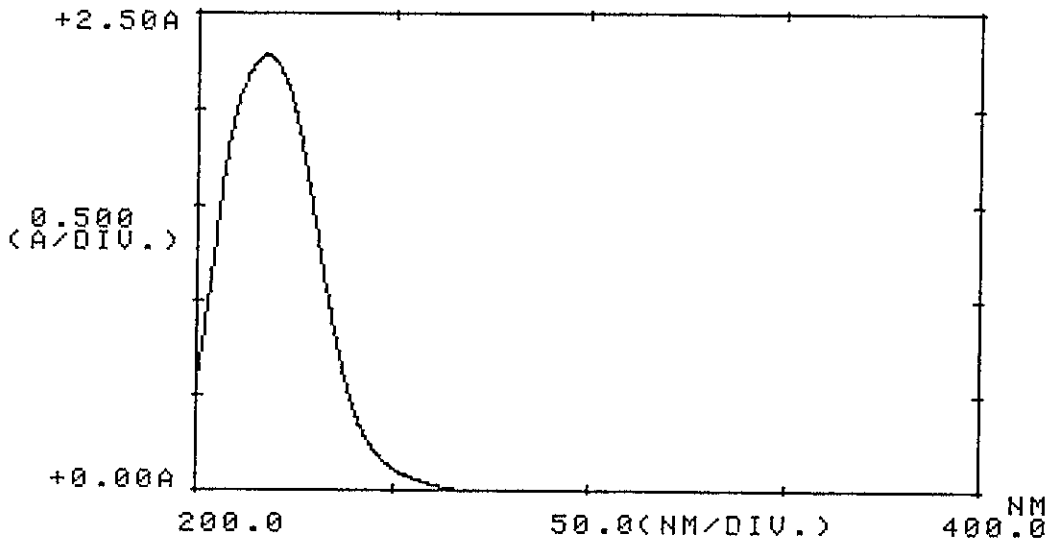


Figure 3.58 UV (MeOH) spectrum of compound AH5-Ac

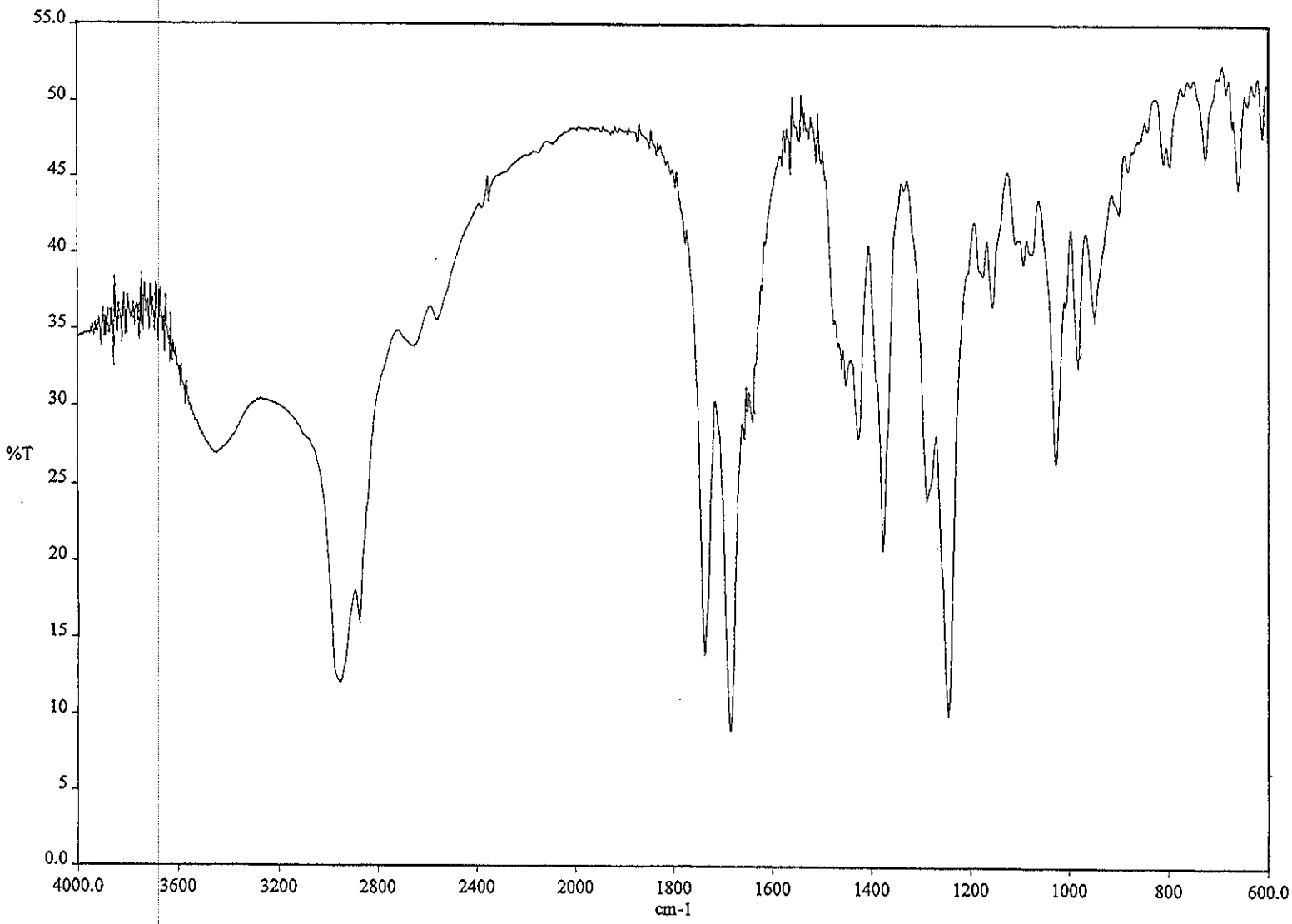


Figure 3.59 FT-IR (KBr) spectrum of compound AH5-Ac

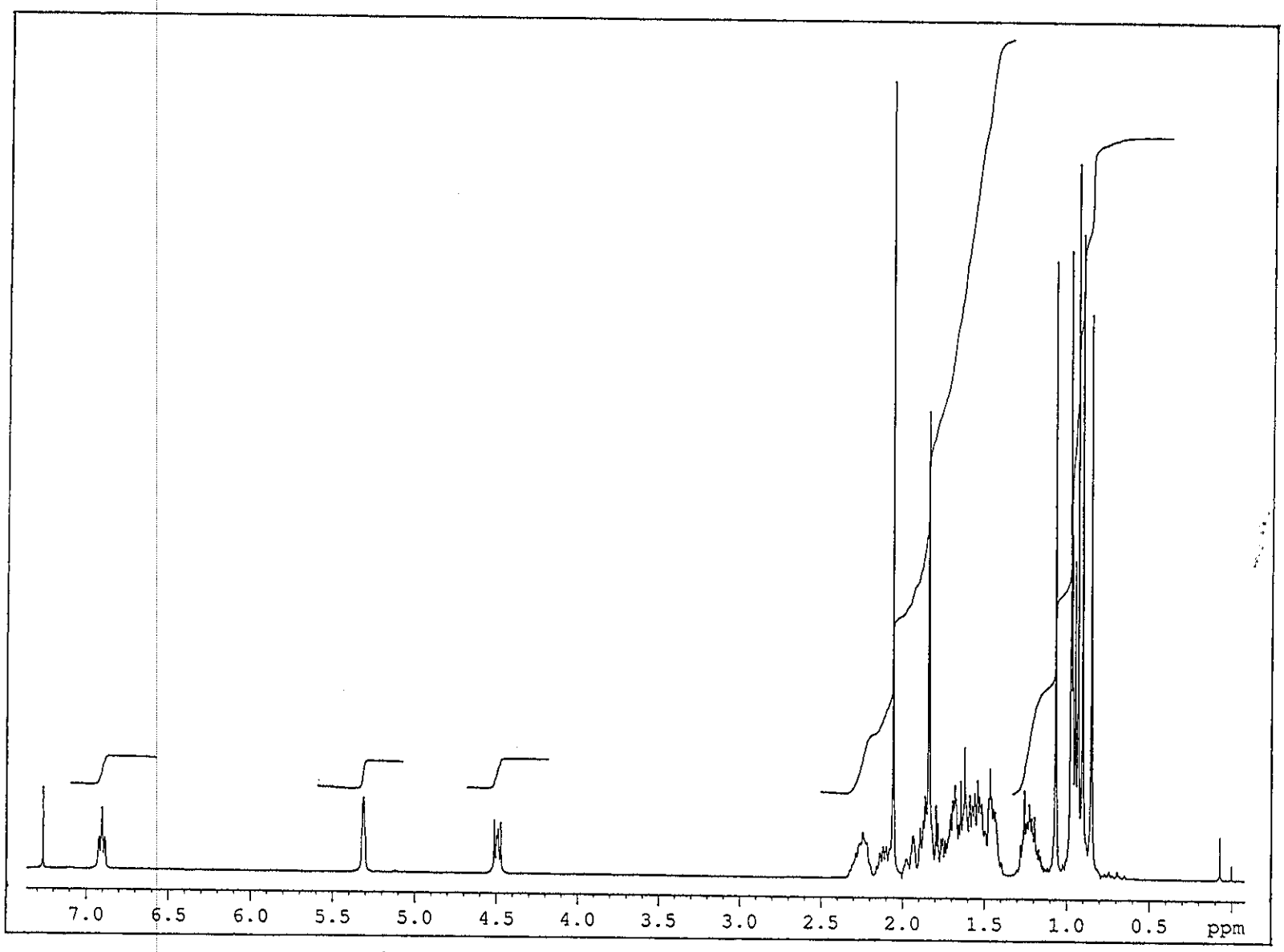


Figure 3.60 ^{1}H NMR (400 MHz)($CDCl_3$) spectrum of compound AH5-Ac

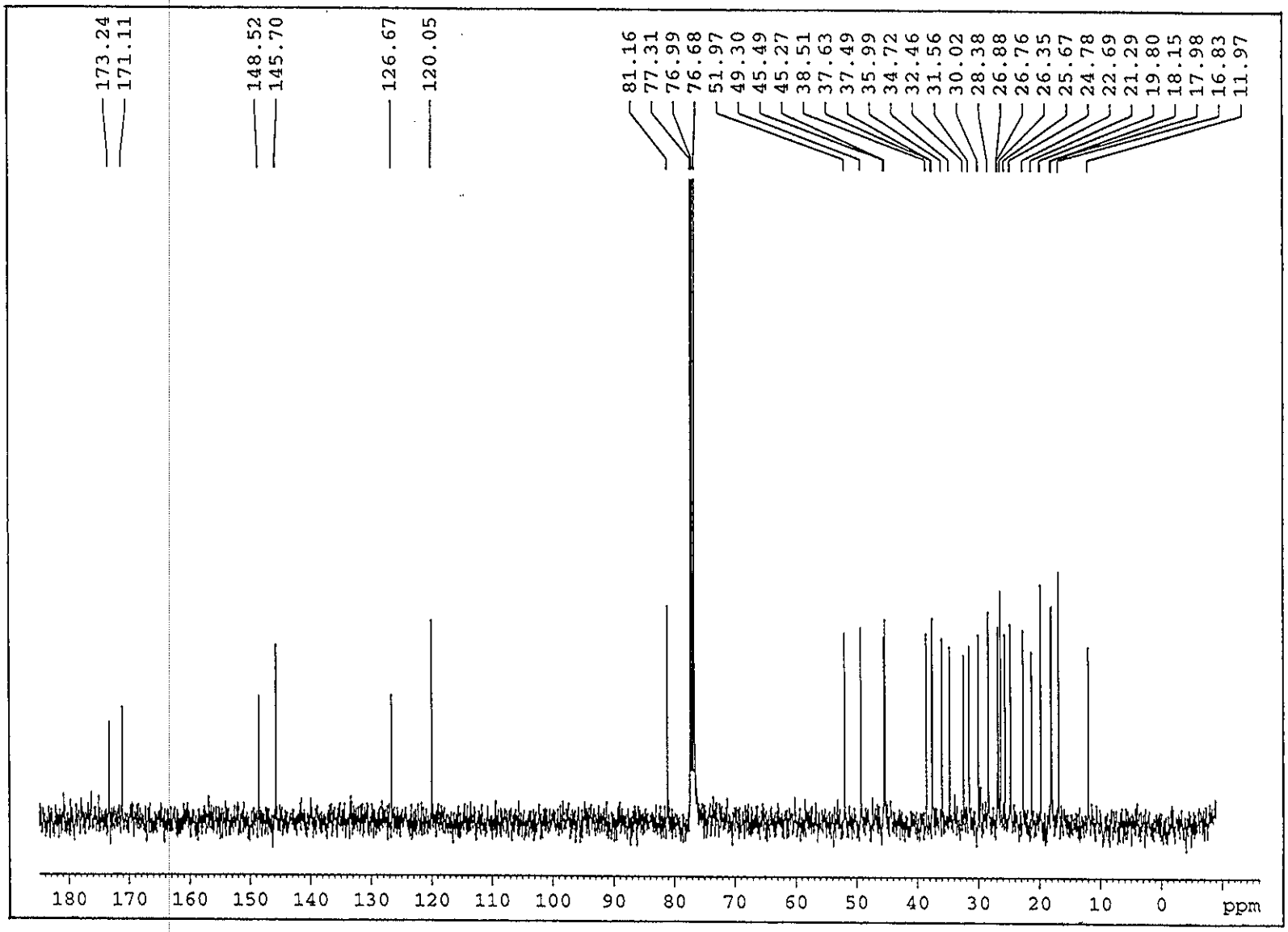


Figure 3.61 ^{13}C NMR (100 MHz)($CDCl_3$) spectrum of compound AH5-Ac

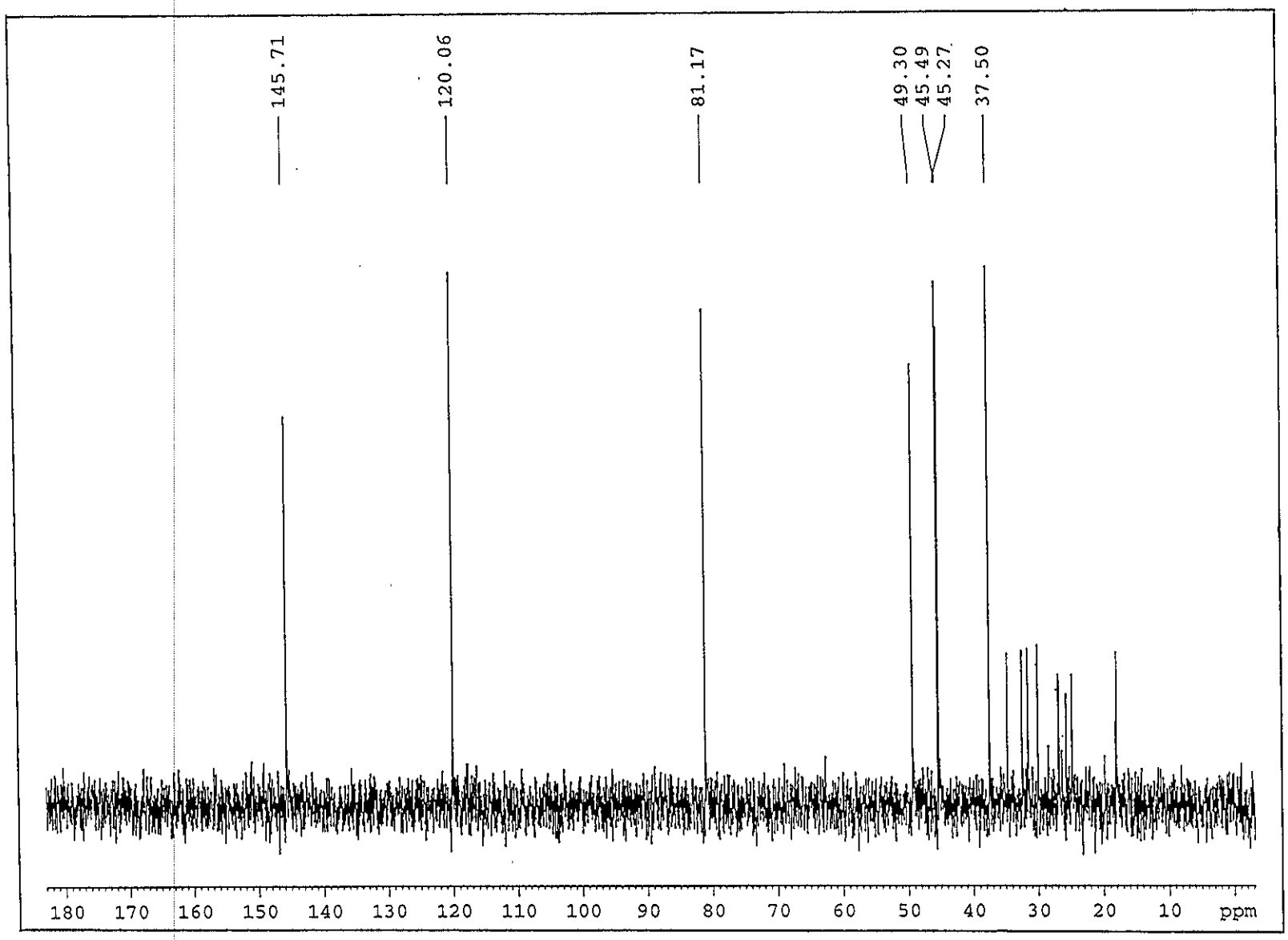


Figure 3.62 DEPT 90° spectrum of compound AH5-Ac

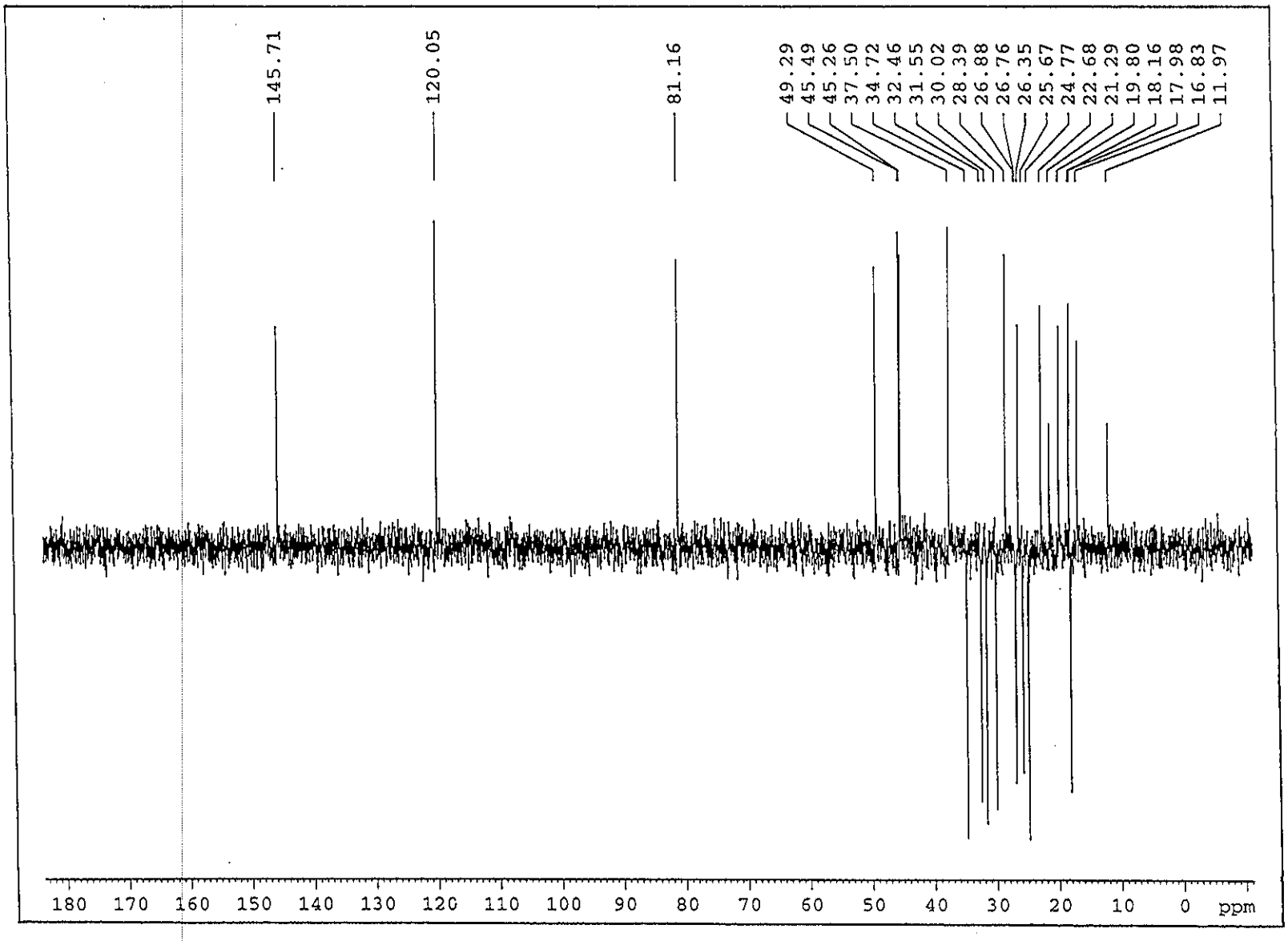


Figure 3.63 DEPT 135° spectrum of compound AH5-Ac

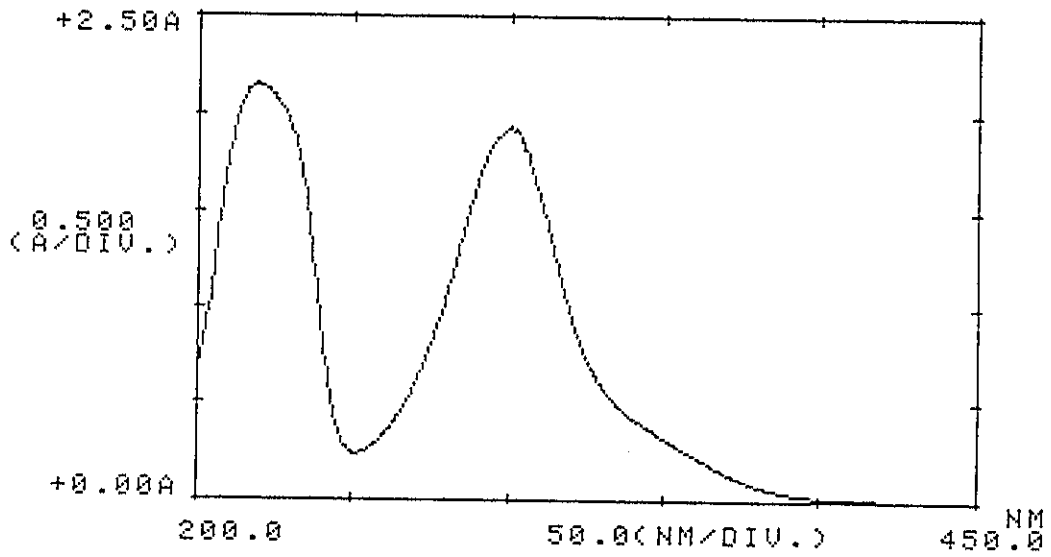


Figure 3.64 UV (MeOH) spectrum of compound AH7

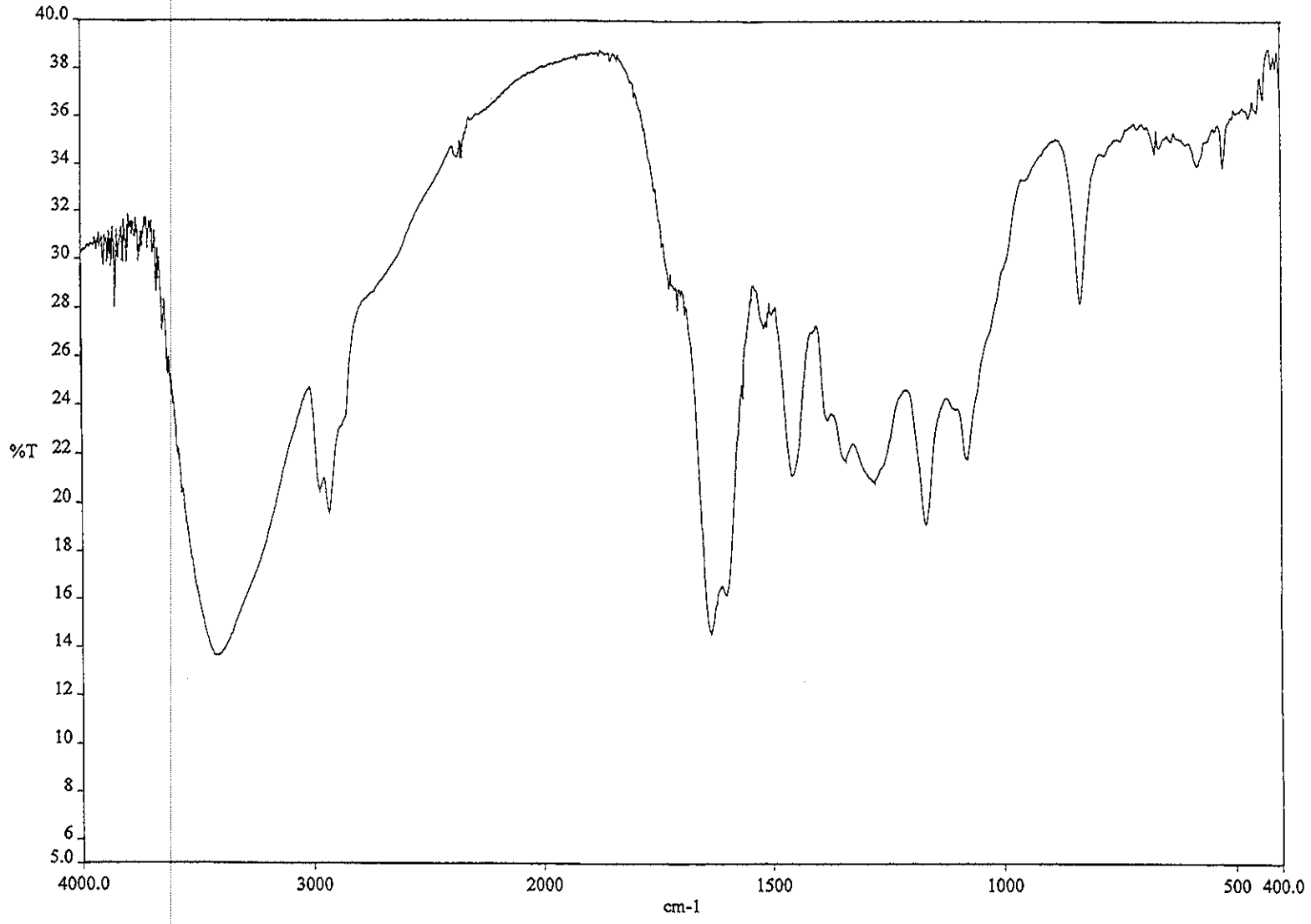


Figure 3.65 FT-IR (KBr) spectrum of compound AH7

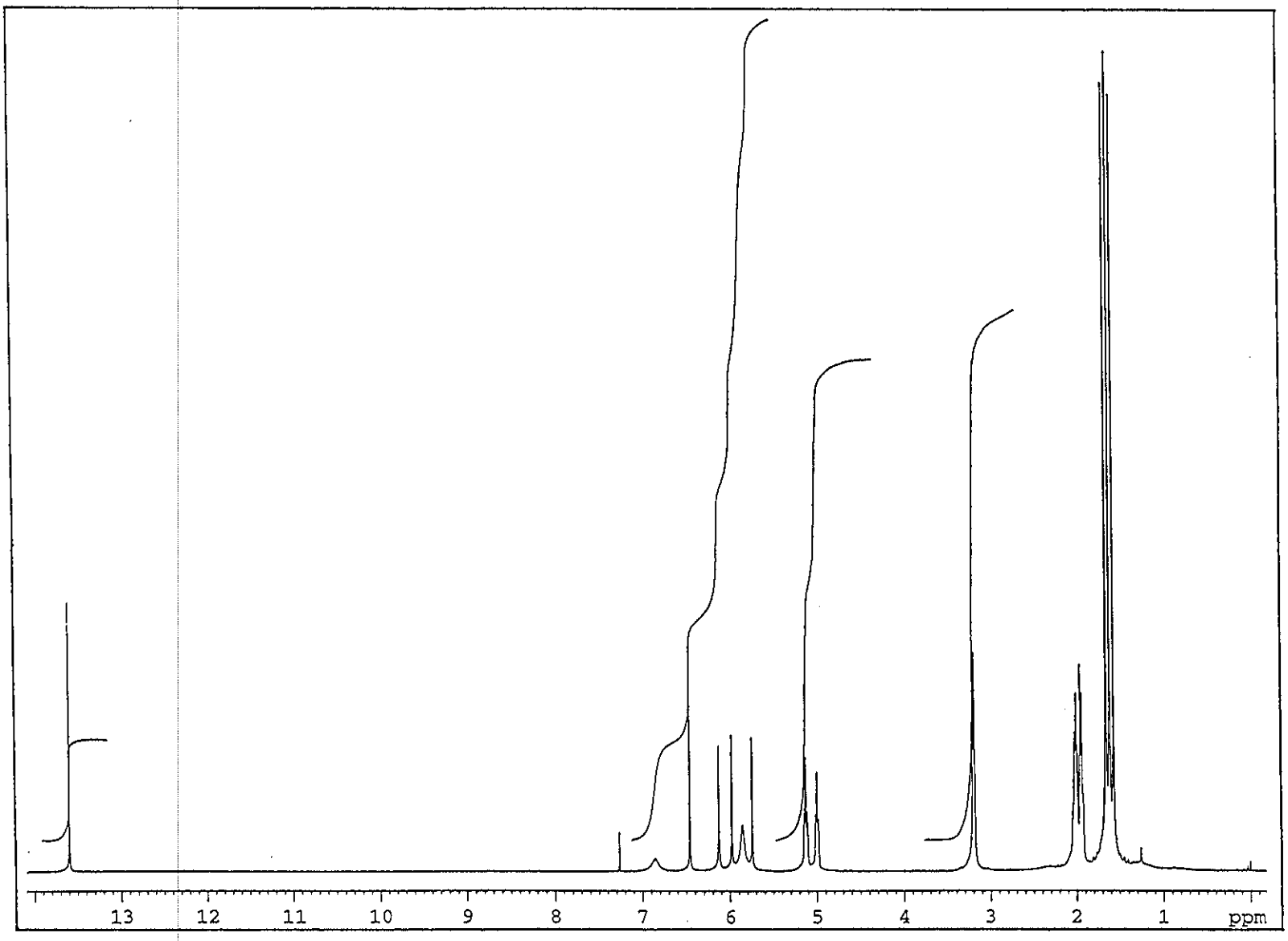


Figure 3.66 ^{1}H NMR (400 MHz)($CDCl_3$) spectrum of compound AH7

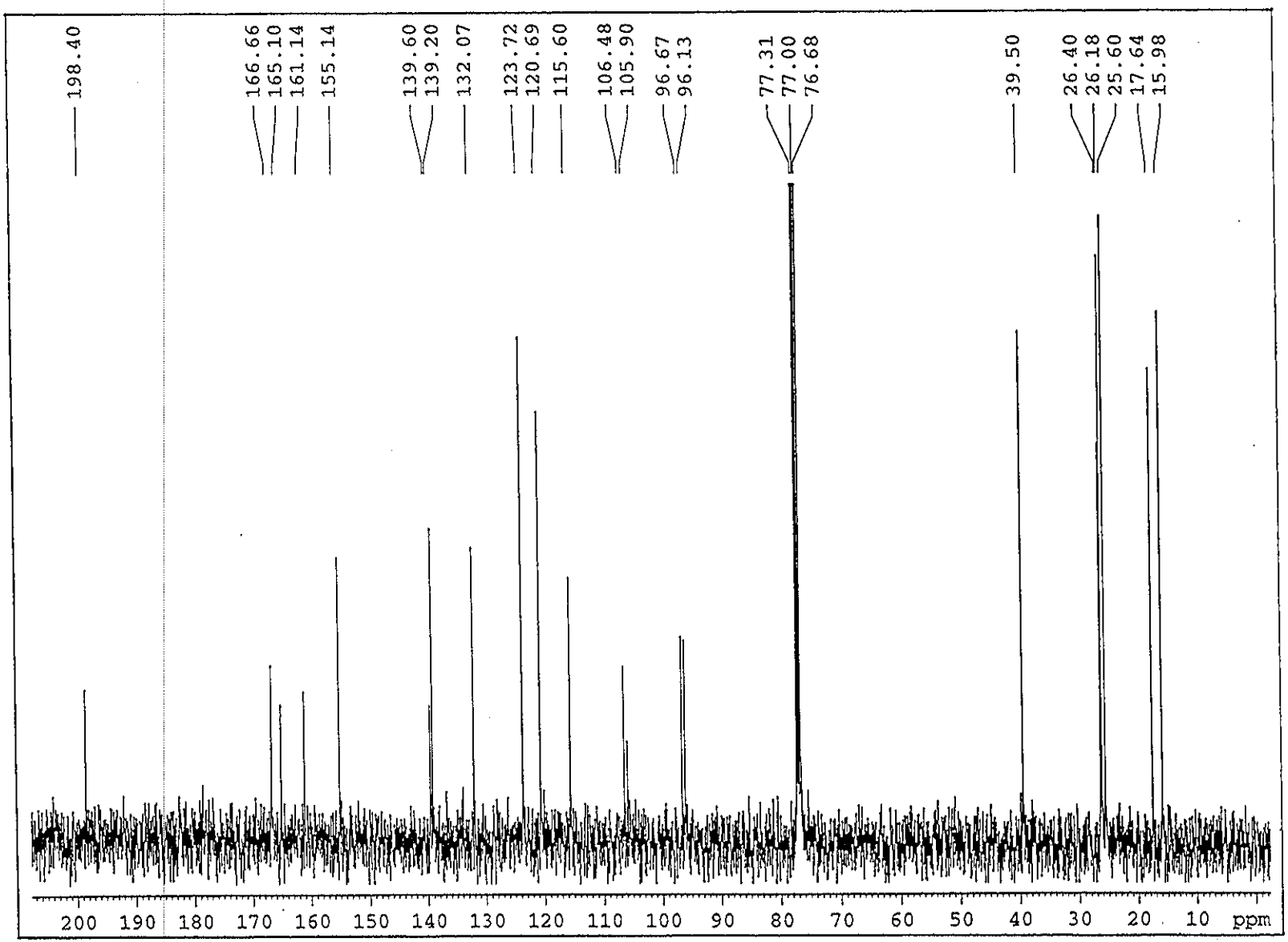


Figure 3.67 ^{13}C NMR (100 MHz)($CDCl_3$) spectrum of compound AH7

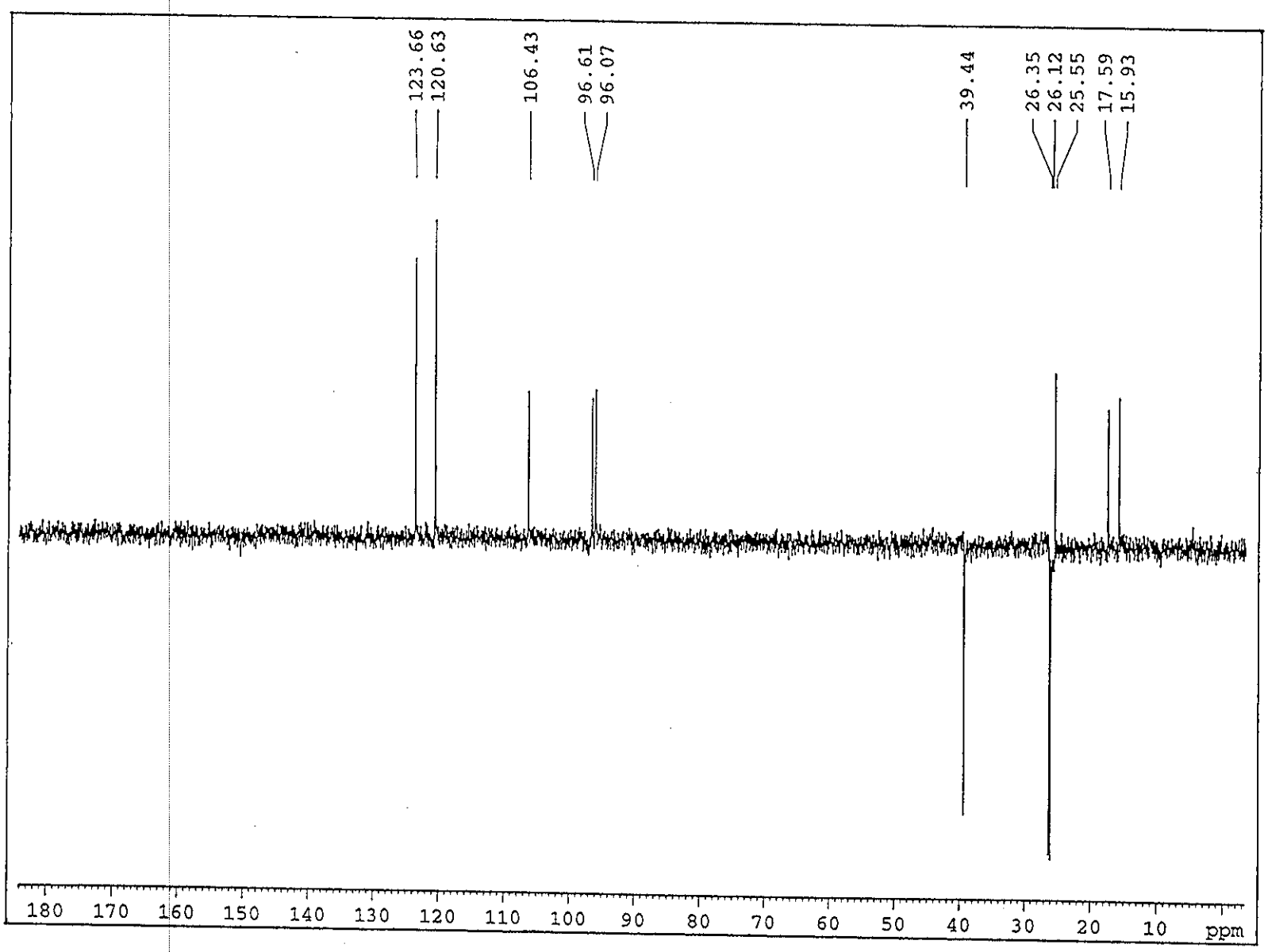


Figure 3.68 DEPT 135° spectrum of compound AH7

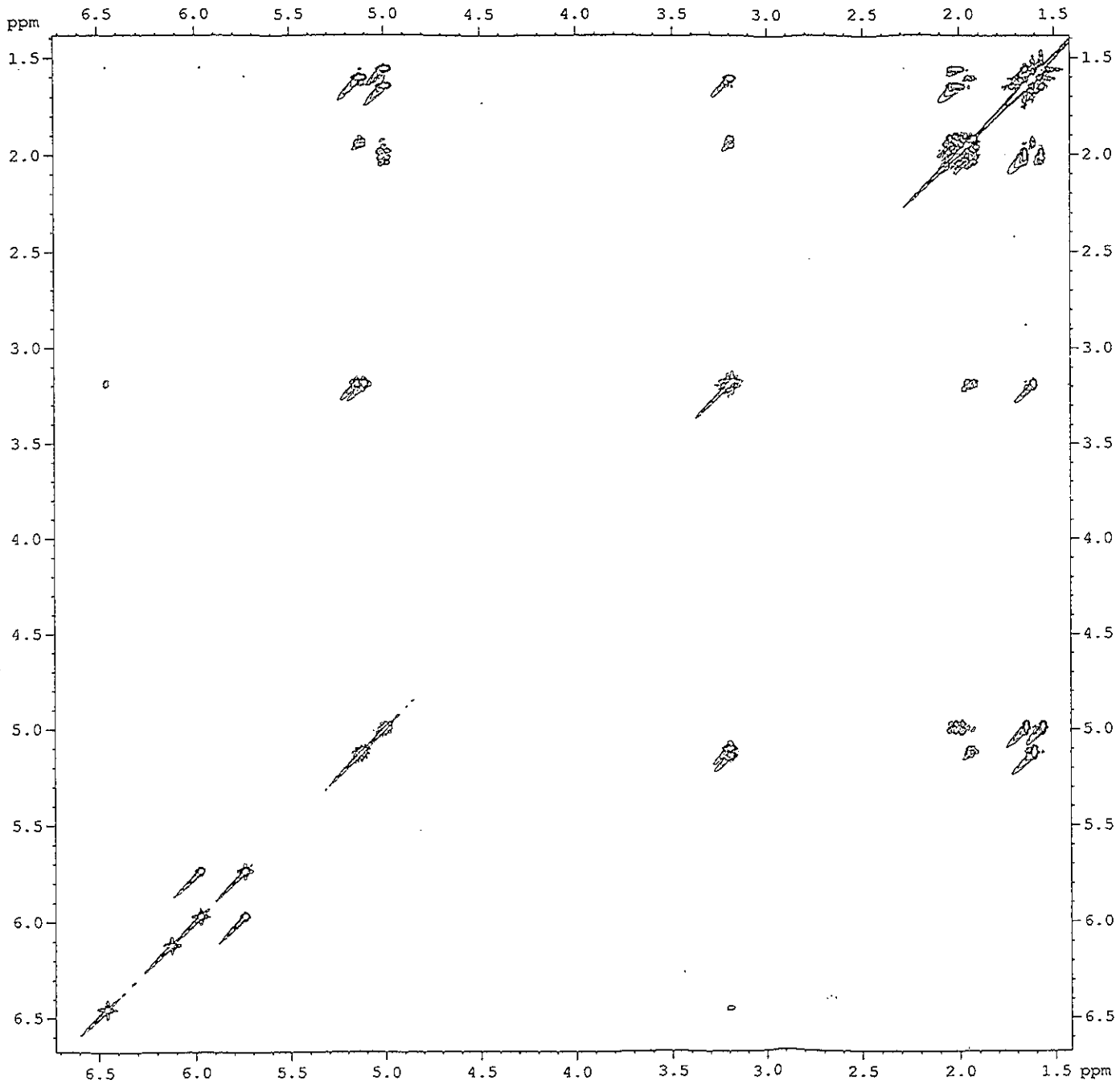


Figure 3.69 ^{1}H-^{1}H COSY spectrum of compound AH7

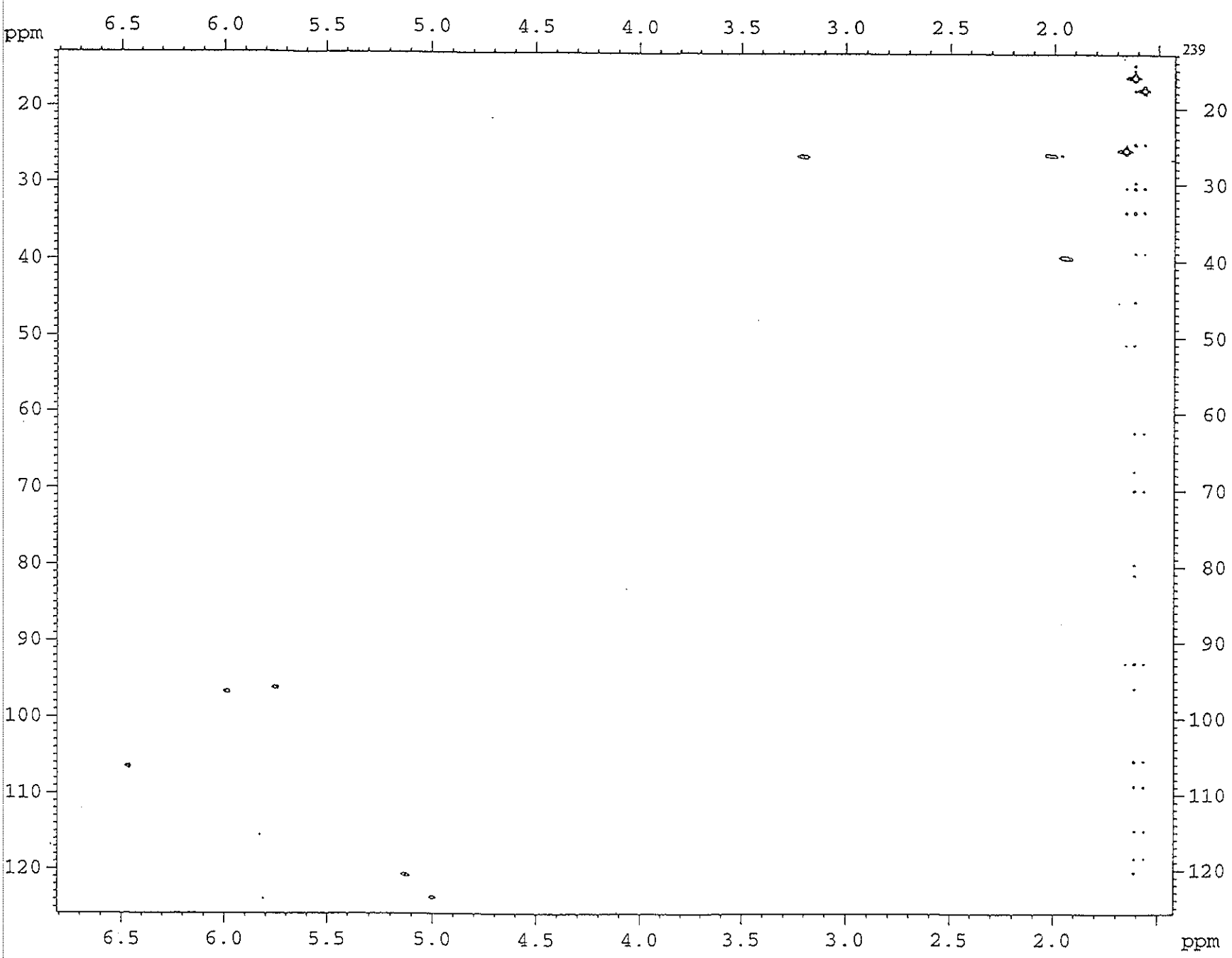


Figure 3.70 2D HMQC spectrum of compound AH7

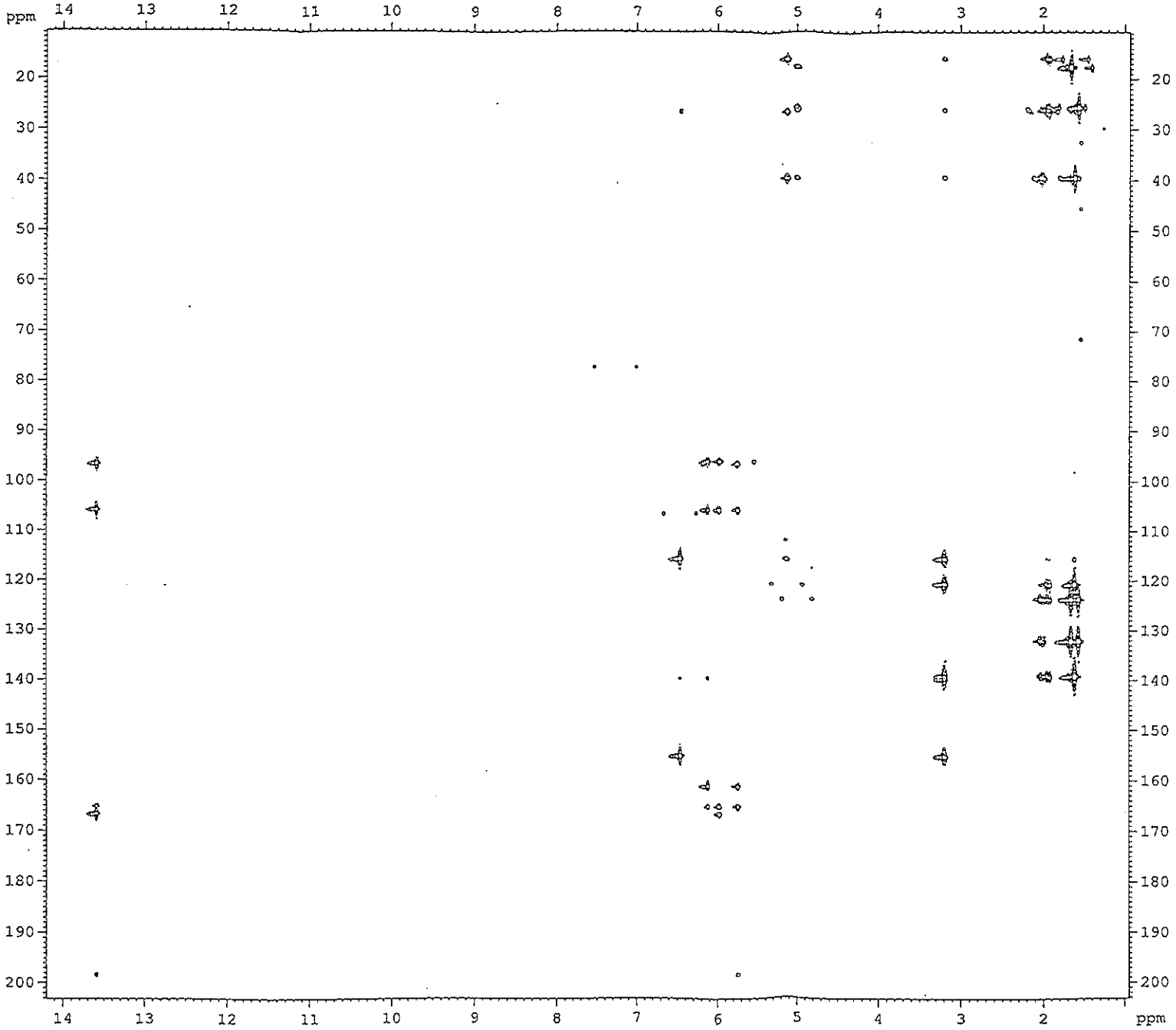


Figure 3.71 2D HMBC spectrum of compound AH7

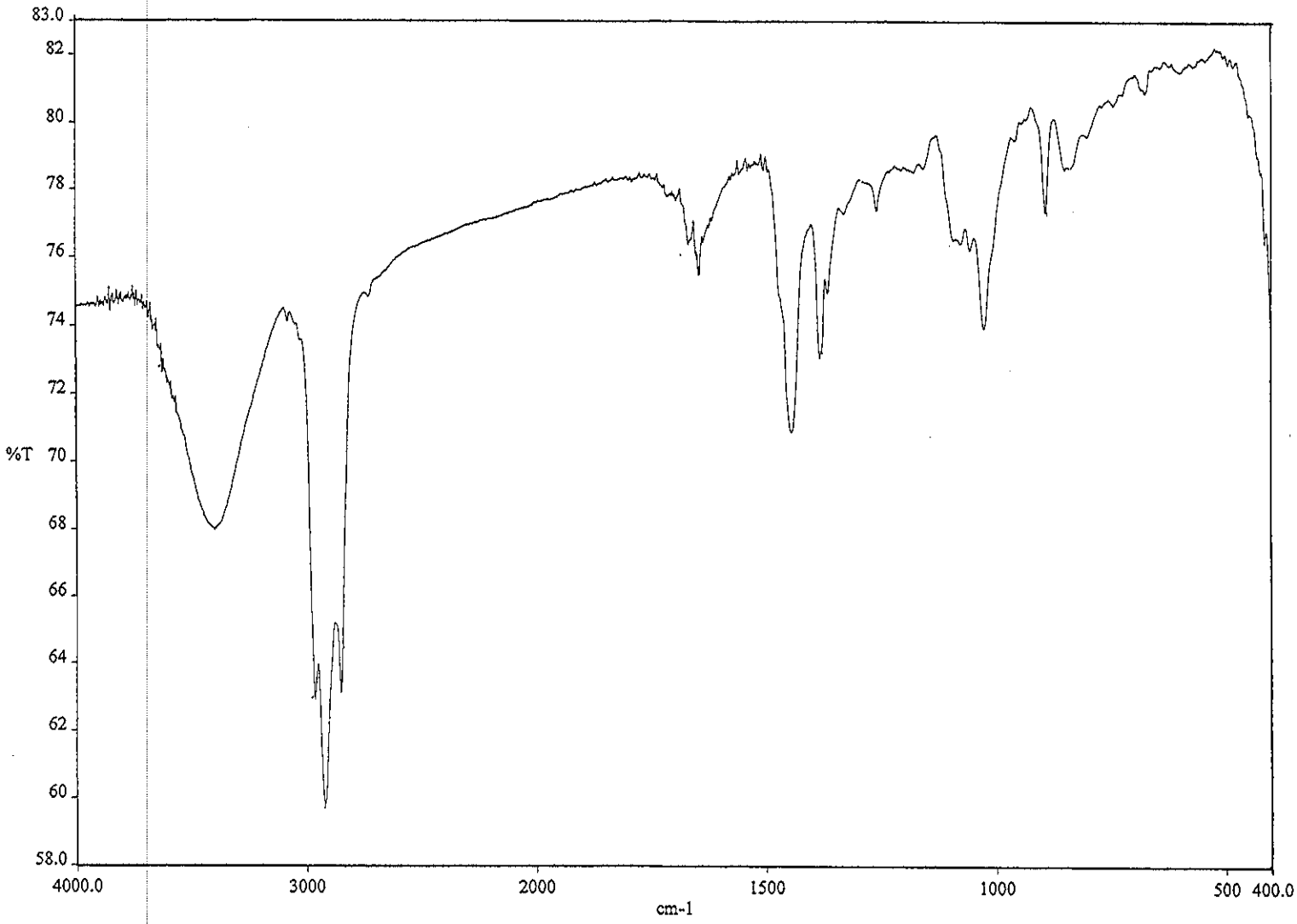


Figure 3.72 FT-IR (neat) spectrum of compound AH1

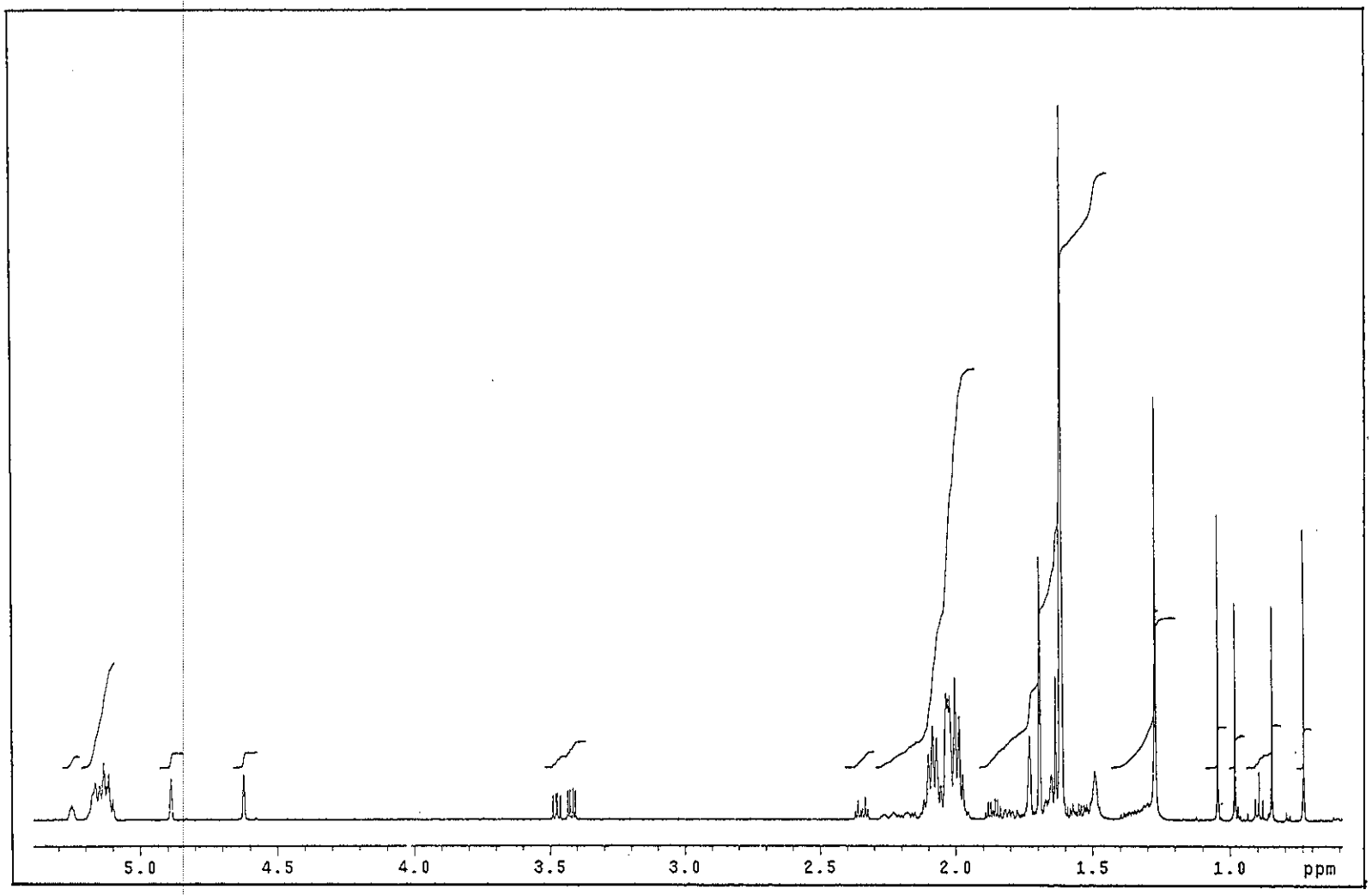


Figure 3.73 ^{1}H NMR (500 MHz)($CDCl_3$) spectrum of compound AH1

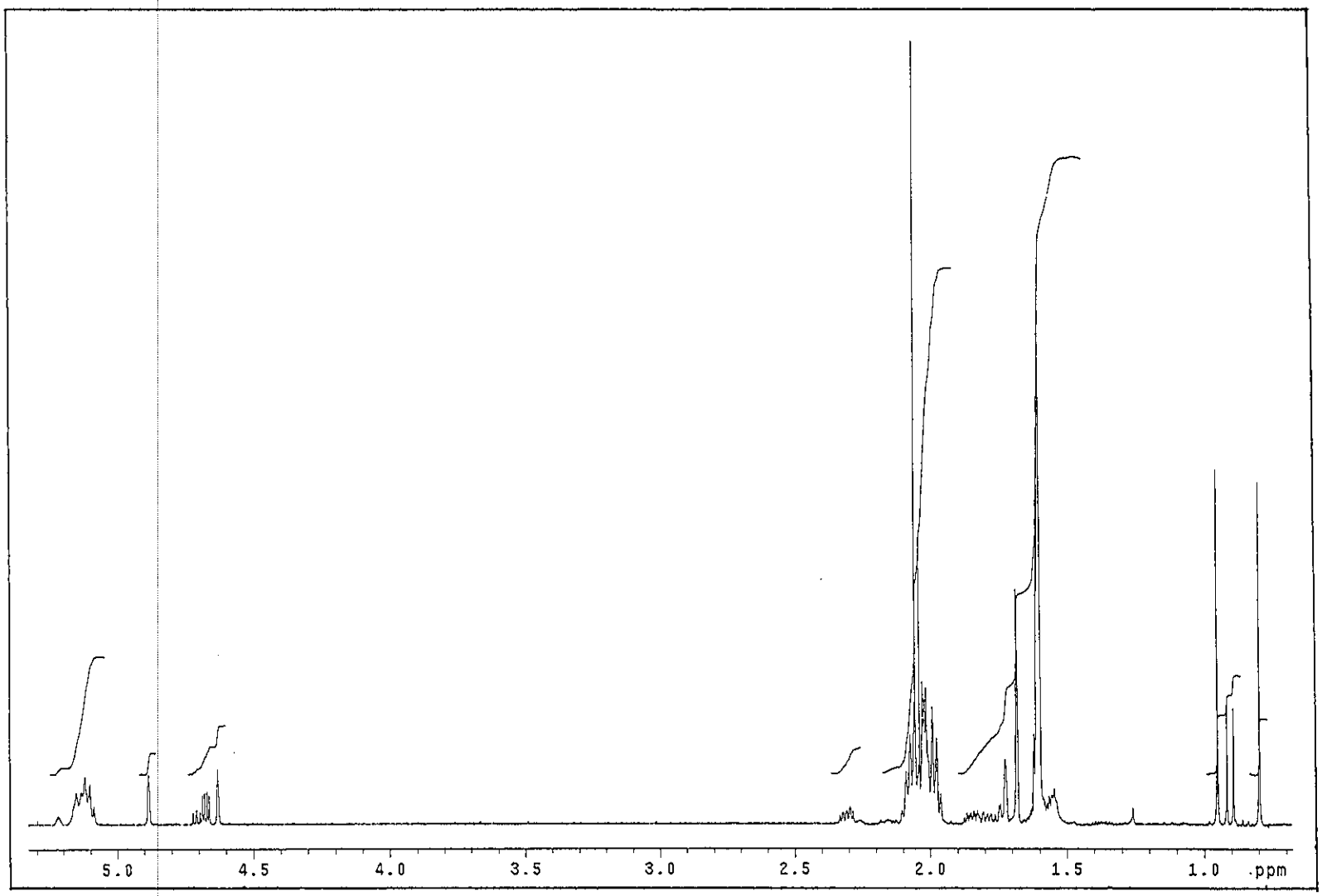


Figure 3.74 ^{1}H NMR (500 MHz)($CDCl_3$) spectrum of compound AH1-Ac

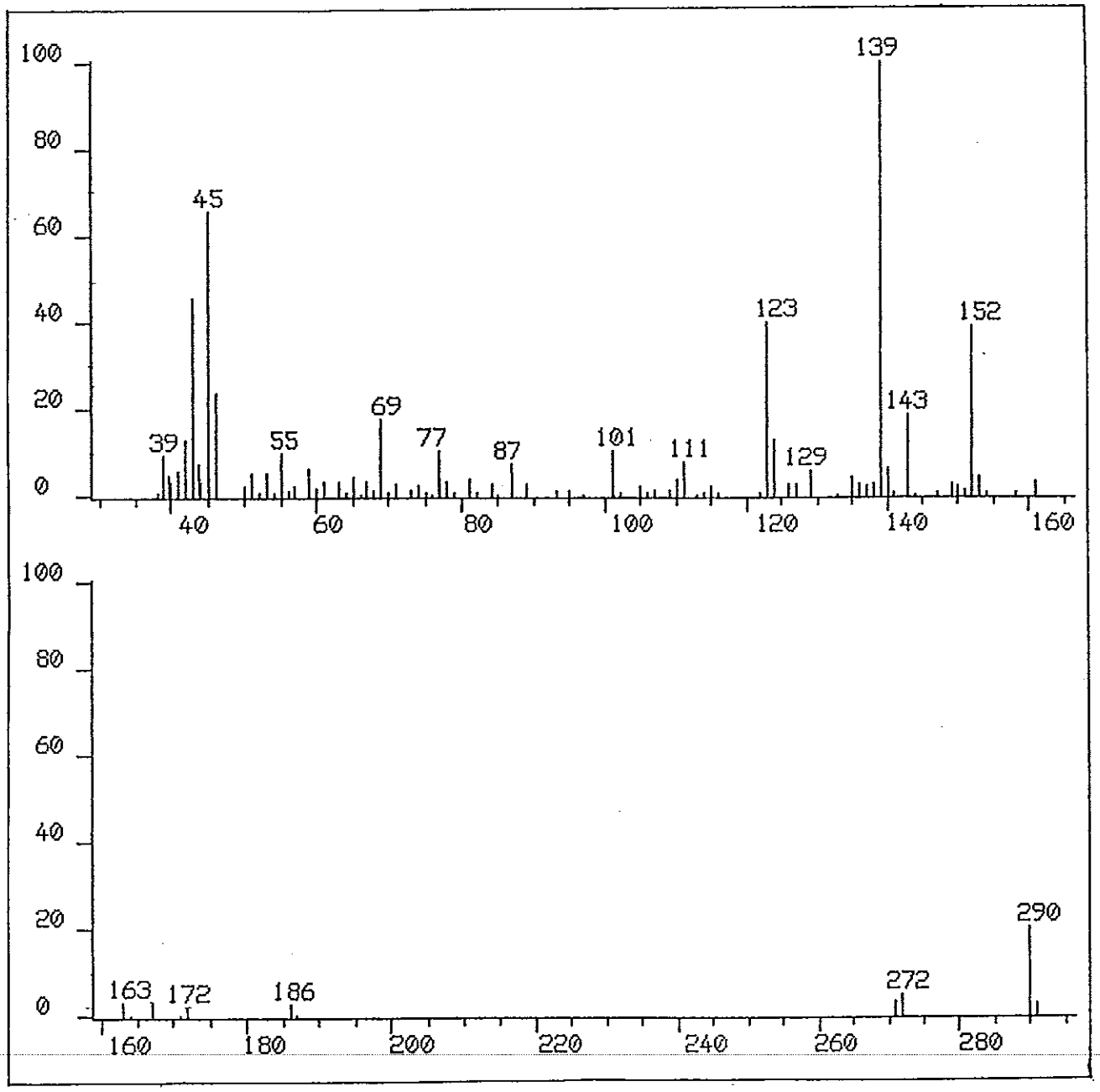


Figure 3.75 Mass spectrum of compound AH6

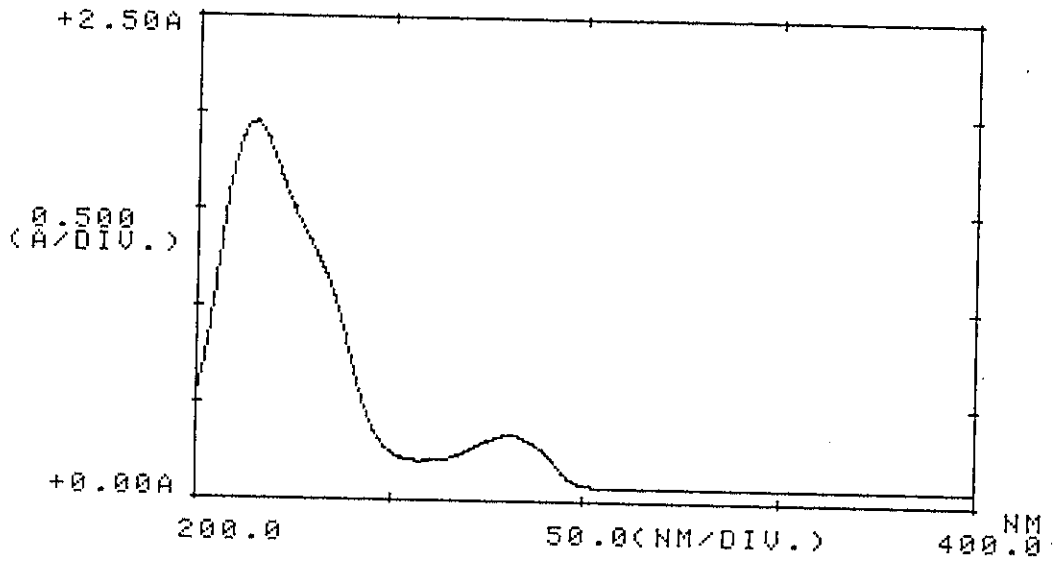


Figure 3.76 UV (MeOH) spectrum of compound AH6

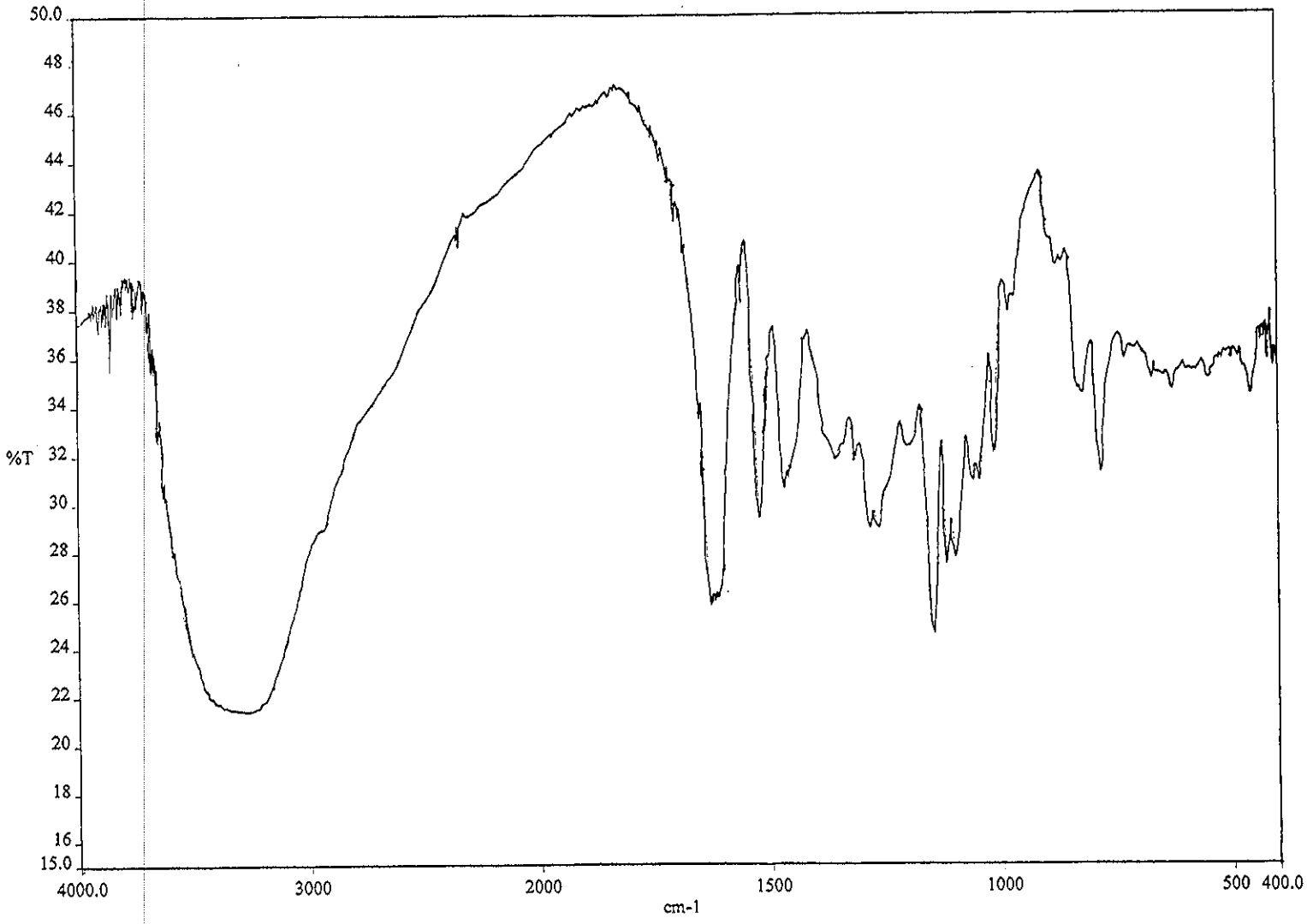


Figure 3.77 FT-IR (KBr) spectrum of compound AH6

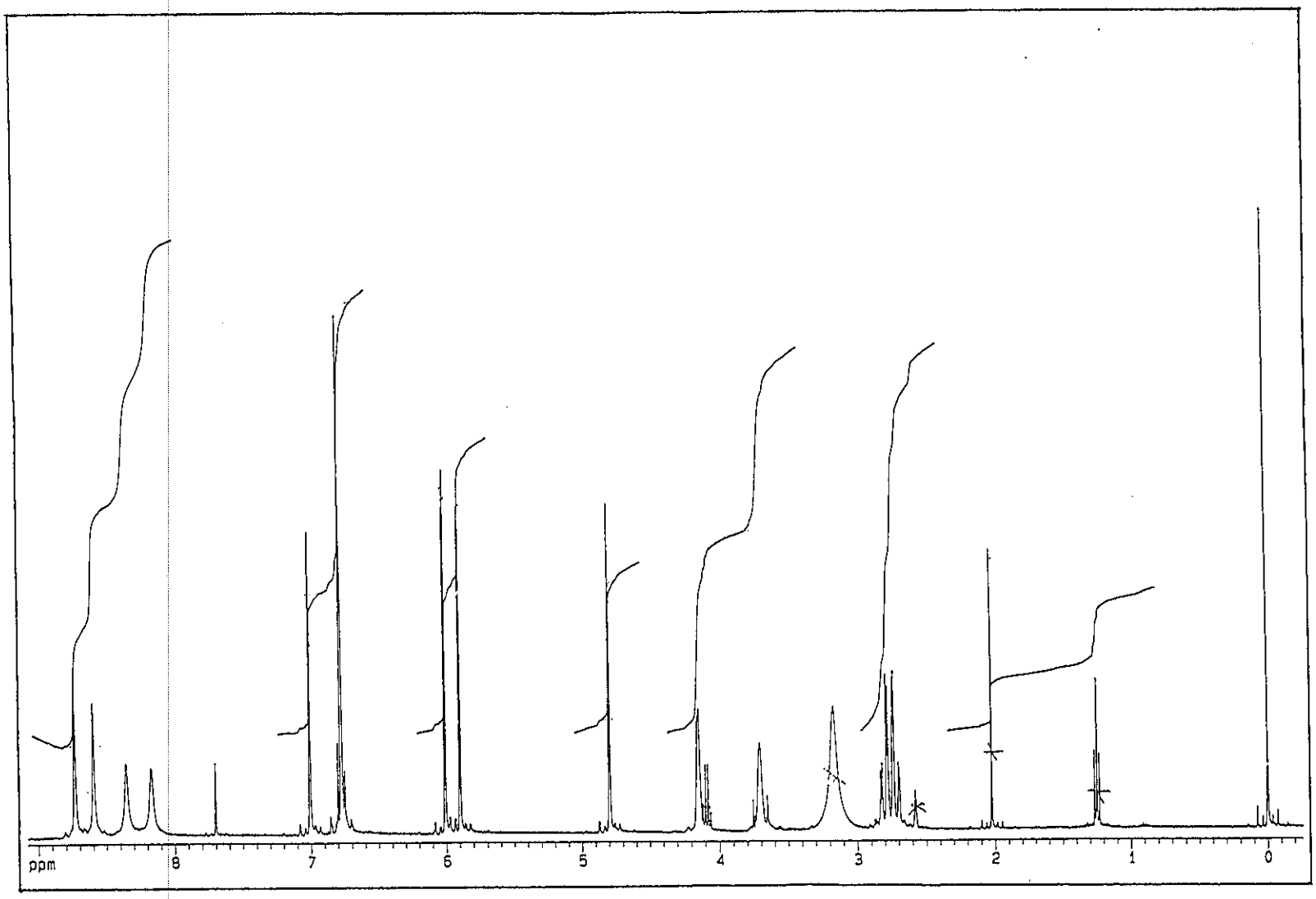


Figure 3.78 ^{1}H NMR (400 MHz)($CDCl_3$+DMSO-d_6) spectrum of compound AH6

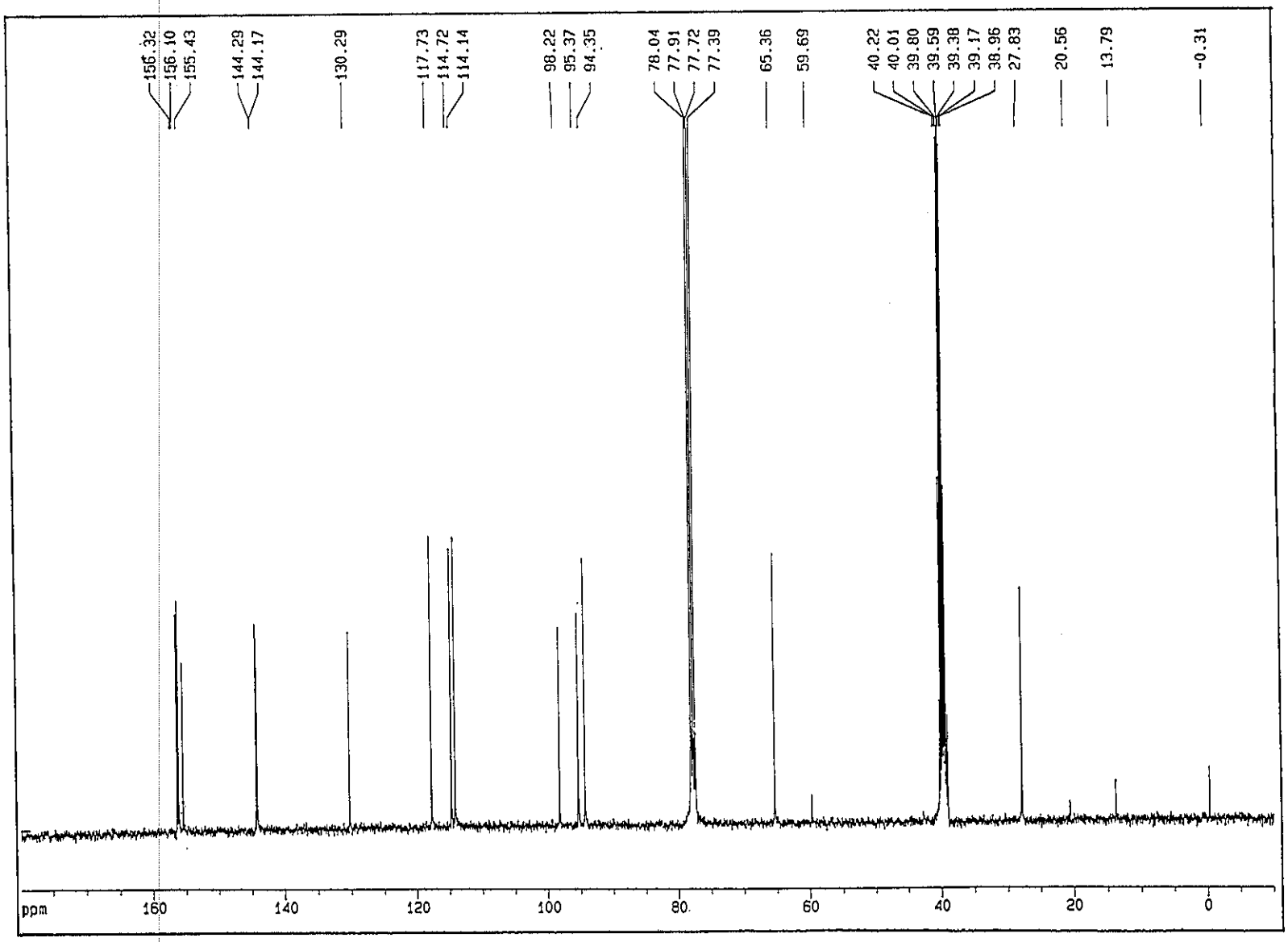


Figure 3.79 ^{13}C NMR (100 MHz)($CDCl_3$+DMSO-d_6) spectrum of compound AH6

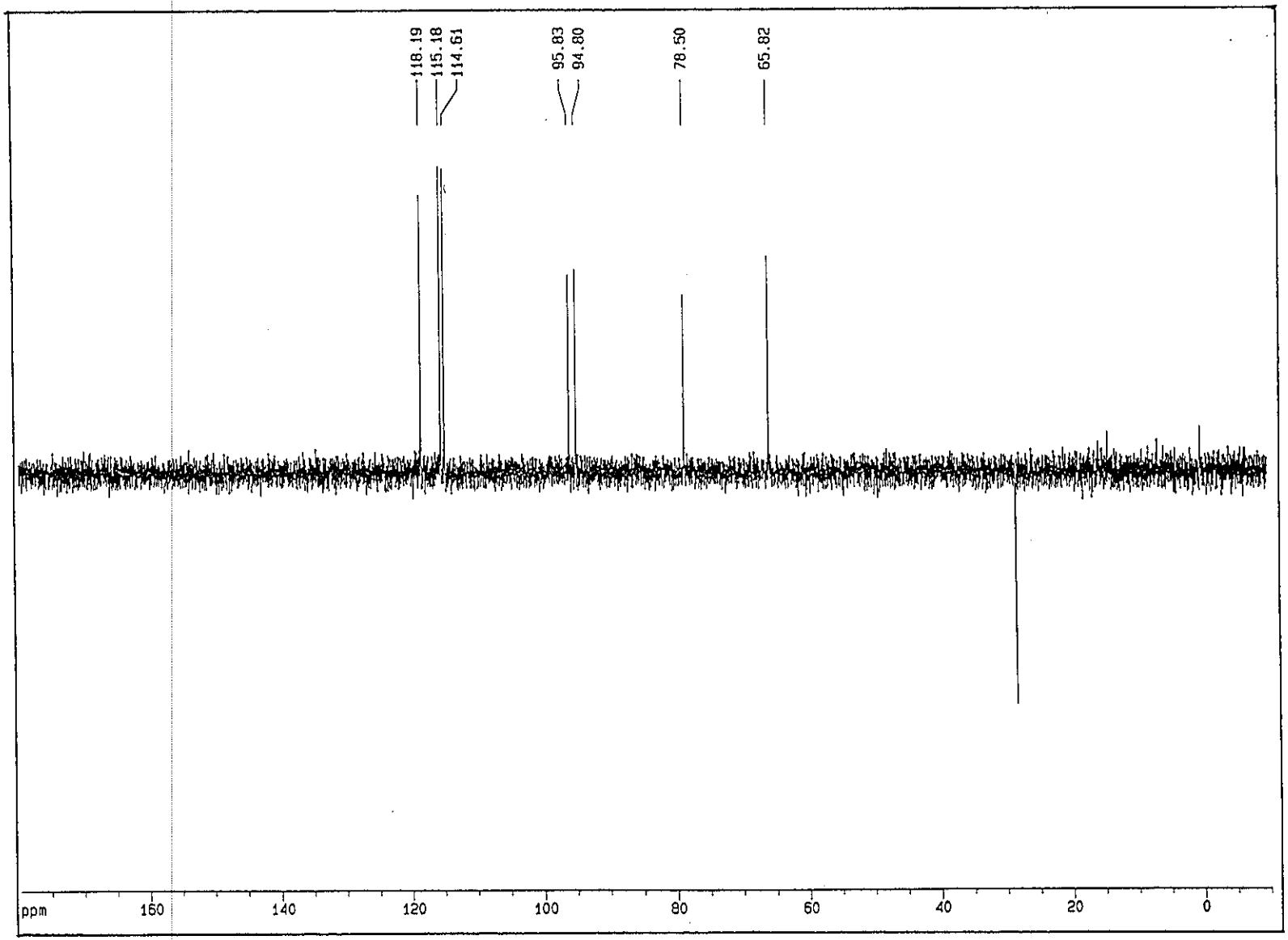


Figure 3.80 DEPT 135° spectrum of compound AH6

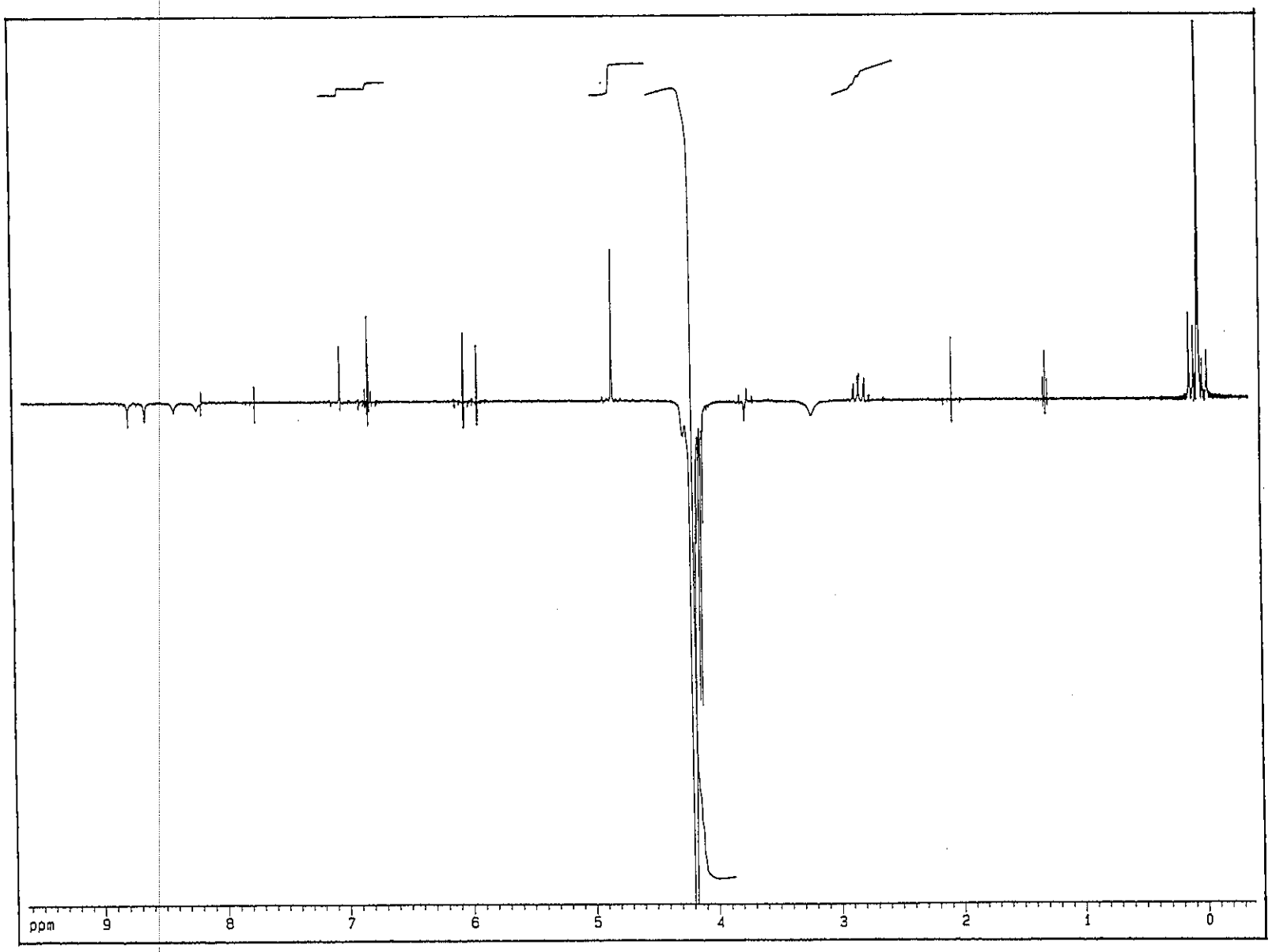


Figure 3.81 NOE difference spectrum of compound AH6 after irradiation at δ_H 4.14 ppm

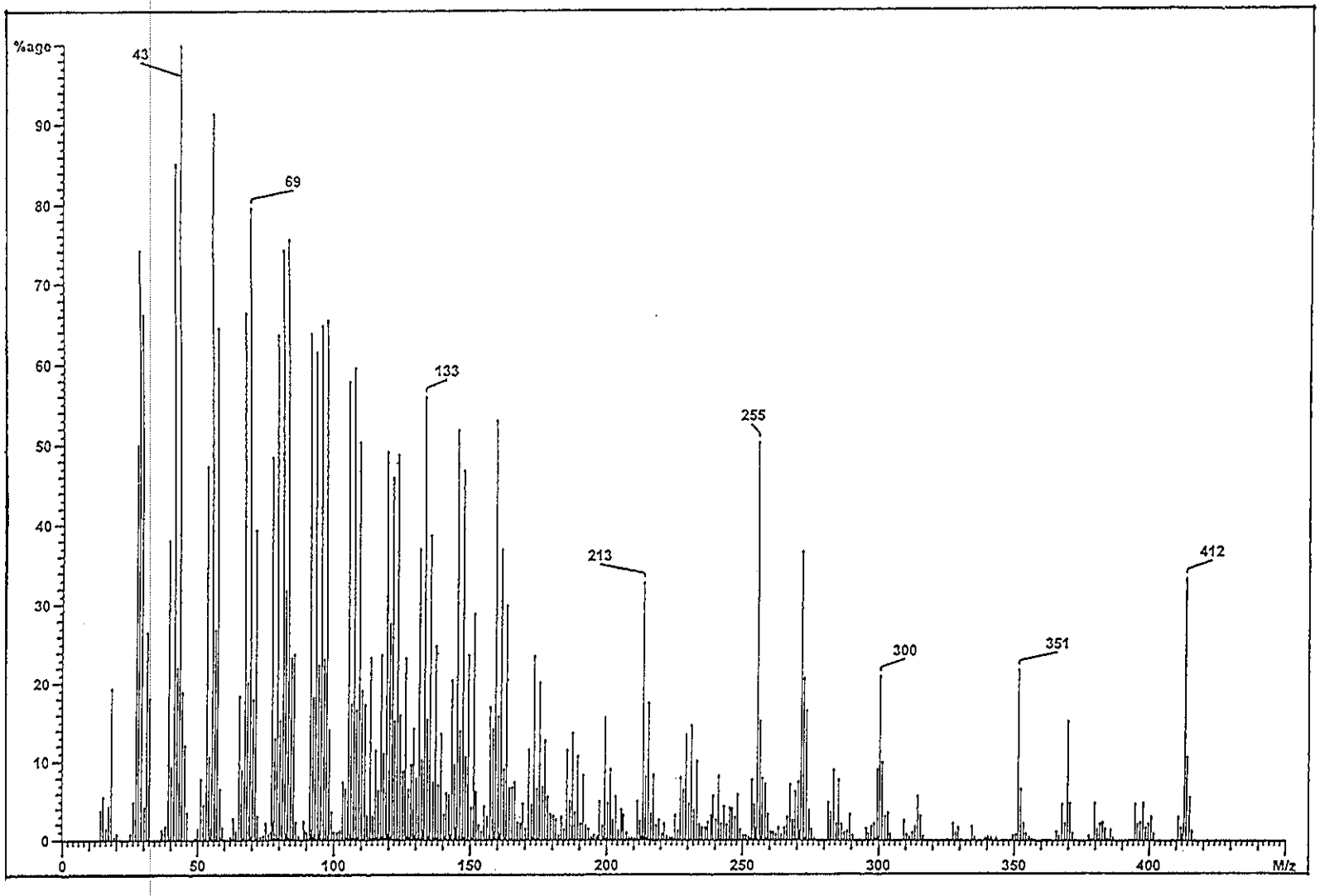


Figure 3.82 Mass spectrum of compound AH8

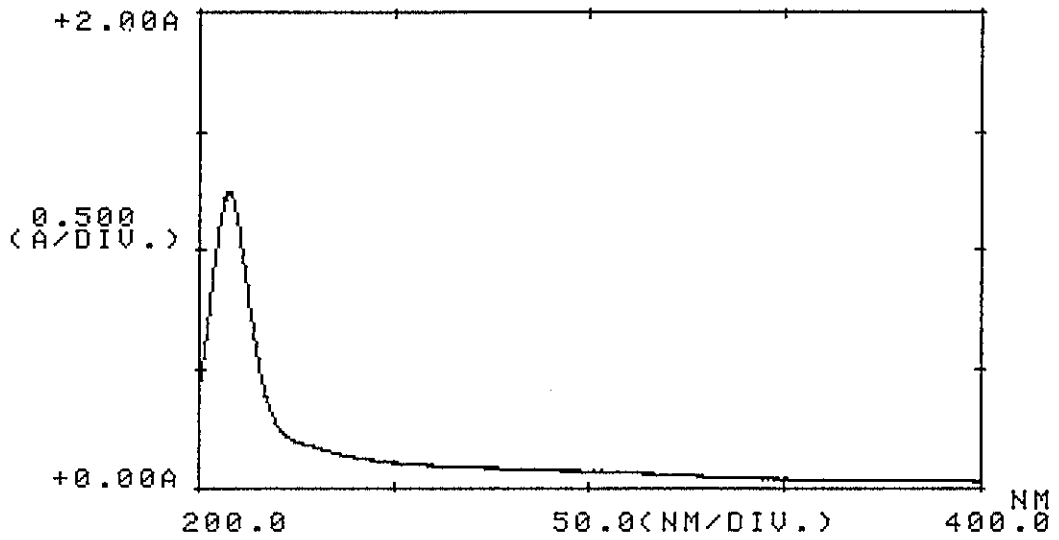


Figure 3.83 UV (MeOH) spectrum of compound AH8

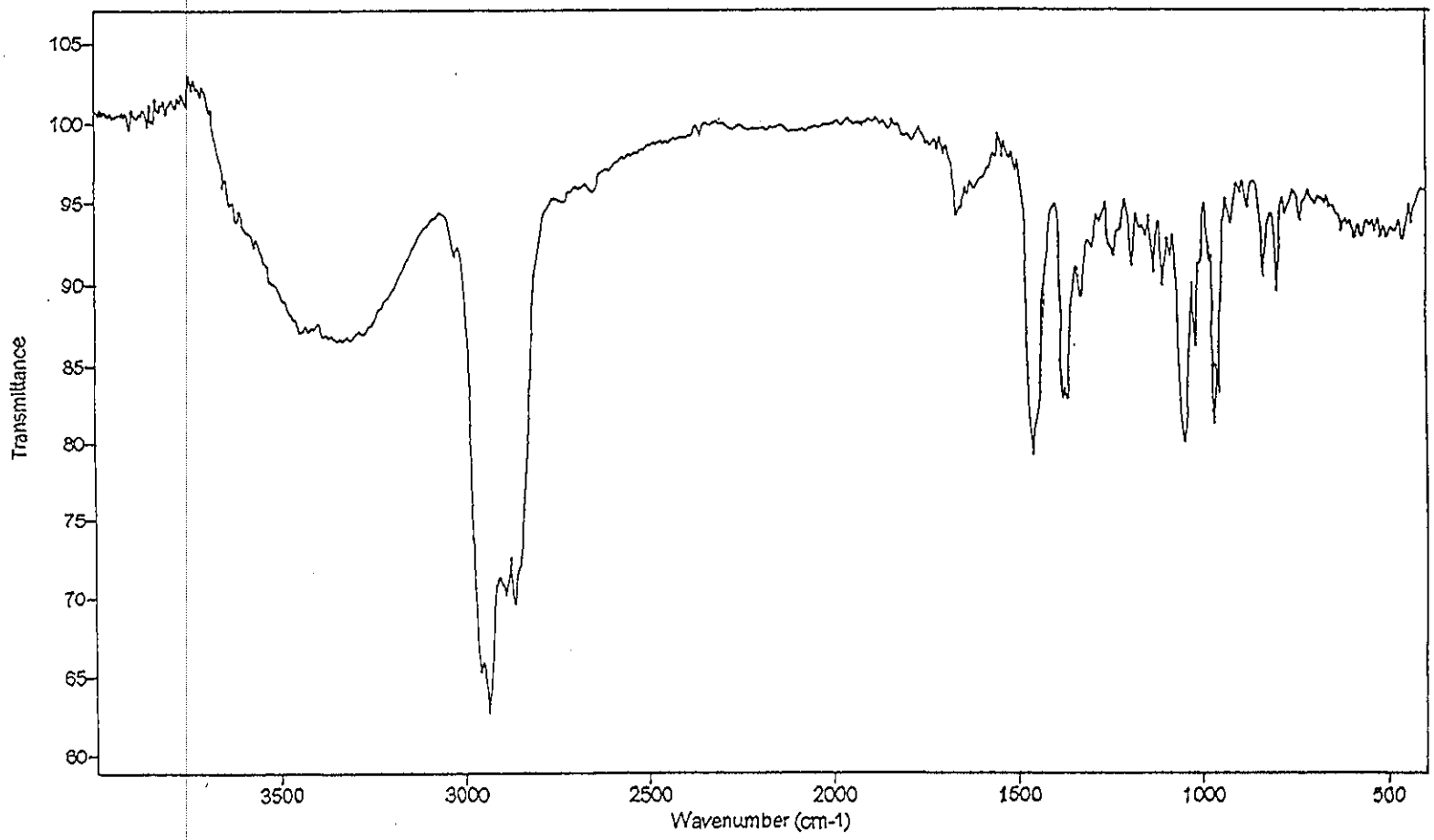


Figure 3.84 FT-IR (KBr) spectrum of compound AH8

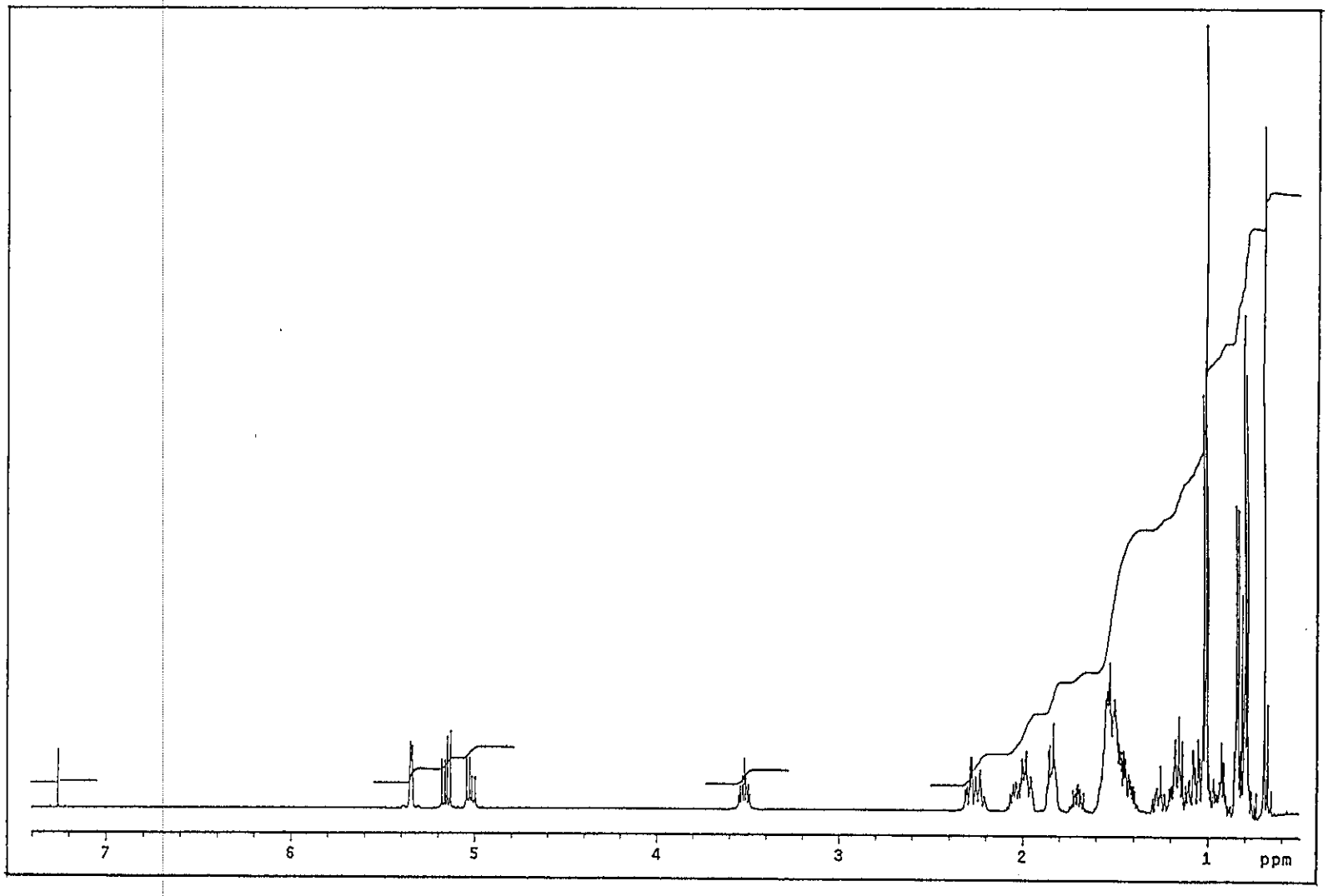


Figure 3.85 ^{1}H NMR (500 MHz)($CDCl_3$) spectrum of compound AH8

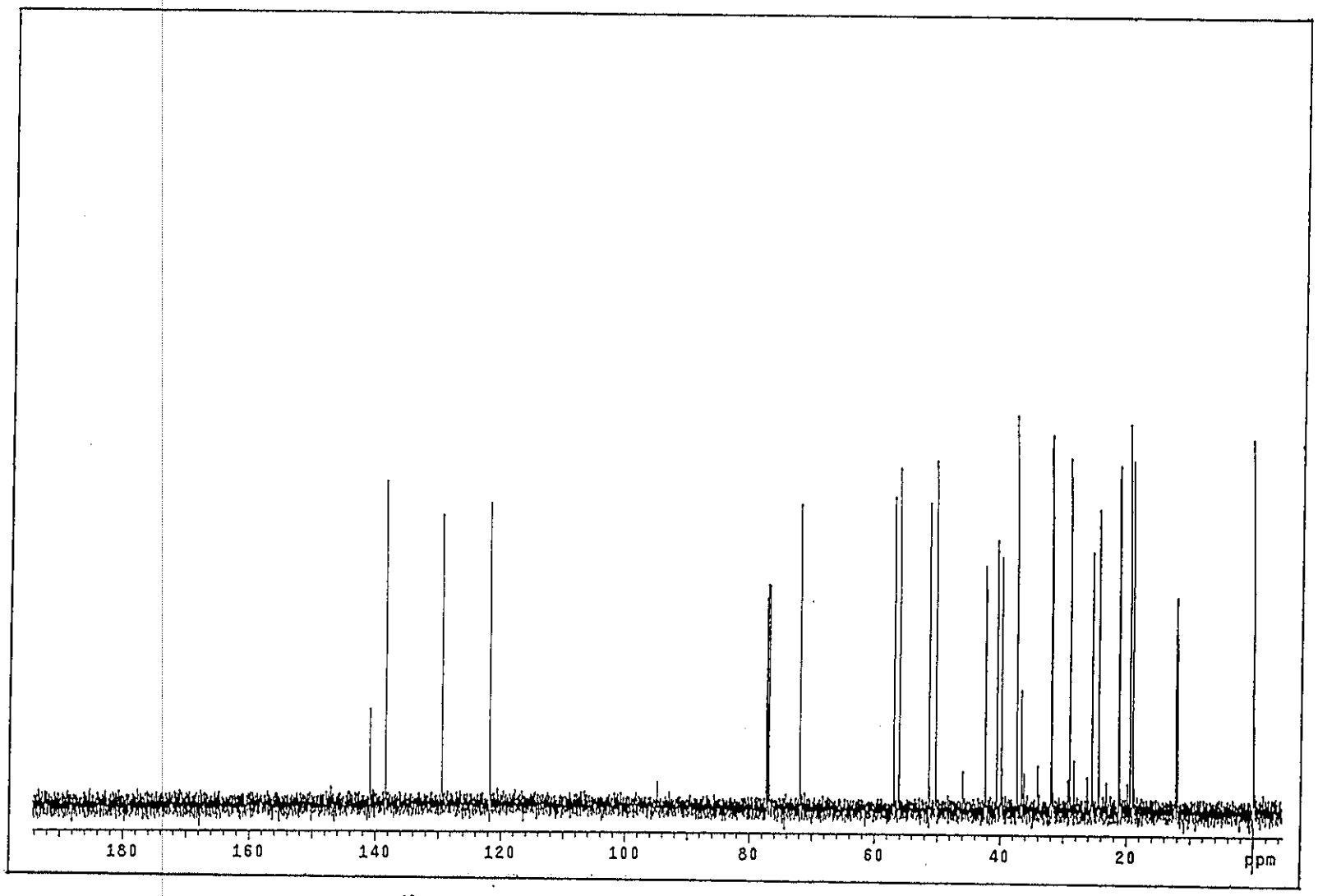


Figure 3.86 ^{13}C NMR (125 MHz)($CDCl_3$) spectrum of compound AH8

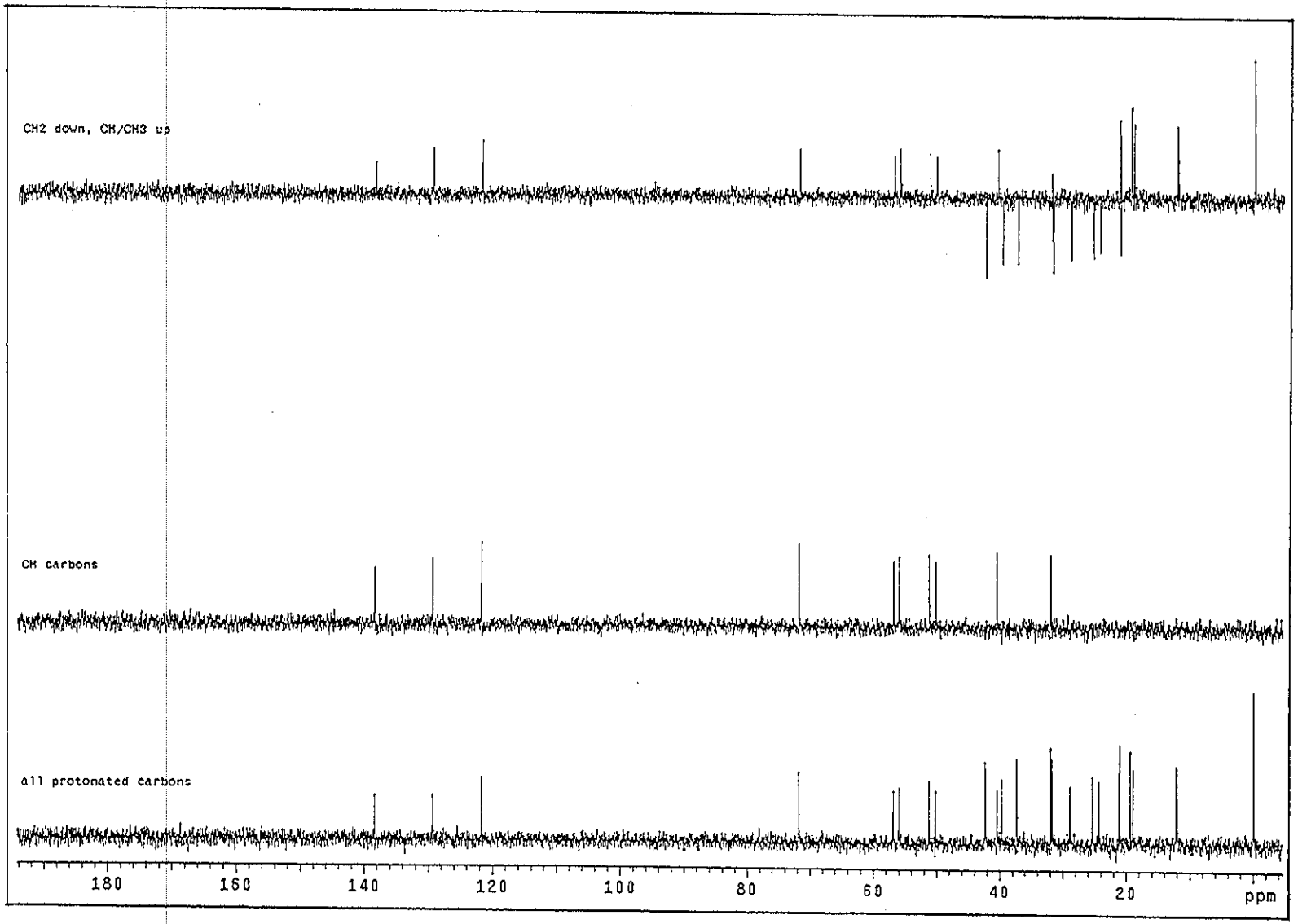


Figure 3.87 DEPT spectrum of compound AH8

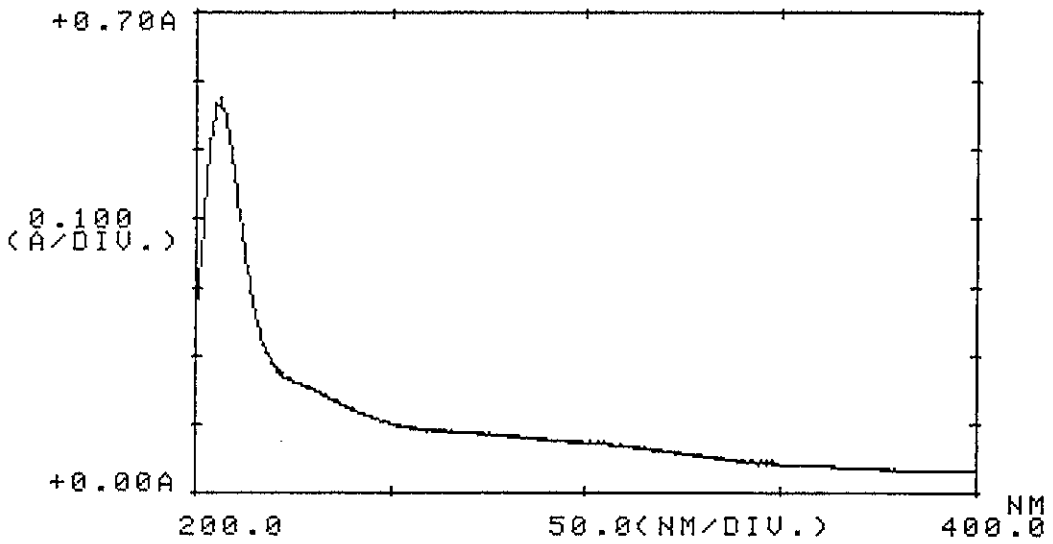


Figure 3.88 UV (MeOH) spectrum of compound AH9

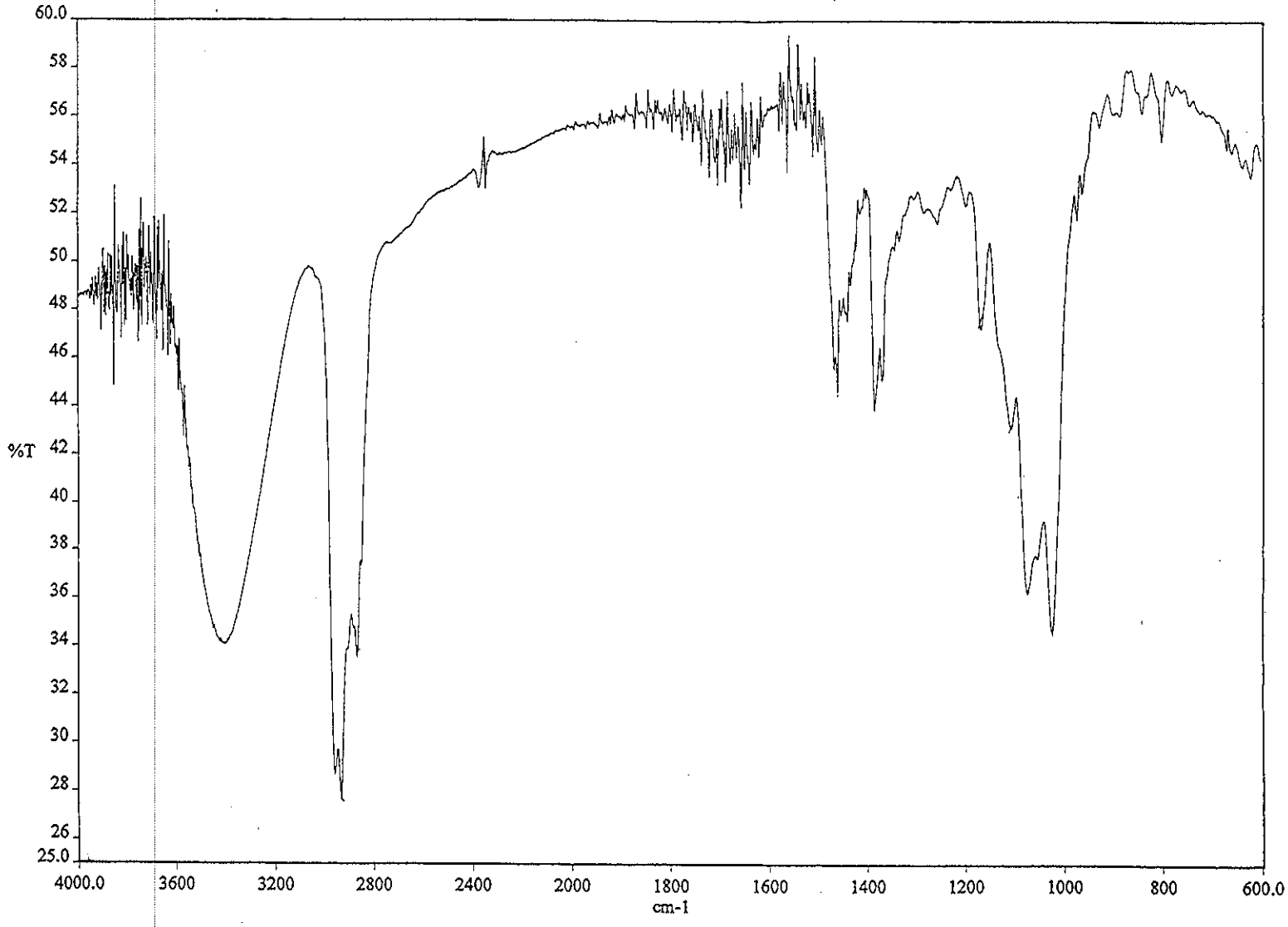


Figure 3.89 FT-IR (KBr) spectrum of compound AH9

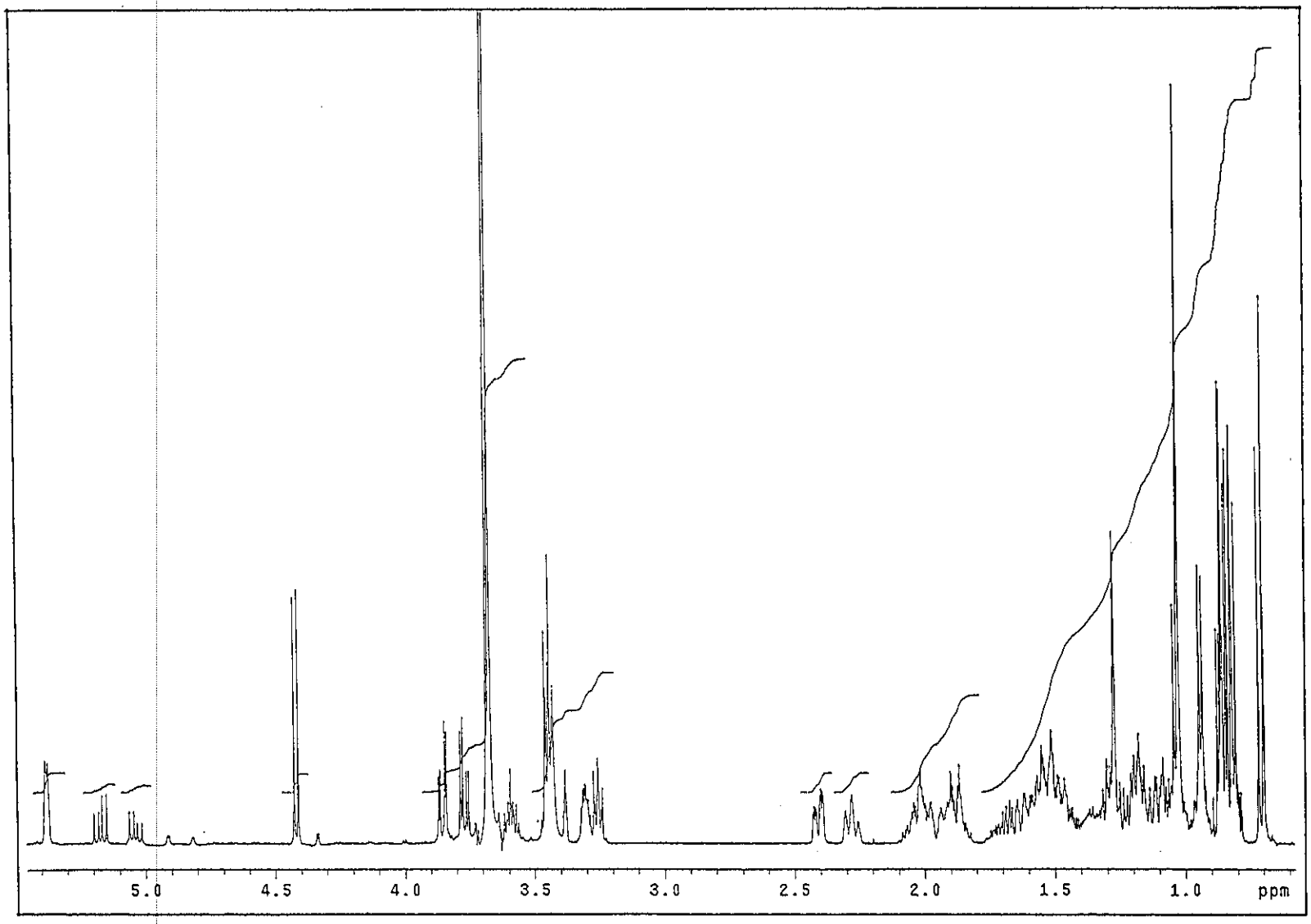


Figure 3.90 ^{1}H NMR (500 MHz)($CDCl_3$+CD_3OD) spectrum of compound AH9

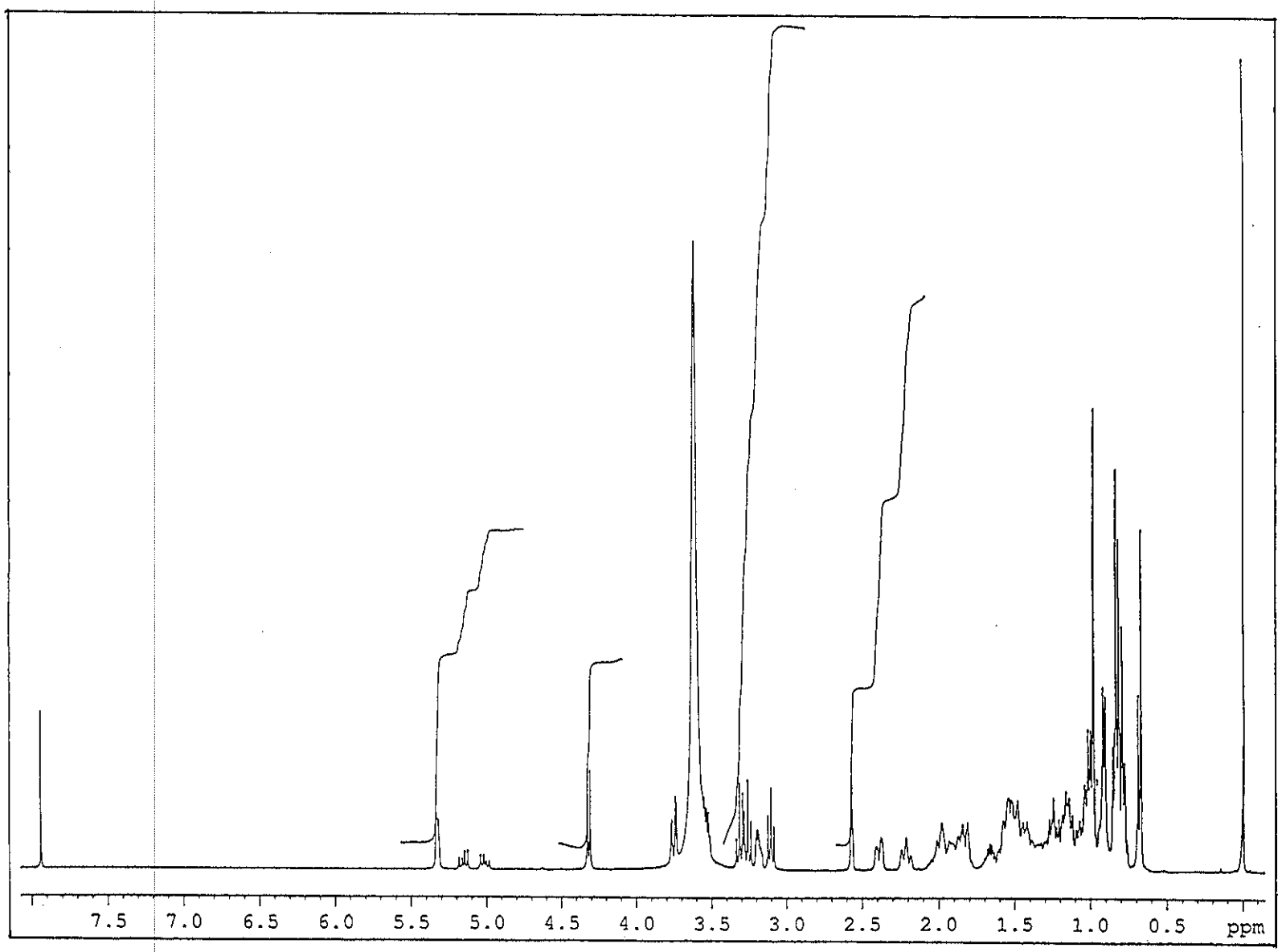


Figure 3.91 ^{1}H NMR (400 MHz)($CDCl_3$+DMSO-d_6) spectrum of a mixture of sitosterol and stigmasterol glucosides

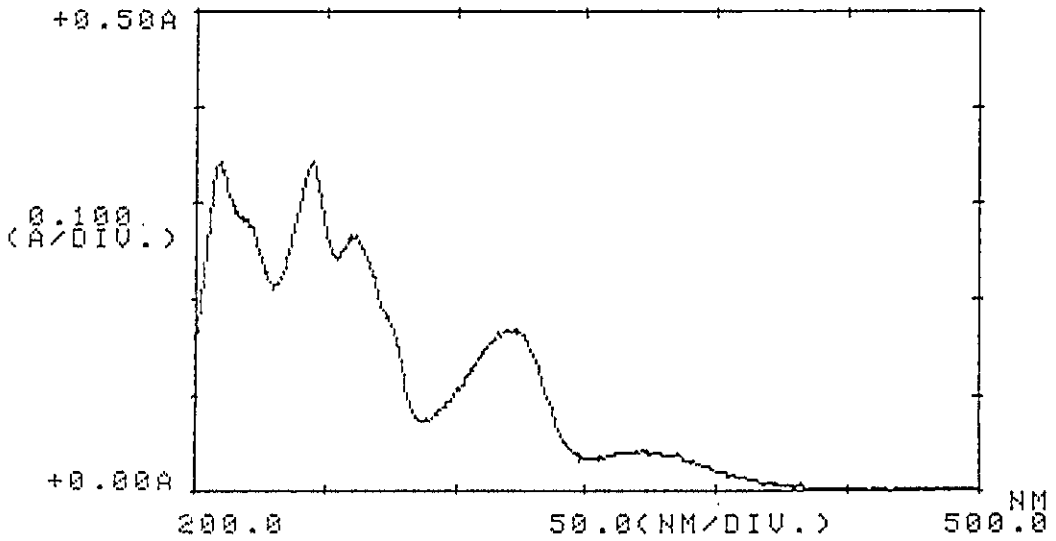


Figure 3.92 UV (MeOH) spectrum of compound AH11

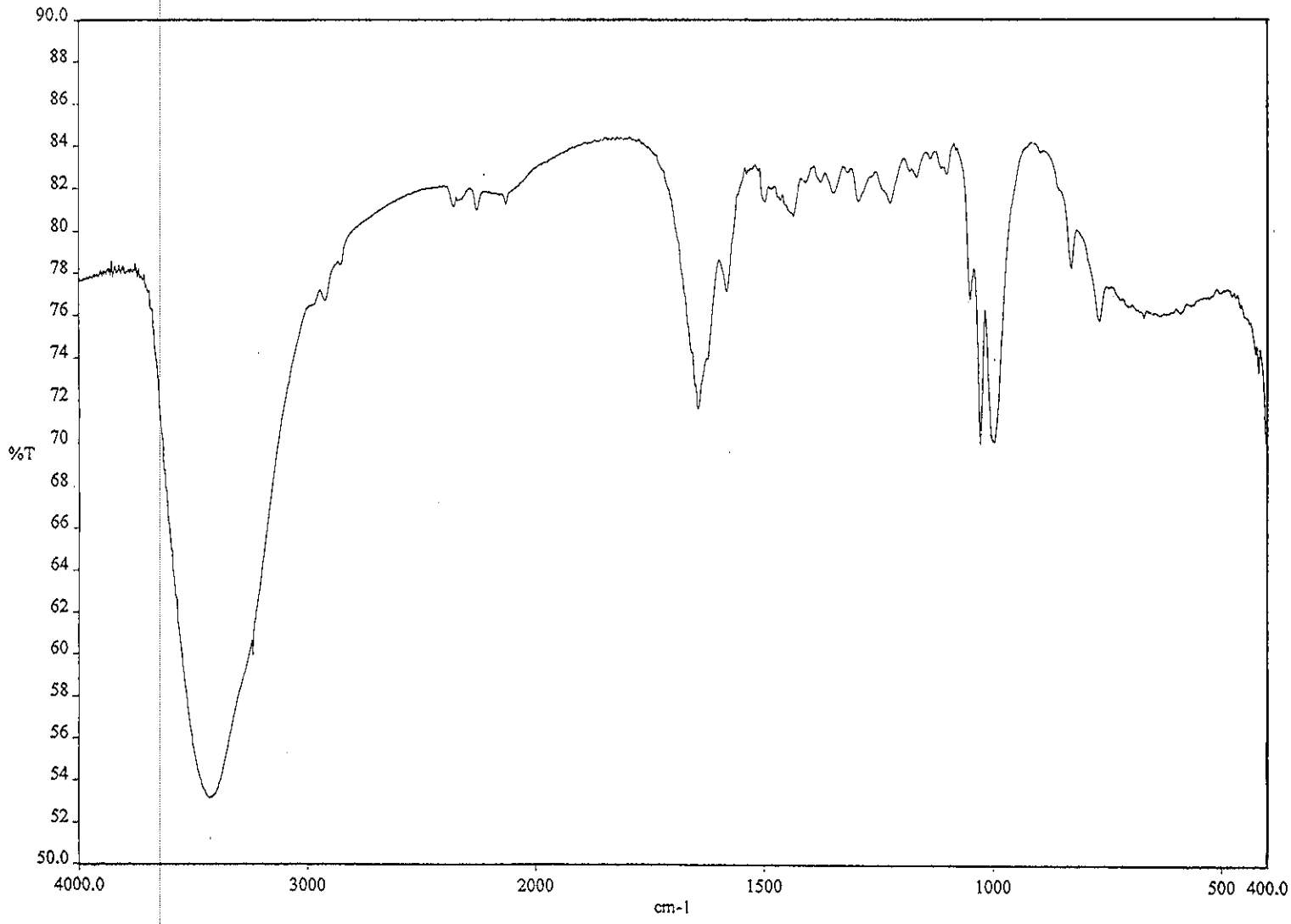


Figure 3.93 FT-IR (KBr) spectrum of compound AH11

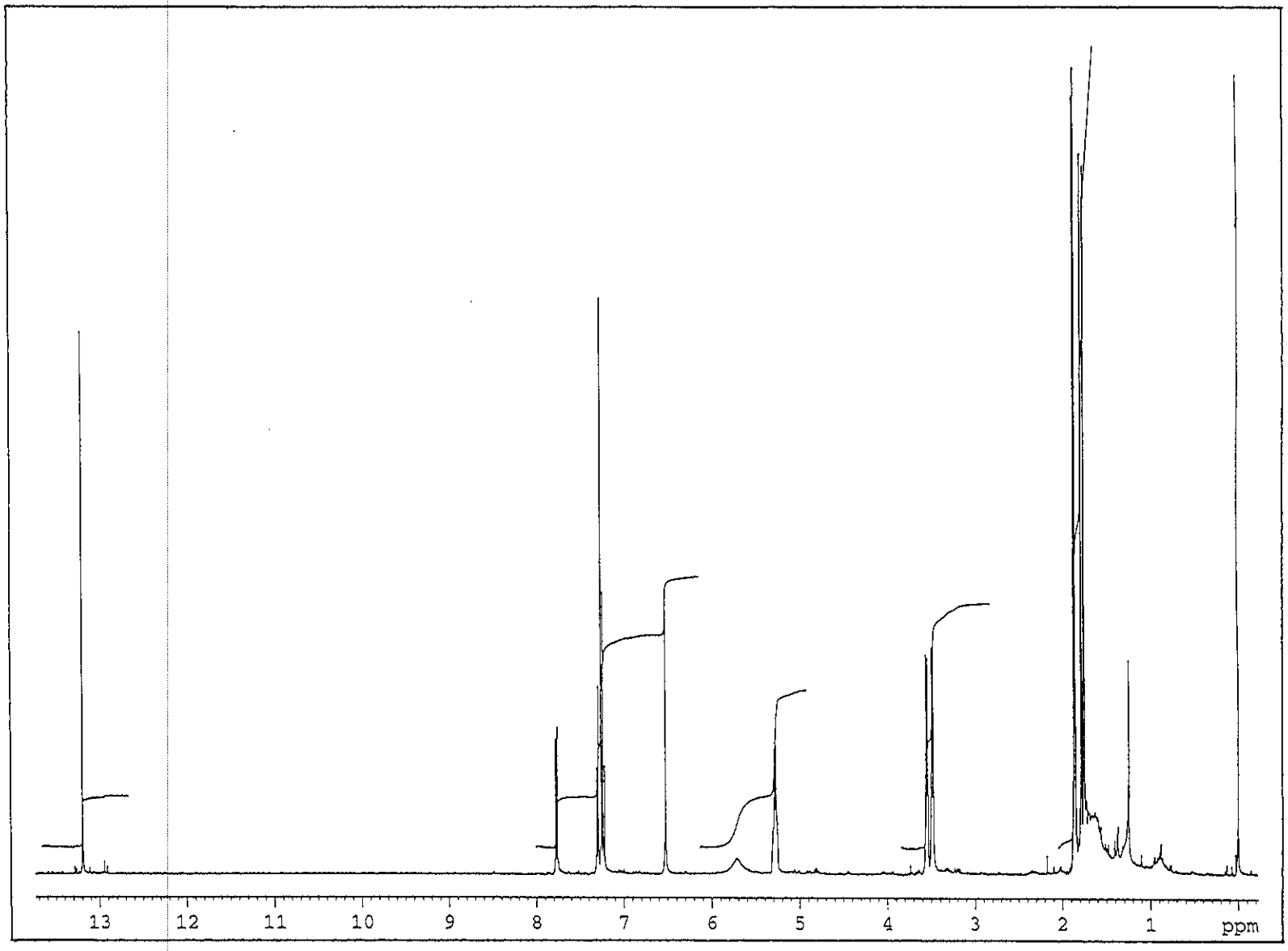


Figure 3.94 ^{1}H NMR (400 MHz)($CDCl_3$) spectrum of compound AH11

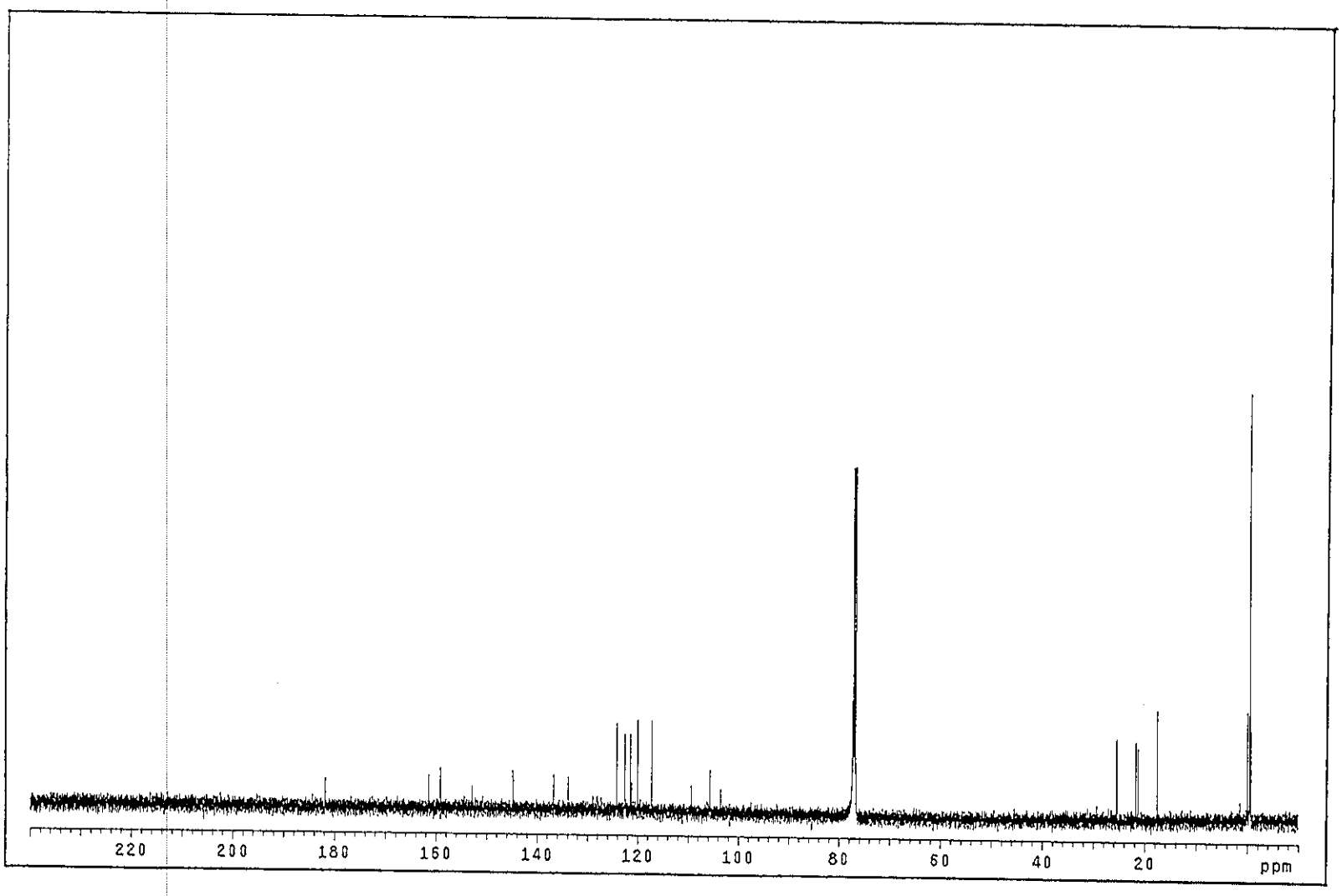


Figure 3.95 ^{13}C NMR (125 MHz)($CDCl_3$) spectrum of compound AH11

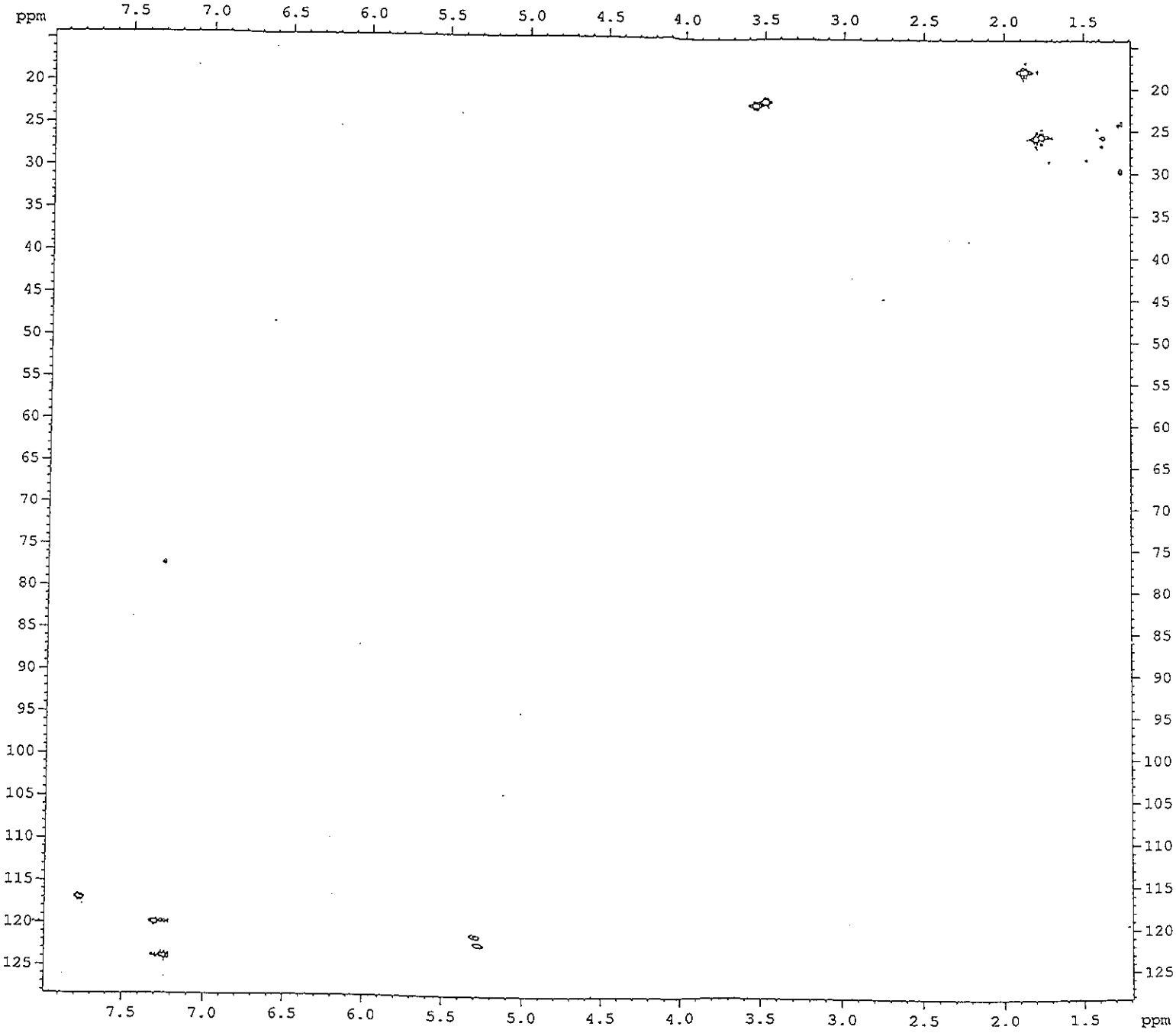


Figure 3.96 2D HMQC spectrum of compound AH11

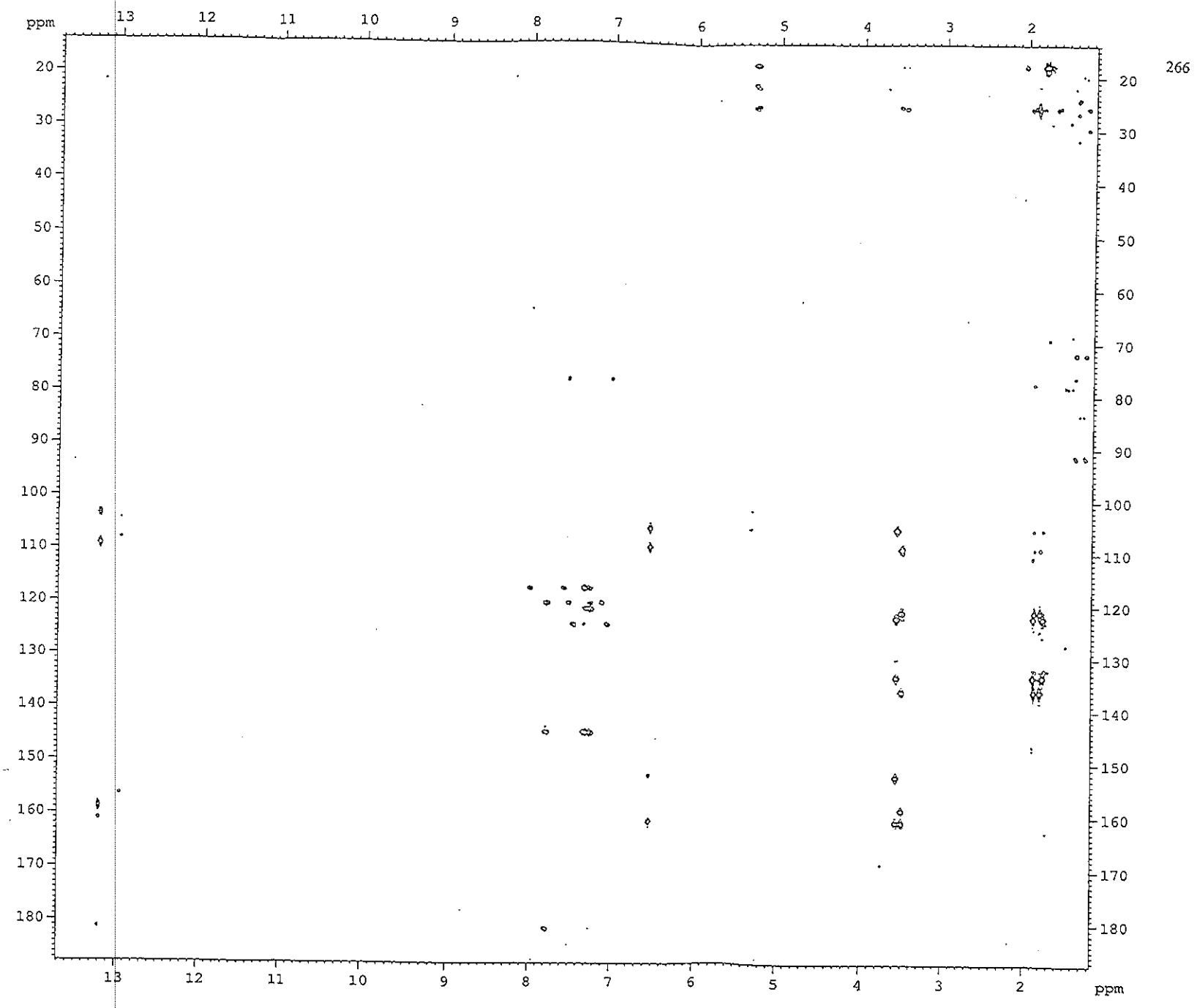


Figure 3.97 2D HMBC spectrum of compound AH11

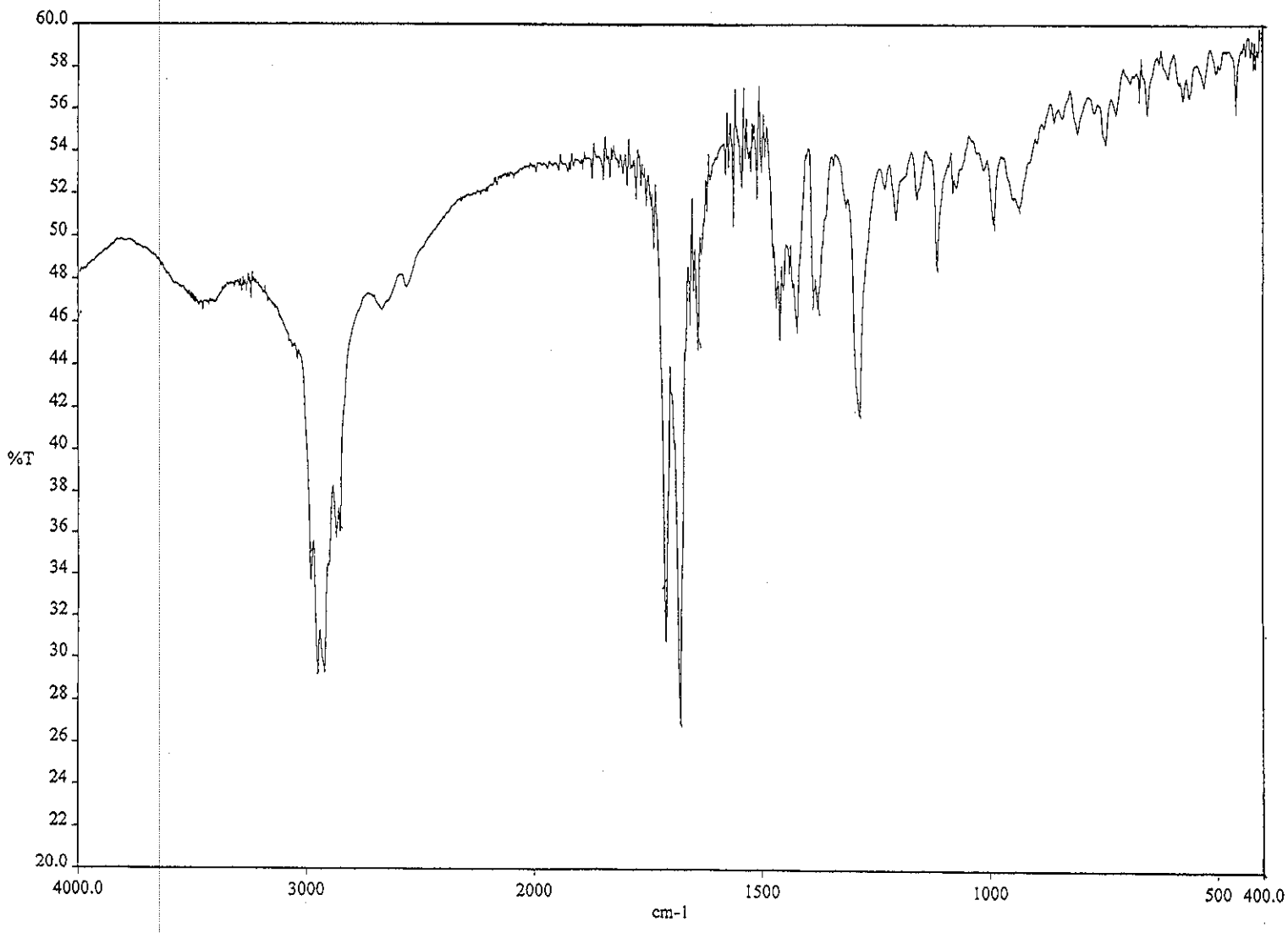


Figure 3.98 FT-IR (KBr) spectrum of 12BS8

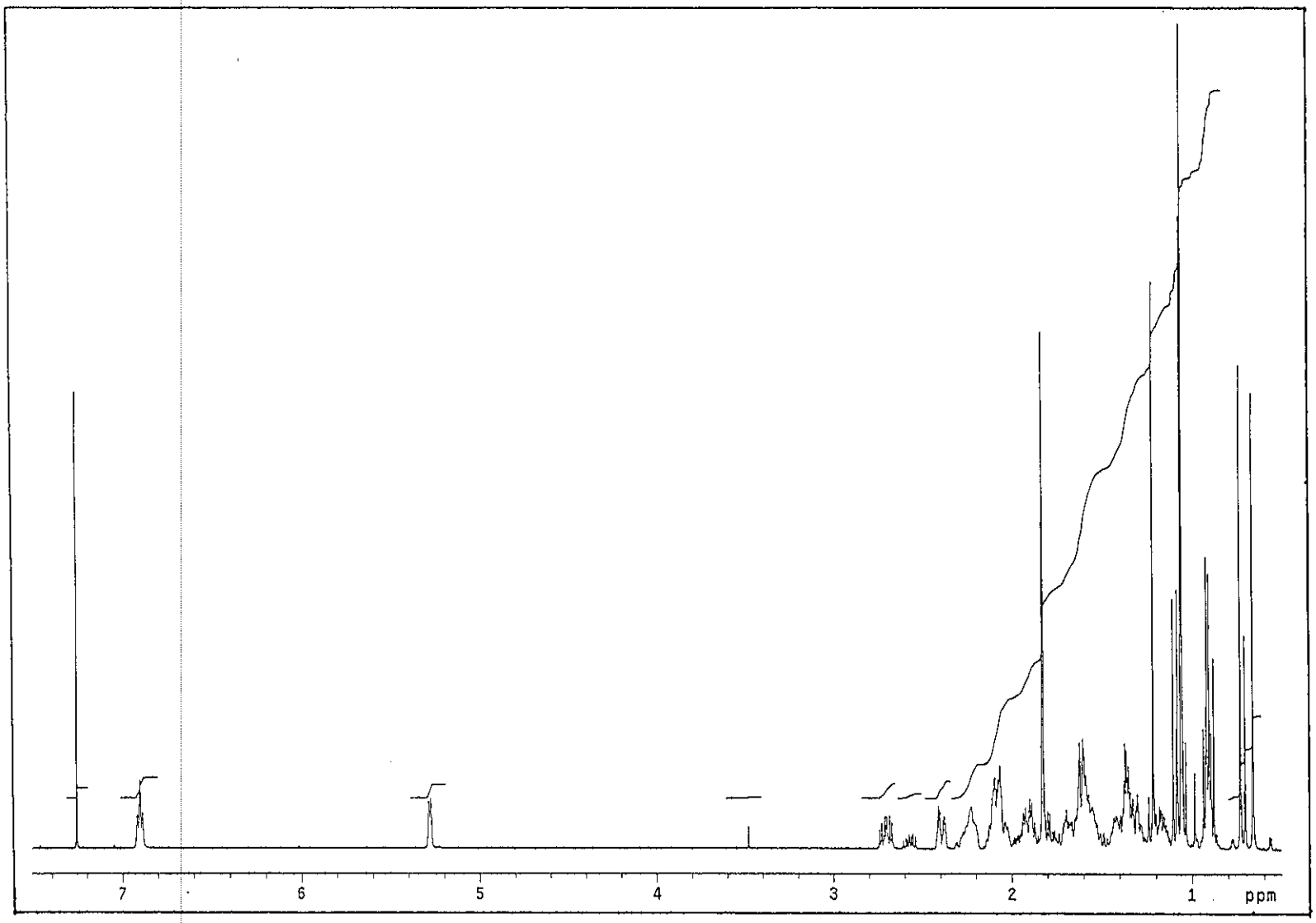


Figure 3.99 ^{1}H NMR (500 MHz)($CDCl_3$) spectrum of 12BS8

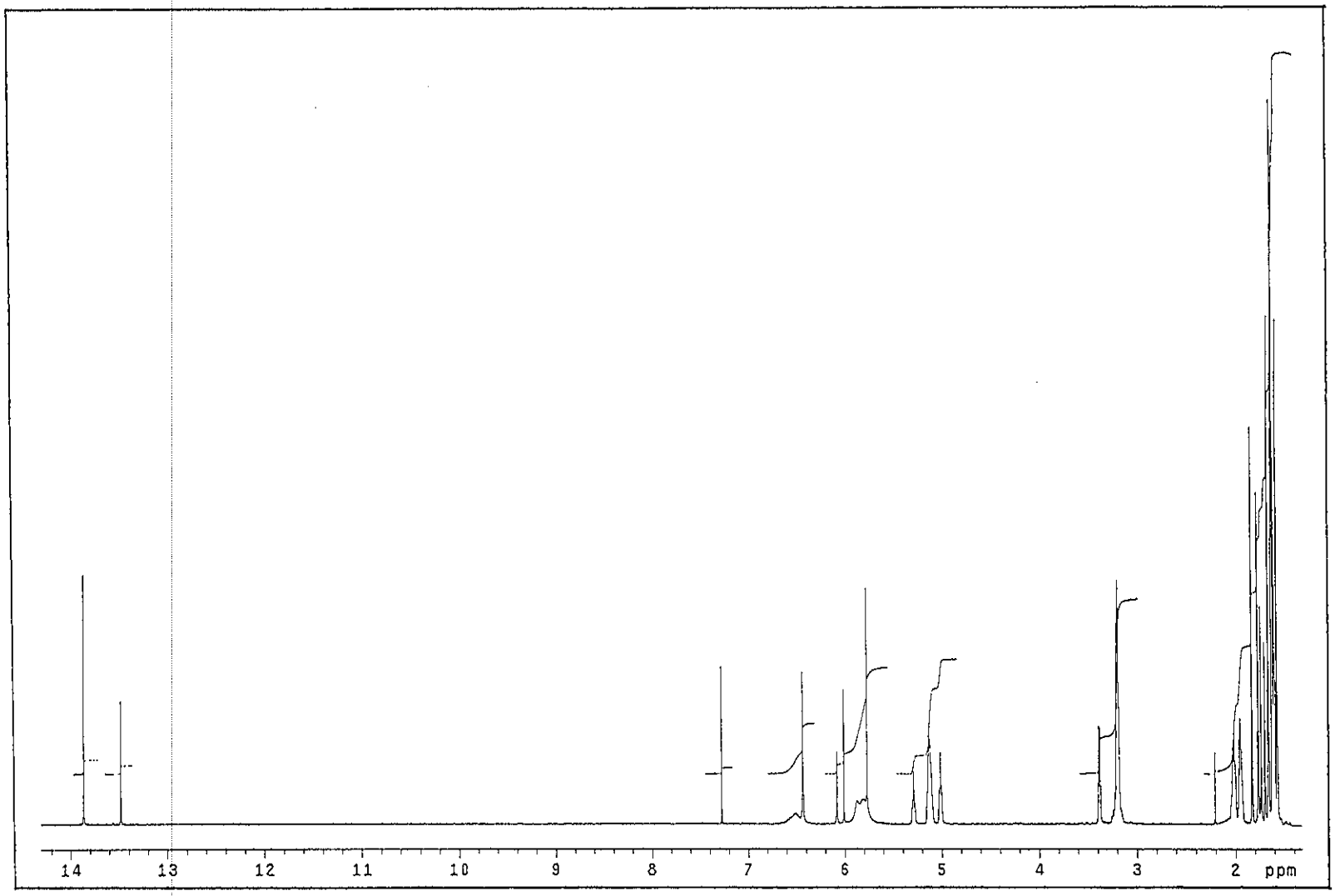


Figure 3.100 ^{1}H NMR (500 MHz)($CDCl_3$) spectrum of K4.7711

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