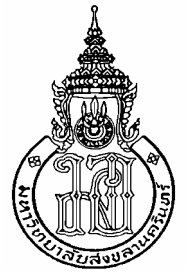

Chemical Constituents from the Roots of *Atalantia monophylla*

Arnon Chukaew

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Organic Chemistry

Prince of Songkla University

2008

Thesis Title	Chemical Constituents from the Roots of *Atalantia monophylla*
Author	Mr. Arnon Chukaew
Major Program	Organic Chemistry

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

…..…..……..

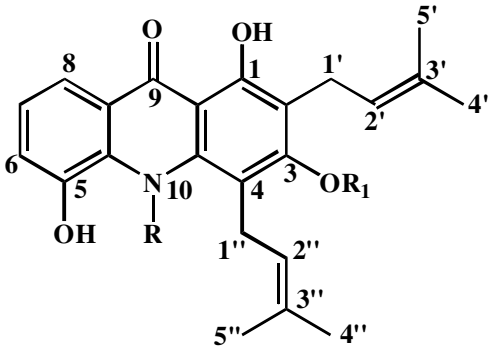
(Assoc. Prof. Dr. Krerkchai Thongnoo)

Dean of Graduate School

ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมีจากรากของต้นมะนาวผี
ผู้เขียน	นายอานนท์ ชูแก้ว
สาขาวิชา	เคมีอินทรีย์
ปีการศึกษา	2551

บทคัดย่อ

การศึกษาองค์ประกอบทางเคมีของส่วนสกัดเมทิลีนคลอไรด์ และอะซีโตนจากรากของต้นมะนาวผี สามารถแยกสารใหม่ได้ 3 สาร เป็นสารประกอบอัลคาลอยด์ชนิดอะครีโดน คือ cycloatalaphylline-A (**AM4**), *N*-methylcycloatalaphylline-A (**AM5**) และ *N*-methylbuxifoliadine-E (**AM9**) นอกจากนี้ยังพบสารที่มีการรายงานแล้ว 17 สาร ประกอบด้วยสารประเภทอะครีโดน อัลคาลอยด์ 8 สาร คือ *N*-methylatalaphylline (**AM1**), atalaphylline (**AM2**), buxifoliadine-A (**AM3**), yukocitrine (**AM6**), *N*-methylataphyllinine (**AM7**), buxifoliadine-E (**AM8**), citrusinine-I (**AM10**) และ junosine (**AM11**) สารประเภทลิโมนอยด์ 4 สาร คือ atalantolide (**AM12**), atalantin (**AM13**), cycloepiatalantin (**AM14**) และ cycloepiatalantin acetate (**AM15**), สารประเภทคูมาริน 2 สาร คือ auraptene (**AM16**) และ 7-*O*-geranylscopoletin (**AM17**) สารประเภทแอนทราควิโนน 1 สาร คือ physcion (**AM18**) และ สารประเภทสเตอรอยด์ 2 สาร คือ สารผสมของ β-sitosterol (**AM19**) และ stigmasterol (**AM20**) โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี และสำหรับสาร **AM7** ใช้ข้อมูลทางเอกซ์เรย์ประกอบการวิเคราะห์อีกด้วย



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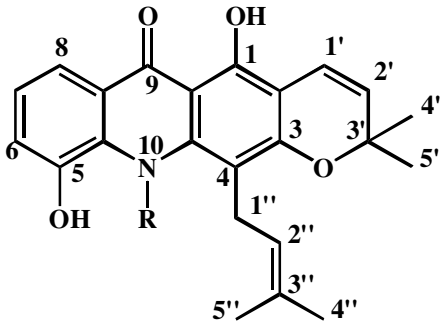

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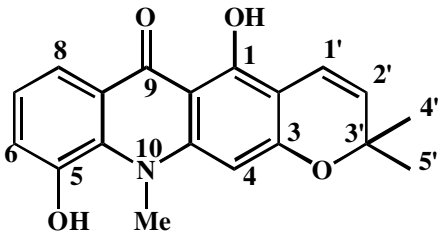

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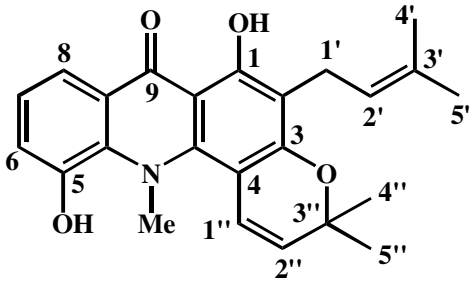

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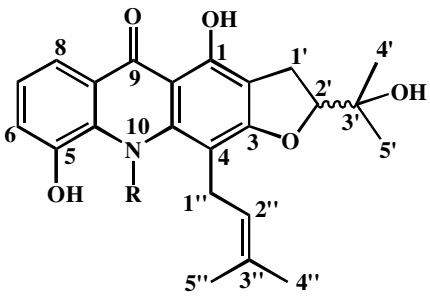

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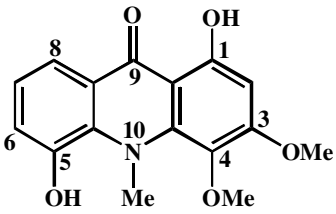

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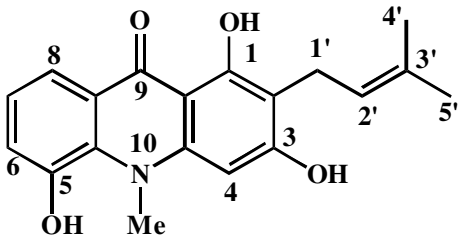

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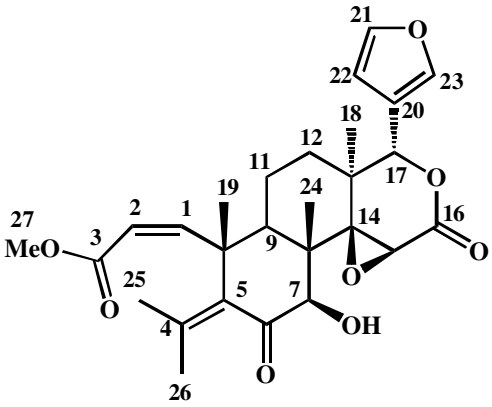

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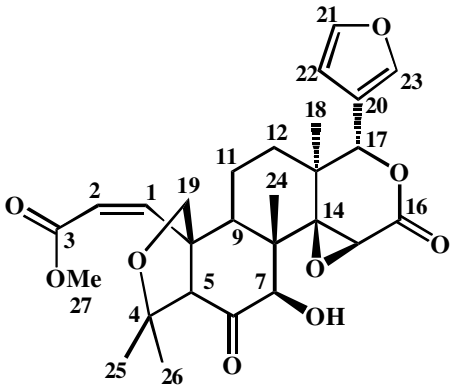

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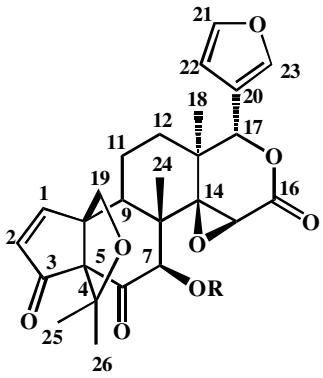

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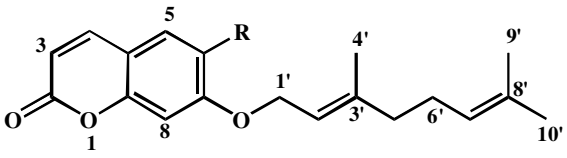

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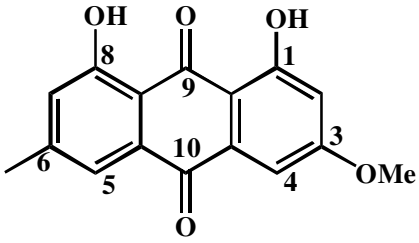

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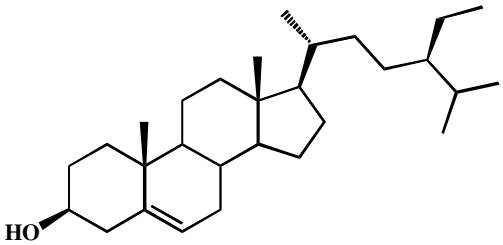

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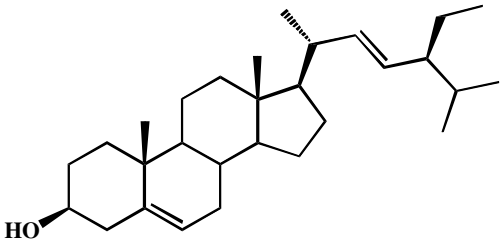

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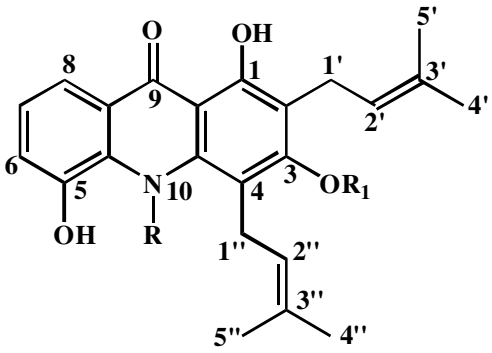
Thesis Title	Chemical Constituents from the Roots of *Atalantia monophylla*
Author	Mr. Arnon Chukaew
Major Program	Organic Chemistry
Academic Year	2008

ABSTRACT

Investigation of the methylene chloride and acetone extracts of the roots of *Atalantia monophylla* resulted in three new acridone alkaloids: cycloatalaphylline-A (**AM4**), *N*-methylcycloatalaphylline-A (**AM5**) and *N*-methylbuxifoliadine-E (**AM9**), together with seventeen known compounds: eight acridones; *N*-methylatalaphylline (**AM1**), atalaphylline (**AM2**), buxifoliadine-A (**AM3**), yukocitrine (**AM6**), *N*-methylataphyllinine (**AM7**), buxifoliadine-E (**AM8**), citrusinine-I (**AM10**) and junosine (**AM11**); four limonoids: atalantolide (**AM12**), atalantin (**AM13**), cycloepiatalantin (**AM14**) and cycloepiatalantin acetate (**AM15**); two coumarins: auraptene (**AM16**) and 7-*O*-geranylscopoletin (**AM17**); one anthraquinone: physcion **(AM18)** and two steroids: a mixture of β-sitosterol (**AM19**) and stigmasterol (**AM20**). Their structures were elucidated by spectroscopic methods. The structure of **AM7** was additionally confirmed by X-ray diffraction analysis.



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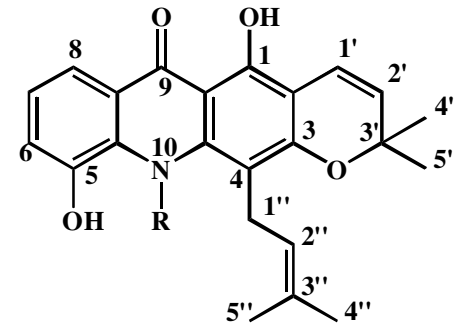
AM2 **R** = H **R_1** = H

AM3 **R** = Me **R_1** = Me



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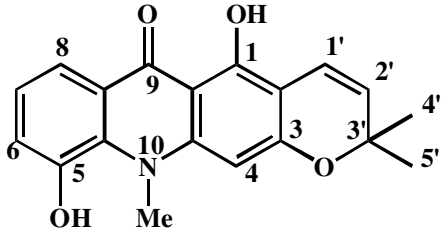

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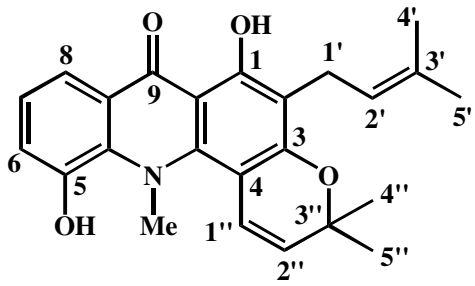

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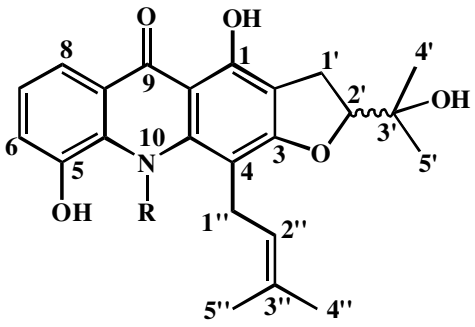

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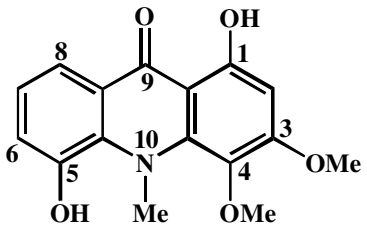

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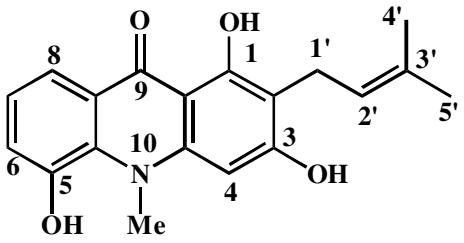

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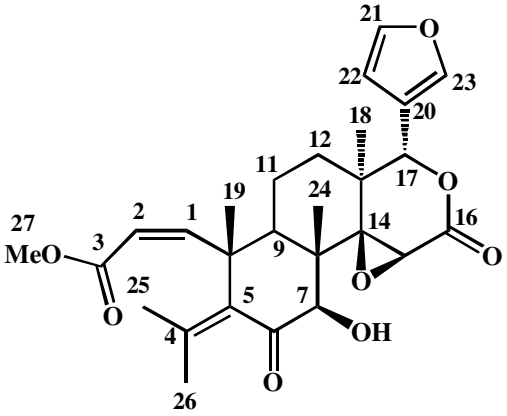

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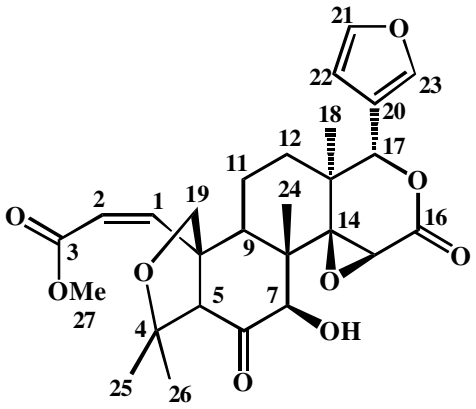

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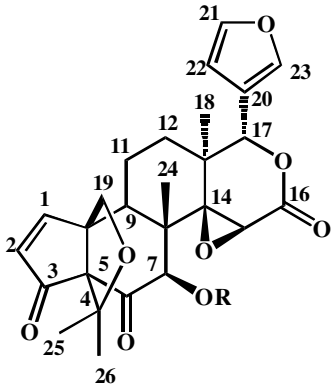

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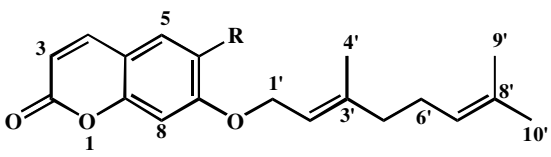

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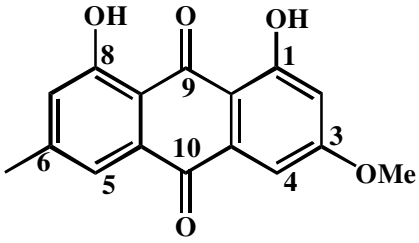

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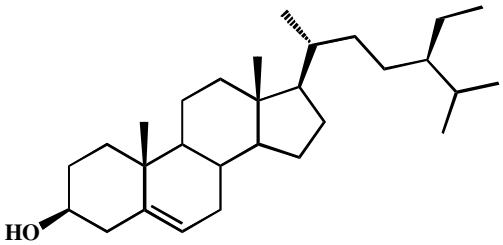

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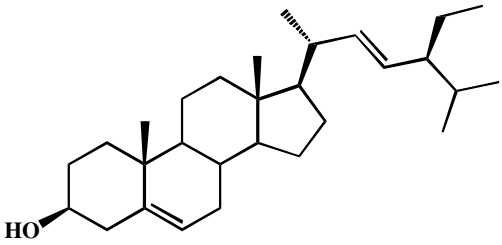

AM19

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I would like to express my appreciation to the staffs of the Department of Chemistry, Faculty of Science, Prince of Songkla University for making this thesis possible. Dr. Yaowapa Sukpondma is highly acknowledged for recording NMR spectral data.

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Arnon Chukaew

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this research is to investigate the chemical constituents of *A. monophylla* in order to exploit potential uses of this plant as a medicinal plant. The chemical investigation of constituents from the roots of *A. monophylla* has led to isolation of eleven acridone alkaloids: **AM1-AM11**, four limonoids: **AM12-AM15**, two coumarins: **AM16-AM17**, one anthraquinone: **AM18** and two steroids: **AM19** and **AM20**. Two acridone alkaloids: buxifoliadine-E (**AM8**) and citrusinine-I (**AM10**) possessed significant anti-allergic activity against cell degranulation in RBL-2H3 cells with an IC_{50} values at 6.1 and 18.7 μM, respectively. All four limonoids isolated: atalantolide (**AM12**), atalantin (**AM13**), cycloepiatalantin (**AM14**) and cycloepiatalantin acetate (**AM15**) were moderately active against MCF-7 (breast adenocarcinoma), HT-29 (human colon cancer), KB (human oral cancer) and HeLa (human cervical cancer) cell lines.

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LIST OF ABBREVIATIONS AND SYMBOLS

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
q	=	*quartet*
m	=	*multiplet*
dd	=	*doublet of doublet*
dt	=	*doublet of triplet*
br s	=	*broad singlet*
qd	=	*quartet of doublet*
g	=	Gram
nm	=	Nanometer
mp	=	Melting point
cm^{-1}	=	Reciprocol centimeter (wave number)
δ	=	Chemical shift relative to TMS
J	=	Coupling constant
$[\alpha]_D$	=	Specific rotation
λ_{max}	=	Maximum wavelength
ν	=	Absorption frequencies

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

$\mathcal{E}$	=	Molar extinction coefficient
m/*z*	=	A value of mass divided by charge
°C	=	Degree celcius
MHz	=	Megahertz
ppm	=	Part per million
C	=	Concentration
FT-IR	=	Fourier Transfrom Infrared
UV-Vis	=	Ultraviolet-Visible
ESI-TOF MS	=	Electrospray Ionization Time-of-Flight Mass Spectrometry
EIMS	=	Electron Ionization Mass Spectrometry
HREIMS	=	High Resolution Electron Ionization Mass Spectrometry
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimensional Nuclear Magnetic Resonance
COSY	=	Correlation Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence

NOE	=	Nuclear Overhauser Effect
NOESY	=	Nuclear Overhauser Effect Correlation Spectroscopy
CC	=	Column Chromatography
QCC	=	Quick Column Chromatography
PLC	=	Preparative Thin Layer Chromatography
DCM	=	Dichloromethane
TMS	=	Tetramethylsilane
$CDCl_3$	=	Deuterochloroform
CD_3OD	=	Deuteromethanol
DMSO	=	Dimethylsulfoxide

CHAPTER 1
INTRODUCTION

1.1 Introduction

Atalantia monophylla (DC.) Corrêa (**Figure**s **1**) is the plant in the Rutaceae family, which is locally known as "Manao Pee (มะนาวผี)". It is a shrub with brown bark and thorny branches distributed in Southeast Asia, East Bengal, South India and Ceylon (Panda 2004). Various parts of this plant has been used as folk medicines for several purposes such as the treatment of chronic rheumatism, paralysis (Basa, 1975), antispasmodic, stimulant and hemiplegia (Panda, 2004). The essential oil from the leaves showed antimicrobial and strong inhibitory activities against some pathogenic fungi (Prasad, 1988), whereas decoction of the leaves is applied in itch and other skin complaints (Panda 2004). In the previous report, limonoids and acridone alkaloids have been isolated from the petroleum ether extract of the root bark (Govindachari *et al*., 1970, Basu and Basa 1972, Kulkarni and Sabata, 1981). Acridone alkaloids have shown several biological activities such as inhibition of Epstein-Barr virus (EBV)-EA induction (Itoigawa *et al*., 2003), induction of human promyelocytic leukemia cell (HL-60) differentiation (Kawaii *et al*., 1999a), and antiproliferative (Kawaii *et al*., 1999b). *Atalantia* genus comprises 12 species: *buxifolia, ceylanica, citroides, guillauminii, hainanensis, macrophylla, monophylla, racemosa, rotandifolia, roxburghiany, simplicifolia and wightii* (http://www.wikimedia.org). *A. monophylla* is the only specie found in Thailand.

A. monophylla is a small to medium-sized, shrubby tree, 8-15 m tall. The bark is distinct ridges and many prickles that is grey brown color. The stem has the character of rut twists. Leaves are single arrange alternate oval, with concave curly end, width 3-5 cm, length 7-12 cm. The flowers are white gathering in a bouquet. The fruits have round character, small-sized with the thick rough skin and an oval-shaped seed. They are found in the mixed forest and seaside forest.

In Thailand, *A. monophylla* has been found in every part of the country. It has many local Thai names: Krut-proei (กรูดเปรย) Khmer-Chanthaburi; Krut

phi (กรูดผี) Surat Thani; Kanao phli (กะนาวพลี) Peninsular; Khi tio (ขี้ติ้ว) Northern; Nang kan (นางกาน) Khon kaen; Manao phi (มะนาวผี) Chiang Mai, Ratchaburi; (Smitinand, 2001).

a. Trees

b. Roots

c. stem

d. Fruits

e. Leaves

f. Flowers

Figure 1 Different parts of *Atalantia monophylla*

1.2 Review of Literatures

Chemical constituents isolated from the six species of this genus were summarized in **Table 1**. Information obtained from Scifinder Scholar copyright in 2007 will be presented and classified into groups: Acridone alkaloids, Alkaloids, Anthraquinones, Aromatics, Coumarins, Flavonoids, Limonoids, Monoterpenoids, Pyropheophorbides, Serverine benzamides, Sesquiterpenoids and Triterpenoids.

Table 1 Compounds from plants of Atalantia genus.

a. Acridone alkaloids
b. Alkaloids
c. Anthraquinones
d. Aromatics
e. Coumarin**s**
f. Flavonoids
g. Limonoids
h. Monoterpenoids
i. Pyropheophorbides
j. Serverine benzamides
k. Sesquiterpenoids
l. Triterpenoids

Scientific name	Part	Compounds	Bibliography
A. buxifolia	Root Bark	*N*-methylseverifoline, **a1** Severifoline, **a2** Atalaphyllinine, **a3** *N*,*O*-Dimethylseverifoline, **a4** *N*-methylataphyllinine, **a5** *N*-methylbicycloatalaphylline, **a6** Noracronycine, **a7** *N*-methylatalaphylline, **a8**	Wu *et al.,* 1982
		Severifoline, **a2** Atalaphyllinine, **a3** Atalaphyllidine, **a9** Citrusinine-I, **a10** Citrusinine-II, **a11** *N*-methylatalaphylline, **a8** 1,2,3-Trihydroxy acridone, **a12** 5-Hydroxy-*N*-methyl-Severifoline, **a7** Glycocitrine-I, **a13** Buxifoliadine-A, **a14** Buxifoliadine-A, **a15**	Wu *et al.,* 2000

Scientific name	Part	Compounds	Bibliography
A. buxifolia	Root Bark	Buxifoliadine-C, **a16** Buxifoliadine-D, **a17** Buxifoliadine-E, **a18** Buxifoliadine-F, **a19** Buxifoliadine-G, **a20** Buxifoliadine-H, **a21**	Wu *et al.,* 2000
		Buxifoliadine-B, **a15** Buxifoliadine-D, **a17** Buxifoliadine-H, **a21** Severifoline, **a2** Citrusinine-I, **a10** Citrusinine-II, **a11** 7-Isovaleroylcycloseverinolide, -**g1** 7-Isovaleroylcycloepiatalantin, -**g2**	Wu *et al.,* 2001
A. ceylanica	Bark	Atalantine, **a22** Ataline, **a23**	Fraser *et al.,* 1973
	Root bark	Xanthoxine, **e7** Racemosin, **e8**	Ahmad *et al.,* 1984
	Heart wood	Ceylantin, **e6**	Murray *et al.,* 1985
	Seed	Cycloatalantin, **g3** Cycloatalantinone, **g4** Cycloatalantin-16-oic acid, **g5** Isocycloatalantin, **g6** Cycloepiatalantin, **g7** Dehydrocycloatalantin, **g8**	Bennett *et al.,* 1994

Scientific name	Part	Compounds	Bibliography
A. ceylanica	Seed	Ataloxime, **b1** Xanthotoxin, **e1** Imperatorin, **e2** Bergapten, **e3** Heraclenin, **e4** Oxypeucedanin, **e5**	Bacher *et al.,* 1999
A missionis	Root and Stem bark	Ostruthine, **e9** Isopimpinellin, **e10**	Barua *et al*., 1974
A. monophylla	Leave Root bark	Benzopyran-6-acrylic acid, **e11** Marmesin, **e12** Sabinene, **h1** Stigmas-5-en-3-ol, **l1** Friedelanone, **l2**, *N*-methylataphyllinine, **a5** Atalaphyllinine, **a3** Obacunoic acid, **g13** Atalaphylline, **a25** *N*-methylataphylline, **a8** *N*-methylbicycloatalaphylline, **a6** *O*-Methylbicycloatalaphylline, **a27** Monomethyl ether atalaphylline, **a28**	Thakar *et al*., 1969 Talapatra *et al*., 1970 Govindachari *et al*., 1970 Basu *et al*., 1972

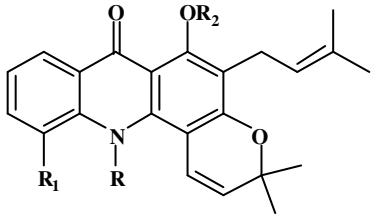
Scientific name	Part	Compounds	Bibliography
A. monophylla	Root bark	Atalaphylline-3,5-dimethyl ether **a29**	Basu *et al*., 1972
		Atalaphyllinine, **a3**	Basa, 1975
		Atalantolide, **g12**	Shringarpure *et al*.,
		Auraptene, **e13**	1975
		Bisabolene, **k1**	
		Trans-*β*-Bergamotenes, **k2**	
		Trans-*α*-Bergamotenes, **k3**	
		Bisabolol, **k4**	
		Norbisabolide, **k5**	
		Bisabols oxide, **k6**	
		Dehydroatalantin, **g8**	
		Atalaphylline, **a25**	
		N-methylatalaphylline, **a8**	
		Atalantine, **a22**	
		physcion, **c1**	
		Atalantin acetate, **g10**	Dreyer *et al.,* 1976
		Rutevin, **g11**	
		Atalaphyllidine, **a9**	Chatterjee *et al.,* 1976
		Atalantin, **g9**	Sabata *et al*., 1977
	Fruit	Severine palmitate, **j1**	Dreyer *et al.,* 1980
		Benzamidate, **j2**	
		Deoxyseverine, **j3**	
		Severine acetate, **j4**	
		Oxodeoxyseverine, **j5**	
		Severinol, **j6**	
	Heart wood	Psoralene, **e14**	Kulkarni *et al*., 1980
		Isopsoralene, **e15**	
		Stigmast-4-en-3-one, **l3**	

Scientific name	Part	Compounds	Bibliography
A. monophylla	Leave	Stigmas-5-en-3-ol, **l1**	Shah *et al.,* 1981
	Root bark	Atalaphylline, **a25**	Kulkarni *et al*., 1981
		N-methylatalaphylline, **a8**	
		N-methylatalaphylline-3,5-dimethyl ether, **a30**	
		N-methyl-tri-*O*-methylatalaphylline, **a31**	
		Cycloatalaphylline 3,5-dimethyl ether, **a32**	
		1,3-Dihydroxy-5-methoxy-acridone, **a24**	Bahar *et al.,* 1982
		Atalaphylline, **a25**	
		5-Hydroxyarborinine, **a26**	Shah *et al.,* 1982
		N-methylataphyllinine**, a5**	
	Leave	Pyropheophorbide a, **i1**	Chansakaow *et al.,*
		Pyropheophorbide b, **i2**	1994
A. racemosa	Heart wood	Xanthoxine, **e7**	Banerji *et al.,* 1988b
		Isoevodionol, **e16**	
		Umbelliferone, **e17**	
		Luvangetin, **e18**	
		Xanthyletin, **e19**	
		Rutaretin, **e20**	
		Rutarin, **e21**	
		Racemosin, **e22**	
		Racemoflavone, **f1**	
		Atalantaflavone, **f2**	

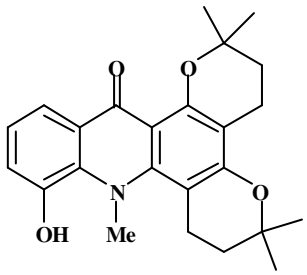
Scientific name	Part	Compounds	Bibliography
A. wightii	Root	Kokusaginin, **b4**	Banerji *et al*., 1982
		Xanthyletin, **e19**	
		Cinnamic acid lactone, **e30**	
		Isoimpinellin, **e31**	
		Ostol, **e32**	
		Marmesin, **e12**	
		Xanthoxine, **e7**	
		Obacylactone, **g14**	
		Atalantin, **g9**	
		Phebalosin, **e27**	
		N-methylatalaphylline, **a8**	
		N-methylataphyllinine, **a5**	
		Auraptene, **e13**	
		Umbelliferone, **e17**	
		Micromelumin, **e28**	
		Murrangatin, **e29**	
	Stem bark	Skimmianin, **b2**	
		Heplopine, **b3**	
		p-Coumaric acid ethyl ester, **d1**	
	Stem bark	Imperatorin, **e2**	Banerji *et al.,* 1988a
		Scopoletol, **e23**	
		Marmin, **e24**	
		Limettin, **e25**	
		Crenyllatin, **e26**	
		Phebalosin, **e27**	

Structures

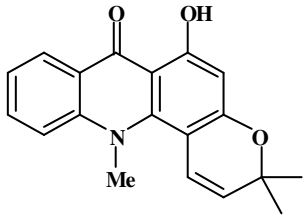
a Acridone alkaloids



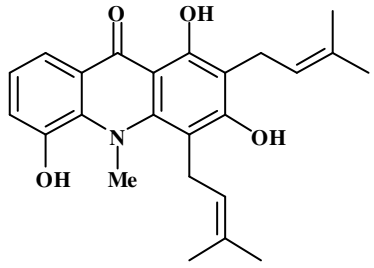
R	R_1	R_2	
Me	H	H :	*N*-Methylseverifoline, **a1**
H	H	H :	Severifoline, **a2**
H	OH	H :	Atalaphyllinine, **a3**
Me	H	Me :	*N*,*O*-Dimethylseverifoline, **a4**
Me	OH	H :	*N*-Methylataphyllinine, **a5**



N-methylbicycloatalaphylline, **a6**



Noracronycine, **a7**



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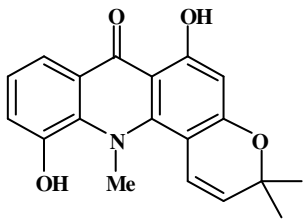
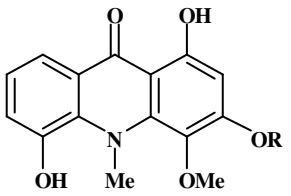
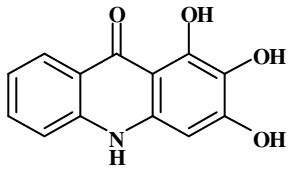


Atalaphyllidine, **a9**

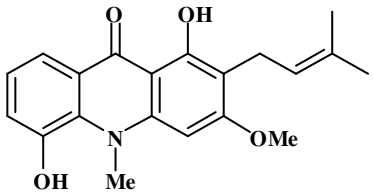


R = Me : Citrusinine-I, **a10**

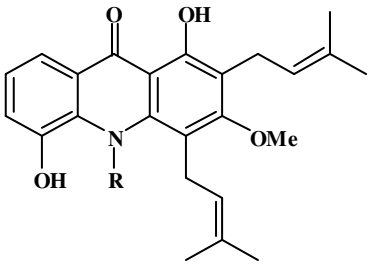
R = H : Citrusinine-II, **a11**



1,2,3-Trihydroxy acridone, **a12**



Glycocitrine-I, **a13**



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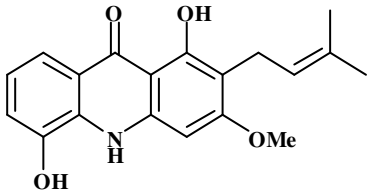
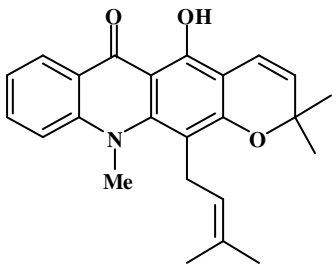
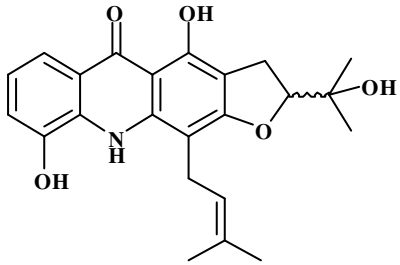


Buxifoliadine-C, **a16**



Buxifoliadine-D, **a17**



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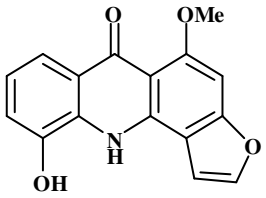

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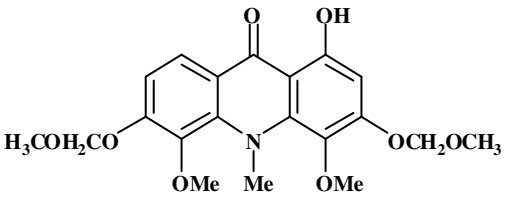
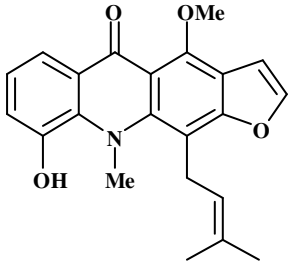
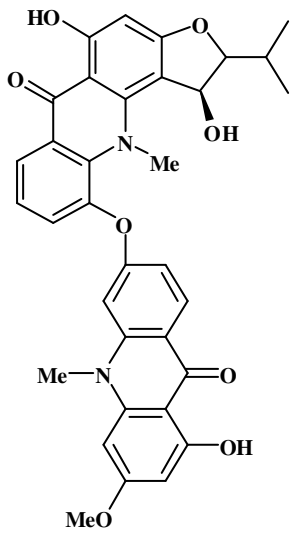


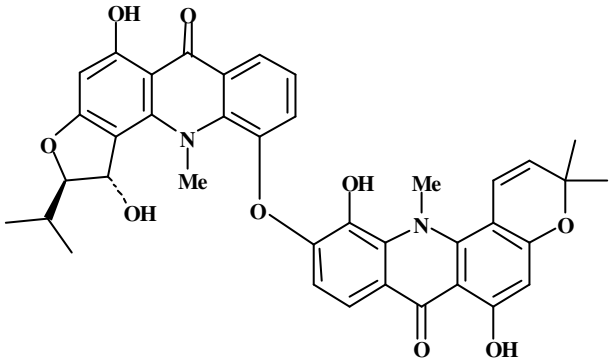
Buxifoliadine-G, **a20**



Buxifoliadine- H, **a21**



Atalantine, **a22**



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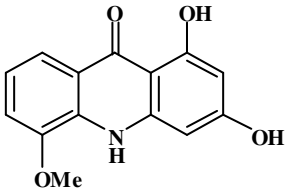
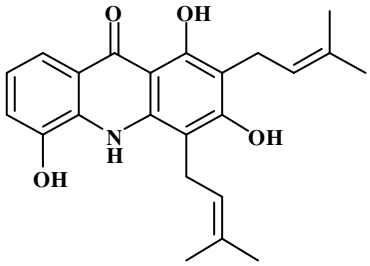
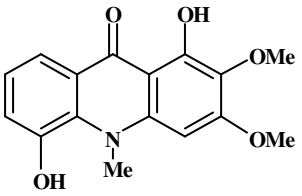


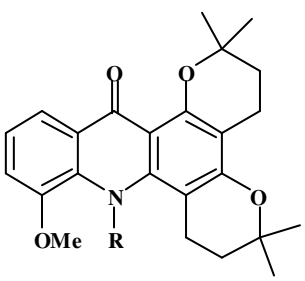
1,3-Dihydroxy-5-methoxy-acridone, **a24**



Atalaphylline, **a25**

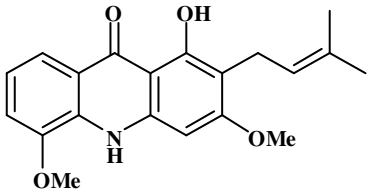


5-Hydroxyarborinine, **a26**

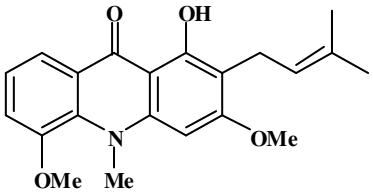


R = H : *O*-Methylbicycloatalaphylline, **a27**

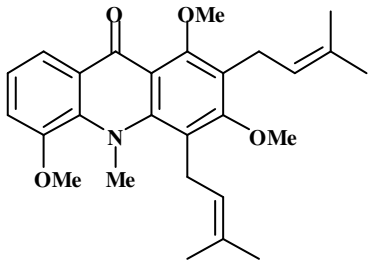
R = Me : Monomethyl ether atalaphylline, **a28**



Atalaphylline 3,5-dimethyl ether, **a29**



N-Methyl-atalaphylline-3,5-dimethyl ether, **a30**



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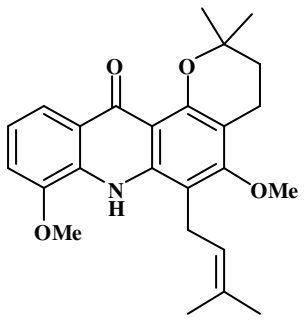
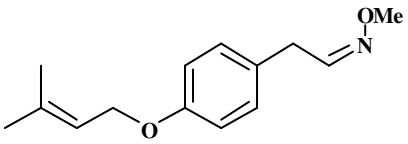


Cycloatalaphylline 3,5-dimethyl ether, **a32**

b. Alkaloids



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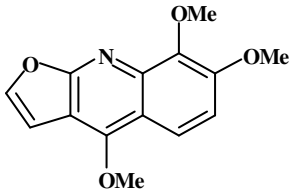
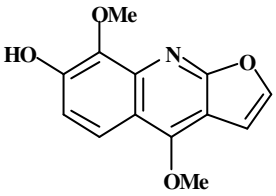


Skimmianin, **b2**



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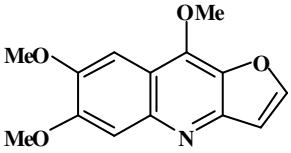
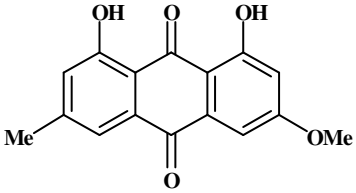


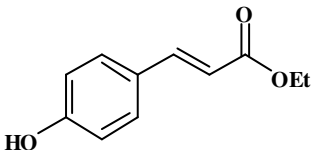
Kokusaginin, **b4**

c. Anthraquinone



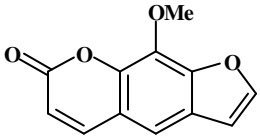
Physcion, **c1**

d. Aromatic



p-Coumaric acid ethyl ester, **d1**

e. Coumarins



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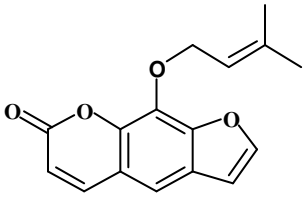

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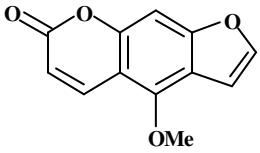
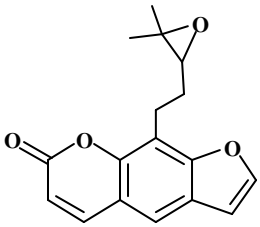


Bergapten, **e3**



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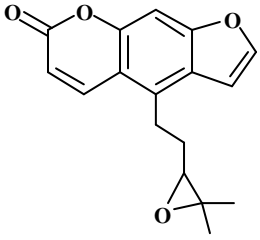

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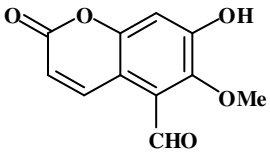

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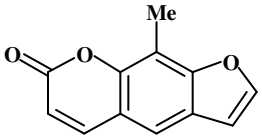
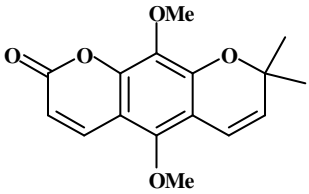
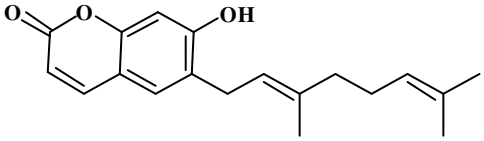


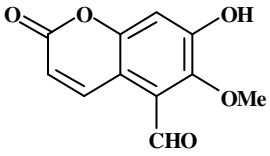
Xanthoxine, **e7**



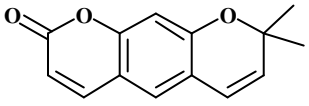
Racemosin, **e8**



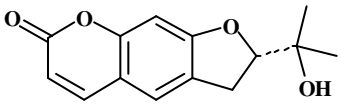
Ostruthine, **e9**



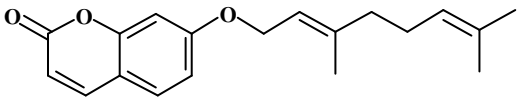
Isoimpinellin, **e10**



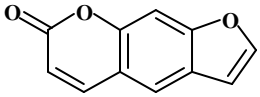
Benzopyran-6-acrylic acid, **e11**



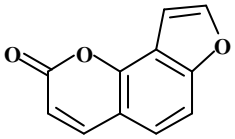
Marmesin, **e12**



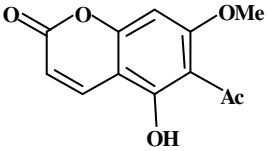
Auraptene, **e13**



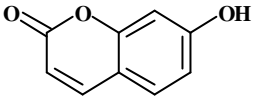
Psoralene, **e14**



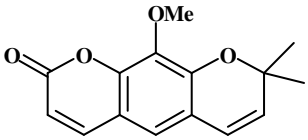
Isopsoralene, **e15**



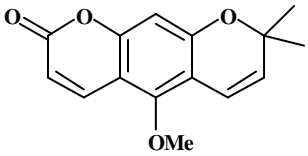
Isoevodionol, **e16**



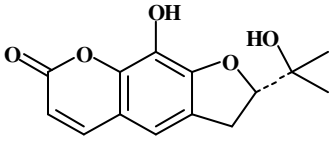
Umbelliferone, **e17**



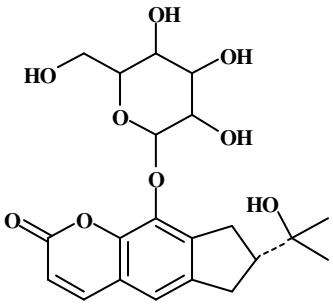
Luvangetin, **e18**



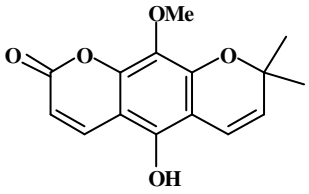
Xanthyletin, **e19**



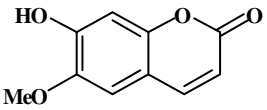
Rutaretin, **e20**



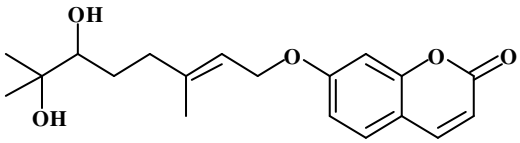
Rutarin, **e21**



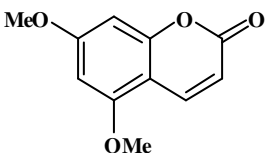
Racemosine, **e22**



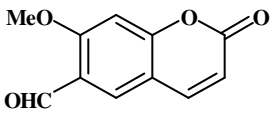
Scopoletol, **e23**



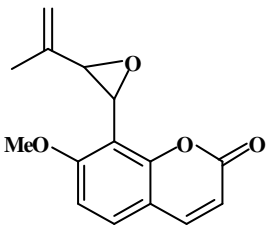
Marmin, **e24**



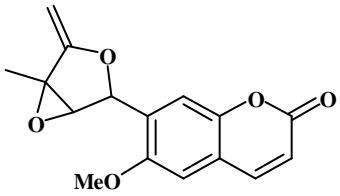
Limettin, **e25**



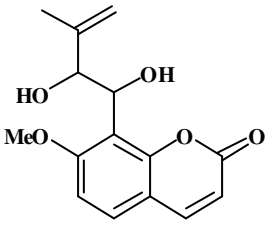
Crenyllatin, **e26**



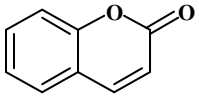
Phebalosin, **e27**



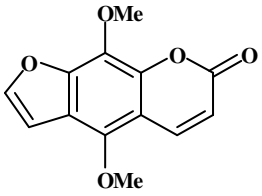
Micromelumin, **e28**



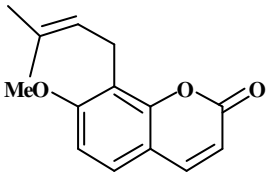
Murrangatin, **e29**



Cinnamic acid lactone, **e30**

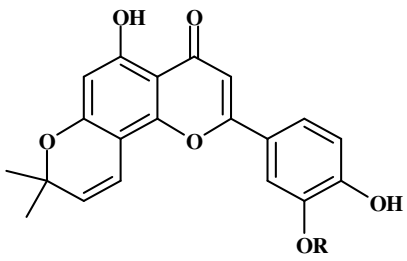


Isoimpinellin, **e31**



Ostol, **e32**

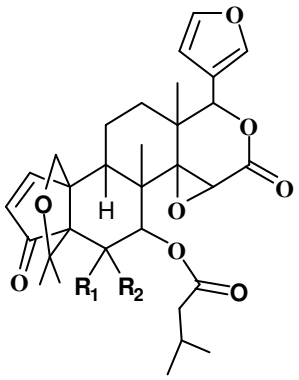
f. Flavonoids



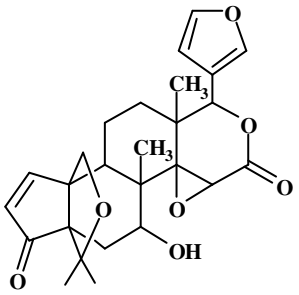
R = OMe : Racemoflavone, **f1**

R = H : Atalantaflavone, **f2**

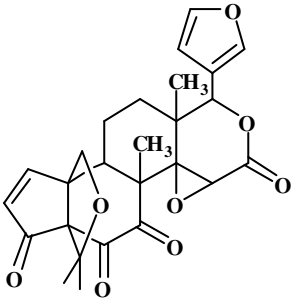
g. Limonoids



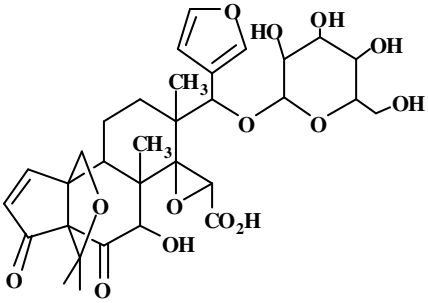
R_1	R_2
OH	H : 7-Isovaleroylcycloseverinolide **g1**
O	O : 7-Isovaleroylcycloepiatalantin, **g2**



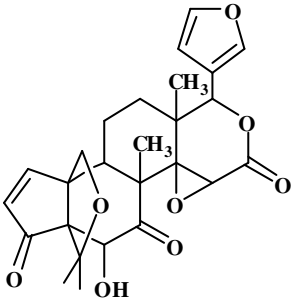
Cycloatalantin, **g3**



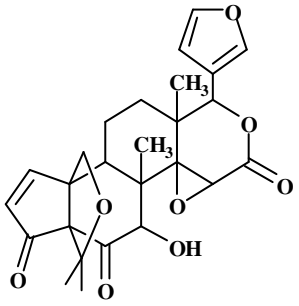
Cycloatalantinone, **g4**



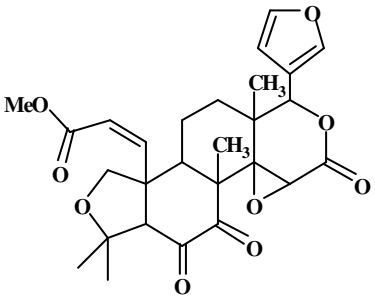
Cycloatalantin-16-oic acid, **g5**



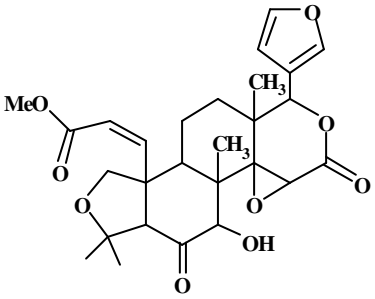
Isocycloatalantin, **g6**



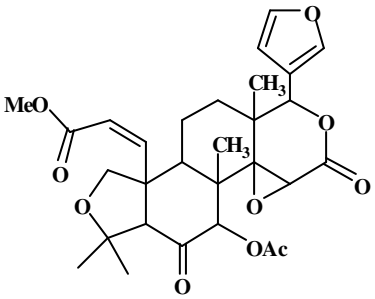
Cycloepiatalantin, **g7**



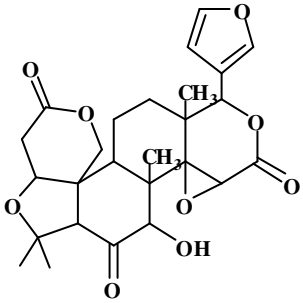
Dehydrocycloatalantin, **g8**



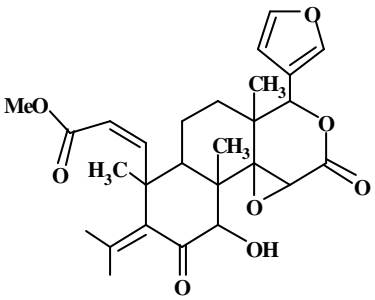
Atalantin, **g9**



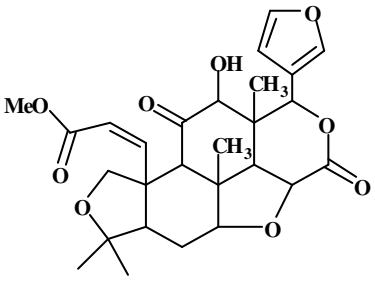
Atalantin acetate, **g10**



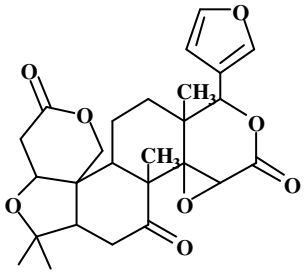
Rutevin, **g11**



Atalantolide, **g12**

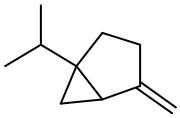


Obacunoic acid, **g13**



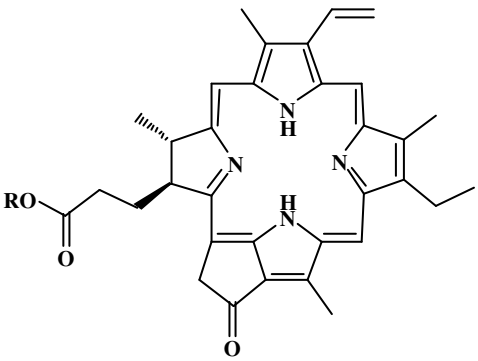
Obacylactone, **g14**

h. Monoterpenoid



Sabinene, **h1**

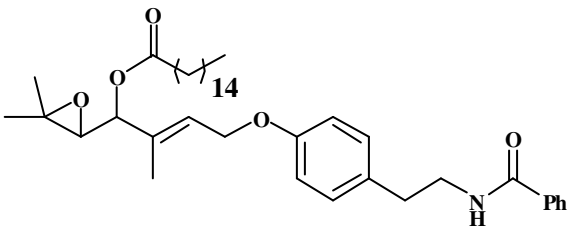
i. Pyropheophorbides



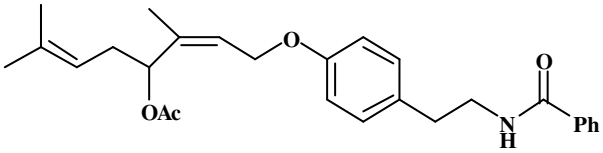
R = H : Pyropheophorbide a, **i1**

R = Me : Pyropheophorbide b, **i2**

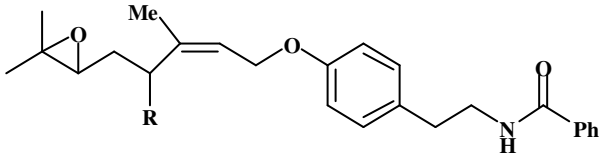
j. Serverine benzamides



Severine palmitate, **j1**



Benzamidate, **j2**



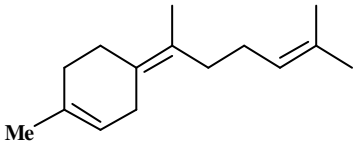
R=H : Deoxyseverine, **j3**

R=OAc : Severine acetate, **j4**

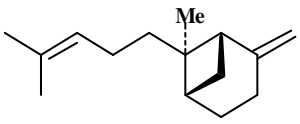
R=Ac : Oxodeoxyseverine, **j5**

R=OH : Severinol, **j6**

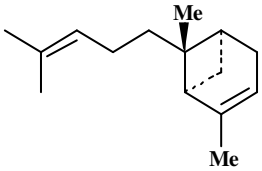
k. Sesquiterpenoids



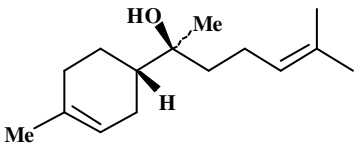
Bisabolene, **k1**



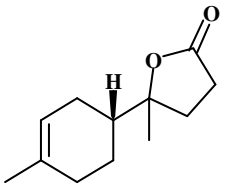
trans-β-Bergamotenes, **k2**



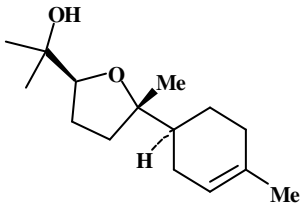
trans-α-Bergamotenes, **k3**



Bisabolol, **k4**

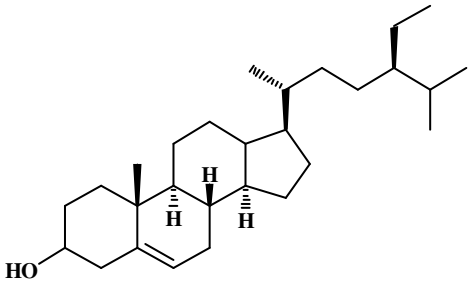


Norbisabolide, **k5**

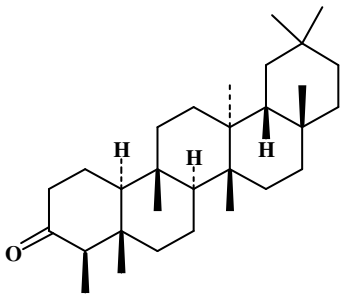


Bisabols oxide, **k6**

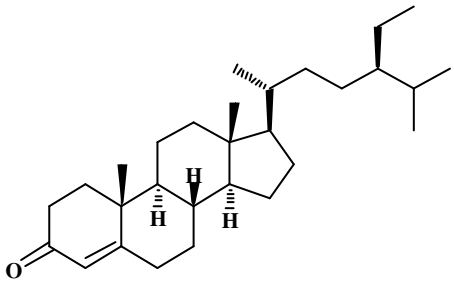
l. Triterpenoids



Stigmast-5-en-3-ol, **l1**



Friedelanone, **l2**



Stigmast-4-en-3-one, **l3**

1.3 Objective

This research work involved isolation, purification and structure elucidation of chemical constituents isolated from the roots of *Atalantia monophylla* and also evaluation of pure compounds for anti-allergic, antibacterial and cytotoxic activities.

CHAPTER 2
EXPERIMENTAL

2.1 Instruments and Chemicals

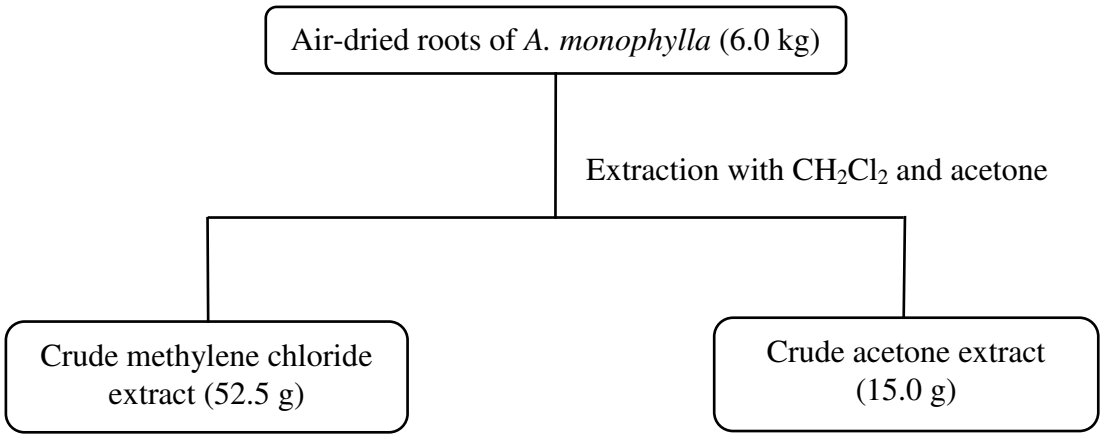
Melting points were determined on the Fisher-John melting point apparatus. The UV spectra were measured with a SPECORD S 100 (Analytikjena) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in MeOH solution. The optical rotation $[\alpha]_D$ was measured in chloroform and methanol solution with Sodium D line (590 nm) on a JASCO P-1020 digital polarimeter. The IR spectra were measured with a Perkin-Elmer FTS FT-IR spectrophotometer. Single Crystal X-ray diffraction measurements were collected using SMART 1-K CDD diffractometer with monochromated Mo-Kα radiation (λ = 0.71073 A) using ω-scan mode and SHELXTL for structure solution and refinement. NMR spectra were recorded using 300 MHz Bruker FTNMR Ultra Shield^{TM} spectrometers in acetone-d_6 and $CDCl_3$ with TMS as the internal standard. Chemical shifts are reported in δ (ppm) and coupling constants (J) are expressed in hertz. EI and HREI mass spectra were measured on a Kratos MS 25 RFA spectrometer. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except chloroform was analytical grade reagent. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (Merck) and silica gel 100 (Merck), respectively.

2.2 Plant Material

Root of *A. monophylla* was collected from Trang province in the southern part of Thailand, in June 2006. Identification was made by Assoc.Prof. Dr.Kitichate Sridith and a specimen (Arnon Chukaew 1) deposited at PSU herbarium, Department of Biology, Faculty of Science, Prince of Songkla University.

2.3 Extraction and Isolation

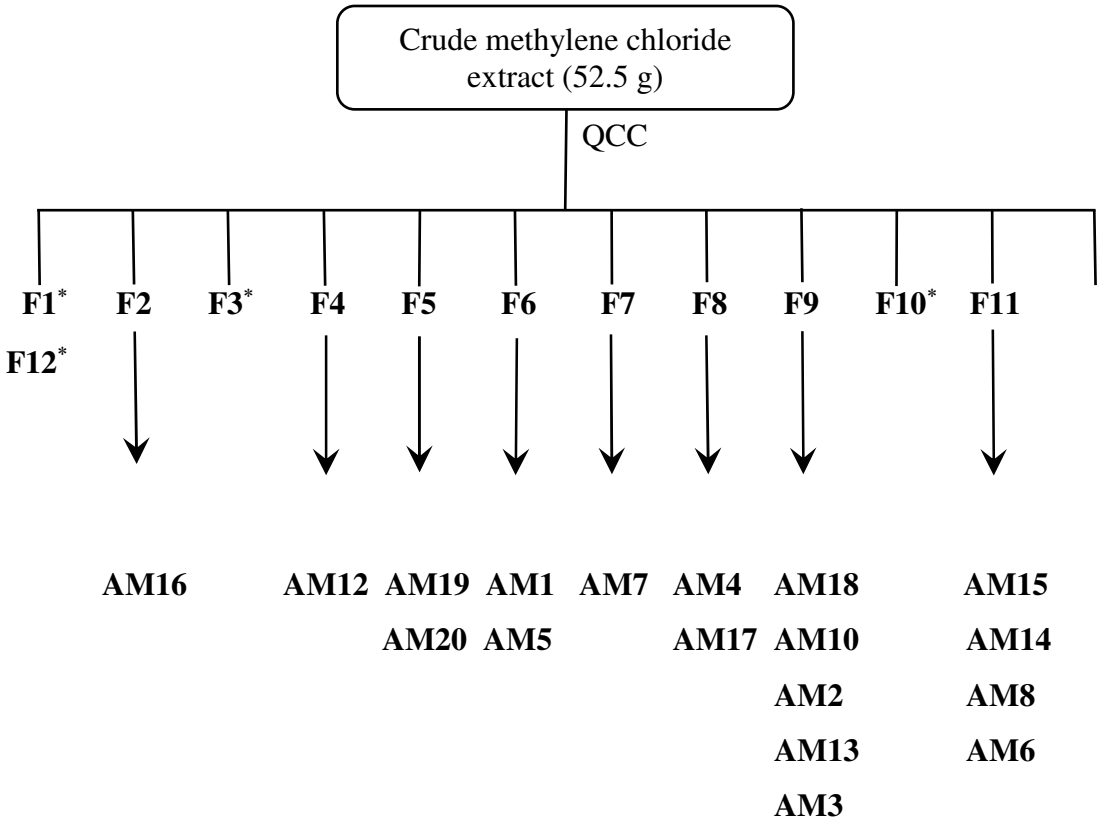
The air-dried and pulverized root (6.0 kg) was successively extracted with methylene chloride and acetone (2 x 20 L for one week for each solvent) at room temperature to furnish a yellow viscous residue of CH_2Cl_2 extract (52.5 g) and brownish acetone extract (15.0 g), respectively. The process of extraction was shown in **Scheme 1**.



Scheme 1 Extraction of the roots of *A. monophylla*

2.4 Isolation and Chemical Investigation

2.4.1 Investigation of the crude methylene chloride extract from the roots of *A. monophylla*



* No further investigation

Scheme 2 Isolation of compounds **AM1-8**, **AM10**, **AM12-20** from the methylene chloride extract.

The crude methylene chloride extract as a yellow viscous residue (52.5 g) was subjected to quick column chromatography over silica gel using solvent of increasing polarity from hexane through ethyl acetate. The eluates were collected and combined based on TLC characteristic to give twelve fractions (F1-F12).

Fraction 2 (1.5 g) was subjected to QCC with a gradient of EtOAc–hexane and followed by CC with acetone–hexane (1:5, v/v) to give **AM16**: auraptene (53.3 mg).

Fraction 4 (3.2 g) was subjected to QCC with a gradient of acetone–hexane and followed by CC with acetone–hexane (1:5, v/v) to give **AM12:** atalantolide (11.3 mg).

Fraction F5 was filtered and washed with hexane to yield a mixture of **AM19**: *β*-sitosterol and **AM20**: stigmasterol (154.0 mg) as a white solid and the mother liquor as yellow viscous oil after evaporation of the solvent.

Fraction F6 (1.5 g) was filtered and washed with hexane to give a yellow crystal (F6A) and followed by CC with acetone–hexane (1:5, v/v) to give **AM1**: *N*-methylataphylline (7.0 mg) and **AM5**: *N*-methylcycloataphylline-A (20.0 mg) and the mother liquor as yellow viscous oil after evaporation of the solvent.

Fraction 7 (1.2 g) was purified by QCC with a gradient of EtOAc-CH_2Cl_2 to give **AM7**: *N*-methylataphyllinine (25.0 mg).

Fraction 8 (2.5 g) was purified by QCC with a gradient of acetone–hexane to afford 8 fractions (8A-8H).

Subfraction 8C (154.0 mg) was separated by CC with acetone-hexane (1:6, v/v) to give **AM4:** cycloataphylline-A (2.3 mg).

Subfraction 8D (147.0 mg) was purified by CC with acetone-hexane (1:5, v/v) to give **AM17:** 7-*O*-geranylscopoletin (3.7 mg).

Fraction 9 (4.3 g) was purified by QCC with a gradient of acetone–hexane to afford 6 fractions (9A-9F).

Subfraction 9A (85.0 mg) was purified by CC with acetone-hexane (1:5, v/v) to give **AM18**: physcion (12.0 mg).

Subfraction 9C (385.0 mg) was purified by QCC with acetone-hexane (1:5, v/v) to give **AM2:** atalaphylline (22.0 mg).

Subfraction 9D (115.0 mg) was purified by CC with acetone-hexane (1:5, v/v) to give **AM3:** buxifoliadine-A (2.8 mg).

Subfraction 9E (250.0 mg) was purified by QCC with a gradient of acetone-CH_2Cl_2 and followed by CC with EtOAc-CH_2Cl_2 (1:25, v/v) to give **AM10:**

citrusinine-I (6.5 mg) and followed by CC with acetone–hexane (1:5, v/v) to give **AM13**: atalantin (26.7 mg).

Fraction 11 (3.1 g) was purified by QCC with a gradient of acetone–hexane to afford 8 fractions (11A-11H).

Subfraction 11A (250.0 mg) was purified by QCC with a gradient of acetone–hexane to give **AM14**: cycloepiatalantin (26.7 mg) and followed by CC with EtOAc-hexane (1.5:5, v/v) to give **AM6:** yukocitrine (2.5 mg).

Subfraction 11D (135.0 mg) was purified by CC with EtOAc-hexane (1:2.5, v/v) to give **AM15:** cycloepiatalantin acetate (20.7 mg).

Subfraction 11F (112.0 mg) was purified by CC with acetone-hexane (1:5, v/v) to give **AM8**: buxifoliadine-E (6.7 mg).

Compound AM1: *N*-methylatalaphylline, orange needles, m.p. 189-192 °C; UV λ_{max} (MeOH) (log ε): 205 (4.18), 272 (4.25), 335 (3.85) and 414 (3.42) nm; IR (KBr) ν_{max} 3350 (O-H stretching), 1633 (>C=O stretching) and 1604 (aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 2**.

Compound AM2: atalaphylline, orange needles, m.p. 245-247 °C; UV λ_{max} (MeOH) (log ε): 205 (7.65), 253 (7.77), 283 (7.73), 305 (7.37) and 404 (6.92) nm; IR (KBr) ν_{max} 3378 (O-H stretching), 1636 (>C=O stretching) and 1605 (aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 3**.

Compound AM3: buxifoliadine-A, yellow needles, m.p. 155-157 °C UV λ_{max} (MeOH) (log ε): 205 (4.11), 272 (4.21), 323 (3.68) and 416 (3.35) nm; IR (neat) ν_{max} 3385 (O-H stretching), 1637 (>C=O stretching) and 1602 (aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 4**.

Compound AM4: cycloatalaphylline-A, yellow needles, m.p. 238-240 °C; UV λ_{max} (MeOH) (log ε): 275 (1.13), 305 (0.98), 334 (0.79), 376 (0.69) and 401 (0.54) nm; IR (KBr) ν_{max}: 3363 (O-H stretching), 1634 (>C=O stretching) and 1607

(aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 7**; EIMS: *m/z* 377 (19) $[M]^+$; 376 (84), 361 (100), 333 (28), 305 (36), 293 (10), 153 (11); HREIMS: *m/z* $[M]^+$ 377.1626 (calcd for $C_{23}H_{23}NO_4$, 377.1627).

Compound AM5: *N*-methylcycloatalaphylline-A, yellow-orange crystals, m.p. 240-241 °C; UV λ_{max} (MeOH) (log ε): 204 (1.24), 272 (1.14), 323 (0.86), 345 (0.73) and 417 (0.37) nm; IR (KBr) ν_{max}: 3369 (O-H stretching), 1639 (>C=O stretching) and 1608 (aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 8**; EIMS: *m/z* 391 (23) $[M]^+$; 390 (96), 375 (100), 347 (50), 335 (54), 321 (30), 317 (18), 279 (13), 119 (17); HREIMS: *m/z* $[M]^+$ 391.1748 (calcd. for $C_{24}H_{25}NO_4$, 391.1784).

Compound AM6: yukocitrine, yellow needles, m.p. 215-217 °C; UV λ_{max} (MeOH) (log ε): 203 (3.57), 295 (3.98), 304 (4.03) and 413 (3.03) nm; IR (neat) ν_{max}: 3385 (O-H stretching), 1638 (>C=O stretching) and 1604 (aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 9**.

Compound AM7: *N*-methylataphyllinine, orange crystals, m.p. 195-196 °C; UV λ_{max} (MeOH) (log ε): 205 (3.97), 290 (4.16), 345 (3.60) and 422 (3.22) nm; IR (neat) ν_{max}: 3374 (O-H stretching), 1635 (>C=O stretching) and 1604 (aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 10**.

Compound AM8: buxifoliadine-E, yellow needles, m.p. 247-249 °C; $[\alpha]^{27}_{D}$ ±0° (*c* 0.12, MeOH), UV λ_{max} (MeOH) (log ε): 205 (4.04), 258 (4.28), 283 (4.24) and 394 (3.43) nm; IR (neat) ν_{max}: 3385 (O-H stretching), 1634 (>C=O stretching) and 1602 (aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 12**.

Compound AM10: citrusinine-I, orange needles, m.p. 206-207 °C; UV λ_{max} (MeOH) (log ε): 203 (3.80), 221 (3.74), 263 (4.19), 319 (3.71) and 416 (3.27) nm; IR (neat) ν_{max}: 3386 (O-H stretching), 1633 (>C=O stretching) and 1604

(aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 16**.

Compound AM12: atalantolide, light yellow crystals, m.p. 228-230 $^{\circ}C$; UV λ_{max} (MeOH) (log ε): 209 (3.90) nm; IR (neat) ν_{max}: 3401 (O-H stretching), 1742, 1717 and 1658 (>C=O stretching) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz,) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 20**.

Compound AM13: atalantin, light yellow crystals, m.p. 182-184 $^{\circ}C$; UV λ_{max} (MeOH) (log ε): 210 (4.01) nm; IR (neat) ν_{max}: 3396 (O-H stretching), 1739 and 1709 (>C=O stretching) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see Table **23**.

Compound AM14: cycloepiatalantin, yellow crystals, m.p. 308-310 $^{\circ}C$; UV λ_{max} (MeOH) (log ε): 211 (3.92) nm; IR (neat) ν_{max}: 3390 (O-H stretching), 1733 and 1693 (>C=O stretching) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 26**.

Compound AM15: cycloepiatalantin acetate, yellow crystals, m.p. 115-117 $^{\circ}C$; UV λ_{max} (MeOH) (log ε): 214 (4.05) nm; IR (neat) ν_{max}: 1736 and 1693 (>C=O stretching) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 29**.

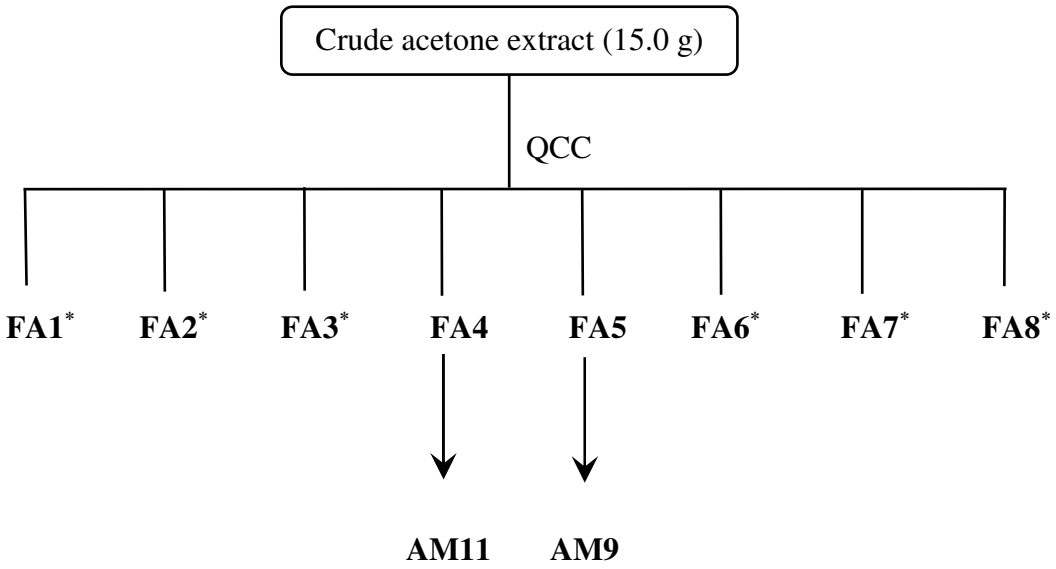
Compound AM16: auraptene, white solid, m.p. 71-73 $^{\circ}C$; UV λ_{max} (MeOH) (log ε): 205 (4.02), 252 (3.13) and 323 (3.95) nm; IR (neat) ν_{max}: 1710, and 1612 (aromatic) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz,) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see **Table 31**.

Compound AM17: 7-*O*-geranylscopoletin, white solid, m.p. 86-88 $^{\circ}C$; UV λ_{max} (MeOH) (log ε): 206 (3.96), 229 (3.64), 253 (3.16), 294 (3.13) and 345 (3.44) nm; IR (neat) ν_{max}: 1725 (>C=O stretching) and 1607 (aromatic) 1553 (C=C) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 33**.

Compound AM18*:* physcion, yellow crystals, m.p. 208-210 °C; UV λ_{max} (MeOH) (log ε): 221 (3.33), 252 (3.05), 264 (3.07), 285 (3.05) and 434 (2.87) nm; IR (neat) ν_{max}: 3380 (O-H stretching) and 1646 (>C=O stretching) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 35**.

The mixture of compound **AM19***:* β-sitosterol and **AM20**: stigmasterol was obtained as colorless crystals, ^{1}H ($CDCl_3$, 300 MHz).

2.4.2 Investigation of the crude acetone extract from the roots of *A. monophylla*



* No further investigation

Scheme 3 Isolation of compounds **AM11 and AM9** from the acetone extract.

The brownish crude acetone extract of *A. monophylla* (15.0 g) was subjected to quick column chromatography and eluted with hexane and ethyl acetate. The eluates were combined on the basis of TLC characteristic to give eight fractions (FA1-FA8).

Fraction FA4 (1.2 g) was purified by QCC with a gradient of acetone–hexane to afford 8 fractions (4A-4H).

Subfraction 4C (112.0 mg) was purified by CC with acetone-hexane (1:5, v/v) to give **AM11**: junosine (2.1 mg).

Fraction FA5 (615.0 mg) was purified by QCC with a gradient of acetone–hexane to afford 8 fractions (5A-5H).

Subfraction 5B (50.0 mg) was purified by CC with EtOAc-CH_2Cl_2 (1:10, v/v) to give **AM9**: *N*-methylbuxifoliadine-E (2.3 mg).

Compound AM9: *N*-methylbuxifoliadine-E, yellow needles, m.p. 250-252 $^{\circ}$C; $[\alpha]^{27}_{D}$ ±0° (*c* 0.12, MeOH); UV λ_{max} (MeOH) (log ε): 252 (1.19), 276 (1.03), 282 (1.29), 327 (0.98) and 395 (0.65) nm; IR (KBr) ν_{max}: 3374 (O-H stretching), 1639 (>C=O stretching) and 1604 (aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 13**; EIMS: *m/z* 409 (23) $[M]^{+}$; 408 (100), 393 (71), 335 (36), 321 (48), 104 (12); HREIMS: *m/z* $[M]^{+}$ 409.1888 (calcd for $C_{24}H_{27}NO_5$, 409.1889).

Compound AM11: junosine, orange needles, m.p. 218-220 $^{\circ}$C; UV λ_{max} (MeOH) (log ε): 205 (3.96), 265 (4.12), 285 (4.06), 305 (3.79) and 407 (3.29) nm; IR (neat) ν_{max}: 3380 (O-H stretching), 1636 (>C=O stretching) and 1604 (aromatic) cm^{-1}. For ^{1}H NMR (acetone-d_6, 300 MHz,) and ^{13}C NMR (acetone-d_6, 75 MHz) spectral data, see **Table 19**.

2.5 Bioassay

2.5.1 Anti-allergic activity assay

2.5.1.1 Inhibitory effects on the release of *β*-hexosaminidase from RBL-2H3 cells.

Inhibitory effects on the release of *β*-hexosaminidase from RBL-2H3 were evaluated by the following method (Matsuda *et al*., 2002).

2.5.1.2 *β*-Hexosaminidase inhibitory activity

In order to clarify that the anti-allergic effects of samples were due to the inhibition of *β*-hexosaminidase release and not *β*-hexosaminidase activity, the following assay was carried out. The cell suspension (5×10^7 cells) in 6 ml of PBS was sonicated. The solution was then centrifuged; and the supernatant diluted with Siraganian buffer and adjusted to equalize the enzyme activity of the degranulation tested above. The enzyme solution (45 µl) and test sample solution (5 µl) were transferred into a 96-well microplate and incubated with 50 µl of the substrate solution at 37 °C for 1 h. The reaction was stopped by adding 200 µl of the stop solution. The absorbance was measured using a microplate reader at 405 nm and the results were expressed as mean ± SEM of four determinations. The IC_{50} values were calculated using the Microsoft Excel program. The statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

2.5.2 Antibacterial assay

The isolated compound from the root of *A. monophylla* were tested for antibacterial activities against *Bacillus subtilis, staphylococcus aureus* TISTR517 and *Candida albicans* (obtained from Department of Industrial Biotechnology, Faculty of Agroindustry, PSU). Vancomycin which was used as a standard showed antibacterial activity of 75 µg/ml.

2.5.3 Cytotoxic assay

The procedure for cytotoxic assay was performed by the sulphorhodamine B (SRB) assay as described by Skehan et al. (Skehan *et al*., 1990). In this study, three cancer cell lines obtained from National Cancer Institute, Bangkok, Thailand, were used: MCF-7 (breast adenocarcinoma), KB (human oral cancer), HT-29 (human colon cancer) and HeLa (human cervical cancer). Camptothecin which was used as a standard showed cytotoxic activity in the range of 0.2-2.0 μg/ml.

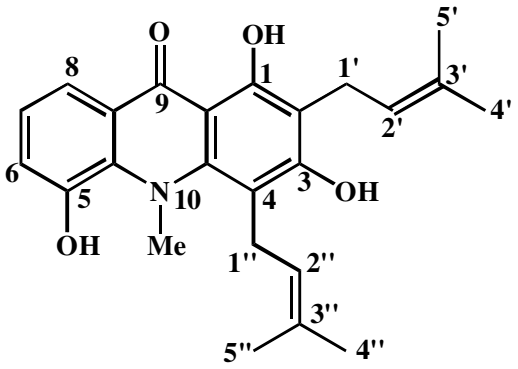
CHAPTER 3
RESULTS AND DISCUSSION

3.1 Structure elucidation of compounds from the roots of *A. monophylla*

The crude methylene chloride and acetone extracts from the root of *A. monophylla* were subjected to repeated quick column and column chromatography over silica gel to furnish three new acridone alkaloids: cycloatalaphylline-A (**AM4**), *N*-methylcycloatalaphylline-A (**AM5**), and *N*-methylbuxifoliadine-E (**AM9**) together with eight known acridones: **AM1**-**AM3**, **AM6**-**AM8**, **AM10** and **AM11**, four known limonoids: **AM12**-**AM15**, two known coumarins: **AM16** and **AM17**, one known anthraquinone: **AM18** and the mixture of compounds **AM19:** β-sitosterol and **AM20**: stigmasterol.

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^{1}H, ^{13}C NMR, DEPT 135°, DEPT 90°, HMQC, HMBC, COSY and NOESY. Mass spectra were determined for the new compounds: **AM4**, **AM5** and **AM9**. The physical data of the known compounds were also compared with the reported values. In addition X-ray crystallographic structure was reported for compound **AM7**.

3.1.1 Compound AM1



Compound **AM1** was isolated as orange needles. The UV-Vis spectrum exhibited the absorption bands at 205, 272, 325 and 414 nm characteristic of a 9-acridone chromophore, which was confirmed by IR absorption maxima indicating the presence of hydroxyl (3350 cm^{-1}) and chelated carbonyl (1633 cm^{-1}) groups.

The ^{1}H NMR spectral data (**Table 2**) of **AM1** exhibited the presence of a chelated phenolic hydroxyl group at C-1 as a singlet signal at δ 14.56. Two broad singlets at δ 9.28 and δ 7.89 indicated two hydroxyl groups in the molecule. One methyl singlet signal at δ 3.67 and together with ^{13}C-NMR spectrum at δ 47.6 was assigned for *N*-methyl group. The ^{13}C NMR and DEPT spectral data (**Table 2**) exhibited 24 carbons, attributable to five methyl, two methylene, five methine and twelve quaternary carbons. In the aromatic region, ABX pattern of ^{1}H NMR at δ 7.77 (1H, *dd*, *J* = 7.8, 1.5 Hz), 7.26 (1H, *dd*, *J* = 7.8, 1.5 Hz), and 7.16 (1H, *t*, *J* = 7.8 Hz) were attributed to H-8, H-6, and H-7, respectively. The lower field proton at δ 7.77 was deshielded by the 9-carbonyl group. In the aliphatic region, two sets of prenyl groups appeared at δ 5.37 (1H, *m*), 3.60 (2H, *br d*, *J* = 6.0 Hz), 1.80 (3H, *br s*), 1.71 (3H, *d,* 1.5 Hz), and 5.25 (1H, *m*), 3.45 (2H, *br d*, *J* = 6.9 Hz), 1.80 (3H, *br s*), 1.67 (3H, *d,* 0.9 Hz). The locations of two prenyl groups at C-2 and C-4, respectively were confirmed by HMBC correlation of H-1′ at δ 3.45 with the carbons at δ 159.7 (C-1) 107.5 (C-2) 123.4 (C-2′) 132.4 (C-3′) and 161.2 (C-3), of H-1′′ at δ 3.60 with the carbons at δ 109.3 (C-4), 161.2 (C-3), 122.4 (C-2′′) and 131.4 (C-3′′). Two hydroxyl groups were placed at C-3 and C-5, respectively from HMBC correlation of 3-OH at δ 7.89 to the carbons at δ 161.2 (C-3), 107.5 (C-2) and 109.3 (C-4) and 5-OH at δ 9.28 with the carbons at 138.2 (C-5a), 119.4 (C-6) and 148.5 (C-5) (**Figure 2**). The

complete HMBC correlations were summarized in **Table 2**. Therefore, compound **AM1** was assigned as *N*-methylatalaphylline (Govindachari *et al*., 1970).

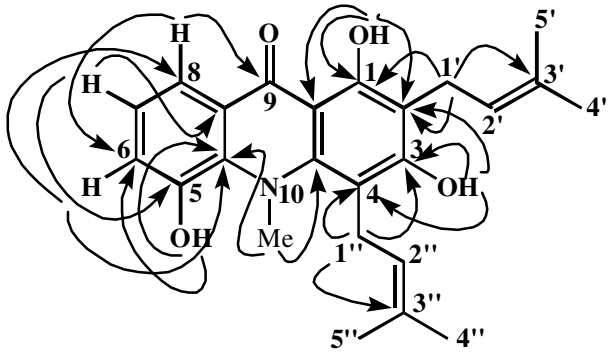
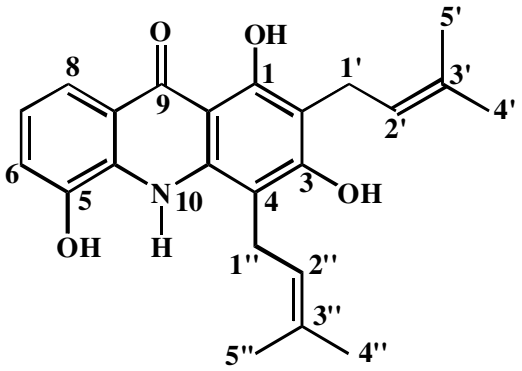


Figure 2 Selected HMBC correlation of **AM1**

Table 2 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM1** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	159.7	C		
1-OH			14.56 (s)	C-9a, C-2, C-1
2	107.5	C		
3	161.2	C		
3-OH			7.89 (br s)	C-2, C-3, C-4
4	109.3	C		
5	148.5	C		
5-OH			9.28 (br s)	C-5a, C-6, C-5
6	119.4	CH	7.26 (dd, *J* = 7.8, 1.5)	C-8, C-5a
7	122.7	CH	7.16 (t, *J* = 7.8)	C-5, C-8a
8	116.2	CH	7.77 (dd, *J* = 7.8, 1.5)	C-6, C-5a, C-9
9	182.6	C		
4a	148.9	C		
5a	138.2	C		
8a	125.0	C		
9a	107.0	C		
1′	21.2	CH_2	3.45 (br d, *J* = 6.9)	C-1, C-2, C-2′, C-3′
2′	123.4	CH	5.37 (m)	
3′	132.4	C		
4′	17.0	CH_3	1.80 (br s)	C-2′, C-3′, C-5′
5′	25.0	CH_3	1.71 (d, *J* = 1.5)	C-2′, C-3′, C-4′
1″	26.2	CH_2	3.60 (br d, *J* = 6.0)	C-3, C-4, C-4a, C-3″
2″	122.4	CH	5.25 (m)	
3″	131.4	C		
4″	17.0	CH_3	1.80 (br s)	C-2″, C-3″, C-5″
5″	25.0	CH_3	1.67 (br s)	C-2″, C-3″, C-4″
10-NMe	47.6	CH_3	3.67 (s)	C-4a, C-5a

3.1.2 Compound AM2



Compound **AM2** was isolated as orange needles. The UV-Vis spectrum exhibited the absorption bands at 205, 253, 283, 305 and 404 nm characteristic of a 9-acridone chromophore which was confirmed by the presence of IR absorption maxima of hydroxyl (3378 cm^{-1}) and chelated carbonyl (1636 cm^{-1}) functionalities.

The ^{1}H and ^{13}C NMR spectral data (**Table 3**) of **AM2** were similar to those of **AM1**, except that *N*-methyl signal (δ_H 3.67, δ_C 47.6) in **AM1** was replaced by an NH proton (δ_H 9.00) in **AM2**. The chelated hydroxyl signal was evidenced at δ 14.65. The locations of two prenyl groups at C-2 and C-4, respectively were confirmed by HMBC correlation of H-1′ at δ 3.48 with the carbons at δ 159.5 (C-1), 107.8 (C-2), 122.7 (C-2′) and 131.2 (C-3′) and of H-1″ (δ 3.65) with the carbons at δ 101.1 (C-4), 138.7 (C-4a), 158.7 (C-3) and 134.0 (C-3″) (**Figure 3**). The complete HMBC correlations were summarized in **Table 3**. Therefore, compound **AM2** was assigned as atalaphylline (Govindachari *et al*., 1970).

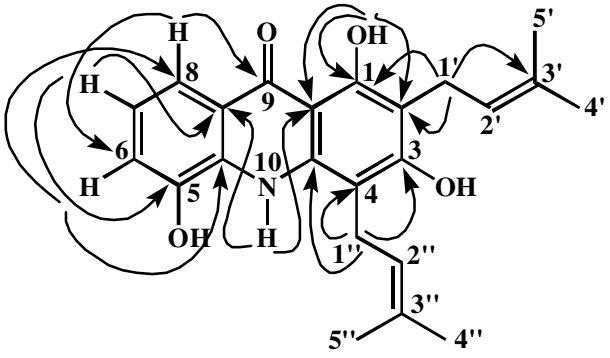
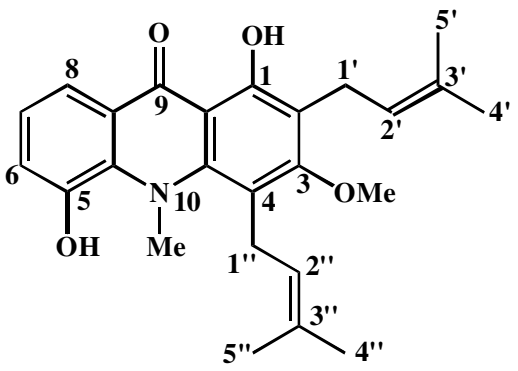


Figure 3 Selected HMBC correlation of **AM2**

Table 3 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM2** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	159.5	C		
1-OH			14.65 (s)	C-9a, C-2, C-1
2	107.8	C		
3	158.7	C		
4	101.1	C		
5	144.6	C		
6	115.6	CH	7.20 (br d, *J* = 7.8)	C-8, C-5, C-5a
7	120.8	CH	7.07 (t, *J* = 7.8)	C-5, C-8a
8	115.8	CH	7.76 (d, *J* = 7.8)	C-6, C-5a, C-9
9	181.2	C		
4a	138.7	C		
5a	131.1	C		
8a	120.0	C		
9a	104.3	C		
1′	21.4	CH_2	3.48 (d, *J* = 7.0)	C-1, C-2, C-2′, C-3′
2′	122.7	CH	5.27 (br t, *J* = 7.0)	C-1′, C-4′, C-5′
3′	131.2	C		
4′	17.1	CH_3	1.81 (s)	C-2′, C-3′, C-5′
5′	25.0	CH_3	1.67 (s)	C-2′, C-3′, C-4′
1″	22.4	CH_2	3.65 (d, *J* = 7.0)	C-3, C-4, C-4a, C-3″
2″	121.9	CH	5.15 (br t, *J* = 7.0)	C-1″, C-4″, C-5″
3″	134.0	C		
4″	17.3	CH_3	1.98 (s)	C-2″, C-3″, C-5″
5″	25.0	CH_3	1.75 (s)	C-2″, C-3″, C-4″
10-NH			9.00 (br s)	C-8a, C-9a

3.1.3 Compound AM3



Compound **AM3** was isolated as yellow needles, m.p. 155-157 °C. The 9-acridone skeleton in the molecule was suggested by ultraviolet (UV) spectroscopic absorptions at 205, 272, 323 and 416 nm and a carbonyl group absorption band at 1637 cm^{-1}.

The ^{1}H and ^{13}C NMR spectral data (**Table 4**) of **AM3** were similar to those of **AM1**. The difference was shown as the replacement of a singlet signal of the hydroxyl group at C-3 (δ 7.89) in **AM1** with a methoxyl group (δ 3.85) in **AM3**. The presence of a chelated phenolic hydroxyl group at C-1 was indicated by the ^{1}H-NMR signal at δ 14.40. One-proton singlet at δ 9.42 indicated another hydroxyl group in the molecule. Two singlet signals at δ 3.85 and δ 3.72 (each 3H) together with ^{13}C-NMR spectra at δ 62.0 and 47.9 were assigned for methoxyl and *N*-methyl groups respectively. The location of two prenyl groups at C-2 and C-4, respectively were confirmed by HMBC correlation of H-1′ at δ 3.40 with the carbons at δ 160.8 (C-1), 116.2 (C-2), 123.7 (C-2′) and 131.4 (C-3′), of H-1″ (δ 3.65) with the carbons at δ 115.2 (C-4), 165.7 (C-3) 124.5 (C-2″) and 134.1 (C-3″). The O-methoxyl group was placed at C-3 due to HMBC correlation of O-Me at δ 3.85 with the carbon at δ 165.7 (C-3) (**Figure 4**). On the basis of the above results, the structure of buxifoliadine-A was assigned as **AM3** (Wu and Chen, 2000).

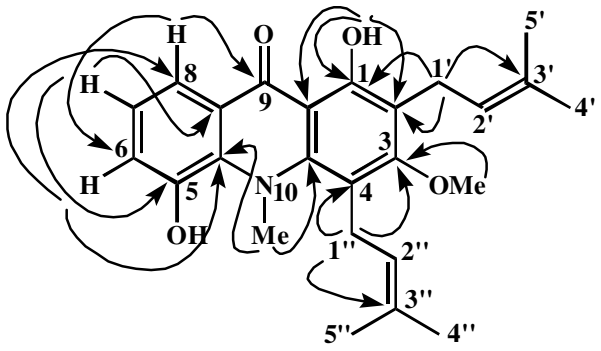


Figure 4 Selected HMBC correlation of **AM3**

Table 4 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM3** (acetone-d_6)

Position	δ_C		δ_H **(mult, *J*, Hz)**	**HMBC**
1	160.8	C		
1-OH			14.40 (s)	C-9a, C-2, C-1
2	116.2	C		
3	165.7	C		
3-OMe	62.0	CH_3	3.85 (s)	C-3
4	115.2	C		
5	150.0	C		
5-OH			9.42 (br s)	
6	120.6	CH	7.30 (dd, *J* = 7.8, 1.5)	C-8, C-5a
7	123.7	CH	7.17 (t, *J* = 7.8)	C-5, C-8a
8	117.2	CH	7.78 (dd, *J* = 7.8, 1.5)	C-5a, C-9
9	184.3	C		
4a	149.5	C		
5a	136.8	C		
8a	125.8	C		
9a	110.3	C		
1′	23.2	CH_2	3.40, (br d, *J* = 6.9)	C-1, C-2, C-2′, C-3′

Table 4 (continued)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
2′	123.7	CH	5.35 (m)	
3′	131.4	C		
4′	17.9	CH_3	1.66 (s)	C-2′, C-3′, C-5′
5′	25.7	CH_3	1.77 (s)	C-2′, C-3′, C-4′
1″	27.5	CH_2	3.65 (d, *J* = 7.0)	C-3, C-4, C-4a, C-3″
2″	124.5	CH	5.30 (br t, *J* = 7.0)	
3″	134.1	C		
4″	18.1	CH_3	1.65 (s)	C-2″, C-3″, C-5″
5″	25.8	CH_3	1.82 (s)	C-2″, C-3″, C-4″
10-NMe	47.9	CH_3	3.72 (s)	C-4a, C-5a

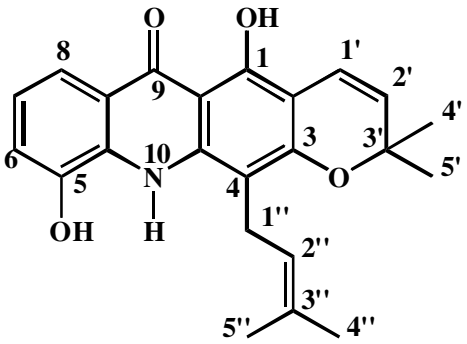
Table 5 Comparison of ^{1}H NMR spectral data between compounds **AM1**, **AM3** and Buxifoliadine-A (**R**, acetone-d_6)

Position	AM1 δ_H (mult, *J*, Hz)	AM3 δ_H (mult, *J*, Hz)	R δ_H (mult, *J*, Hz)
1-OH	14.56 (s)	14.40 (s)	14.38 (s)
2			
3-OH	7.89 (br s)		
3-OMe		3.85 (s)	3.84 (s)
4			
5-OH	9.28 (br s)	9.42 (br s)	9.23 (s)
6	7.26 (dd, *J* = 7.8, 1.5)	7.30 (dd, *J* = 7.8, 1.5)	7.28 (dd, *J* = 8.0, 1.6)
7	7.16 (t, *J* = 7.8)	7.17 (t, *J* = 7.8)	7.16 (t, *J* = 8.0)
8	7.77 (dd, *J* = 7.8, 1.5)	7.78 (dd, *J* = 7.8, 1.5)	7.78 (dd, *J* = 8.0, 1.6)
9			
4a			
5a			
8a			
9a			
1′	3.45 (br d, *J* = 6.9)	3.40, (br d, *J* = 6.9)	3.39 (d, *J* = 6.8)
2′	5.37 (m)	5.35 (m)	5.28 (t, *J* = 6.8)
3′			
4′	1.80 (br s)	1.66 (s)	1.65 (br s)
5′	1.71 (d, *J* = 1.5)	1.77 (s)	1.75 (br s)
1″	3.60 (br d, *J* = 6.0)	3.65 (d, *J* = 7.0)	3.64 (d, *J* = 6.2)
2″	5.25 (m)	5.30 (br t, *J* = 7.0)	5.33 (br t, *J* = 6.2)
3″			
4″	1.80 (br s)	1.65 (s)	1.66 (br s)
5″	1.67 (s)	1.82 (s)	1.79 (br s)
10-NMe	3.67 (s)	3.72 (s)	3.71 (s)

Table 6 Comparison of ^{13}C NMR spectral data between compounds **AM1**, **AM3** and Buxifoliadine-A (**R**, acetone-d_6)

Position	δ_C, AM1	δ_C, AM3	δ_C, R
1	159.7	160.8	160.8
2	107.5	116.2	116.1
3	161.2	165.7	165.6
3-OMe		62.0	62.0
4	109.3	115.2	115.2
5	148.5	150.0	149.5
6	119.4	120.6	120.5
7	122.7	123.7	124.0
8	116.2	117.2	117.2
9	182.6	184.3	184.2
4a	148.9	149.5	149.2
5a	138.2	136.8	138.2
8a	125.0	125.8	125.8
9a	107.0	110.3	105.3
1′	21.2	23.2	23.2
2′	123.4	123.7	123.7
3′	132.4	131.4	131.4
4′	17.0	17.9	17.9
5′	25.0	25.7	25.7
1″	26.2	27.5	27.1
2″	122.4	124.5	124.4
3″	131.4	134.1	132.1
4″	17.0	18.1	18.1
5″	25.0	25.8	25.8
10-NMe	47.6	47.9	47.9

3.1.4 Compound AM4



Compound **AM4** was isolated as yellow needles. It showed [M^+] at *m/z* 377.1626 ($C_{23}H_{23}NO_4$) in the HREIMS spectrum. The UV-Vis spectrum exhibited the absorption bands at 275, 305, 334, 376 and 401 nm characteristic of a 9-acridone chromophore which was confirmed by the presence of IR absorption maxima of hydroxyl (3363 cm^{-1}) and chelated carbonyl (1634 cm^{-1}) groups.

The ^{13}C NMR and DEPT spectral data (**Table 7**) exhibited 23 carbons, attributable to four methyl, one methylene, six methine and twelve quaternary carbons. In the aromatic region of the ^1H NMR spectrum, three mutually coupling ABX signals at δ 7.76 (1H, *d*, *J* = 7.8 Hz), 7.25 (1H, *br d*, *J* = 7.8 Hz), and 7.12 (1H, *t*, *J* = 7.8 Hz), were attributed to H-8, H-6 and H-7, respectively. A prenyl group in the molecule was inferred by the signals at δ 5.17 (1H, *br t*, *J* = 7.2 Hz, H-2″), 3.61 (2H, *d*, *J* = 7.2 Hz, 2H-1″), 1.99 and 1.76 (each 3H, *s*, Me-5″, Me-4″ respectively). The remaining signals at δ 6.77, 5.71 (each 1H, *d*, *J* = 9.9 Hz, H-1′, H-2′, respectively), and 1.49 (6H, *s*, Me-4′, Me-5′) represented the presence of a 2,2-dimethylpyrano moiety. The HMBC correlation of H-1″ at δ 3.61 with the carbons at δ 156.2 (C-3) and 139.9 (C-4a), and its NOESY cross peak with the N-H proton at δ 9.05 supported the attachment of a prenyl group at C-4 (**Table 7**). Additional HMBC correlation of H-1′ (δ 6.77) with the carbon at δ 157.1 (C-1), of H-2′ (δ 5.71) with δ 102.1 (C-2) (**Figure 5**) suggested that the 2,2-dimethyl pyran ring was fused to the acridone nucleus with linear orientation. On the basis of the above analysis, the structure of **AM4** was identified and named as cycloatalaphylline-A, a new compound.

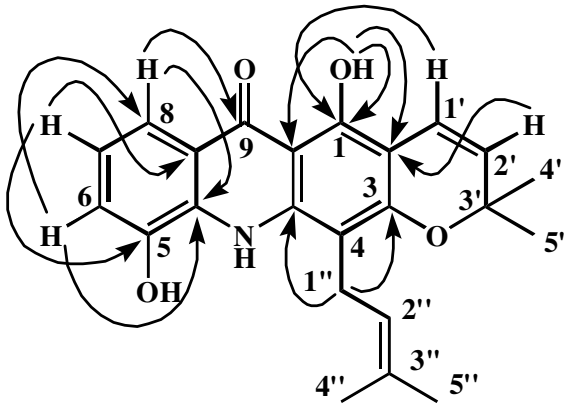


Figure 5 Selected HMBC correlation of **AM4**

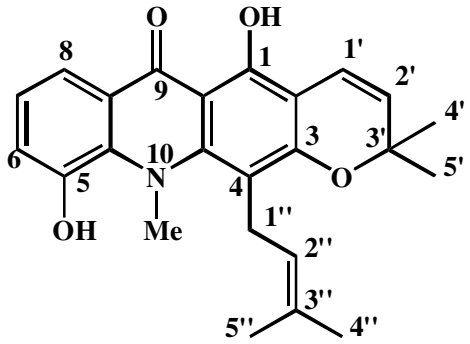
Table 7 ^{1}H, ^{13}C NMR, HMBC and NOESY spectral data of compound **AM4** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**	**NOESY**
1	157.1	C			
1-OH			14.74 (s)	C-9a, C-2, C-1	
2	102.1	C			
3	156.2	C			
4	102.2	C			
5	144.7	C			
5-OH			9.82 (br s)		
6	116.0	CH	7.25 (br d, *J* = 7.8)	C-5a	7
7	121.3	CH	7.12 (t, *J* = 7.8)	C-5, C-8a	6, 8
8	115.7	CH	7.76 (d, *J* = 7.8)	C-6, C-5a, C-9	7
9	181.4	C			
4a	139.9	C			
5a	130.8	C			
8a	120.3	C			
9a	104.2	C			
1′	115.9	CH	6.77 (d, *J* = 9.9)	C-1, C-3′	2′
2′	126.6	CH	5.71 (d, *J* = 9.9)	C-2, C-3′	1′, 4′, 5′

Table 7 (continued)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC	NOESY
3′	77.5	C			
4′/5′	27.5	CH_3×2	1.49 (s)	C-2′, C-3′	2′
1″	21.5	CH_2	3.61 (d, *J* = 7.2)	C-2″, C3″, C-4a, C-3	2″, 10
2″	121.8	CH	5.17 (br t, *J* = 7.2)	C-4″, C-5″	1″, 4″
3″	133.8	C			
4″	25.0	CH_3	1.76 (s)	C-2″, C-3″, C-5″	2″
5″	17.2	CH_3	1.99 (s)	C-2″, C-3″, C-4″	
10-NH			9.05 (br s)		1″

3.1.5 Compound AM5



Compound **AM5** was isolated as yellow-orange crystals. The UV-Vis spectrum exhibited the absorption bands at 204, 272, 323, 345 and 417 nm characteristic of a 9-acridone chromophore which was confirmed by the presence of IR absorption maxima of hydroxyl (3369 cm^{-1}) and chelated carbonyl (1639 cm^{-1}) groups.

Compound **AM5**, showed $[M]^+$ at *m/z* 391.1748 $C_{24}H_{25}NO_4$ whose MW was 14 mass units more than that of **AM4**. The ^1H and ^{13}C NMR spectra were closely related to those of **AM4**, except that the N-H proton signal at δ 9.05 in **AM4** was replaced by *N*-methyl signal in **AM5** at δ_H 3.71 : δ_C 47.7. A prenyl group was placed at C-4 due to HMBC correlation of H-1″ (δ 3.51) with the carbons at δ 108.5 (C-4), 150.0 (C-4a) and 158.8 (C-3), and NOESY cross peak between N-Me (δ 3.71)

and H-2″ (δ 5.36). Hence, **AM5** was an *N*-methyl derivative of **AM4**, a new compound and named as *N*-methylcycloatalaphylline-A.

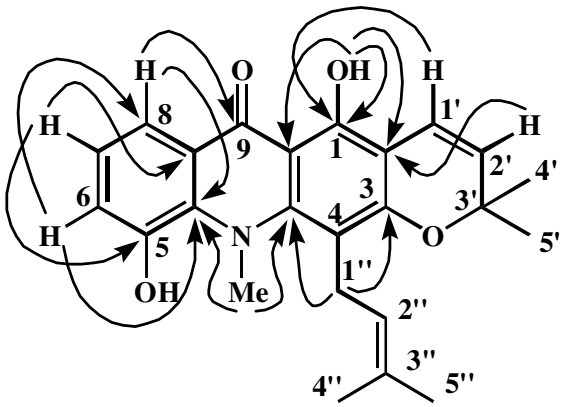
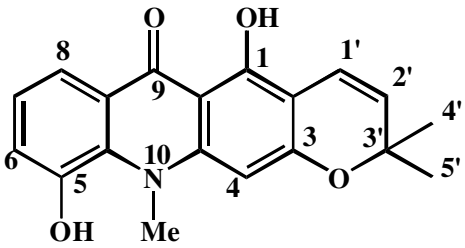


Figure 6 Selected HMBC correlation of **AM5**

Table 8 ^{1}H, ^{13}C NMR, HMBC and NOESY spectral data of compound **AM5** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**	**NOESY**
1	157.5	C			
1-OH			14.63 (s)	C-9a, C-1, C-2	
2	103.4	C			
3	158.8	C			
4	108.5	C			
5	148.6	C			
5-OH			9.41 (br s)		
6	119.7	CH	7.29 (br d, J = 7.5)	C-8, C-5a	7
7	123.1	CH	7.18 (t, J = 7.5)	C-5, C-8a	6, 8
8	116.1	CH	7.76 (d, J = 7.5)	C-5a, C-9	7
9	182.7	C			
4a	150.0	C			
5a	138.0	C			
8a	124.9	C			
9a	106.9	C			
1′	115.6	CH	6.73 (d, J = 9.9)	C-3	2′
2′	126.9	CH	5.70 (d, J = 9.9)		1′, 4′, 5′
3′	77.7	C			
4′/5′	27.6	CH_3×2	1.48 (s)	C-3′, C-2′	2′
1″	25.7	CH_2	3.51 (br d, J = 6.3)	C-2″, C-3″, C-4, C-4a	
2″	123.9	CH	5.36 (m)		10
3″	130.8	C			
4″	24.9	CH_3	1.70 (s)	C-2″, C-3″, C-5″	
5″	17.3	CH_3	1.80 (s)	C-2″, C-3″, C-4″	
10-NMe	47.7	CH_3	3.71 (s)	C-4a, C-5a	2″

3.1.6 Compound AM6



Compound **AM6** was isolated as yellow needles, m.p. 215-217 °C. The UV-Vis spectrum exhibited the absorption bands at 203, 295, 304 and 413 nm characteristic of a 9-acridone chromophore which was confirmed by the presence of IR absorption maxima of hydroxyl (3385 cm^{-1}) and chelated carbonyl (1638 cm^{-1}) groups.

The ^{1}H NMR spectral data (**Table 9**) of **AM6** indicated the presence of a chelated phenolic hydroxyl group at C-1 by the singlet signal at δ 15.22. One-proton broad singlet at δ 9.65 indicated another hydroxyl group in the molecule and one methyl singlet signal at δ_H 4.08 : δ_C 40.5 was assigned for *N*-methyl group. In the aromatic region, signals of ABX pattern at δ 7.89 (1H, *dd*, *J* = 8.1, 1.5 Hz), 7.32 (1H, *dd*, *J* = 8.1, 1.5 Hz) and 7.16 (1H, *t*, *J* = 8.1 Hz) were attributed to H-8, H-6, and H-7, respectively**.** The spectral data of **AM6** were comparable to **AM5**, except that a singlet signal of an aromatic proton at δ 6.38 in **AM6** replaced signals of a prenyl group in **AM5**. Its location was placed at C-4 due to HMBC correlations to δ 101.9 (C-2), 158.8 (C-3), 147.1 (C-4a) and 104.7 (C-9a). On the basis of the above results, the structure of yukocitrine was assigned as **AM6** (Auzi *et al*., 1996).

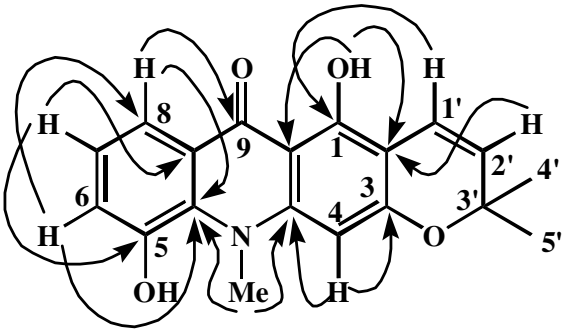


Figure 7 Selected HMBC correlation of **AM6**

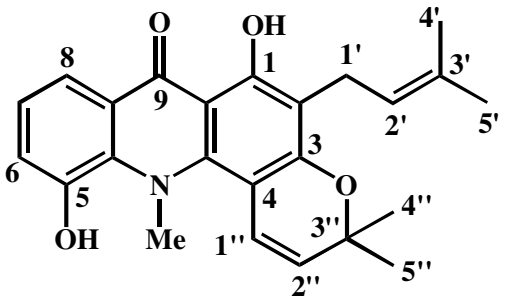
Table 9 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM6** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	159.2	C		
1-OH			15.22 (s)	C-9a, C-2
2	101.9	C		
3	158.8	C		
4	91.9	CH	6.38 (s)	C-2, C-3, C-4a, C-9a
5	147.0	C		
5-OH			9.65 (br s)	
6	120.0	CH	7.32 (dd, *J* = 8.1, 1.5)	C-8, C-5a
7	122.3	CH	7.16 (t, *J* = 8.1)	C-5, C-8a
8	116.8	CH	7.89 (dd, *J* = 8.1, 1.5)	C-5a, C-9, C-6
9	180.6	C		
4a	147.1	C		
5a	136.6	C		
8a	123.0	C		
9a	104.7	C		
1′	115.6	CH	6.72 (d, *J* = 10.1)	C-3, C-3′
2′	126.0	CH	5.69 (d, *J* = 10.1)	C-1, C-3′, C-4′, C-5′

Table 9 (continued)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
3′	77.6	C		
4′/5′	27.7	CH_3×2	1.48 (s)	C-3′, C-2′
10-NMe	40.5	CH_3	4.08 (s)	C-4a, C-5a

3.1.7 Compound AM7



Compound **AM7** was isolated as orange crystals, m.p. 195-196 °C. The UV-Vis spectrum exhibited the absorption bands at 205, 290, 345 and 422 nm characteristic of a 9-acridone chromophore which was confirmed by the presence of IR absorption maxima of hydroxyl (3374 cm^{-1}) and chelated carbonyl (1635 cm^{-1}) groups. The X-ray structure of **AM7** (**Figure 8**) (Chukaew *et al*., 2007) confirmed a structure with an acridone skeleton.

The ^{1}H NMR spectral data (**Table 10**) of **AM7** were similar to those of **AM5**. Signals of a chelated hydroxyl group appeared at δ 14.43 (*s*, 1-OH) and three adjacent aromatic proton signals with ABX pattern were shown at δ 7.72 (1H, *dd*, *J* = 8.1, 1.5 Hz), 7.32 (1H, *dd*, *J* = 8.1, 1.5 Hz) and 7.16 (1H, *t*, *J* = 8.1 Hz) attributable to H-8, H-6 and H-7, respectively. A prenyl group was shown as signals at δ 3.27 (2H, *br d*, *J* = 7.0 Hz, H-1′), 5.20 (1H, *br t, J* = 7.0 Hz, H-2′), 1.75, 1.60 (each, *s*, Me-4′, Me-5′), whose HMBC correlation of H-1′ at δ 3.27 with the carbons at δ 160.2 (C-1), 159.3 (C-3) indicated a connection of a prenyl group at C-2. Signals of a 2,2-dimethyl pyran ring were shown at δ 6.90 (1H, *d, J* = 9.5 Hz, H-1″), 5.43 (1H, *d, J* = 9.5 Hz, H-2″) and 1.43 (6H, *s*, Me-4″, Me-5″). HMBC correlation of H-1″ at δ 159.3 (C-3) and 146.0 (C-4a), of H-2″ at δ 5.43 with the carbon at δ 102.6 (C-4) suggested that a 2,2-

dimethyl pyran ring was fused to the acridone nucleus with angular orientation. Therefore, compound **AM7** was assigned as *N*-methylataphyllinine (Auzi *et al*., 1996).

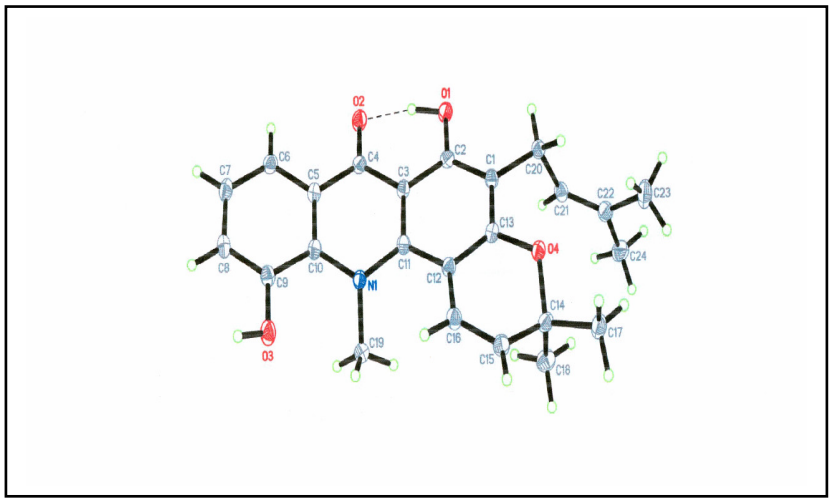


Figure 8 X-ray ORTEP diagram of compound **AM7**

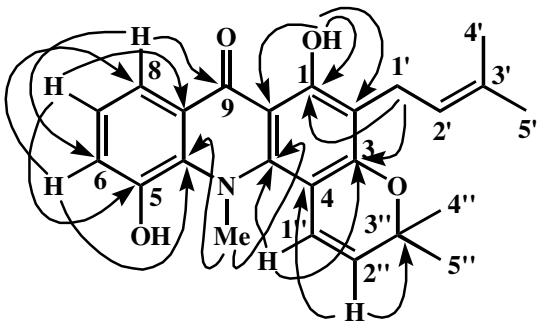


Figure 9 Selected HMBC correlation of **AM7**

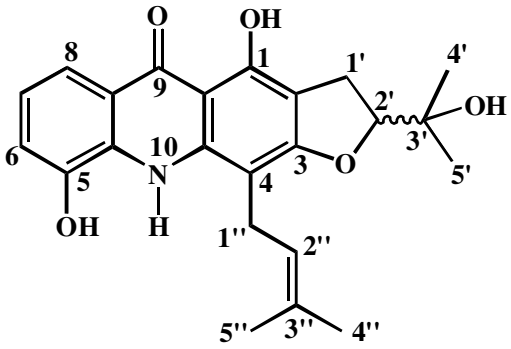
Table 10 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM7** (acetone-d_6)

Position	δ_C	**Type of carbon**	δ_H **(mult, *J*, Hz)**	**HMBC**
1	160.2	C		
1-OH			14.43 (s)	C-9a, C-1, C-2
2	106.4	C		
3	159.3	C		
4	102.6	C		
5	147.8	C		
5-OH			9.45 (br s)	
6	119.5	CH	7.32 (dd, *J* = 8.1, 1.5)	C-8, C-5a
7	123.0	CH	7.16 (t, *J* = 8.1)	C-5, C-8a
8	116.5	CH	7.72 (dd, *J* = 8.1, 1.5)	C-5a, C-9
9	180.8	C		
4a	146.0	C		
5a	137.9	C		
8a	124.9	C		
9a	110.1	C		
1′	21.2	CH_2	3.27 (br d, *J* = 7.0)	C-1, C-3, C-2′, C-3′
2′	122.5	CH	5.20 (br t, *J* = 7.0)	
3′	131.0	C		
4′	26.0	CH_3	1.75 (s)	C-2′, C-3′, C-5′
5′	18.0	CH_3	1.60 (s)	C-2′, C-3′, C-4′
1″	121.0	CH	6.90 (d, *J* = 9.5)	C-3″, C-3, C-4a
2″	123.5	CH	5.43 (d, *J* = 9.5)	C-3″, C-4, C-4″/5″
3″	76.2	C		
4″/5″	27.3	CH_3×2	1.43 (s)	C-3″, C-2″
10-NMe	48.3	CH_3	3.66 (s)	C-4a, C-5a

Table 11 Comparison of ^{1}H NMR spectral data between compounds **AM5**, **AM7** and *N*-methylataphyllinine (**R**, $CDCl_3$)

Position	AM5 δ_H (mult, *J*, Hz)	AM7 δ_H (mult, *J*, Hz)	R δ_H (mult, *J*, Hz)
1-OH	14.63 (s)	14.43 (s)	14.32 (s)
2			
3			
4			
5-OH	9.41 (br s)	9.45 (br s)	
6	7.29 (br d, *J* = 7.5)	7.32 (dd, *J* = 8.1, 1.5)	7.32 (dd, *J* = 7.0, 3.0)
7	7.18 (t, *J* = 7.5)	7.16 (t, *J* = 8.1)	7.06 (t, *J* = 7.0)
8	7.76 (d, *J* = 7.5)	7.72 (dd, *J* = 8.1, 1.5)	7.80 (dd, *J*= 7.0, 3.0)
9			
4a			
5a			
8a			
9a			
1′	6.73 (d, *J* = 9.9)	3.27 (br d, *J* = 7.0)	3.37 (d, *J* = 7.0)
2′	5.70 (d, *J* = 9.9)	5.20 (br t, *J* = 7.0)	5.30 (m)
3′			
4′	1.48 (s)	1.75 (s)	1.82 (s)
5′	1.48 (s)	1.60 (s)	1.68 (s)
1″	3.51 (br d, *J* = 6.3)	6.90 (d, *J* = 9.5)	6.63 (d, *J* = 10.0)
2″	5.36 (m)	5.43 (d, *J* = 9.5)	5.51 (d, *J* = 10.0)
3″			
4″	1.70 (s)	1.43 (s)	1.52 (s)
5″	1.80 (s)	1.43 (s)	1.52 (s)
10-NMe	3.71 (s)	3.66 (s)	3.78 (s)

3.1.8 Compound AM8



Compound **AM8** was isolated as optically inactive yellow needles, m.p. 247-249 °C. The UV-Vis spectrum exhibited the absorption bands at 205, 258, 283 and 394 nm characteristic of a 9-acridone chromophore which was confirmed by the presence of IR absorption maxima of hydroxyl (3385 cm^{-1}) and chelated carbonyl (1634 cm^{-1}) groups.

A proton singlet signal of a phenolic hydroxyl was displayed at δ 14.50 and that of N-H proton at δ 9.01. In the aromatic region, three mutually coupling signals at δ 7.74 (1H, *d*, *J* = 7.8 Hz), 7.20 (1H, *br d*, *J* = 7.8 Hz) and 7.08 (1H, *t*, *J* = 7.8 Hz) were attributed to H-8, H-6 and H-7, respectively. Signals at δ 5.21 (1H, *br t*, *J* = 7.2 Hz), 3.54 (2H, *br d*, *J* = 7.2 Hz), 1.77 (3H, *s*) and 1.97 (3H, *s*) indicated the presence of a prenyl group in the molecule. The ^{1}H and ^{13}C NMR spectral data of **AM8** were partly comparable with those of **AM4** and **AM5**, suggesting an acridone chromophore with a prenyl side chain attached at C-4 from HMBC correlation of H-1″ (δ 3.54) with the carbons at δ 96.4 (C-4), 140.6 (C-4a) and C-3 (164.6). The ^{1}H NMR data different from those of **AM4** and **AM5** were shown as signals at δ 4.80 (1H, *dd*, *J* = 9.0, 8.1 Hz, H-2′), 3.22 and 3.15 (each 1H, *dd*, *J* = 15.6, 8.1 Hz, and 15.6, 9.0 Hz, respectively, 2H-1′), 1.30 and 1.27 (6H, *s*, Me-4′, 5′). These data were consistent with a hydroxyisopropyldihydrofurano moiety whose location was placed between C-2 and C-3 due to HMBC correlation of H-1′ (δ 3.22) with the carbon at δ 105.1 (C-2), of H-1′ (δ 3.15) with δ 164.6 (C-3). Based on these data, **AM8** was assigned as buxifoliadine-E previously isolated from *Severinia buxifolia* (Wu and Chen, 2000).

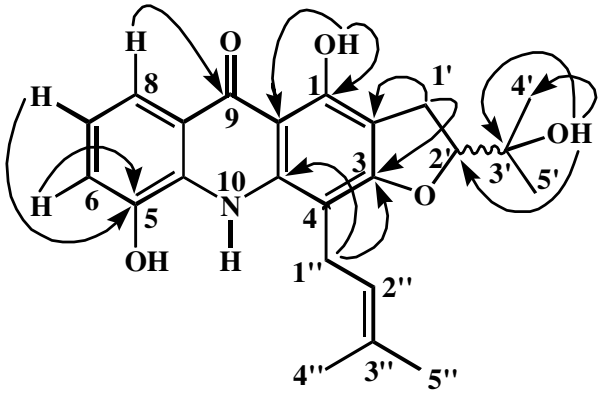


Figure 10 Selected HMBC correlation of **AM8**

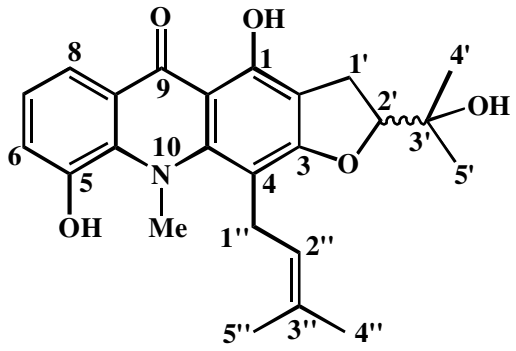
Table 12 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM8** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	157.0	C		
1-OH			14.50 (s)	C-9a, C-1, C-2
2	105.1	C		
3	164.6	C		
4	96.4	C		
5	144.6	C		
5-OH			10.01 (br s)	
6	115.5	CH	7.20 (br d, *J* = 7.8)	C-5a, C-8
7	121.0	CH	7.08 (t, *J* = 7.8)	C-5, C-8a
8	115.7	CH	7.74 (d, *J* = 7.8)	C-5a, C-9, C-6
9	181.0	C		
4a	140.6	C		
5a	130.8	C		
8a	120.1	C		
9a	104.9	C		
1′	26.7	CH_2	3.15 (dd, *J* = 15.6, 9.0)	C-3′, C-3
			3.22 (dd, *J* = 15.6, 8.1)	C-3′, C-2′, C-2

Table 12 (continued)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
2′	91.1	CH	4.80 (dd, *J* = 9.0, 8.1)	
3′-OH	70.7	C		
4′	25.0	CH_3	1.30 (s)	C-3′, C-2′
5′	25.0	CH_3	1.27 (s)	C-3′, C-2′
1″	22.5	CH_2	3.54 (br d, *J* = 7.2)	C-4, C-4a, C-3, C-2″, C-3″
2″	121.6	CH	5.21 (br t, *J* = 7.2)	C-4″, C-5″
3″	134.3	C		
4″	17.2	CH_3	1.97 (s)	C-2″, C-3″, C-5″
5″	24.5	CH_3	1.77 (s)	C-2″, C-3″,
10-NH			9.01 (s)	

3.1.9 Compound AM9



Compound **AM9** was isolated as yellow needles. The UV-Vis spectrum exhibited the absorption bands at 252, 276, 282, 327 and 395 nm characteristic of a 9-acridone chromophore which was confirmed by the presence of IR absorption maxima of hydroxyl (3374 cm^{-1}) and chelated carbonyl (1639 cm^{-1}) groups.

Its molecular formula $C_{24}H_{27}NO_5$ was suggested on the basis of HREIMS (*m/z* 409.1888). The ^1H and ^{13}C NMR spectral data (**Table 13**) of **AM9** were similar to those of **AM8**, except that an *N*-methyl signal (δ_H 3.73, δ_C 47.2) in **AM9** replaced an NH signal (δ_H 9.01) in **AM8**. A proton singlet signal of a phenolic

hydroxyl was displayed at δ 14.45. The location of a prenyl group at C-4 was confirmed by HMBC correlation of H-1″ (δ 3.53) with the carbons at δ 103.0 (C-4), 150.0 (C-4a) and C-3 (167.0). A hydroxyisopropyldihydrofurano moiety was placed between C-2 and C-3 due to HMBC correlation of H-1′ (δ 3.22) with the carbon at δ 106.7 (C-2), of H-1′ (δ 3.15) with δ 167.0 (C-3). Based on these data, **AM9** was identified as *N*-methyl derivative of buxifoliadine-E and named as *N*-methylbuxifoliadine-E, a new compound.

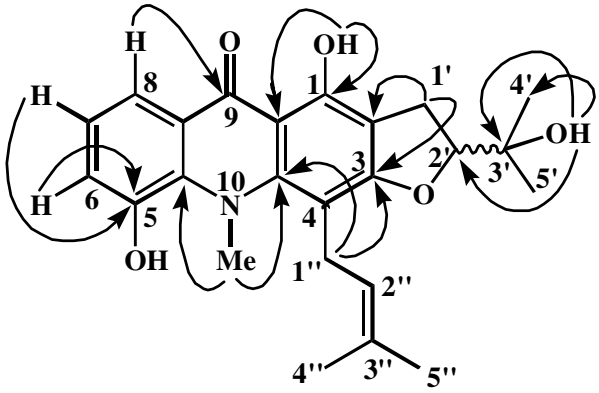


Figure 11 Selected HMBC correlation of **AM9**

Table 13 ^{1}H, ^{13}C NMR, HMBC and NOESY spectral data of compound **AM9** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**	**NOESY**
1	157.2	C			
1-OH			14.45 (s)	C-9a, C-1	
2	106.7	C			
3	167.0	C			
4	103.0	C			
5	148.4	C			
5-OH			9.32 (br s)		
6	119.4	CH	7.27 (dd, *J* = 7.8, 1.5)	C-5, C-5a, C-8	7
7	122.8	CH	7.17 (t, *J* = 7.8)	C-5, C-8a	6, 8
8	116.2	CH	7.77 (dd, *J* = 7.8, 1.5)	C-5a, C-9	7
9	182.3	C			
4a	150.0	C			
5a	138.0	C			
8a	125.0	C			
9a	107.0	C			
1′	26.7	CH_2	3.15 (dd, *J* = 15.6, 9.3)	C-3, C-2′, C-3′	2′
			3.22 (dd, *J* = 15.6, 7.5)	C-2	
2′	91.0	CH	4.82 (dd, *J* = 9.3, 7.5)		1′, 4′/5′
3′-OH	70.8	C	3.76 (s)	C-4′, C-5′, C-2′, C-3′	4′/5′
4′/5′	25.3	CH_3×2	1.28 (s)	C-3′, C-2′	2′, 3′-OH
1″	25.9	CH_2	3.53 (br d, *J* = 6.3)	C-4, C-4a, C-3, C-2″, C-3″	2″, 5″
2″	123.1	CH	5.39 (m)		1″, 4″, 10
3″	131.2	C			

Table 13 (continued)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**	**NOESY**
4″	17.2	CH_3	1.78 (s)	C-2″, C-3″, C-5″	2″
5″	24.9	CH_3	1.69 (s)	C-2″, C-3″, C-4″	1″
10-NMe	47.2	CH_3	3.73 (s)	C-4a, C-5a	2″

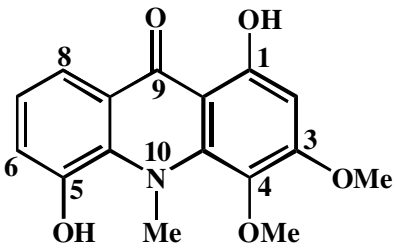
Table 14 Comparison of ^{1}H NMR spectral data between compounds **AM8**, **AM9** and Buxifoliadine-E (**R**, acetone-d_6)

Position	AM8 δ_H (mult, *J*, Hz)	AM9 δ_H (mult, *J*, Hz)	R δ_H (mult, *J*, Hz)
1-OH	14.50 (s)	14.45 (s)	14.49 (s)
2			
3			
4			
5-OH	10.01 (br s)	9.32 (br s)	9.82 (s)
6	7.20 (br d, *J* = 7.8)	7.27 (dd, *J* = 7.8, 1.5)	7.20 (dd, *J* = 8.0, 1.2)
7	7.08 (t, *J* = 7.8)	7.17 (t, *J* = 7.8)	7.08 (t, *J* = 8.0)
8	7.74 (d, *J* = 7.8)	7.77 (dd, *J* = 7.8, 1.5)	7.74 (dd, *J* = 8.0, 1.2)
9			
4a			
5a			
8a			
9a			
1′	3.15 (dd, *J* = 15.6, 9.0)	3.15 (dd, *J* = 15.6, 9.3)	3.16 (dd, *J* = 15.2, 9.2)
	3.22 (dd, *J* = 15.6, 8.1)	3.22 (dd, *J* = 15.6, 7.5)	3.21 (dd, *J* = 15.2, 8.0)
2′	4.80 (dd, *J* = 9.0, 8.1)	4.82 (dd, *J* = 9.3, 7.5)	4.79 (dd, *J* = 9.2, 8.0)
3′-OH		3.76 (s)	3.82 (s)
4′	1.30 (s)	1.28 (s)	1.28 (br s)
5′	1.27 (s)	1.28 (s)	1.25 (br s)
1″	3.54 (br d, *J* = 7.2)	3.53 (br d, *J* = 6.3)	3.55 (d, *J* = 6.8)
2″	5.21 (br t, *J* = 7.2)	5.39 (m)	5.21 (m)
3″			
4″	1.97 (s)	1.78 (s)	1.99 (br s)
5″	1.77 (s)	1.69 (s)	1.68 (br s)
10	9.01 (s)	3.73 (s)	9.02 (s)

Table 15 Comparison of ^{13}C NMR spectral data between compounds **AM8**, **AM9** and Buxifoliadine-E (**R**, acetone-d_6)

Position	δ_C, AM8	δ_C, AM9	δ_C, R
1-OH	157.0	157.2	158.1
2	105.1	106.7	108.2
3	164.6	167.0	165.5
4	96.4	103.0	97.3
5-OH	144.6	148.4	145.3
6	115.5	119.4	116.5
7	121.0	122.8	121.9
8	115.7	116.2	116.8
9	181.0	182.3	181.9
4a	140.6	150.0	141.5
5a	130.8	138.0	131.7
8a	120.1	125.0	121.1
9a	104.9	107.0	106.0
1′	26.7	26.7	27.7
2′	91.1	91.0	92.0
3′	70.7	70.8	71.6
4′	25.0	25.3	25.9
5′	25.0	25.3	29.5
1″	22.5	25.9	23.4
2″	121.6	123.1	122.5
3″	134.3	131.2	135.2
4″	17.2	17.2	18.1
5″	24.5	24.9	25.4
10-NMe		47.2	

3.1.10 Compound AM10



Compound **AM10** was obtained as orange needles**,** m.p. 206-207 °C. The UV-Vis spectrum exhibited the absorption bands at 203, 263, 319 and 416 nm characteristic of a 9-acridone chromophore. An infrared (IR) absorption maxima indicated the presence of hydroxyl (3386 cm^{-1}) and chelated carbonyl (1633 cm^{-1}) groups.

The ^{1}H-NMR spectrum showed a singlet signal at δ 14.22 indicating the presence of a chelated hydroxyl group. Three sharp singlets (each 3H) at δ 3.76, 3.83, and 3.98 were due to methoxyl, *N*-methyl and methoxyl groups, respectively. Signals of three adjacent aromatic protons at δ 7.78 (1H, *d*, J = 7.8 Hz), 7.30 (1H, *br d,* J = 7.8 Hz) and 7.16 (1H, *t,* J = 7.8 Hz) were assigned to H-8, H-6, and H-7, respectively. The deshielding of H-8 is reasonable because it lies in the *peri*-position with respect to the 9-carbonyl moiety. A sharp one-proton singlet signal at δ 6.41 could be attributed to an aromatic proton at C-2 which was confirmed by HMBC correlation of H-2 (δ 6.41) with the carbon at δ 105.9 (C-9a), 160.0 (C-3) and 130.3 (C-4). Two singlet signals at δ 3.76 and δ 3.98 (each 3H) were assigned for methoxyl group at C-3 and C-4 respectively due to HMBC correlations (**Figure 12**) of 3-OMe with the carbon at δ 160.0 (C-3) and 4-OMe with the carbon at δ 130.3 (C-4). NOESY cross peak of O-Me (δ 3.76) at C-4 with N-Me (δ 3.83) supported the assigned structure. On the basis of the above analysis, the structure of **AM10** was identified as citrusinine-I (Wu and Furukawa, 1983).

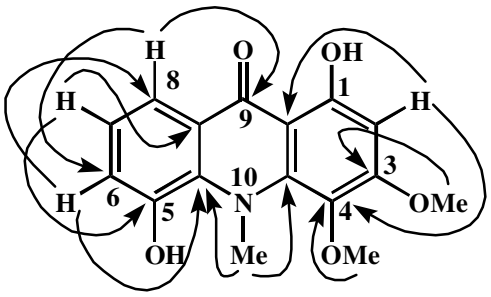


Figure 12 Selected HMBC correlation of **AM10**

Table 16 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM10** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	160.3	C		
1-OH			14.22 (s)	
2	93.7	CH	6.41 (s)	C-9a, C-4, C-3
3	160.0	C		
3-OMe	55.7	CH_3	3.98 (s)	C-3
4	130.3	C		
4-OMe	59.5	CH_3	3.76 (s)	C-4
5	148.0	C		
5-OH			9.42 (br s)	
6	119.9	CH	7.30 (br d, *J* = 7.8)	C-5a, C-8, C-5
7	122.5	CH	7.16 (t, *J* = 7.8)	C-5, C-8a
8	116.3	CH	7.78 (d, *J* = 7.8)	C-5a, C-9, C-6
9	182.2	C		
4a	142.2	C		
5a	137.4	C		
8a	124.5	C		
9a	105.9	C		
10-NMe	45.9	CH_3	3.83 (s)	C-4a, C-5a

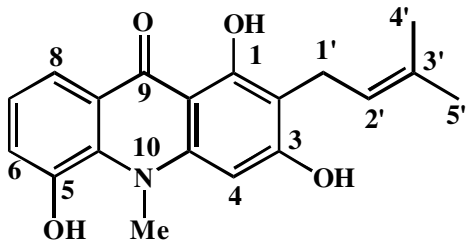
Table 17 Comparison of ^{1}H NMR spectral data between compounds **AM10** and citrusinine-I (**R**, DMSO-d_6+$CDCl_3$)

Position	**AM10** δ_H **(mult, *J*, Hz)**	**R** δ_H **(mult, *J*, Hz)**
1-OH	14.22 (s)	14.05 (s)
2	6.41 (s)	6.30 (s)
3		
3-OMe	3.98 (s)	3.92 (s)
4		
4-OMe	3.76 (s)	3.77 (s)
5-OH	9.42 (br s)	9.16 (br s)
6	7.30 (br d, *J* = 7.8)	7.19 (dd, *J* = 8.0, 2.0)
7	7.16 (t, *J* = 7.8)	7.04 (t, *J* = 8.0)
8	7.78 (d, *J* = 7.8)	7.68 (dd, *J* = 8.0, 2.0)
9		
4a		
5a		
8a		
9a		
10-NMe	3.83 (s)	3.71 (s)

Table 18 Comparison of ^{13}C NMR spectral data between compounds **AM10** and citrusinine-I (**R**, DMSO-d_6+$CDCl_3$)

Position	δ_C, AM10	δ_C, R
1-OH	160.3	159.9
2	93.7	93.4
3	160.0	159.4
3-OMe	55.7	55.9
4	130.3	129.7
4-OMe	59.5	59.9
5-OH	148.0	148.1
6	119.9	119.9
7	122.5	122.4
8	116.3	115.7
9	182.2	181.9
4a	142.2	141.8
5a	137.4	137.1
8a	124.5	124.1
9a	105.9	105.8
10-NMe	45.9	45.9

3.1.11 Compound AM11



Compound **AM11** was obtained as orange needles**,** m.p. 218-220 °C. The UV-Vis spectrum exhibited the absorption bands at 205, 265, 285, 305 and 407 nm characteristic of a 9-acridone chromophore. An infrared (IR) absorption maxima indicated the presence of hydroxyl (3380 cm^{-1}) and chelated carbonyl (1636 cm^{-1}) groups.

The ^{1}H and ^{13}C NMR spectral data of **AM11** were comparable with **AM1**, except that **AM1** has two prenyl groups attached at C-2 and C-4 but only one prenyl group at C-2 in **AM11.** The HMBC correlation of H-1′ at δ 3.38 with the carbons at δ 107.8 (C-2), 162.0 (C-1) and 162.3 (C-3) supported the connection of a prenyl group at C-2. An aromatic proton singlet signal was displayed at δ 6.50 which was assigned as H-4 due to its HMBC correlation to the carbons at δ 104.7 (C-9a), 107.8 (C-2), 140.8 (C-4a) and 162.3 (C-3). The complete HMBC data were summarized in **Table 19**. Based on these data, **AM11** was assigned as junosine (Auzi *et al*., 1996).

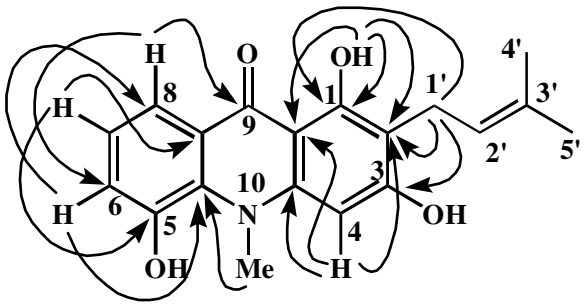
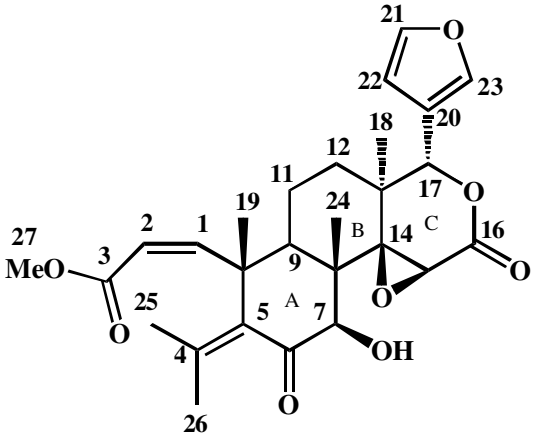


Figure 13 Selected HMBC correlation of **AM11**

Table 19 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM11** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	162.0	C		
1-OH			14.98 (s)	C-9a, C-2, C-1
2	107.8	C		
3	162.3	C		
4	90.5	CH	6.50 (s)	C-9a, C-2, C-3, C-4a
5	146.8	C		
6	119.5	CH	7.28 (dd, *J* = 7.8, 1.5)	C-8, C-5a, C-5
7	121.7	CH	7.12 (t, *J* = 7.8)	C-5, C-8a
8	116.9	CH	7.90 (dd, *J* = 7.8, 1.5)	C-5a, C-9, C-6
9	180.2	C		
4a	140.8	C		
5a	133.8	C		
8a	123.7	C		
9a	104.7	C		
1′	21.0	CH_2	3.38 (br d, *J* = 7.2)	C-1, C-2, C-2′, C-3, C-3′
2′	122.9	CH	5.52 (m)	C-4′, 5′
3′	130.2	C		
4′	17.0	CH_3	1.79 (s)	C-3′, C-2′
5′	25.0	CH_3	1.65 (s)	C-3′, C-2′
10-NMe	40.2	CH_3	4.02 (s)	C-5a, C-4, C-5

3.1.12 Compound AM12



Compound **AM12** was obtained as yellow crystals, m.p. 228-230 °C. The IR spectrum of compound **AM12** indicated the presence of hydroxyl at 3401 cm^{-1} and three carbonyl absorptions at 1742, 1717 and 1658 cm^{-1}, the last band being due to an α, β-unsaturated carbonyl.

The ^1H NMR spectrum (**Table 20**) suggested the presence of a β-substituted furan at δ 7.40 (1H, *br s*), 7.38 (1H, *br s*) and 6.37 (1H, *br s*). It was further established that compound **AM12** was a limonoid with five tertiary C-methyl groups resonating as singlets at δ 2.02, 1.78, 1.40, 1.34 and 0.66 and a COOMe as a singlet at δ 3.55. Two of the five C-methyl groups at δ 2.02 and 1.78 were ascribed to two methyl groups connecting to a double bond, suggesting a seco-limonoid. The presence of an epoxy lactone moiety was revealed by the characteristic H-15 and H-17 singlet signals at δ 4.22 and 5.49 respectively. The ^1H NMR also had two protons of a conjugated double bond absorbing as two AB doublets at δ 6.32 (1H, J = 12.3 Hz) and 5.72 (1H, J = 12.3 Hz). This large coupling constant (J > 10 Hz) for H-1 and H-2 combined with their chemical shifts showed that compound **AM12** belonged to the obacunone type (dreyer, *et al*., 1965) limonoids with seco-ring A of the methyl obacunoate rather than the gedunin-type (J ~ 10 Hz). This result was also supported by a HMBC experiment (**Figure 14**). Based on these data, the structure of atalantolide was assigned as **AM12** (Okorie *et al.*, 1982).

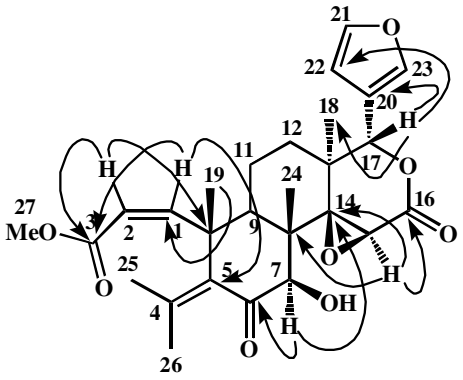


Figure 14 Selected HMBC correlation of **AM12**

Table 20 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM12** ($CDCl_3$)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
1	158.3	CH	6.32 (d, *J* = 12.3)	C-5, C-3, C-10
2	118.0	CH	5.72 (d, *J* = 12.3)	C-1, C-3, C-10
3	166.1	C		
4	152.6	C		
5	135.8	C		
6	201.0	C		
7	79.7	CH	4.81 (s)	C-8, C-24, C-14, C-6
8	45.0	C		
9	44.3	CH	3.36 (br d, *J* = 10.8)	C-10, C-11, C-12
10	45.2	C		
11	20.3	CH_2	1.68 (m)	C-9, C-10, C-12
12	32.5	CH_2	1.84 (br d, *J* = 6.9)	C-11, C-9
13	37.8	C		
14	67.3	C		
15	51.3	CH	4.22 (s)	C-8, C-14, C-16
16	167.6	C		

Table 20 (continued)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
17	78.2	CH	5.49 (s)	C-12, C-13, C-18, C-20, C-22
18	20.0	CH_3	1.34 (s)	C-12, C13, C-14, C-17
19	24.4	CH_3	1.40 (s)	C-10, C-5
20	120.5	C		
21	142.8	CH	7.38 (br s)	C-22, C-20, C-23
22	110.0	CH	6.37 (br s)	C-20, C-23
23	140.9	CH	7.40 (br s)	C-22, C-20, C-21
24	13.0	CH_3	0.66 (s)	C-7, C-8, C-14
25	29.1	CH_3	1.78 (s)	C-4, C-5, C-25
26	25.5	CH_3	2.02 (s)	C-4, C-5, C-24
27	51.4	CH_3	3.55 (s)	C-3

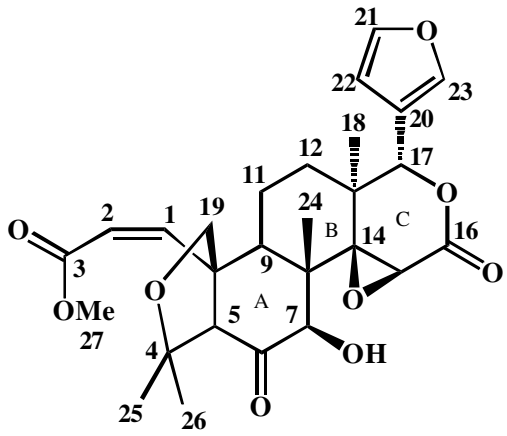
Table 21 Comparison of ^{1}H NMR spectral data between compounds **AM12** and atalantolide (**R**, $CDCl_3$)

Position	δ_H (mult, *J*, Hz), AM12	δ_H (mult, *J*, Hz), R
1	6.32 (d, *J* = 12.3)	6.37 (d, *J* = 12.4)
2	5.72 (d, *J* = 12.3)	5.75 (d, *J* = 12.4)
3		
4		
5		
6		
7	4.81 (s)	4.83 (d, *J* = 2.9)
8		
9	3.36 (br d, *J* = 10.8)	3.39 (m)
10		
11	1.68 (m)	1.63 (m)
12	1.84 (br d, *J* = 6.9)	1.81 (m)
13		
14		
15	4.22 (s)	4.24 (s)
16		
17	5.49 (s)	5.51 (s)
18	1.34 (s)	1.36 (s)
19	1.40 (s)	1.41 (s)
20		
21	7.38 (br s)	7.40 (m)
22	6.37 (br s)	6.39 (dd, *J* = 1.8, 1.0)
23	7.40 (br s)	7.40 (m)
24	0.66 (s)	0.68 (s)
25	1.78 (s)	1.77 (s)
26	2.02 (s)	2.04 (s)
27	3.55 (s)	3.57 (s)

Table 22 Comparison of ^{13}C NMR spectral data between compounds **AM12** and atalantolide (**R**, $CDCl_3$)

Position	δ_C, AM12	δ_C, R
1	158.3	158.4
2	118.0	118.0
3	166.1	166.3
4	152.6	152.8
5	135.8	135.8
6	201.0	201.1
7	79.7	79.8
8	45.0	45.0
9	44.3	44.3
10	45.2	45.2
11	20.3	20.3
12	32.5	32.4
13	37.8	37.9
14	67.3	67.4
15	51.3	51.3
16	167.6	167.7
17	78.2	78.3
18	20.0	20.0
19	24.4	24.4
20	120.5	120.5
21	142.8	141.0
22	110.0	110.0
23	140.9	142.9
24	13.0	13.0
25	29.1	29.1
26	25.5	25.6
27	51.4	51.4

3.1.13 Compound AM13



Compound **AM13** was obtained as yellow crystals, mp 182-184 °C. The IR spectrum of compound **AM13** indicated the presence of hydroxyl at 3396 cm^{-1} and two carbonyl bands at 1739 and 1709 cm^{-1}.

Compound **AM13**, the second limonoid isolated, has spectroscopic properties similar to those of atalantolide, compound **AM12** (**Table 23**). Immediately recognizable are the β-substituted furan, H-17, H-15, α, β-unsaturated methyl ester, H-9 (δ 3.24) and a singlet signal at δ 4.63 attributable to H-7. The appearance of four tertiary methyl ^{1}H NMR signals and two doublets (δ 3.78 and 4.13, J = 10.0 Hz, 2H-19) suggested a carbon skeleton related to that of a limonin with an ether bridge from C-19 to C-4. This arrangement was further supported by a one-proton singlet at δ 3.01, attributable to H-5. Consideration of the chemical shift of H-15 (δ 4.19) and H-17 (δ 5.43) in compound **AM13** and comparison with the data for atalantolide (compound **AM12**) also suggested an epoxy lactone moiety. The ^{13}C NMR spectrum of atalantin (see **Table 23**) was in accord with this assignment. The ^{13}C NMR signals at δ 67.8 (C-14), 52.1 (C-15), 166.9 (C-16) and 77.7 (C-17) confirmed the presence of a ring D epoxy lactone system. This result was also supported by a HMBC experiment (**Figure 15**). Based on these data, the structure of **AM13** was assigned as atalantin (Sabata *et al*., 1977).

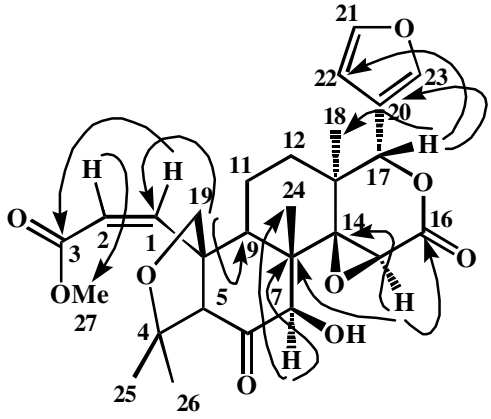


Figure 15 Selected HMBC correlation of **AM13**

Table 23 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM13** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	163.4	CH	6.74 (d, *J* = 12.5)	C-3
2	119.5	CH	5.89 (d, *J* = 12.5)	C-10, C-27
3	166.2	C		
4	83.8	C		
5	65.0	CH	3.01 (br s)	C-4, C-19, C-25
6	208.8	C		
7	80.0	CH	4.63 (s)	C-8, C-14, C-24
8	43.7	C		
9	41.0	CH	3.24 (m)	C-5, C-8, C-11, C-12
10	54.0	C		
11	20.3	CH_2	1.85 (m), 1.74 (m)	C-8, C-9, C-12
12	31.0	CH_2	1.88 (m), 1.33 (m)	C-11, C-13, C-18
13	37.8	C		
14	67.8	C		
15	52.1	CH	4.39 (s)	C-8, C-14, C-16
16	166.9	C		
17	77.7	CH	5.43 (s)	C-18, C-20, C-22

Table 23 (continued)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
18	19.5	CH_3	1.17 (s)	C-12, C-13, C-17
19	74.4	CH_2	4.13, 3.78, (d, *J* = 10.0)	C-1, C-5, C-9
20	120.7	C		
21	143.1	CH	7.48 (br s)	C-20, C-22, C-23
22	110.1	CH	6.42 (br s)	C-20, C-23
23	141.4	CH	7.54 (br s)	C-20, C-21, C-22
24	11.3	CH_3	0.81 (s)	C-8, C-9, C-14
25	29.9	CH_3	1.14 (s)	C-4, C-5, C-26
26	24.6	CH_3	1.20 (s)	C-4, C-5, C-25
27	51.2	CH_3	3.59 (s)	C-2, C-3

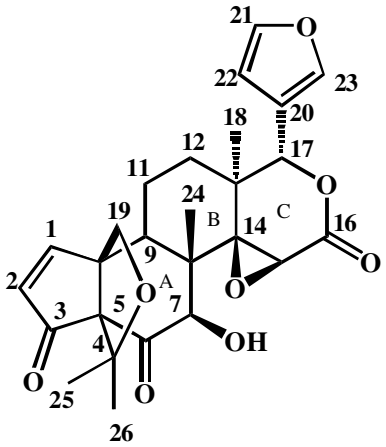
Table 24 Comparison of ^{1}H NMR spectral data between compounds **AM13** and atalantin (**R**, $CDCl_3$)

Position	δ_H (mult, *J*, Hz), AM13	δ_H (mult, *J*, Hz), R
1	6.74 (d, *J* = 12.5)	6.62 (d, *J* = 12.0)
2	5.89 (d, *J* = 12.5)	5.90 (d, *J* = 12.0)
3		
4		
5	3.01 (s)	3.11 (br s)
6		
7	4.63 (s)	4.77 (br s)
8		
9	3.24 (m)	3.34 (m)
10		
11	1.85 (m), 1.74 (m)	1.68 (m)
12	1.88 (m), 1.33 (m)	1.83 (m)
13		
14		
15	4.39 (s)	4.44 (s)
16		
17	5.43 (s)	5.53 (s)
18	1.17 (s)	1.24 (s)
19	4.13, 3.78, (each d, *J* = 10.0)	4.17, 3.79, (each d, *J* = 9.5)
20		
21	7.48 (br s)	7.41 (m)
22	6.42 (br s)	6.36 (m)
23	7.54 (br s)	7.41 (m)
24	0.81 (s)	0.89 (s)
25	1.14 (s)	1.30 (s)
26	1.20 (s)	1.36 (s)
27	3.59 (s)	3.57 (s)

Table 25 Comparison of ^{13}C NMR spectral data between compounds **AM13** and atalantin (**R**, $CDCl_3$)

Position	δ_C, AM13	δ_C, R
1	163.4	163.3
2	119.5	120.1
3	166.2	165.9
4	83.8	84.4
5	65.0	64.5
6	208.8	209.1
7	80.0	80.0
8	43.7	43.9
9	41.0	40.2
10	54.0	52.7
11	20.3	20.4
12	31.0	30.6
13	37.8	38.1
14	67.8	68.5
15	52.1	52.9
16	166.9	167.7
17	77.7	78.1
18	19.5	19.7
19	74.4	75.0
20	120.7	120.5
21	143.1	141.1
22	110.1	110.0
23	141.4	143.0
24	11.3	12.5
25	29.9	31.3
26	24.6	25.0
27	51.2	51.9

3.1.14 Compound AM14



Compound **AM14** was obtained as yellow crystals, m.p. 308-310 °C. The IR spectrum of compound **AM14** indicated the presence of a hydroxyl at 3390 cm^{-1} and two carbonyl absorptions at 1733 and 1693 cm^{-1}.

The ^{1}H and ^{13}C NMR spectral data of **AM14** were comparable with **AM13**, except that **AM13** had an *α, β*-unsaturated methyl ester group attached at C-10 but compound **AM14**, had a cyclopent-2-enone ring. The ^{1}H NMR signals of an *α, β*-unsaturated methyl ester group in **AM13** shown as two doublets at δ 6.74 and 5.89 (J = 12.5 Hz) and O-Me at δ 3.59 were replaced by signals of a cyclopent-2-enone ring in **AM14** which appeared as two doublets at δ 7.99 and 6.15 (J = 5.7 Hz) and no evidence of O-Me singlet signal. Besides a proton singlet signal at δ 3.01 (H-5) as shown in **AM13** was not shown in **AM14**. The ^{13}C NMR ester signal in **AM13** at δ 166.2 was replaced by a carbonyl signal at δ 200.6 in **AM14**. Compound **AM14** could be formed from cyclization of **AM13**. This result was also supported by a HMBC experiment (**Figure 15**). Based on these data, the structure of **AM14** was assigned as cycloepiatalantin (dreyer *et al.,* 1976).

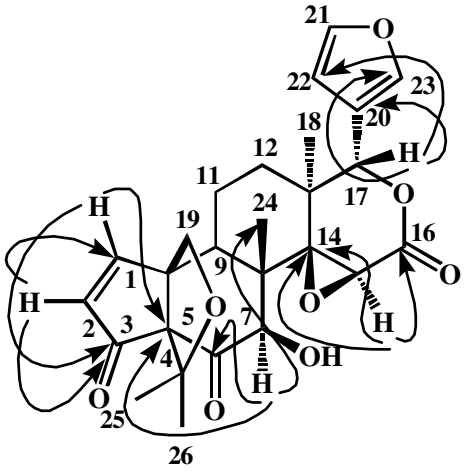


Figure 16 Selected HMBC correlation of **AM14**

Table 26 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM14** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	169.4	CH	7.99 (d, *J* = 5.7)	C-2, C-3, C-5, C-9
2	129.4	CH	6.15 (d, *J* = 5.7)	C-10, C-27
3	200.6	C		
4	84.8	C		
5	71.9	C		
6	200.0	C		
7	76.2	CH	3.39 (s)	C-8, C-14, C-24
8	43.3	C		
9	33.0	CH	3.02 (q, *J* = 6.6)	C-5, C-8, C-11, C-12
10	61.0	C		
11	15.9	CH_2	2.27 (m), 1.93 (m)	C-8, C-9, C-12
12	25.7	CH_2	1.88 (m), 1.50 (m)	C-11, C-13, C-18
13	38.0	C		
14	69.1	C		
15	56.9	CH	3.86 (s)	C-8, C-14, C-16

Table 26 (continued)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
16	166.9	C		
17	77.7	CH	5.60 (s)	C-18, C-20, C-22
18	17.3	CH_3	1.22 (s)	C-12, C-13, C-17
19	69.5	CH_2	4.02, 3.89 (eachd, J = 9.6)	C-1, C-5, C-9
20	120.9	C		
21	143.2	CH	7.59 (br s)	C-20, C-22, C-23
22	110.0	CH	6.50 (br s)	C-20, C-23
23	141.6	CH	7.68 (br s)	C-20, C-21, C-22
24	15.1	CH_3	1.10 (s)	C-8, C-9, C-14
25	28.3	CH_3	1.13 (s)	C-4, C-5, C-26
26	24.5	CH_3	1.31 (s)	C-4, C-5, C-25

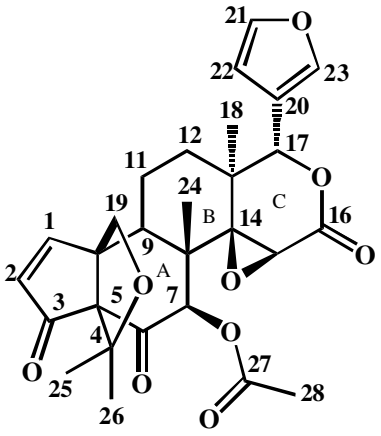
Table 27 Comparison of ^{1}H NMR spectral data between compounds **AM14** and cycloepiatalantin (**R**, $CDCl_3$)

Position	δ_H (mult, *J*, Hz), AM14	δ_H (mult, *J*, Hz), R
1	7.99 (d, *J* = 5.7)	7.72 (d, *J* = 6.0)
2	6.15 (d, *J* = 5.7)	6.24 (d, *J* = 6.0)
3		
4		
5		
6		
7	3.39 (s)	3.46 (s)
8		
9	3.02 (q, *J* = 6.6)	3.02 (m)
10		
11	2.27 (m), 1.93 (m)	2.18 (m)
12	1.88 (m), 1.50 (m)	1.85 (m)
13		
14		
15	3.86 (s)	3.89 (s)
16		
17	5.60 (s)	5.58 (s)
18	1.22 (s)	1.24 (s)
19	4.02, 3.89 (each d, *J* = 9.6)	4.01, 3.86 (each d, *J* = 10.0)
20		
21	7.59 (br s)	7.42 (t, *J* = 1.0)
22	6.50 (br s)	6.33 (d, *J* = 1.0)
23	7.68 (br s)	7.45 (m)
24	1.10 (s)	1.10 (s)
25	1.13 (s)	1.20 (s)
26	1.31 (s)	1.36 (s)

Table 28 Comparison of ^{13}C NMR spectral data between compounds **AM14** and cycloepiatalantin (**R**, $CDCl_3$)

Position	δ_C, AM14	δ_C, R
1	169.4	169.5
2	129.4	129.8
3	200.6	201.3
4	84.8	85.2
5	71.9	71.9
6	200.0	200.3
7	76.2	75.9
8	43.3	43.1
9	33.0	32.8
10	61.0	60.9
11	15.9	16.2
12	25.7	25.5
13	38.0	37.9
14	69.1	69.3
15	56.9	56.9
16	166.9	167.4
17	77.7	77.8
18	17.3	17.7
19	69.5	69.6
20	120.9	120.6
21	143.2	143.3
22	110.0	110.1
23	141.6	141.5
24	15.1	15.5
25	28.3	28.8
26	24.5	25.1

3.1.15 Compound AM15



Compound **AM15** was obtained as yellow crystals, m.p. 115-117 $^{\circ}$C. The IR spectrum of compound **AM15** indicated the presence of a two carbonyl bands at 1736 and 1693 cm^{-1}.

The ^{1}H and ^{13}C NMR spectra of compound **AM15** (**Table 29**) were closely related to those of **AM14**, except that the hydroxyl group at C-7 in **AM14** was replaced by an acetate group in **AM15** shown as a methyl singlet at δ 1.91 and carbons signals at δ 19.7 (C-28) and 167.6 (C-27). The carbinol resonance (H-7) previously observed at δ 3.39 in **AM14** was shifted downfield to δ 4.59 in compound **AM15.** An acetate group was placed at C-7 due to HMBC correlation of H-7 (δ 4.59) with the carbons at δ 167.6 (C-27) and protons of methyl acetate (δ 1.91, 3H-28,) with the carbons at δ 76.1 (C-7) (**Table 29**). Based on these data, the structure of **AM15** was assigned as cycloepiatalantin acetate (dreyer *et al.,* 1976).

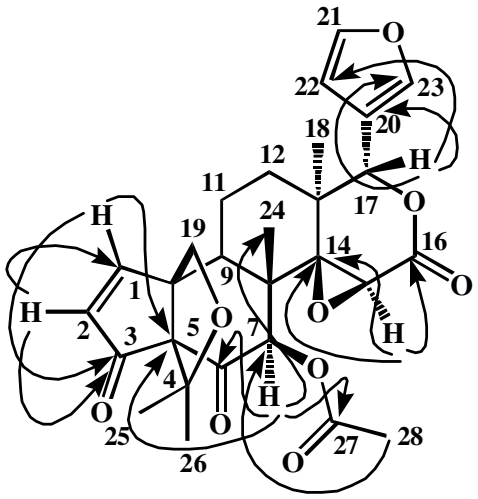


Figure 17 Selected HMBC correlation of **AM15**

Table 29 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM15** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1	170.5	CH	8.13 (d, *J* = 5.4)	C-2, C-3, C-5, C-9
2	129.3	CH	6.27 (d, *J* = 5.4)	C-10, C-27
3	200.6	C		
4	84.9	C		
5	73.0	C		
6	195.3	C		
7	76.1	CH	4.59 (s)	C-8, C-14, C-24, C-27
8	43.0	C		
9	34.4	CH	3.01 (m)	C-5, C-8, C-11, C-12
10	61.3	C		
11	15.9	CH_2	2.34 (m), 2.05 (m)	C-8, C-9, C-12
12	25.4	CH_2	1.96 (m), 1.57 (m)	C-11, C-13, C-18
13	38.3	C		
14	68.6	C		

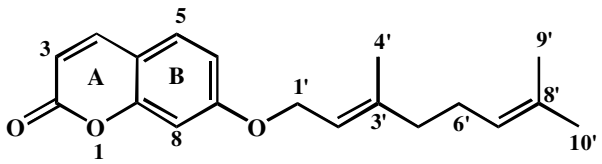
Table 29 (continued)

Position	δ_C		δ_H (mult, *J*, Hz)	HMBC
15	56.1	CH	3.67 (s)	C-8, C-14, C-16
16	166.2	C		
17	77.7	CH	5.64 (s)	C-18, C-20, C-22
18	17.6	CH_3	1.21 (s)	C-12, C-13, C-17
19	69.4	CH_2	4.05, 3.96 (each d, J = 9.9)	C-1, C-5, C-9
20	120.6	C		
21	143.3	CH	7.59 (br s)	C-20, C-22, C-23
22	110.0	CH	6.49 (br s)	C-20, C-23
23	141.7	CH	7.62 (br s)	C-20, C-21, C-22
24	14.8	CH_3	1.11 (s)	C-8, C-9, C-14
25	27.9	CH_3	1.13 (s)	C-4, C-5, C-26
26	24.3	CH_3	1.33 (s)	C-4, C-5, C-25
27	167.6	C		
28	19.7	CH_3	1.91 (s)	C-7

Table 30 Comparison of ^{1}H NMR spectral data between compounds **AM15** and cycloepiatalantin acetate (**R**, $CDCl_3$)

Position	δ_H (mult, *J*, Hz), AM15	δ_H (mult, *J*, Hz), R
1	8.13 (d, *J* = 5.4)	7.76 (d, *J* = 6.0)
2	6.27 (d, *J* = 5.4)	6.26 (d, *J* = 6.0)
3		
4		
5		
6		
7	4.59 (s)	4.62 (s)
8		
9	3.01 (m)	2.91 (m)
10		
11	2.34 (m), 2.05 (m)	2.21 (m)
12	1.96 (m), 1.57 (m)	1.85 (m)
13		
14		
15	3.67 (s)	3.67 (s)
16		
17	5.64 (s)	5.58 (s)
18	1.21 (s)	1.23 (s)
19	4.05, 3.96 (each d, *J* = 9.9)	4.00, 3.88 (each d, *J* = 10.0)
20		
21	7.59 (br s)	7.42 (t, *J* = 1.0)
22	6.49 (br s)	6.30 (d, *J* = 1.0)
23	7.62 (br s)	7.45 (m)
24	1.11 (s)	1.15 (s)
25	1.13 (s)	1.17 (s)
26	1.33 (s)	1.38 (s)
27		
28	1.91 (s)	1.94 (s)

3.1.16 Compound AM16



Compound **AM16** was obtained as white crystals, m.p. 71-73 °C. The UV-Vis spectrum exhibited the absorption bands at 205, 252 and 323 nm typical of a coumarin nucleus. The IR absorption indicated the presence of a carbonyl (1710 cm^{-1}) group.

The ^1H NMR spectrum of compound **AM16** showed two AB systems of ring A at δ 7.64, 6.25 (1H each, *d, J* = 8.0 Hz, H-4, H-3, respectively) and three aromatic proton signals of ring B, ABM pattern at δ 7.37 (1H, *d*, *J* = 8.0 Hz), 6.85 (1H, *dd*, *J* = 8.0, 3.0 Hz) and 6.82 (1H, *d*, *J* = 3.0 Hz) attributing to H-5, H-6, and H-8, respectively, which were characteristic of the 7-substituted coumarin skeleton. The substituent was identified by ^1H NMR spectroscopy as the oxy-geranyl group according to these signals at δ 5.47 (1H, br t, *J* = 6.6 Hz, H-2′), 5.10 (1H, m, H-7′), 4.61 (1H, br d, *J* = 6.6 Hz, H-1′), 2.11 (4H, m, 2H-5′ and 2H-6′), 1.76 (3H, s, H-9′), 1.66 (3H, s, H-10′) and 1.60 (3H, s, H-4′). The oxy-geranyl side chain was placed at C-7 due to HMBC correlation of H-1′ (δ 4.61) with the carbon at δ 162.5 (C-7). The assignment was also supported by a HMBC experiment (**Table 31**). Based on these data, the structure of **AM16** was assigned as auraptene (Muñoz *et al.,* 1982, Jiménez *et al*., 2000).

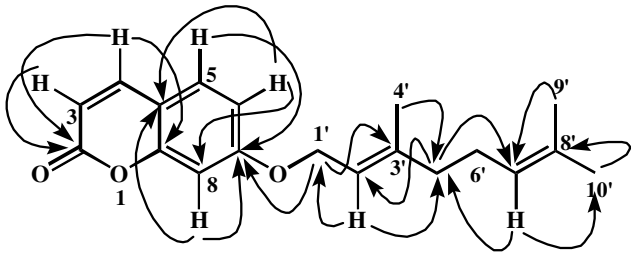


Figure 18 Selected HMBC correlation of **AM16**

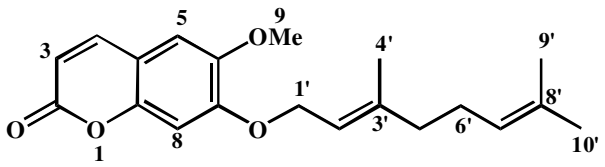
Table 31 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM16** ($CDCl_3$)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
2	161.0	C		
3	113.5	CH	6.25 (d, *J* = 8.0)	C-2, C-4a
4	143.5	CH	7.64 (d, *J* = 8.0)	C-2, C-4a, C-8a
5	129.6	CH	7.37 (d, *J* = 8.0)	C-6, C-7
6	112.0	CH	6.85 (dd, *J* = 8.0, 3.0)	C-4a, C-7, C-8
7	162.5	C		
8	101.5	CH	6.82 (d, *J* = 3.0)	C-4a, C-6, C-7, C-8a
4a	112.5	C		
8a	156.0	C		
1′	65.5	CH_2	4.61 (br d, *J* = 6.6)	C-7, C-2′, C-3′
2′	118.6	CH	5.47 (br t, *J* = 6.6)	C-1′, C-5′
3′	142.5	C		
4′	18.8	CH_3	1.60 (s)	C-2′, C-3′, C-5′
5′	39.0	CH_2	2.11 (m)	C-2′, C-3′, C-7′,
6′	26.5	CH_2	2.11 (m)	C-3′, C-5′, C-8′
7′	124.0	CH	5.10 (m)	C-5′, C-6′, C-10′
8′	132.1	C		
9′	17.5	CH_3	1.76 (s)	C-7′, C-8′, C-10′
10′	25.5	CH_3	1.66 (s)	C-7′, C-8′, C-9′

Table 32 Comparison of ^{1}H NMR spectral data between compounds **AM16** and auraptene (**R**, $CDCl_3$)

Position	AM16 δ_H (mult, *J*, Hz)	R δ_H (mult, *J*, Hz)
2		
3	6.25 (d, *J* = 9.0)	6.25 (d, *J* = 10.0)
4	7.64 (d, *J* = 9.0)	7.65 (d, *J* = 10.0)
5	7.37 (d, *J* = 8.0)	7.43 (d, *J* = 7.0)
6	6.85 (dd, *J* = 8.0, 3.0)	6.87 (dd, *J* = 7.0, 3.0)
7		
8	6.82 (d, *J* = 3.0)	6.82 (d, J = 3.0)
4a		
8a		
1′	4.61 (br d, *J* = 6.6)	4.63 (d, *J* = 7.0)
2′	5.47 (br t, *J* = 6.6)	5.50 (t, *J* = 7.0)
3′		
4′	1.60 (s)	1.80 (s)
5′	2.11 (m)	2.20 (m)
6′	2.11 (m)	2.20 (m)
7′	5.10 (m)	5.10 (m)
8′		
9′	1.76 (s)	1.75 (s)
10′	1.66 (s)	1.65 (s)

3.1.17 Compound AM17



Compound **AM17** was obtained as white solid, m.p. 86-88 °C. The UV-Vis spectrum exhibited the absorption bands at 206, 229, 253, 294 and 345 nm typical of a coumarin nucleus. The IR absorption indicated the presence of a carbonyl (1725 cm^{-1}) group.

The ^{1}H and ^{13}C NMR spectral data of **AM17** (**Table 33**) were similar to those of **AM16** (**Table 33**) except for the disappearance of the signal of the aromatic proton at δ 6.85 (1H, *dd*, 8.0, 3.0 Hz) and the appearance of O-Me singlet signal at δ 3.86 indicating that this aromatic proton was replaced by a methoxyl group. The location of the methoxyl group at C-6 was assigned by HMBC correlations (**Figure 18**) of the methoxyl protons at δ_H 3.86 (3H-9) to the carbons at δ_C 146.8 (C-6) and 109.0 (C-5). The complete HMBC data were summarized in **Table 33**. Therefore, compound **AM17** was identified as 7-*O*-geranylscopoletin (Rubal *et al*., 2007, Torres, *et al*., 1979).

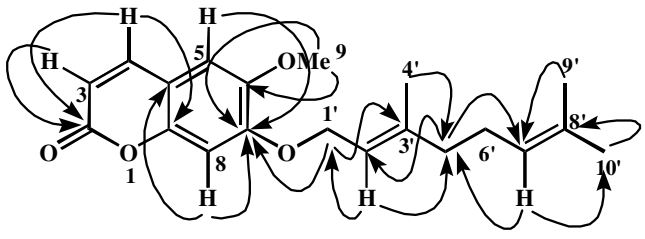


Figure 19 Selected HMBC correlation of **AM17**

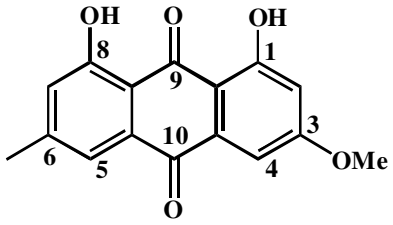
Table 33 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM17** (acetone-d_6)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
2	160.4	C		
3	112.8	CH	6.21 (d, *J* = 9.3)	C-2, C-4a
4	143.7	CH	7.87 (d, *J* = 9.3)	C-2, C-5, C-8a
5	109.0	CH	7.18 (s)	C-4, C-6, C-7, C-8a
6	146.8	C		
7	152.3	C		
8	101.0	CH	6.94 (s)	C-4a, C-6, C-7, C-8a
9	55.6	CH_3	3.86 (s)	C-5, C-6
4a	111.3	C		
8a	149.9	C		
1′	65.7	CH_2	4.73 (br d, *J* = 6.6)	C-7, C-2′, C-3′
2′	119.2	CH	5.52 (br t, *J* = 6.6)	C-1′, C-5′, C-4′
3′	141.2	C		
4′	15.8	CH_3	1.80 (s)	C-2′, C-3′, C-5′
5′	39.2	CH_2	2.13 (m)	C-2′, C-3′, C-7′,
6′	26.0	CH_2	2.13 (m)	C-3′, C-5′, C-8′
7′	123.7	CH	5.11 (m)	C-6′
8′	131.2	C		
9′	16.8	CH_3	1.60 (s)	C-7′, C-8′, C-10′
10′	24.6	CH_3	1.63 (s)	C-7′, C-8′, C-9′

Table 34 Comparison of ^{1}H NMR spectral data between compounds **AM17** and 7-*O*-geranylscopoletin (**R**, $CDCl_3$)

Position	AM17 δ_H (mult, *J*, Hz)	R δ_H (mult, *J*, Hz)
2		
3	6.21 (d, *J* = 9.3)	6.26 (d, *J* = 9.0)
4	7.87 (d, *J* = 9.3)	7.63 (d, *J* = 9.5)
5	7.18 (s)	6.85 (s)
6		
7		
8	6.94 (s)	6.82 (s)
9	3.86 (s)	3.83 (s)
4a		
8a		
1′	4.73 (br d, *J* = 6.6)	4.71 (d, *J* = 6.5)
2′	5.52 (br t, *J* = 6.6)	5.47 (t, *J* = 6.5)
3′		
4′	1.80 (s)	1.76 (d, *J* = 1.0)
5′	2.13 (m)	2.20 (m)
6′	2.13 (m)	2.20 (m)
7′	5.11 (m)	5.10 (m)
8′		
9′	1.60 (s)	1.61 (d, *J* = 1.0)
10′	1.63 (s)	1.65 (d, *J* = 1.0)

3.1.18 Compound AM18



Compound **AM18** was isolated as an yellow crystals. The UV-Vis spectrum exhibited the absorption bands at 221, 252, 264, 285 and 434 nm, characteristic of a conjugated quinone system, which was supported by IR absorption maxima indicating the presence of hydroxyl (3380 cm^{-1}) and a chelated carbonyl (1646 cm^{-1}) groups.

The ^1H NMR spectral data of **AM18** (**Table 35**) showed two chelated hydroxyl groups at δ 12.31 and 12.10, which were assigned to carbons at C-1 and C-8 from HMBC experiment (**Table 35**). The appearance of two broad singlet aromatic protons at δ_H 7.60 and 7.07 were attributed to *meta* splitting of H-5 and H-7 and long range coupling with an aromatic methyl protons at δ_H 2.45 (3H, *s*, Me-6). The COSY cross-peaks were shown between H-5/H-7 and Me-6 (**Table 35**). The lower-field aromatic proton at δ_H 7.60 was assigned to H-5 due to its location in the deshielding region of carbonyl functionality. The ^1H NMR spectral data also showed two signals of *meta*-coupled aromatic protons at δ_H 7.35 (1H, *d*, 2.4 Hz) and 6.67 (1H, *d*, 2.4 Hz) and the lower-field aromatic proton was assigned to H-4 due to the anisotropic effect from a carbonyl group. Moreover, the ^1H NMR spectral data (**Table 35**) showed a singlet signal of a methoxyl group at δ 3.88 (3H, *s*, 3-OMe), whose location at C-3 was assigned by its HMBC correlation (**Figure 19**) to a carbon at δ 166.6 (C-3). The complete HMBC data were summarized in **Table 35**. Therefore, compound **AM18** was identified as physcion (Chu, 2005).

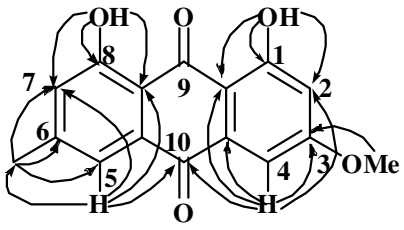
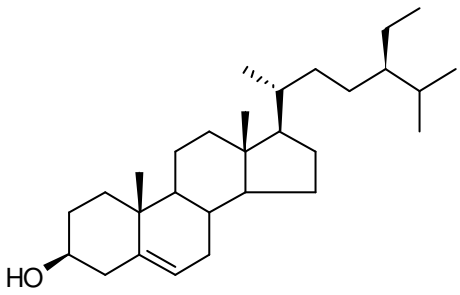


Figure 20 Selected HMBC correlations of **AM18**

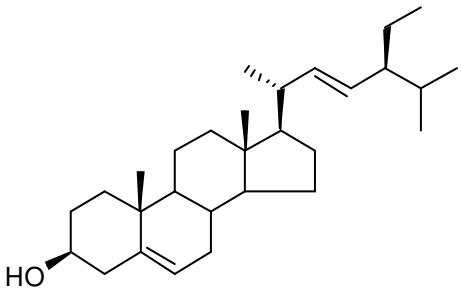
Table 35 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **AM18** ($CDCl_3$)

Position	**δ_C**		**δ_H (mult, *J*, Hz)**	**HMBC**
1-OH	165.2	C	12.31 (s)	C-1, C-2, C-9a
2	106.8	CH	6.67 (d, *J* = 2.4)	C-1, C-3, C-4, C-9a
3	166.6	C		
4	108.2	CH	7.35 (d, *J* = 2.4)	C-2, C-3, C-10,C-4a, C-9a
5	121.3	CH	7.60 (br d, *J* = 1.5)	C-7, C-10, C-8a, 6-Me
6	148.4	C		
7	124.5	CH	7.07 (br s)	C-5, C-8, C-8a, 6-Me
8-OH	162.5	C	12.10 (s)	C-6, C-7, C-8, C-8a
9	190.8	C		
10	182.0	C		
4a	135.3	C		
5a	133.2	C		
8a	113.7	C		
9a	110.3	C		
3-OMe	56.1	CH_3	3.88 (s)	C-3
6-Me	22.2	CH_3	2.45 (br s)	C-5, C-6, C-7

3.1.19 Compounds AM19 and AM20



AM19



AM20

The mixture of **AM19** and **AM20** was obtained as colorless crystals. The ^{1}H NMR spectra showed an oxymethine proton at δ 3.57-3.47 (*m*) and three olefinic protons at δ 5.36-5.34 (*d*, *J* = 5.1 Hz), 5.16 (*dd*, *J* = 8.4, 15.1 Hz) and 5.01 (*dd*, *J* = 8.4, 15.1 Hz). The ^{1}H NMR spectral data of this compound corresponded to a previous reported data (Thongdeeying 2005). Thus, the mixture was identified as β-sitosterol (**AM19**) and stigmasterol (**AM20**).

3.2 Bioactivities of isolated compounds from the roots of *A. monophylla*

In this research, several compounds belonging to acridone alkaloids, limonoids and coumarins groups have been isolated. This plant has been reported to exhibit several biological activities (Panda 2004). However, only anti-allergic, antibacterial and cytotoxic activities were chosen according to positive activity of the crude extracts.

3.2.1 Anti-allergic activity

The results were shown in **Table 36**. Of all metabolites evaluated, buxifoliadine-E (**AM8**) possessed the most potent anti-allergic activity against cell degranulation in RBL-2H3 cells with an IC_{50} value of 6.1 μM, followed by citrusinine-I (**AM10**, IC_{50} = 18.7 μM), whereas other compounds displayed moderate effects (IC_{50} = 34.0-40.1 μM) or inactive (IC_{50} >100 μM). Buxifoliadine-E (**AM8**, IC_{50} = 6.1 μM) displayed six-fold higher effect than ketotifen fumarate (IC_{50} = 47.5 μM), a clinically used drug. The compounds were also tested on β-hexosaminidase activity to clarify whether their effects were due to the inhibition of enzyme activity or of degranulation. As a result, these isolated compounds were inactive against the enzyme activity of β-hexosaminidase (**Table 36**).

Table 36 Anti-allergic activities of compounds (**AM1**, **AM2**, **AM5**, **AM7**, **AM8**, **AM10**, **AM12**, **AM16**-**AM18**) from the roots of *A. monophylla*

Compounds	**IC_{50} (μM)**	**Enzyme inhibition at 100 μM**
N-methylataphylline (**AM1**)	>100	22.5
atalaphylline (**AM2**)	>100	19.6
N-methylcycloatalaphylline-A (**AM5**)	40.1	23.4
N-methylataphyllinine (**AM7**)	>100	18.9
buxifoliadine-E (**AM8**)	6.1	21.3
citrusinine-I (**AM10**)	18.7	19.9
atalantolide (**AM12**)	35.1	22.1
auraptene (**AM16**)	73.2	18.2
7-*O*-geranylscopoletin (**AM17**)	>100	21.7
physcion (**AM18**)	34.0	18.0
ketotifen fumarate	47.5	15.8

Each value represents mean ± S.E.M. of four determinations.

3.2.2 Antibacterial activity

The results of antibacterial activity of the tested compound were given in **Table 37**. Only compound **AM7** exhibited significant antibacterial activity against *B. subtilis* and *S. aureus* whereas compound **AM1** was moderately active against *S. aureus*. Compounds **AM2**, **AM9**, **AM12**-**AM16**, and **AM18** were inactive against all microorganisms tested.

3.2.3 Cytotoxic activity

From cytotoxicity result shown in **Table 38**, all limonoids isolated, compounds **AM12**-**AM15** were moderately active against all cancer cell lines tested as compare to camptothecin. Compounds **AM1**, **AM2**, **AM7**, **AM9**, **AM16** and **AM18** were found to be inactive (**Table 38**).

Table 37 Antibacterial activity of the compounds isolated from the roots of *A. monophylla*

Compound	Minimum Inhibitive Concentration (µg/ml)						
	B. subtilis	*S. aureus*	*E. faecalis*	*S. thypi*	*S. sonnei*	*P. aeruginosa*	*C. albicans*
AM1	-	31.25	-	-	-	-	-
AM2	-	-	-	-	-	-	-
AM7	7.8	7.8	-	-	-	-	-
AM9	-	-					-
AM12	-	-	-	-	-	-	-
AM13	-	-	-	-	-	-	-
AM14	-	-	-	-	-	-	-
AM15	-	-	-	-	-	-	-
AM16	-	-					-
AM18	-	-	-	-	-	-	-
Vancomycin	<3.906	<3.906	-	2500	625	78.12	-

- = Inactive at > 50.1 µg/ml

MIC < 1.1 µg/ml	highly active
MIC = 1.25-5.0 µg/ml	very active
MIC = 5.1-10.0 µg/ml	active
MIC = 10.1-35.0 µg/ml	moderately active
MIC = 35.1-50.0 µg/ml	weakly active
MIC > 50.1 µg/ml	inactive

Table 38 In vitro cytotoxic activity of the compounds isolated from the roots of *A. monophylla*

Compound	Cell lines			
	IC_{50} (μg/ml)			
	MCF-7	HeLa	HT-29	KB
AM1	-	-	-	-
AM2	-	-	-	-
AM7	-	-	-	-
AM9	-	-	-	-
AM12	25.4	25.7	26.3	23.4
AM13	10.9	11.2	11.6	10.9
AM14	22.7	25.3	25.6	20.2
AM15	24.2	25.7	26.3	23.4
AM16	-	-	-	
AM18	-	-	-	-
camptothecin	0.2-2.0	0.2-2.0	0.2-2.0	0.2-2.0

- = Inactive at > 50.1 μg/ml

MIC < 1.1 μg/ml	highly active
MIC = 1.25-5.0 μg/ml	very active
MIC = 5.1-10.0 μg/ml	active
MIC = 10.1-35.0 μg/ml	moderately active
MIC = 35.1-50.0 μg/ml	weakly active
MIC > 50.1 μg/ml	inactive

CHAPTER 4
CONCLUSION

Three new acridone alkaloids, named cycloatalaphylline-A (**AM4**), *N*-methylcycloatalaphylline-A (**AM5**) and *N*-methylbuxifoliadine-E (**AM9**) and seven teen known compounds, eight acridones: *N*-methylatalaphylline (**AM1**), atalaphylline (**AM2**), buxifoliadine-A (**AM3**), yukocitrine (**AM6**), *N*-methylataphyllinine (**AM7**), buxifoliadine-E (**AM8**), citrusinine-I (**AM10**) and junosine (**AM11**), four limonoids: atalantolide (**AM12**), atalantin (**AM13**), cycloepiatalantin (**AM14**) and cycloepiatalantin acetate (**AM15**); two coumarins: auraptene (**AM16**) and 7-*O*-geranylscopoletin (**AM17**); one anthraquinone: physcion **(AM18)** and two steroids: a mixture of β-sitosterol (**AM19**) and stigmasterol (**AM20**) were isolated from the roots of *A. monophylla*. Their structures were elucidated by spectroscopic methods. The structure of **AM7** was additionally confirmed by X-ray diffraction analysis. It was found that two acridone alkaloids : buxifoliadine-E (**AM8**) possessed the most potent anti-allergic activity against cell degranulation in RBL-2H3 cells with an IC_{50} value of 6.1 μM, followed by citrusinine-I (**AM10**, IC_{50} = 18.7 μM), whereas physcion (**AM18**), atalantolide (**AM12**), *N*-methylcycloatalaphylline-A (**AM5**) and auraptene (**AM16**) displayed moderate effects with IC_{50} values of 34.0, 35.1, 40.1 and 73.2, respectively. Compounds **AM1**, **AM2**, **AM7** and **AM17** were found inactive. Only *N*-methylataphyllinine (**AM7**) exhibited significant antibacterial activity against *B. subtilis* and *S. aureus*. For cytotoxic activity, limonoids: atalantolide (**AM12**), atalantin (**AM13**), cycloepiatalantin (**AM14**) and cycloepiatalantin acetate (**AM15**) were moderately active against MCF-7, HeLa, HT-29 and KB cell lines.

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APPENDIX

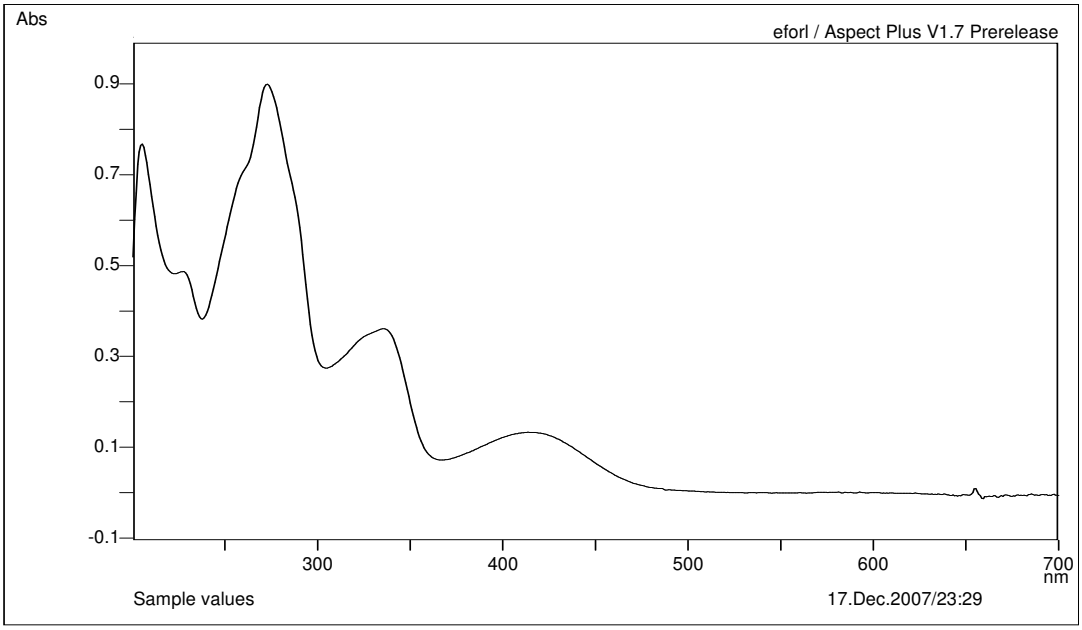

Figure 21 UV (MeOH) spectrum of compound **AM1**

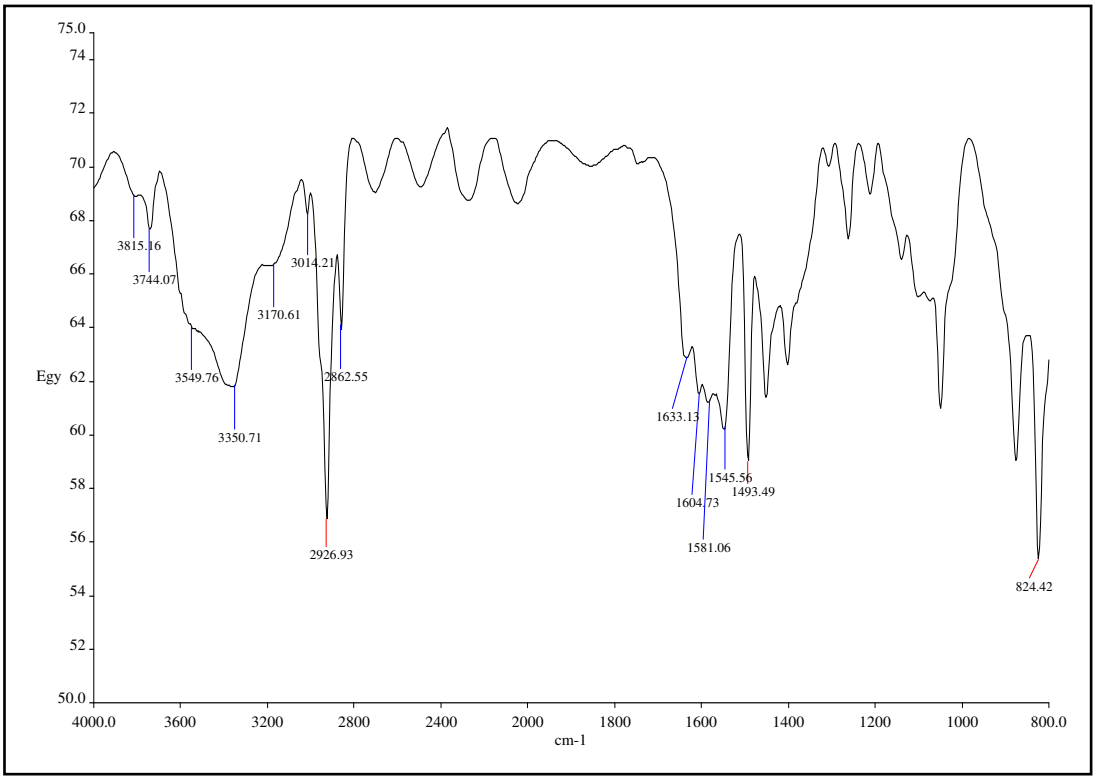

Figure 22 IR (KBr) spectrum of compound **AM1**

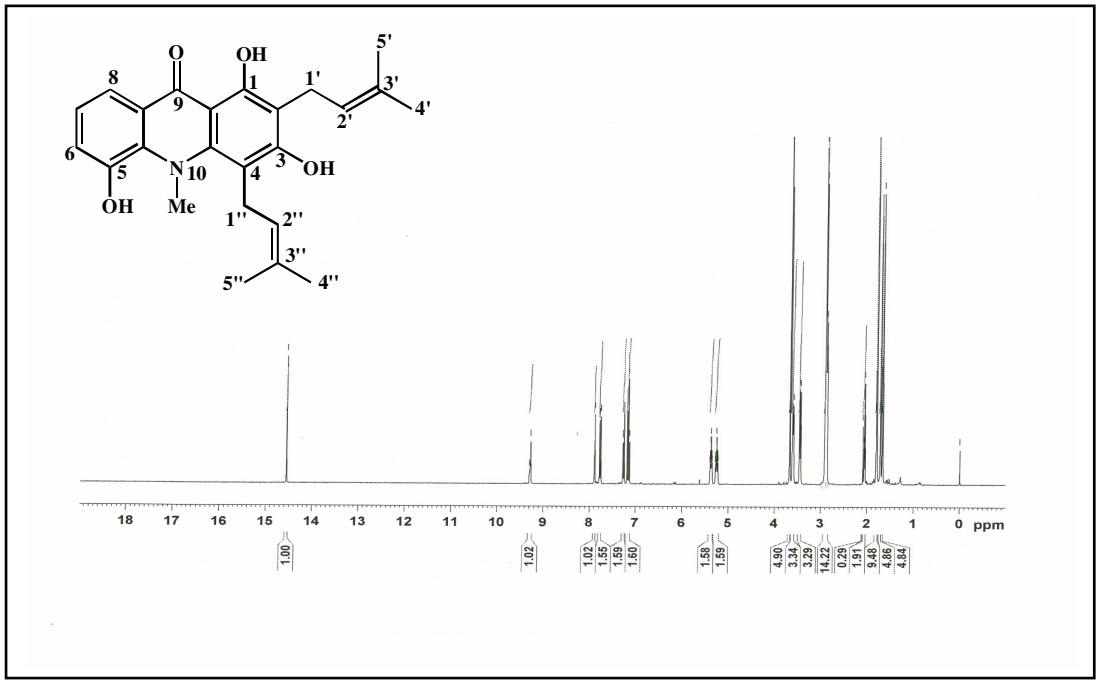

Figure 23 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM1**

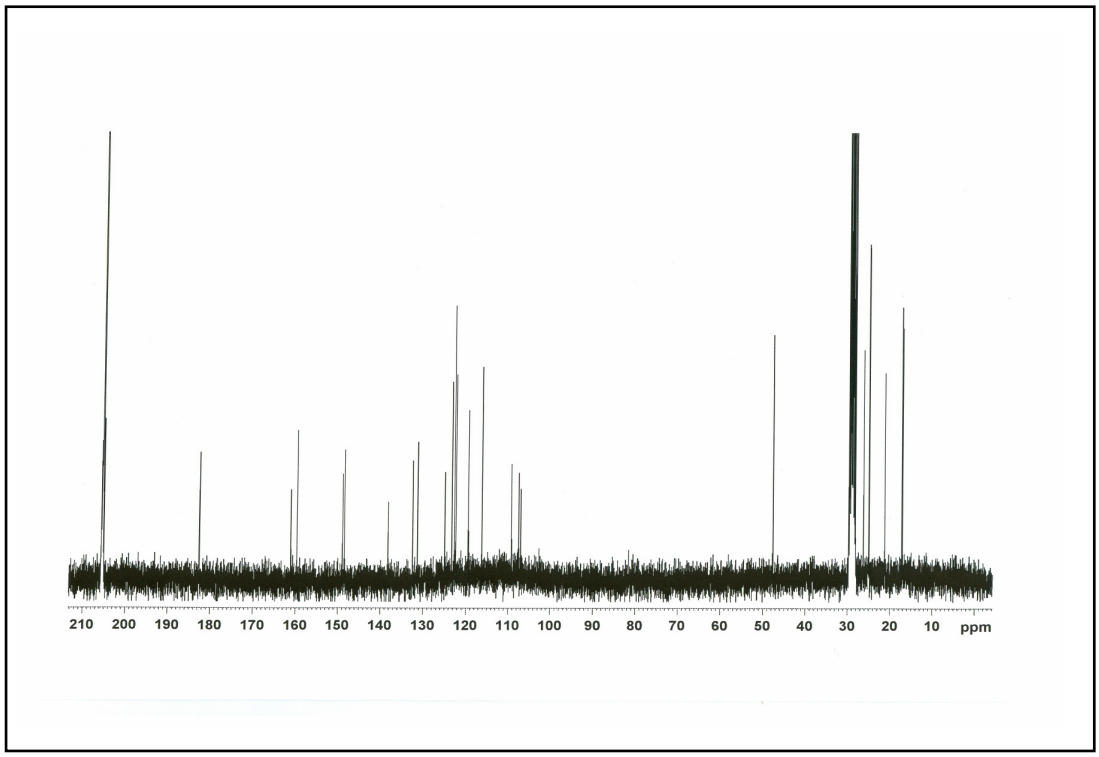

Figure 24 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM1**

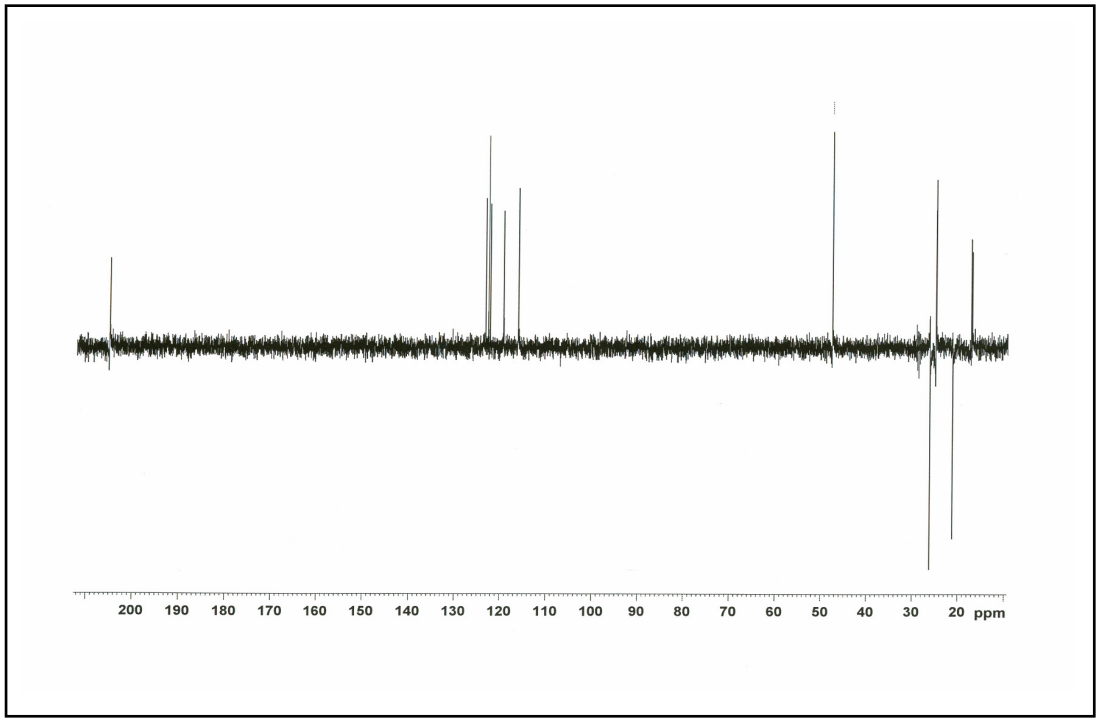

Figure 25 DEPT 135° (acetone-d_6) spectrum of compound **AM1**

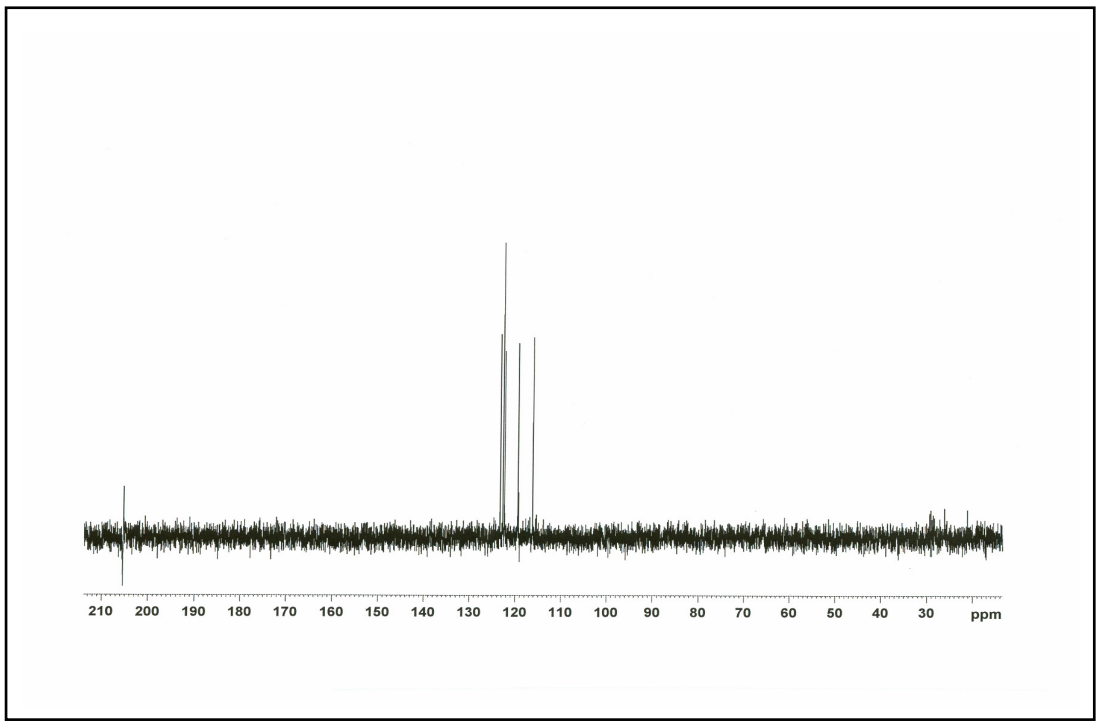

Figure 26 DEPT 90° (acetone-d_6) spectrum of compound **AM1**

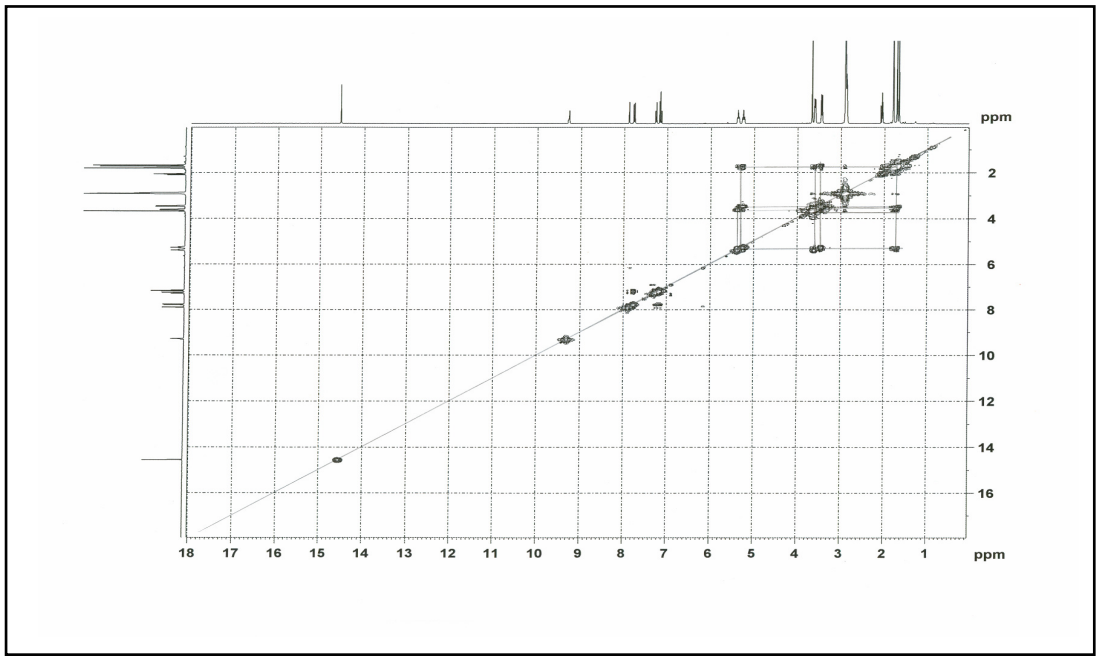

Figure 27 2D COSY (acetone-d_6) spectrum of compound **AM1**

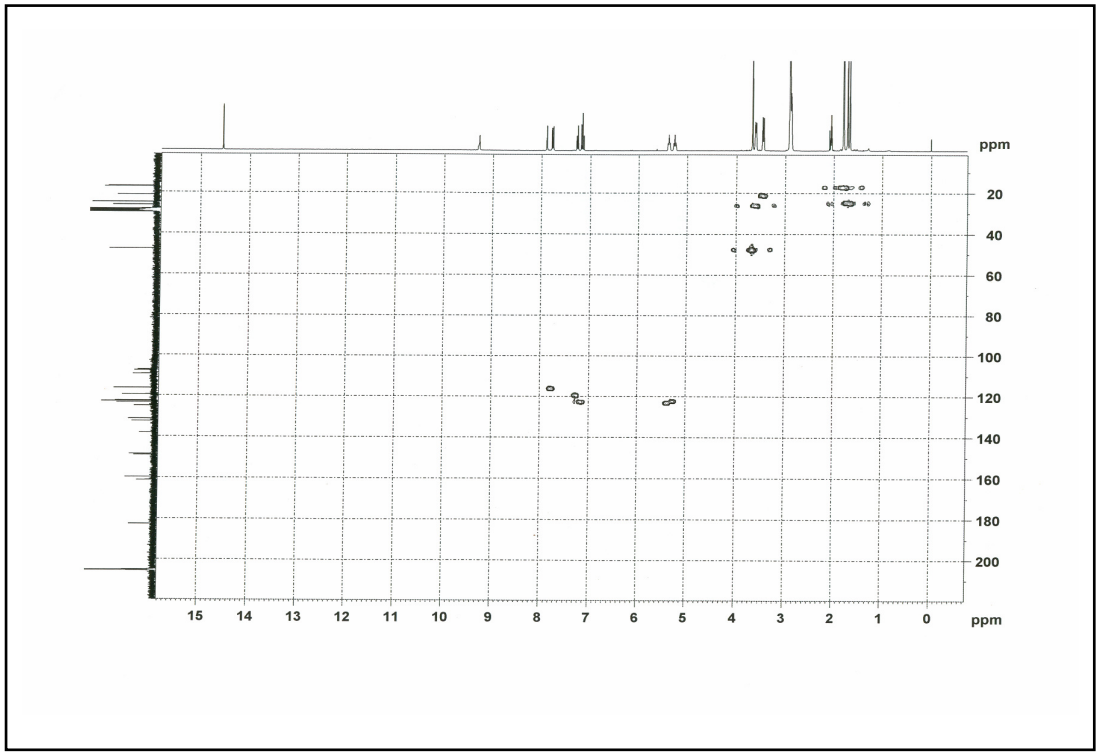

Figure 28 2D HMQC (acetone-d_6) spectrum of compound **AM1**

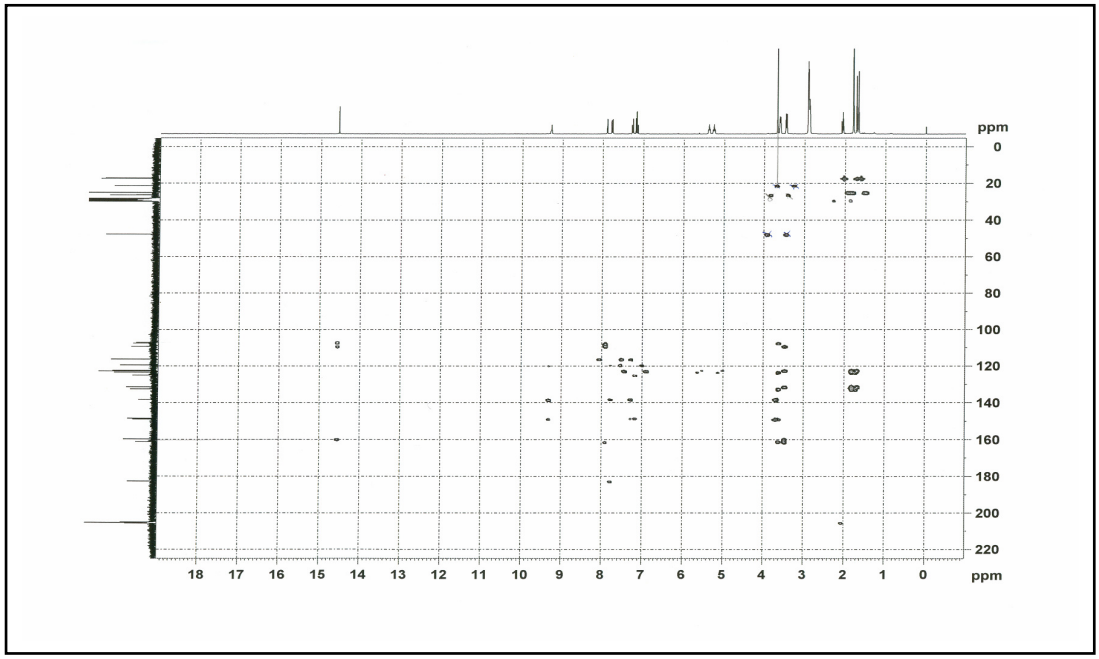

Figure 29 2D HMBC (acetone-d_6) spectrum of compound **AM1**

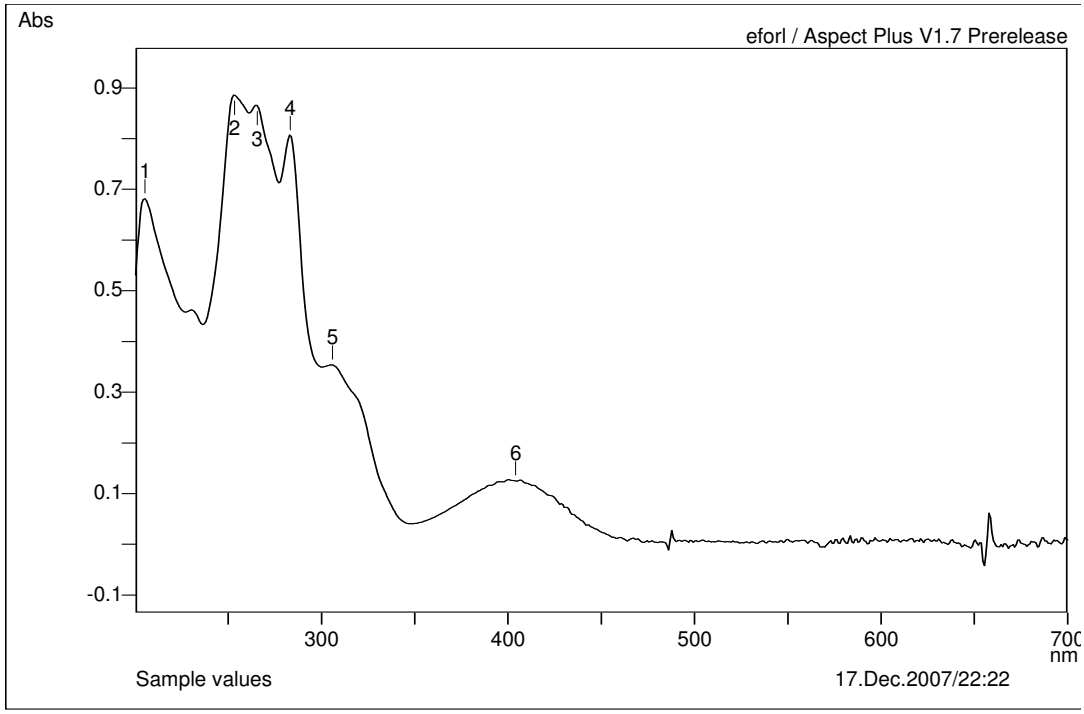

Figure 30 UV (MeOH) spectrum of compound **AM2**

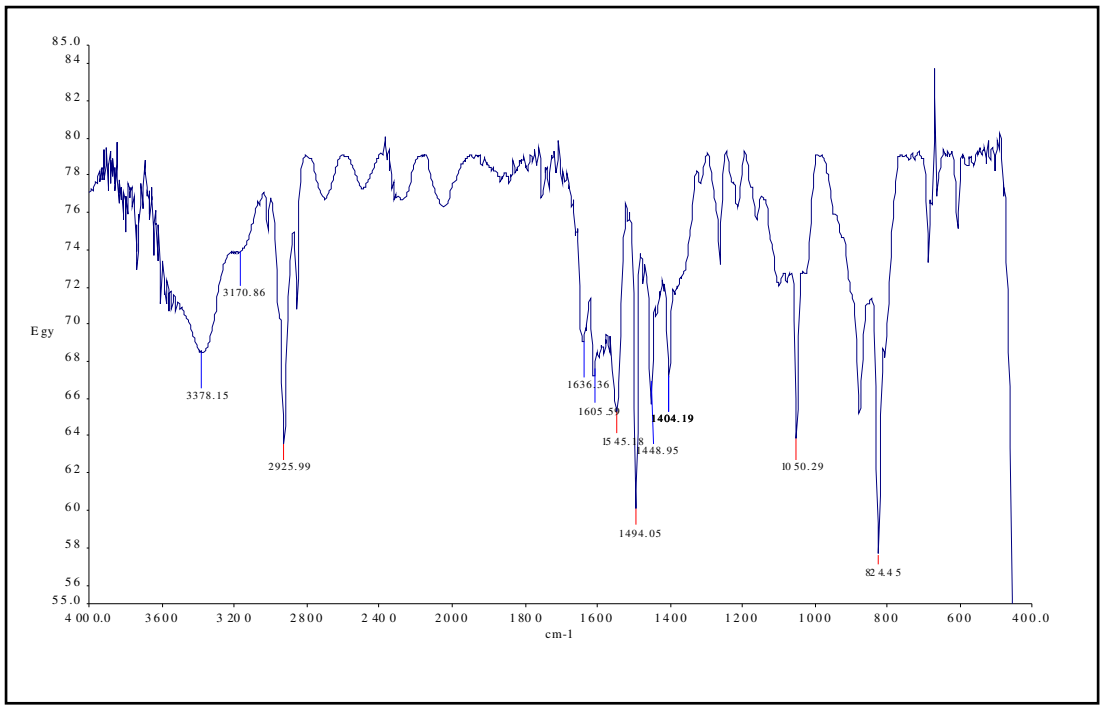

Figure 31 IR (KBr) spectrum of compound **AM2**

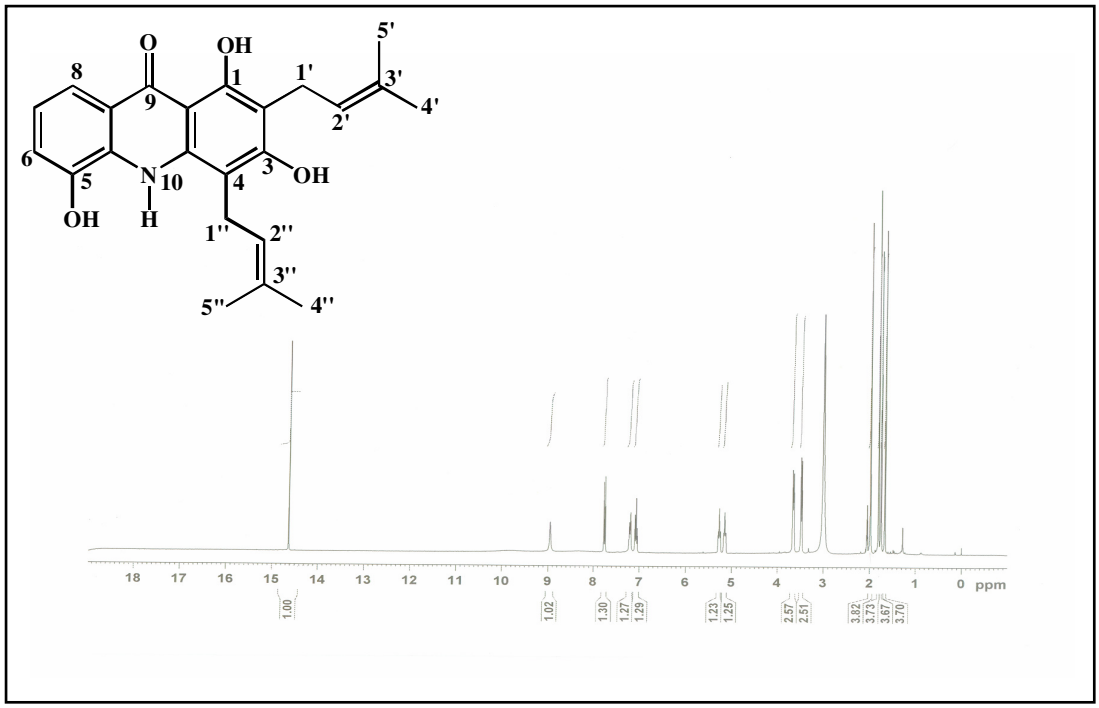

Figure 32 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM2**

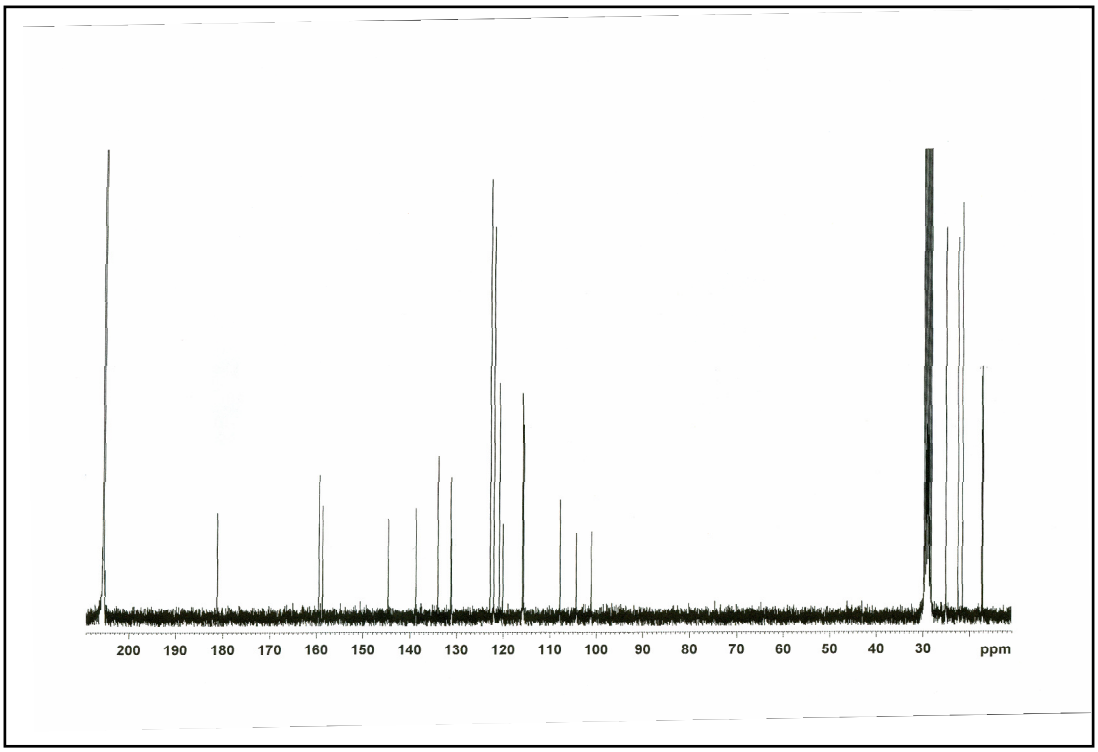

Figure 33 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM2**

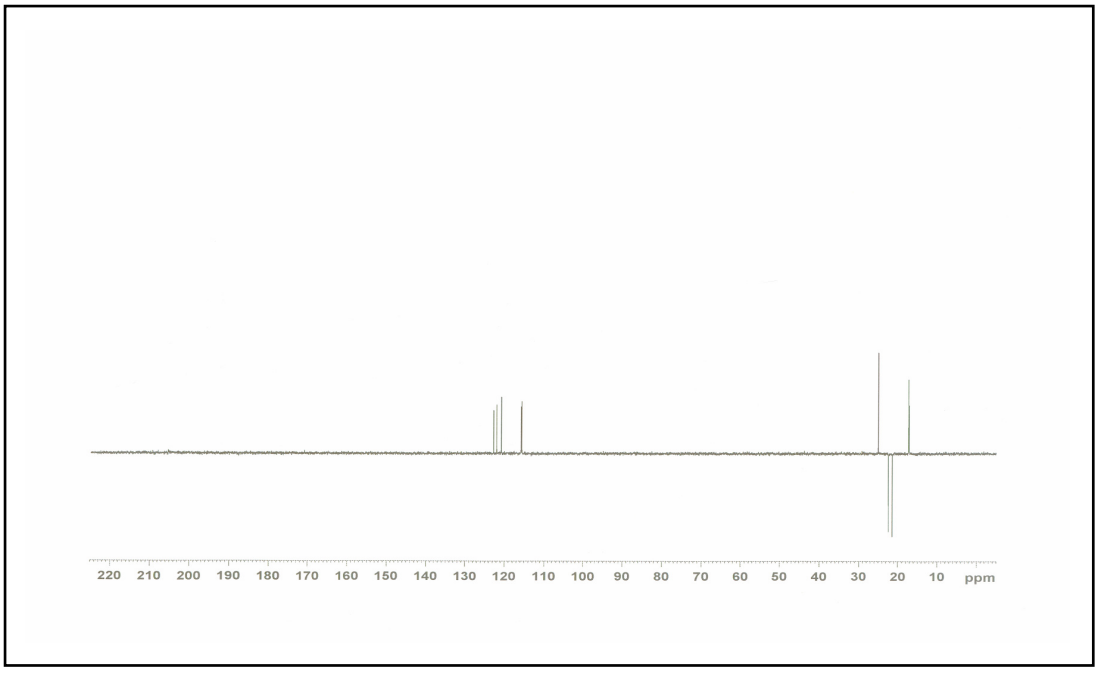

Figure 34 DEPT 135° (acetone-d_6) spectrum of compound **AM2**

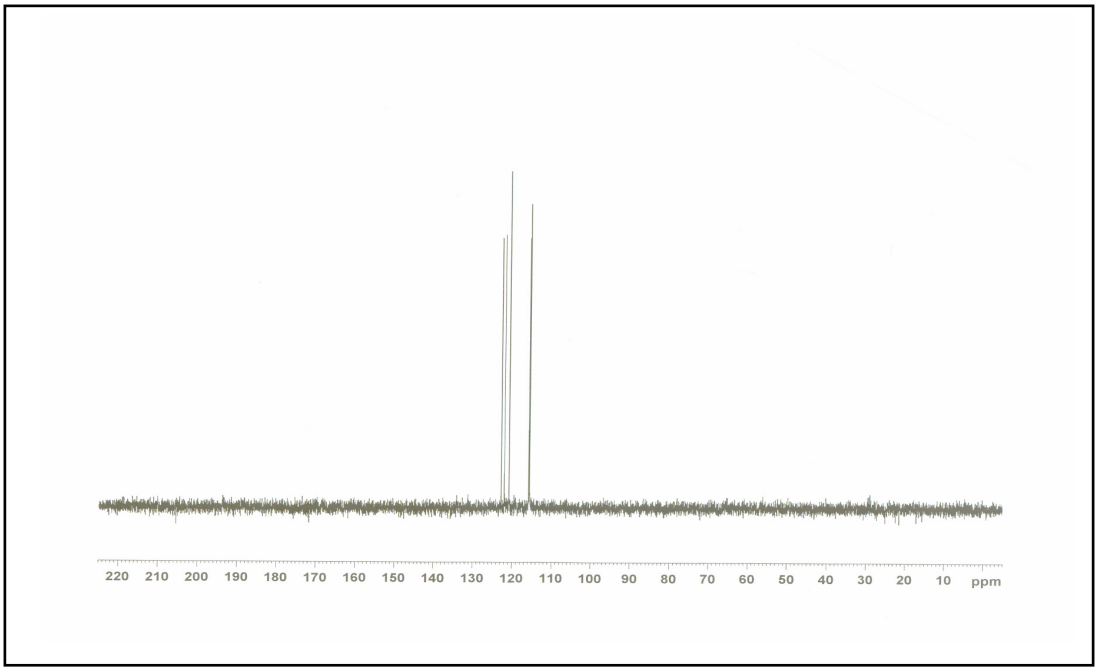

Figure 35 DEPT 90° (acetone-d_6) spectrum of compound **AM2**

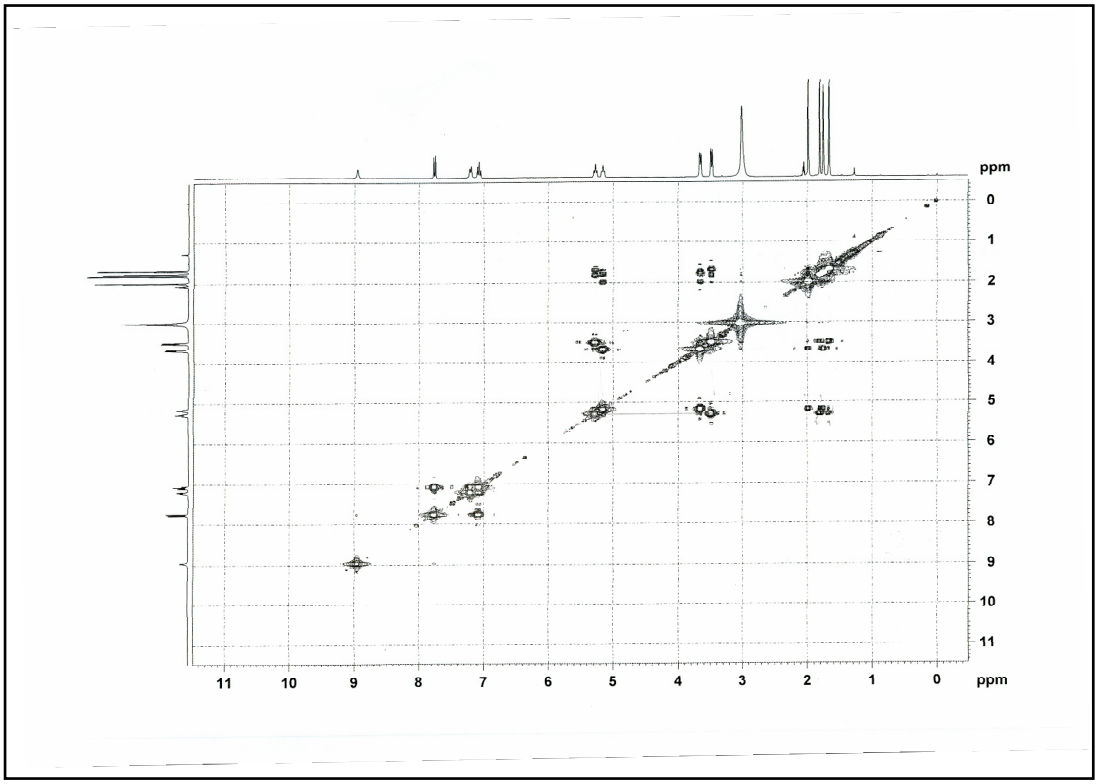

Figure 36 2D COSY (acetone-d_6) spectrum of compound **AM2**

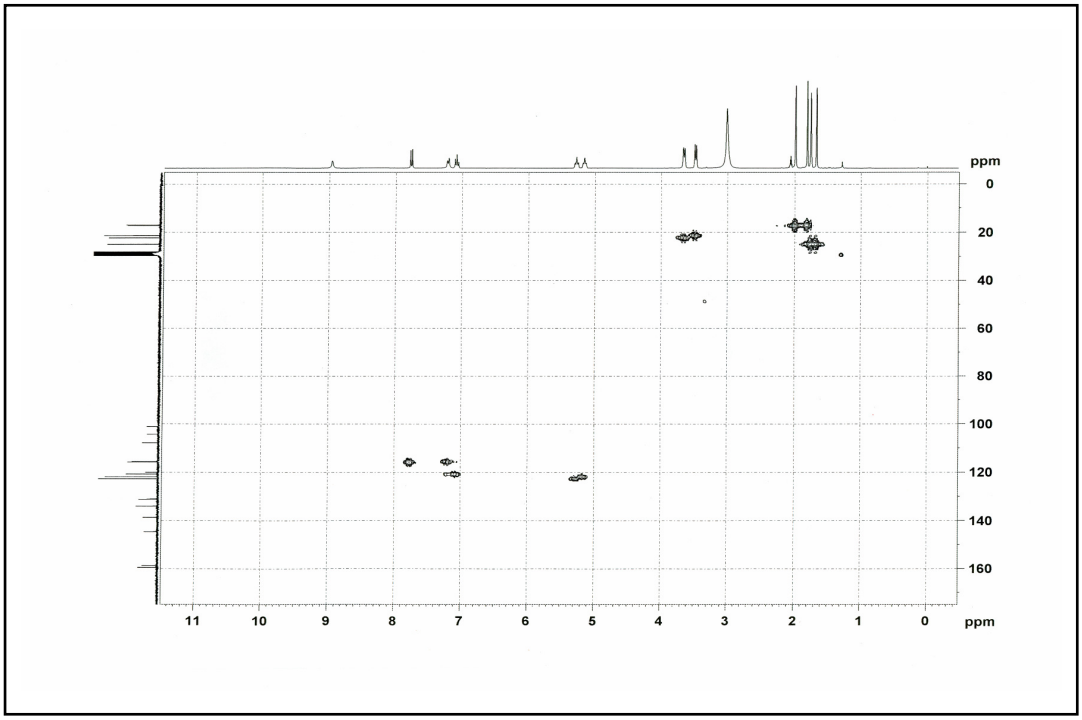

Figure 37 2D HMQC (acetone-d_6) spectrum of compound **AM2**

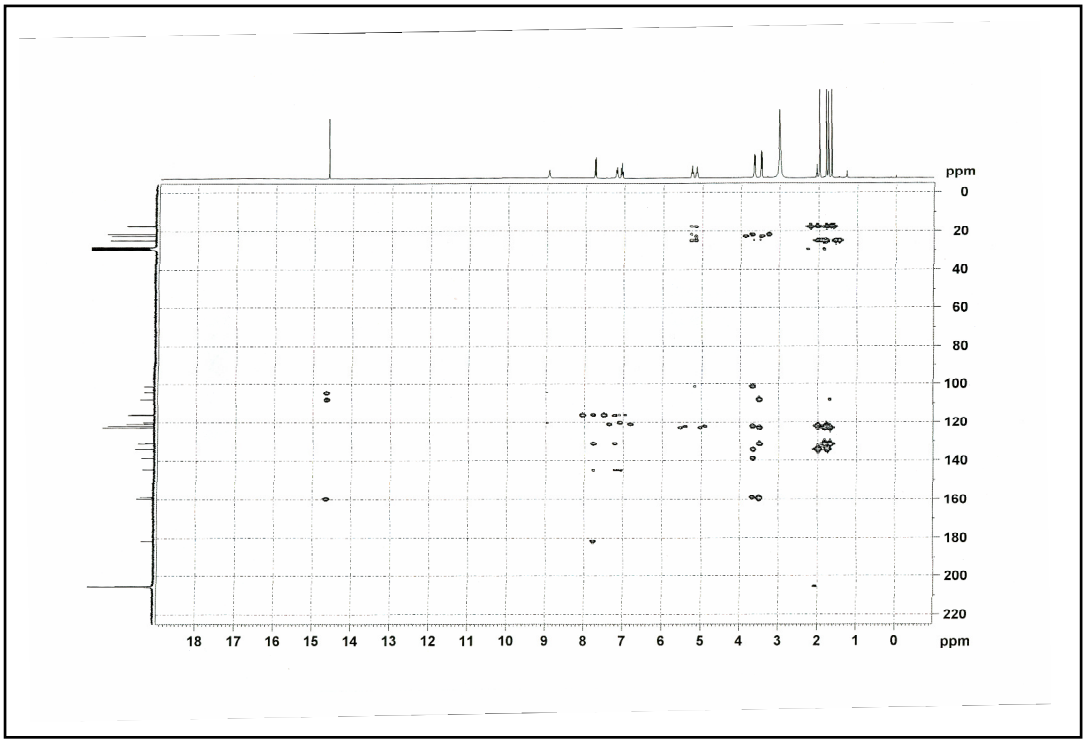

Figure 38 2D HMBC (acetone-d_6) spectrum of compound **AM2**

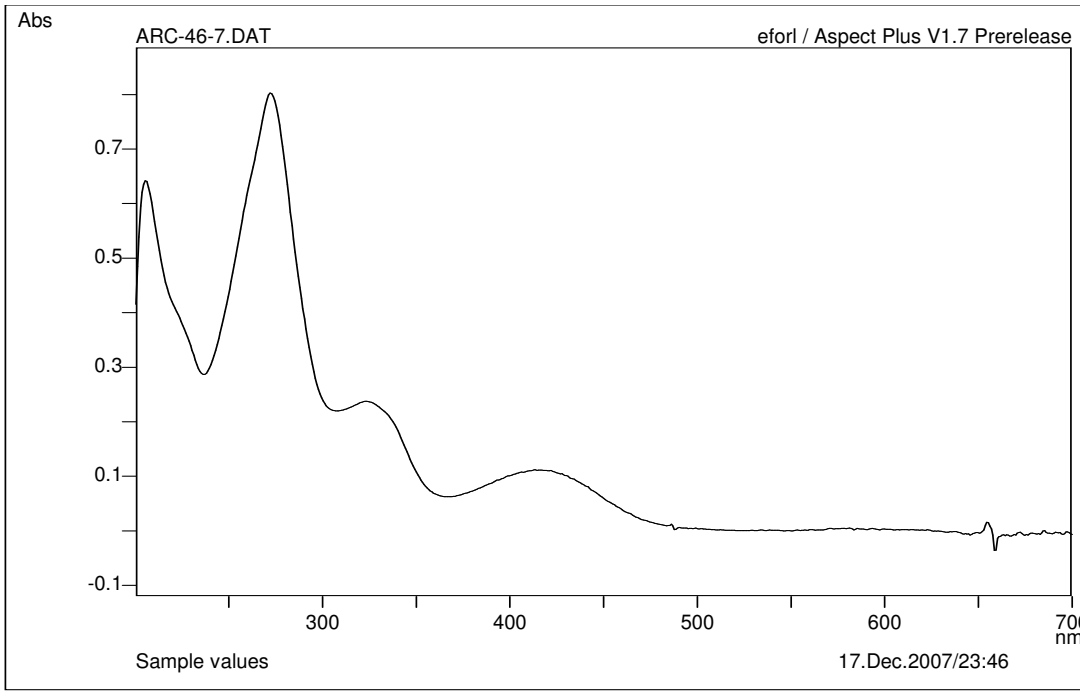

Figure 39 UV (MeOH) spectrum of compound **AM3**

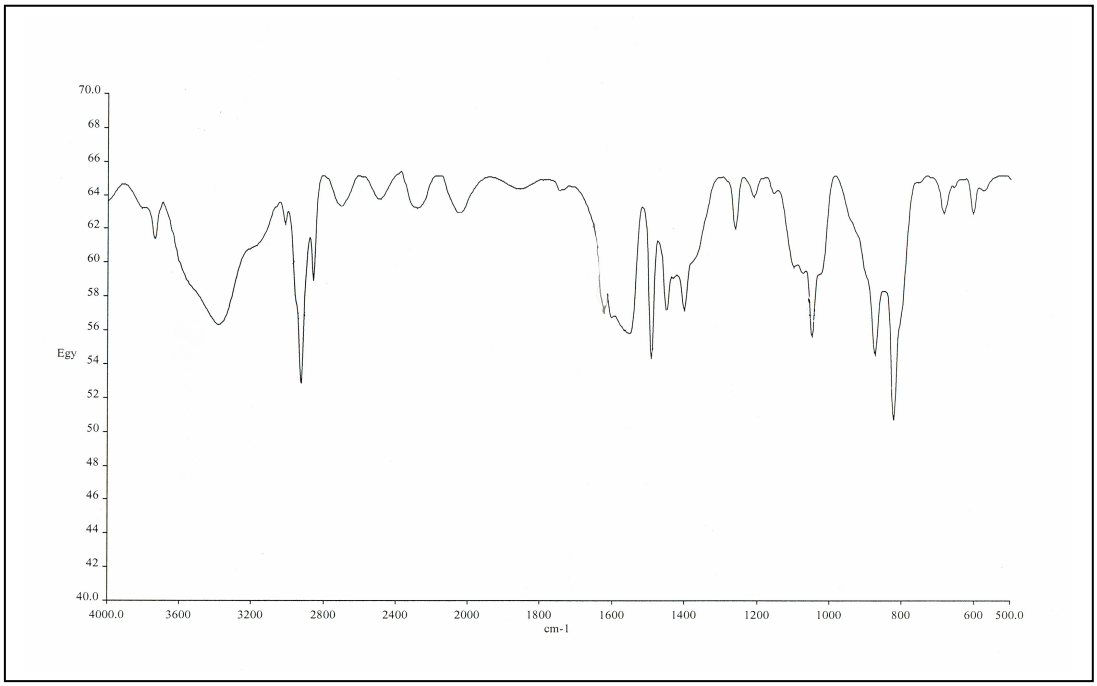

Figure 40 IR (neat) spectrum of compound **AM3**

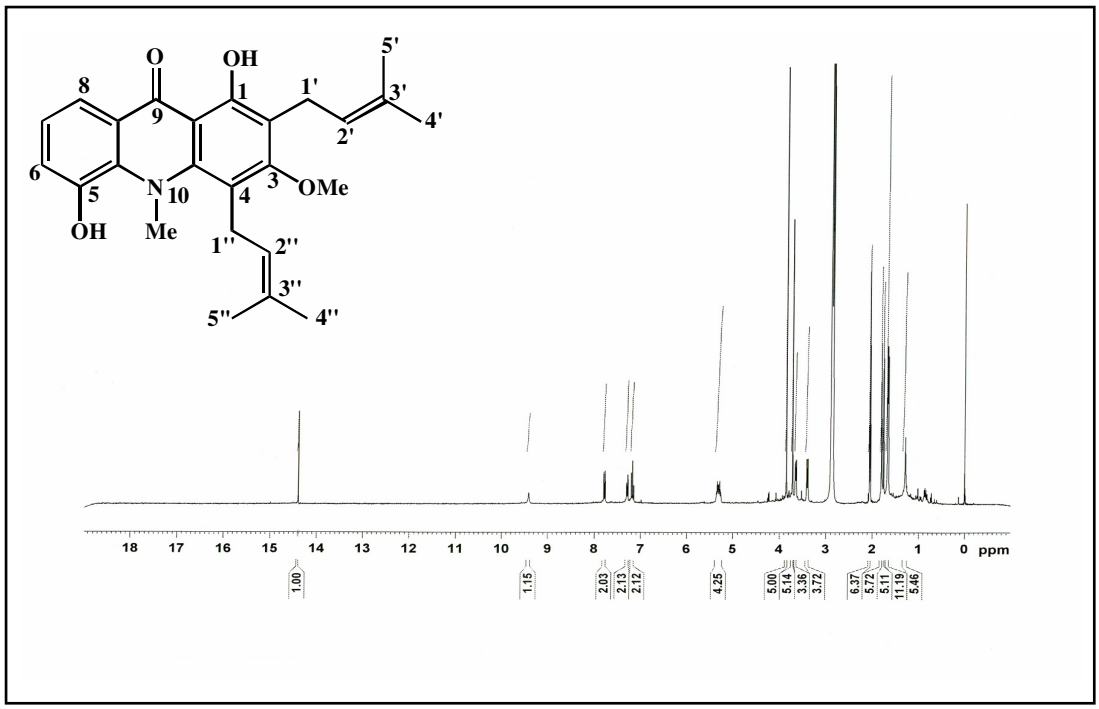

Figure 41 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM3**

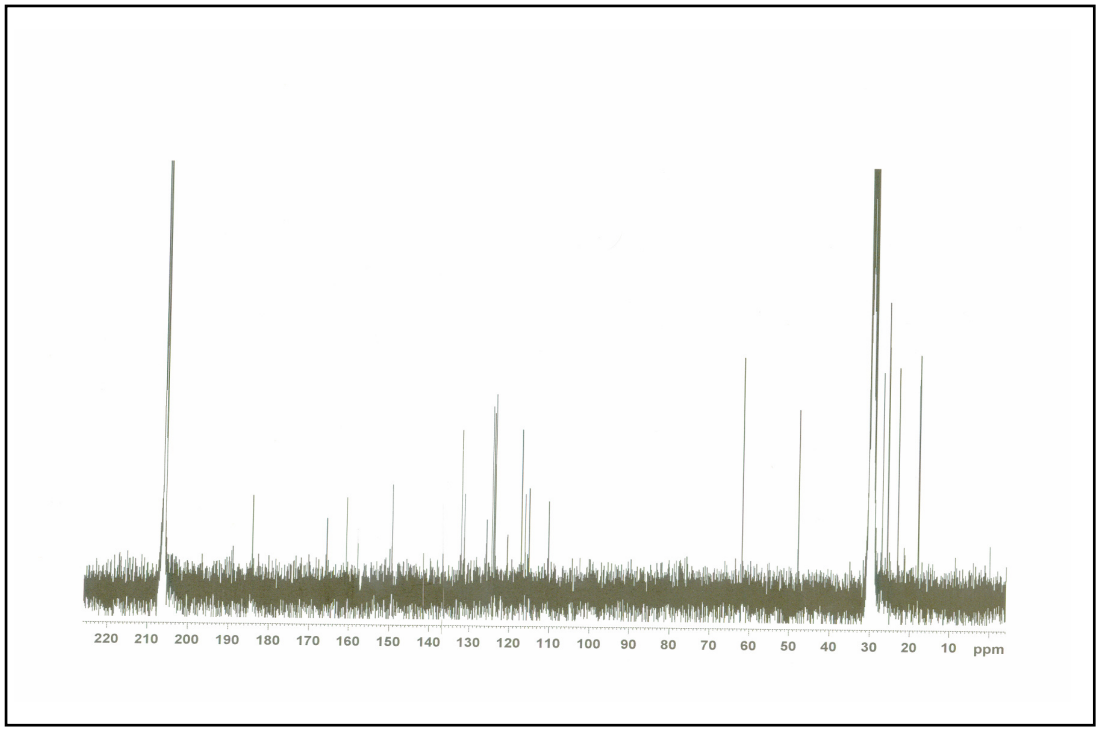

Figure 42 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM3**

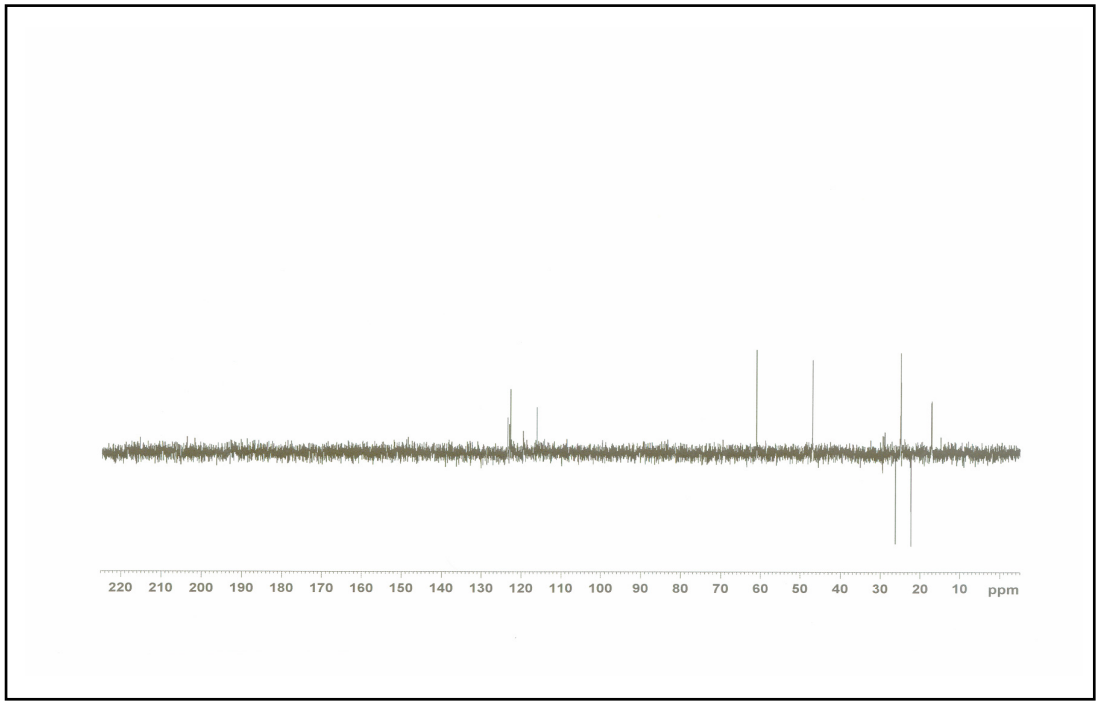

Figure 43 DEPT 135° (acetone-d_6) spectrum of compound **AM3**

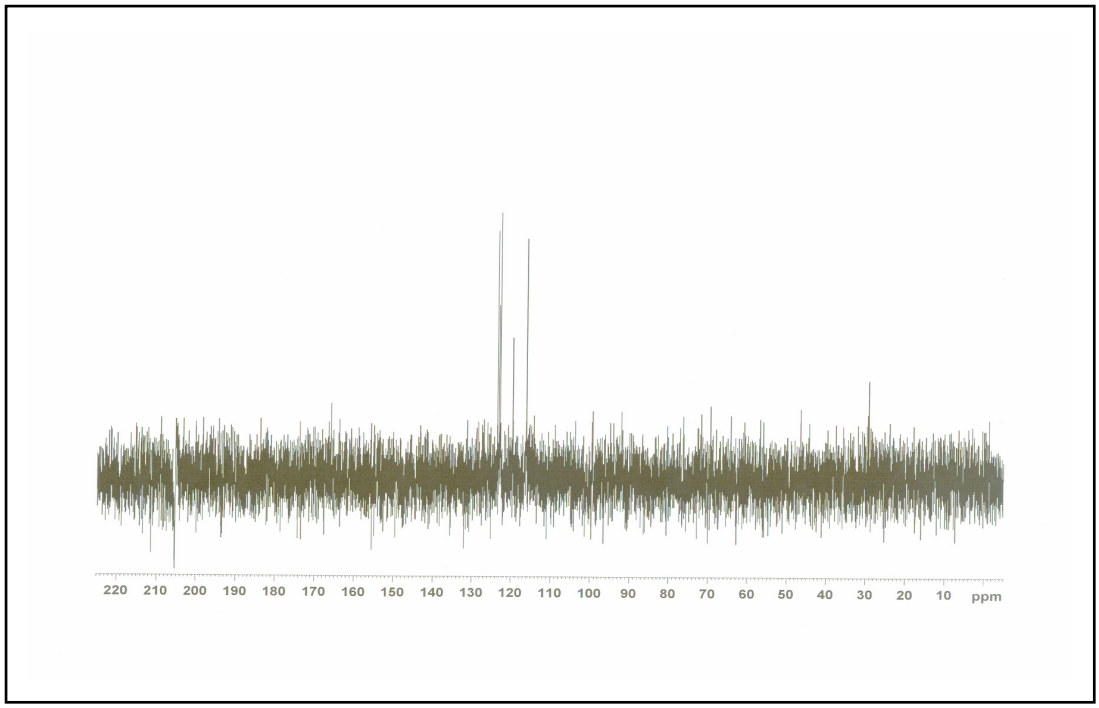

Figure 44 DEPT 90° (acetone-d_6) spectrum of compound **AM3**

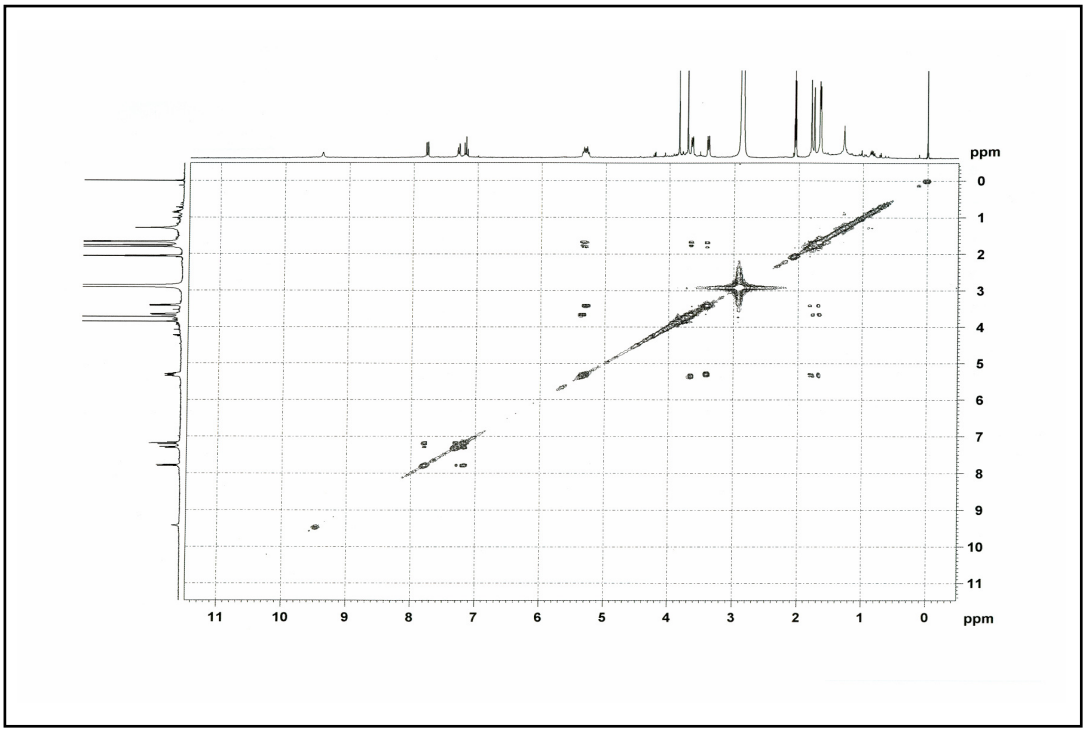

Figure 45 2D COSY (acetone-d_6) spectrum of compound **AM3**

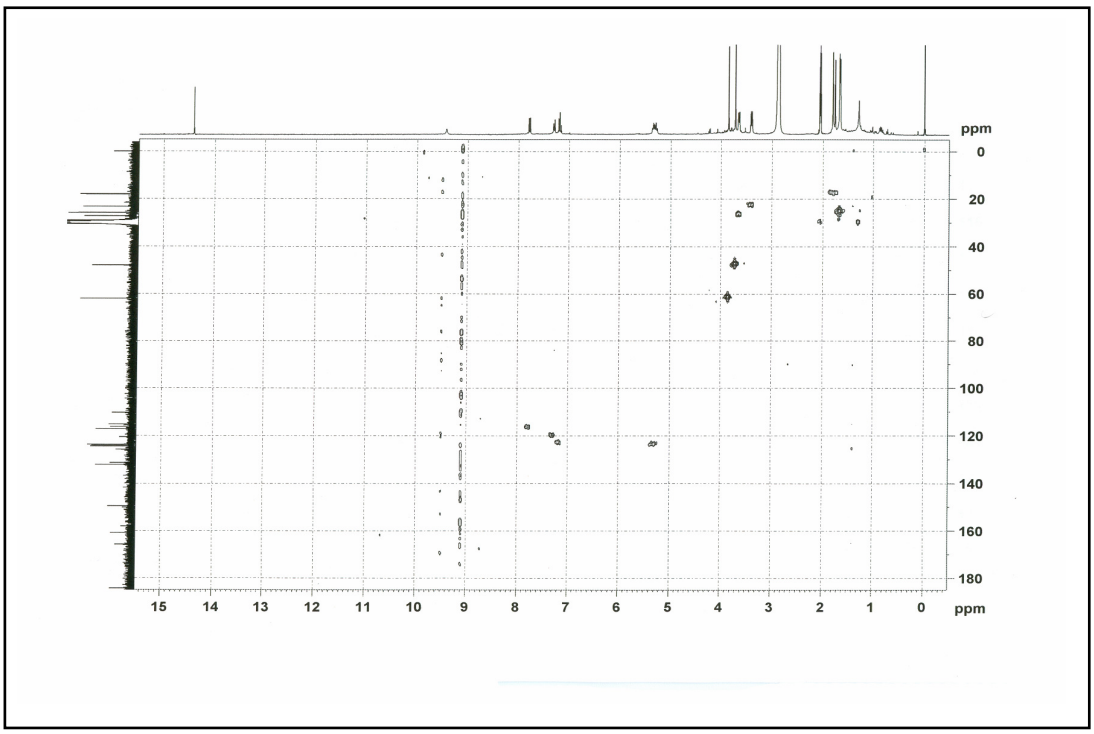

Figure 46 2D HMQC (acetone-d_6) spectrum of compound **AM3**

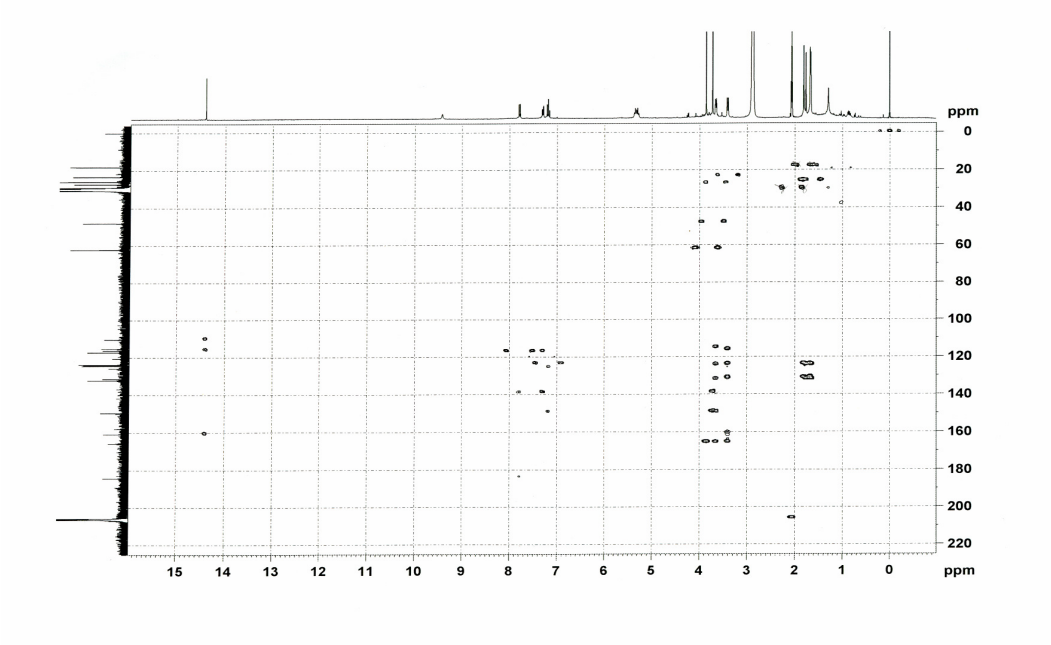

Figure 47 2D HMBC (acetone-d_6) spectrum of compound **AM3**

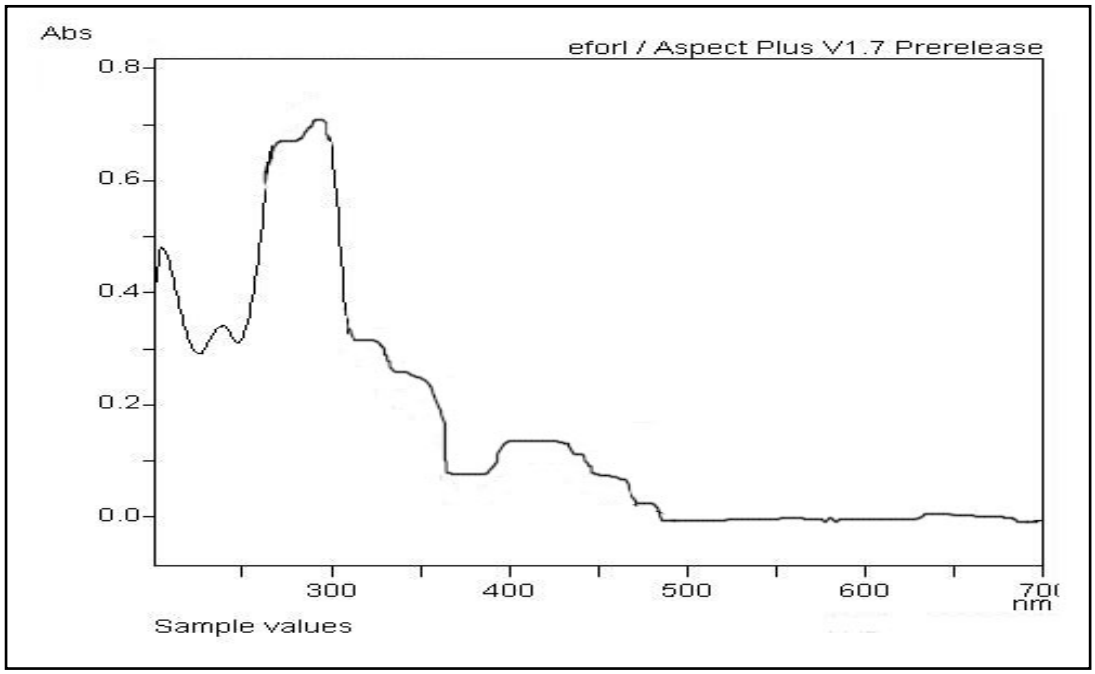

Figure 48 UV (MeOH) spectrum of compound **AM4**

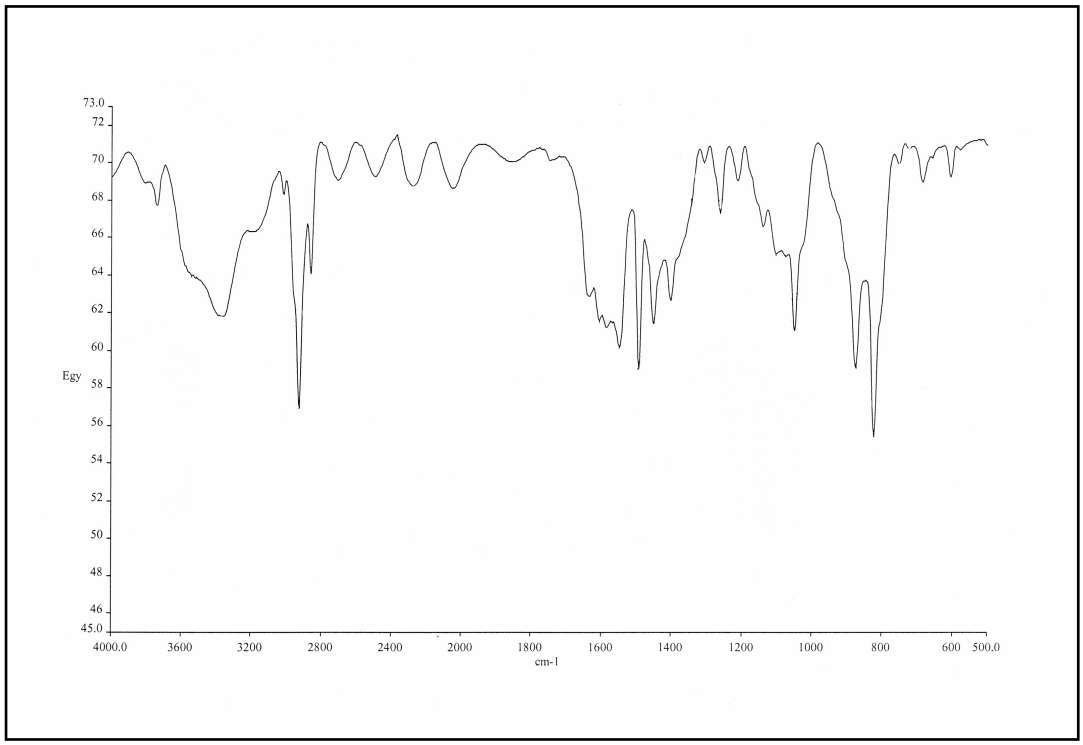

Figure 49 IR (KBr) spectrum of compound **AM4**

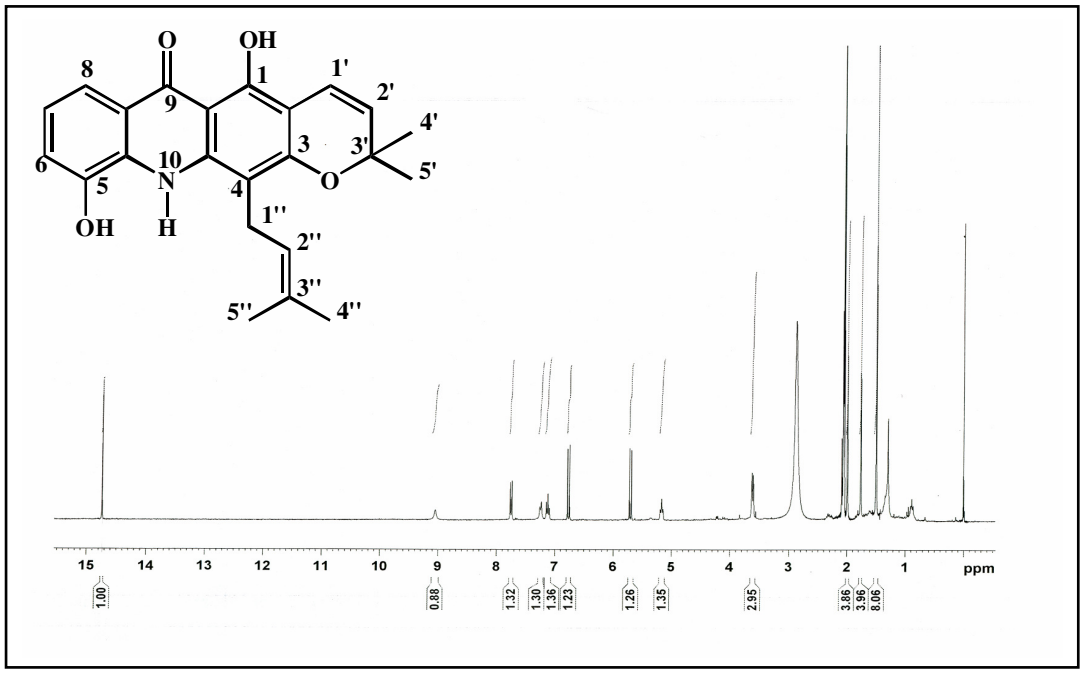

Figure 50 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM4**

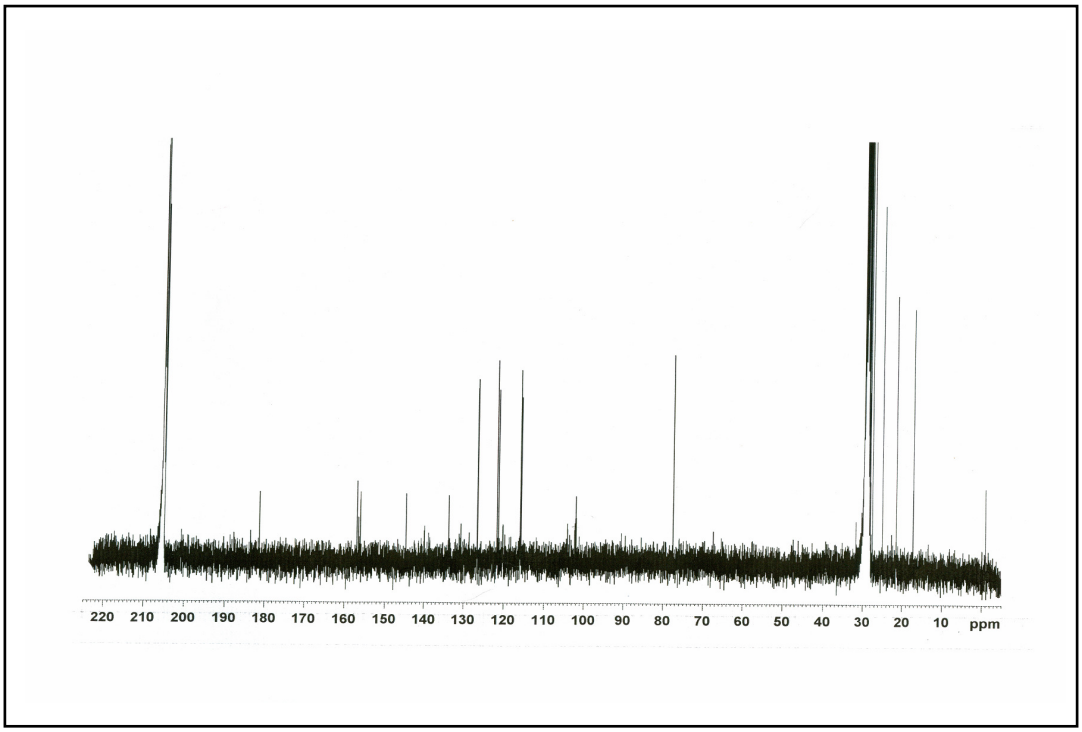

Figure 51 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM4**

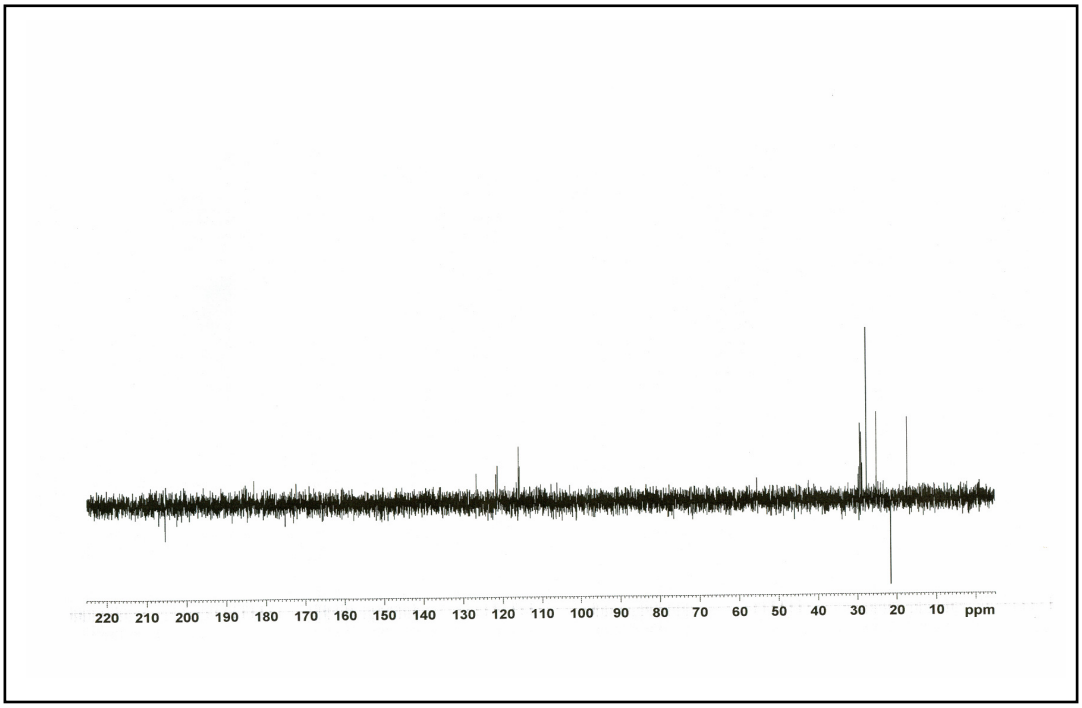

Figure 52 DEPT 135° (acetone-d_6) spectrum of compound **AM4**

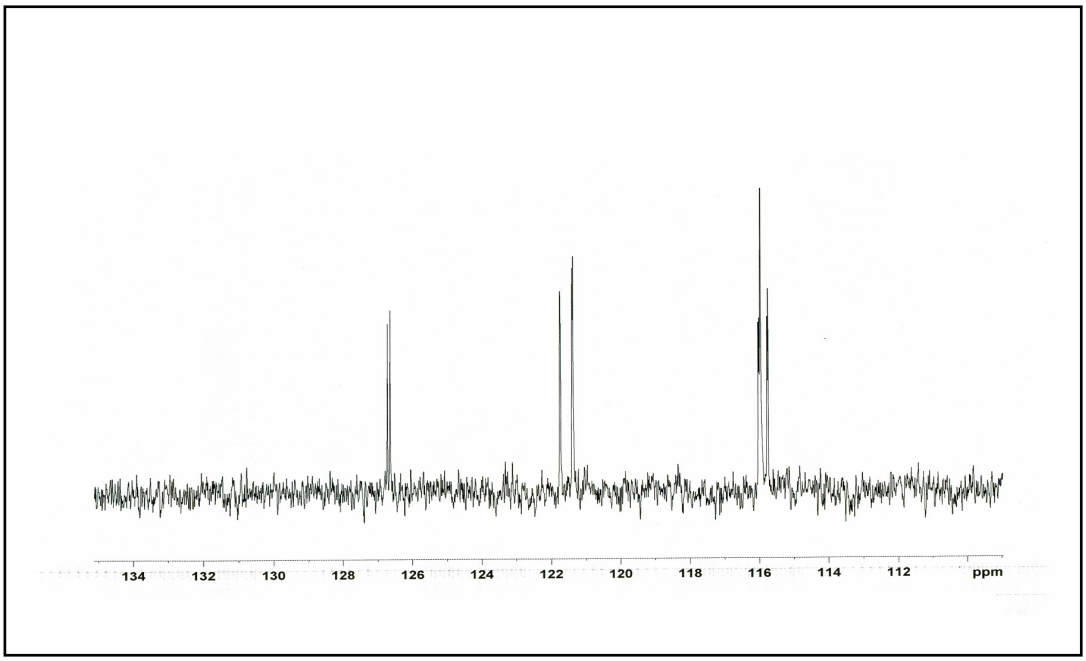

Figure 53 DEPT 90° (acetone-d_6) spectrum of compound **AM4**

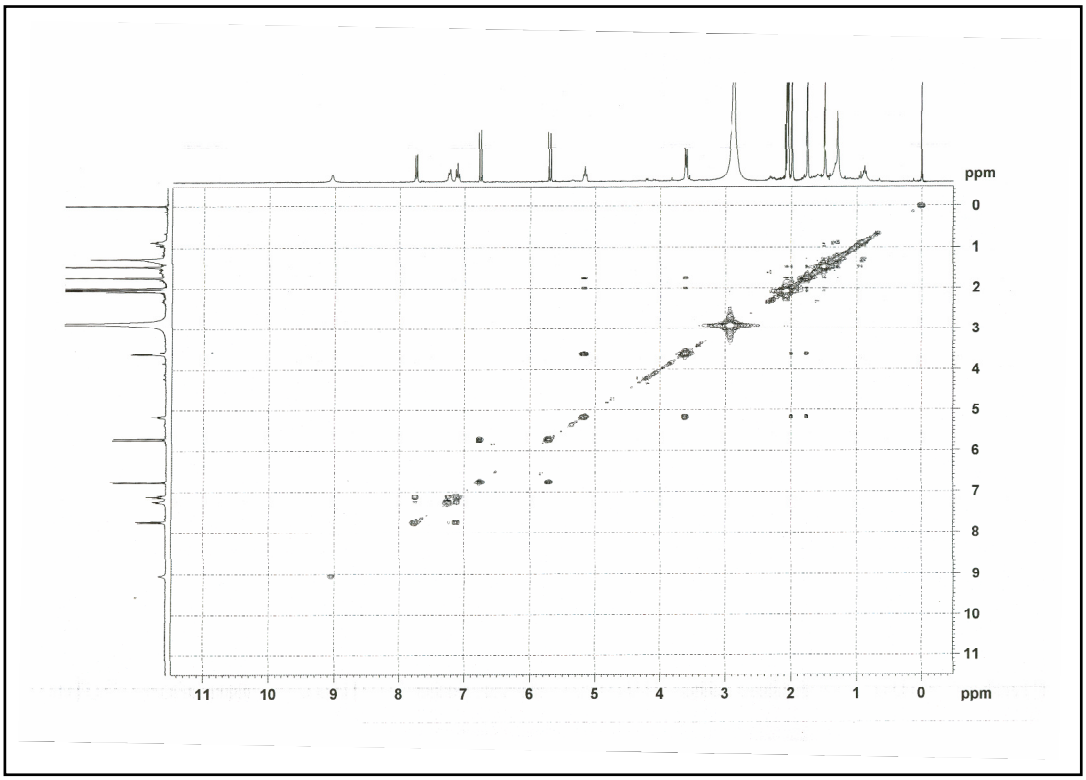

Figure 54 2D COSY (acetone-d_6) spectrum of compound **AM4**

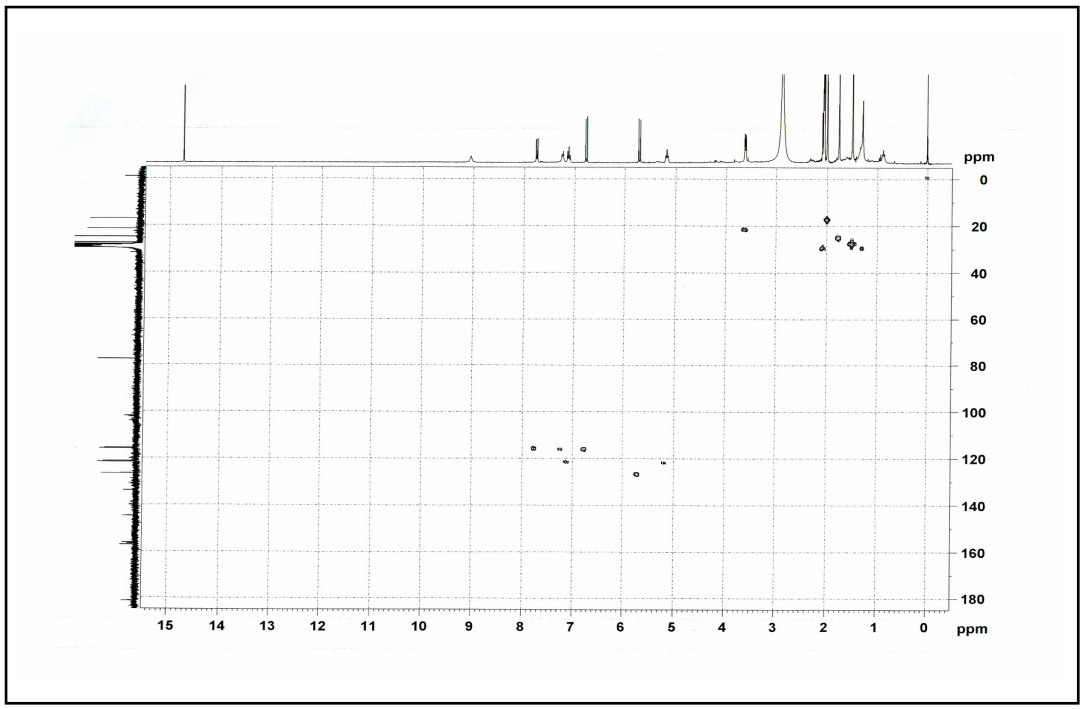

Figure 55 2D HMQC (acetone-d_6) spectrum of compound **AM4**

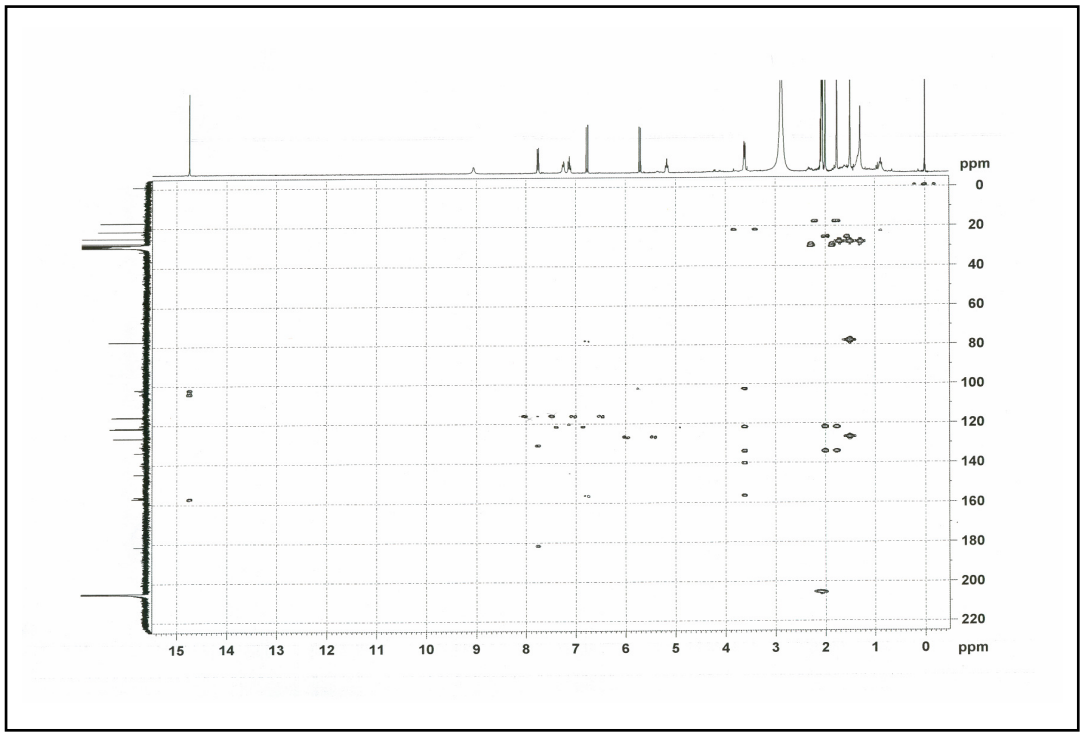

Figure 56 2D HMBC (acetone-d_6) spectrum of compound **AM4**

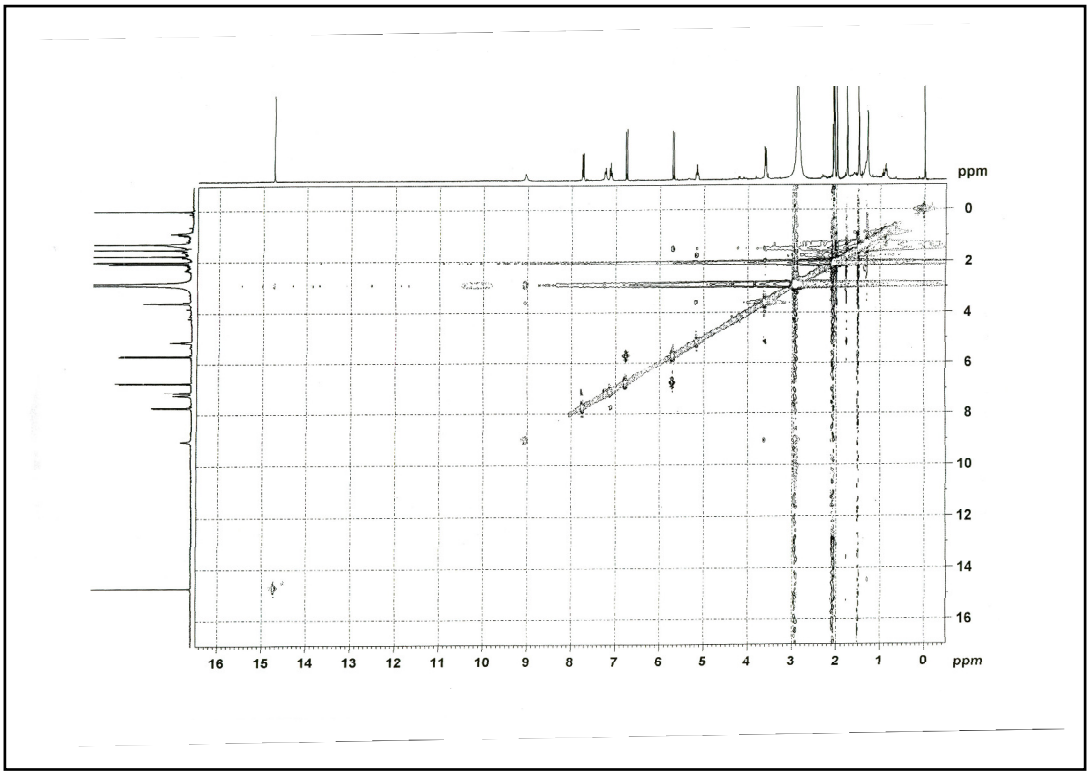

Figure 57 2D NOESY (acetone-d_6) spectrum of compound **AM4**

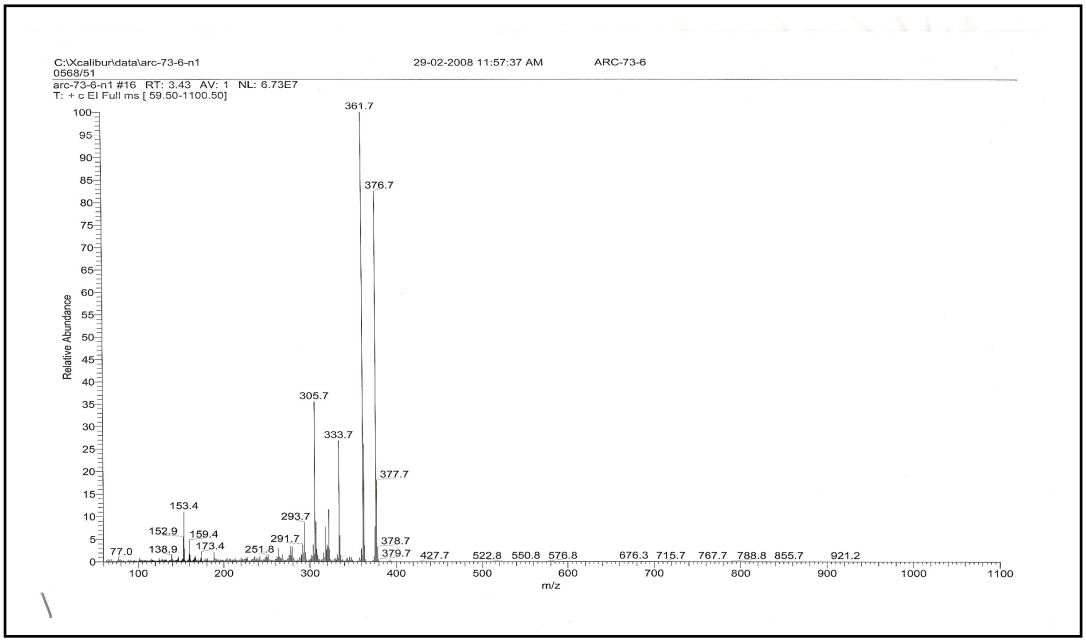

Figure 58 EIMS spectrum of compound **AM4**

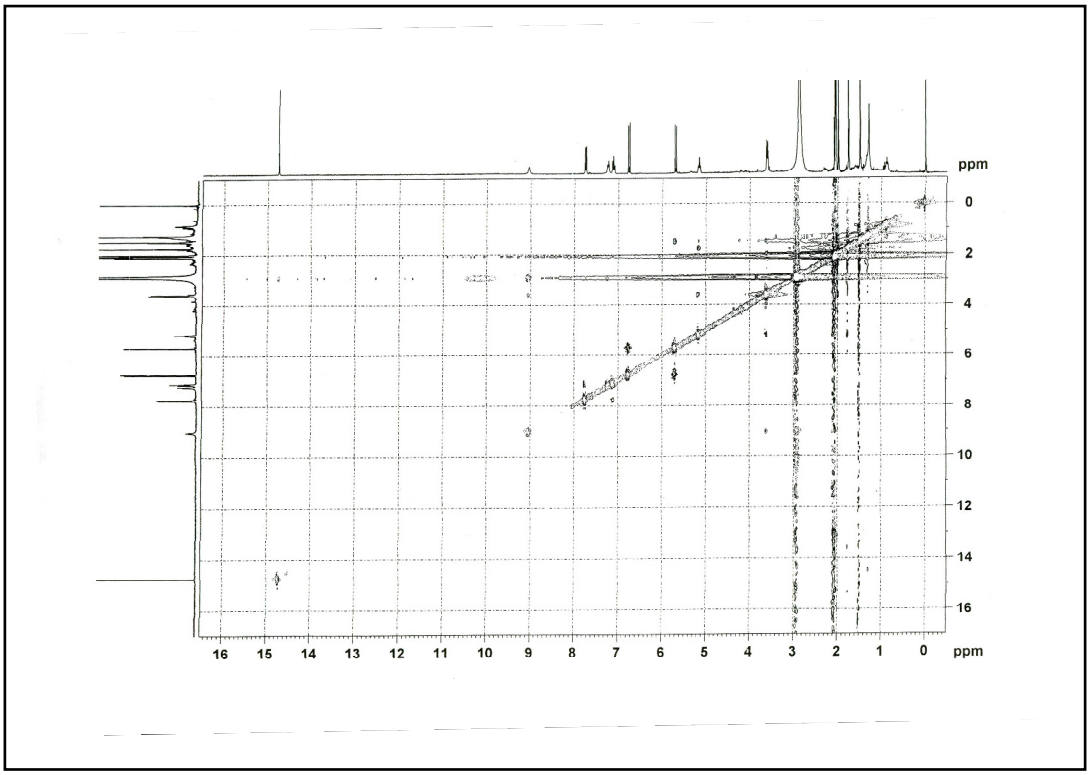

Figure 57 2D NOESY (acetone-d_6) spectrum of compound **AM4**

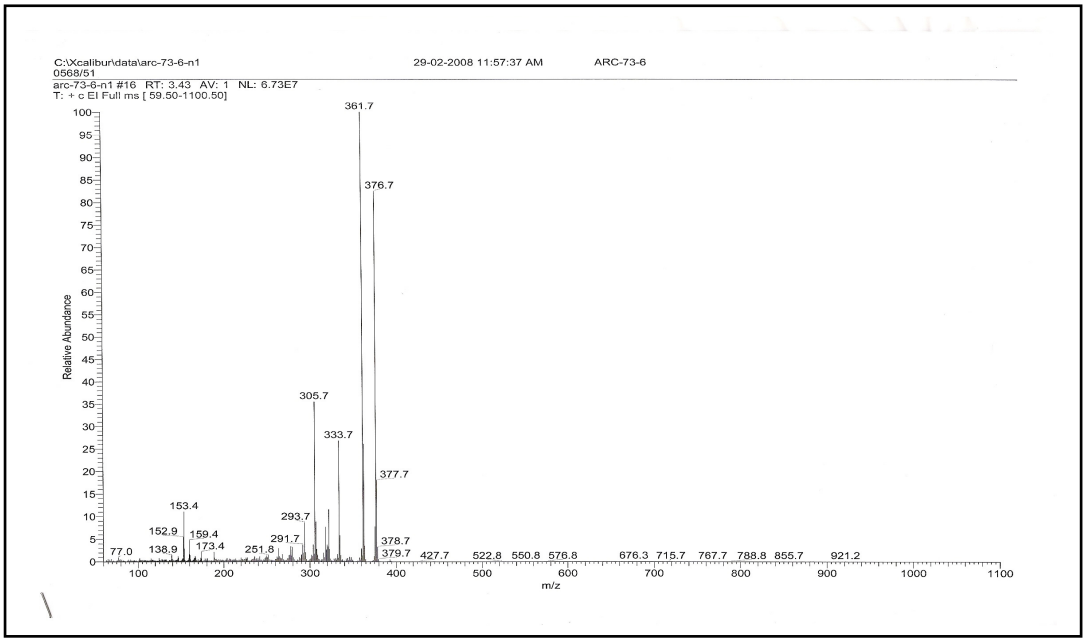

Figure 58 EIMS spectrum of compound **AM4**

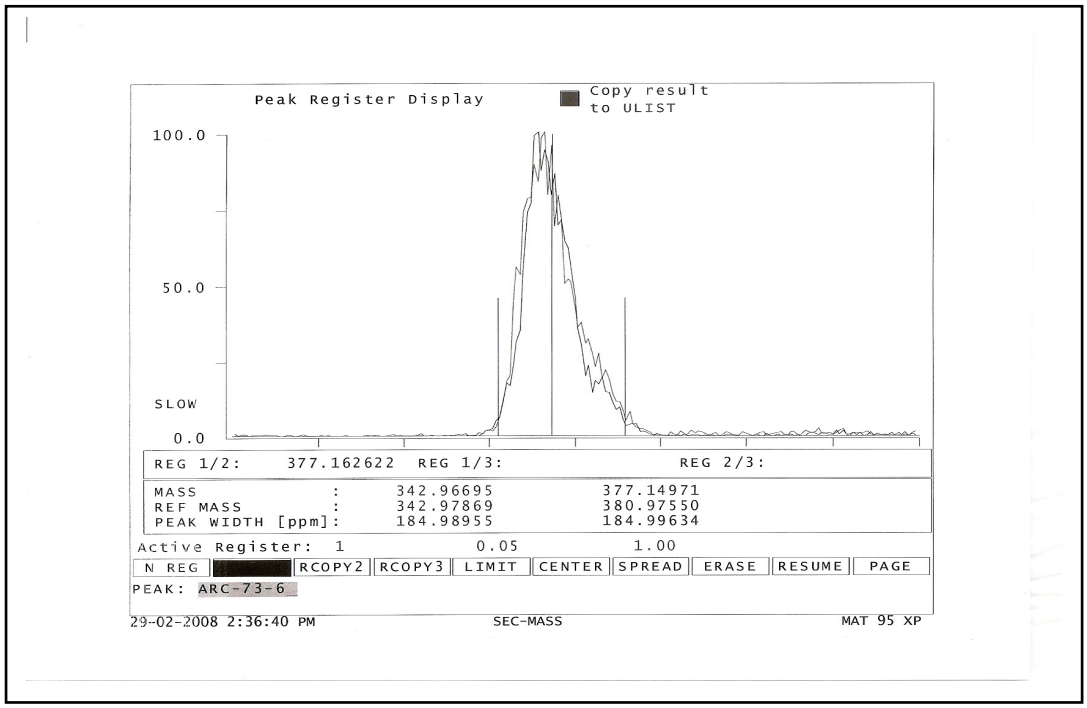

Figure 59 HREIMS spectrum of compound **AM4**

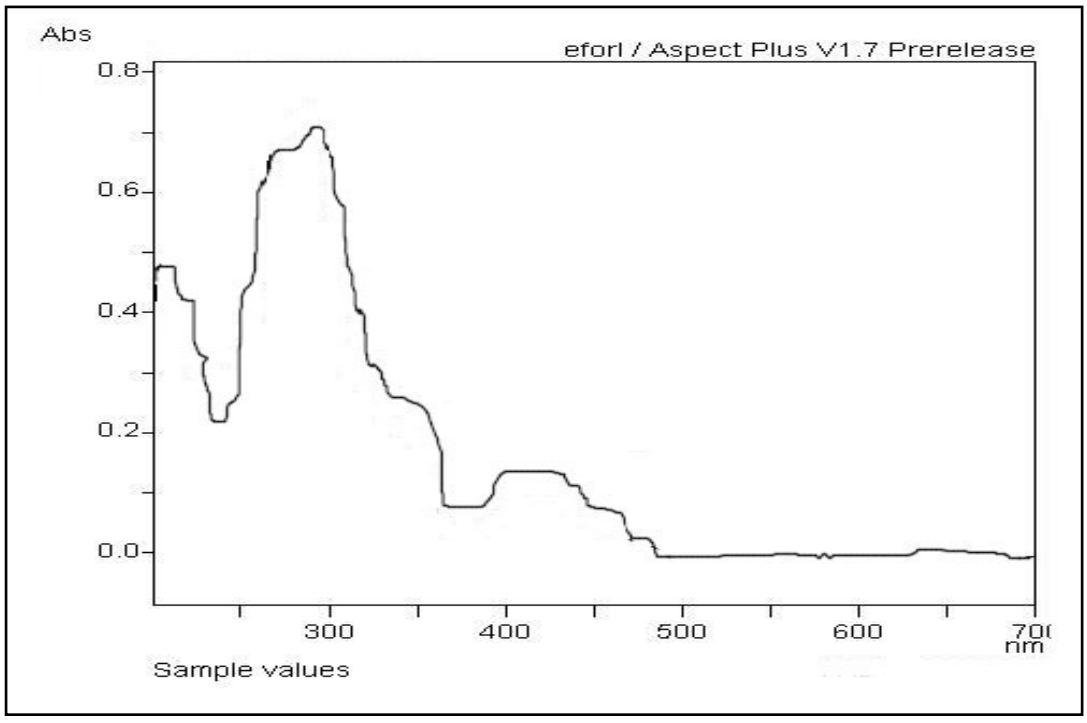

Figure 60 UV (MeOH) spectrum of compound **AM5**

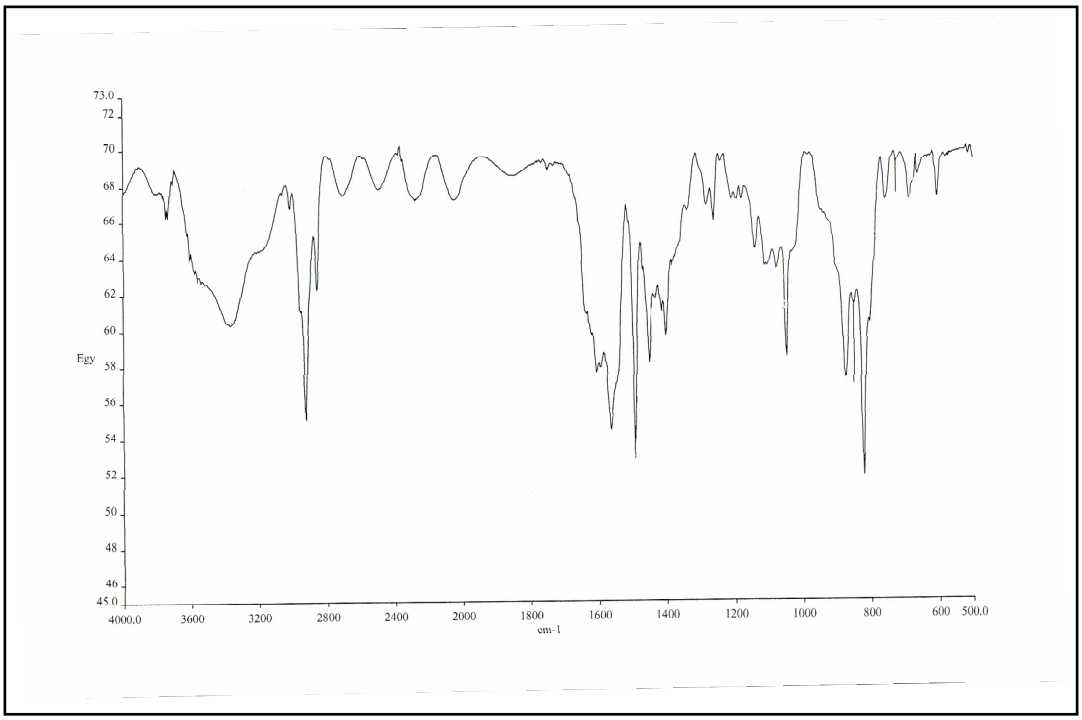

Figure 61 IR (KBr) spectrum of compound **AM5**

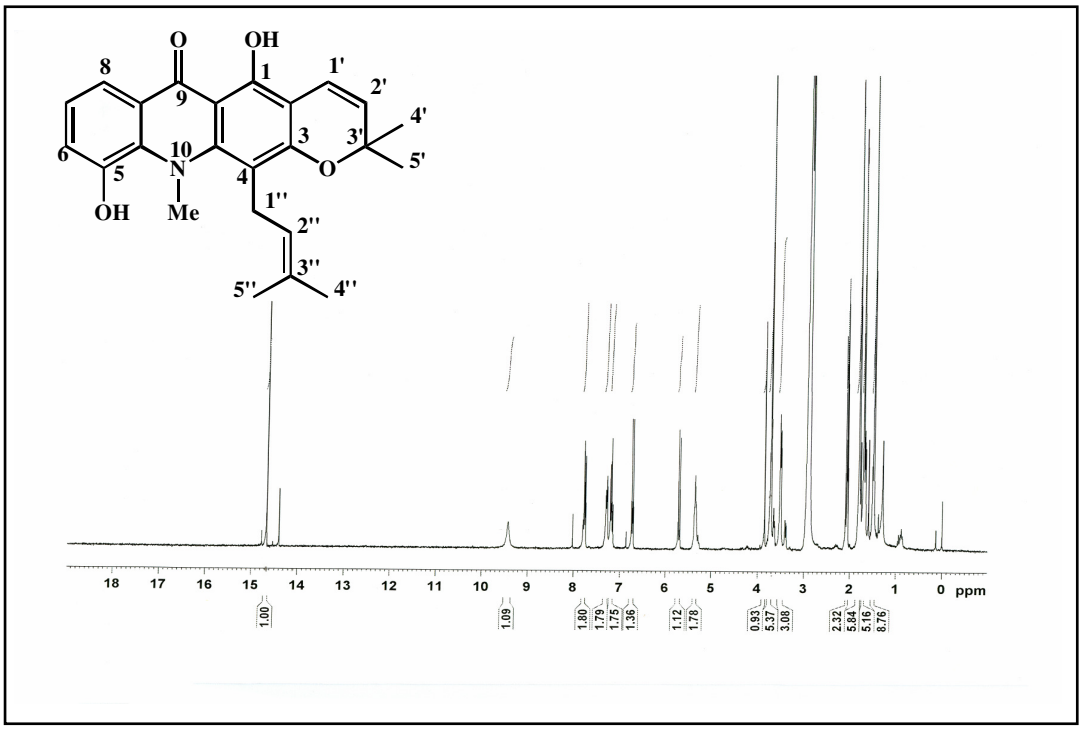

Figure 62 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM5**

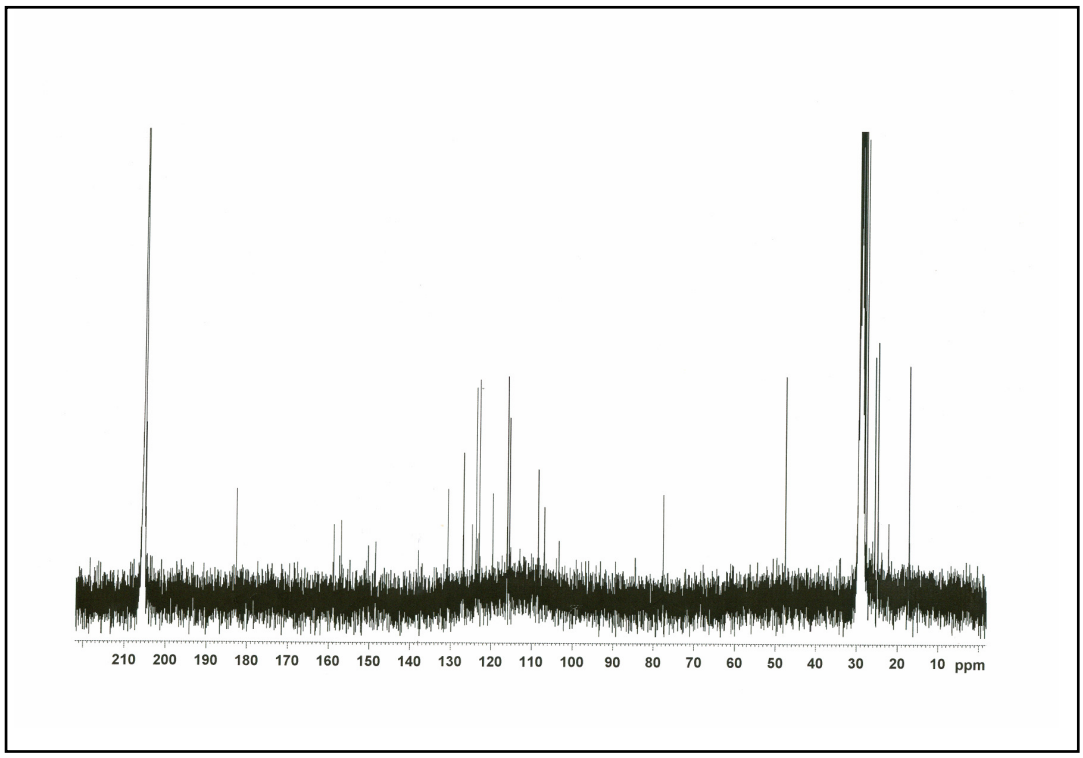

Figure 63 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM5**

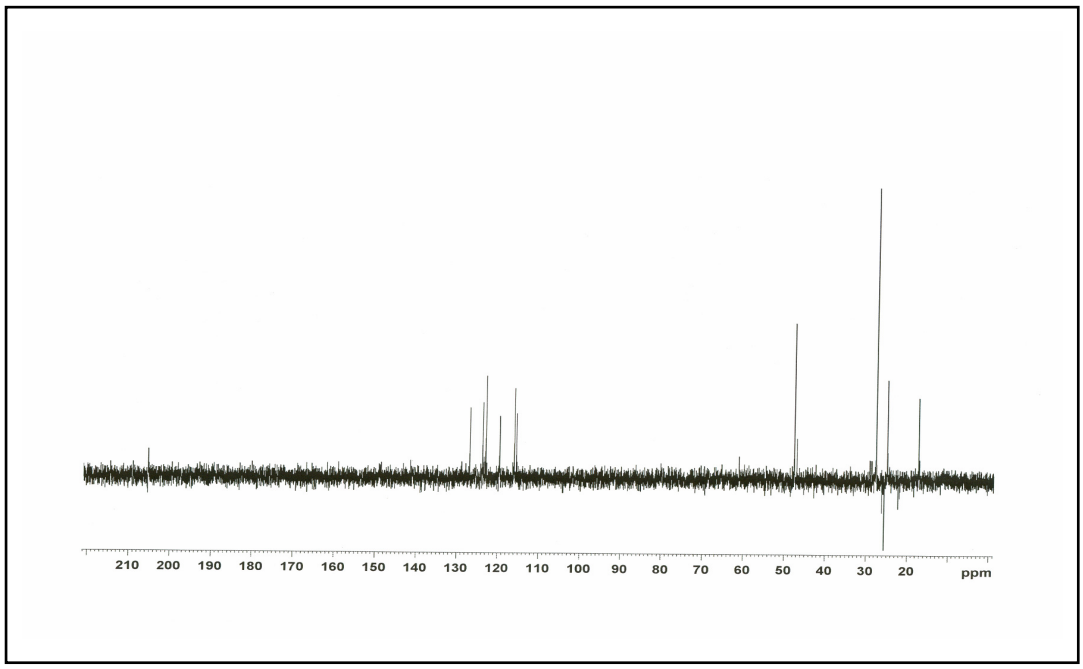

Figure 64 DEPT 135° (acetone-d_6) spectrum of compound **AM5**

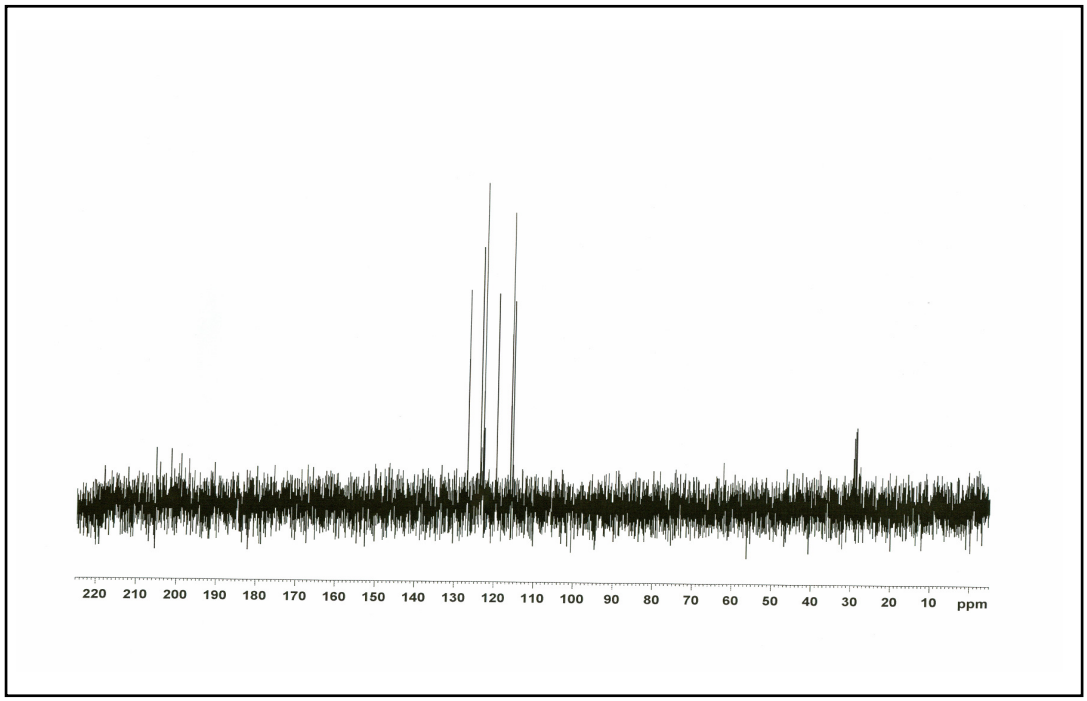

Figure 65 DEPT 90° (acetone-d_6) spectrum of compound **AM5**

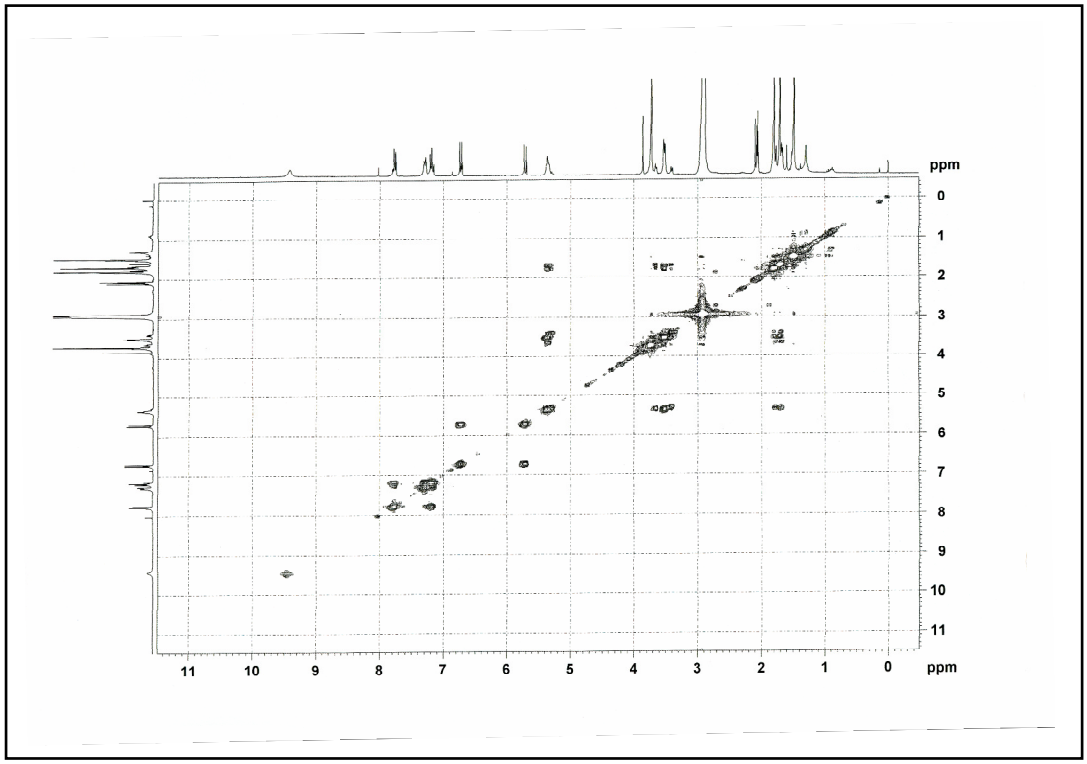

Figure 66 2D COSY (acetone-d_6) spectrum of compound **AM5**

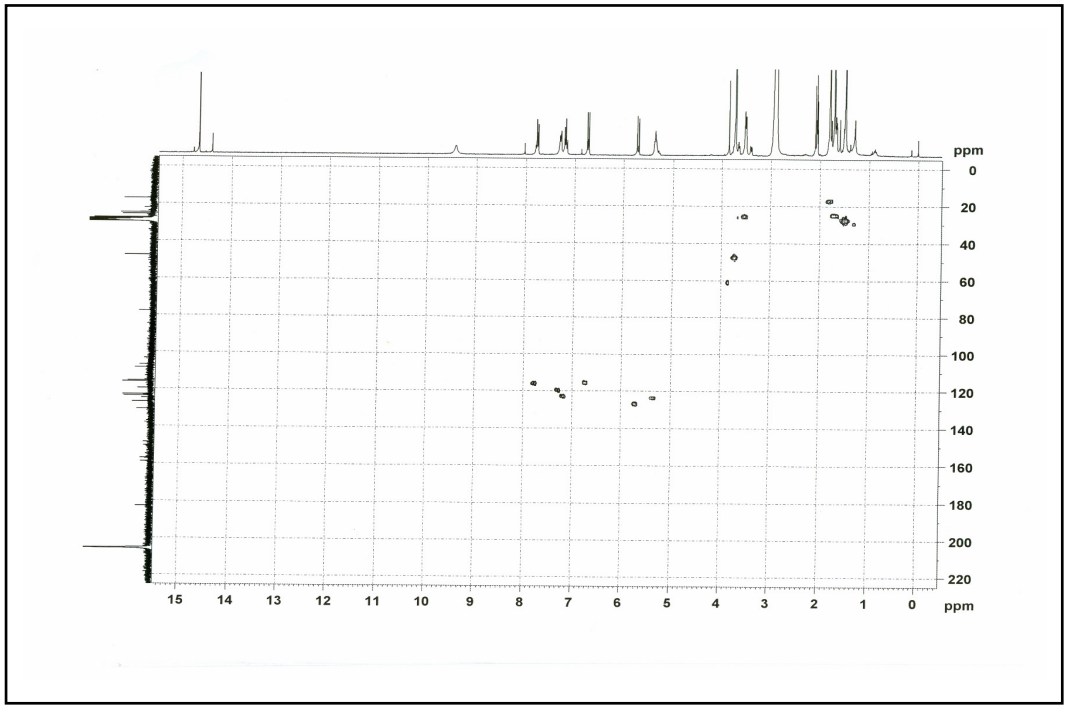

Figure 67 2D HMQC (acetone-d_6) spectrum of compound **AM5**

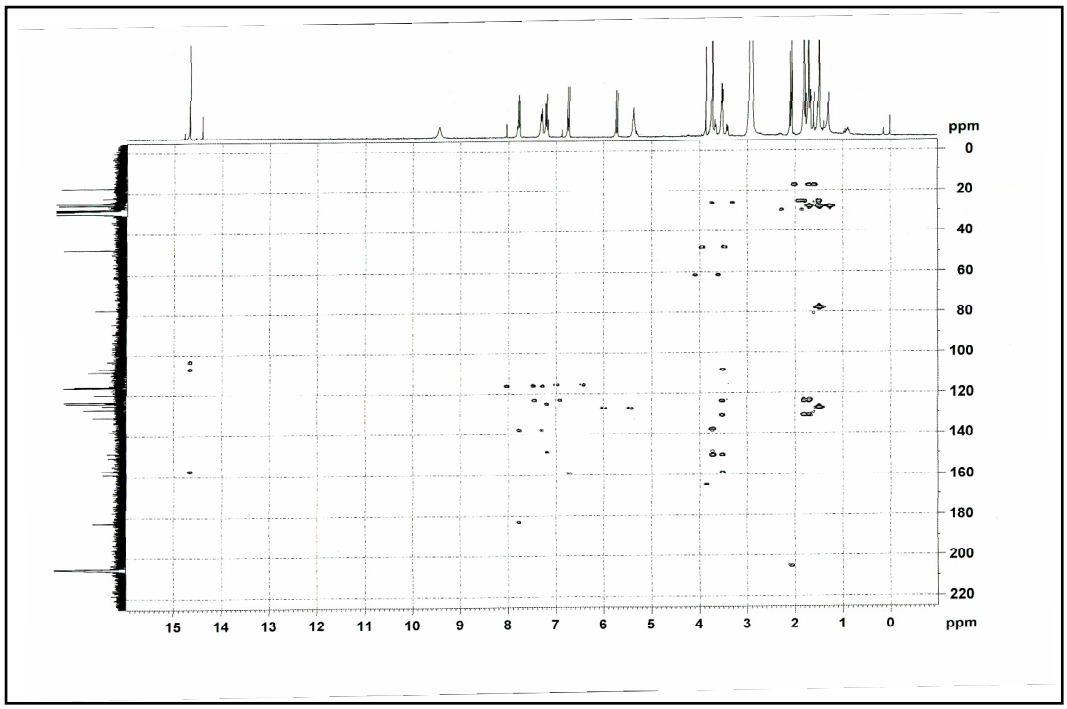

Figure 68 2D HMBC (acetone-d_6) spectrum of compound **AM5**

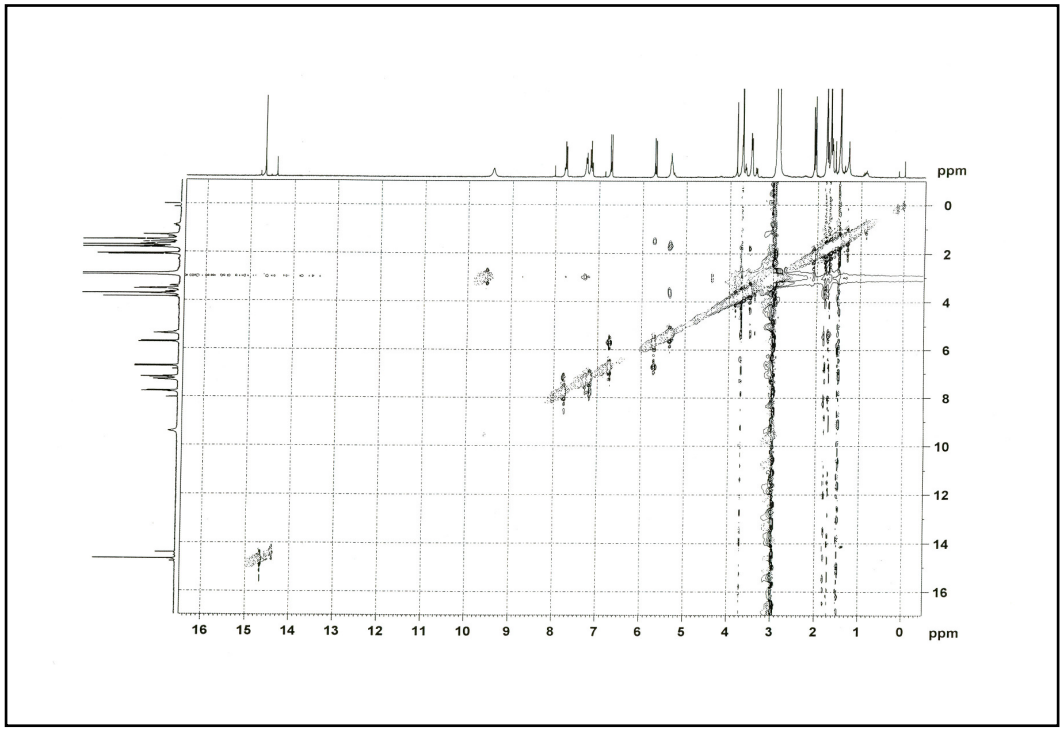

Figure 69 2D NOESY (acetone-d_6) spectrum of compound **AM5**

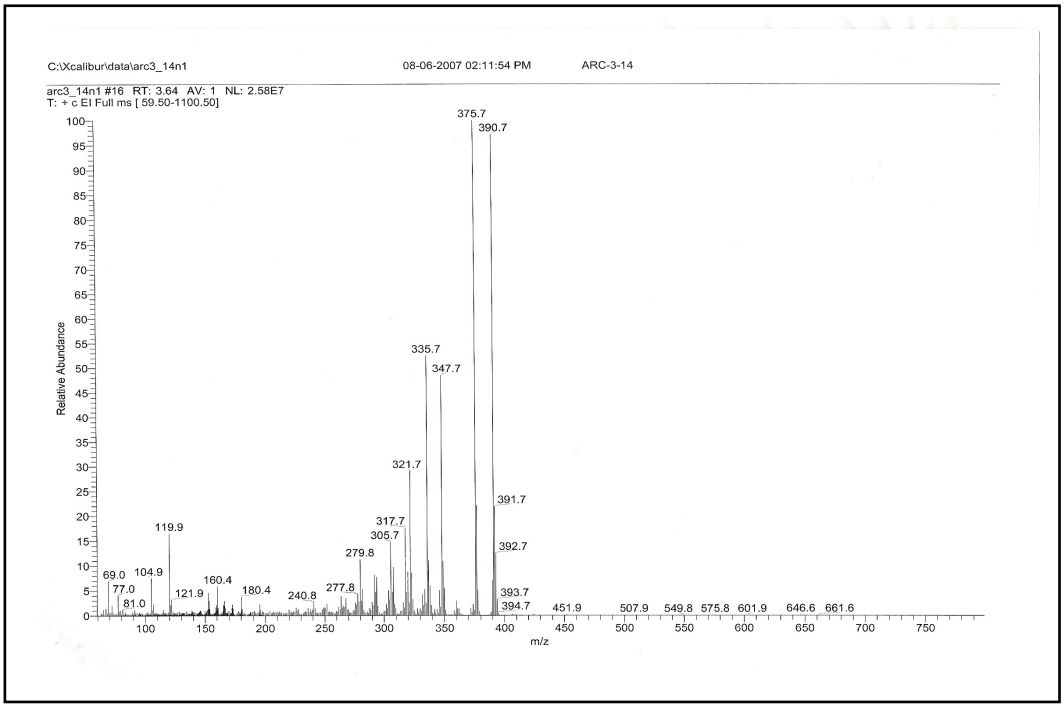

Figure 70 EIMS spectrum of compound **AM5**

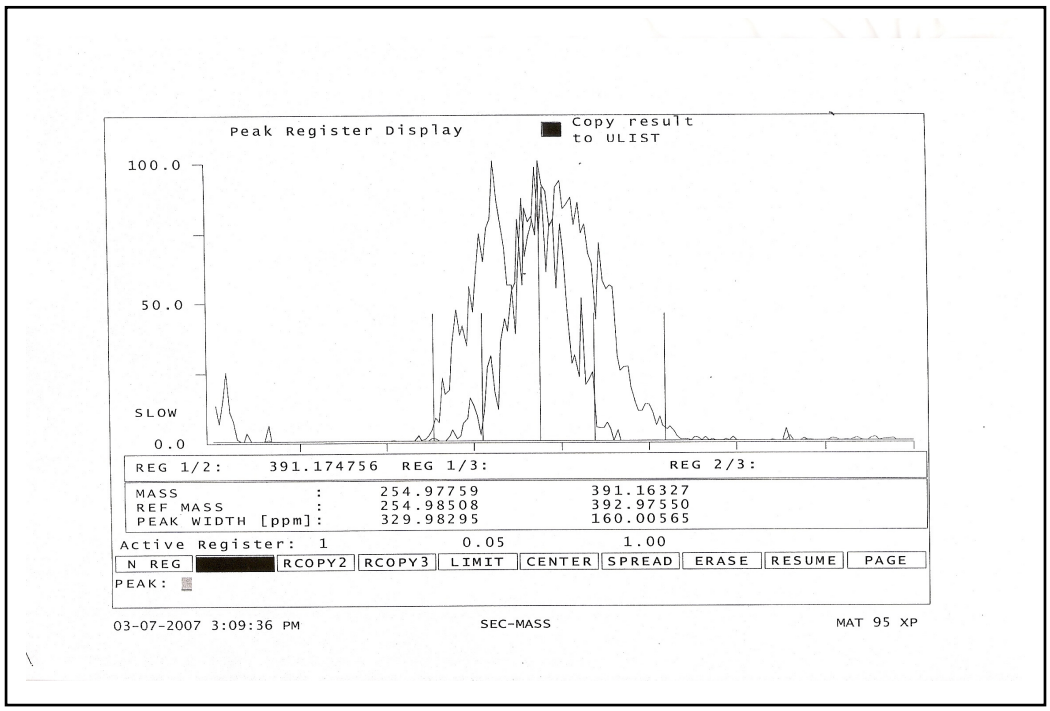

Figure 71 HREIMS spectrum of compound **AM5**

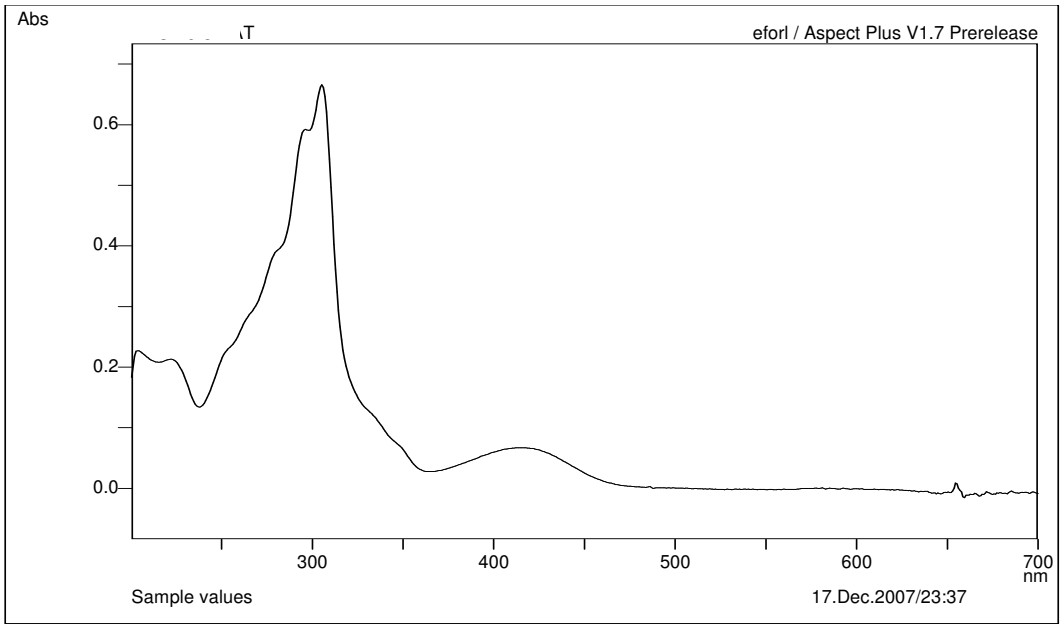

Figure 72 UV (MeOH) spectrum of compound **AM6**

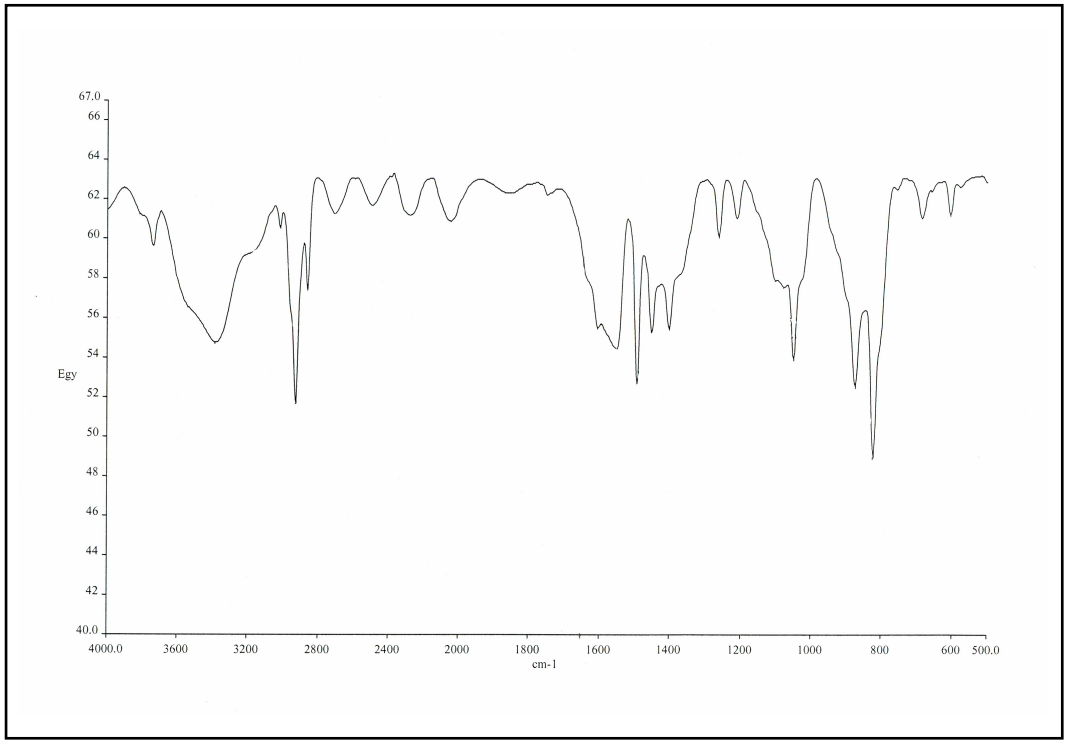

Figure 73 IR (neat) spectrum of compound **AM6**

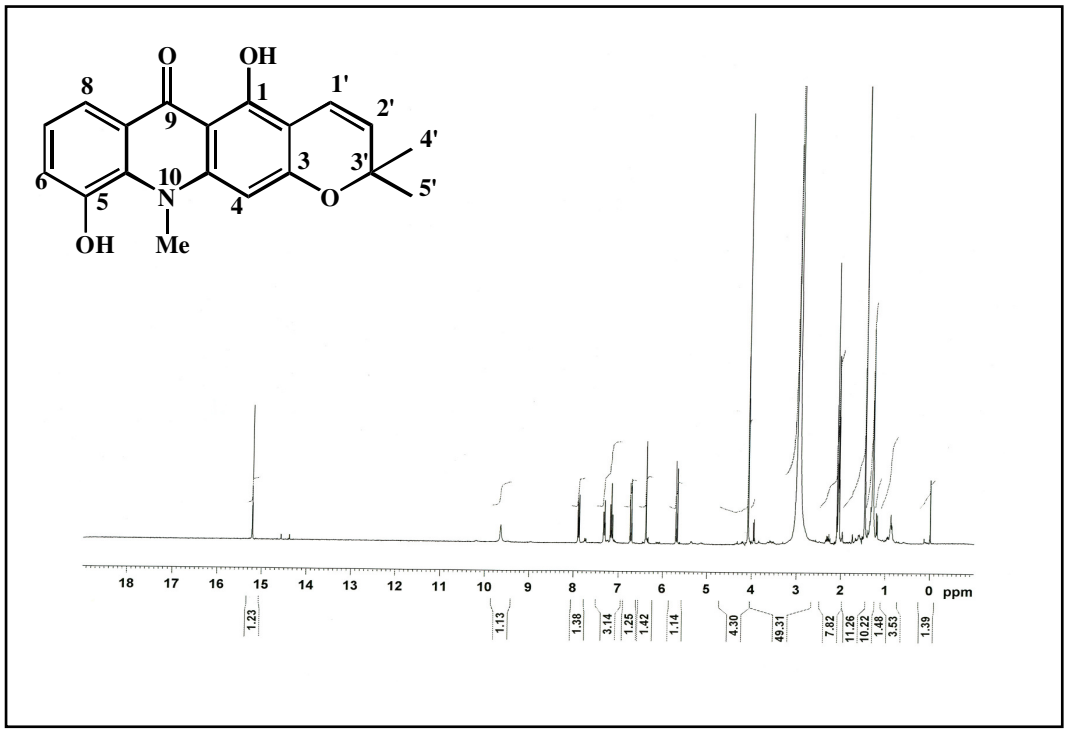

Figure 74 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM6**

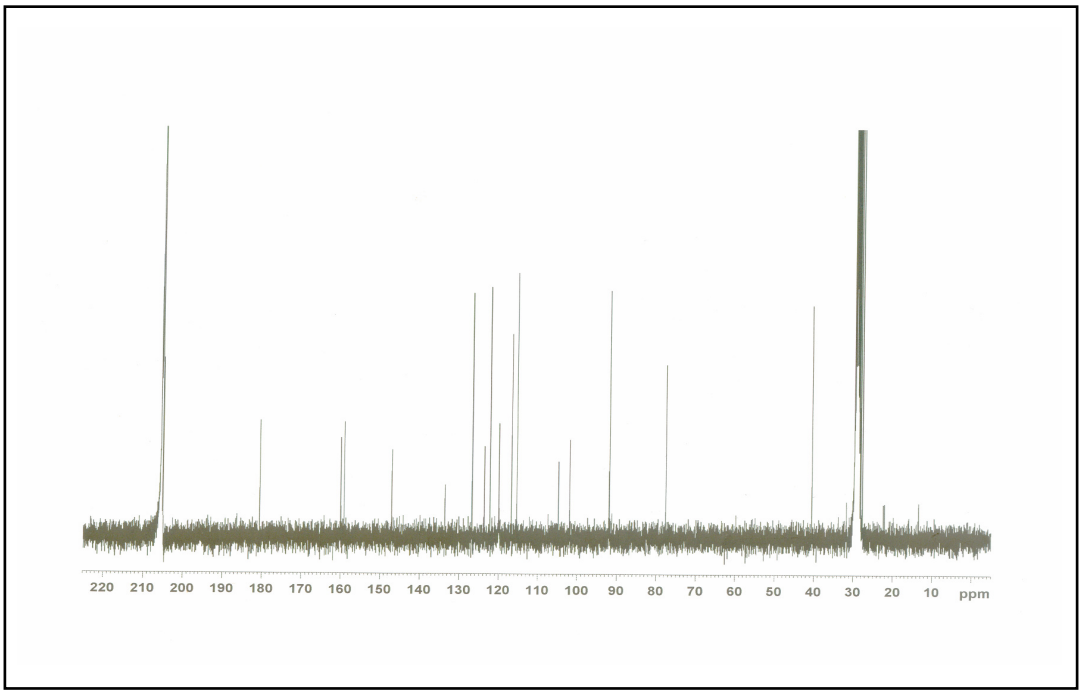

Figure 75 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM6**

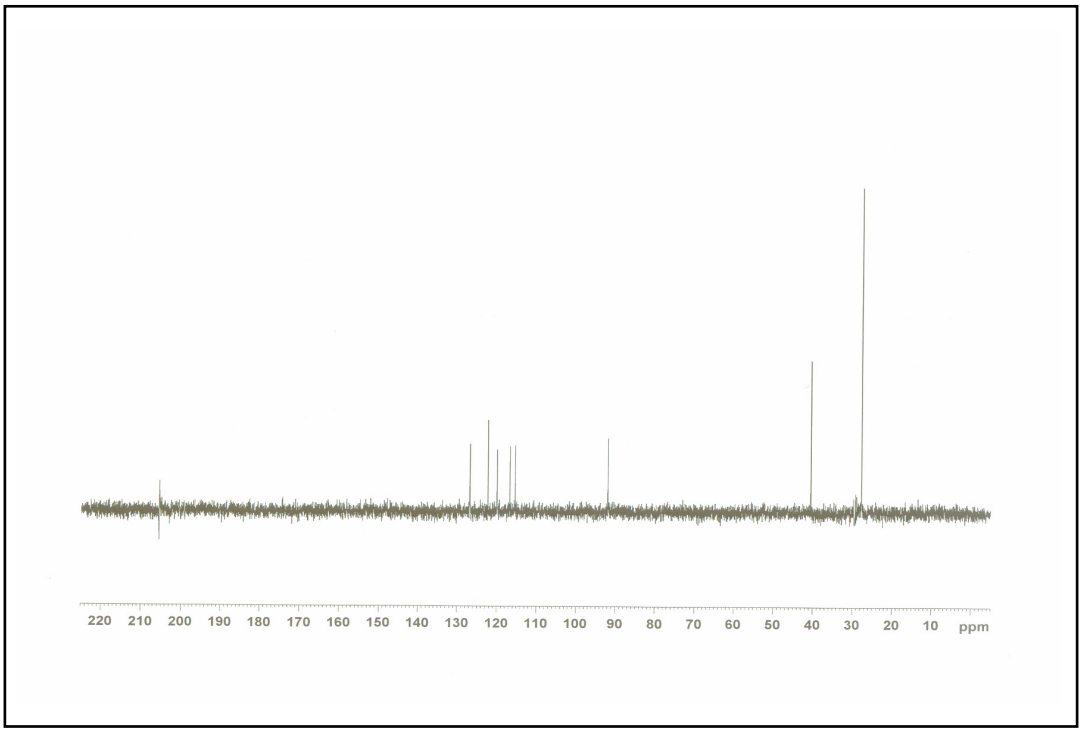

Figure 76 DEPT 135° (acetone-d_6) spectrum of compound **AM6**

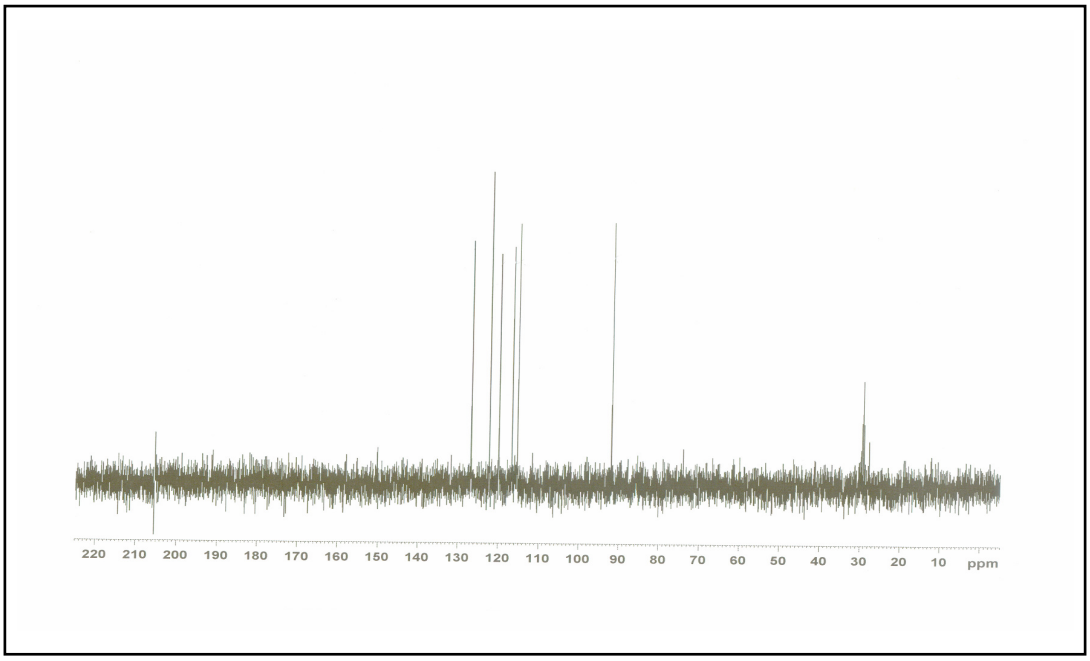

Figure 77 DEPT 90° (acetone-d_6) spectrum of compound **AM6**

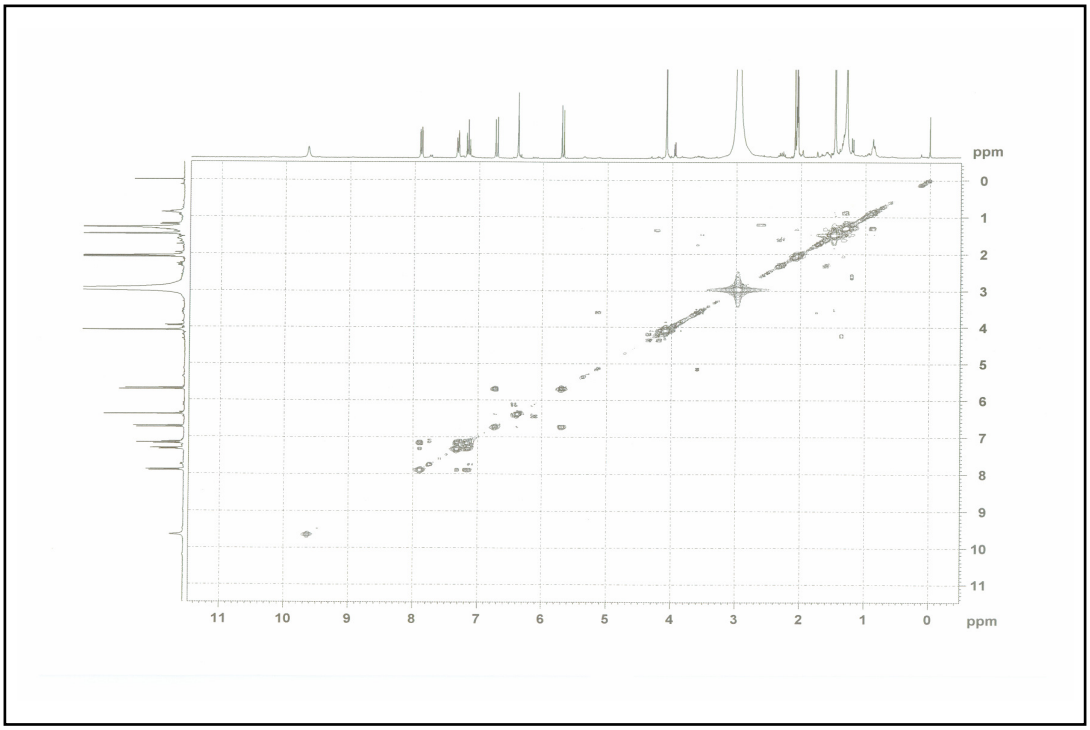

Figure 78 2D COSY (acetone-d_6) spectrum of compound **AM6**

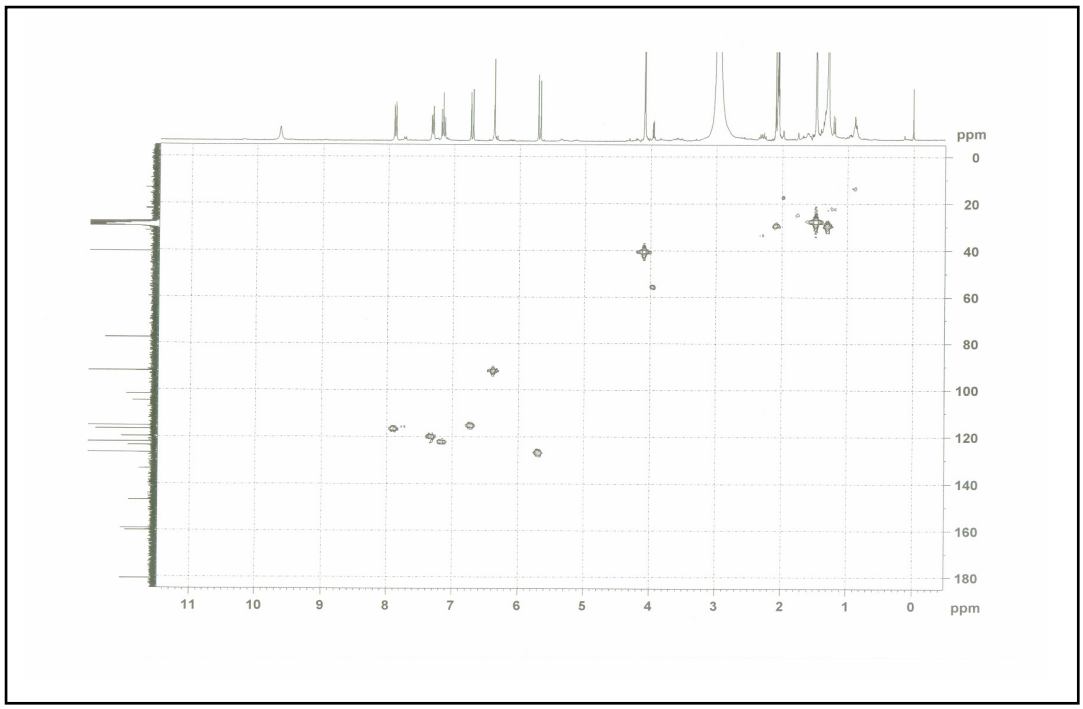

Figure 79 2D HMQC (acetone-d_6) spectrum of compound **AM6**

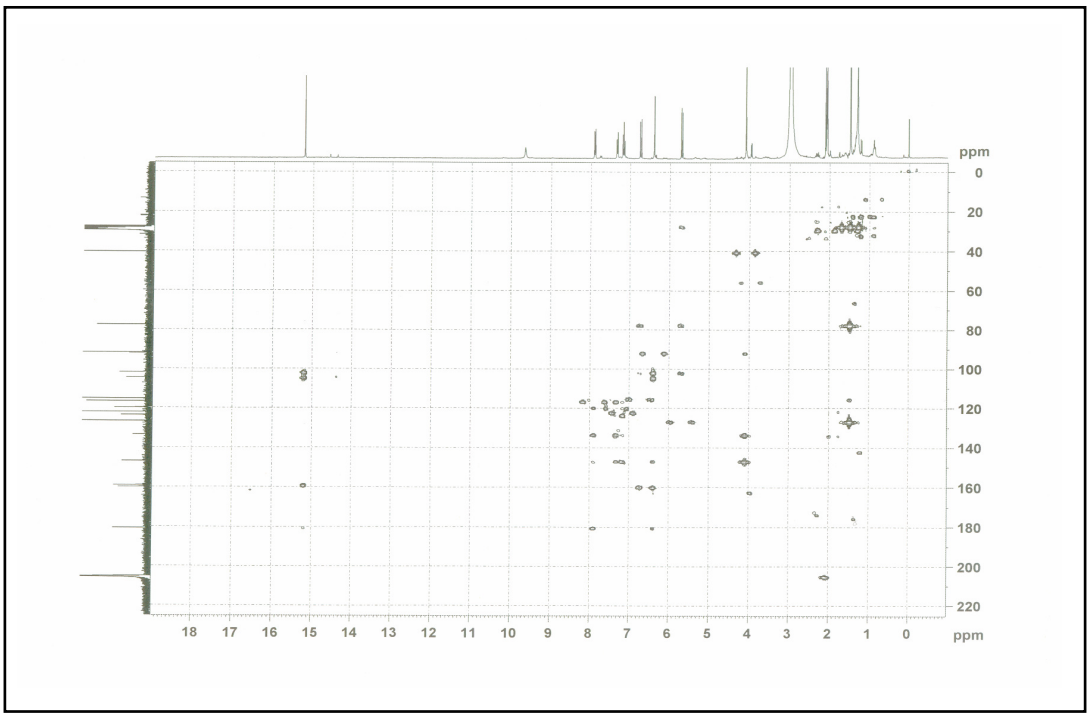

Figure 80 2D HMBC (acetone-d_6) spectrum of compound **AM6**

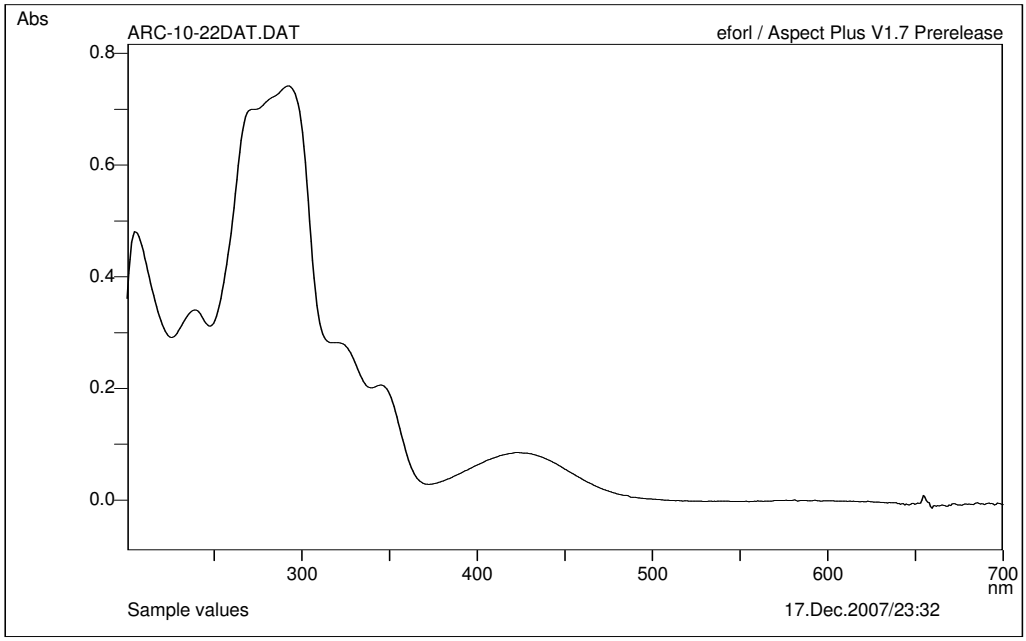

Figure 81 UV (MeOH) spectrum of compound **AM7**

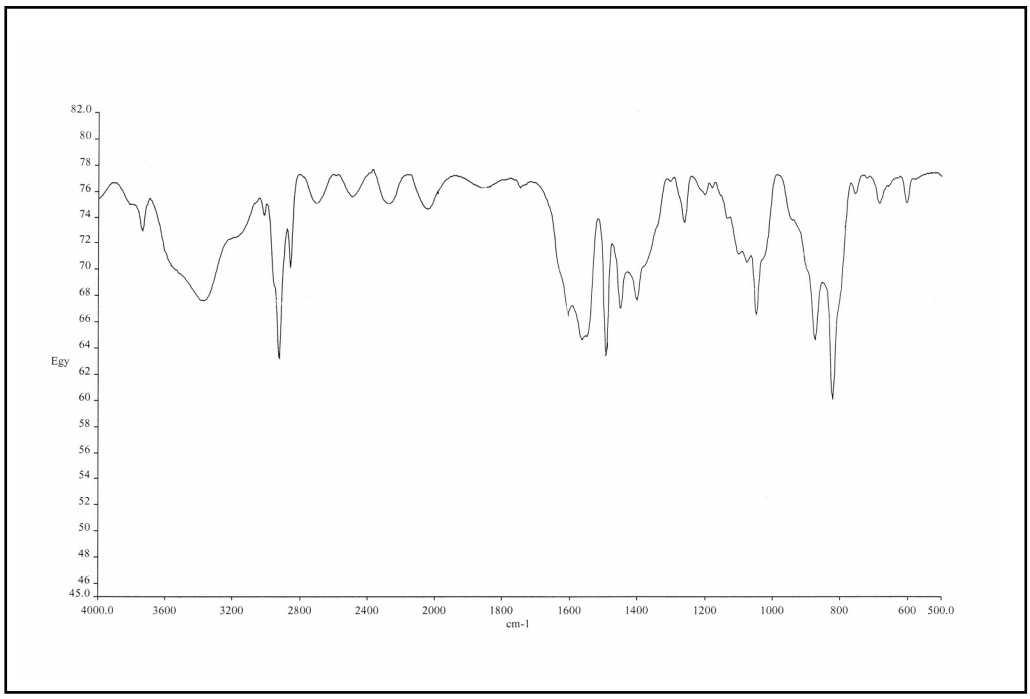

Figure 82 IR (neat) spectrum of compound **AM7**

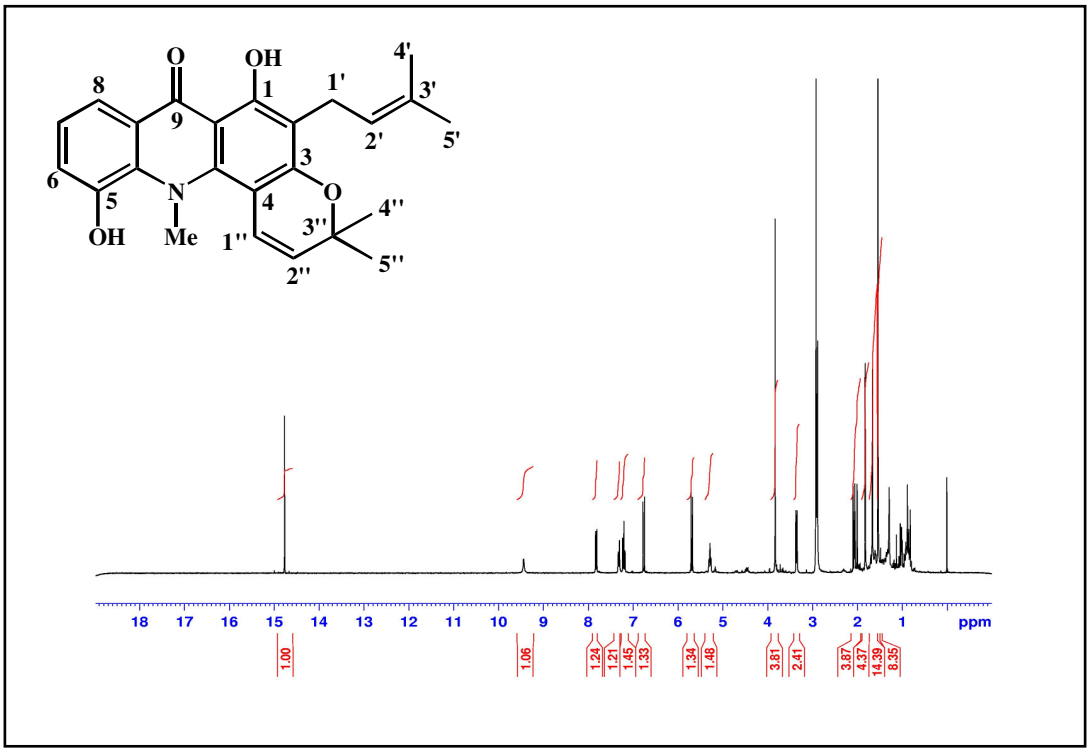

Figure 83 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM7**

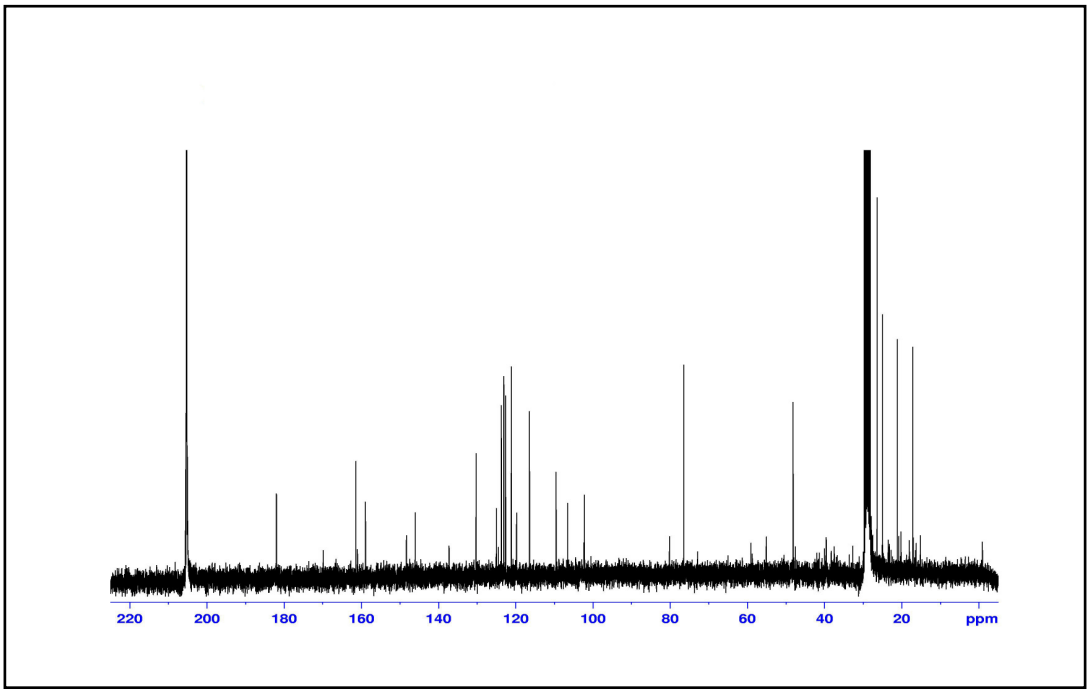

Figure 84 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM7**

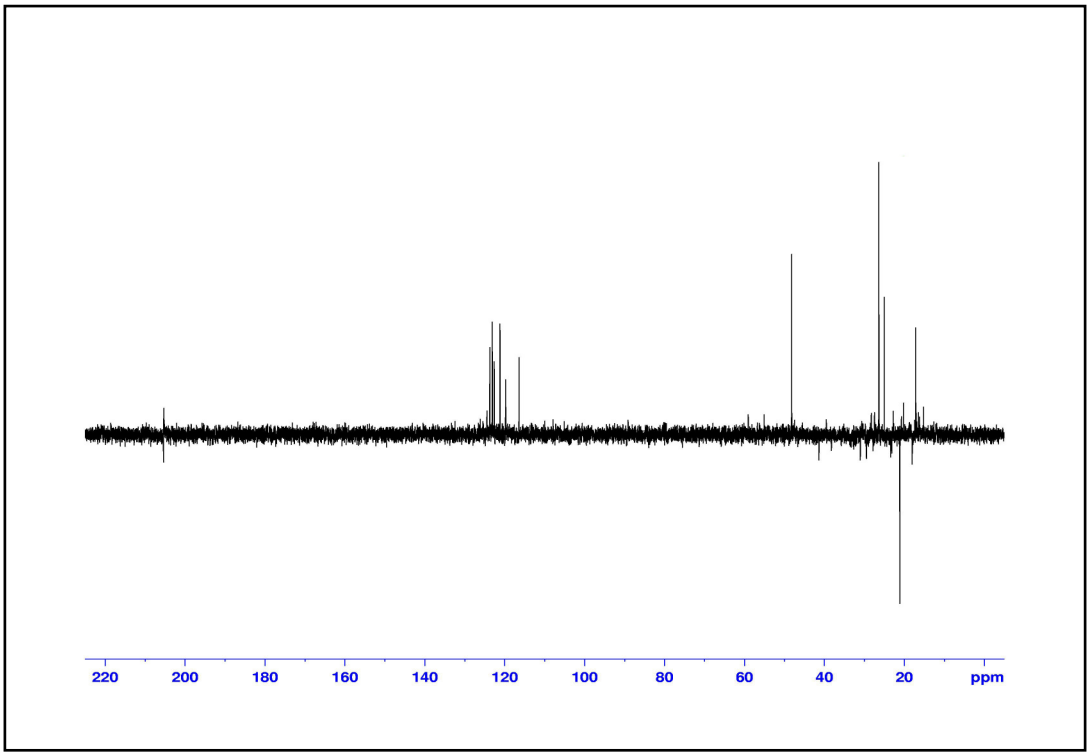

Figure 85 DEPT 135° (acetone-d_6) spectrum of compound **AM7**

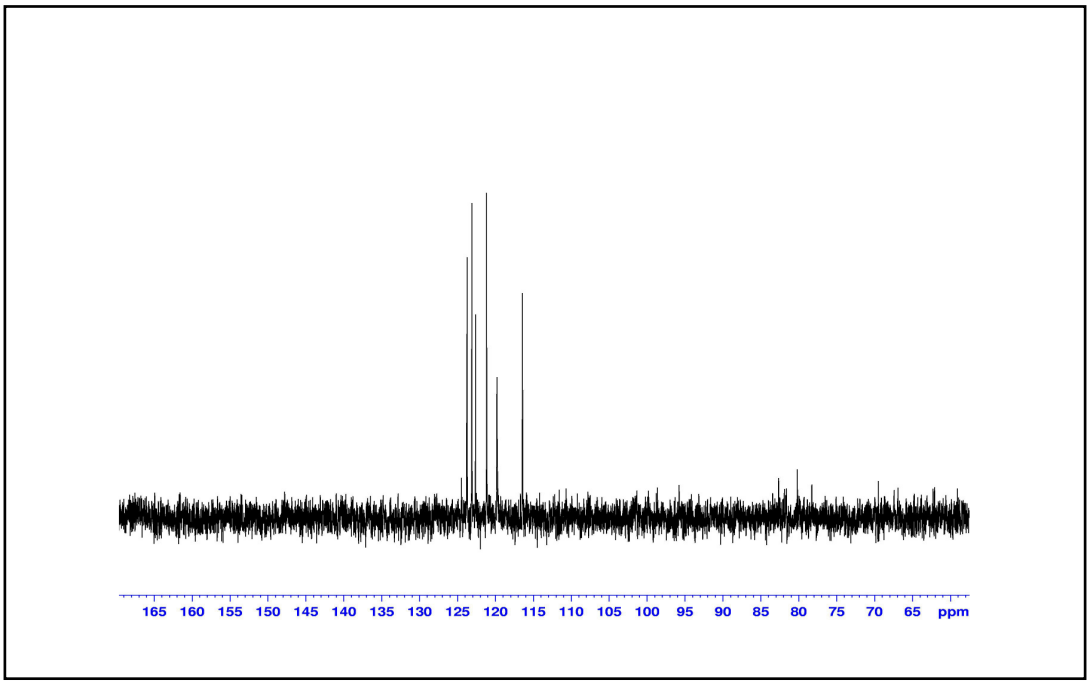

Figure 86 DEPT 90° (acetone-d_6) spectrum of compound **AM7**

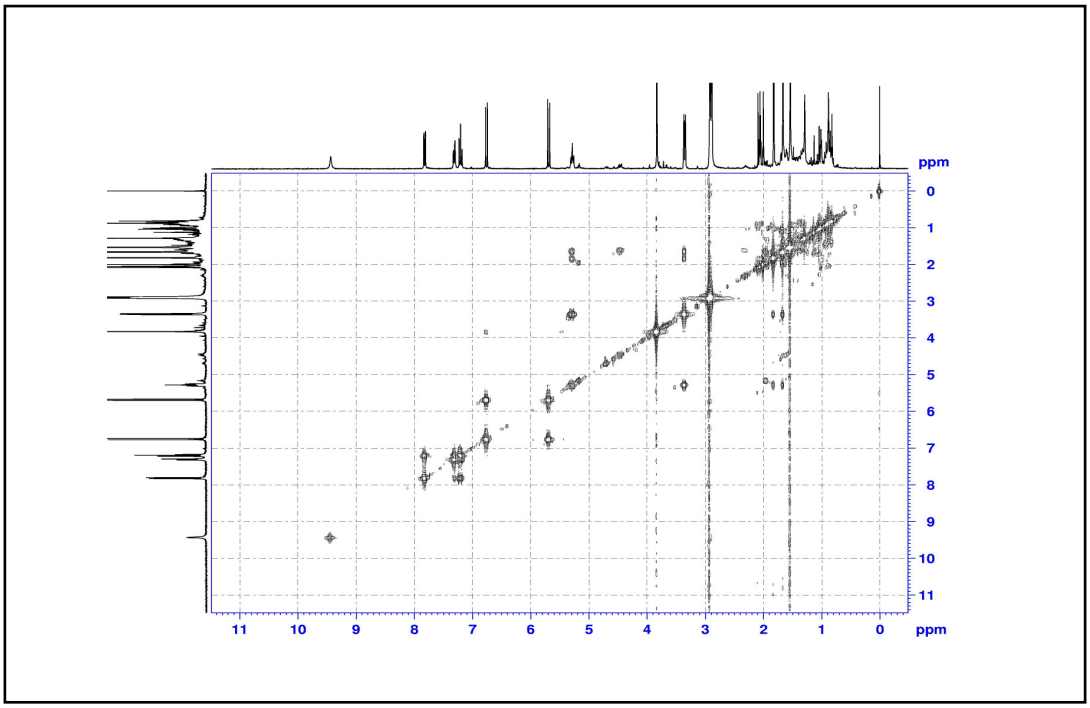

Figure 87 2D COSY (acetone-d_6) spectrum of compound **AM7**

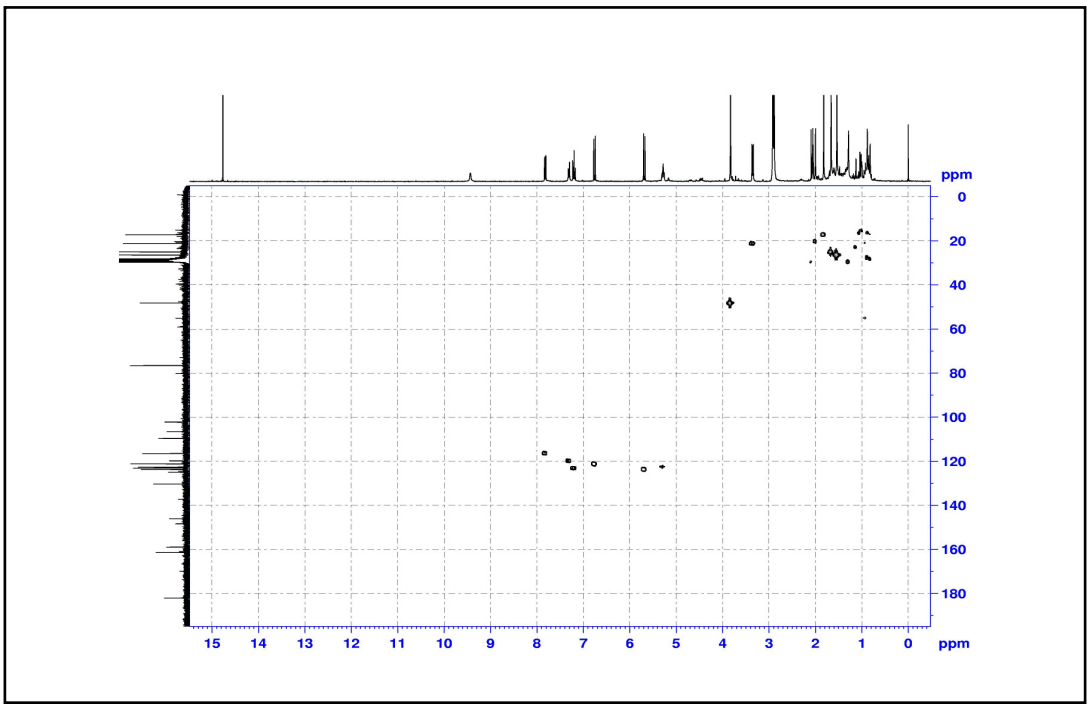

Figure 88 2D HMQC (acetone-d_6) spectrum of compound **AM7**

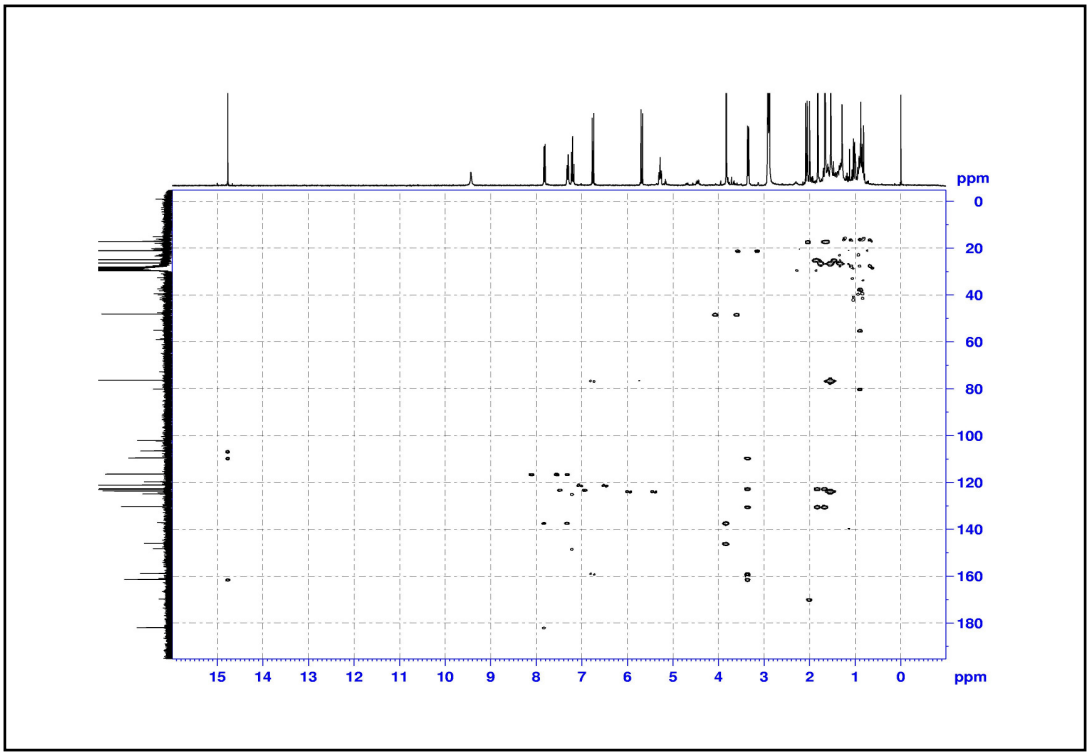

Figure 89 2D HMBC (acetone-d_6) spectrum of compound **AM7**

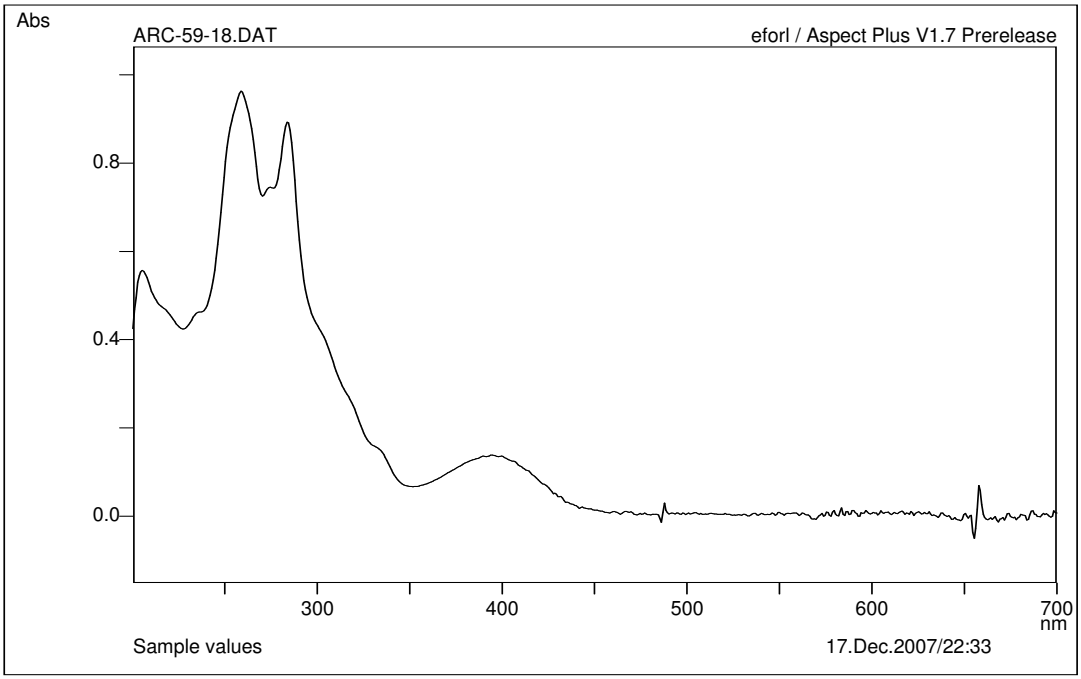

Figure 90 UV (MeOH) spectrum of compound **AM8**

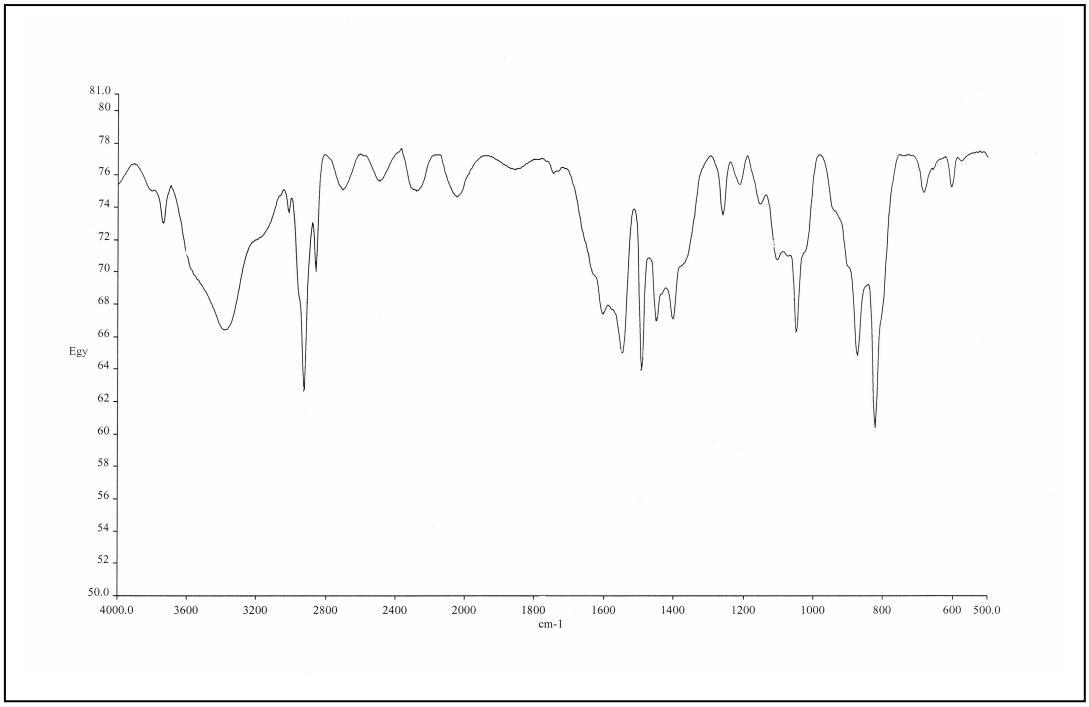

Figure 91 IR (neat) spectrum of compound **AM8**

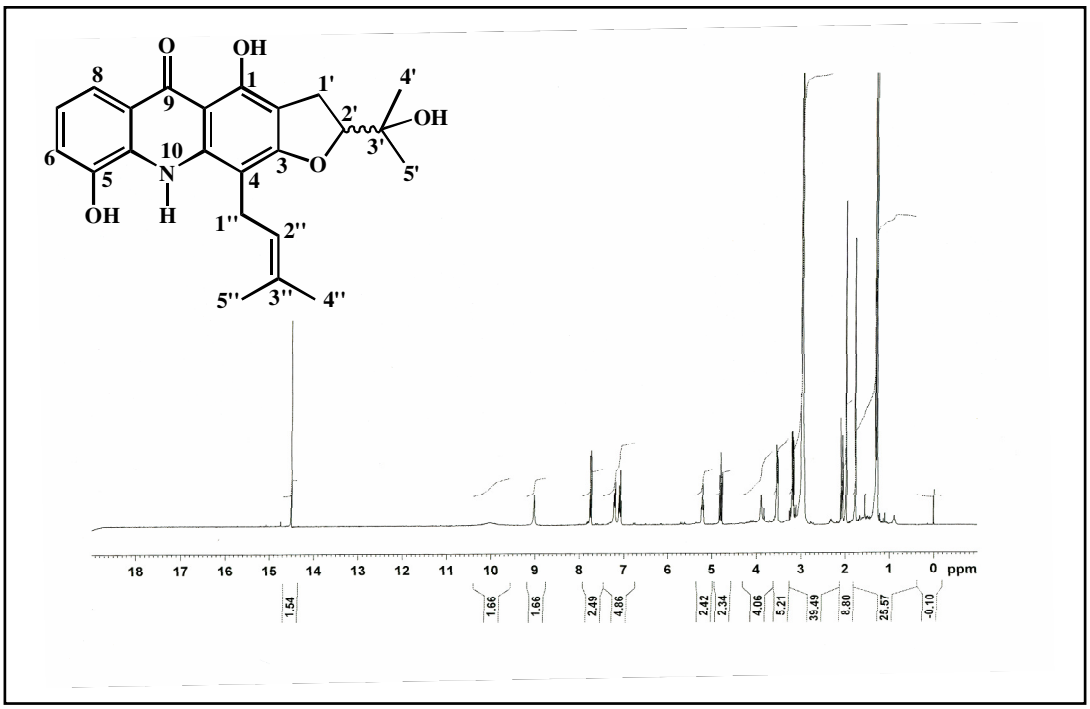

Figure 92 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM8**

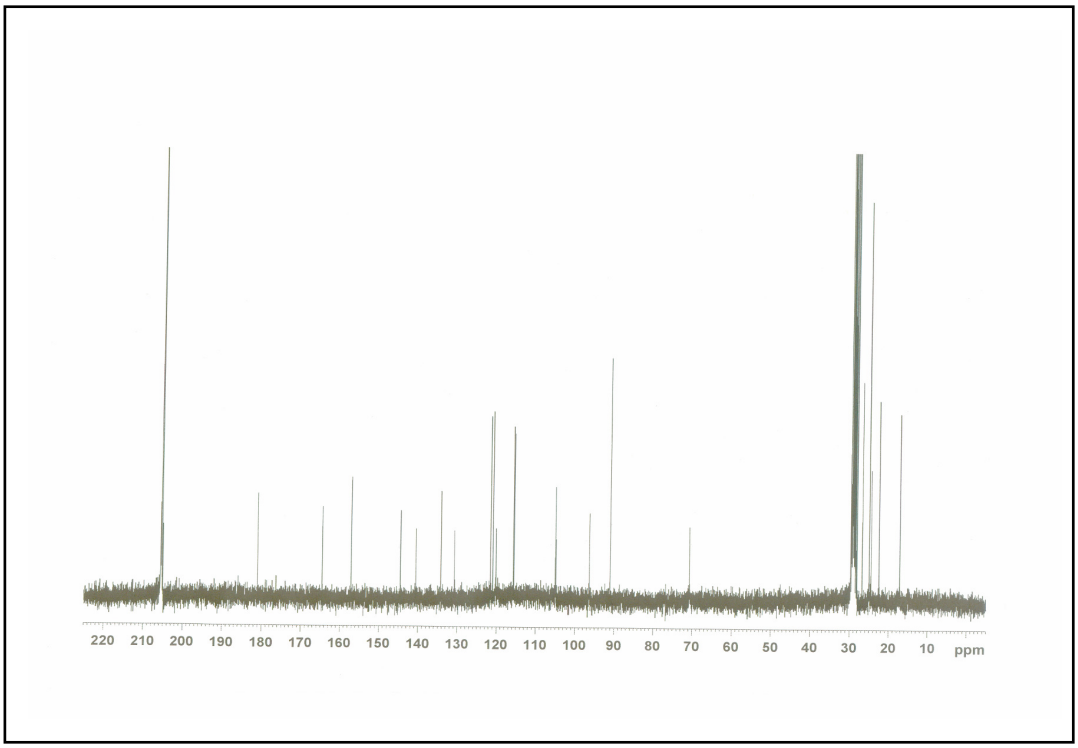

Figure 93 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM8**

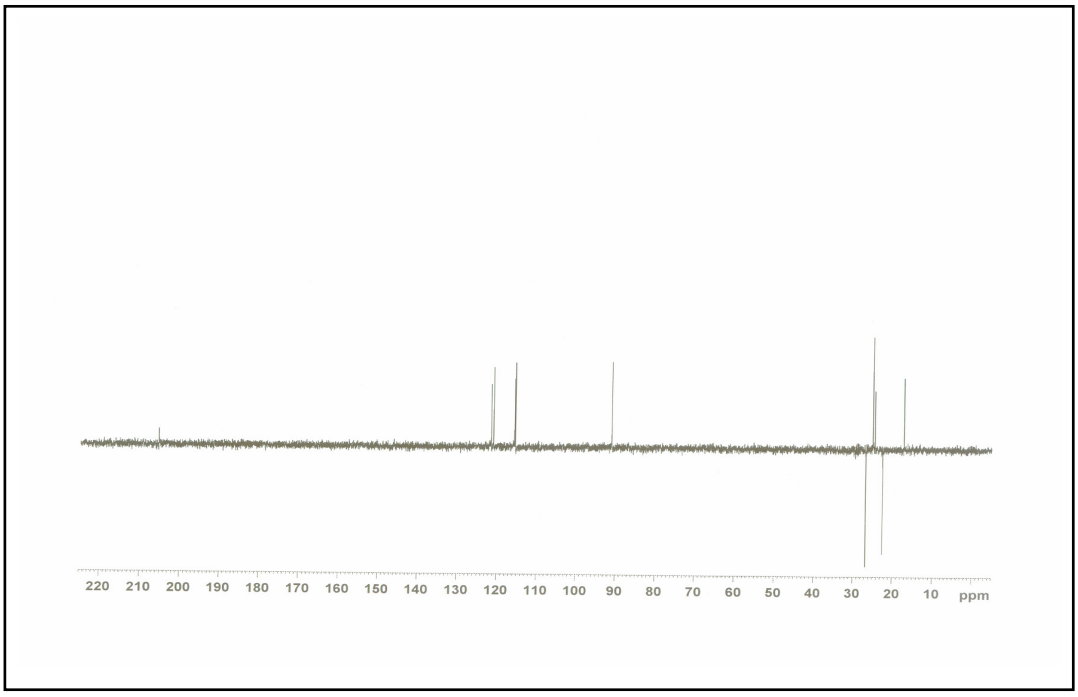

Figure 94 DEPT 135° (acetone-d_6) spectrum of compound **AM8**

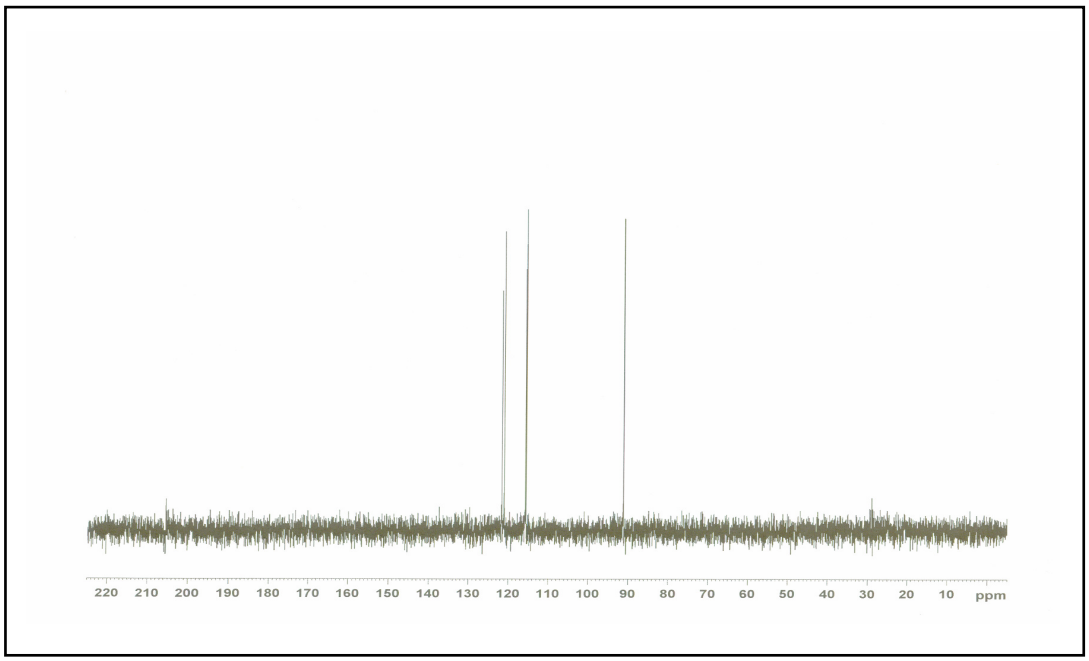

Figure 95 DEPT 90° (acetone-d_6) spectrum of compound **AM8**

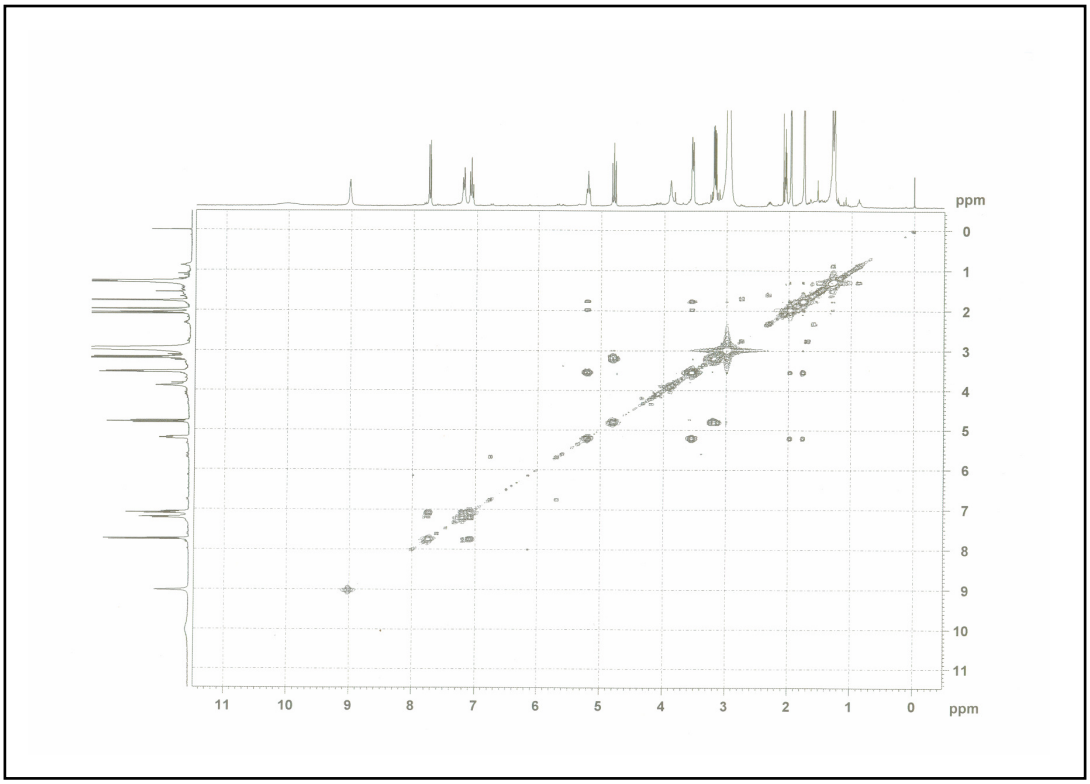

Figure 96 2D COSY (acetone-d_6) spectrum of compound **AM8**

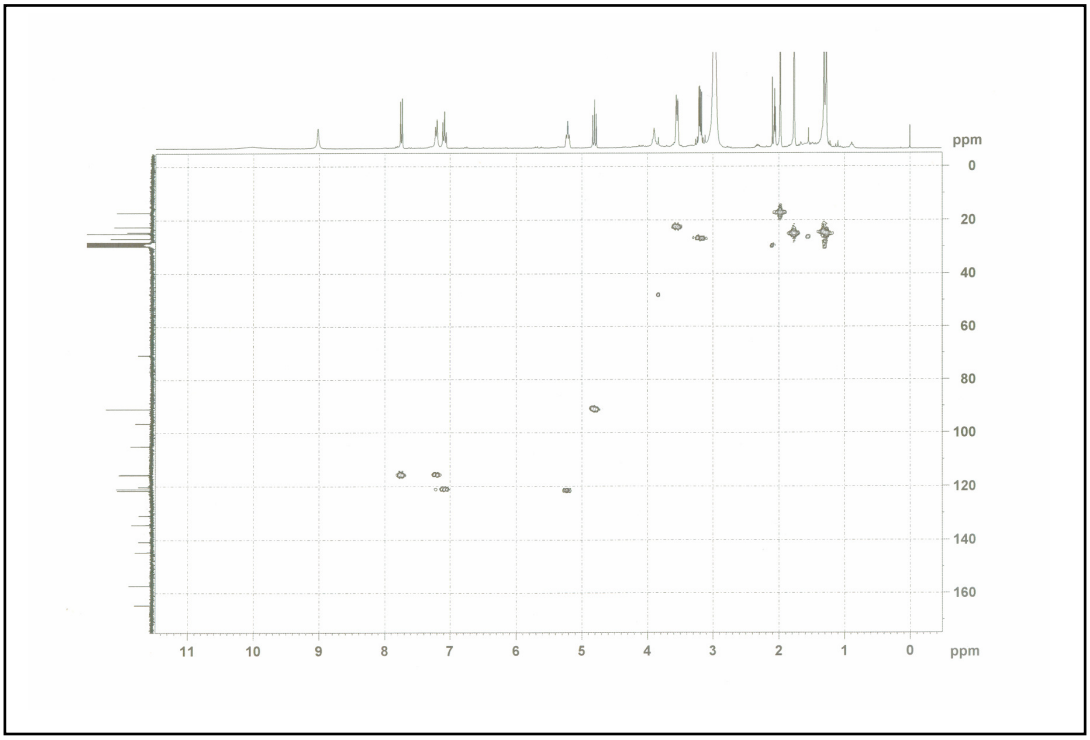

Figure 97 2D HMQC (acetone-d_6) spectrum of compound **AM8**

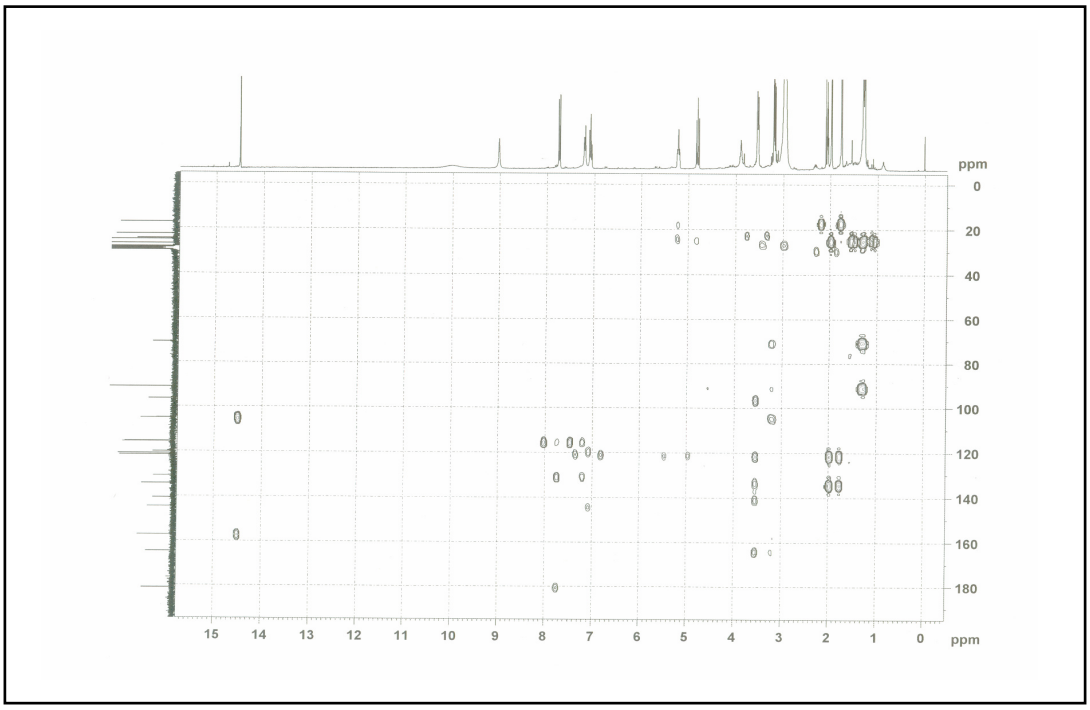

Figure 98 2D HMBC (acetone-d_6) spectrum of compound **AM8**

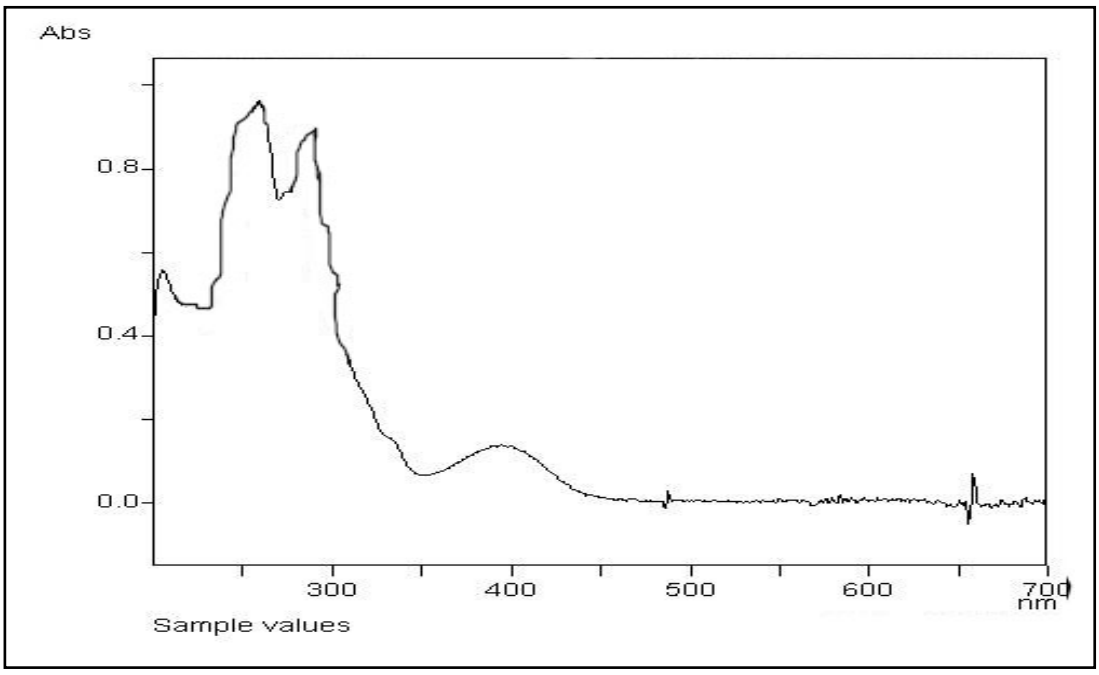

Figure 99 UV (MeOH) spectrum of compound **AM9**

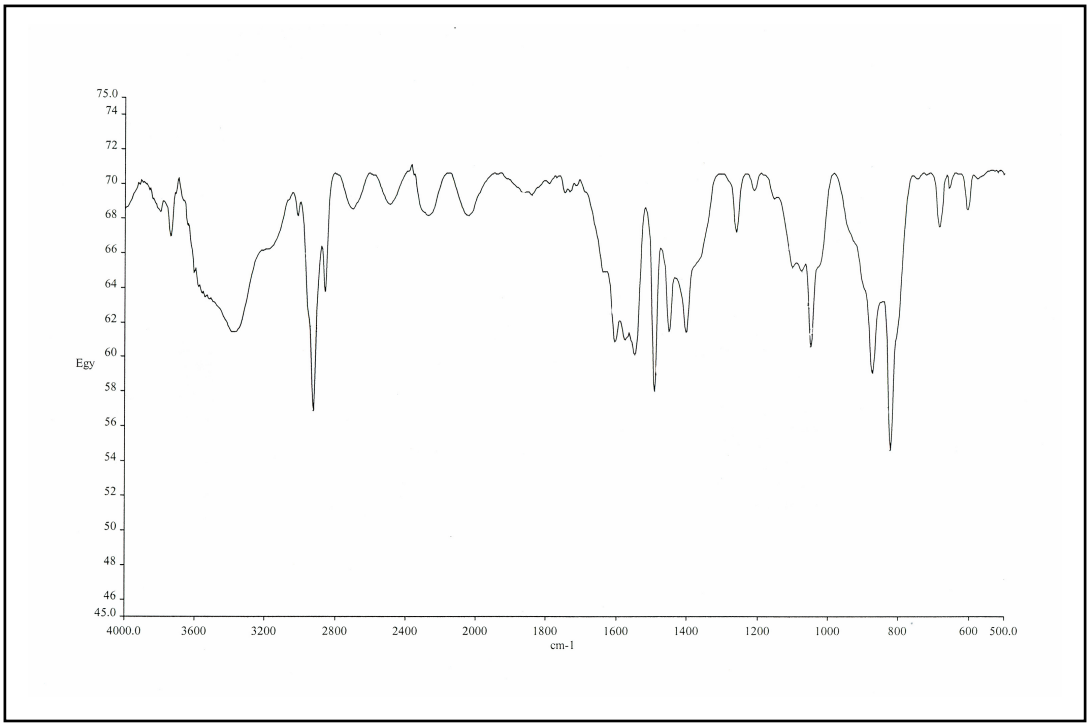

Figure 100 IR (KBr) spectrum of compound **AM9**

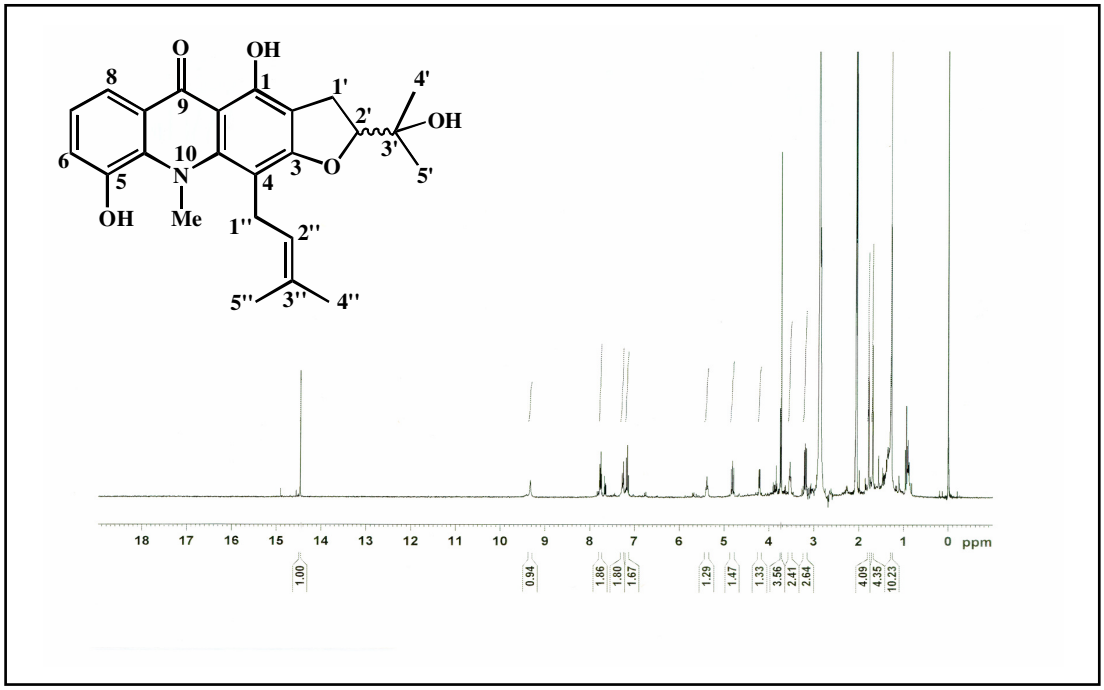

Figure 101 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM9**

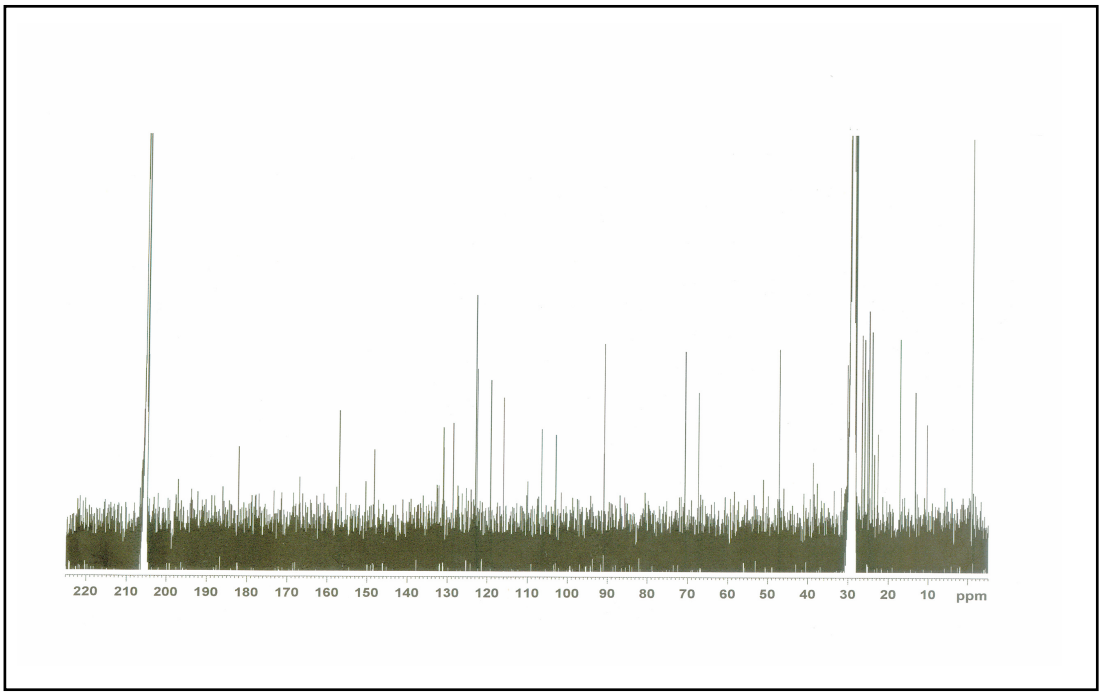

Figure 102 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM9**

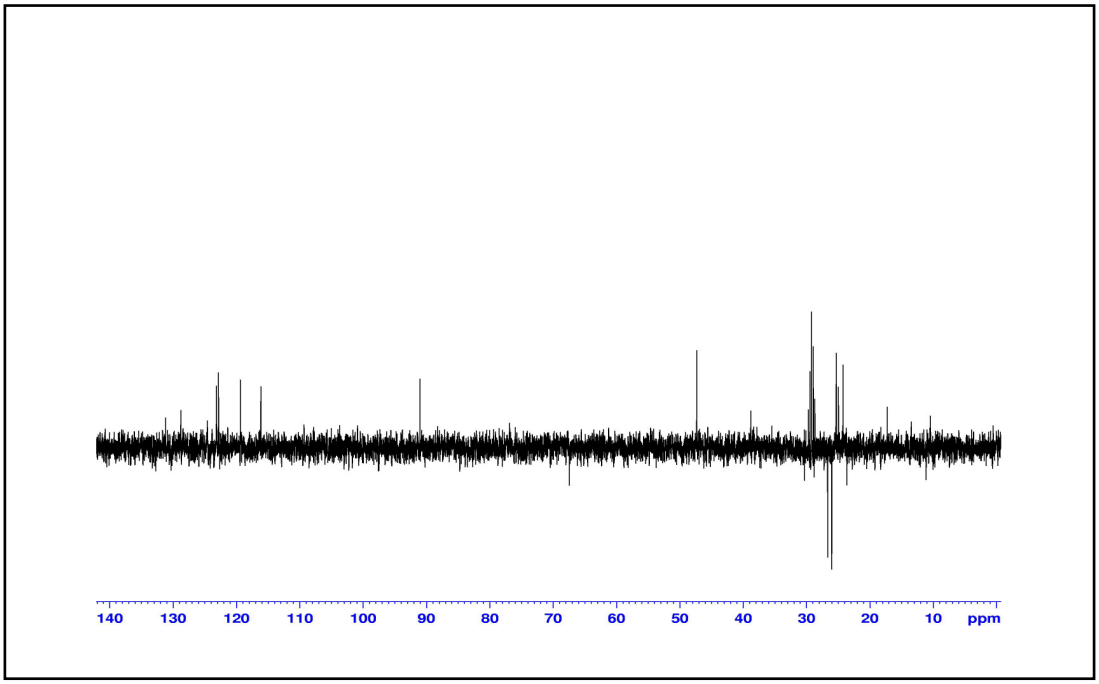

Figure 103 DEPT 135° (acetone-d_6) spectrum of compound **AM9**

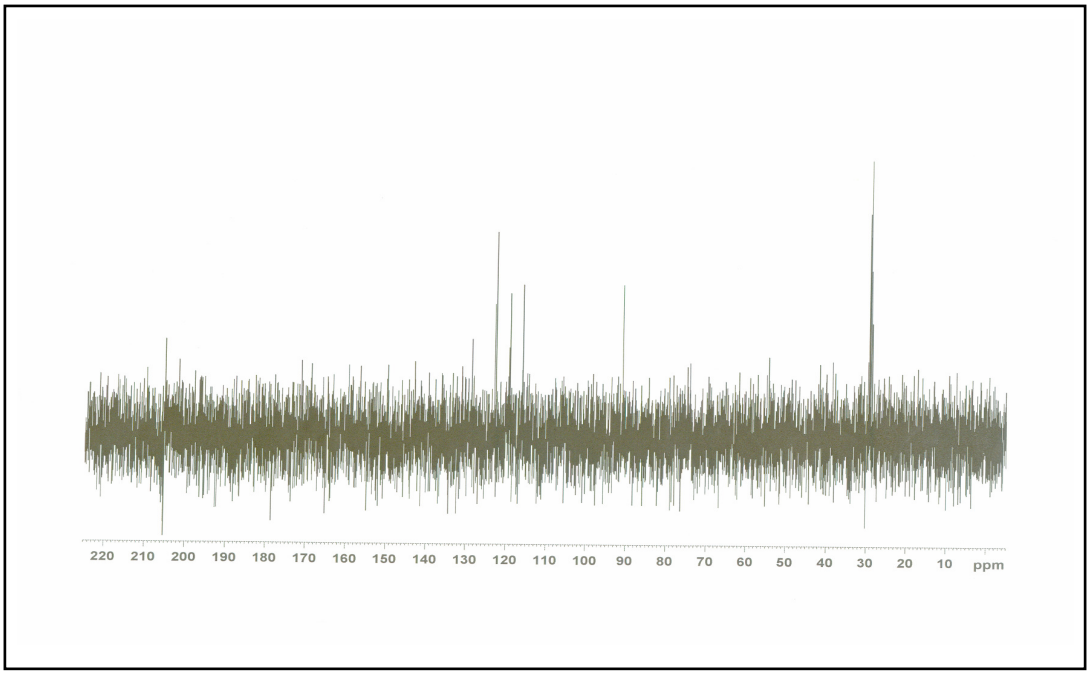

Figure 104 DEPT 90° (acetone-d_6) spectrum of compound **AM9**

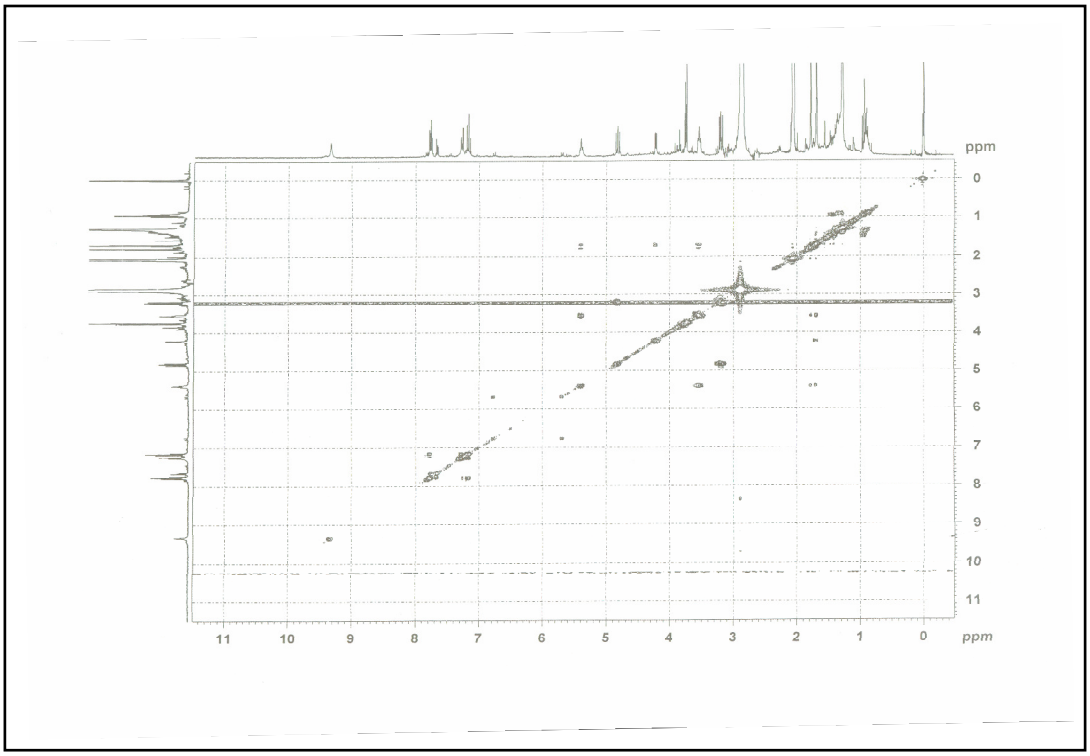

Figure 105 2D COSY (acetone-d_6) spectrum of compound **AM9**

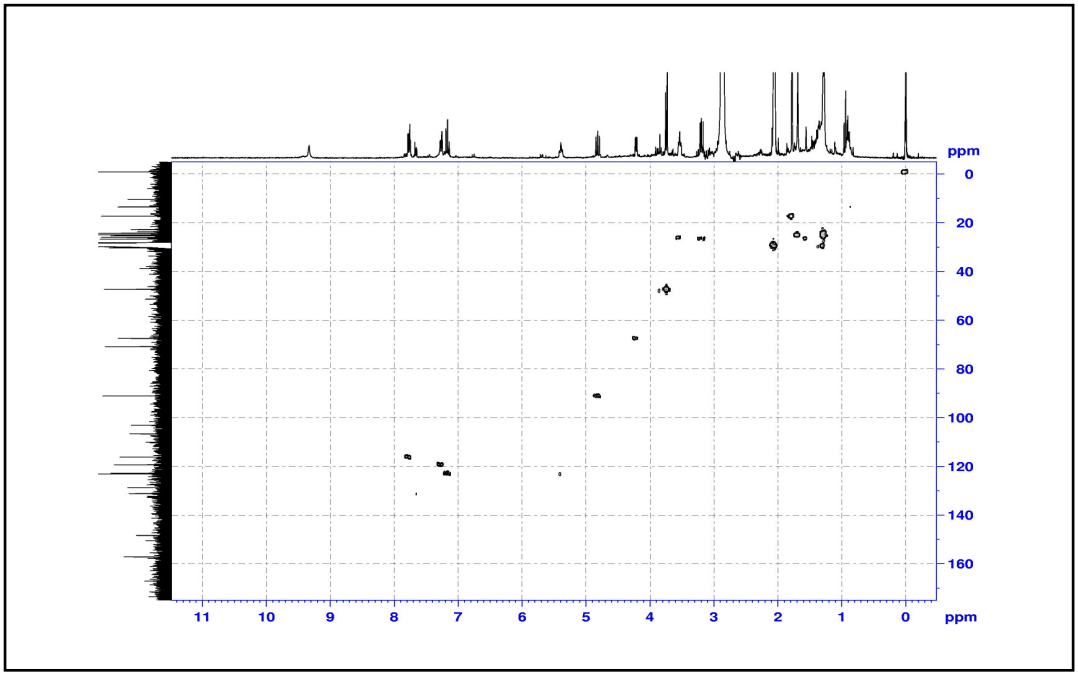

Figure 106 2D HMQC (acetone-d_6) spectrum of compound **AM9**

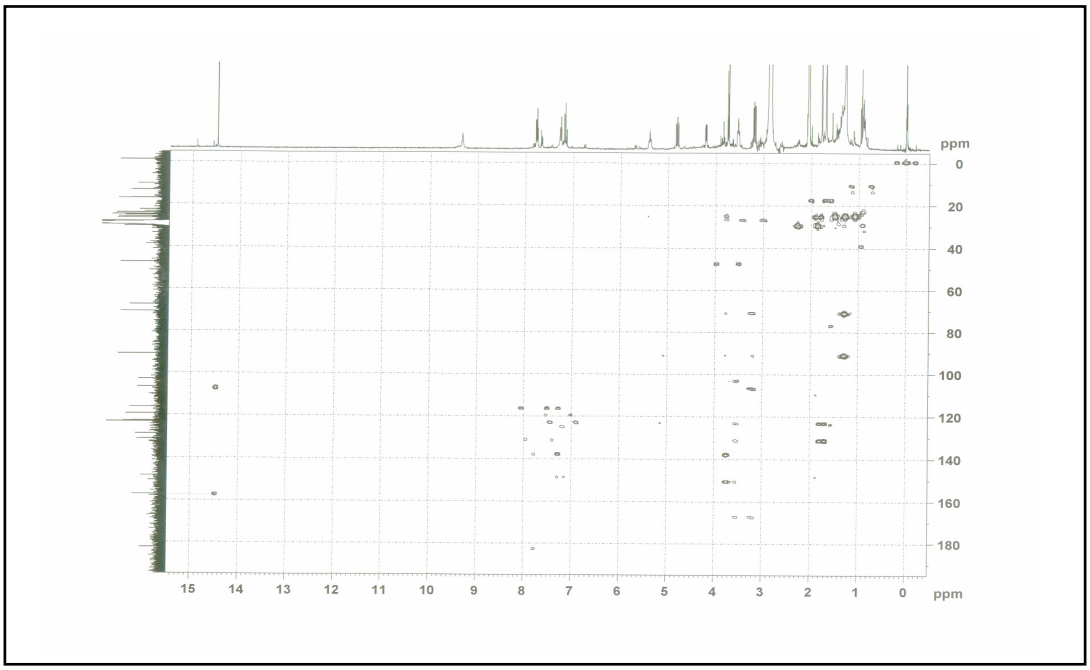

Figure 107 2D HMBC (acetone-d_6) spectrum of compound **AM9**

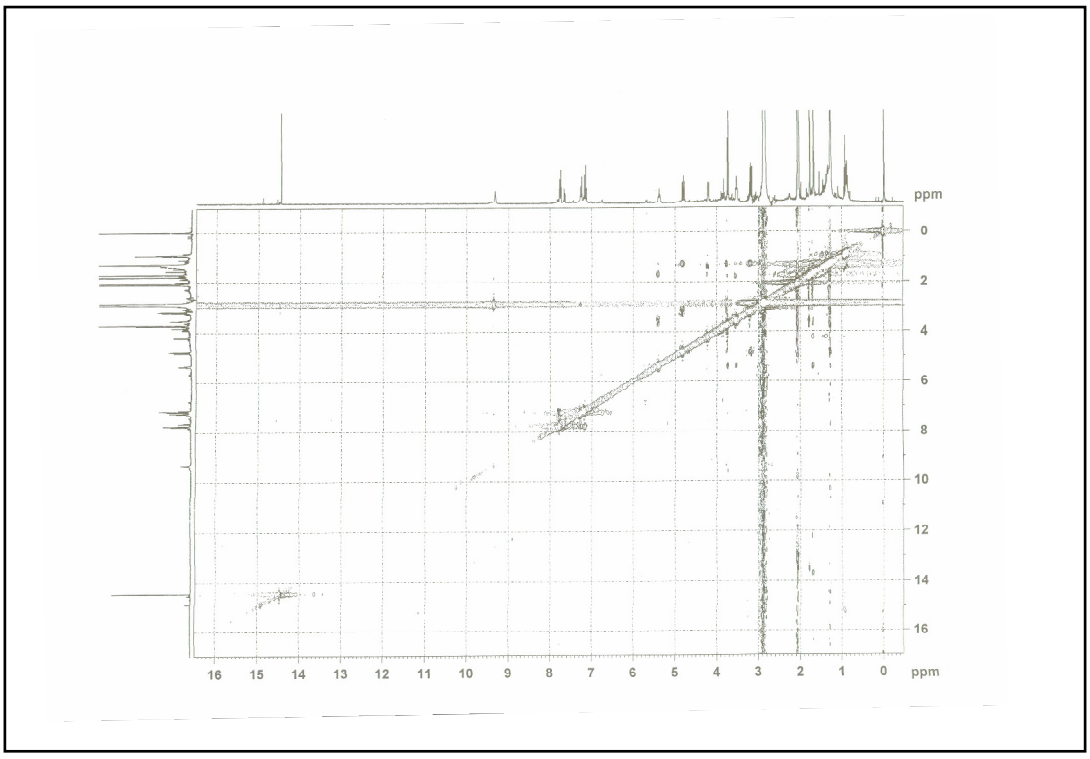

Figure 108 2D NOESY (acetone-d_6) spectrum of compound **AM9**

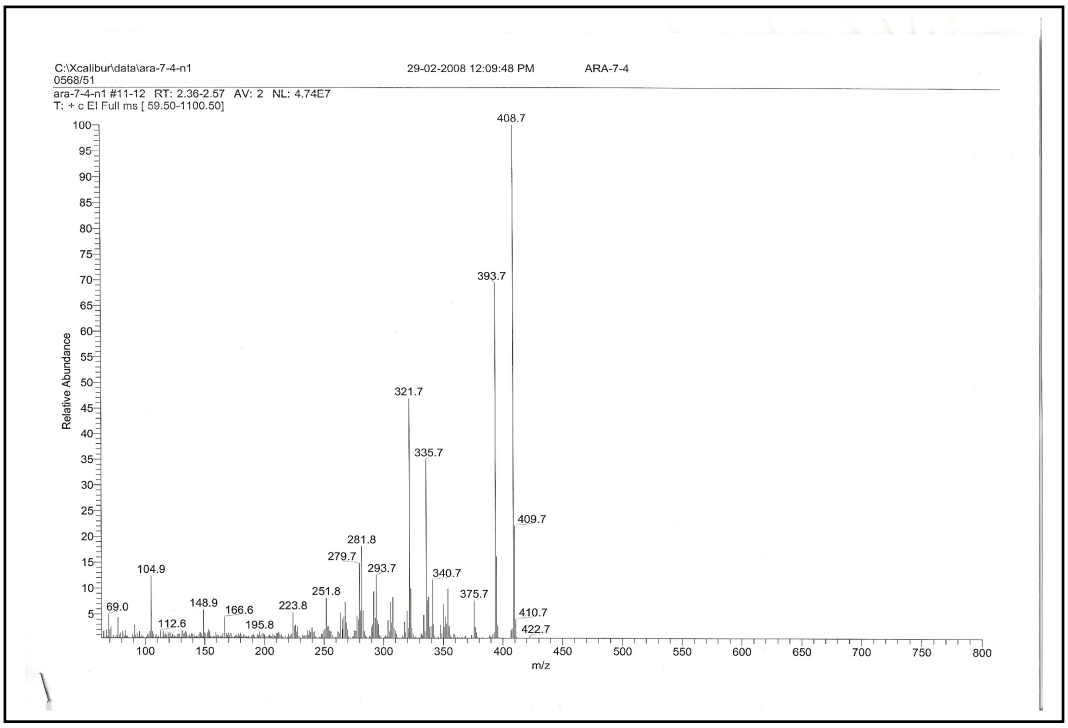

Figure 109 EIMS spectrum of compound **AM9**

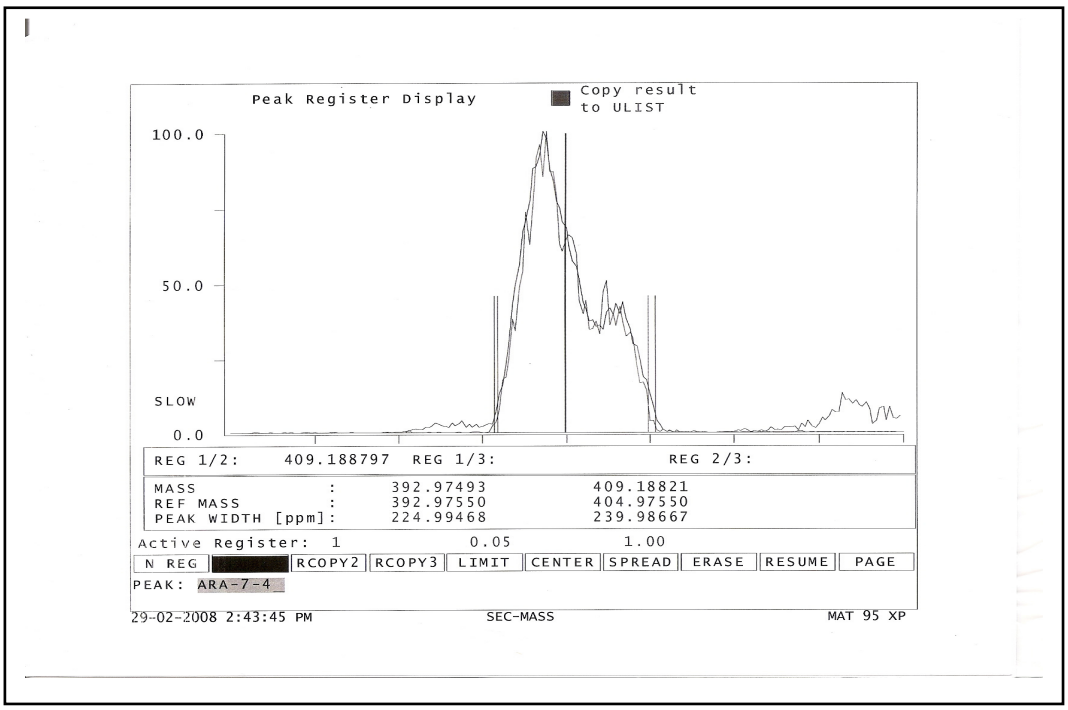

Figure 110 HREIMS spectrum of compound **AM9**

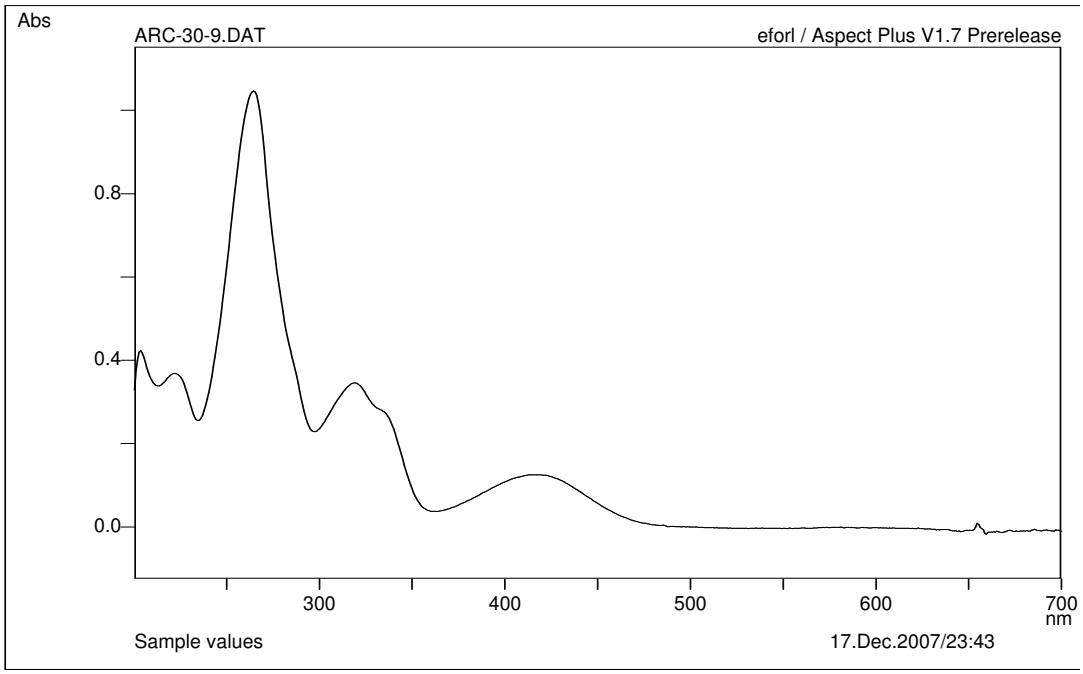

Figure 111 UV (MeOH) spectrum of compound **AM10**

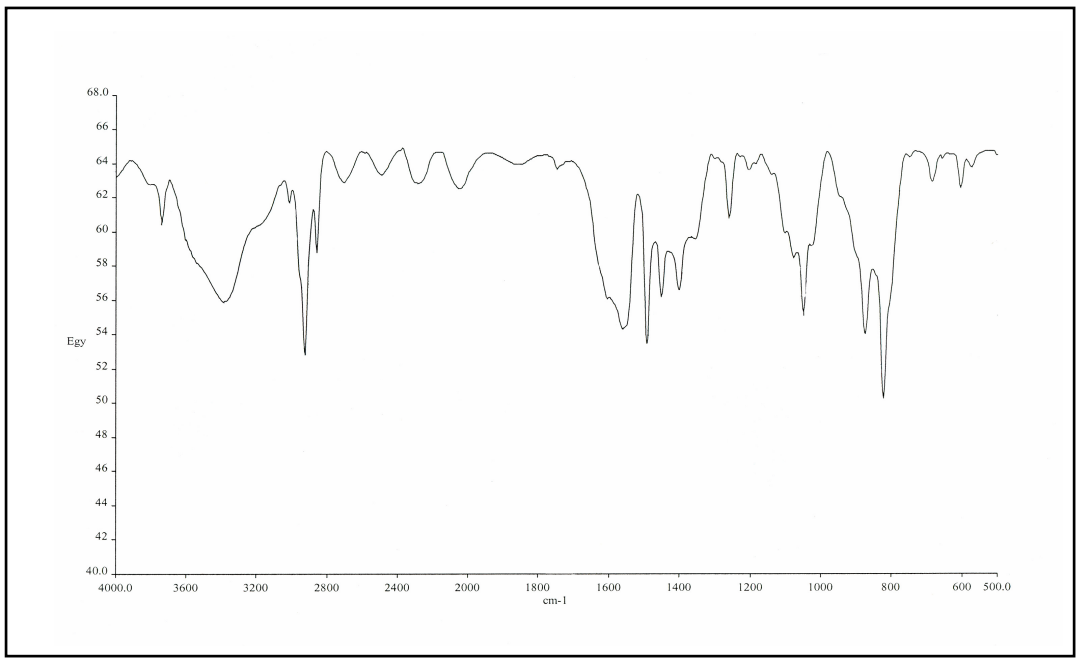

Figure 112 IR (neat) spectrum of compound **AM10**

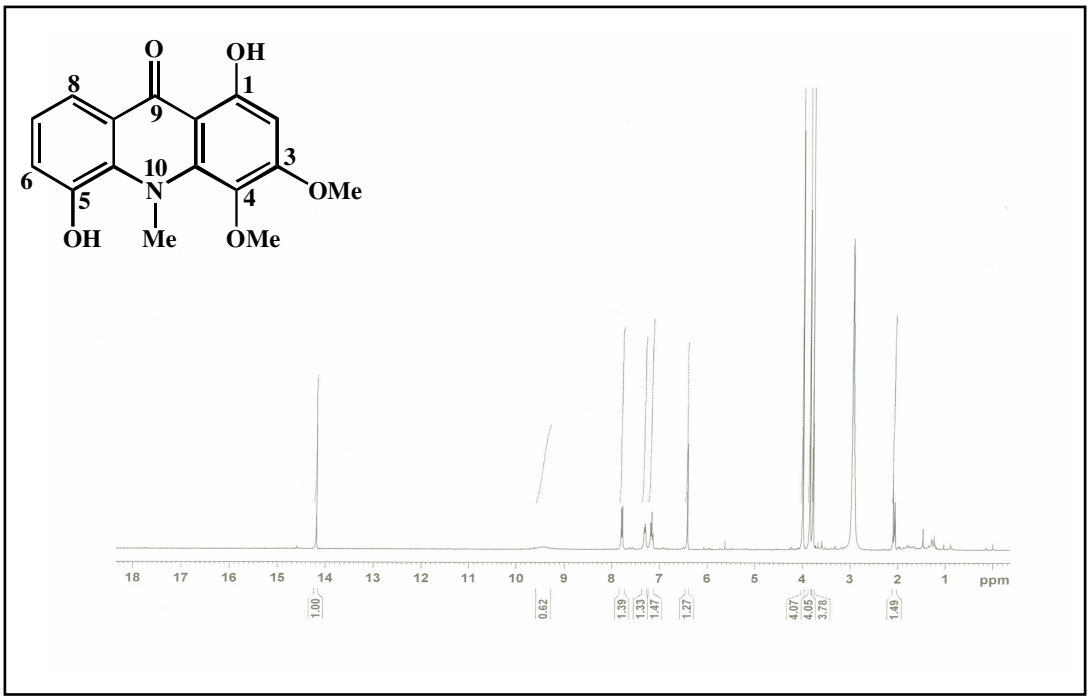

Figure 113 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM10**

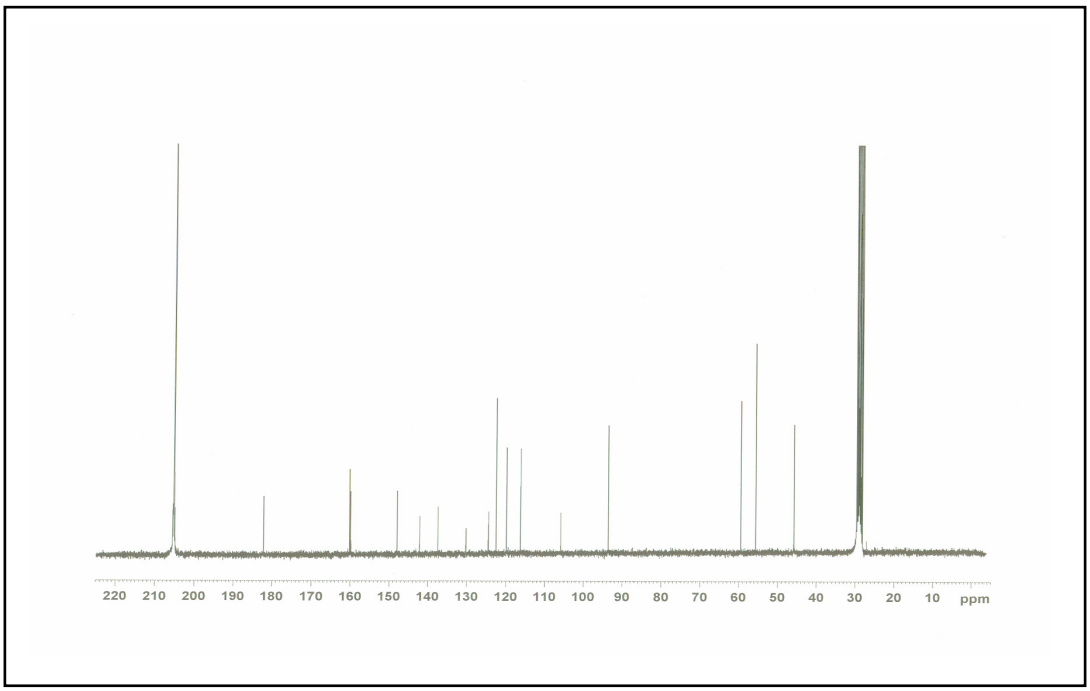

Figure 114 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM10**

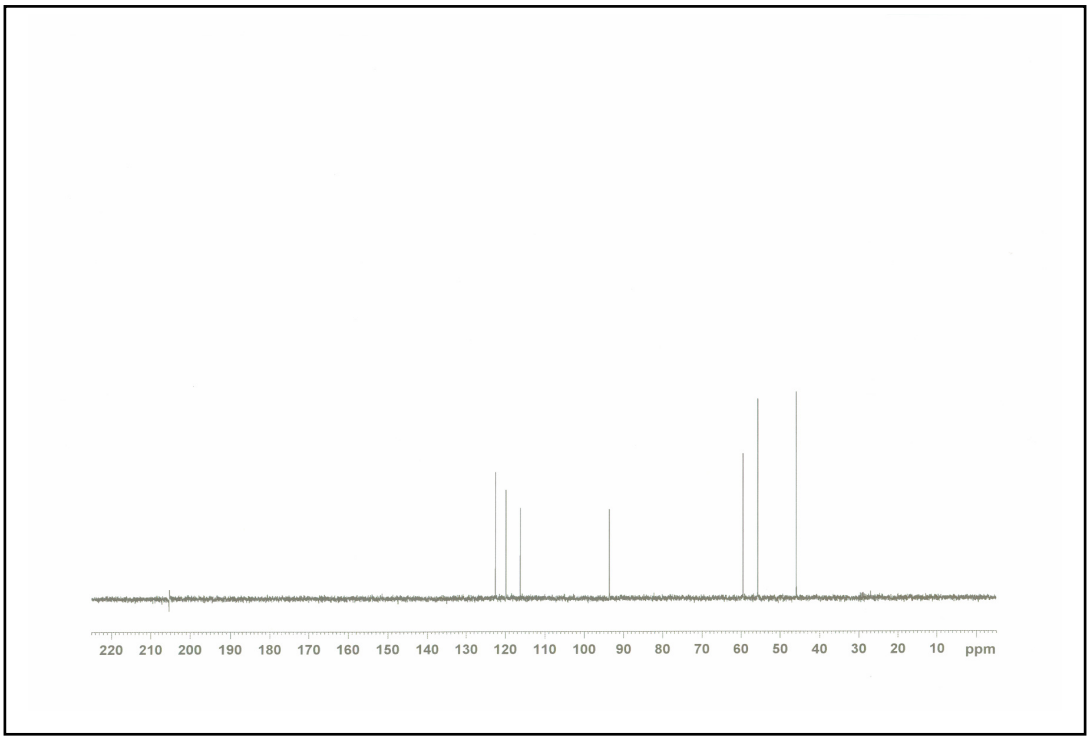

Figure 115 DEPT 135° (acetone-d_6) spectrum of compound **AM10**

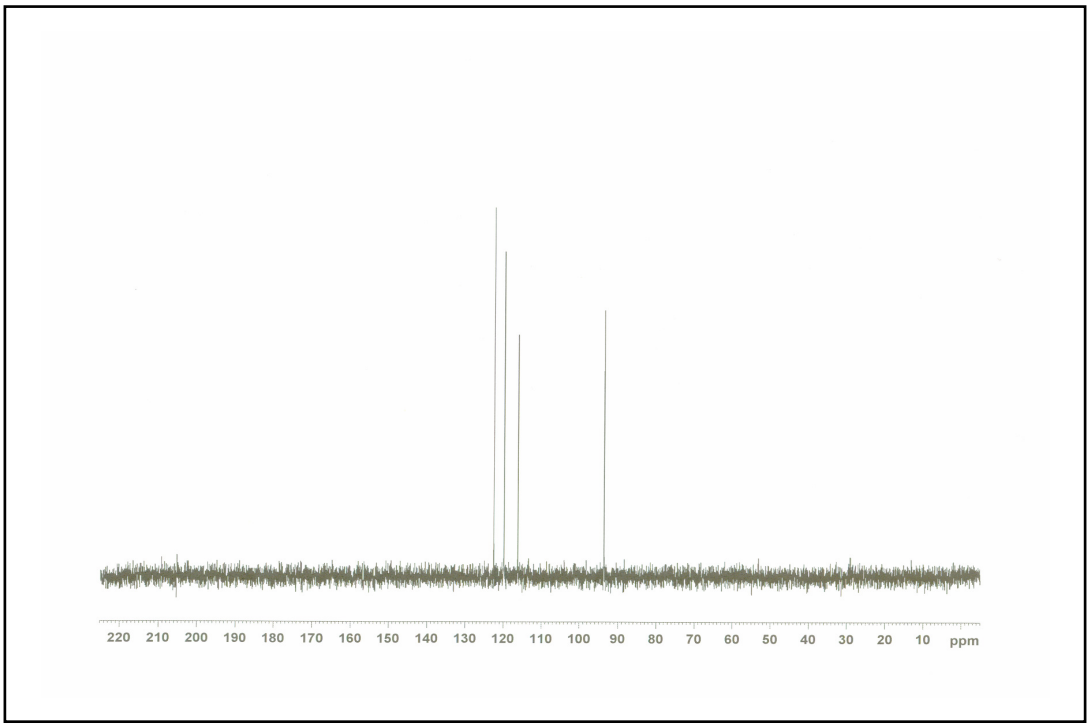

Figure 116 DEPT 90° (acetone-d_6) spectrum of compound **AM10**

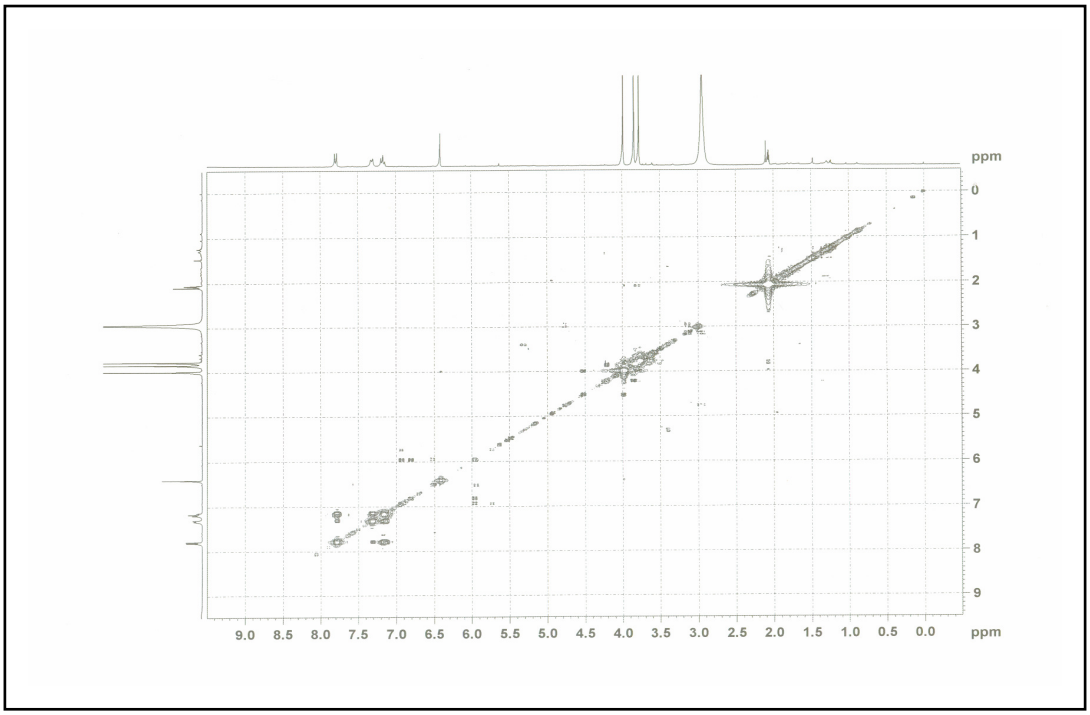

Figure 117 2D COSY (acetone-d_6) spectrum of compound **AM10**

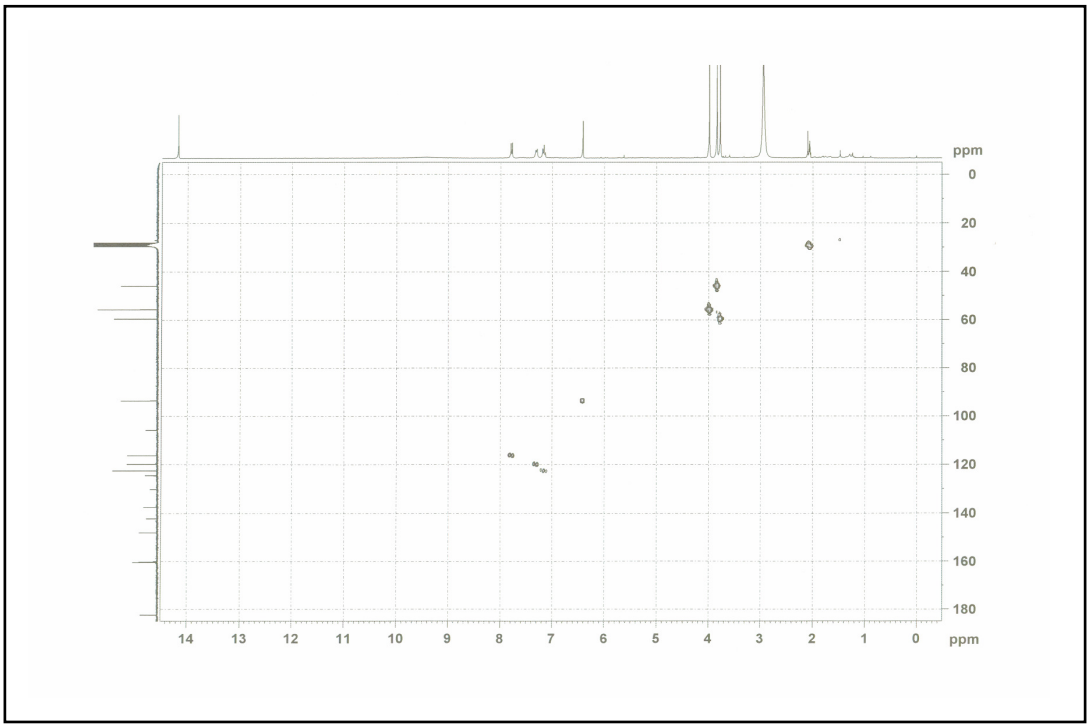

Figure 118 2D HMQC (acetone-d_6) spectrum of compound **AM10**

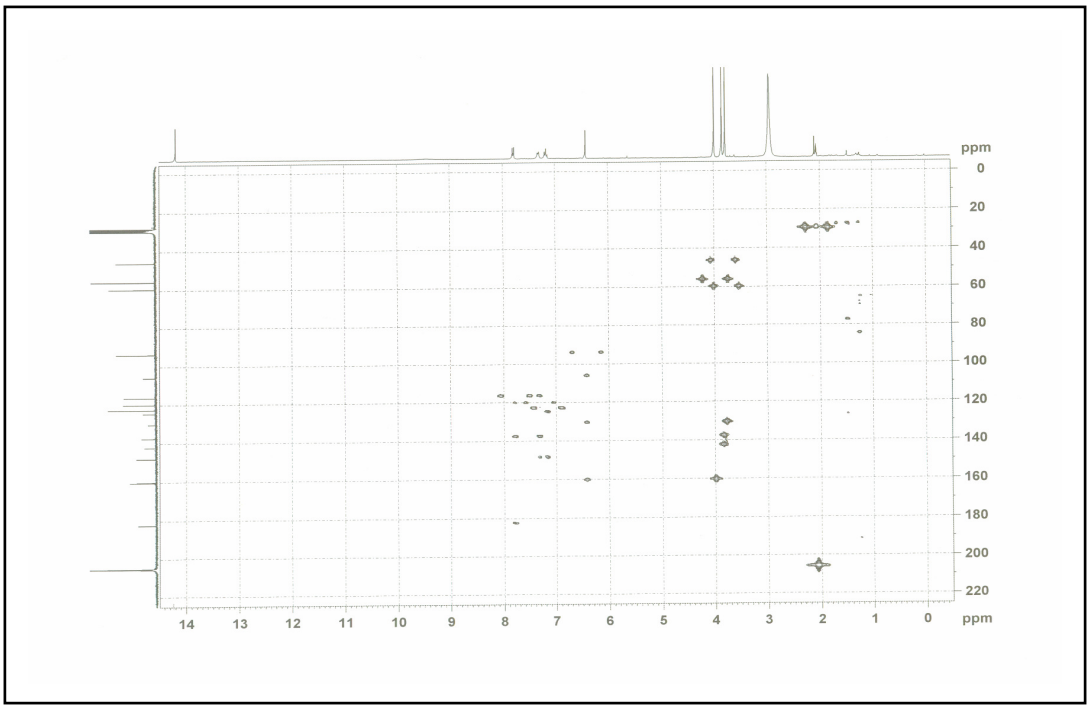

Figure 119 2D HMBC (acetone-d_6) spectrum of compound **AM10**

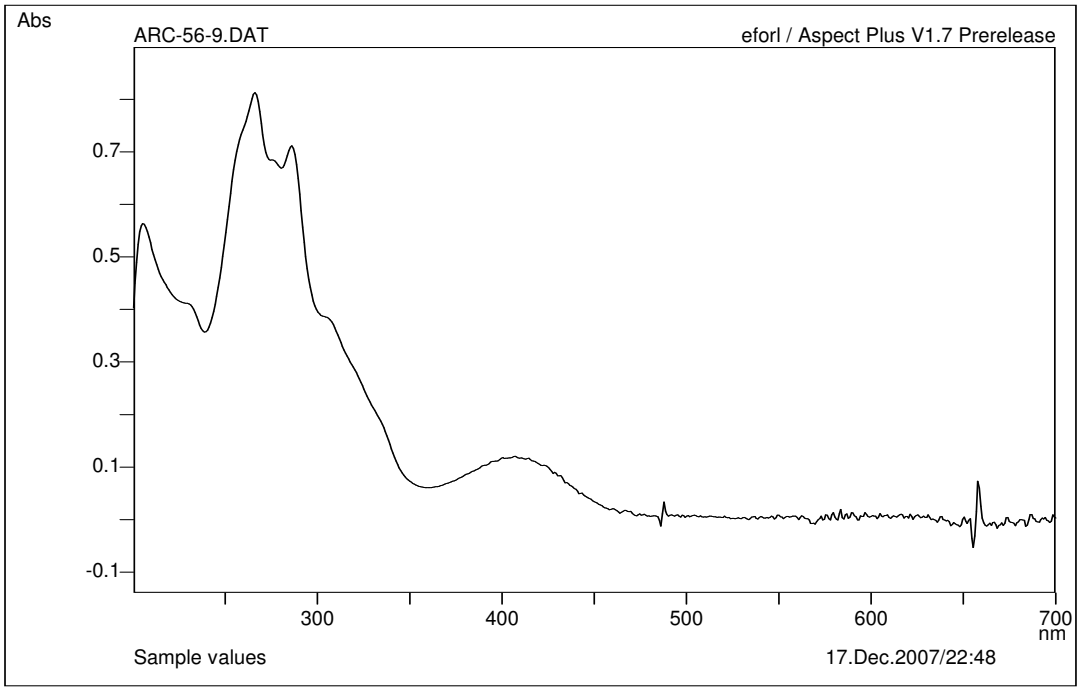

Figure 120 UV (MeOH) spectrum of compound **AM11**

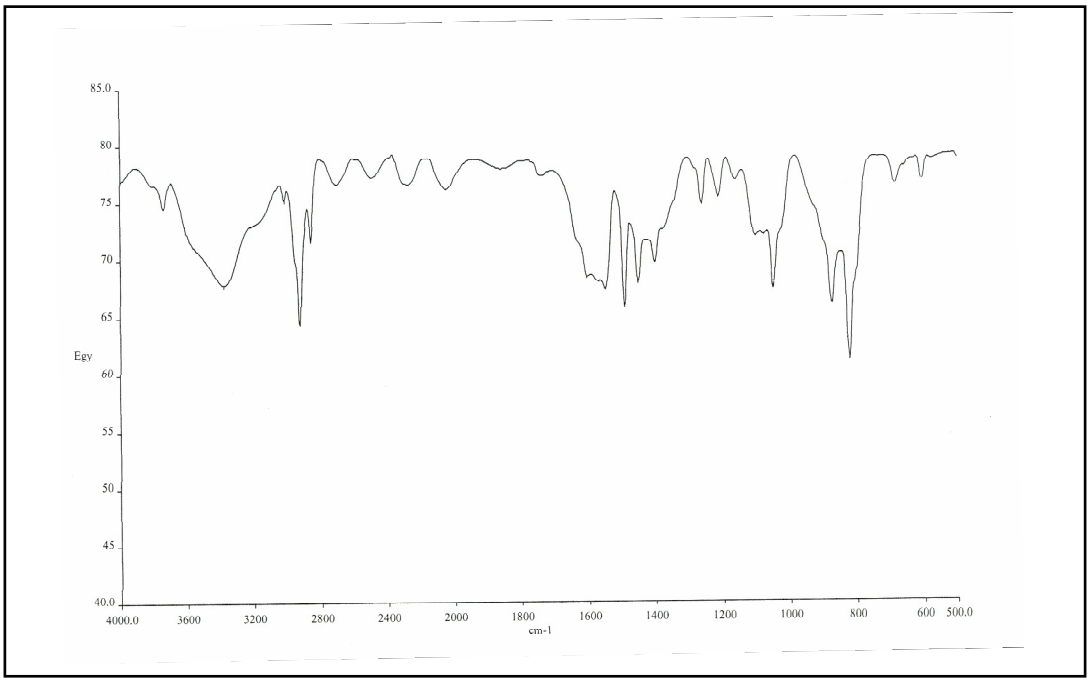

Figure 121 IR (neat) spectrum of compound **AM11**

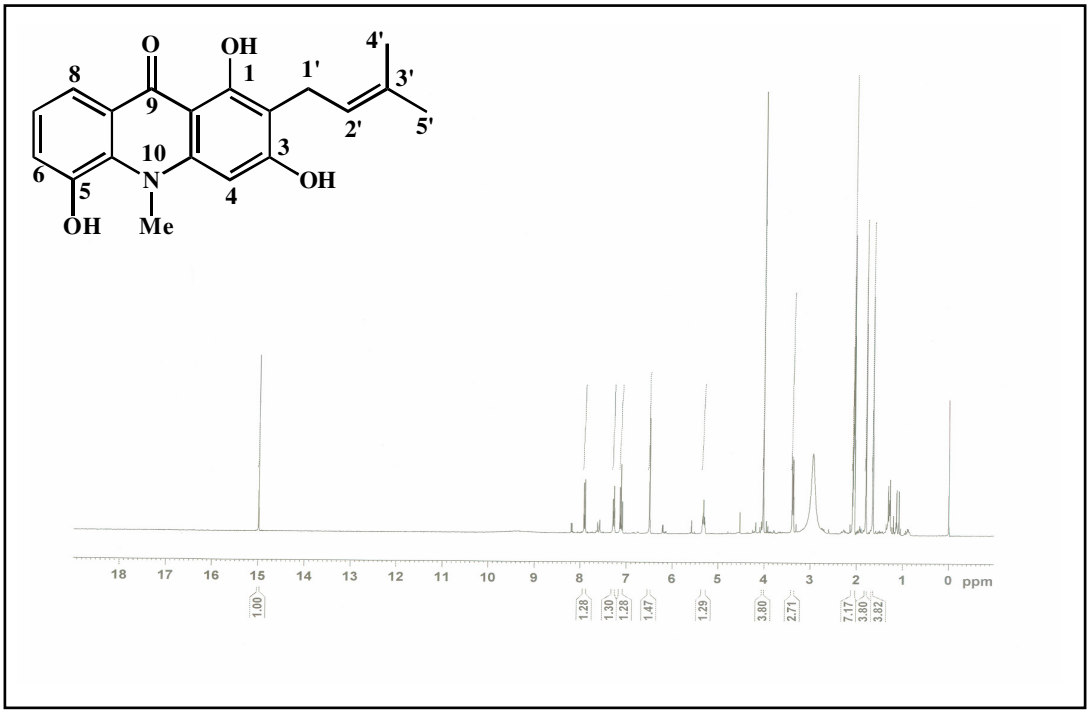

Figure 122 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM11**

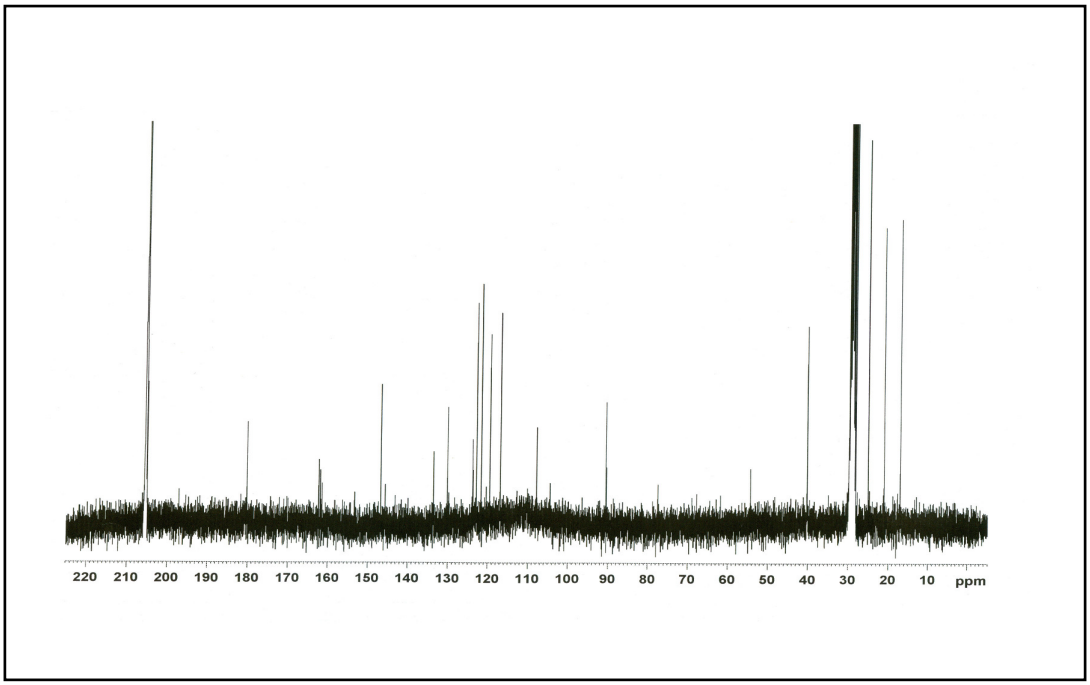

Figure 123 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM11**

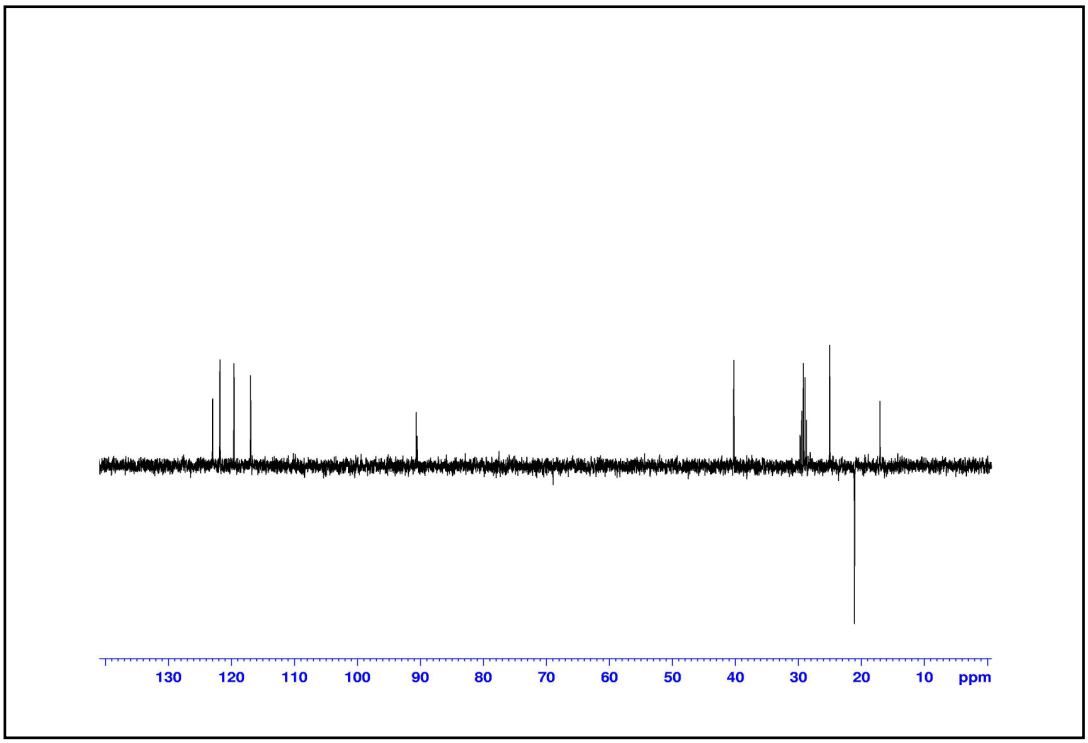

Figure 124 DEPT 135° (acetone-d_6) spectrum of compound **AM11**

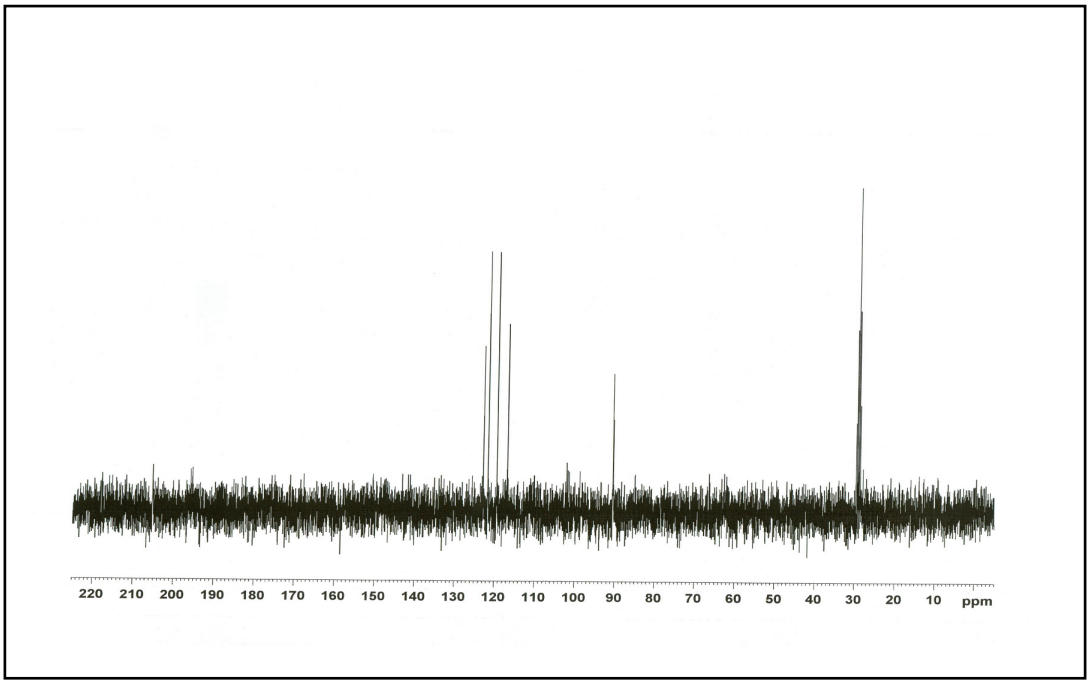

Figure 125 DEPT 90° (acetone-d_6) spectrum of compound **AM11**

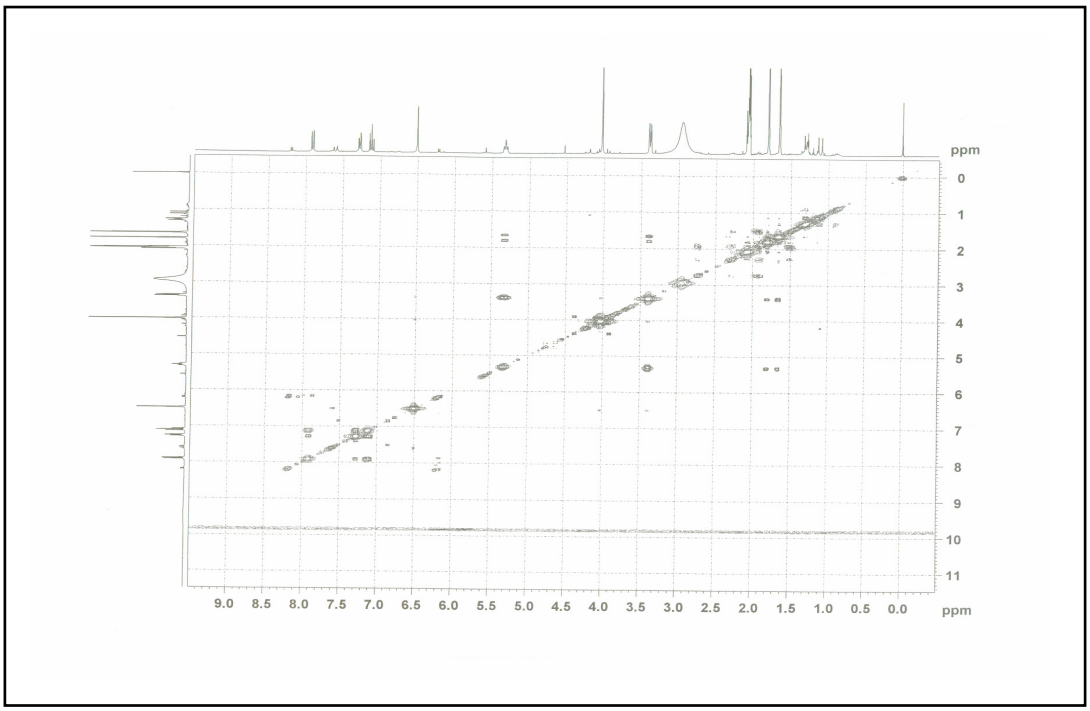

Figure 126 2D COSY (acetone-d_6) spectrum of compound **AM11**

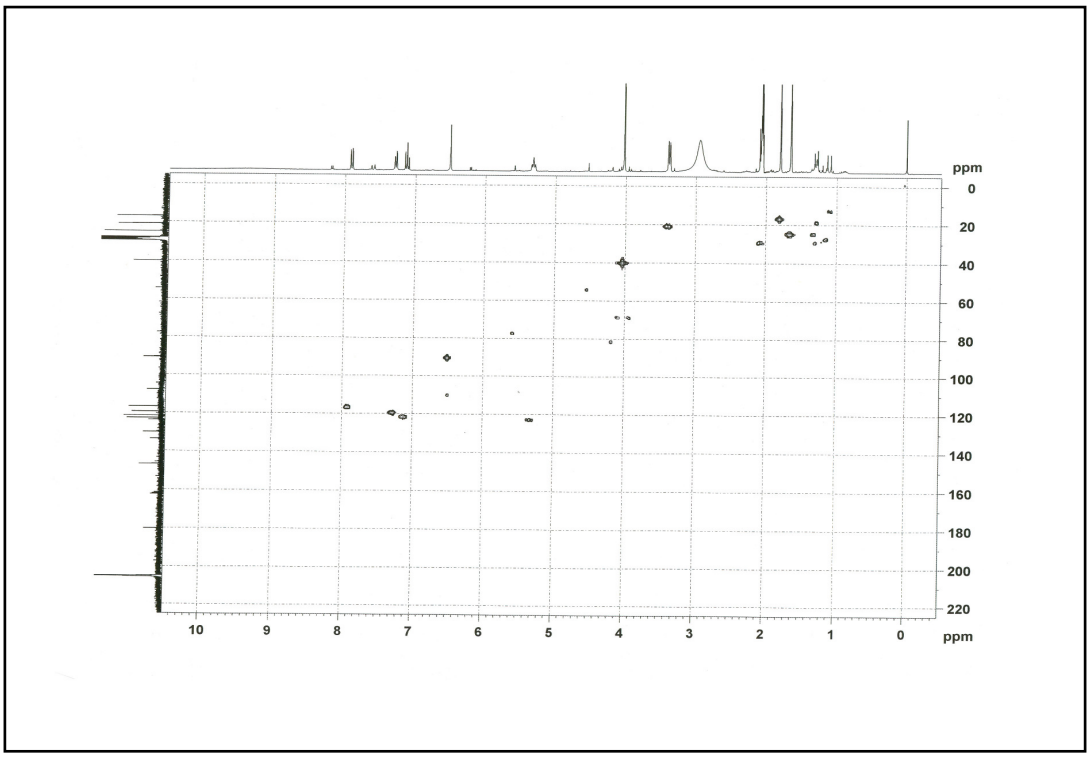

Figure 127 2D HMQC (acetone-d_6) spectrum of compound **AM11**

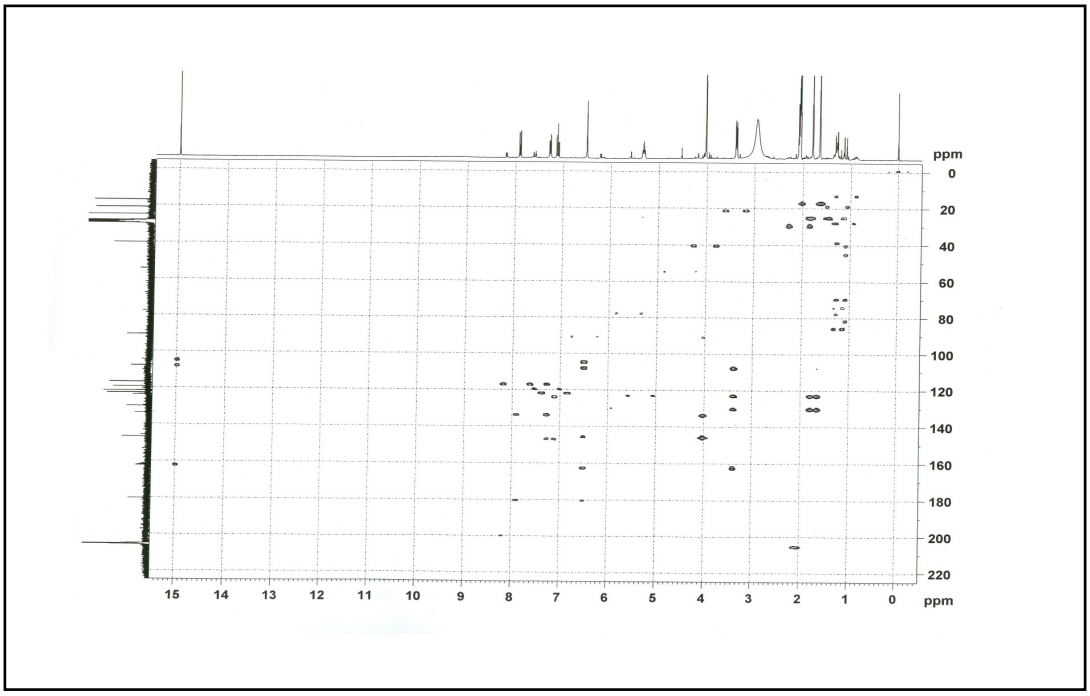

Figure 128 2D HMBC (acetone-d_6) spectrum of compound **AM11**

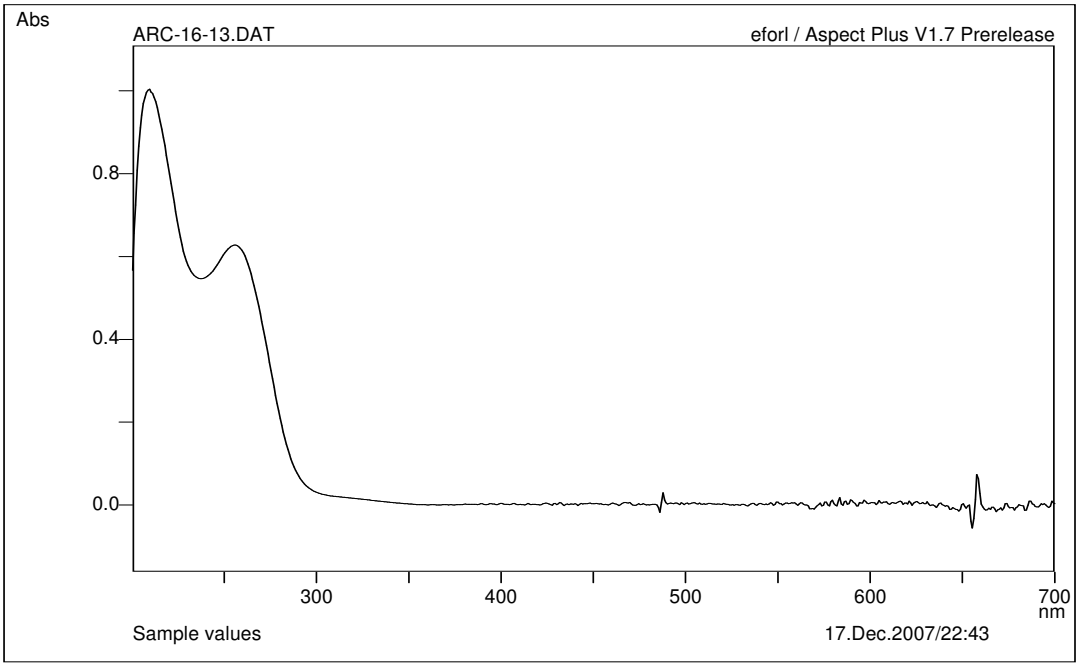

Figure 129 UV (MeOH) spectrum of compound **AM12**

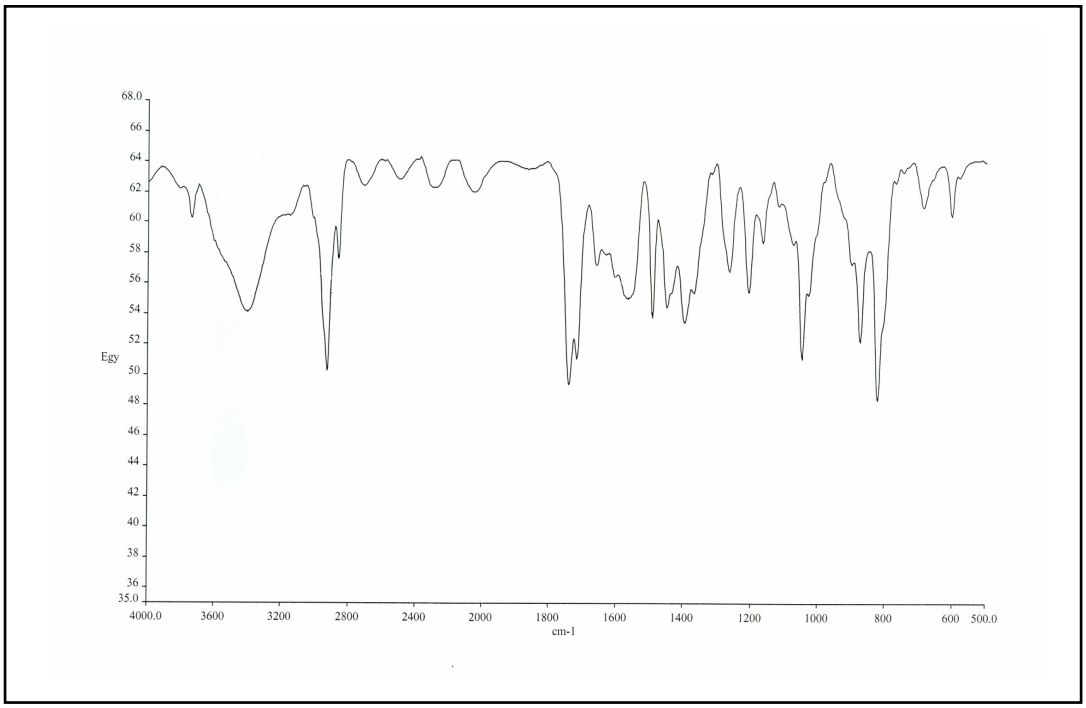

Figure 130 IR (neat) spectrum of compound **AM12**

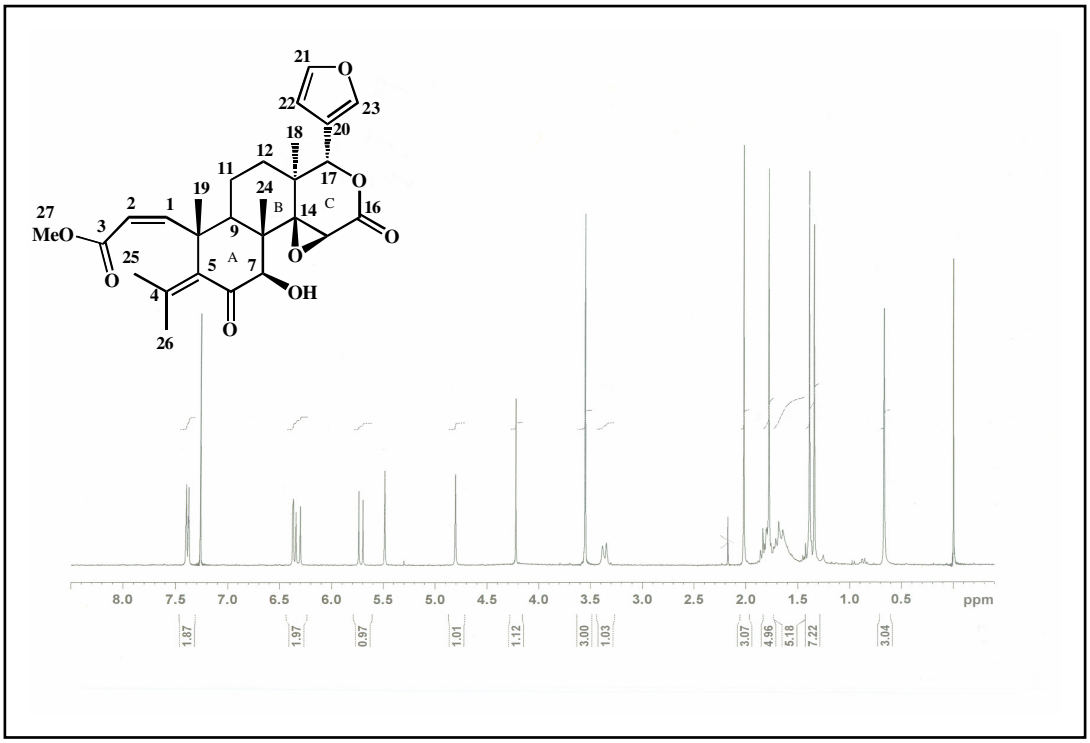

Figure 131 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **AM12**

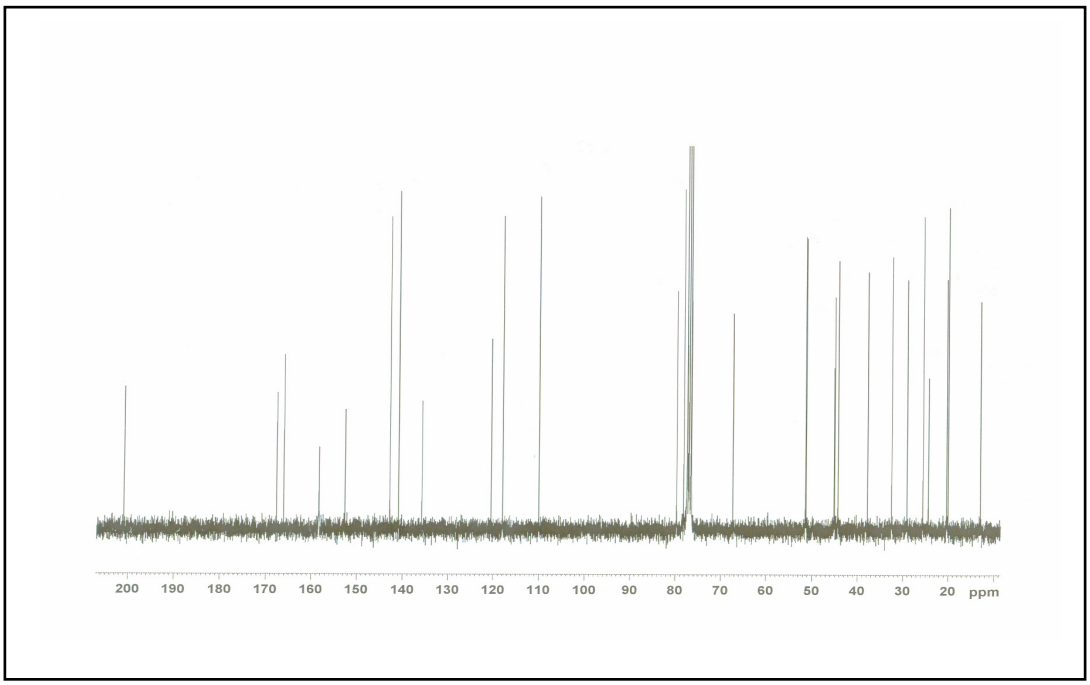

Figure 132 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **AM12**

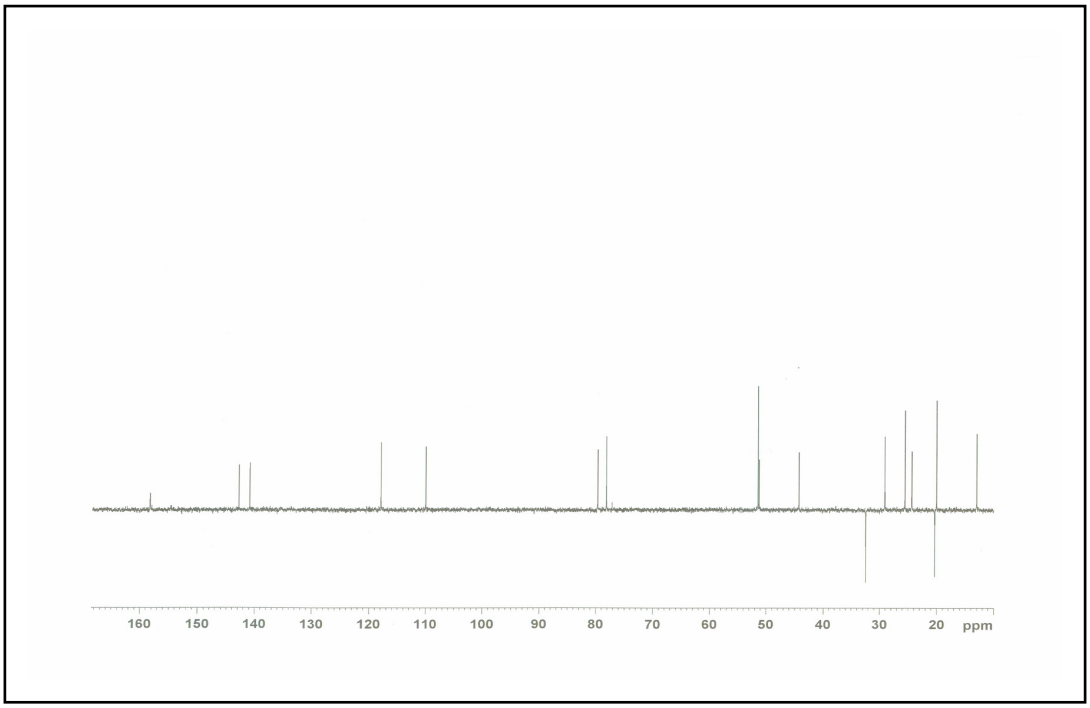

Figure 133 DEPT 135° ($CDCl_3$) spectrum of compound **AM12**

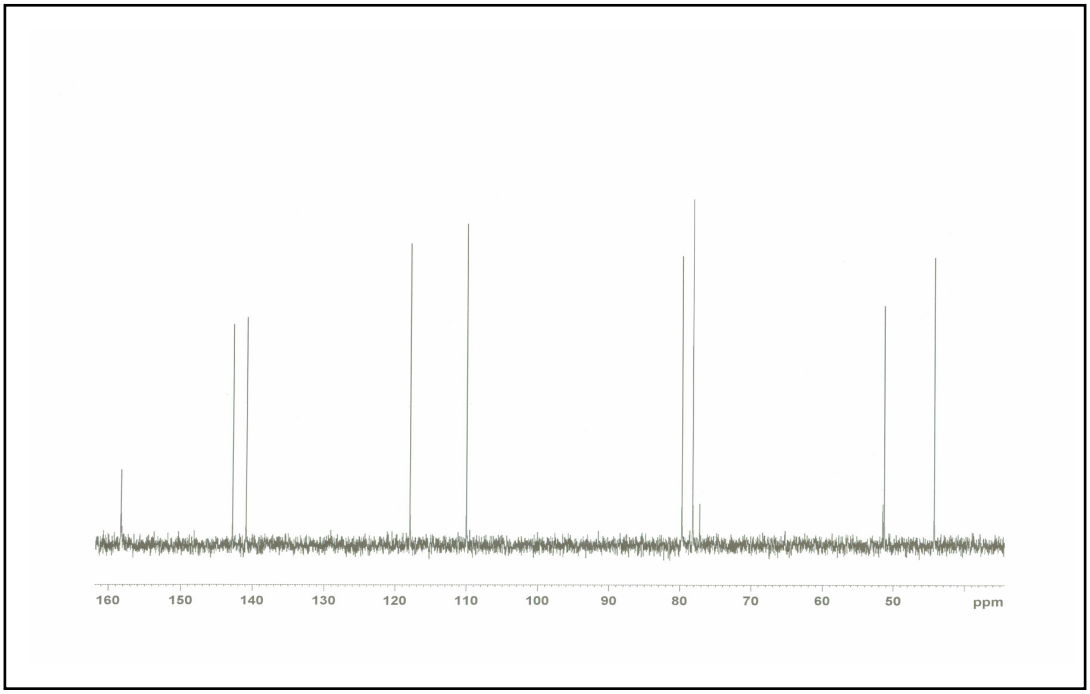

Figure 134 DEPT 90° ($CDCl_3$) spectrum of compound **AM12**

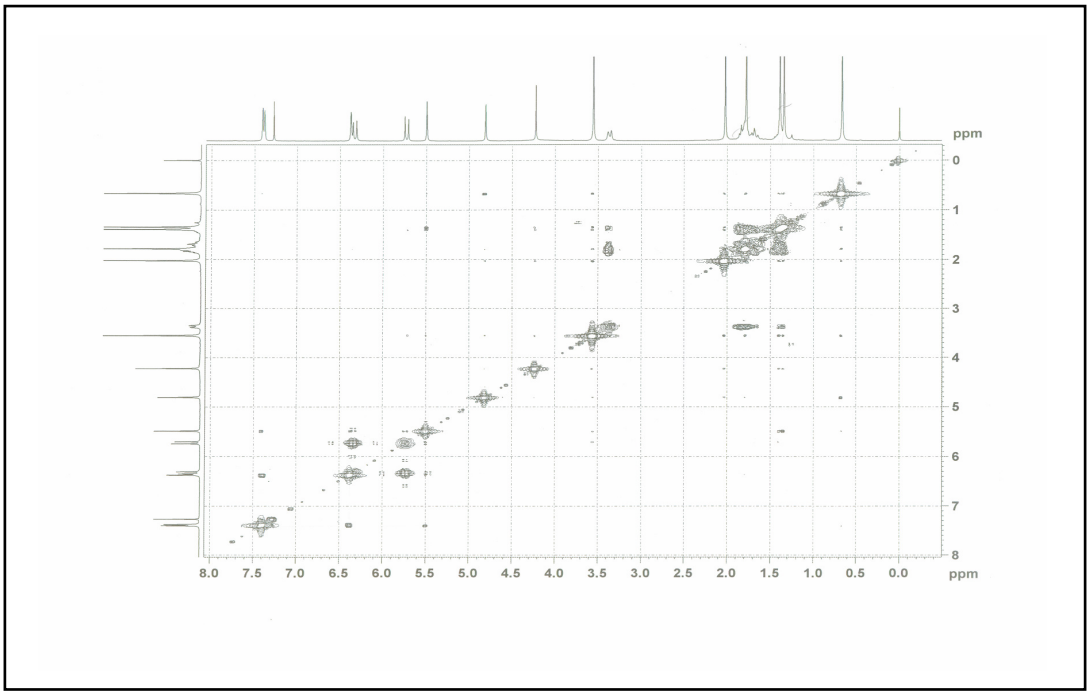

Figure 135 2D COSY ($CDCl_3$) spectrum of compound **AM12**

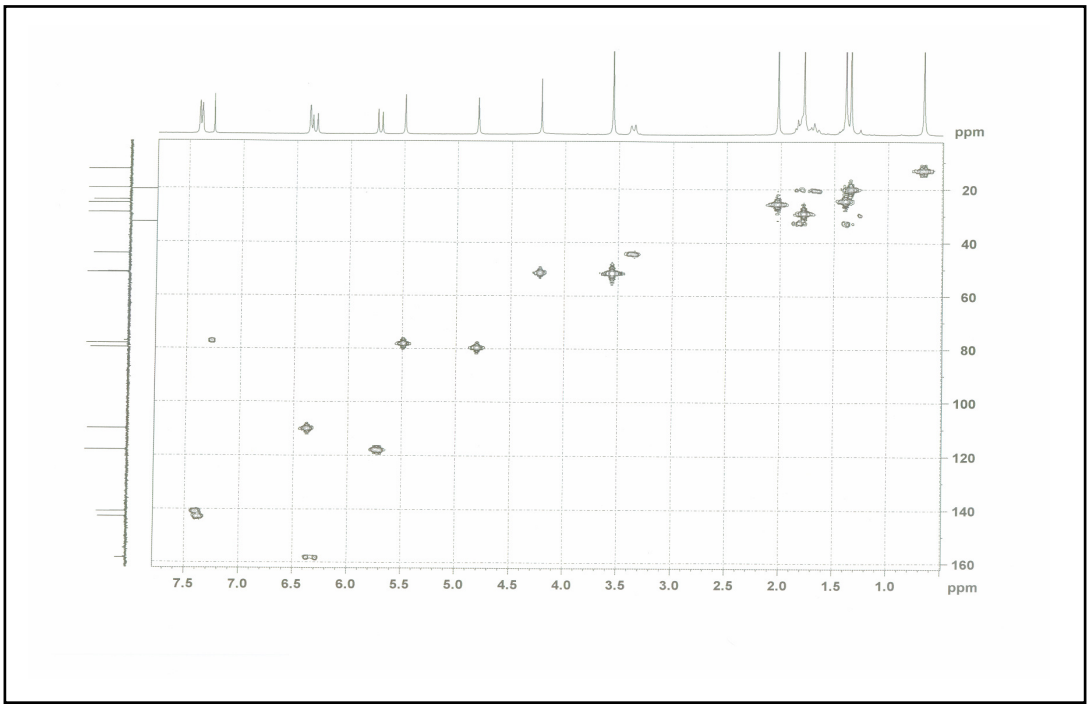

Figure 136 2D HMQC ($CDCl_3$) spectrum of compound **AM12**

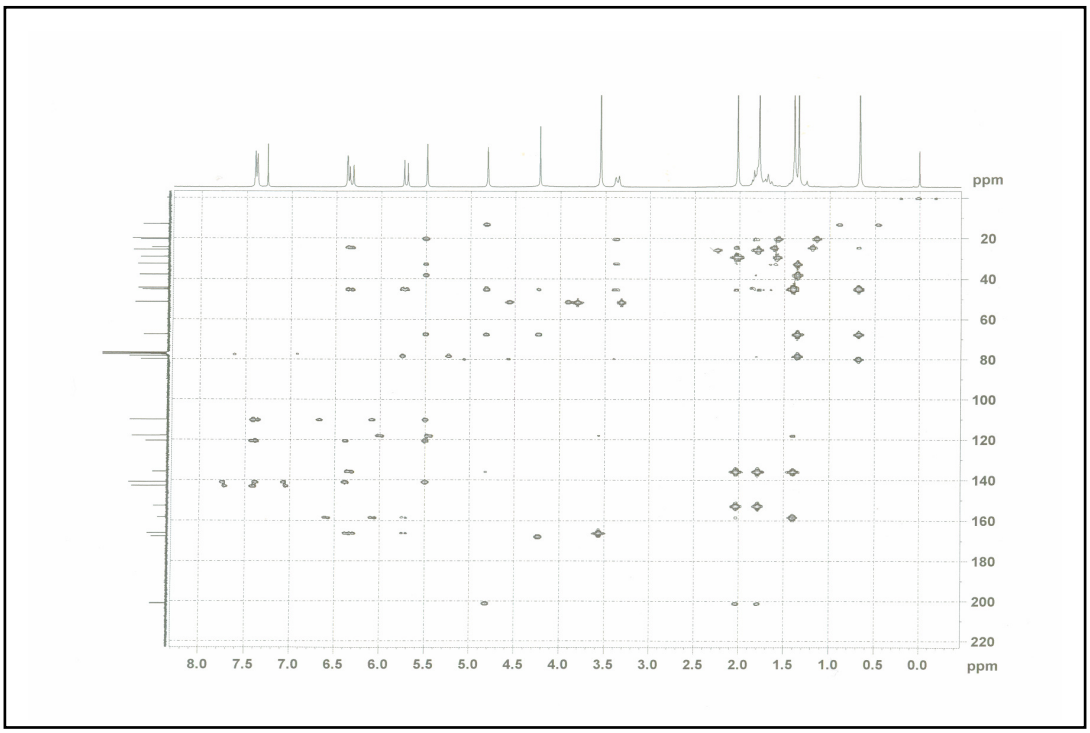

Figure 137 2D HMBC ($CDCl_3$) spectrum of compound **AM12**

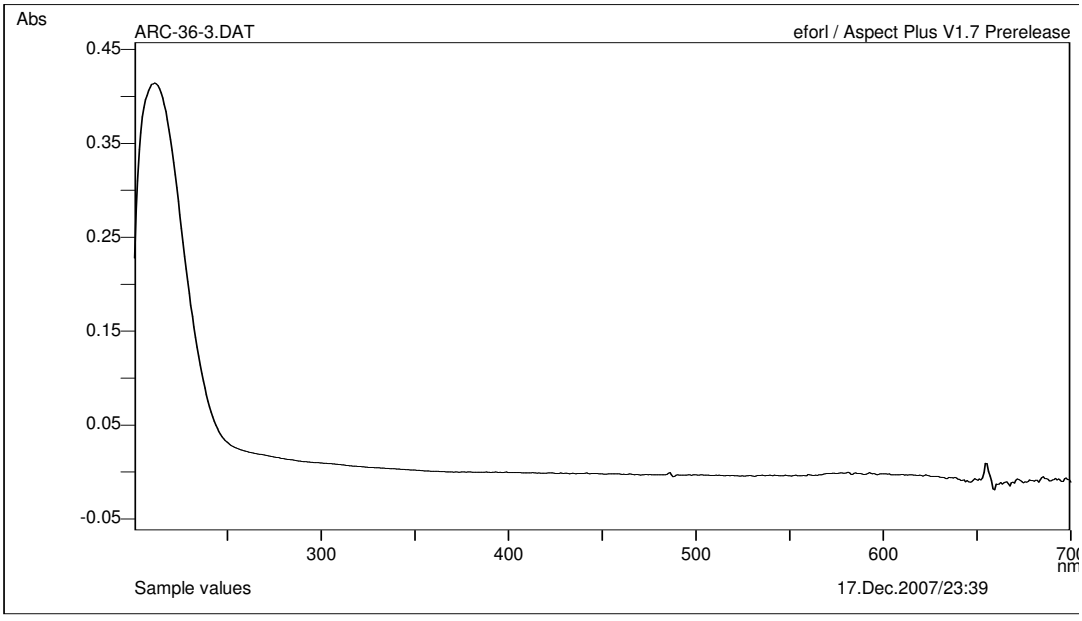

Figure 138 UV (MeOH) spectrum of compound **AM13**

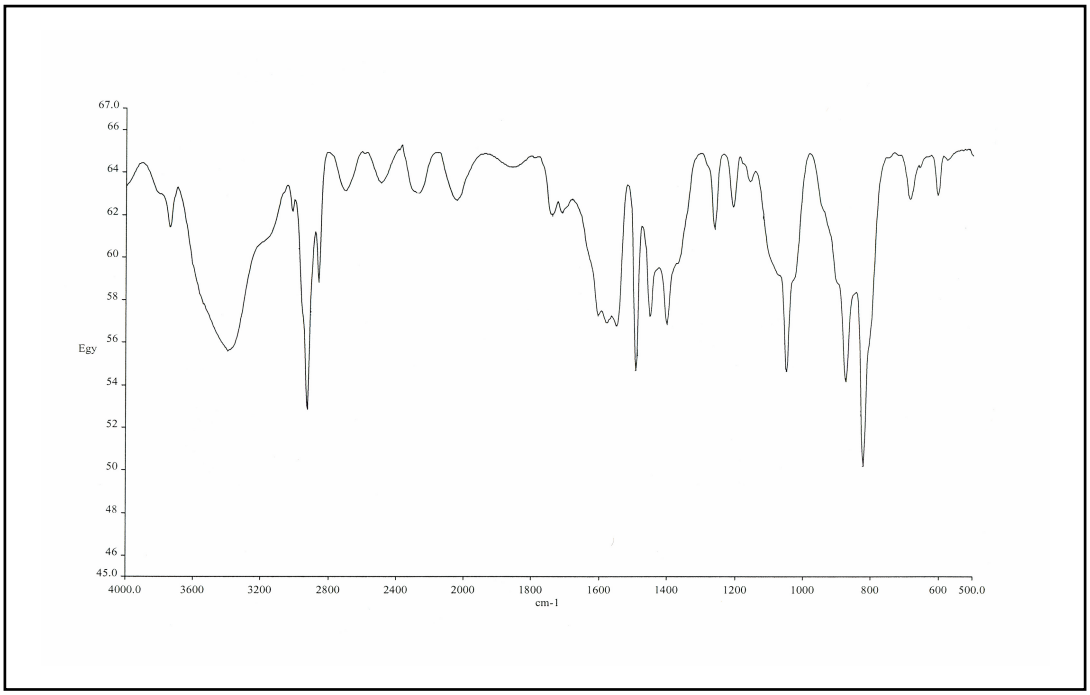

Figure 139 IR (neat) spectrum of compound **AM13**

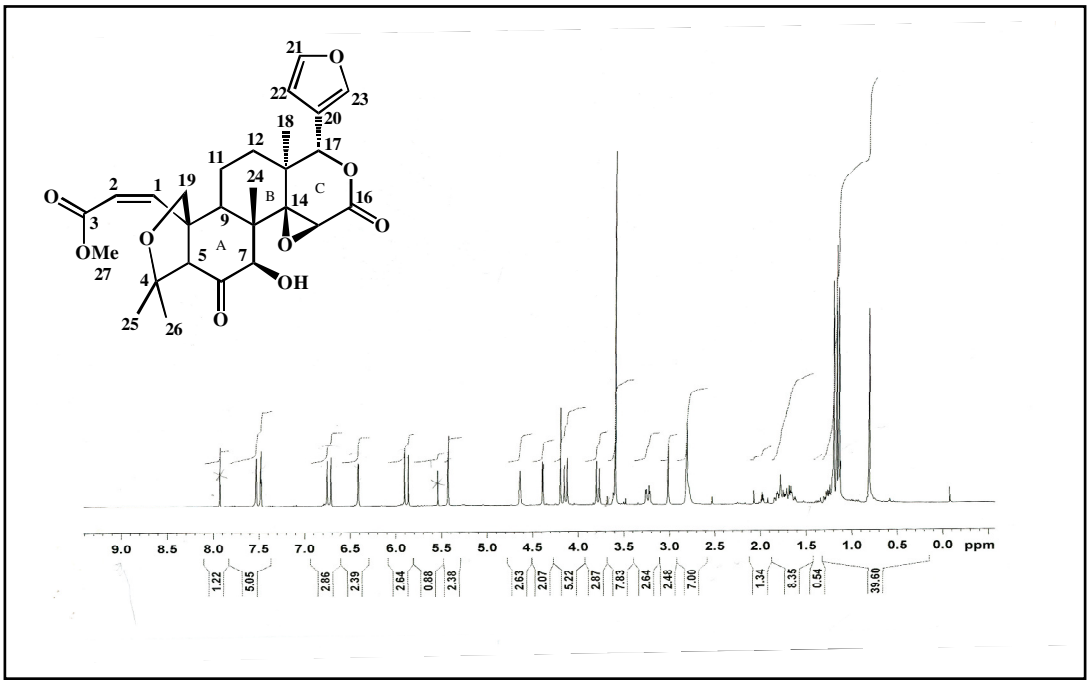

Figure 140 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM13**

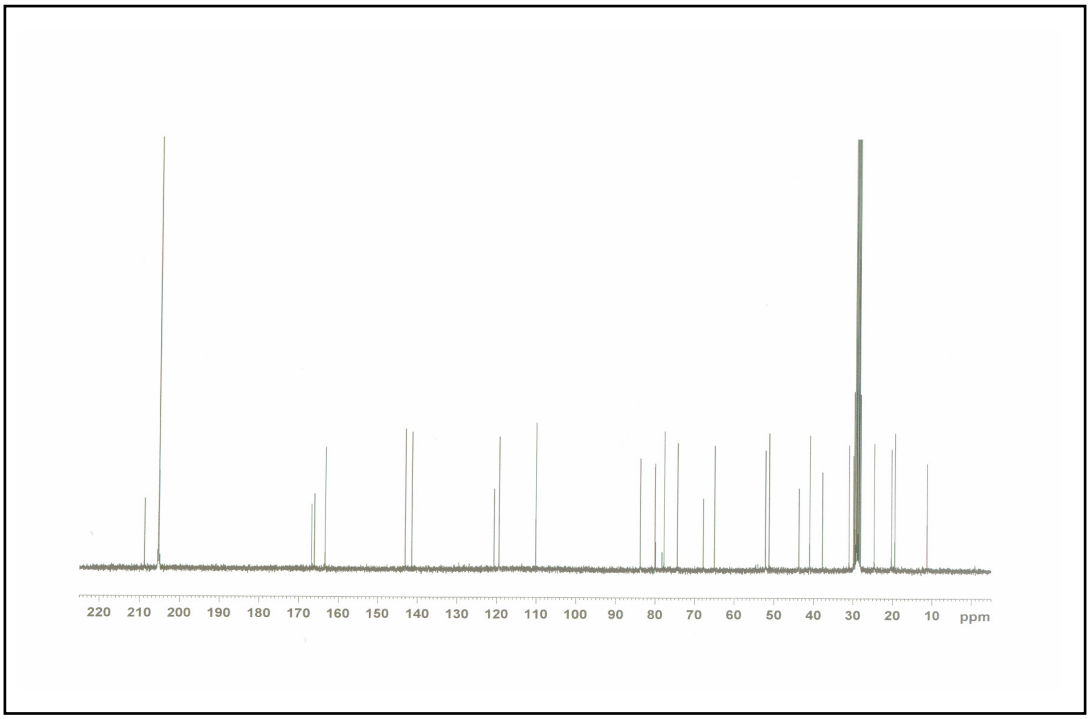

Figure 141 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM13**

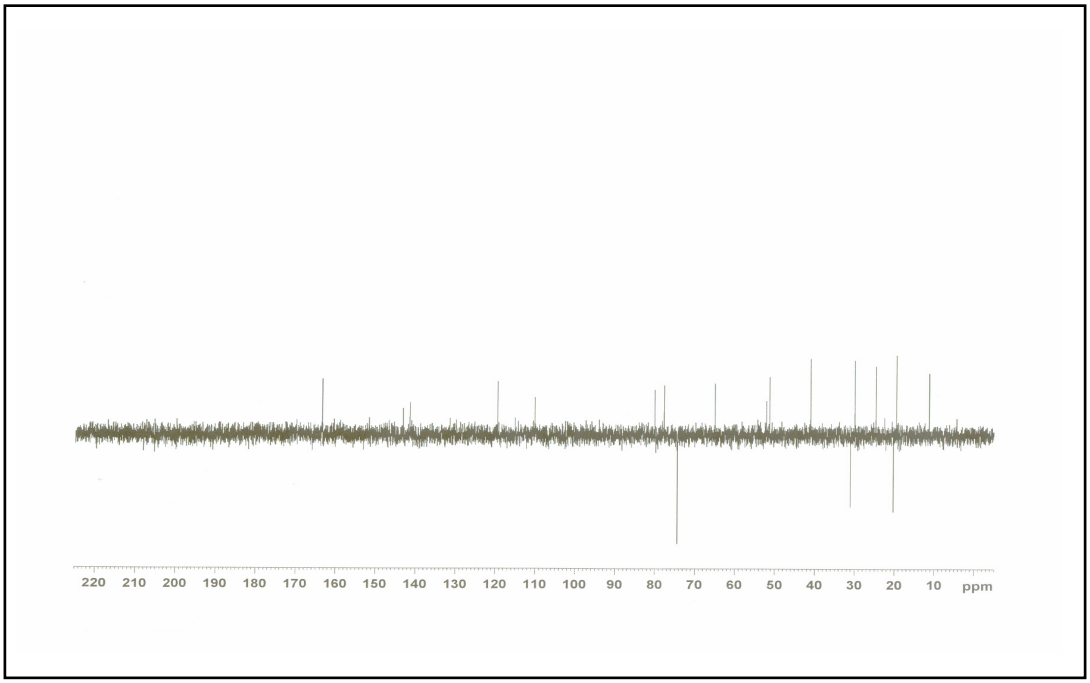

Figure 142 DEPT 135° (acetone-d_6) spectrum of compound **AM13**

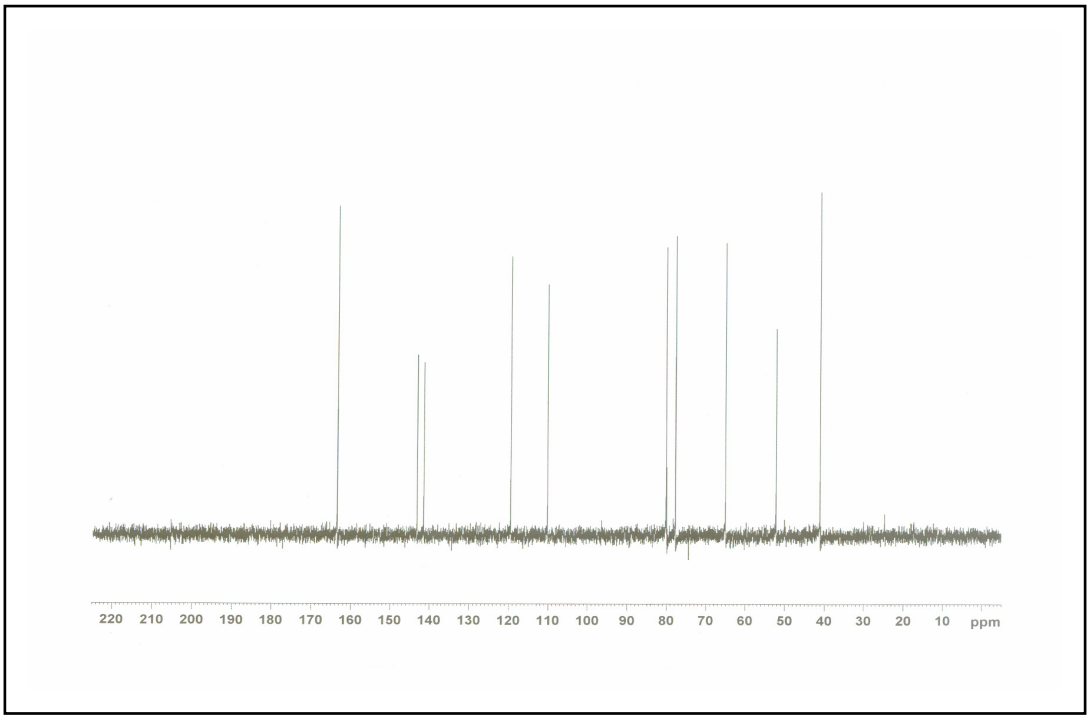

Figure 143 DEPT 90° (acetone-d_6) spectrum of compound **AM13**

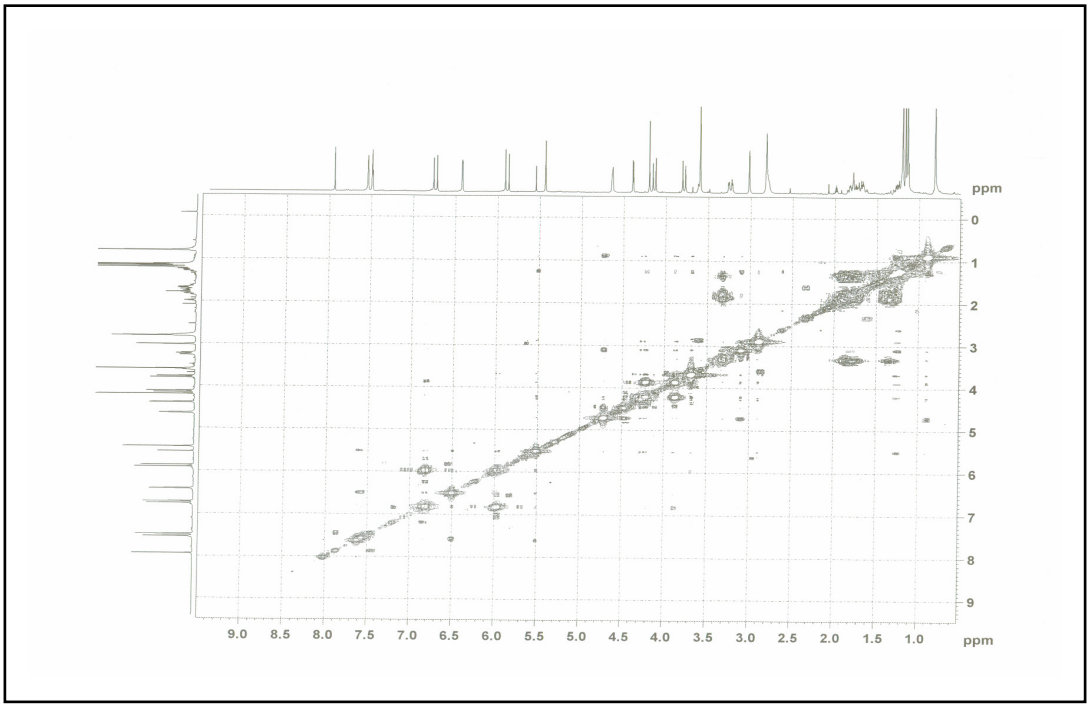

Figure 144 2D COSY (acetone-d_6) spectrum of compound **AM13**

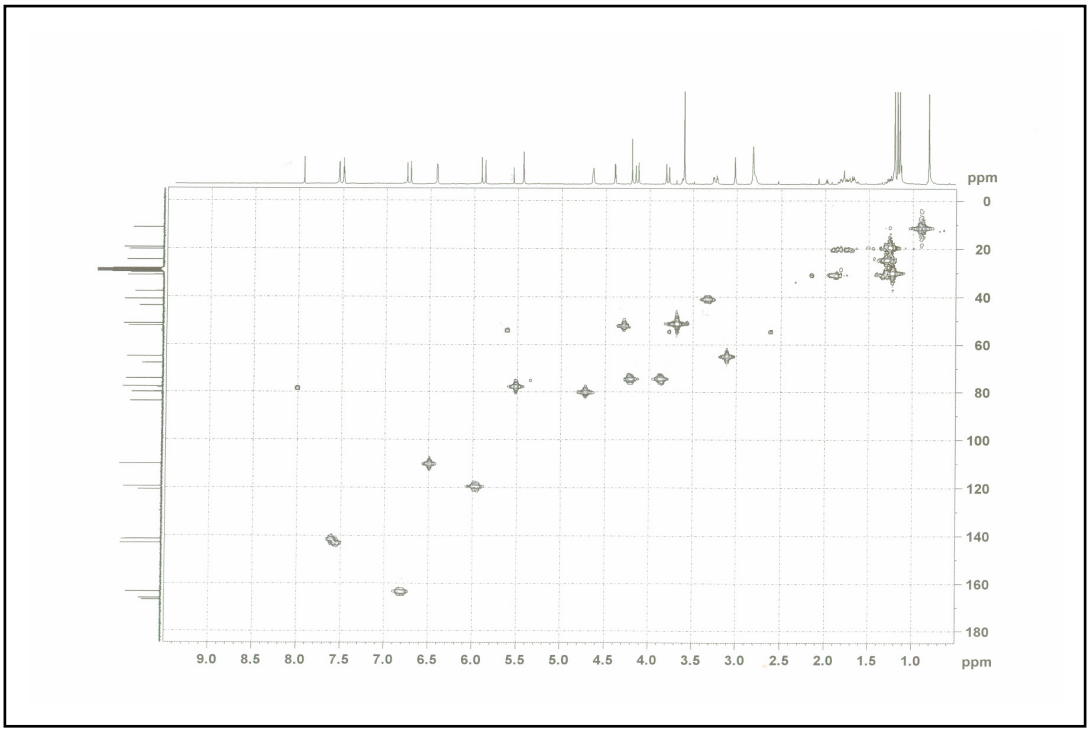

Figure 145 2D HMQC (acetone-d_6) spectrum of compound **AM13**

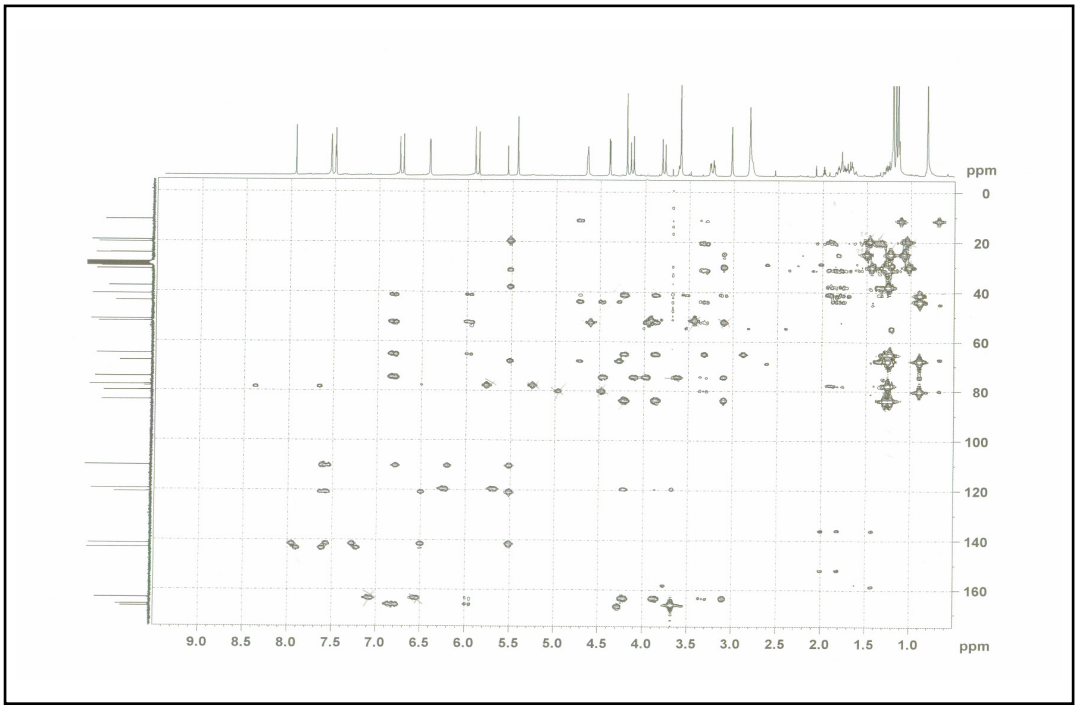

Figure 146 2D HMBC (acetone-d_6) spectrum of compound **AM13**

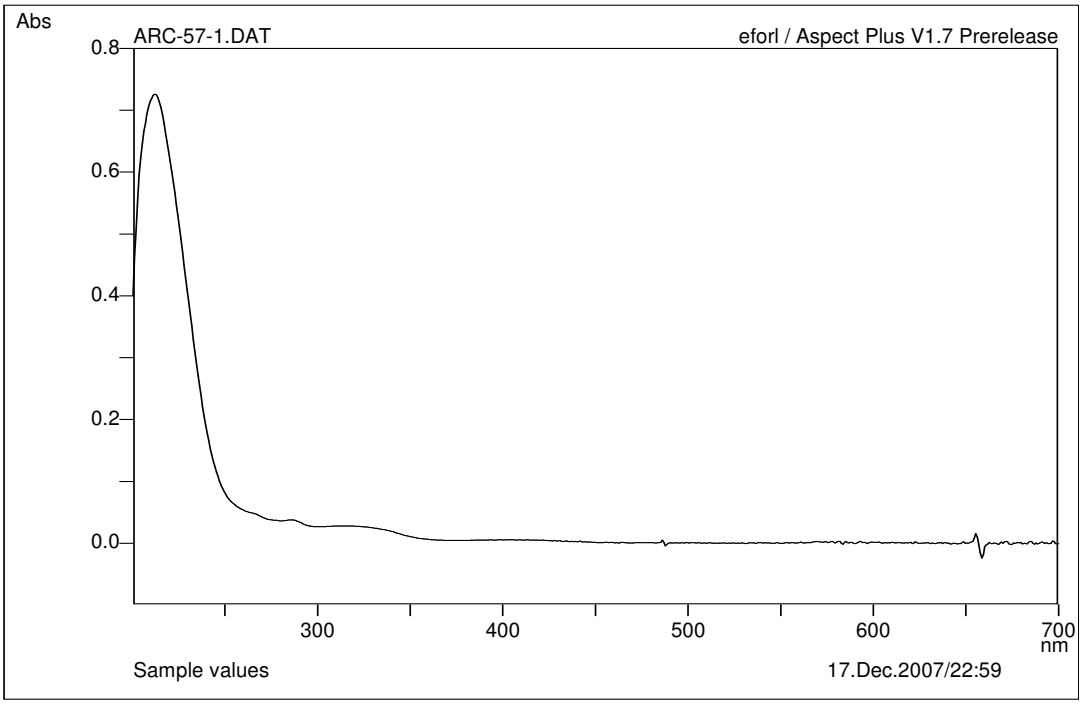

Figure 147 UV (MeOH) spectrum of compound **AM14**

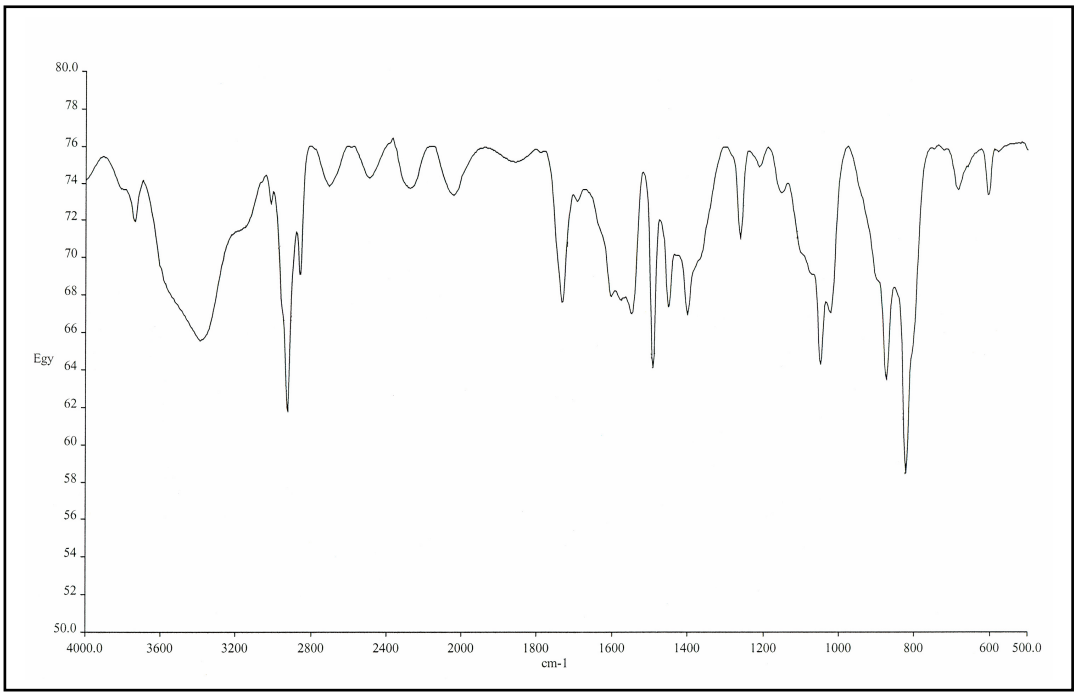

Figure 148 IR (neat) spectrum of compound **AM14**

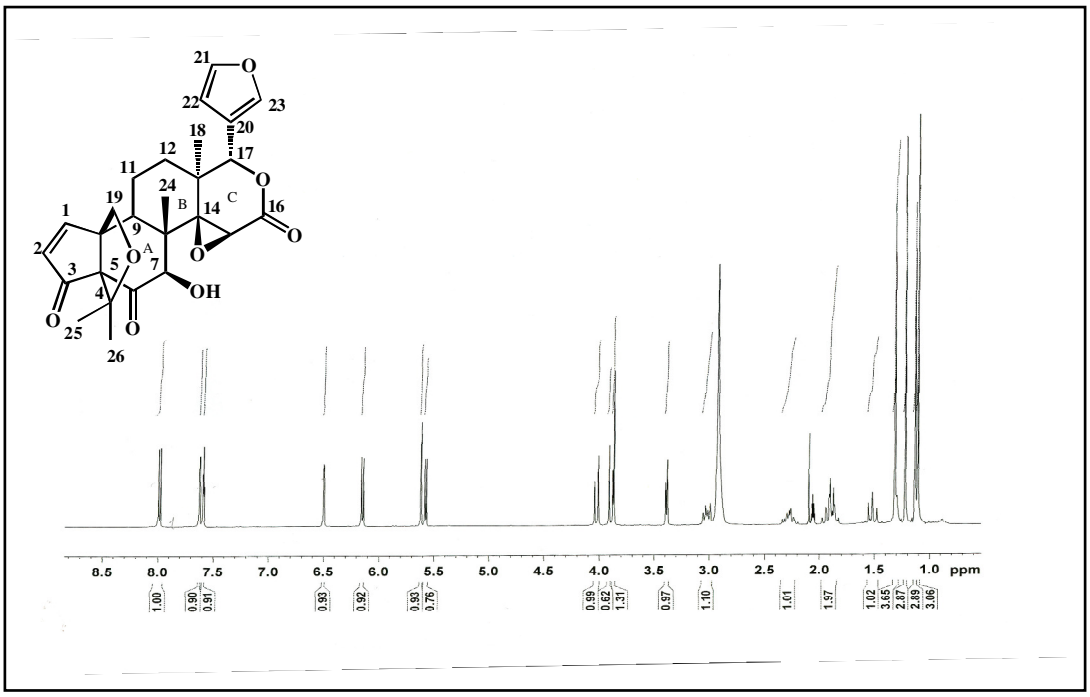

Figure 149 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM14**

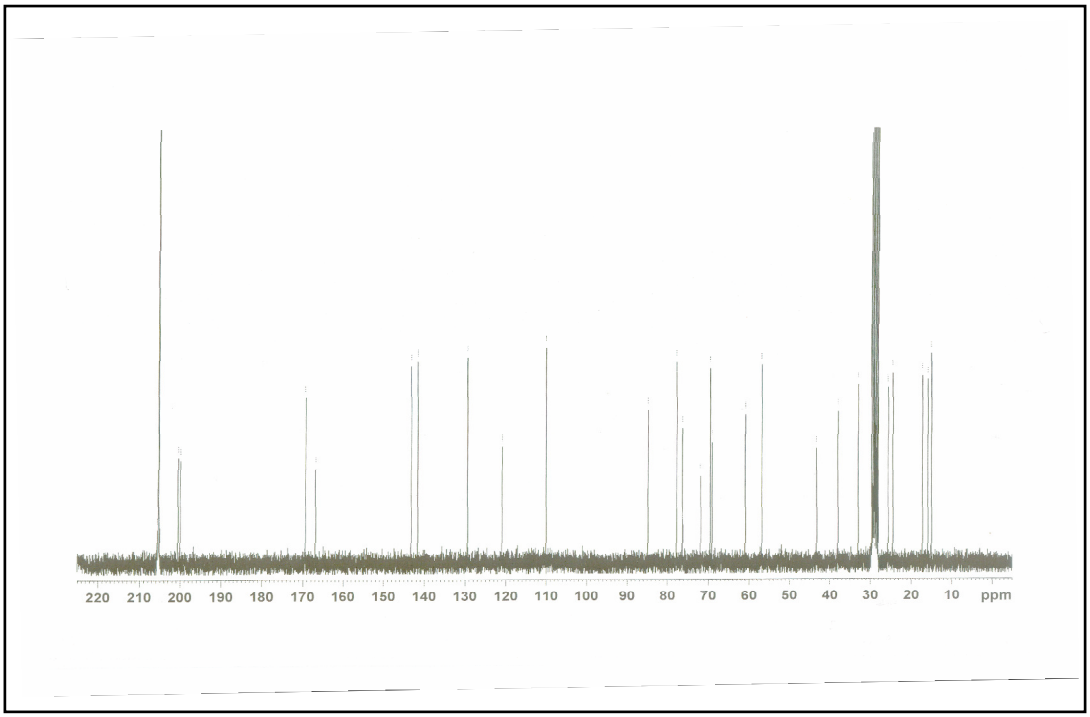

Figure 150 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM14**

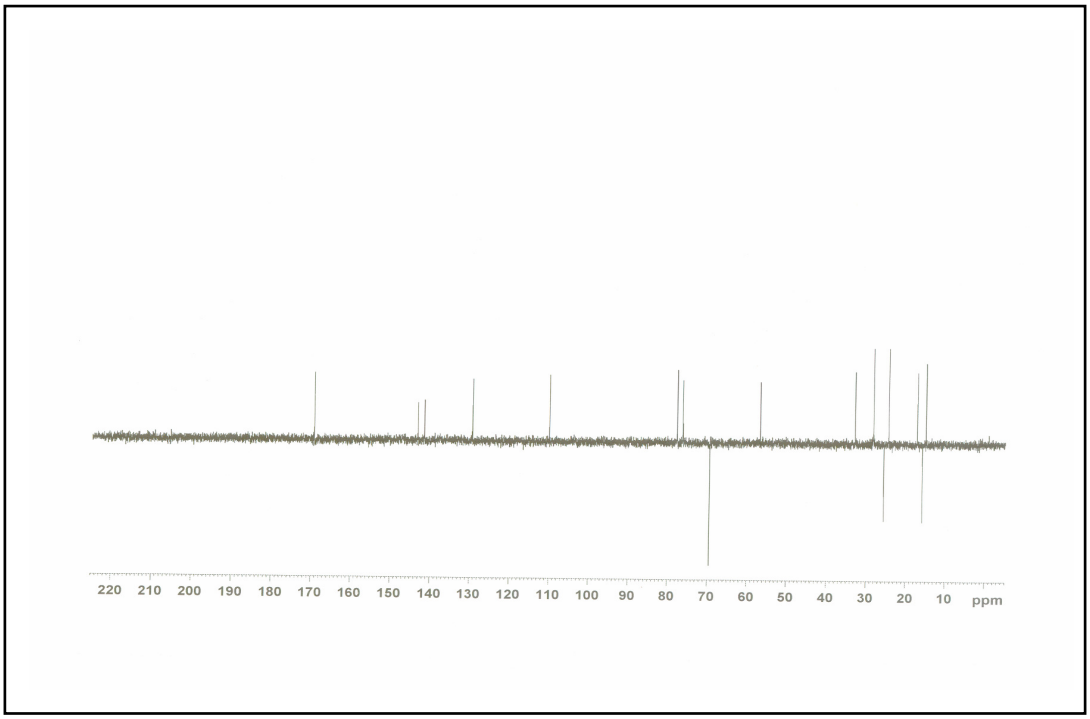

Figure 151 DEPT 135° (acetone-d_6) spectrum of compound **AM14**

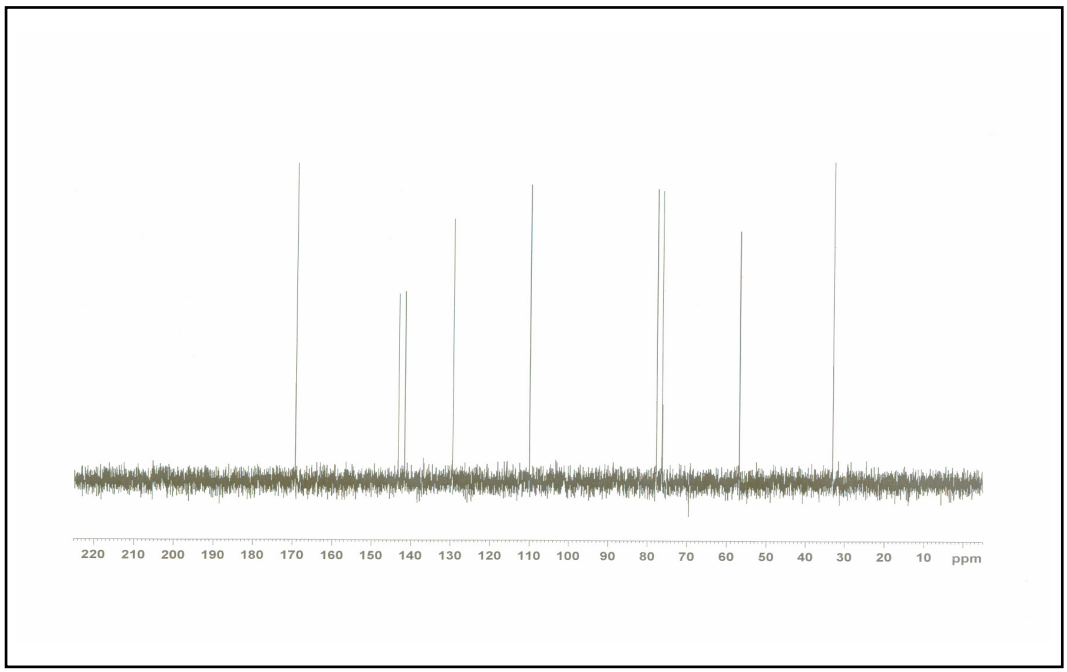

Figure 152 DEPT 90° (acetone-d_6) spectrum of compound **AM14**

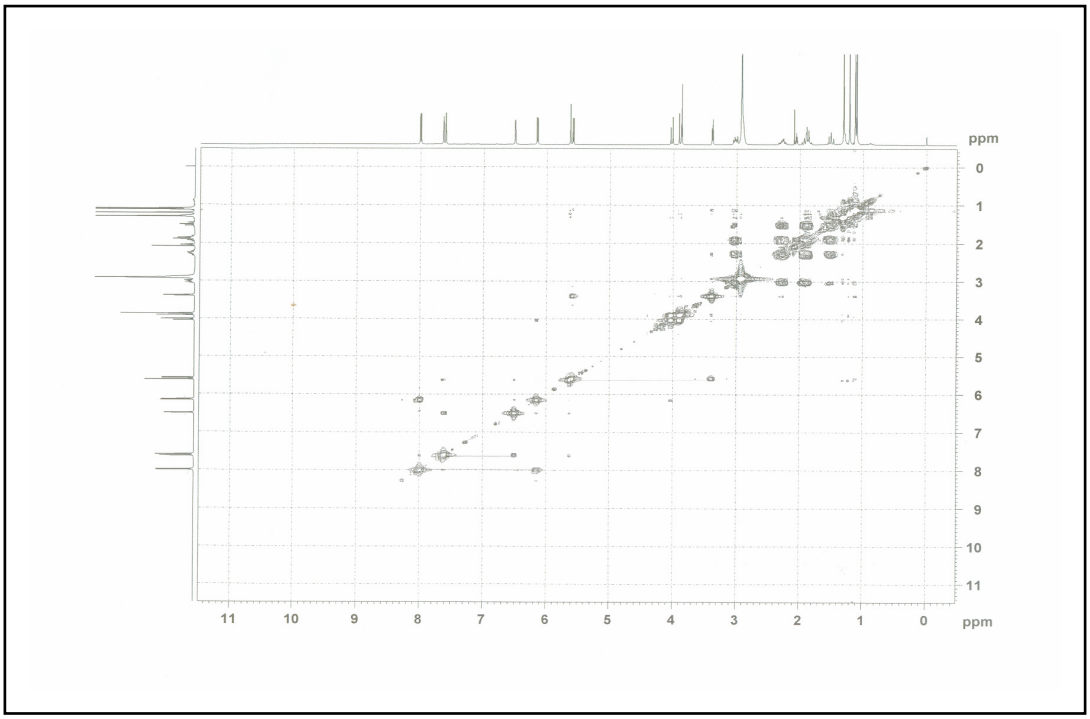

Figure 153 2D COSY (acetone-d_6) spectrum of compound **AM14**

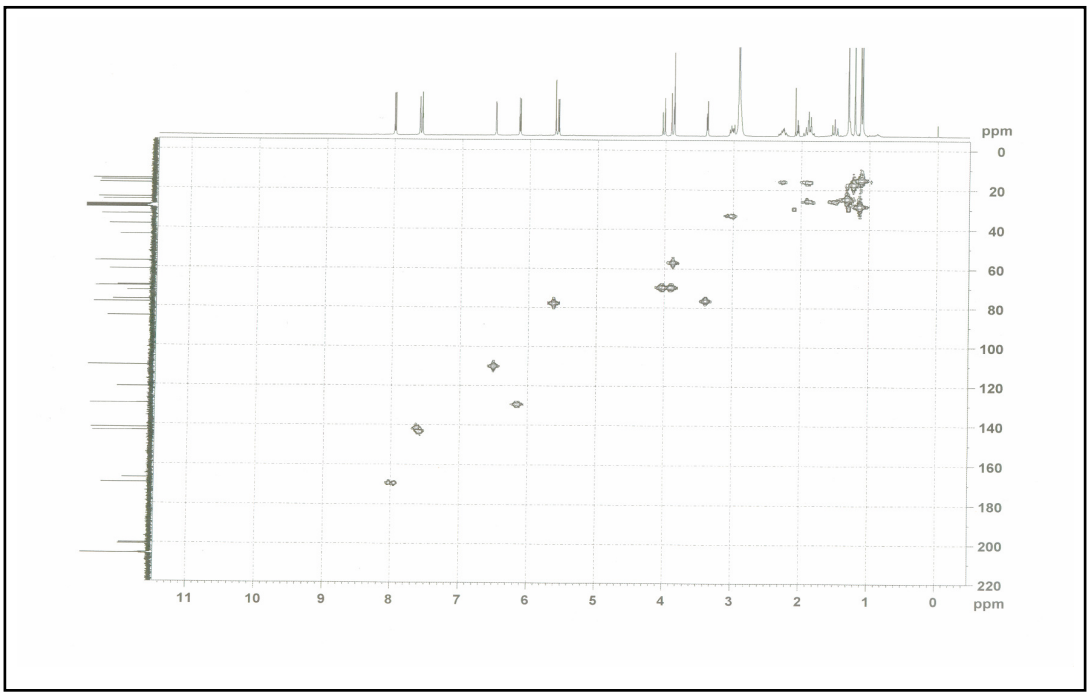

Figure 154 2D HMQC (acetone-d_6) spectrum of compound **AM14**

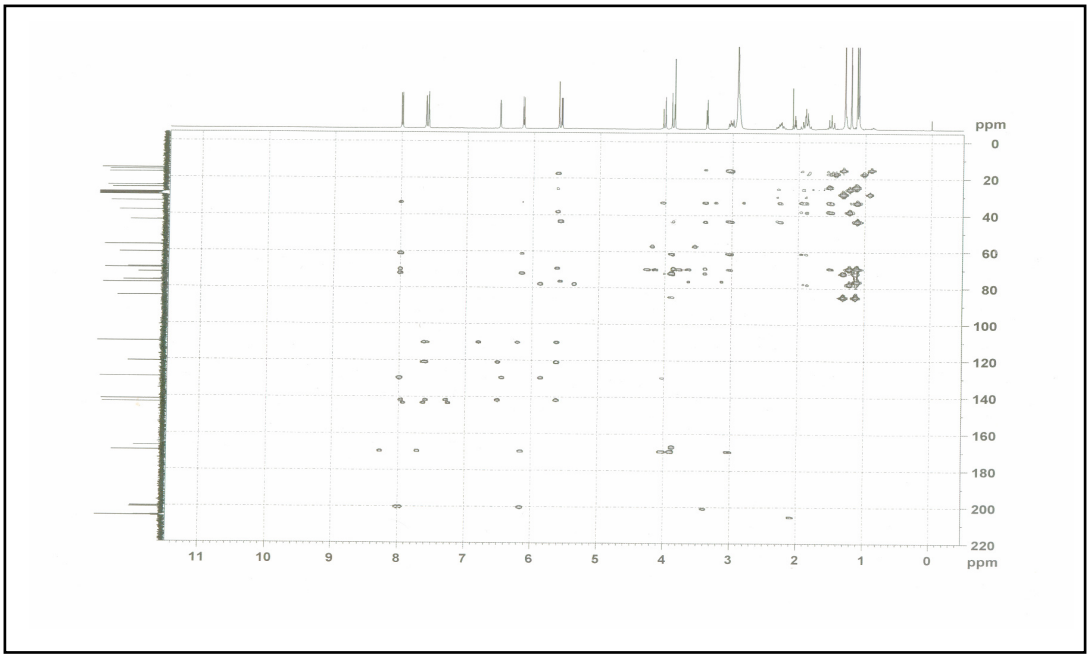

Figure 155 2D HMBC (acetone-d_6) spectrum of compound **AM14**

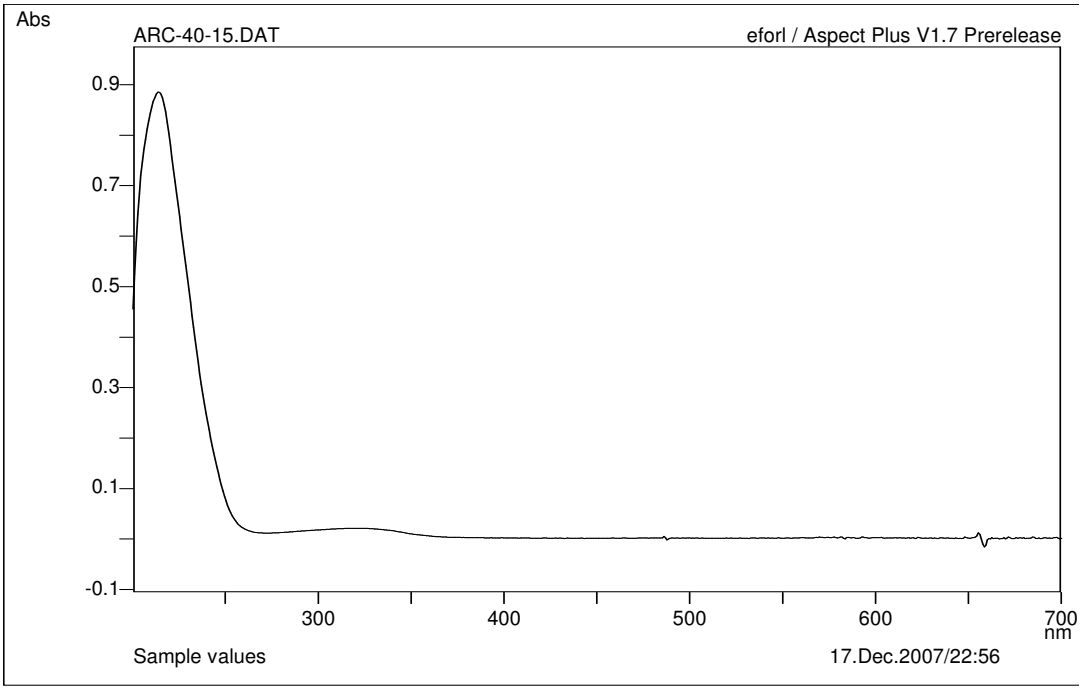

Figure 156 UV (MeOH) spectrum of compound **AM15**

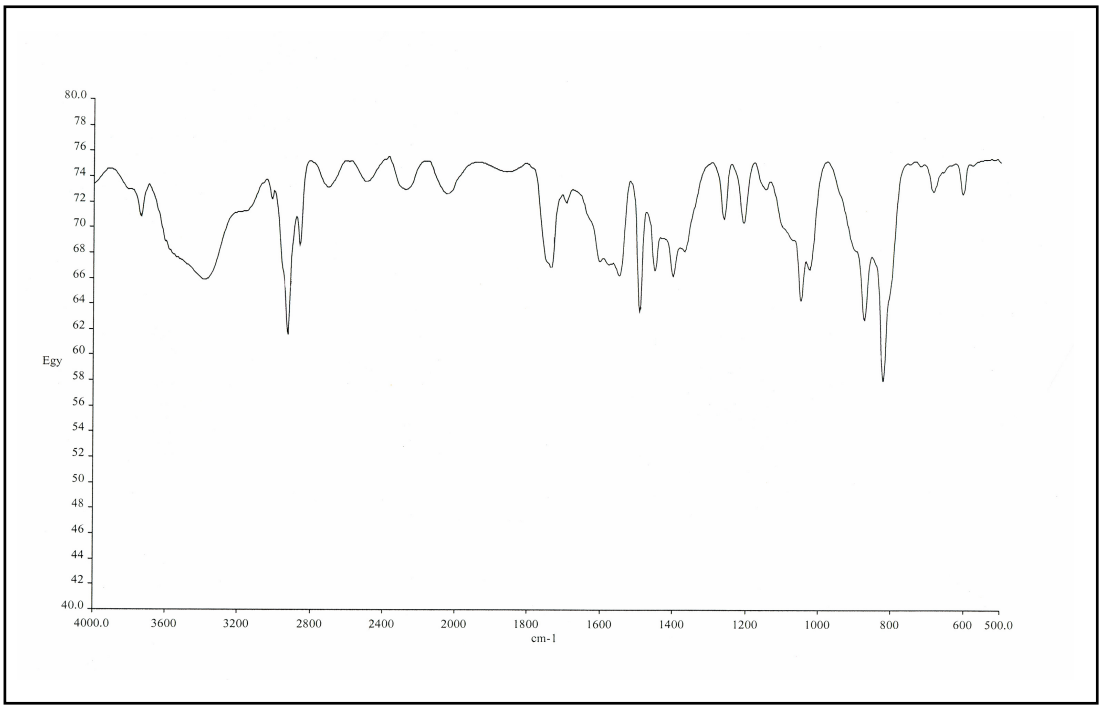

Figure 157 IR (neat) spectrum of compound **AM15**

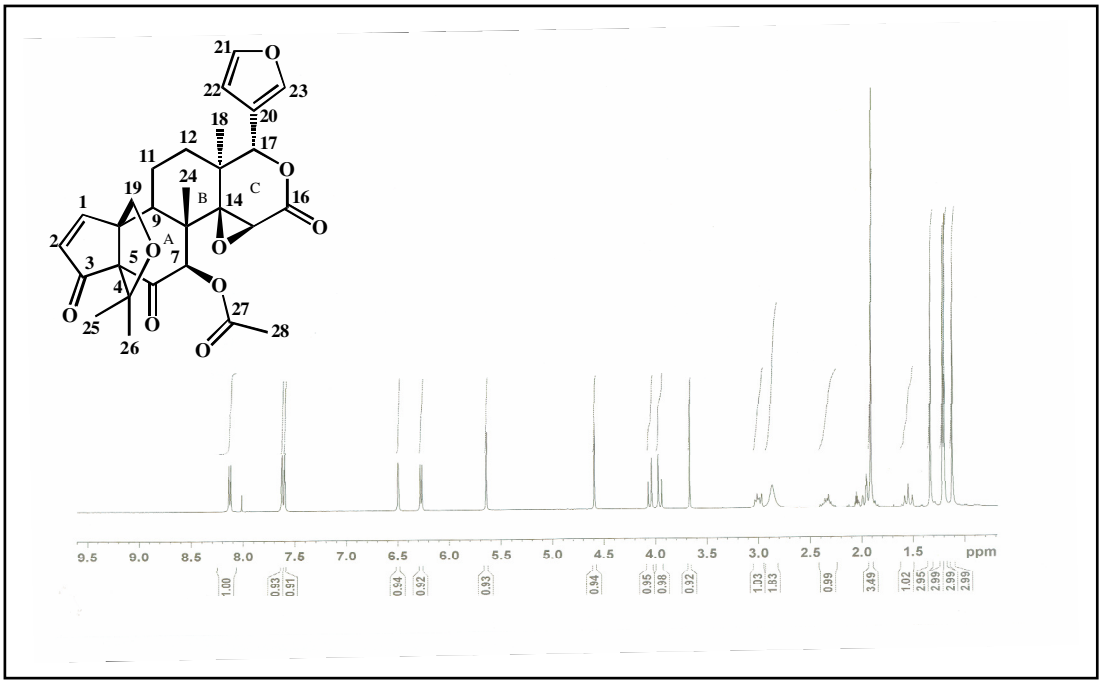

Figure 158 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM15**

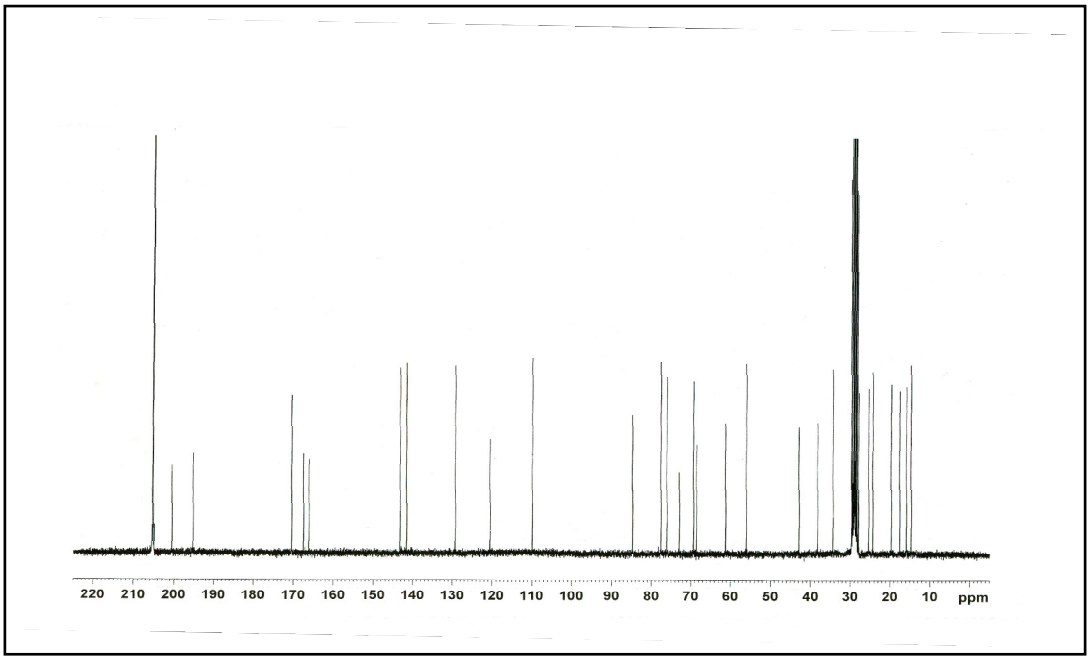

Figure 159 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM15**

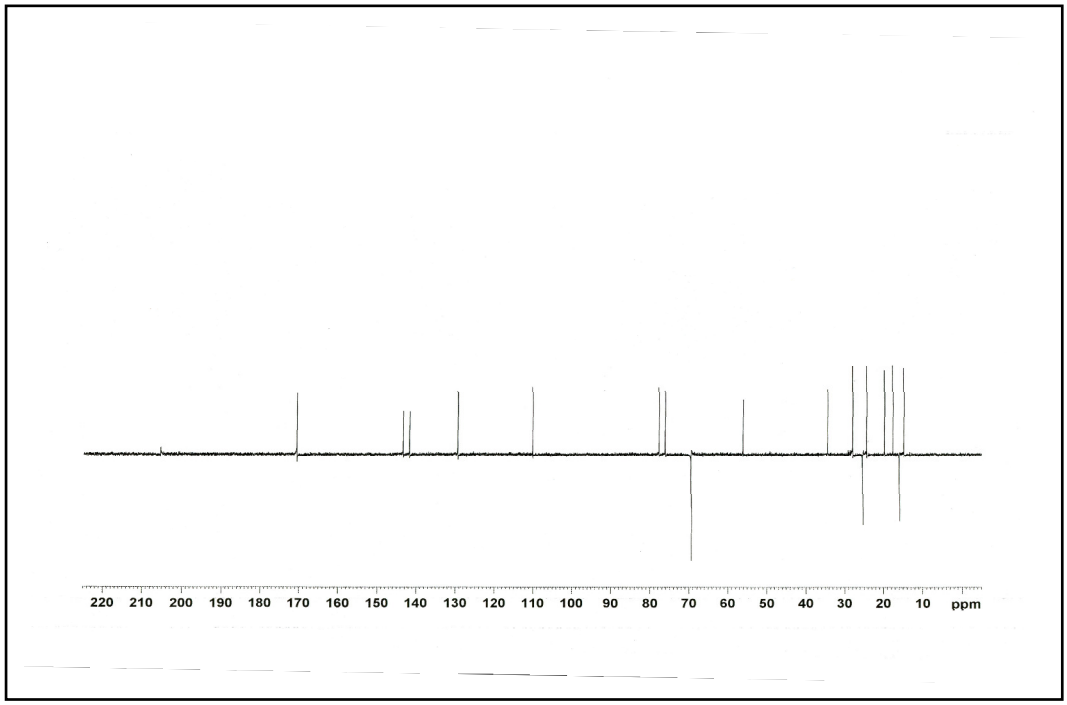

Figure 160 DEPT 135° (acetone-d_6) spectrum of compound **AM15**

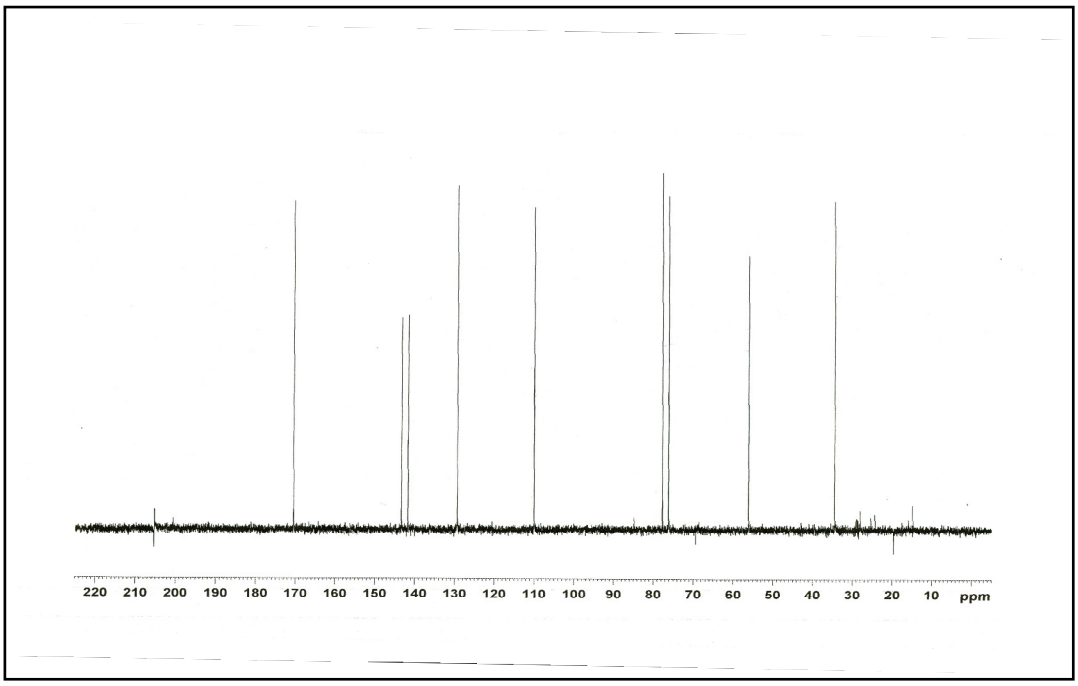

Figure 161 DEPT 90° (acetone-d_6) spectrum of compound **AM15**

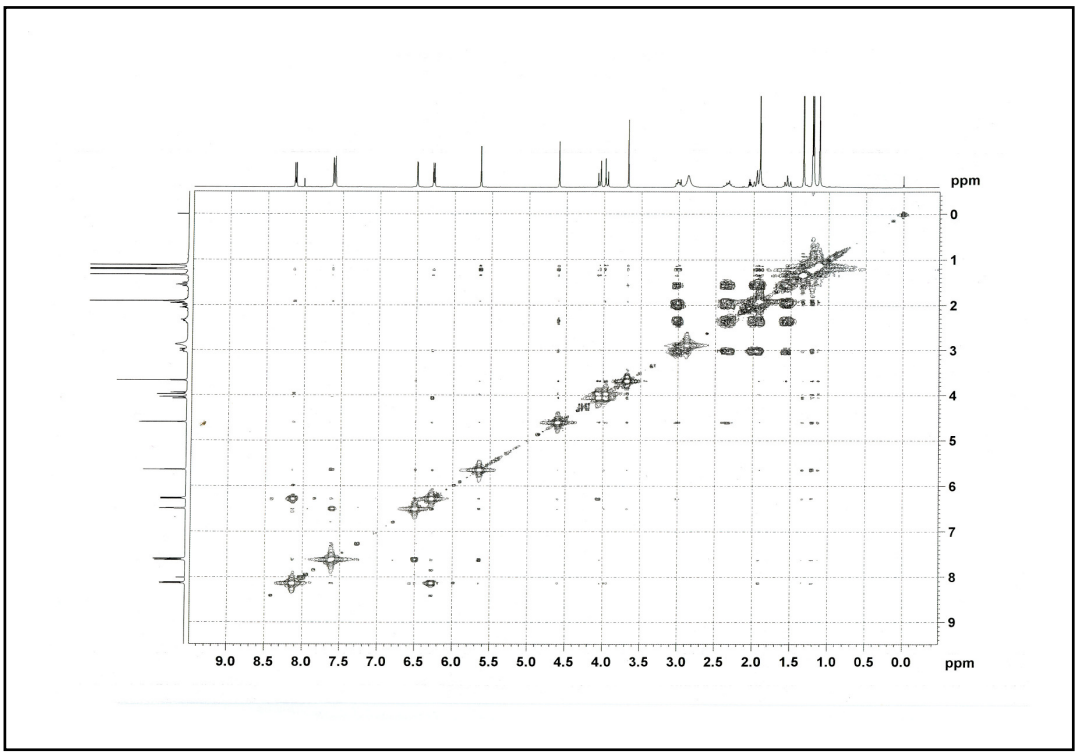

Figure 162 2D COSY (acetone-d_6) spectrum of compound **AM15**

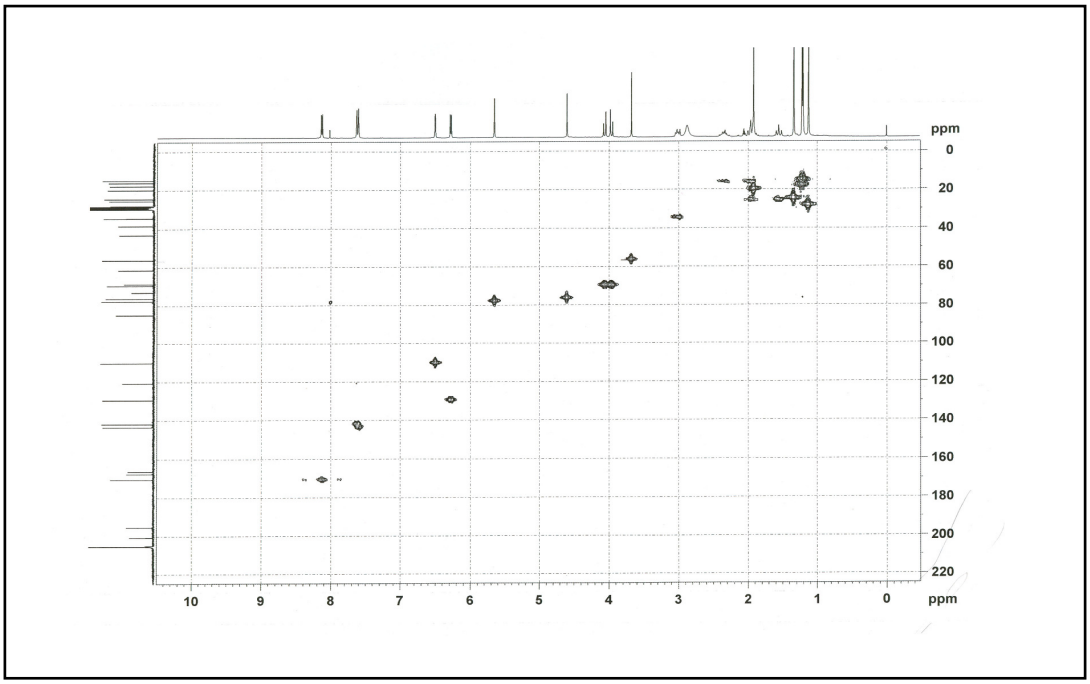

Figure 163 2D HMQC (acetone-d_6) spectrum of compound **AM15**

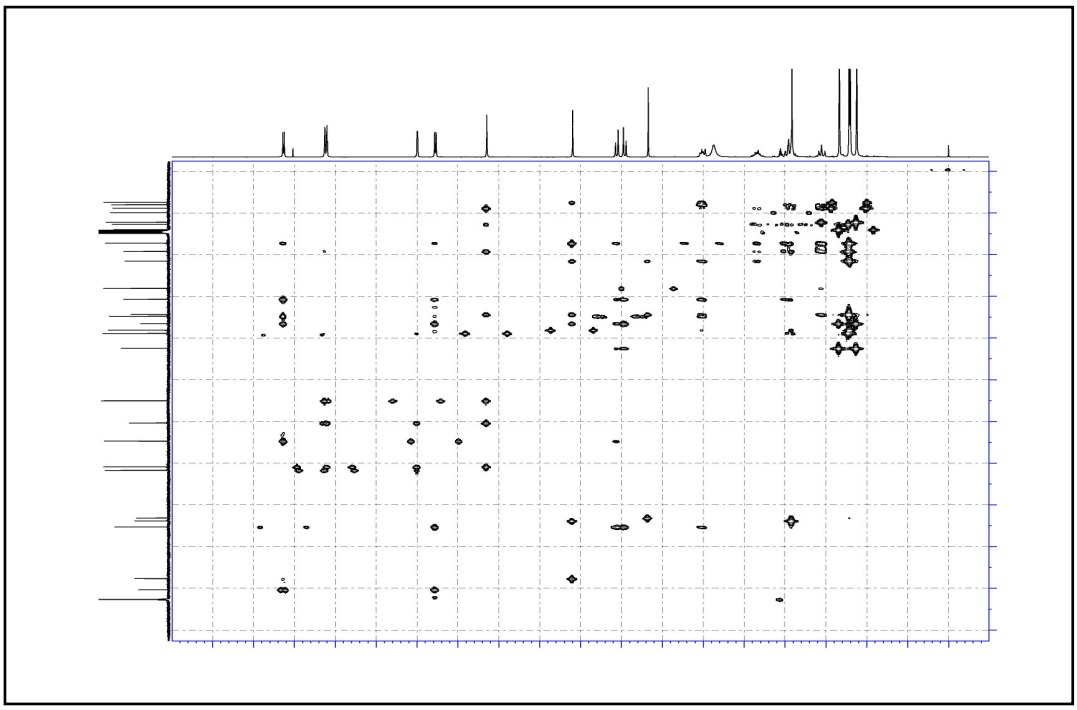

Figure 164 2D HMBC (acetone-d_6) spectrum of compound **AM15**

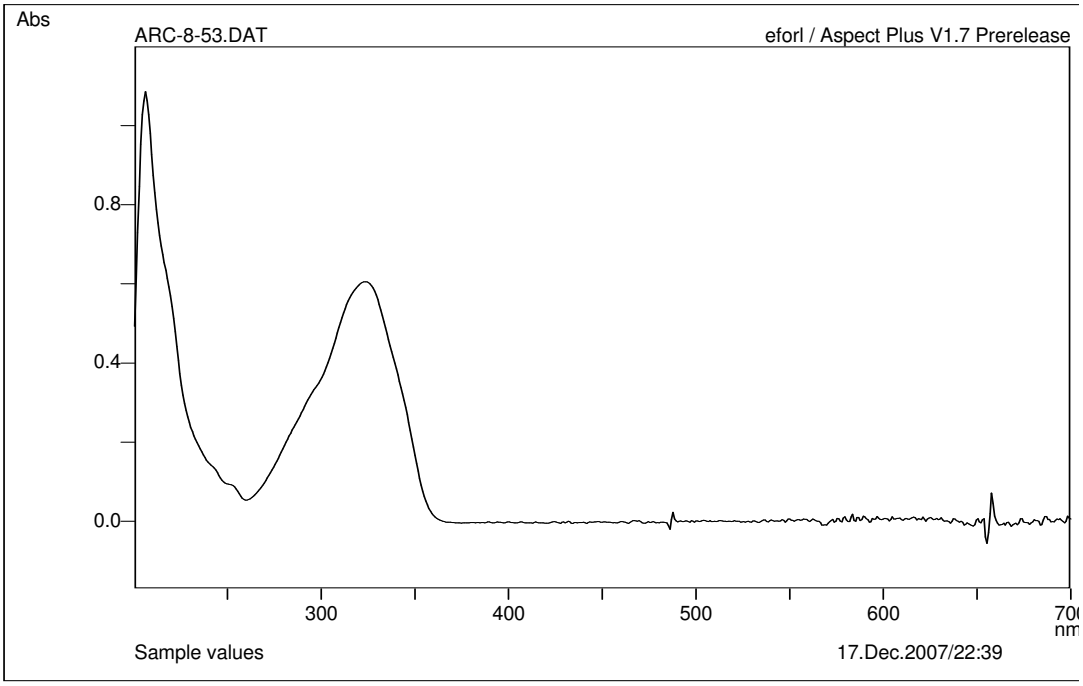

Figure 165 UV (MeOH) spectrum of compound **AM16**

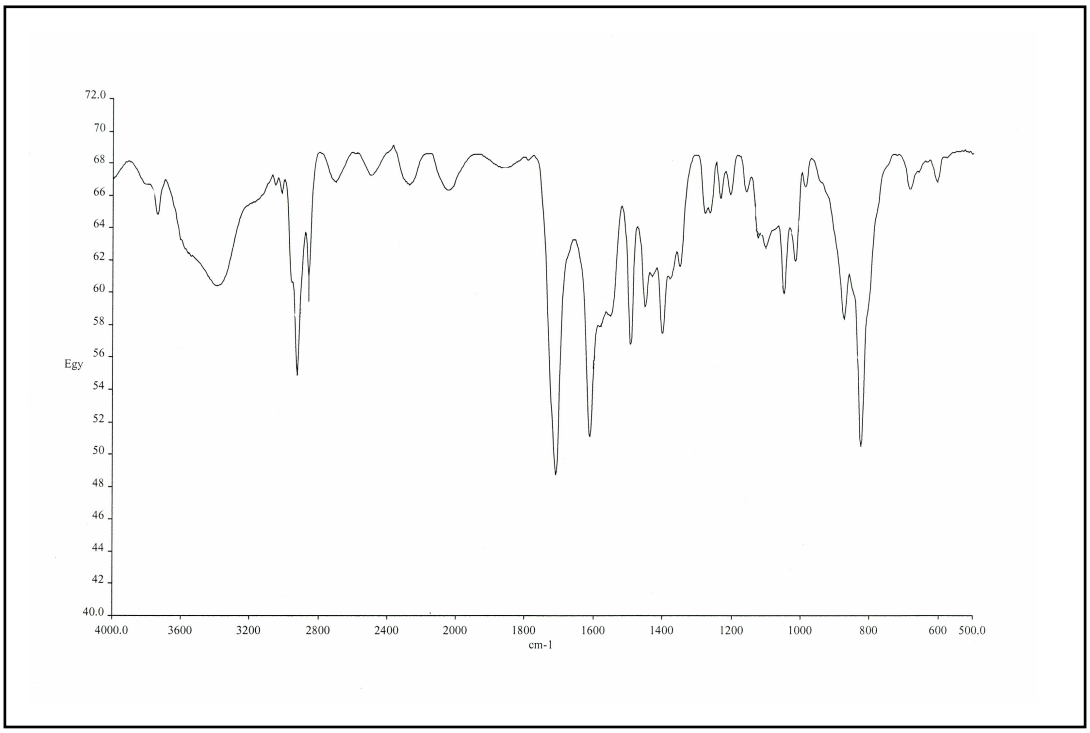

Figure 166 IR (neat) spectrum of compound **AM16**

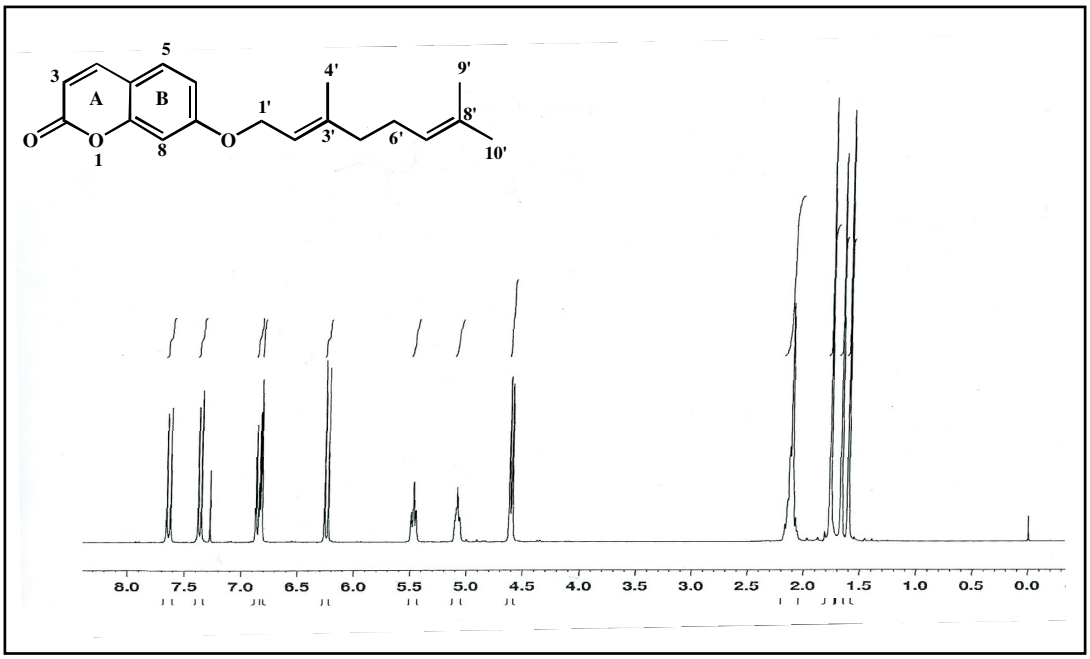

Figure 167 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **AM16**

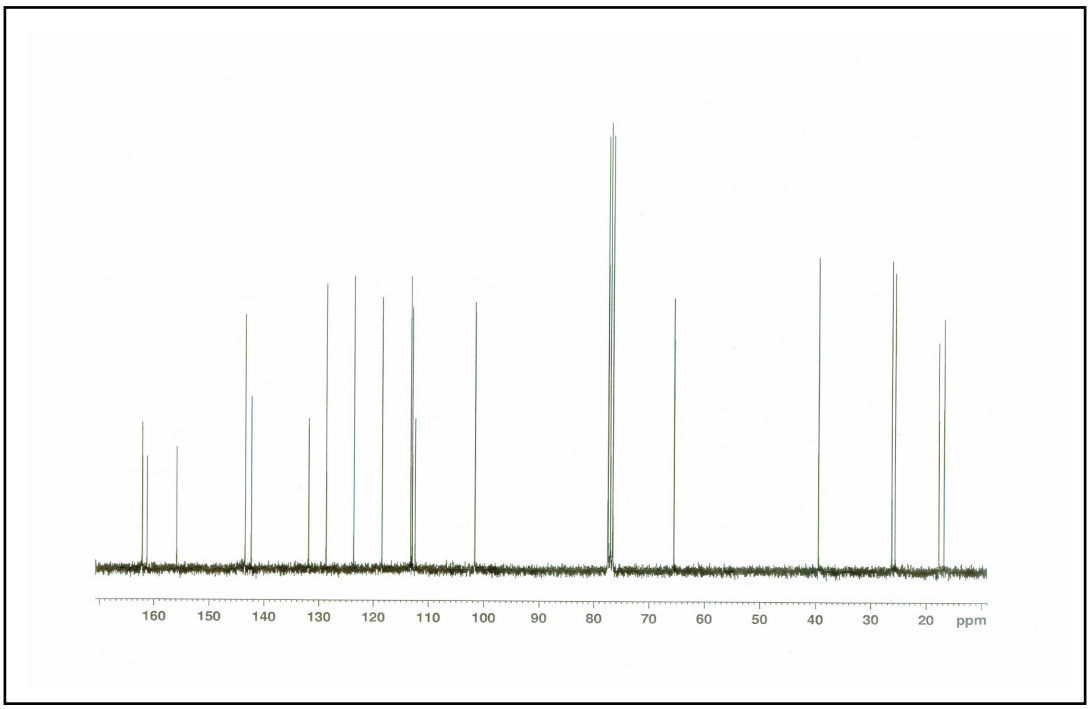

Figure 168 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **AM16**

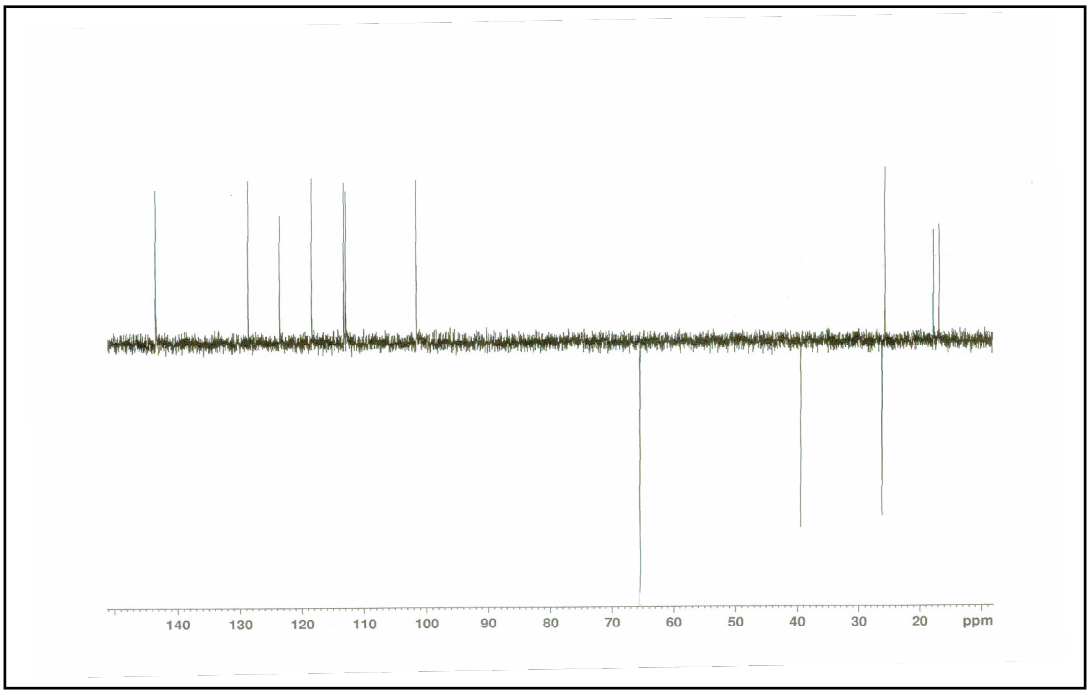

Figure 169 DEPT 135° ($CDCl_3$) spectrum of compound **AM16**

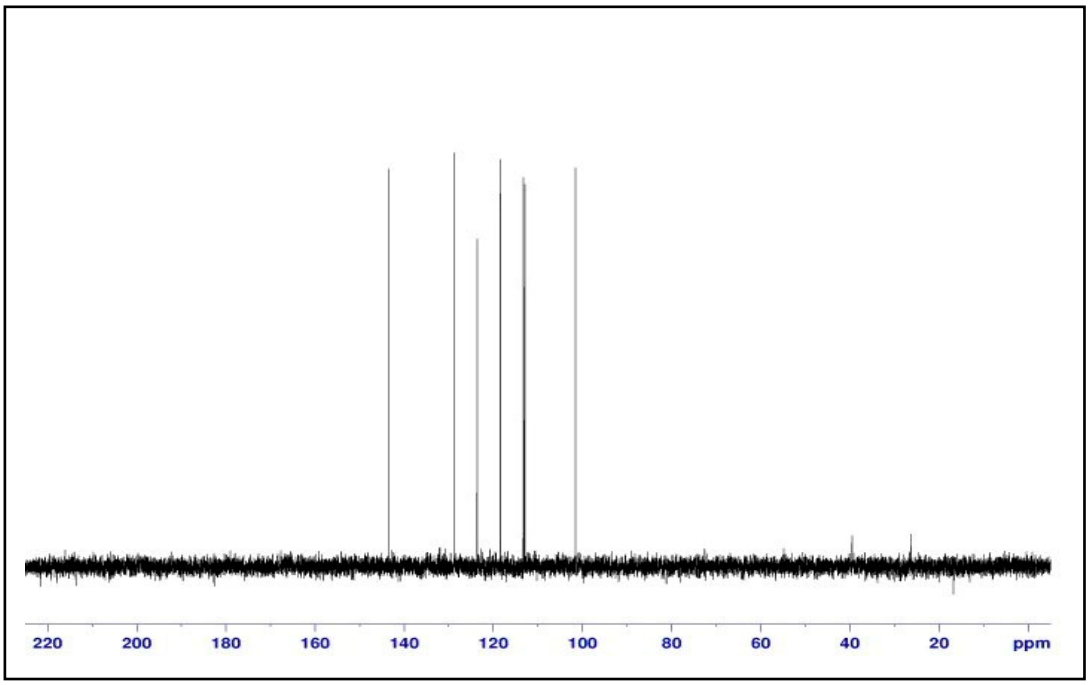

Figure 170 DEPT 90° ($CDCl_3$) spectrum of compound **AM16**

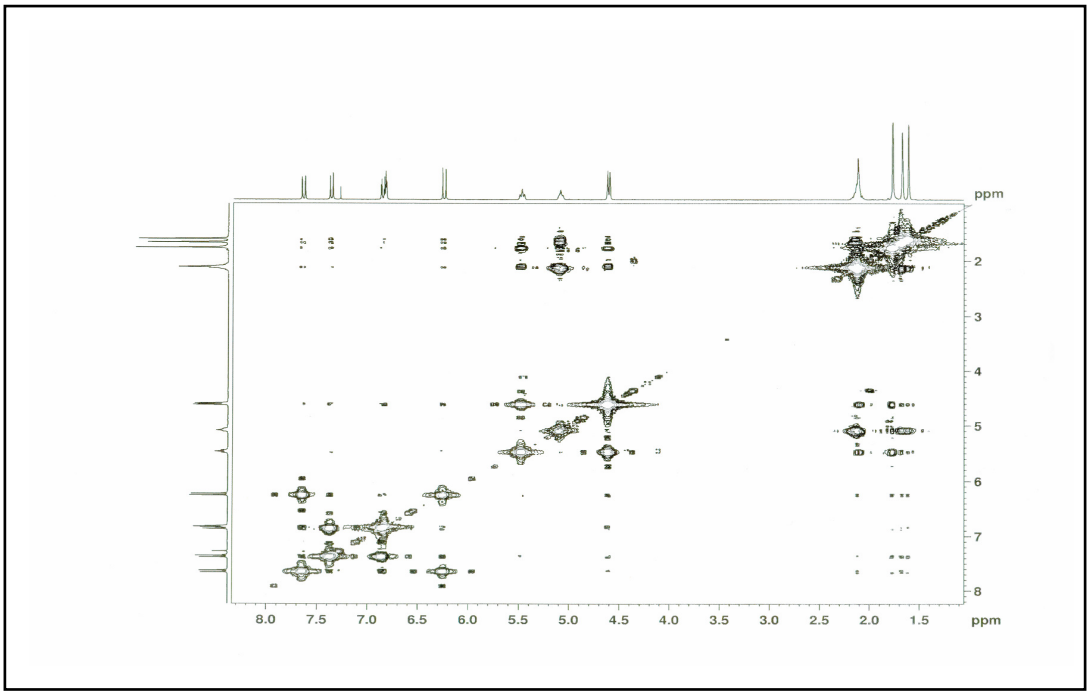

Figure 171 2D COSY ($CDCl_3$) spectrum of compound **AM16**

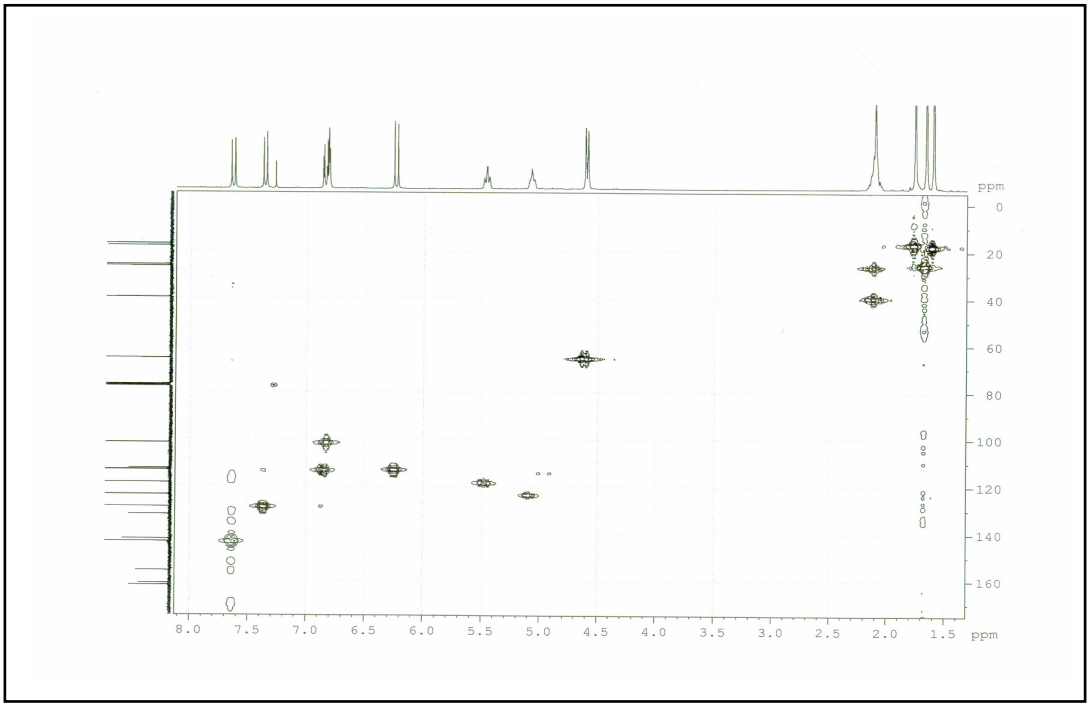

Figure 172 2D HMQC ($CDCl_3$) spectrum of compound **AM16**

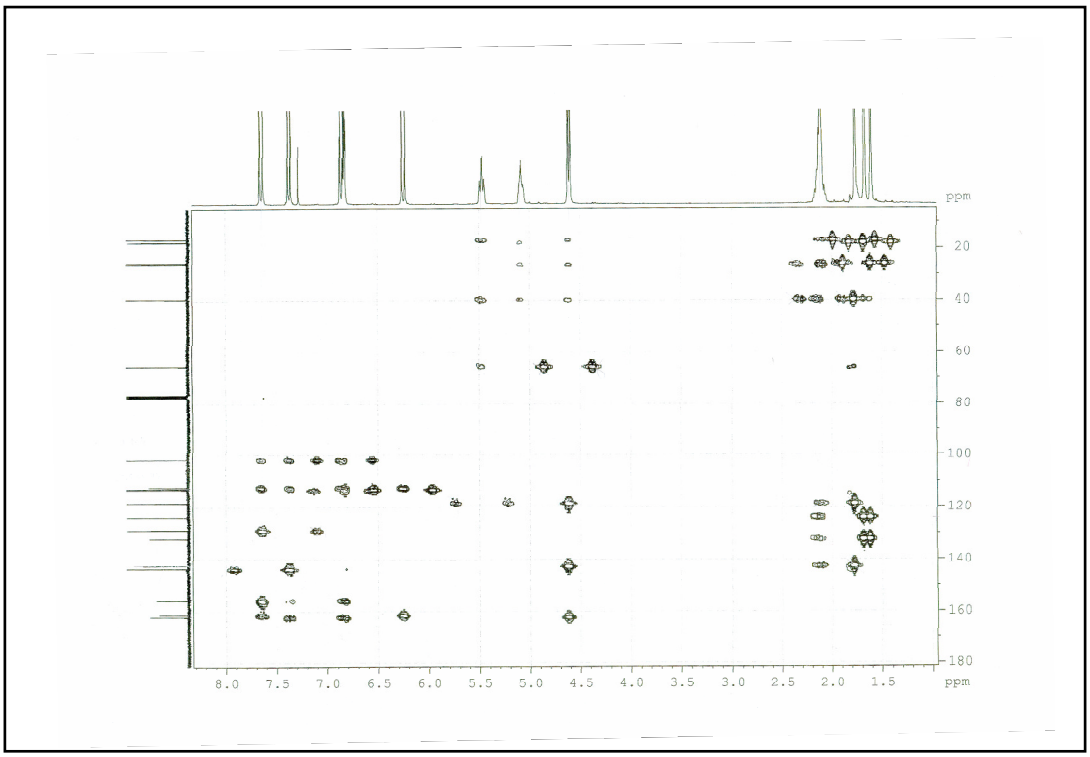

Figure 173 2D HMBC ($CDCl_3$) spectrum of compound **AM16**

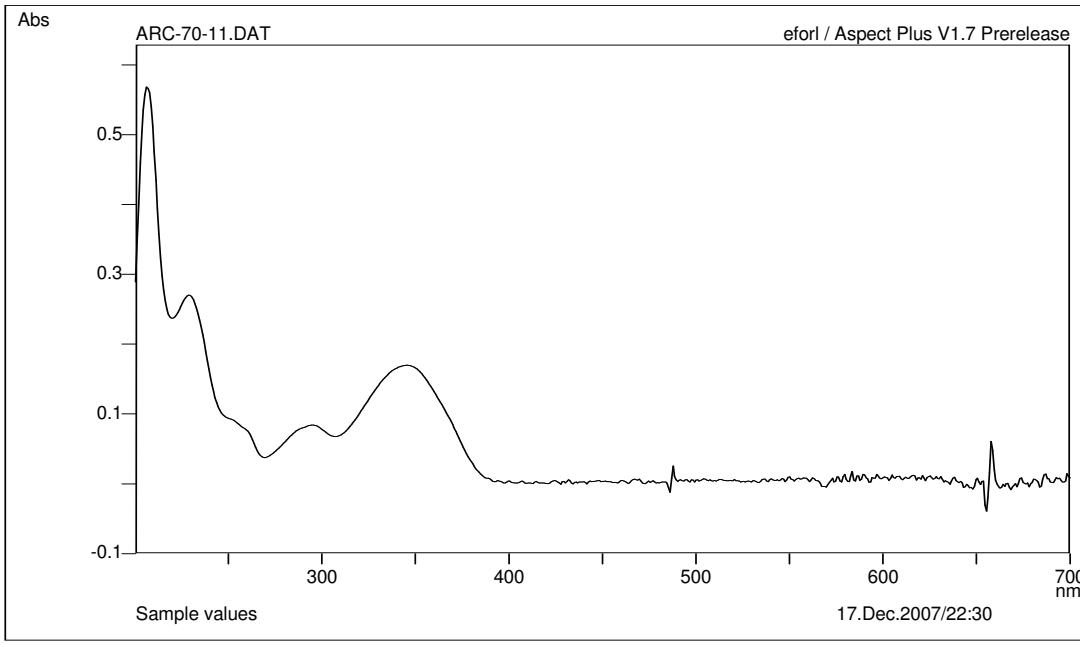

Figure 174 UV (MeOH) spectrum of compound **AM17**

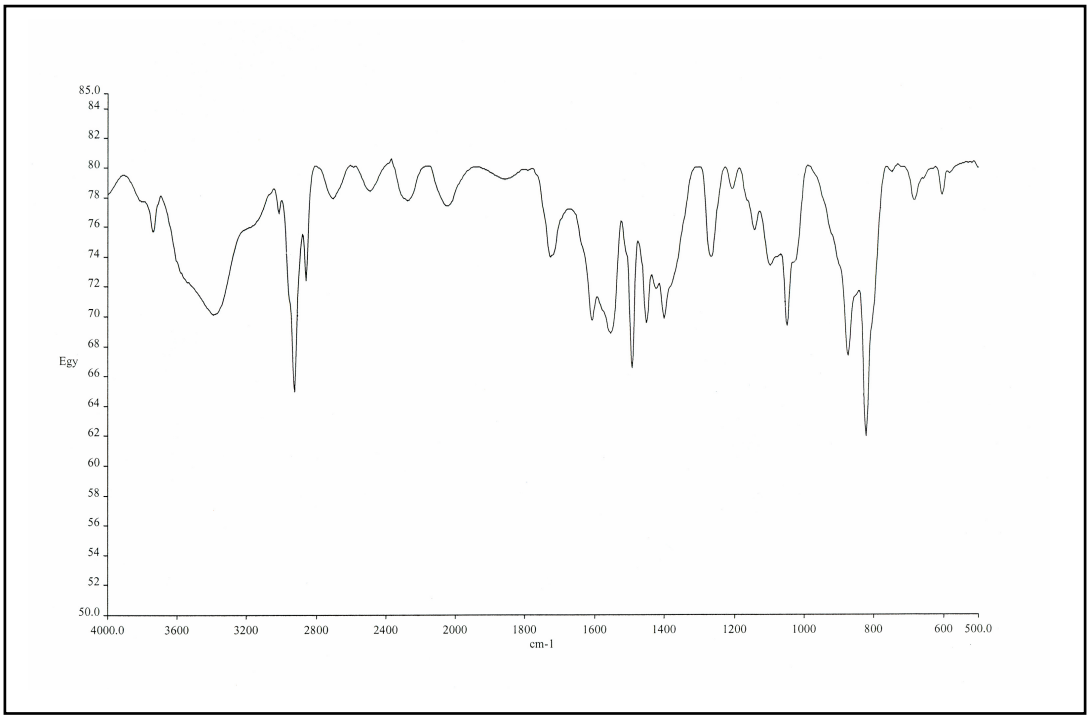

Figure 175 IR (neat) spectrum of compound **AM17**

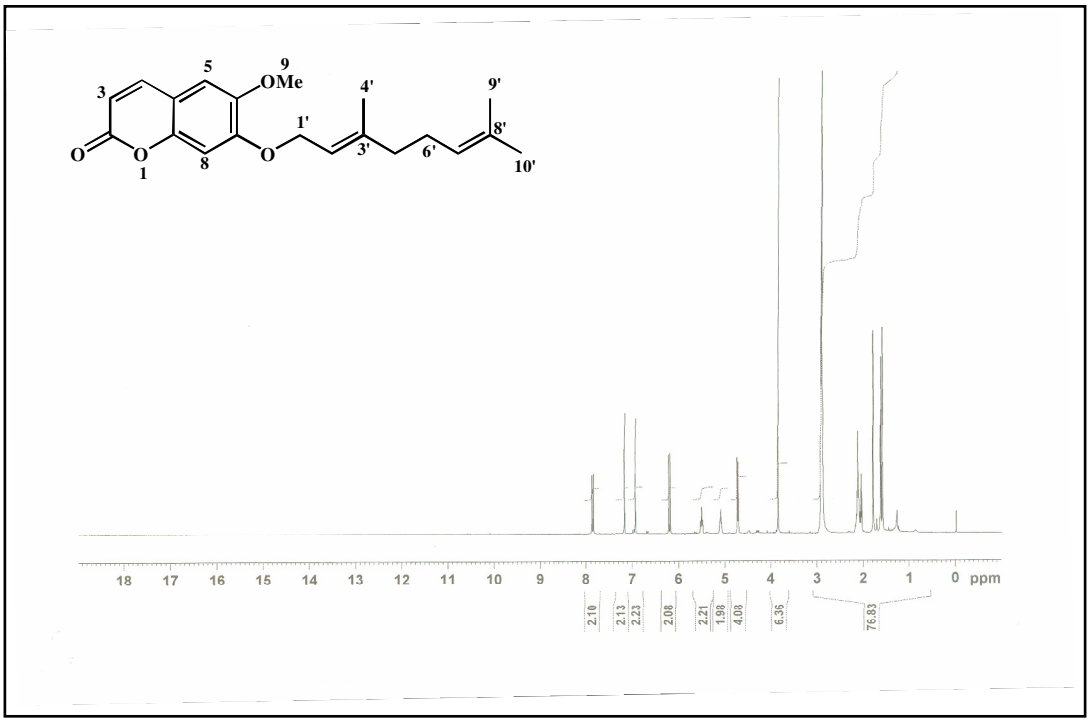

Figure 176 ^{1}H NMR (300 MHz) (acetone-d_6) spectrum of compound **AM17**

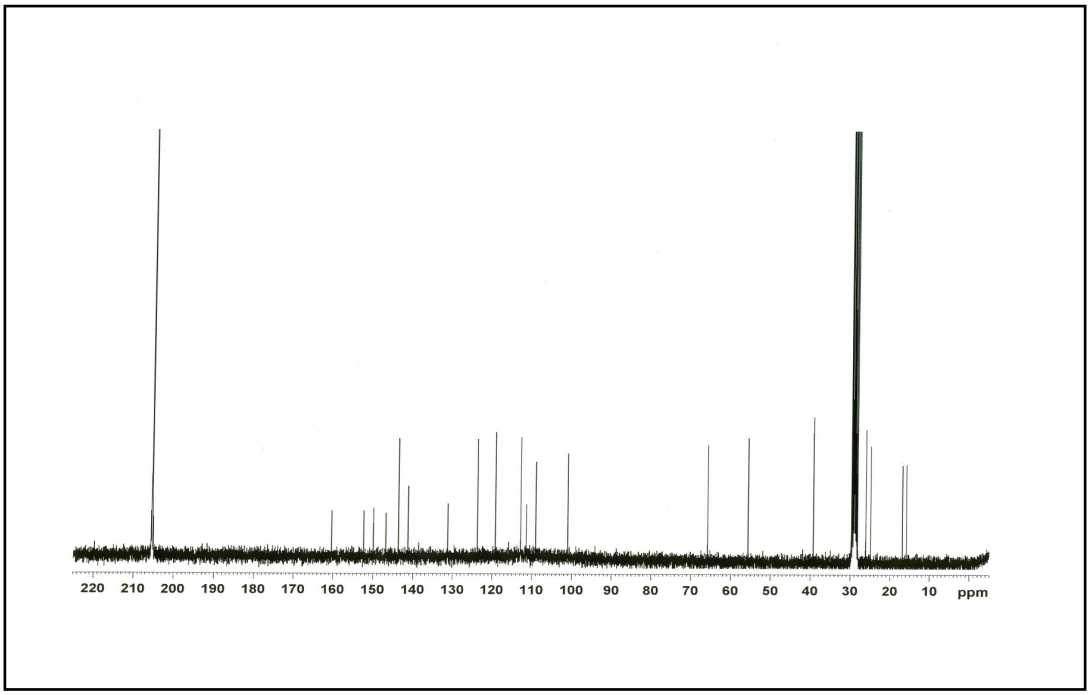

Figure 177 ^{13}C NMR (75 MHz) (acetone-d_6) spectrum of compound **AM17**

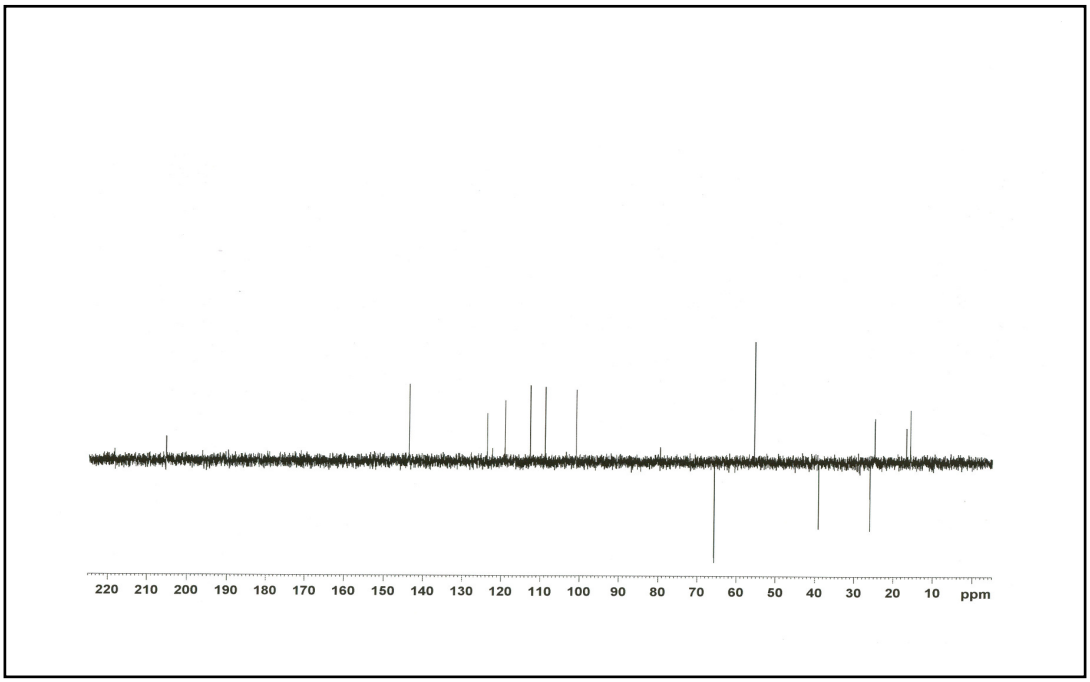

Figure 178 DEPT 135° (acetone-d_6) spectrum of compound **AM17**

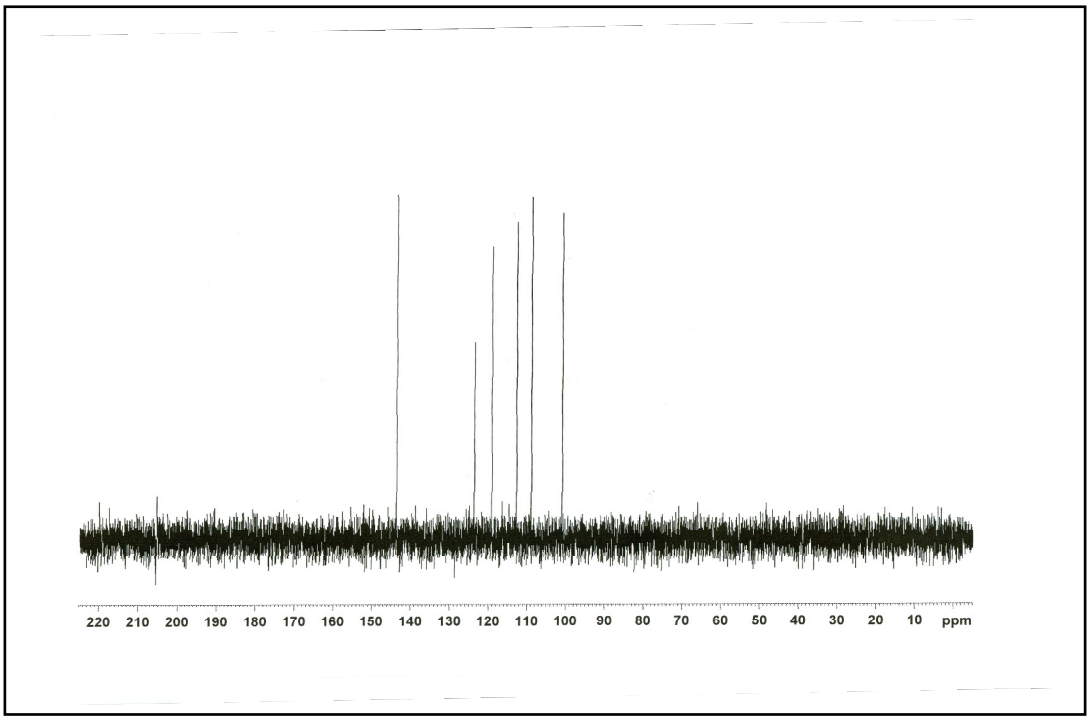

Figure 179 DEPT 90° (acetone-d_6) spectrum of compound **AM17**

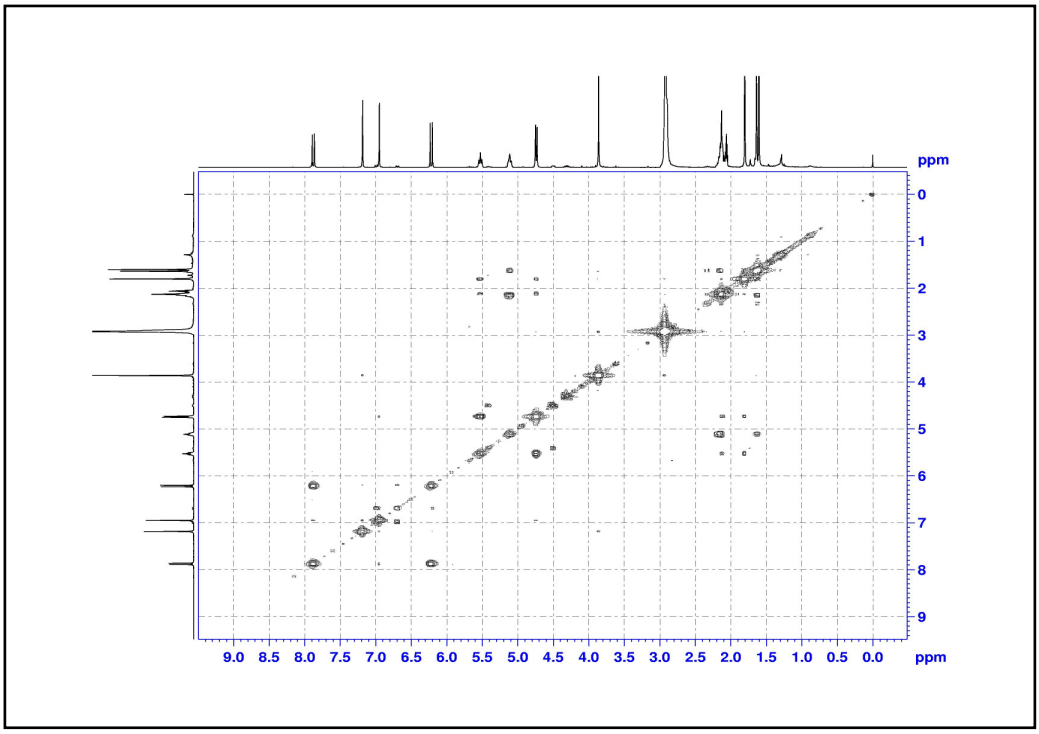

Figure 180 2D COSY (acetone-d_6) spectrum of compound **AM17**

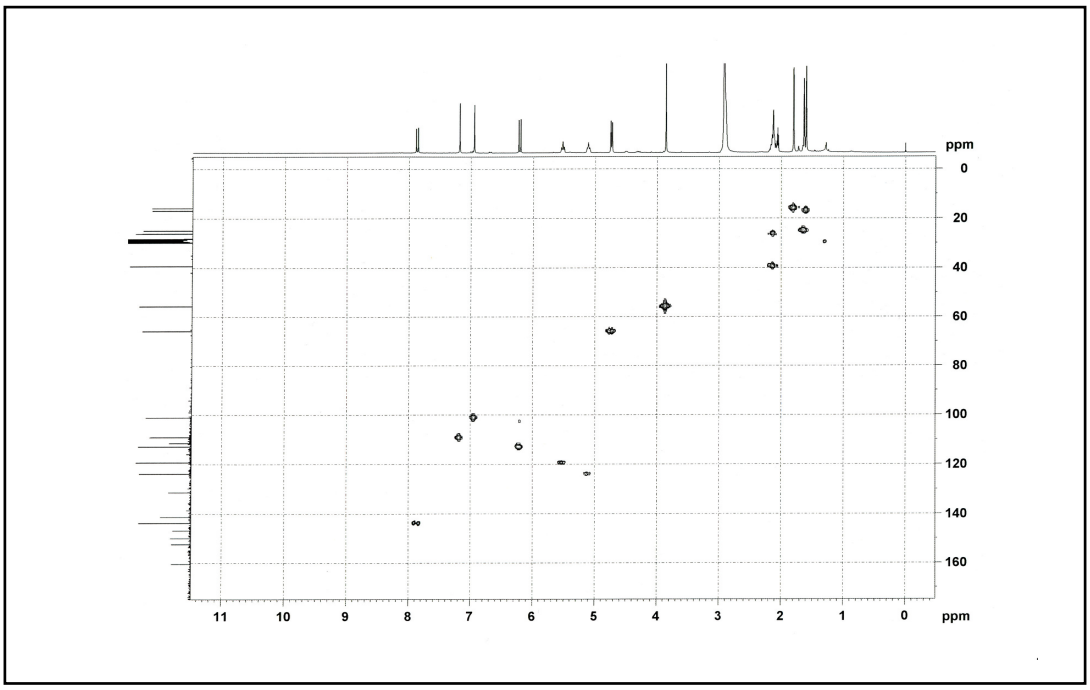

Figure 181 2D HMQC (acetone-d_6) spectrum of compound **AM17**

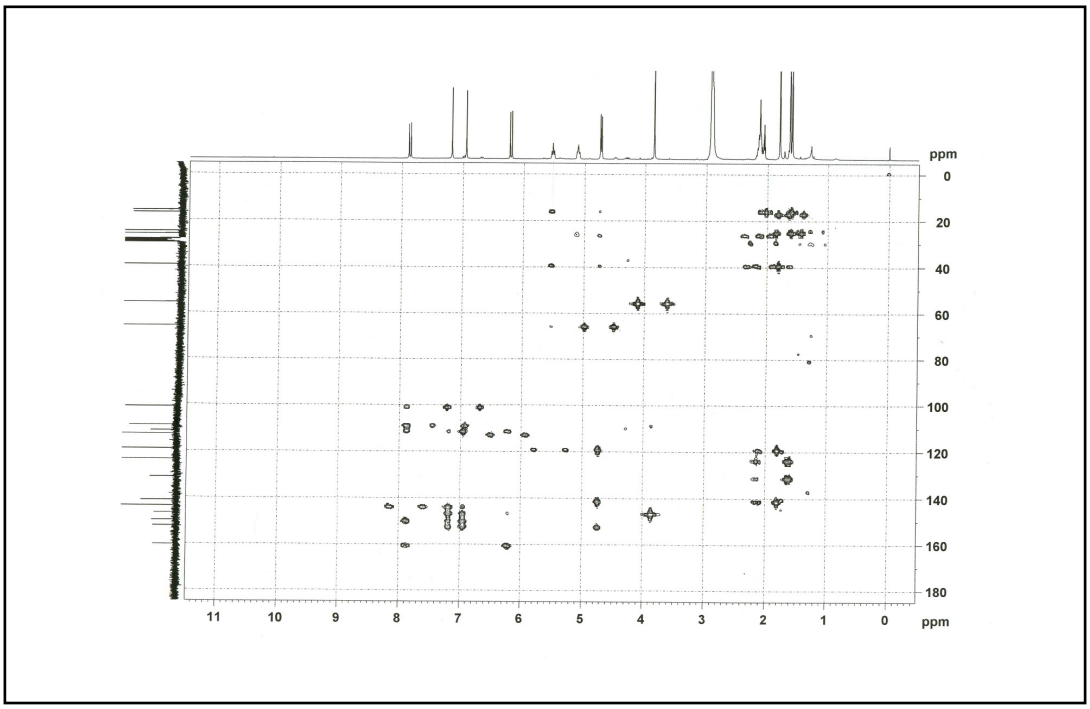

Figure 182 2D HMBC (acetone-d_6) spectrum of compound **AM17**

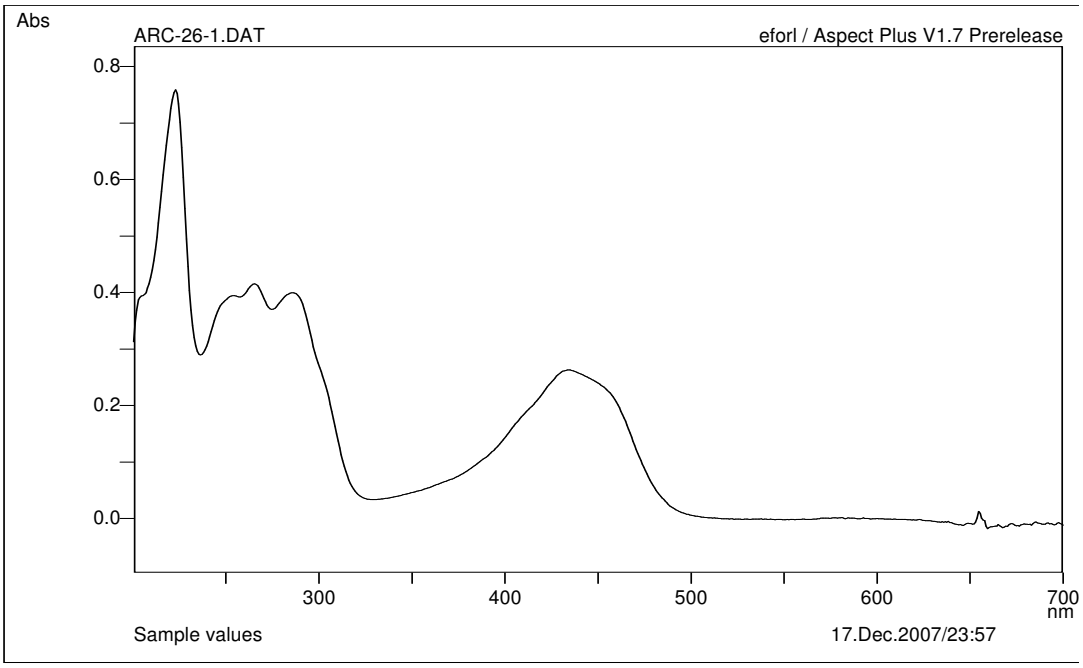

Figure 183 UV (MeOH) spectrum of compound **AM18**

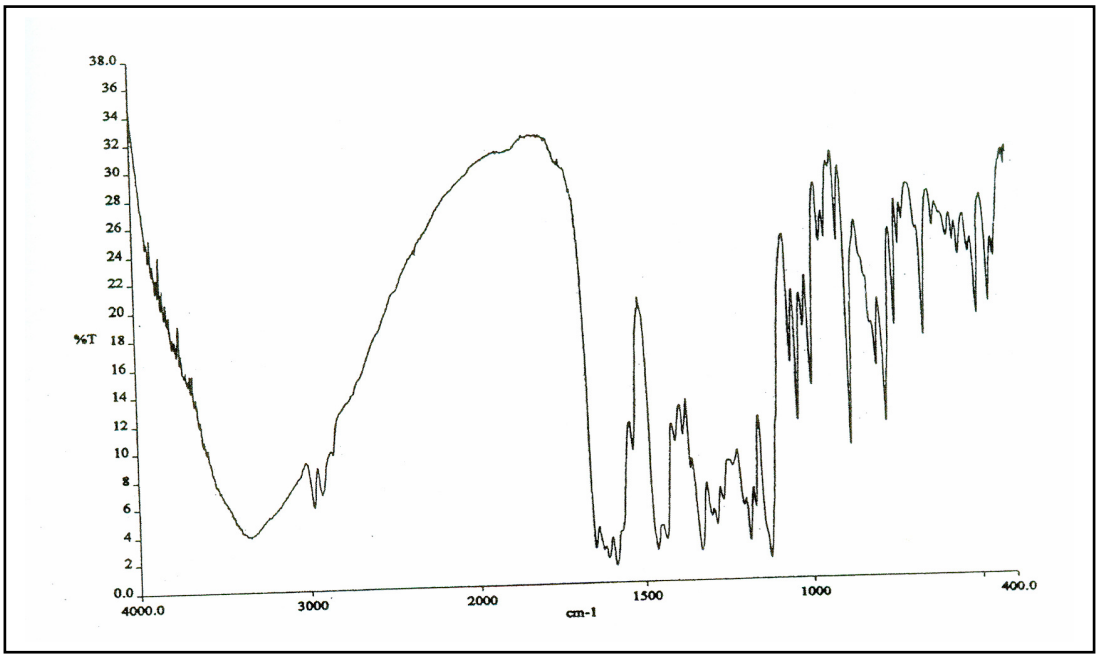

Figure 184 IR (neat) spectrum of compound **AM18**

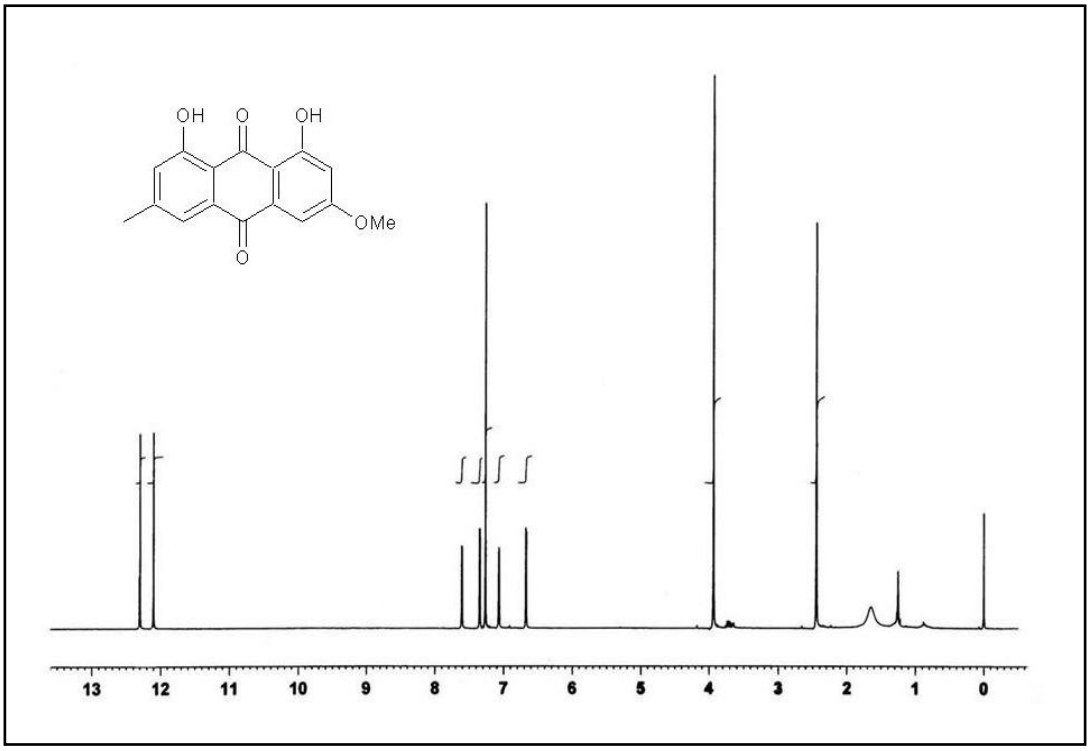

Figure 185 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **AM18**

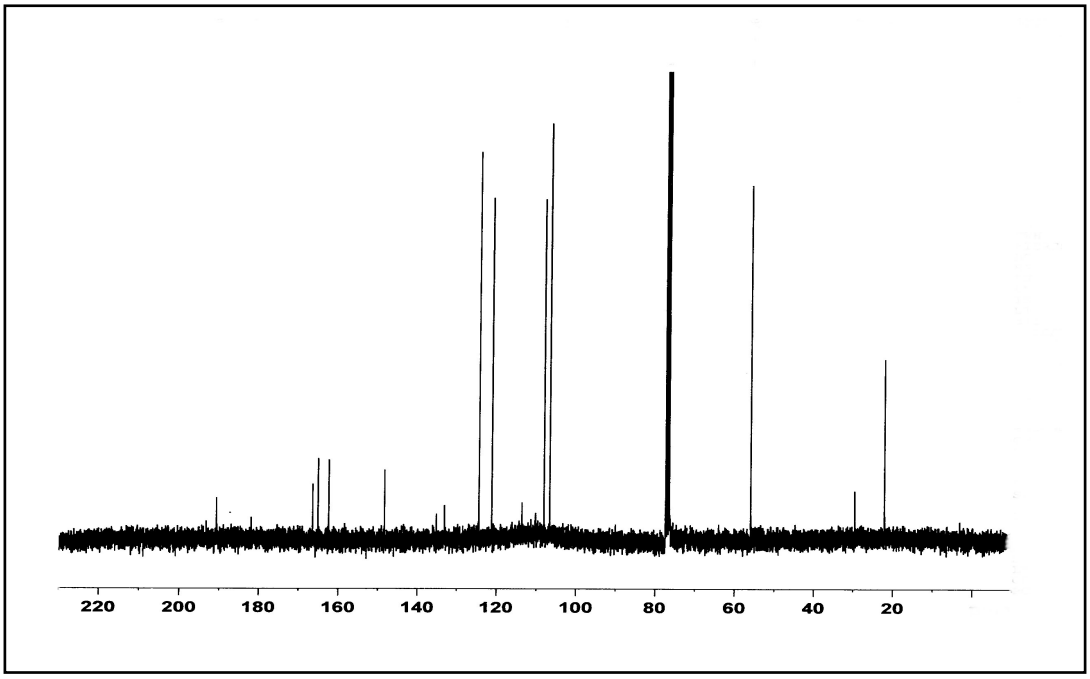

Figure 186 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **AM18**

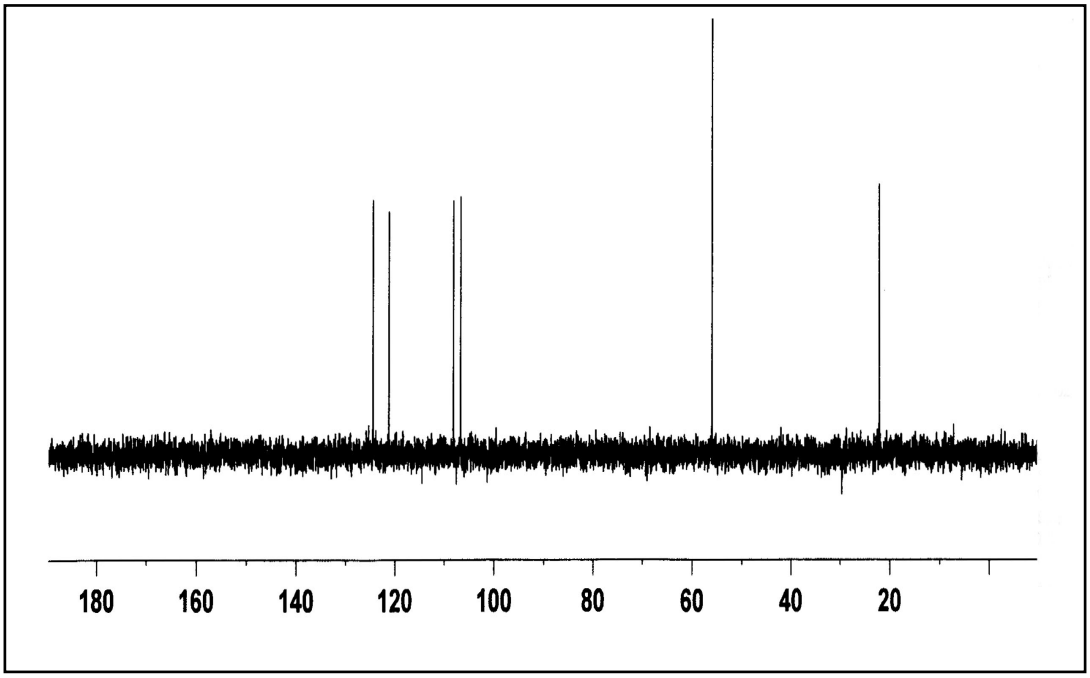

Figure 187 DEPT 135° ($CDCl_3$) spectrum of compound **AM18**

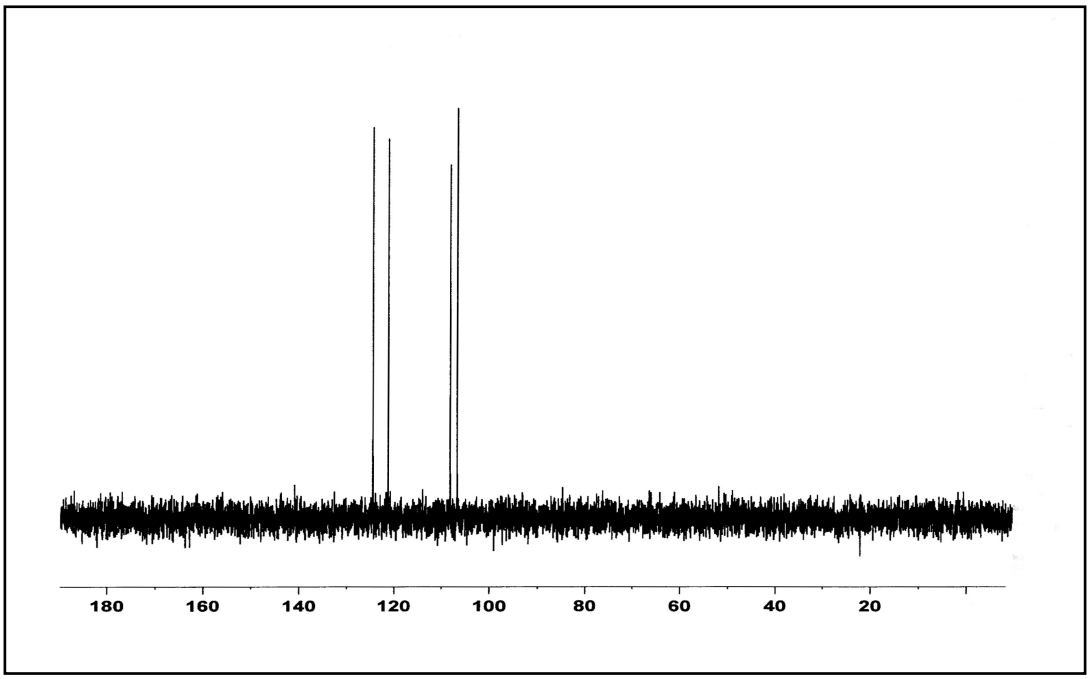

Figure 188 DEPT 90° ($CDCl_3$) spectrum of compound **AM18**

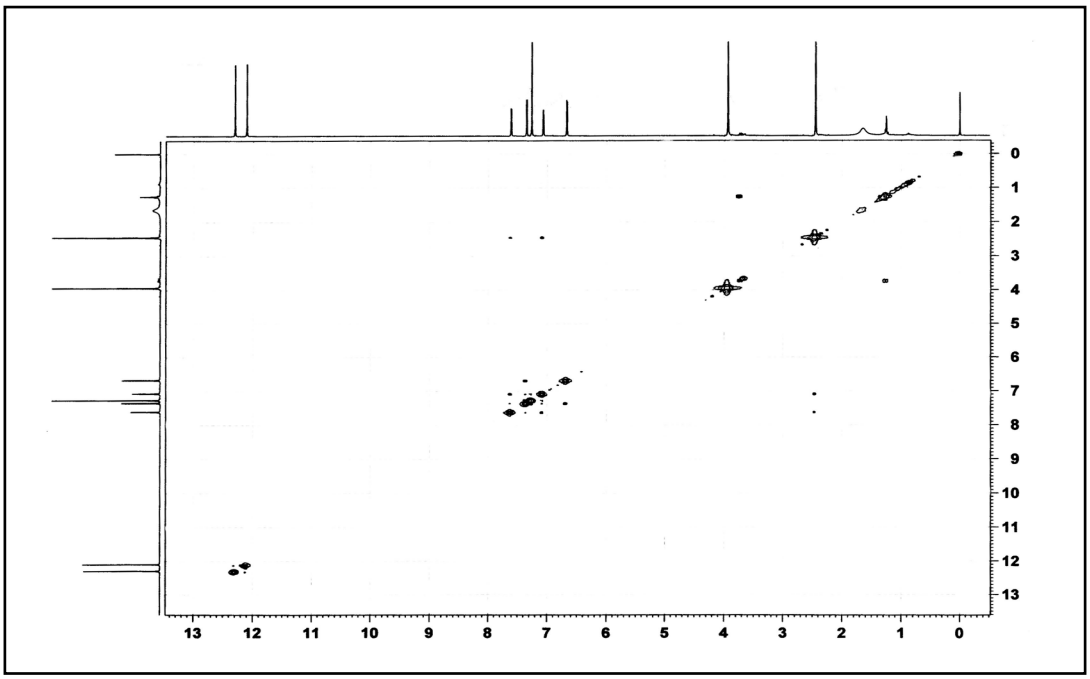

Figure 189 2D COSY ($CDCl_3$) spectrum of compound **AM18**

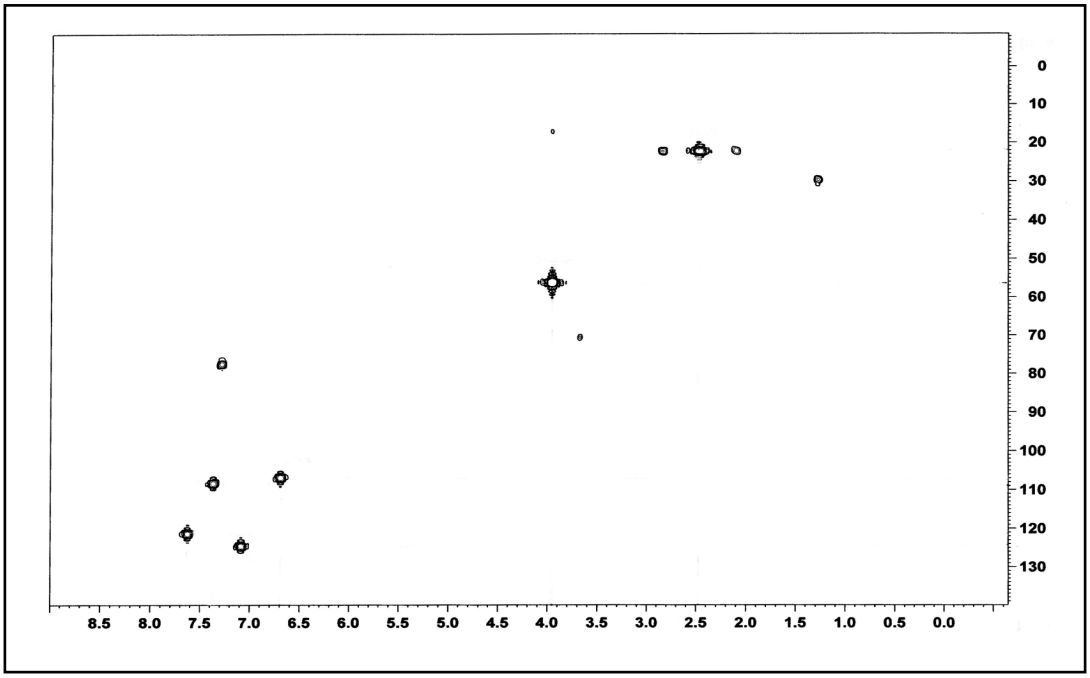

Figure 190 2D HMQC ($CDCl_3$) spectrum of compound **AM18**

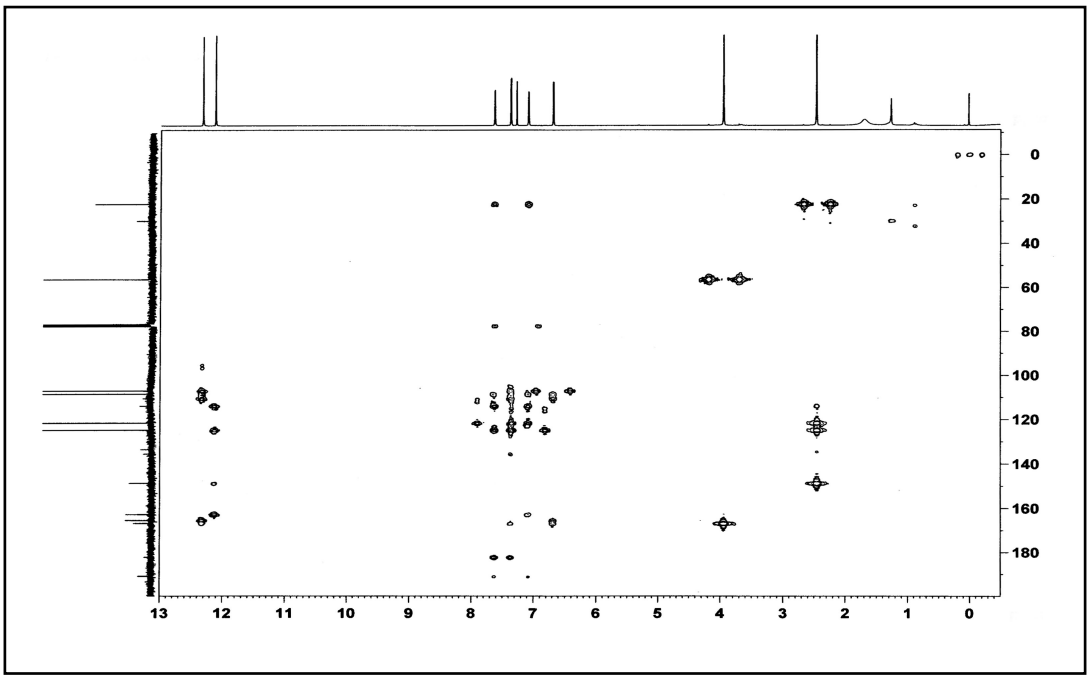

Figure 191 2D HMBC ($CDCl_3$) spectrum of compound **AM18**

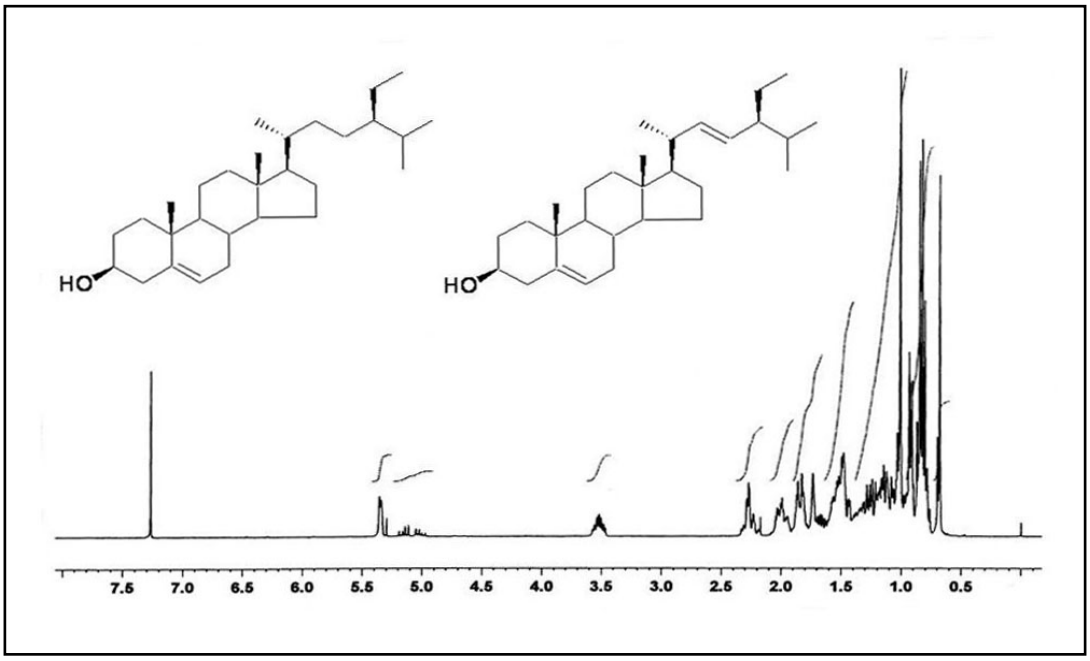

Figure 192 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **AM19-AM20**