CHEMICAL CONSTITUENTS OF AGLAIA EXIMA

LOONG XE-MIN

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
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CHEMICAL CONSTITUENTS OF *AGLAIA EXIMA*

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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE

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ABSTRACT

A preliminary study on the chemical constituents of *Aglaia exima* was performed. The leaves of *Aglaia exima* was extracted in three different solvents: hexane, dichloromethane and methanol.

The fractionation of the hexane extract of the leaves of *Aglaia exima* collected in Terengganu led to the isolation of one new cycloartane triterpenoid; cycloart-24-ene-26-ol-3-one **203** and seven known compounds, which are four cycloartane triterpenoids; 24(*E*)-cycloart-24-ene-3*β*,26-diol **199**, vaticinone **200**, schizandronic acid **201**, 24(*E*)-3*β*-hydroxycycloart-24-ene-26-al **204**, one dammarane triterpenoid; cabraleahydroxylactone **202** and two steroids; *β*-sitosterol **205** (sterol) and stigmast-5-ene-28-one **207** (sterol). The structures of compounds were analyzed and elucidated by using spectroscopic methods; 1D and 2D NMR, mass spectrometry, infrared spectrometry and X-ray.

The hexane, dichloromethane and methanol extracts from leaves of *Aglaia exima* were subjected to biological activity screening. All isolated compounds were measured *in vitro* for their cytotoxic activities against eight cancer cell; lung (A549), prostate (DU-145), skin (SK-MEL-5), pancreatic (BxPC-3), liver (Hep G2), colon (HT-29), breast (MCF-7) and (MDA-MB-231). The new cycloartane triterpenoid, cycloart-24-ene-26-ol-3-one **203**, showed potent cytotoxic activity against colon (HT-29) cancer cell line (IC_{50} 11.5 μM).

ABSTRAK

Kajian awal atas juzuk-juzuk kimia *Aglaia exima* telah dilaksanakan. Daun *Aglaia exima* diekstrak dalam tiga pelarut yang berbeza: heksana, diklorometana, metanol.

Pemisahan pada ekstrak heksana dari daun *Aglaia exima* di Terrengganu menghasilkan satu sikloartana triterpenoid yang baru 24(*E*)-sikloart-24-en-26-ol-3-on **203**, tujuh sebatian iaitu, empat sikloartana triterpenoid sikloart-24-en-3*β*,26-diol **199**, vatikinon **200**, asid schizandronik **201**, 24(*E*)-3*β*-hidroksisikloart-24-en-26-al **204**, satu dammarane triterpenoid; cabraleahidroksilakton **202** dan dua steroid *β*-sitosterol **205** dan stigmast-5-en-28-on **206**. Struktur- struktur sebatian telah dianalisis dan ditentukan dengan menggunakan teknik spektroskopi; spektrometer resonansi magnet inti (NMR), jisim spektrometri, spektrofotometer inframerah (IR) dan sinar x.

Eksperimen kesan aktiviti biologi terhadap ekstrak heksana, diklorometana dan metanol daripada daun *Aglaia exima* telah diuji. Semua sebatian yang dikenalpasti telah diukur in vitro untuk aktiviti sitotoksik terhadap lapan barisan sel kanser, paru-paru (A549), prostat (DU-145), kulit (SK-MEL-5), pankreas (BxPC-3), hati (Hep G2), kolon (HT-29), payudara (MCF-7) and (MDA-MB-231). sikloartana triterpenoid yang baru 24(*E*)-sikloart-24-en-26-ol-3-on **203** menunjukkan aktiviti sitotoksik berpotensi pada barisan sel kanser kolon (HT-29) (IC_{50} 11.5 μM).

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ABBREVIATIONS

α	Alpha
Å	Armstrong
β	Beta
γ	Gamma
br s	Broad singlet
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
CH_3	Methyl group
cm^{-1}	Per centimeter
COSY	H-H correlation spectroscopy
δ	Chemical shift
DEPT	Distortioness Enchancement by Polarisation Transfer
db	Double bond
dd	Doublet of doublet
ddd	Doublet of doublet of doublet
dt	Doublet of triplet
DMEM	Dulbecco's Modified Eagle Medium
er	*erythro*
ε	Molar absorptivity
Ed_{50}	Effective dose of 50% activity
FT-NMR	Fourier Transform-Nuclear Magnetic Resonance
^1H	Proton NMR
g	Gram
GCMS	Gas Chromatography Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quantum Coherance
Hz	Hertz
IC_{50}	Concentration required to inhibit of 50% activity
IR	Infrared
J	Coupling constant (Hz)
L	Litre
LCMS	Liquid Chromatography Mass Spectrometry
λ	Lambda (maximum wavelength)
m	Metre
m	Multiplet
m/z	Mass to charge ratio
MeOH	Methanol
MHz	Mega Hertz
MS	Mass spectrum
mL	Mililitre
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)

μg/mL	Microgram per mililitre
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
OMe	Methoxyl group
OCH_2O	Methylenedioxy group
OH	Hydroxyl group
ppm	Parts per million
q	Quartet
quin	Quintet
s	Singlet
t	Triplet
th	*threo*
TLC	Thin Layer Chromatography
UV	Ultraviolet
^{13}C	Carbon-13 NMR
2D NMR	Two dimensional NMR
%v/v	Percentage volume per volume

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

The tropical rain forest of Malaysia is known to be one of the most diversified ecosystems on earth[1]. The diversity of flora in Malaysia is estimated to comprise of 7900 species of flowering plants and 1500 genera of seed plants (Whitmore, 1984). The high amount of rainfall, which is over 2200 mm a year, favorably supports the growth of trees, tall shrubs, ferns and climbers[1]. The richness of plants contributes to the existence of enormous variety of natural products which have strong impact towards human culture. Hence, it provides scientist an interesting resource for research.

Natural products are commonly termed as 'secondary metabolites' which have pharmacological properties and biological effect to human. Many are insecticidal, fungicidal, phytotoxic, anti-inflammatory, anti-cancer and anti-bacterial. Some plays a protecting role to the plants from herbivores and microbial infection. They can also act as defense compounds against herbivores and pathogens as flower pigments that attract pollinators, or as hormone or signal molecules. Some are used as medicine to treat human disorders and infections due to their pharmacological properties[2].

Due to the high medicinal value and biological effects to human, natural products play a highly significant role in drug discovery until now and was proved by the analysis of the number of natural products derived drugs present in the total drugs launched from the 1981 to 2002 demonstrated by Newman *et al*[3]. According to the study, natural products were concluded as significant source of new drugs and have enormous influence especially in the anti-hypertensive area. In addition, another study showed that more than 80% of drug molecules are natural products or natural compounds inspired[4].

In addition, natural products derived drugs were also proven to contribute significantly to the profitability of many pharma and biotech companies. They are well represented as top 35 of worldwide selling ethical drug sales of 2000, 2001 and 2002[5]. Table 1.1 shows the ranking of natural product- derived drugs in top 35 worldwide selling ethical drug sales for 2000, 2001 and 2002.

Table 1.1: Ranking and Function of Natural Product-Derived Drugs in Top 35 Worldwide Ethical Drug Sales for 2000, 2001 and 2002

NP-derived drugs[a]	**Rank**		
	2000	**2001**	**2002**
Atorvastatin - lower blood cholesterol (Hochadel, 2006)	2	1	1
Simvastatin - lower blood cholesterol (Hochadel, 2006)	3	3	2
Estrone - estrogen replacement therapy (Ravina, 2011)	16	16	25
Amoxicillin + Clavulanic acid - kills bacteria growth caused by infection (Hochadel, 2006)	17	17	27
Enalapril - controls high blood pressure by relaxing blood vessels (Hochadel, 2006)	18	-	-
Pravastatin (BMS) - lower blood cholesterol (Hochadel, 2006)	19	15	15
Pravastatin (Sankyo) - lower blood cholesterol (Hochadel, 2006)	23	31	-
Paclitaxel - disrupting cancer cell growth, affective in late stage ovarian, breast and lung cancer (Dubin, 2001)	25	-	-
Azithromycin - treat bacterial infections in different part of body (Hochadel, 2006)	27	29	33
Ribavirin - block virus replication (St. Georgiv, 2010)	28	30	11
Gabapentin - treat epilepsy (Wyllie, 2006)	30	23	14

NP-derived drugs[a]	Rank		
	2000	**2001**	**2002**
Fluticasone propionate - maintenance treatment of asthma (Spratto, 2009)	31	33	-
Clarithromycin - treatment of pneumonia (Yaffe, 2010)	32	-	-
Cyclosporine - prevent rejection with kidney, liver and heart transplantation (Lahita, 2004)	34	-	-
Lisinopril - controls high blood pressure by relaxing blood vessels (Hochadel, 2006)	35	-	-
Salmeterol + Fluticasone propionate - treat asthma (Page, 2004)	-	-	13
Oxycodone HCl - relieve or control moderate to severe pain (Freye, 2008)	-	-	32

[a] NP-derived drug indicates that the drug is either a NP, a semisynthetic derivative of a NP, or a synthetic drug that is modeled on a NP pharmacophore.

In view of the importance of natural products in the pharmaceutical industry, the author has conducted a study on *Aglaia exima* from the family Meliaceae collected from Terengganu. The chemical constituents of the plant were sent for cytotoxic activity tests against various cell lines such as skin, colon, pancreas, lung, prostate, breast and liver.

1.2 OBJECTIVES OF THE STUDY:

The objectives of this study are as follows:

i. To isolate the chemical constituents of *Aglaia exima*.

ii. To elucidate and identify the structure of chemical constituents isolated from *Aglaia exima* by spectroscopic methods: NMR, UV, IR, mass spectrometry and X-ray analysis.

iii. To evaluate the cytotoxicity of the isolated compounds against selected cancer cell lines.

1.3 MELIACEAE

The Meliaceae or Mahogany family comprises 50-52 genera with about 550 species, mostly found in the tropical region of Asia, Africa, Australia, and South American[6]. Some aspects of Meliaceae are discussed briefly in the following paragraphs.

1.3.1 DISTRIBUTIONS[7,8]

Plants of Meliaceae are very common trees or shrubs with flowers and under storey of lowland primary forest. The plants belonging to Meliaceae were best represented in the Malesian region, the Malay peninsula alone has more species (91 species in 17genera) than the whole of Africa (84 species). Plants from this family usually utilized for medicine and other purpose. Some plants from genera Melia were effective against insects.

1.3.2 GENERAL APPEARANCE AND MORPHOLOGY[9]

Meliaceae belongs to Order Rutales s. str. comprising Rutaceae, Meliaceae, Simaroubaceae, Cneoraceae and Burseraceae (Waterman & Grundon 1983). It can be classified from the different family by its specific features of leaves mostly pinnate, not glandular punctuate, exstipulate, spirally arranged, trifoliolate, with a single blade or rarely bipinnate. Leaflets usually entire rarely lobed or serrate. Flower usually unisexual, with well developed rudiments of opposite sex, in cymose panicles, petals are three to six but usually five, sometimes joined at the base, usually is one whorl, green, white, cream, pink to claret and violet or yellow. Calyx usually lobed, sometimes with 3-5 sepals. Anthers in 1 or, rarely, 2 or more whorls, sometimes locellate, at tips of filaments or at the margin of the tube or within its throat. Stamens are usually eight to ten, in a filamental tube on base of the disc with or without lobes. Ovary is locular, each

locule with one to many ovules. Fruits are often a drupe, berry or a capsule. Seeds are few, large, usually arillate or winged.

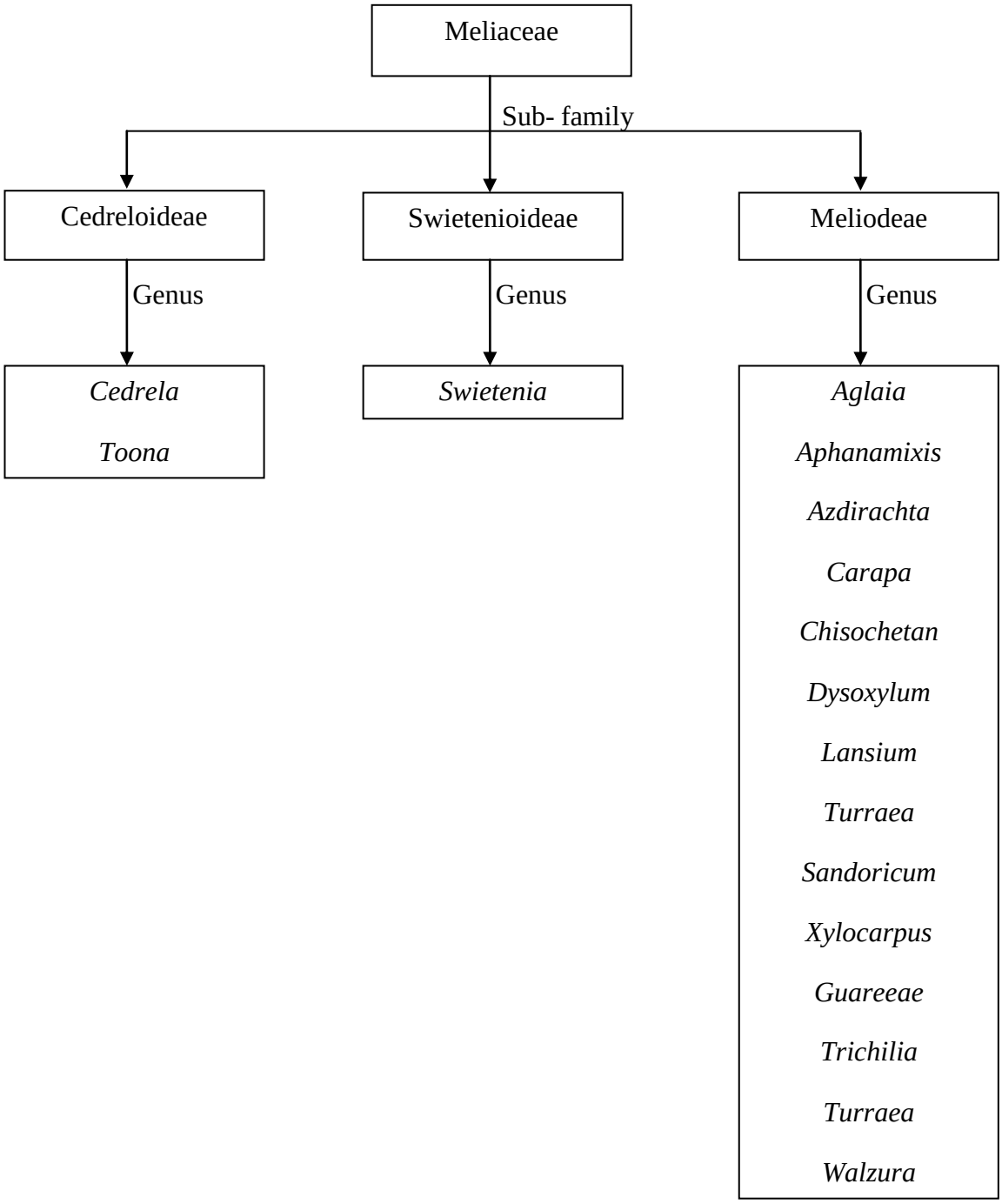
1.3.3 THE ECONOMIC AND BIOLOGICAL IMPORTANCE[9]

Recently, many phytochemists and biologists are highly interested on Meliaceae, because many compounds with insect-antifeeding, insect-repellent or and insecticidal properties were found, and many members of this family are highly esteemed in traditional medicine of most continents. *Walsura monophylla* is commercially tested for treatment of cancer. Besides, some are edible tropical fruits. *Aglaia dookkoo* Griff. or known as *Lansium domesticum* Jack is the well known tropical fruit in Malaysia. Many other species from this family have different and useful economic usage and table below lists the use of some Meliaceous plants.

Table 1.2: List of the Usage of Some Meliaceous Plants

Species	**Usage**
Chisocheton and *Aphanamixis sp*.	The oil from seeds used as an illuminant
Azadirachta excelsa	Leaves as green vegetables
Melia azedarach	Foliage and fruits used as a febrifuge and vermifuge
Azadirachta indica	Neem from leaves used as a powerful insecticides

1.3.4 CLASSIFICATION OF FAMILY MELIACEAE



Scheme 1.1: Classification of family Meliaceae[8]

1.4 GENUS; *AGLAIA*[10]

Genus *Aglaia* has a total of 105 species belonging to family Meliaceae. It is the largest genus of Meliaceae. The trees are mainly found in subtropical and tropical forest of southern mainland China, Indo-Malaysian region and the Pacific Island.

1.4.1 DISTRIBUTION AND HABITAT[9]

Some of the species are tall trees and occasionally emergent, some are undergrowth treelets, which may be unbranched, and others are rheophytes. At least two species, *A. elaeagnoidea* and *A. brownie*, are frequently and *A. lawii* is sometimes littoral; *A. cucullata* is found in tidal estuaries and mangrove swamps.

1.4.2 GENERAL APPEARANCE AND MORPHOLOGY[9,11,12]

Leaves from this genus are alternate, lanceolate, oblanceolate, ovate, obovate, elliptical or oblong, the lamina which usually of moderate thickness, sometimes membraneous or coriaceous. Inflorescence usually axillary or supra-axillary. Flowers unisexual with well developed rudiments of the opposite sex. The small different between female and male is female inflorescence usually smaller and less-branched but flower always larger than in the male. It has only five anthers. Calyx is in a cup-shaped and it consists of 5 free sepals which are slightly grown together at the base. Corolla is with 3-5 petals, free or united at the base, free from the staminal tube or partially united to it, usually yellow, sometimes pink or white. Disk is absent in the plant from genus *Aglaia*. Ovary is locular, superior, depressed-globose or ovoid with dense stellate hair or peltate scales, locules with 1 or 2 collateral or superposed ovules, placentation axial, style 1, stigma ovoid. Fruits are in subglobose, obovoid or ellipsoid, indehiscent or a loculicidal capsule. Seeds are large, with an aril or sarcotesta covering the seed.

1.4.3 KNOWN ECONOMIC AND BIOLOGICAL IMPORTANCE[11]

Many of the species of *Aglaia* have traditionally been used for medicinal properties in several countries (Table 1.3). Plants of *Aglaia* are used for the treatment of inflammatory skin diseases and allergic inflammatory disorders such as asthma in Vietnam[13]. In several of Southeast Asia countries including China and Vietnam, *Aglaia* plants are traditionally used as insecticides which kill insects[14]. Besides, the wood of *Aglaia* sometimes can be used for construction or making furniture. The fleshly layer around the seeds of some species is edible, *Aglaia dookkoo* Griff. or known as *Lansium domesticum* is the tropical fruits, langsat. *Aglaia korthalsii* are widely grown for their fruits in villages in the north of Peninsular Malaysia, Kelantan. The flowers of *A. odorata* Lour. are known to be used as tea flavour.

Table 1.3: List of some *Aglaia* species used in traditional treatments of several countries

***Aglaia* species**	**Parts of Plant**	**Treatments**
A. elliptica[15]	Bark	- In Philippines, it was boiled to treat tumors
	Leaves	- In Philippines, it was applied to wounds
A. roxburghiana[16]		- Treats inflammation, leprosy, throat infections, billous and febrile complaints
A. odorata[17]		- Used as heart stimulant, febrifuge, for the treatment of coughs, inflammations and injuries
A. lawii[18]		- In Vietnam, it was used to treat bacterial infection, liver and tumor diseases

CHAPTER 2

GENERAL ASPECTS OF *AGLAIA* SPECIES

2.1 GENERAL

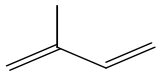
The previous investigation on the isolation of chemical compounds and biological activity of the plants from family Meliaceae has been performed by scientists from worldwide due to the richness of active natural products, triterpenoids, limonoid, sesquiterpenes and sterols. Many of these compounds have strong biological activities such as anticancer, anti-inflammatory and insecticidal. A plenty of studies on *Aglaia* species were performed, an immense amount of active biological compounds have been found, as genus *Aglaia* is the largest genus in family Meliaceae[5]. The studies of general characteristic and chemical constituents found in the family Meliaeceae and genus *Aglaia* will be shown in this section.

2.2 TERPENES[19,20,21,22]

Terpenes are a class of hydrocarbons in which structures are multiples of five-carbon isoprene units (2-methyl-1,3-butadiene) **1**. Therefore, its general formula is $(C_5H_8)_n$. Terpenoids are oxygen-containing analogs of the terpenes, it has been further functionalized. Both terpenes and terpenoids comprise the largest group of natural products in plants and animals. All terpenes are formed by the condensation of two or more isoprene units and characterized by the number of five-carbon isoprene unit which they contain. For example, triterpenes are C_{30} compounds that contain six isoprenes units. Squalene, an acyclic triterpenes which is formed by a head-to-tail condensation of two sesquiterpenes (farnesylpyrophosphate) units plays a significant role as precursor of sterols[23]. Terpenes can be classified into hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterpenes, triterpenes, tetraterpenes, polyterpenes. The table below shows the classifications and example of terpenes.

Table 2.1: Classification of Terpenes According to Isoprene Units and Their Examples

Class	Molecular formula	Isoprene units	Examples
Hemiterpenes	C_5H_8	1	Isoprene **1**
Monoterpenes	$C_{10}H_{16}$	2	Linalool **2**, Myrcene **3**
Sesquiterpenes	$C_{15}H_{24}$	3	Zingiberene **4**
Diterpenes	$C_{20}H_{32}$	4	Carnosol **5**, Cembrene **6**
Sesterterpenes	$C_{25}H_{40}$	5	Geranylfarnesol **7**
Triterpenes	$C_{30}H_{48}$	6	Squalene **8**
Tetraterpenes	$C_{40}H_{64}$	8	β-carotene **9**
Polyterpenes	$(C_5H_8)_n$	>8	Natural rubber **10**



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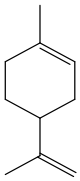

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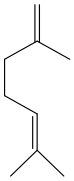

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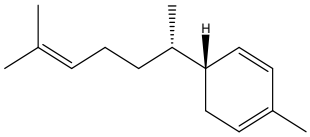

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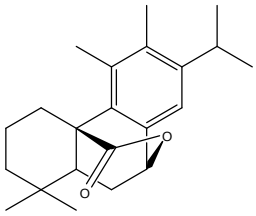

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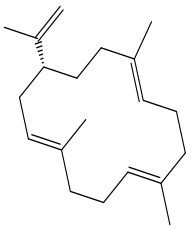

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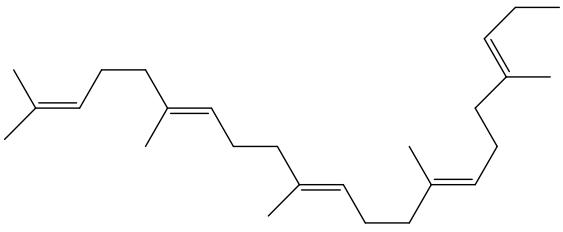

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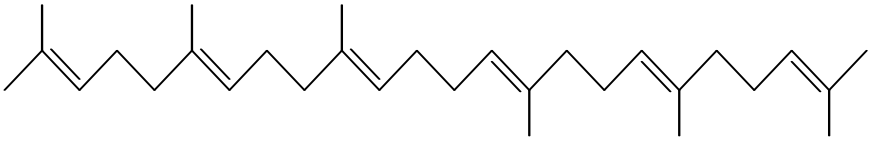

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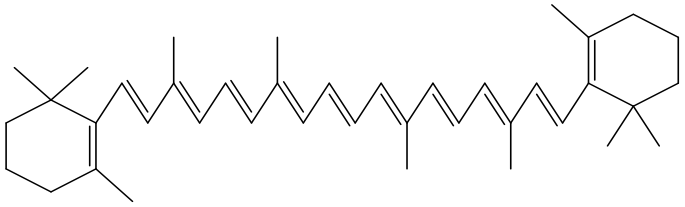

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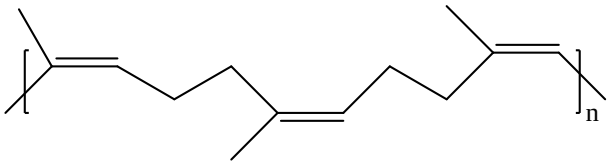

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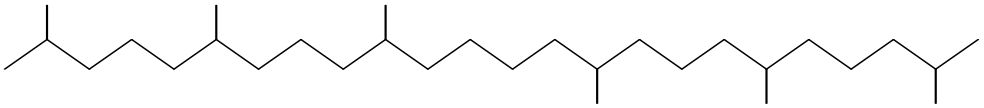
2.3 TRITERPENOIDS[24,25,26]

Triterpenoids are the largest group among various terpenoid groups with 4000 different molecules and 40 skeletal types. They are compounds with a carbon skeleton based on six-isoprene units which are derived from the squalene **8** biosynthetically. The existence of more than one type of triterpenoids was due to the different type of ring closure in this acyclic intermediate, squalene **8**. They are further classified as acyclic, monocyclic, bicyclic, tricyclic, tetracyclic, pentacyclic triterpenoids according to the number of rings present in the molecule (see Table 2.2). Tetracyclic as steroidal types (C-27) and pentacyclic (C-30) were the major groups. Most of the triterpenoids are either tetracyclic or pentacyclic. The tetracyclic triterpenoid belongs to the important class of compound (Chapter 2.4), the steroids, which are considered separately. It will be discussed in the following section. Pentacyclic triterpenoids with six-membered ring are of higher plant origin. The table below shows the general structures of each types and classes of triterpenoids found in Meliaceae.

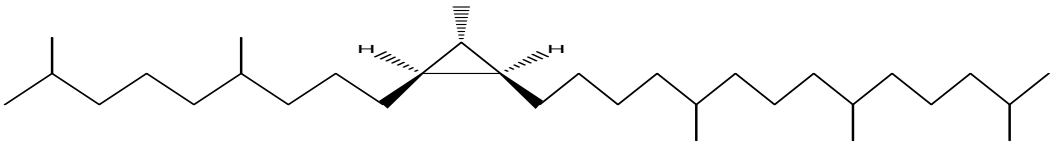
Table 2.2: Types and Classes of Triterpenoids Found in Meliaceae[27]

Acyclic

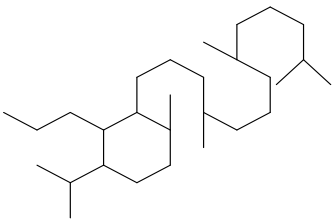


Squalene **8**

Monocyclic

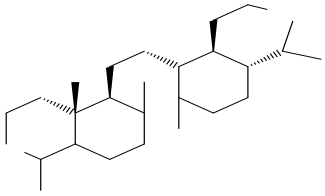


Presqualene **11**



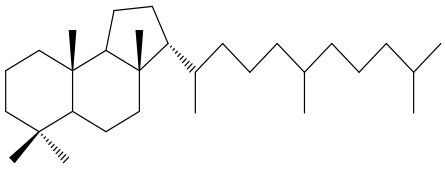
Iridogermane **12**

Bicyclic

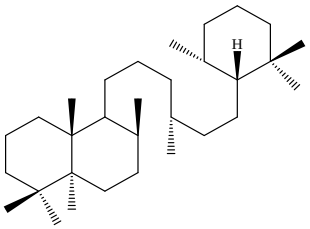


Lansane **13**

Tricyclic

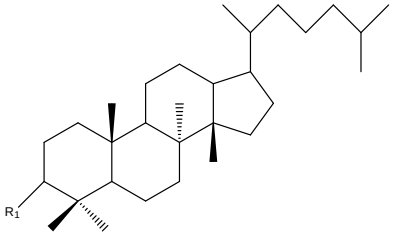


Malabaricane **14**



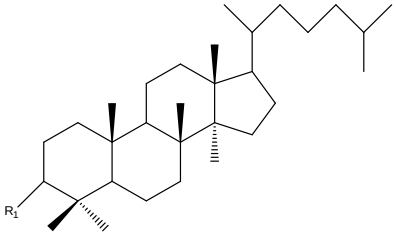
Ambrane **15**

Tetracyclic



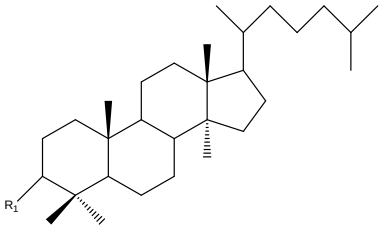
R_1 = OH or O

Protostane **16**



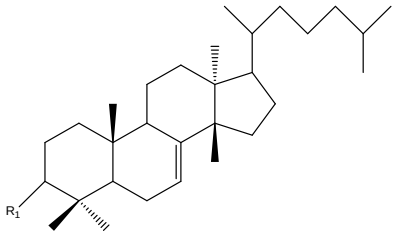
R_1 = OH or O

Dammarane **17**



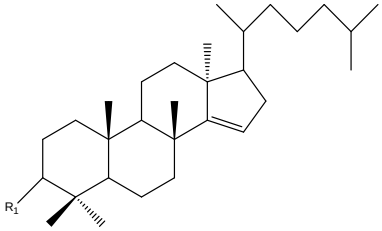
R_1 = OH or O

Lanostane **18**



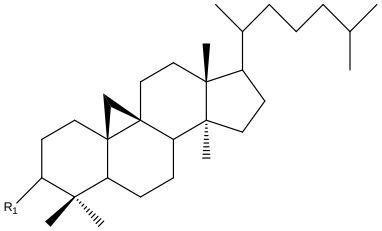
R_1 = OH or O

Tirucallane/ euphane **19**



R_1 = OH or O

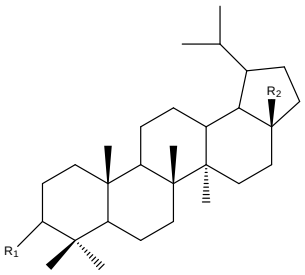
Apotirucallane **20**



R_1 = OH or O

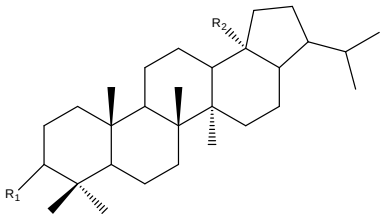
Cycloartane **21**

Pentacyclic



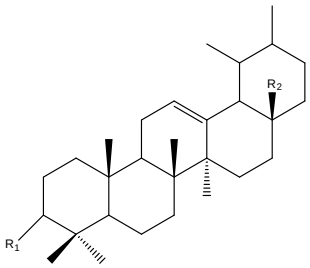
R_1 = OH or O
R_2 = CH_3 or COOH

Lupane **22**



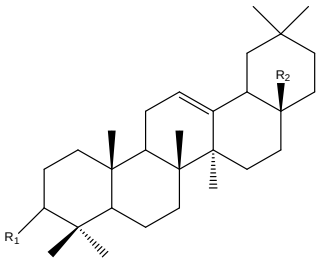
R_1 = OH or O
R_2 = CH_3 or COOH

Moretane **23**



R_1 = OH or O
R_2 = CH_3 or COOH

Ursane **24**

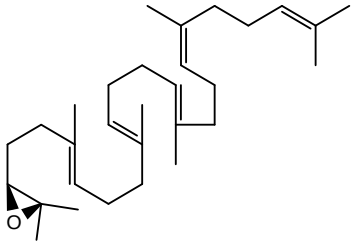


R_1 = OH or O
R_2 = CH_3 or COOH

Oleanane **25**

2.3.1 BIOSYNTHESIS OF TRITERPENOIDS[25,26,27,28,29,30,31,32]

Squalene **8** is the precursor of all triterpenoids and steroids. A variety of triterpenoids is formed depends on the stereoselectively cyclization and structurally rearrangement of squalene **8** or squalene 2,3-epoxide **26** in a single enzyme-catalyzed reaction. The product of squalene epoxide **26** depends on the conformation that squalene epoxide **26** assumes in binding to the cyclase and the nature and position of the nucleophile or base in the enzyme.



26

Table 2.3: The Major Groups of Terpenoids as the Important Precursors of All Steroids in Plants Which Produced from the Cyclization.

Tetracyclic triterpenoids	**Pentacyclic triterpenoids**
Dammarane **17**	Lupane **22**
Tirucallane/ Euphane **19**	Ursane **24**
Cycloartane **21**	Oleanane **25**

Hereunder the biosynthesis of these triterpenoids is described starting from the acetylcoenzyme A (acetyl-CoA) **28** (Scheme 2.1).

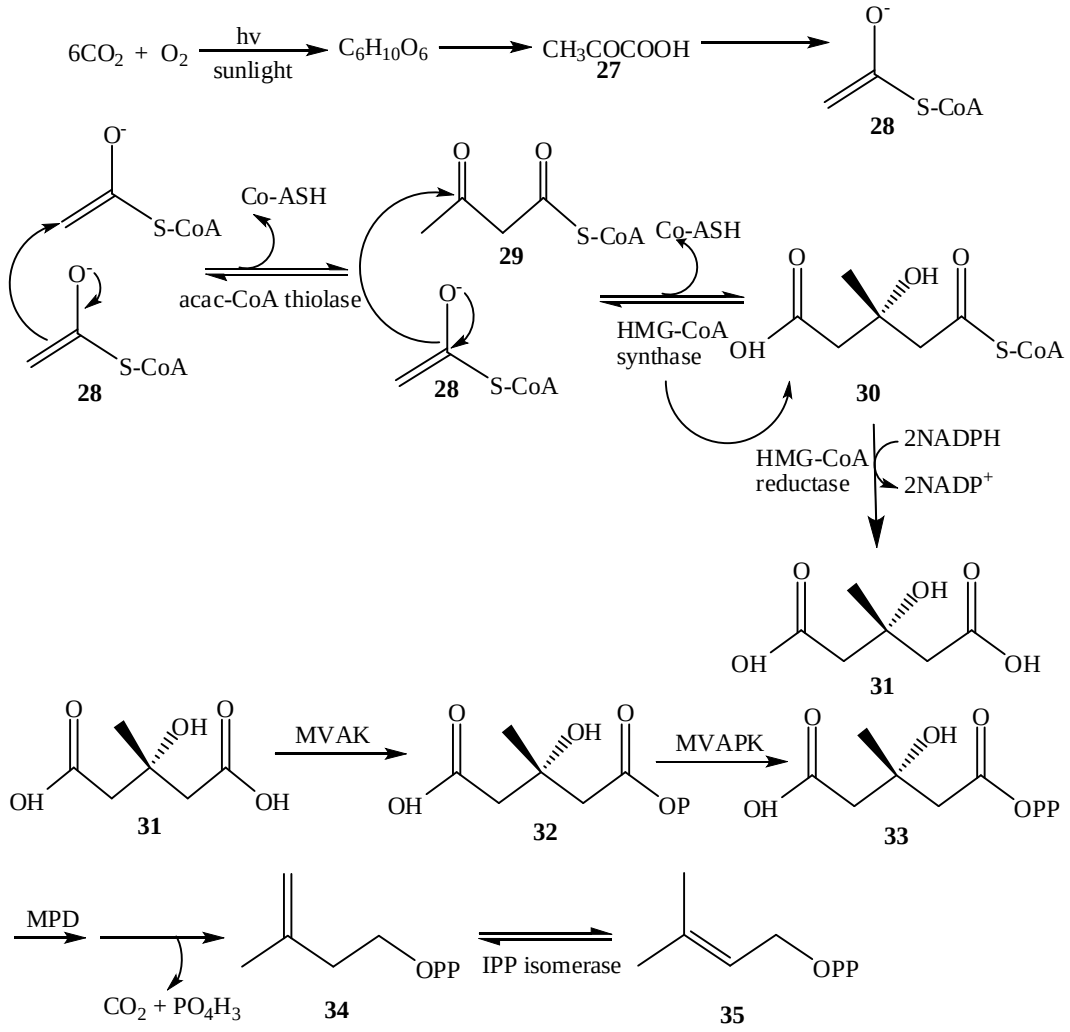
The biosynthesis of squalene **8** was initiated by converting six molecules carbon dioxide and six molecules of oxygen under sunlight to the glucose molecule, which break down through glycolysis to pyruvic acid **27**. The pyruvic acid **27** is converted to enolate acetyl coenzyme A **28** which will couple with another enolate acetyl coenzyme via Claisen-type condensation mediated by the enzyme acetoacetyl-CoA thiolase to form acetoacetyl coenzyme A **29**. The acetoacetyl coenzyme A **29** react with enolate

acetylcoenzyme A **28** via aldol condensation to form 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) **30**, which is then forms the key intermediate, (3R)-mevalonic acid (MVA) **31** by NADPH and catalysed by enzyme hydroxymethylglutaryl-CoA reductase. The (3R)-mevalonic acid (MVA) **31** is then converted into isopentylpyrophosphate (IPP) **34** through three successive phosphorylation progressing via (3R)-mevalonate-5-phosphate (MVAP) **32** and (3R)-mevalonate-5-pyrophosphate (MVAPP) **33**, which are catalysed by the enzyme mevalonate kinase (MVAK), mevalonate 5-phosphate kinase (MVAPK), and mevalonate 5-pyrophosphate decarboxylase (MPD), respectively. It followed by the decarboxylative elimination to yield the IPP **34**. It is then undergoes isomerisation to its allylic isomer, dimethylallylpyrophosphate (DMAPP) **35** catalysed by the enzyme isopentyldiphosphate isomerase (Scheme 2.1).

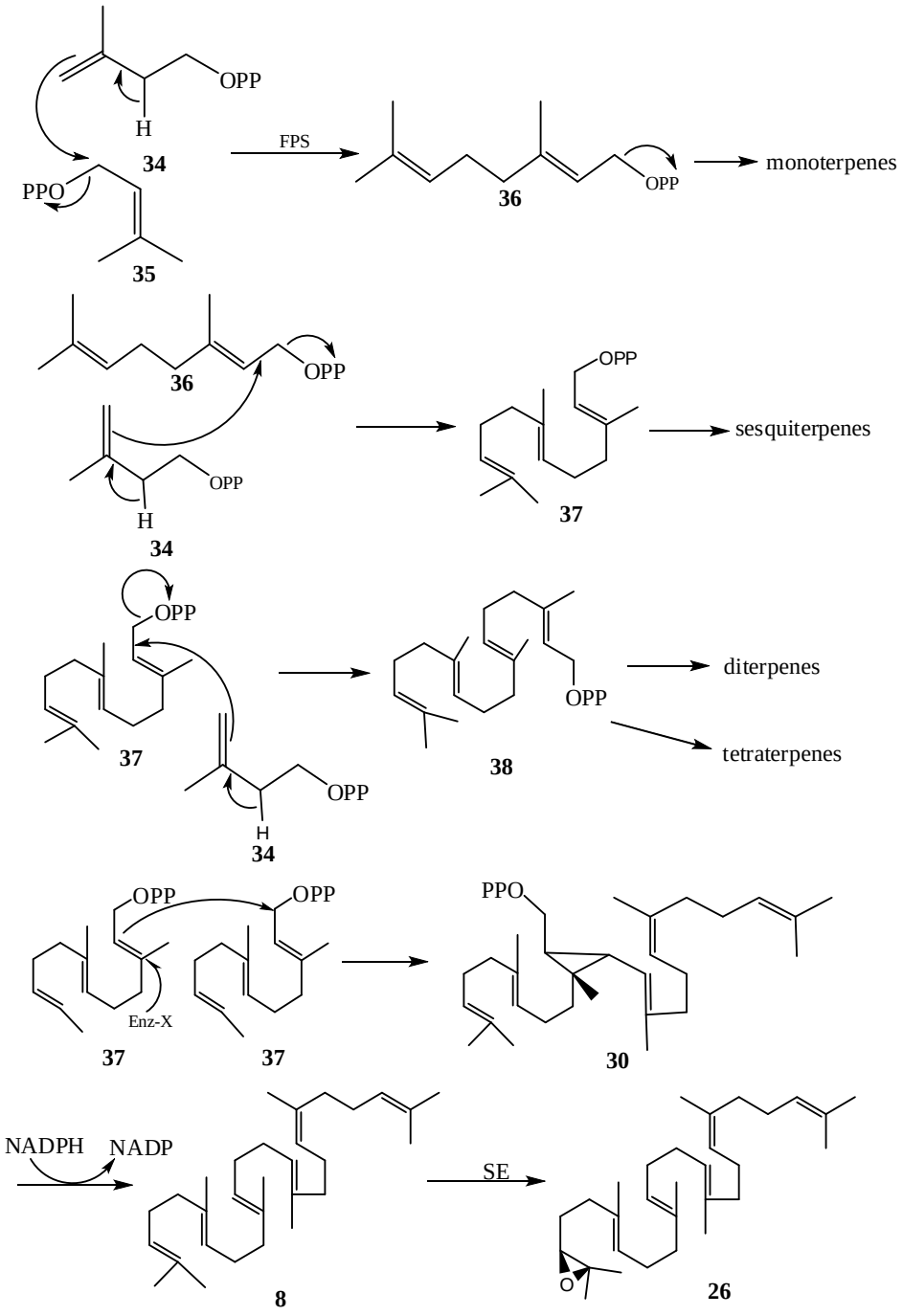
Condensation of the DMAPP **35** and IPP **34** gives geranyl pyrophosphate (GPP) **36**, a precursor of monoterpenes which then couples with IPP **34** to give farnesyl pyrophosphate (FPP) **37**. The biosynthesis of farnesyl pyrophosphate (FPP) **37** is catalysed by the enzyme farnesyl pyrophosphate synthase (FPS). The FPP **37** and GGPP **38** are precursors of sesquiterpenes and diterpenes respectively. Two molecules of farnesyl pyrophosphate (FPP) **37** couple with each other to form presqualene-pyrophosphate (PSPP) **39**, which undergoes reductive arrangement to furnish squalene **8**. Squalene-2,3-oxide **26** is formed from the epoxidation of squalene catalysed by the enzyme squalene epoxidase (SE). Different types of tetracyclic and pentacyclic triterpenoids are formed according to the free rotation of five C-C bonds when binding the squalene **8** or squalene-2,3-oxide **26** in conformation to the active site of the cyclase (Scheme 2.2).

The dammarane type triterpenoids are furnished through the chair-chair-chair-boat cyclization of squalene-2,3-epoxide **26** which leads to a dammarenyl cation **40**. This cation then undergoes a series of Wagner-Meerwein migration with the sequence of 1,2-hydride followed by 1,2-methyl shifts and then terminated by the loss of proton to form euphol **41**, a triterpenoid where dammarane skeleton found (Scheme 2.3).

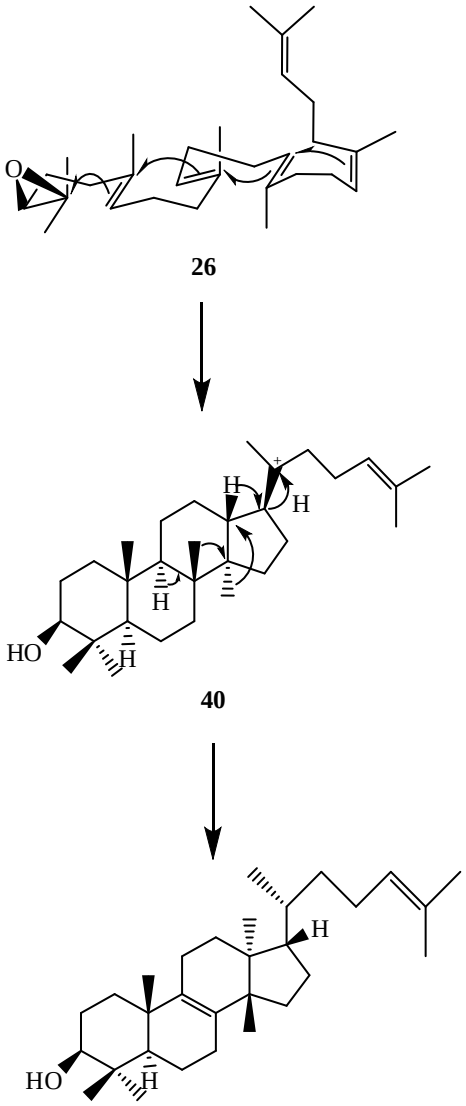
Besides this, chair-boat-chair-boat cyclization leads to the cycloartane group and steroids in plants (Scheme 2.3). Upon the cyclization, a transient protosteryl cation **42** is formed and then undergoes a series of Wagner-Meerwein firstly 1,2-hydride shifts then 1,2-methyl shifts until a proton loss from C-10 methyl forming a cyclopropane ring and hence creating cycloartenol **43** (Scheme 2.4).



Scheme 2.1 Biosynthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP)

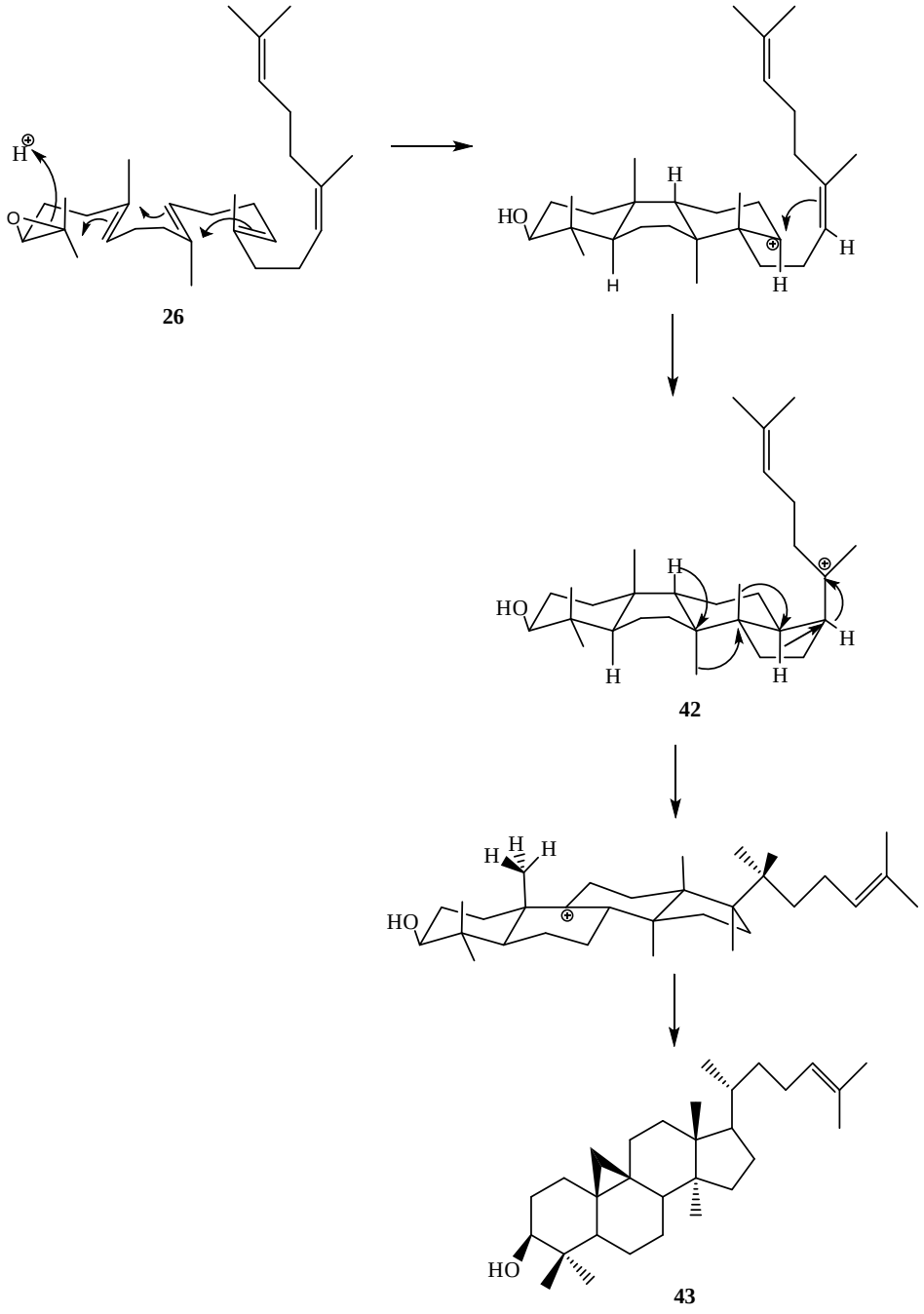


Scheme 2.2 Biosynthesis of squalene **8** and squalene-2,3-oxide **26**, the precursor of steroids and triterpenoids



26 **40** **41**

Scheme 2.3 Cyclization of squalene oxide to dammarane-type triterpenoids **40** and euphol **41** (chair-chair-chair-boat cyclization)

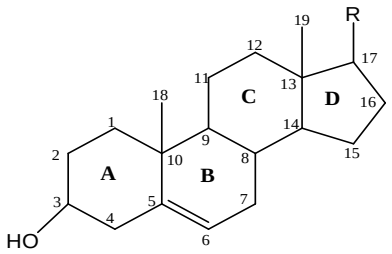


26 **42** **43**

Scheme 2.4 Cyclization of squalene epoxide **26** to cycloartane- type triterpenoids (chair-boat-chair-boat cyclization)

2.4 STRUCTURES AND BIOSYNTHESIS OF STEROIDS[26,33,34,35]

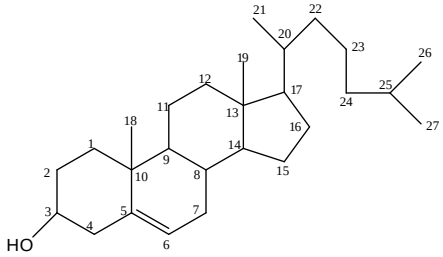
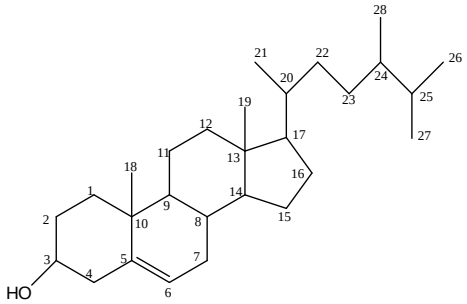
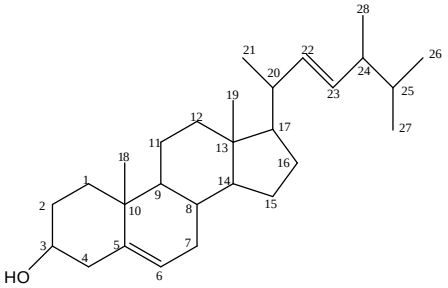
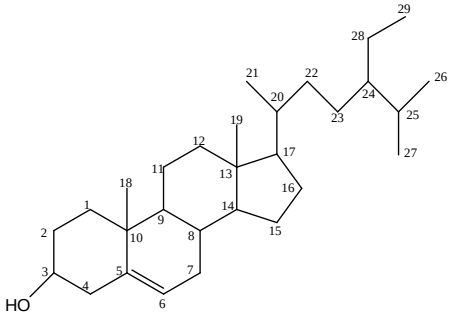
Steroids are plant metabolites considered to be a tetracyclic terpenes-derived natural products synthesized via the mevalonic acid (MVA) **31** in the cytoplasm of plants and animals. Each has a complex basic ring and side chain structure which made up by four rings of three cyclohexane and a cyclopentane ring. With the characteristic of four ring structure, they are used as the starting material for the production of many hormones. Hereunder the four rings designated by A, B, C and D that made up of the skeleton of steroids.

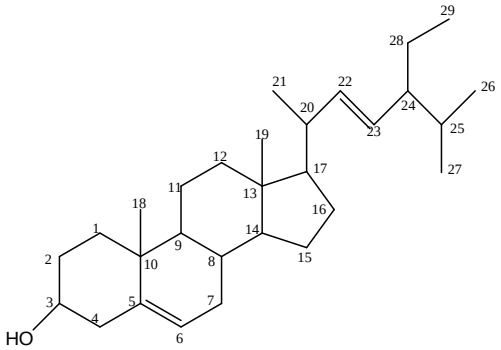


All steroids have the same basic tetracyclic structure but differ in their substituent group, R, sometimes aliphatic or functionalized attached to ring D. The steroids molecules are flat, two methyl groups at position 10 and 13 on the ring located above the molecule plane. On the other hand, some steroids have more complicated ring and side chain structures.

Steroids can be found in both animals and plants. For example, cholesterol **44** is the main steroid found in vertebrates but it also can be found in plants and in small quantity in some algae. In higher plants, mainly C_{28} (total of 28 carbons) and C_{29} (total of 29 carbons) sterols which mainly comprise of sitosterol **47** and stigmasterol **48** are found. Both sterols can be termed as phytosterol. Besides, invertebrates possess a mixture of C_{26}, C_{27}, C_{28} and C_{29} sterols. Red algae contain C_{27} sterols while brown and green algae contain C_{29} sterols. No steroids were appeared in prokaryotes.

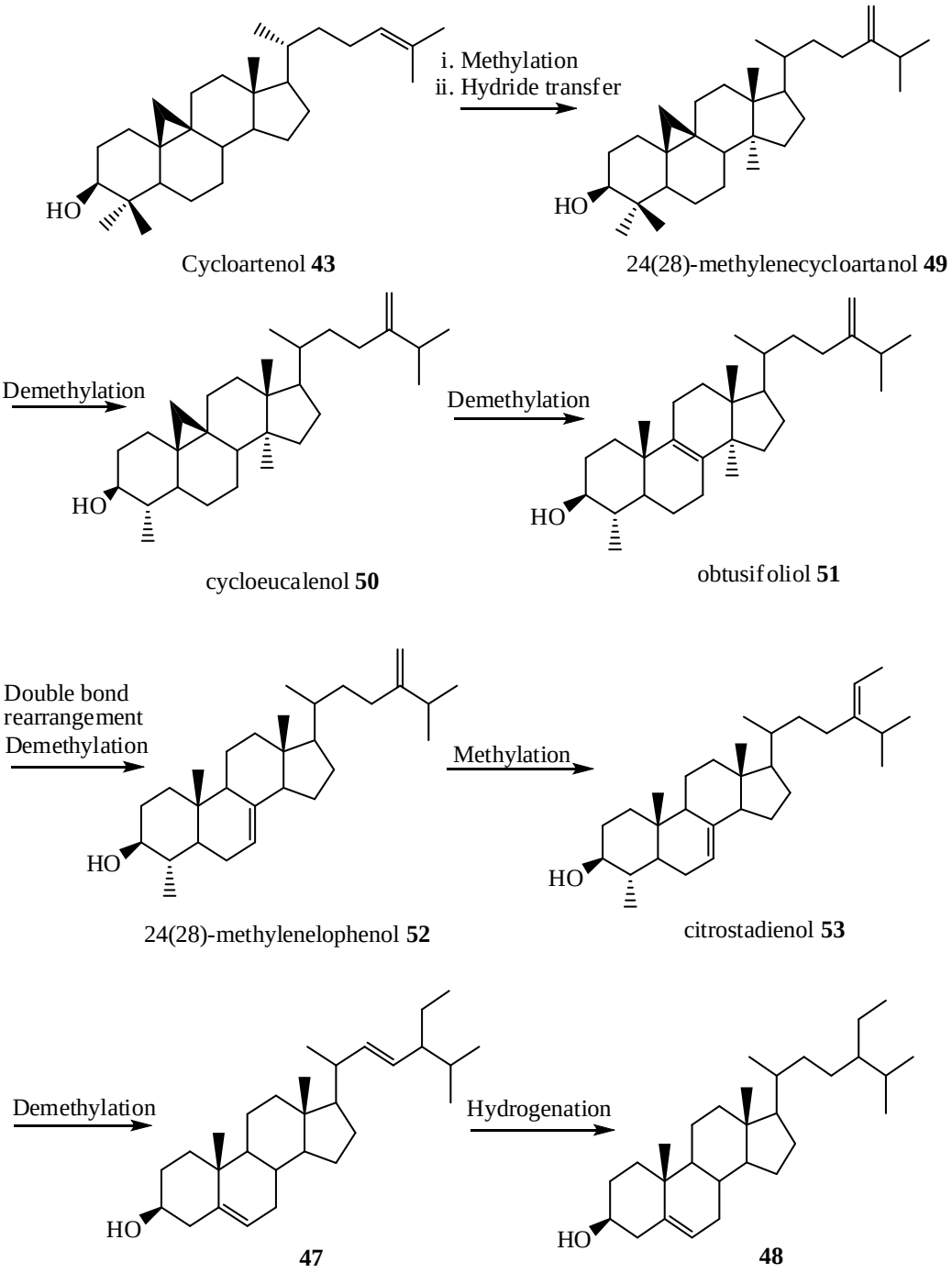
Table 2.4: Some typical steroids in plants.

Name of Compounds	Structures
Cholesterol **44** C_{27}	 **44**
Campesterol **45** C_{28}	 **45**
Brassicasterol **46** C_{28}	 **46**
Sitosterol **47** C_{29}	 **47**

Stigmasterol **48** C_{29}	 **48**

Squalene **8** is a precursor of cycloartenol **43** and cholesterol **44** which in turn is the precursor of other steroids. The commonest phytosterol, sitosterol **47** and stigmasterol **48** were found in plant *Aglaia exima*.

Hereunder the plausible biosynthesis route of steroids and phytosterol. The opening of the cyclopropane ring of cycloartenol **43** probably occurs after loss of the 4α-methyl group and before loss of the C-14 methyl group in the same order as found in the biosynthesis of cholesterol (animal). It also involves the C- methylation of the sterol side chain at C-24 which is an important area of biochemical difference between animals and fungi, as well as plants. 24(28)-methylenecycloartanol **49** formed from cycloartenol **43** through the first C- methylation reaction and the hydride transfer from C-24 to C-25 is catalyzed by sterol methyl transferase. The loss of C-14 methyl converted 24(28)-methylenecycloartanol **49** to obtusifoliol followed by the double bond rearrangement which furnish 24(28)-methylenelophenol **52**. It was continued by C-methylation, loss of 4α- methyl group and double bond rearrangement occurred to form stigmasterol **48** and sitosterol **47** from citrastadienol **53** (Scheme 2.5).



Scheme 2.5 Biosynthesis of steroids and phytosterol (sitosterol **47** and stigmasterol **48**)

2.5 CHEMICAL CONSTITUENTS OF *AGLAIA* SPECIES

Many biologically active compounds were found from *Aglaia* species by extensive research of scientists. Below are the compounds that obtained from different part of plants of *Aglaia* sp..

Table 2.5: Chemical Constituents Found in *Aglaia* sp.

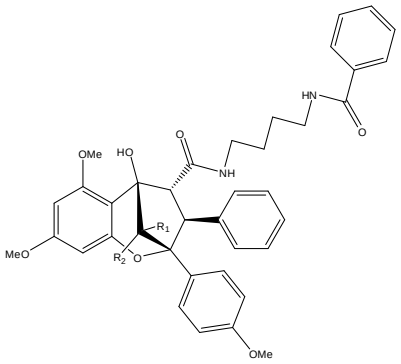
Types of Compounds	***Aglaia* sp.**	**Compounds**
Cyclopenta[bc] benzopyran	*A. forbesii*[36,37]	Desacetylpyramidaglains A **54** Desacetylpyramidaglains C **55** Desacetylapyramidaglains D **56**
		Aglaforbesins A **58** Aglaforbesins B **59**
	A. forbesii[37] *A. argentea*[37]	Aglains A **57**
	A. edulis[38]	Thapsakin B **60** Isothapsakin B **61** Homothapsakin A **62** Thapsakin A acetate **63** Thapsakon A **64** Thapsakon B **65**
	A. testiculari[39]	Aglaxiflorin D **66**
	A. argentea[37]	Aglains B **67** Aglains C **68**
	A. ponapensin[40]	Ponapensin **69**
	A. grandis[41]	Grandiamides A **70**
	A. foveolata[42]	Foveoglin A **71** Foveoglin B **72** Isofoveoglin **73** Cyclofoveoglin **74** Secofoveoglin **75**
Benzo[b] oxepine	*A. edulis*[38,43]	Edulisones A **76** Edulisones B **77** Thapoxepine A **78** Homothapoxepine A **79** Thapoxepine B **80**
	A. forbesii[37]	Forbaglin A **81**

Types of Compounds	***Aglaia* sp.**	**Compounds**
Cycopenta[b] benzofuran	*A. edulis*[38]	Aglaroxin A **82** Pannellin **83**
	A. tomentosa[44] *A. forbesii*[37] *A.crassinervia*[45] *A. ferruginaea*[46] *A. oligophylla*[47]	Rocaglaol **84**
	A. forbesii[37]	Ethylrocaglaol **85**
	A. argentea[37] *A. elliptica*[48]	Didesmethylrocaglamide **86**
	A. odorata[49]	C-3'-hydroxyaglain C **87** C-19,C-3'-dihydroxyaglain C **88** C-19-hydroxy,C-3'-methoxyaglain C **89**
	A. elliptica[48] *A. elaeagnoidea*[50]	Rocaglamide **90** Methyl rocaglate **91**
	A. duperreana[51]	C-1-*O*-acetyldemethylrocaglamide **92**
	A. spectabilis[52]	C-1-*O*-acetyl-4'-demethoxy-3',4'-methylenedioxy derivative of methylrocaglate **93**
	A. elliptica[51,52]	4'-demethoxy-3',4'-methylene-dioxy-methyl rocaglate **94** 1-*O*-formyl-4'-demethoxy-3',4'-methylenedioxy-methyl rocaglate **95** 4'-demethoxy-3',4'-methylenedioxyrocaglaol **96** 1-oxo-4'-demethoxy-3',4'-methylenedioxyrocaglaol **97**
	A. spectabilis[52]	4C-3'methoxy derivative of methylrocaglate **98**
	A. odorata[49]	C-3'-methoxyrocaglamide **99** C-3'-methoxylrocaglaol **100** C-1-oxime **101** C-3'-hydroxydidemethylrocaglamide **104**
	A. duperreana[51]	C-3'-hydroxyrocaglamide **102** C-1-*O*-acetyl-3'-hydroxyrocaglamide **103**
	A. foveolata[42]	Silvestrol **105**
Cycloartane	*A. forbesii*[36]	23,24,25-trihydroxycycloartan-3-one **106**

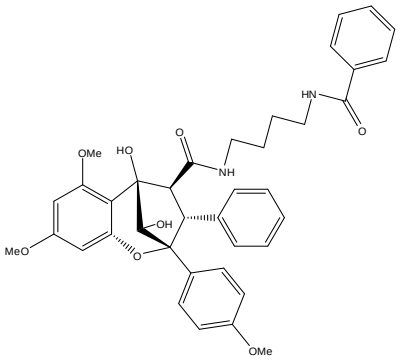
Types of Compounds	***Aglaia* sp.**	**Compounds**
Cycloartane	*A. harmsiana*[53]	(24*R*)-cycloartane-3*β*,24,25-triol **107** (24*R*)-cycloartane-3*β*,24,25,28-tetrol **108** (24*R*)-cycloartane-3*α*,24,25-triol **109**
	A. roxburghiana[54]	Roxburghiadiol A **110** Roxburghiadiol B **111**
	A. argentea[55]	Argenteanones A **112** Argenteanones B **113** Argenteanol **114**
Pregnane steroids	*A. forbesii*[36]	2β,3β -dihydroxy-5α-pregn-17(Z)-en-16-one **115**
	A. tomentosa[44]	Aglatomin A **116**
	A. lawii[56]	(E)-Aglawone **117**
	A. tomentosa[44]	Aglatomine B **118**
	A. silvestris[57]	Pregnacetal **119**
	A. ponapensin[40]	*E*-volkendousin **120**
	A. grandis[58]	2β,3β-dihydroxy-5α-pregnane-16-one **121** 2β,3β-dihydroxy-5α-pregn-17(20)-(Z)-en-16-one **122**
Bisamides	*A. forbesii*[36] *A. foveolata*[42]	Pyramidatine **123**
	A. edulis[59]	Aglaiduline **124** Aglaithioduline **125** Aglaidithioduline **126**
	A. tenuicaulis[60]	Pyrrolotenin **127** Secopyrrolotenin **128**
	A. testicularis[39] *A. odorata*[49]	Piriferine **129** Odorinol **130**
	A. argentea[37] *A. oligophylla*[47]	Odorine **131**
	A. oligophylla[47]	2'-epi-odorine **132**
	A. grandis[41]	Grandiamides B **133** Grandiamides C **134**
Amide ester	*A. tenuicaulis*[62]	Tenucaulin A **135** Isotenucaulin A **136** Tenucaulin B **137** Aglatenin **138** Tenaglin **139**

Types of Compounds	***Aglaia* sp.**	**Compounds**
Amide ester	*A. tenuicaulis*[62]	Caulitenin **140**
Amide alcohol	*A. tenuicaulis*[62]	Aglatenol **141**
Dammaranes	*A. lawii*[44]	Aglinins A **142** Aglinins B **144**
	A. lawii[44] *A. foveolata*[61]	Eichlerianic acid **143** Foveolins A **145**
	A. oligophylla[47]	Cabraleone **146**
	A. lawii[58] *A. tomentosa*[44]	Cabraleadiol monoacetate **149** Cabraleadiol **150**
	A. tomentosa[44] *A. foveolata*[63]	3-epiocotillol **148**
	A. tomentosa[44]	Cabraleadiol 3-acetate **147** Aglinin D **151** Aglinin C **152**
	A. oligophylla[47]	Ocotillone **153** Ocotillol **154**
	A. elaeagnoidea[50]	20*S*,24*S*-epoxy-25-hydroxydammaran-3-one **155** 20*S*,24*S*-epoxy-25-hydroxy-methyldammaran-3-one **156**
	A. tomentosa[44]	Cabralealactone **157** Cabralealactone 3-acetate **158**
	A. crassinervia[45]	3-*epi*-Cabraleahydroxylactone **159**
	A. silvestris[59,62]	Silvaglenamin **160** Silvaglin A **161** Isosilvaglin A **162** Desoxysilvaglin **163** Methylisofoveolate B **164** Methylfoveolate B **165** Aglasilvinic acid **166** Isoeichlerianic acid **167** Methylisoeichleriate **168**
	A. ignea[63]	Dammarenolic acid **169**
	A. oligophylla[47]	Dipterocarpol **170** 20(*S*),24(*S*)-dihydroxydammar-25-en-3-one **171** 20*S*,25-epoxy-24*R*-hydroxy-3-dammaranone **172** 20*S*,25-epoxy-24*R*-hydroxydammarane-3α-ol **173**

Types of Compounds	***Aglaia* sp.**	**Compounds**
Dammaranes	*A. foveolata*[42,63]	Foveolins B **174** Dymalol **175** 17,24-epoxy-25-hydroxy-21-methoxy-3,4-seco-baccharane **176**
Tirucallane	*A. leucophylla*[64]	(-)-leucophyllone **177** (-)-niloticin **178** (-)-bourjortinolone **179** (-)-piscidinol **180**
Lignan	*A. testicularis*[39]	Secoisolariciresinol dimethyl ether **181**
	A. elaeagnoidea[50]	*Trans*-3,4-bis(3,4,5-trimethoxy-benzyl)-tetrahydrofuran **182** *Trans*-3,4-bis(3,4,5-trimethoxy-benzyl)-1,4-butanediol diacetate **183**
	A. cordata[65]	Aglacins E **184** Aglacins F **185** Aglacins G **186** Aglacins H **187**
Glabretal	*A. crassinervia*[45]	Aglaiaglabretol A **188** Aglaiaglabretol B **189** Aglaiaglabretol C **190**
Aminopyrrolidine	*A. odorata*[49]	Syringaresinol **191**
Aglaialactone	*A. ponapensin*[40]	5,6-desmethylenedioxy-5-methoxy-aglalactone **192**
Limonoid	*A. elaeagnoidea*[50]	6α,11β-diacetoxygedunin **193**
Sesquiterpenes	*A. silvestris*[59]	α-muurolene **194**
	A. lawii[44]	Spathulenol **195**
	A. silvestris[59]	Viridiflorol **196**
	A. leucophylla[66]	(-)-caryophyllene oxide **197**
	A. grandis[41]	4β,10α-dihydroxyaromadendrane **198**



54 R_1 = OH; R_2= H
55 R_1 = H; R_2= OH



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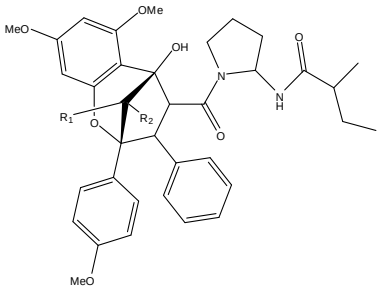
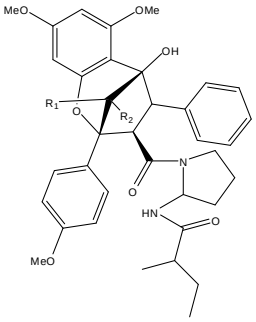
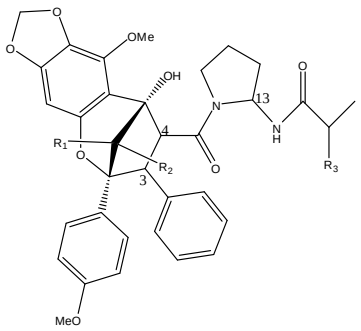


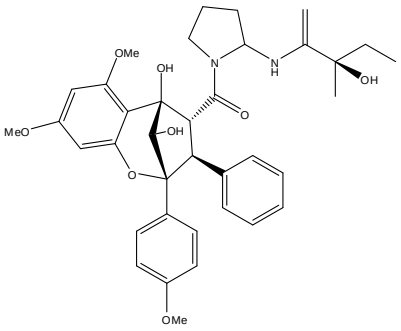
57 R_1 = AcO; R_2= H



58 R_1 = OH; R_2= H
59 R_1 = H; R_2= OH



60 R_1 = H; R_2 = OH; R_3= CH_3
61 R_1 = OH; R_2 = H; R_3= CH_3
62 R_1 = H; R_2 = OH; R_3= CH_2CH_3
63 R_1 = H; R_2 = $OCOCH_3$; R_3= CH_3
64 R_1 = R_2 = O; R_3= CH_3, H-3α, H-4β, 13*S*
65 R_1 = R_2 = O; R_3= CH_3, H-3β, H-4α, 13*S*



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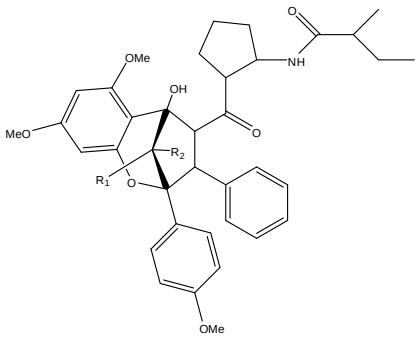
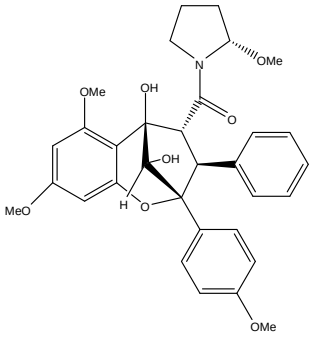


67 R_1 = H; R_2 = OH; H-3β, H-4α, 13*S*

68 R_1 = H; R_2= OH_3; H-3α, H-4β, 13*S*



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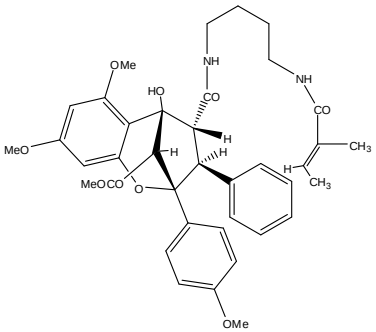

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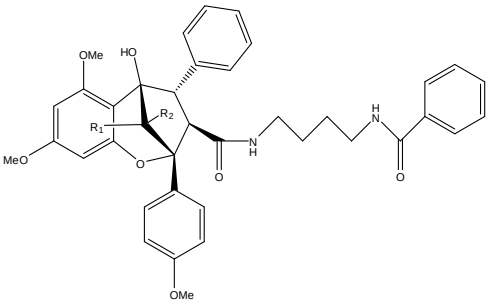
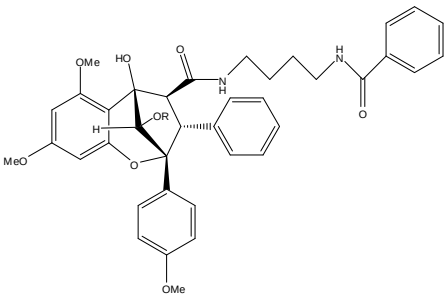
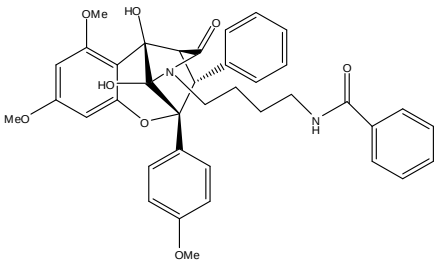


71 R_1 = OH; R_2 = H

72 R_1 = H; R_2 = OH



73 R = H



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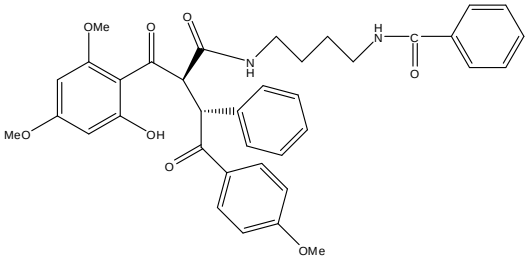

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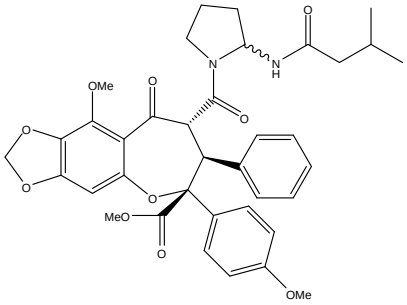
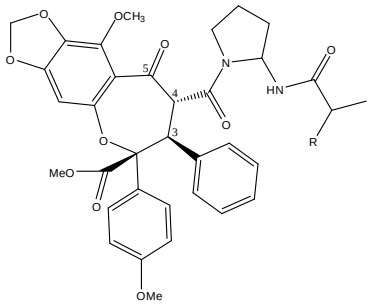
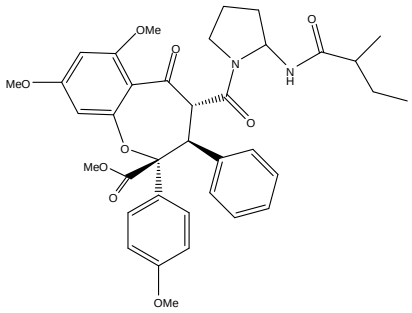


76 13*R*
77 13*S*



78 R = CH_3; H-3α, H-4β
79 R = CH_2CH_3
80 R= CH_3; H-3β, H-4α



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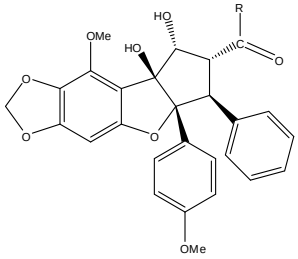
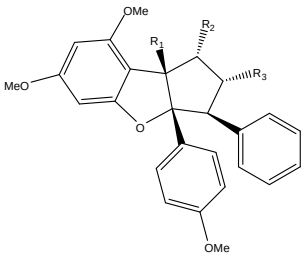
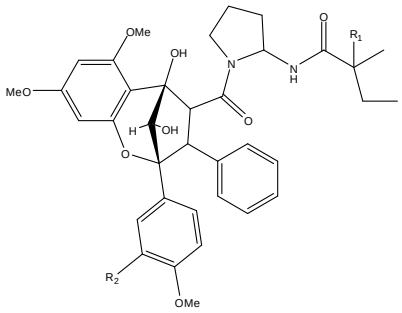


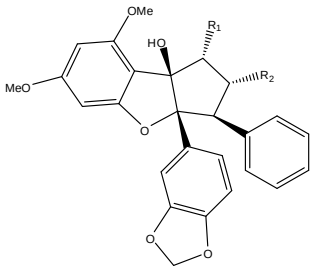
82 R = $N(CH_3)_2$
83 R = OCH_3



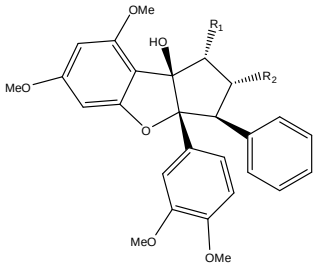
84 R_1 = OH; R_2 = OH; R_3 = H
85 R_1 = OEt; R_2 = OH; R_3 = H
86 R_1 = OH; R_2 = OH; R_3 = $CONH_2$
90 R_1 = OH; R_2 = OH; R_3 = $CON(CH_3)_2$
91 R_1 = OH; R_2 = OH; R_3 = COOMe
92 R_1 = OH; R_2 = $OCOCH_3$; R_3 = $CONHCH_3$



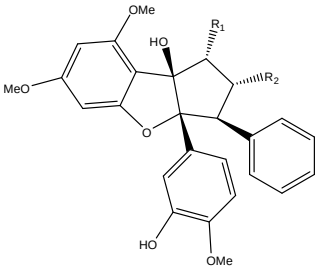
87 R_1 = OH; R_2 = H; R_3 = OH
88 R_1 = OH; R_2 = OH; R_3 = OH
89 R_1 = OH; R_2 = OH; R_3 = OCH_3



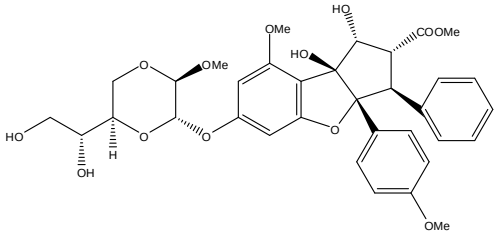
93 R_1 = $OCOCH_3$; R_2 = $COOCH_3$
94 R_1 = OH; R_2 = COOMe
95 R_1 = OCHO; R_2 = COOMe
96 R_1 = OH; R_2 = H
97 R_1 = O; R_2 = H



98 R_1 = OH; R_2 =$COOCH_3$
99 R_1 = OH; R_2 = $CON(CH_3)_2$
100 R_1 = OH; R_2 = H
101 R_1 = NOH; R_2 = $COOCH_3$



102 R_1 = OH; R_2 = $CON(CH_3)_2$
103 R_1 = $OCOCH_3$; R_2 = $CON(CH_3)_2$
104 R_1 = OH; R_2 = $CONH_2$



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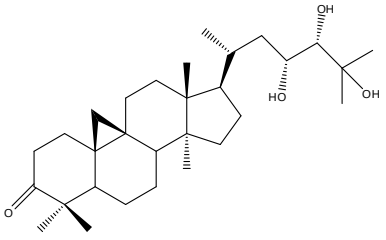

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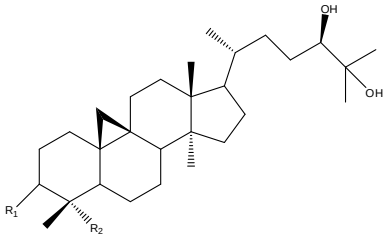
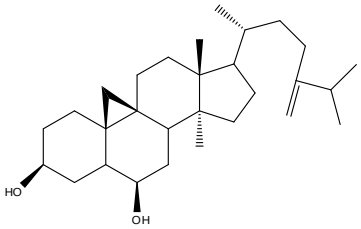


107 R_1 = β-OH, α-H; R_2 = CH_3
108 R_1 = β-OH, α-H; R_2 = CH_2OH
109 R_1 = α-OH, β-H; R_2 = CH_3



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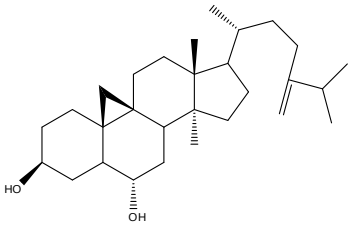

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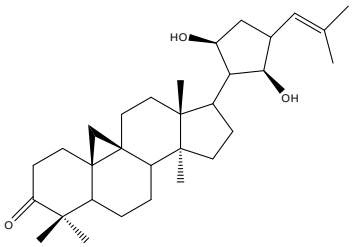

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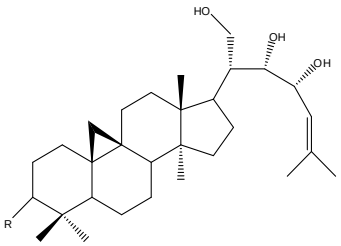
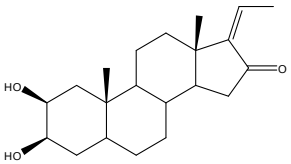


113 R = O
114 R = OH



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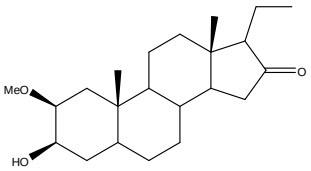

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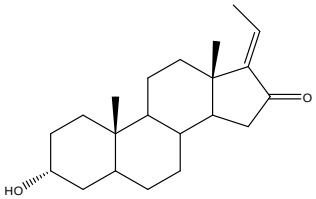

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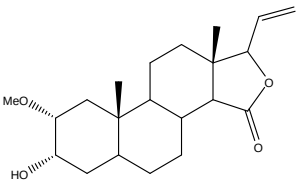

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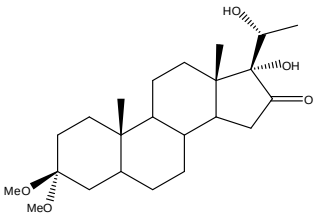

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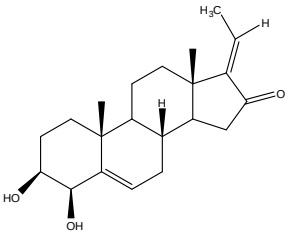

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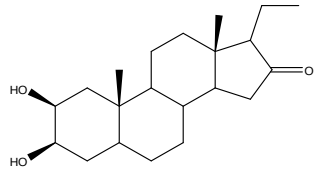

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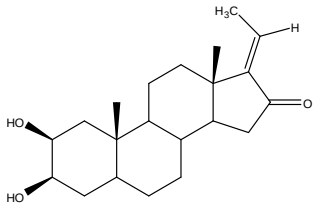

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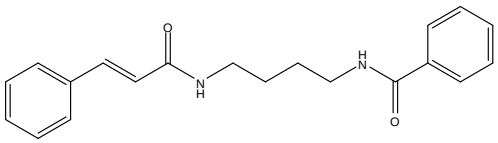

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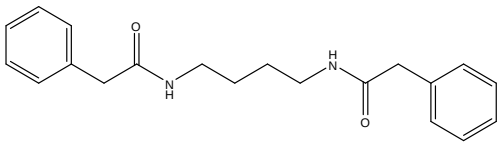

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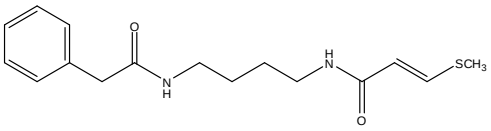

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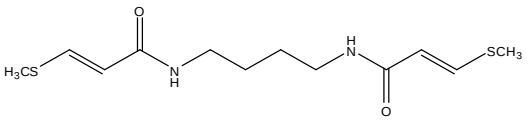

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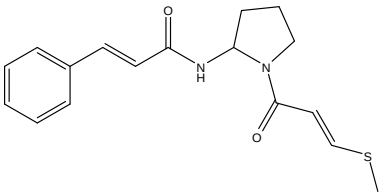

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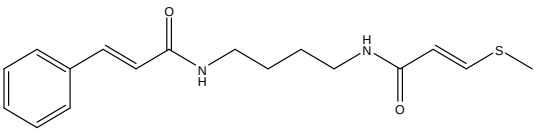

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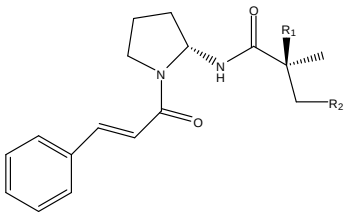
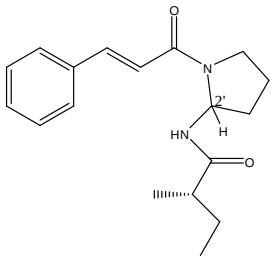
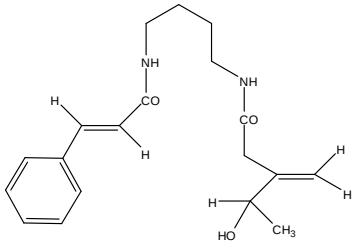


129 R_1 = H; R_2 = H
130 R_1 = OH; R_2 = CH_3



131 2'Hβ
132 2'Hα



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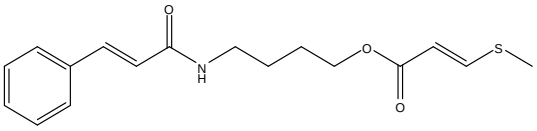

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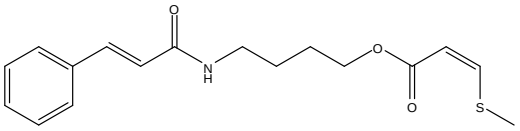

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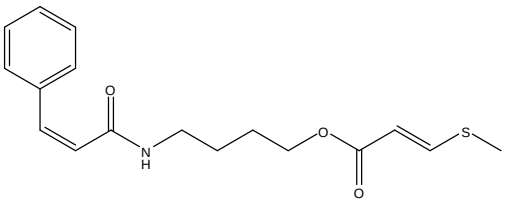

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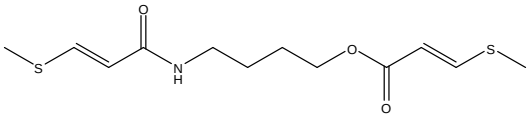

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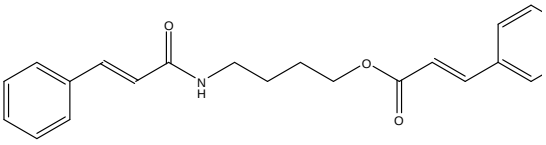

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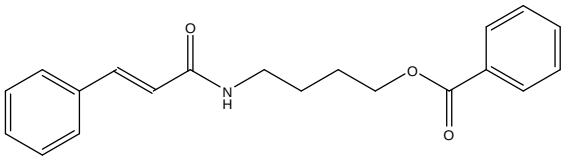

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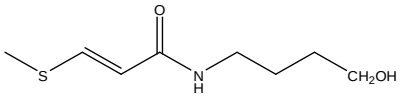

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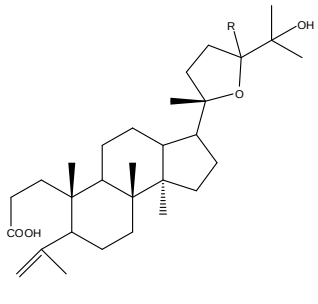
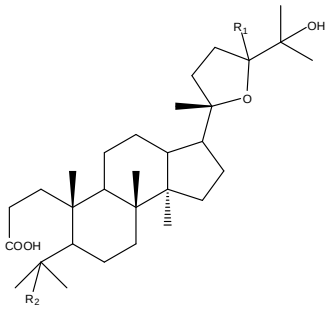
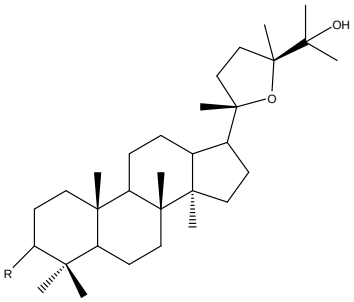


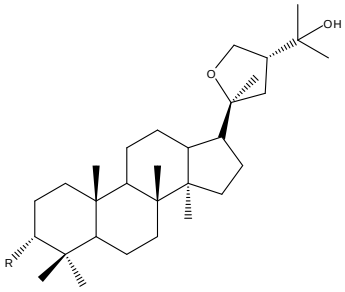
142 R = OH
143 R = H



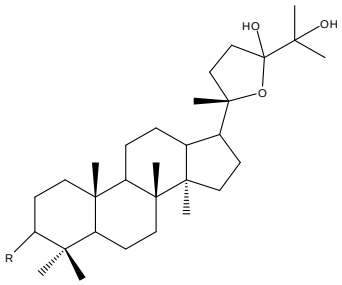
`144 R_1 = OH; R_2 = H
145 R_1 = H; R_2 = OH



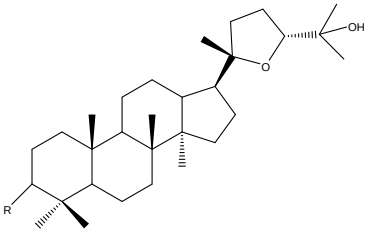
146 R = O
147 R = OAc
148 R = OH



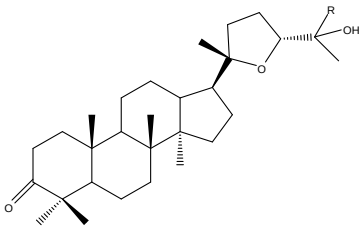
149 R = OAc
150 R = OH



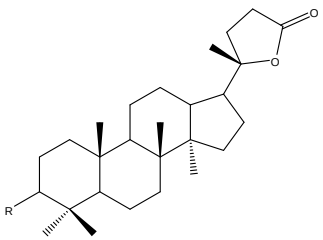
151 R = O
152 R = OH



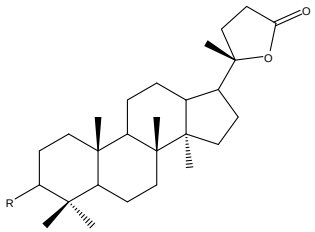
153 R = O
154 R = OH



155 R = Me
156 R = CH_2OH



157 R = O
158 R = OAc



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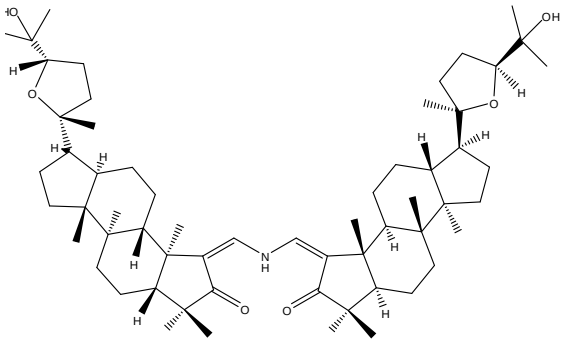

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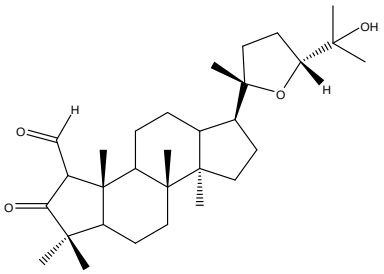

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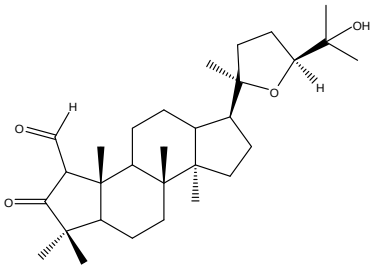

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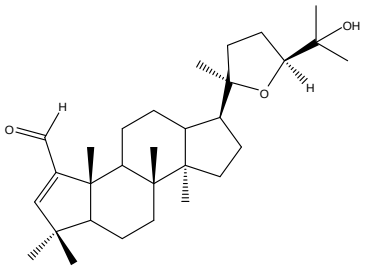

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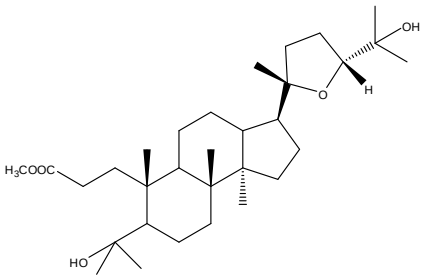

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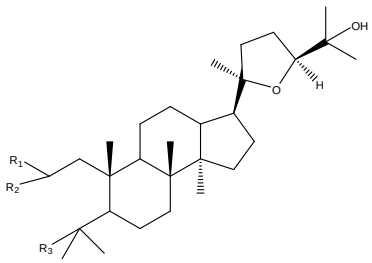
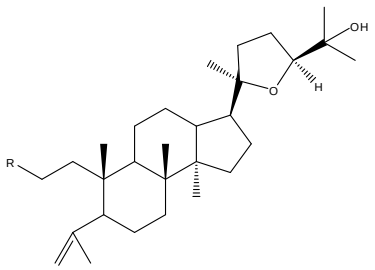
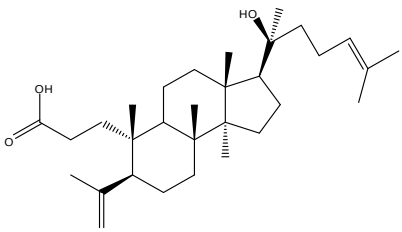


165 $R_1 = COOCH_3$; $R_2 = H$; $R_3 = OH$
166 $R_1 = OCH_3$; $R_2 = OCH_3$; $R_3 = COOH$



167 R = COOH
168 R = $COOCH_3$



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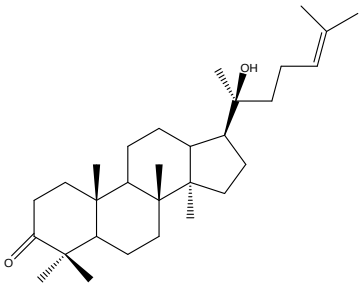

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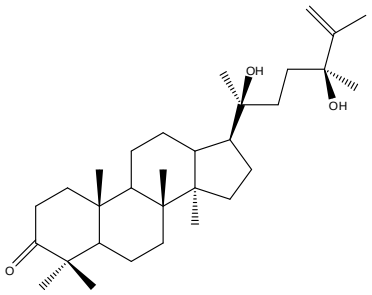

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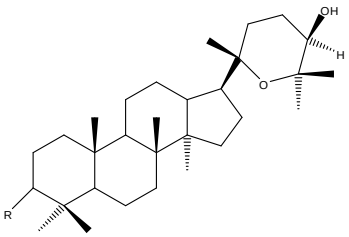
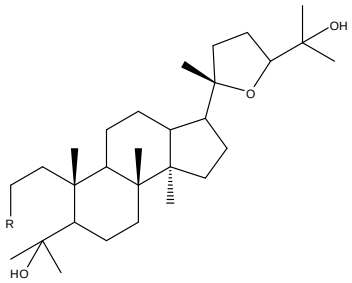
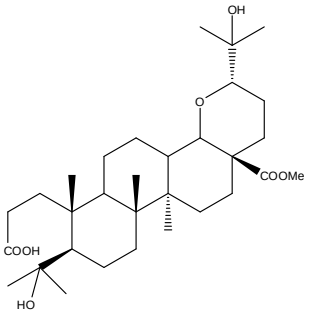


172 R = O
173 R = OH



174 R = COOH
175 R = COOMe



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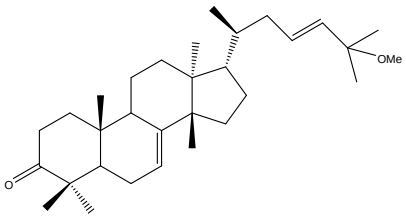

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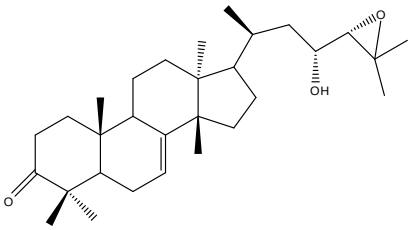

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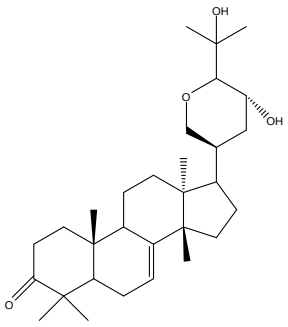

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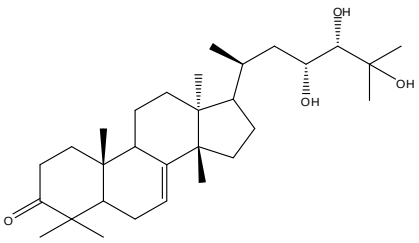

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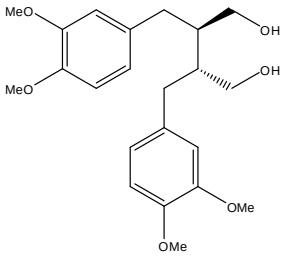

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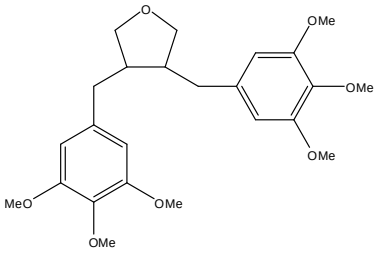

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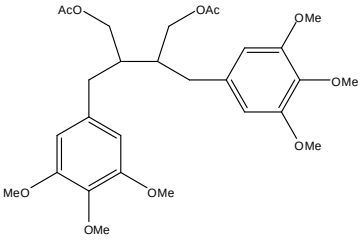

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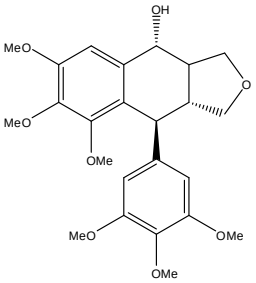

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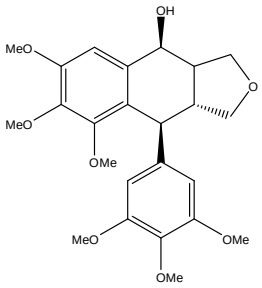

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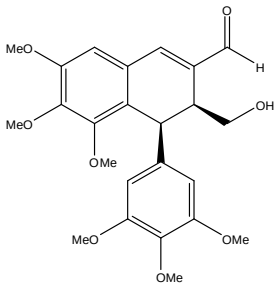

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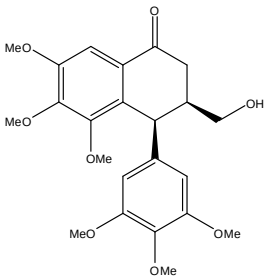

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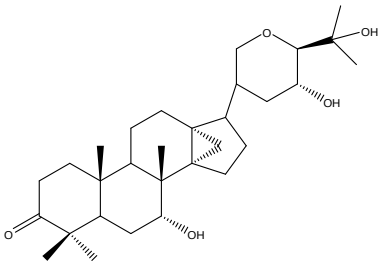

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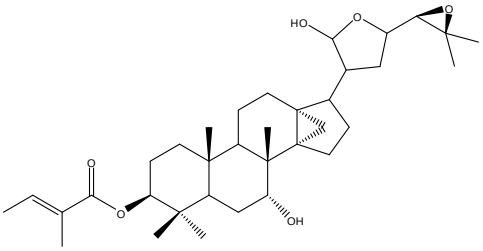

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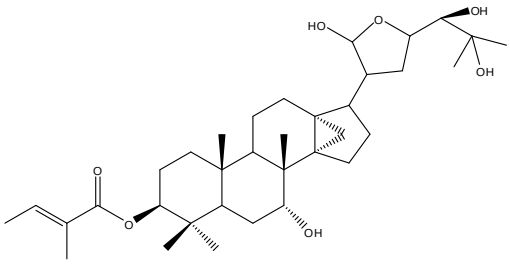

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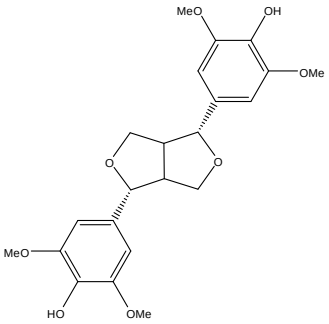

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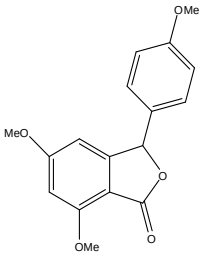

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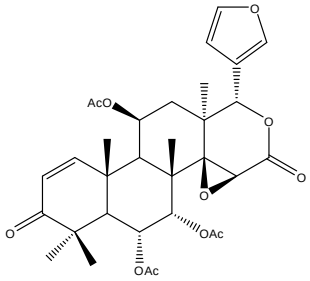

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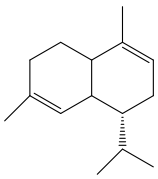

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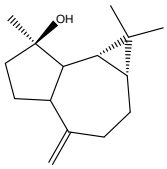

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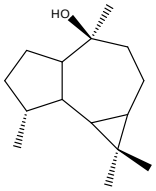

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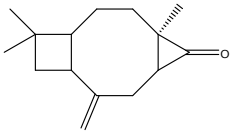

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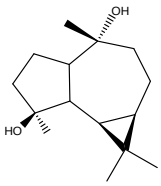

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CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 COMPOUNDS OF *AGLAIA EXIMA*

Eight compounds were obtained from *Aglaia exima* (KL 4762) which was collected from H.S Kepayang, Pahang, Malaysia on 26 November 1997 and has been deposited at the Herbarium of Department of Chemistry, University of Malaya. All of the compounds were separated by using the chromatographic techniques which are thin layer chromatography (TLC), column chromatography (CC), high performance liquid chromatography (HPLC) and preparative thin layer chromatography (PTLC). Repeated chromatographic separation of the hexane crude extract led to the isolation of eight pure compounds (Table 3.1). One of the compound, 24(*E*)- cycloart-24-ene-26-ol-3-one **203,** is a new compound.

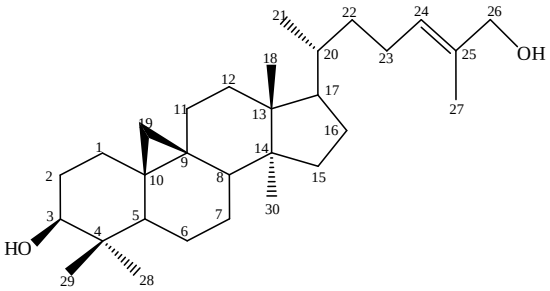
The structures of the isolated compounds were then elucidated through the nuclear magnetic resonance spectroscopy (NMR), ^{1}H, ^{13}C, DEPT, HSQC, HMBC, COSY, mass spectroscopy (MS), ultraviolet spectroscopy (UV) and infrared spectroscopy (IR). Majority of compounds were identified based on the spectral characteristics depicted in NMR spectra data for complete assignments of carbons and protons.

This chapter discussed herein the analysis and elucidation of eight compounds that were isolated from the hexane crude extract of the leaves of *Aglaia exima*.

Table 3.1: Isolated compounds from the hexane crude extract of *Aglaia exima*

Name of Compounds	Type of compound	Yield (mg)	Percentage of compounds from hexane crude (%)
Cycloart-24-ene-3*β*,26-diol **199**	Cycloartane	93.6	0.62
Vaticinone **200**	Cycloartane	6.5	0.04
Schizandronic acid **201**	Cycloartane	16.3	0.11
Cabraleahydroxylactone **202**	Dammarane	2.9	0.02
24(*E*)- cycloart-24-ene-26-ol-3-one **203**	Cycloartane	19.1	0.13
24(*E*)-3*β*-hydroxycycloart-24-ene-26-al **204**	Cycloartane	25.6	0.17
β-sitosterol **205**	Sterol	34.2	0.23
Stigmast-5-ene-28-one **206**	Sterol	5.1	0.03

Compound A: Cycloart-24-ene-3*β*,26-diol **199**



Compound A was isolated as a colorless crystal with a molecular formula of $C_{30}H_{50}O_2$ and its melting point was 154-155 ^oC. It was then confirmed by the literature spectroscopic data[66] and X-ray analysis (see Apendix). The optical rotation, $[\alpha]_D^{23.8}$ = +48.4^o (c 0.00062, CH_3OH) shows β-configuration of OH at C-3. A molecular ion peak at m/z 442 was shown in EI-MS spectra and the UV spectrum showed absorption band at 210 nm. Besides, its IR spectrum showed IR absorption at 3400 cm^{-1} suggesting the presence of hydroxyl group.

The ^1H NMR spectrum in Figure 3.3 showed a characteristic signal of cyclopropane methylene, a pair of doublet at upfield region of δ 0.55 (1H, *d*, *J*= 4.2Hz) and δ 0.33 (1H, *d*, *J*= 4.2Hz). There were five methyls assigned by the peak between δ 0.80 to δ 0.97 and a methyl attached to a double bond emerged as a singlet at more downfield position δ 1.59 (3H, *s*). The ^1H NMR also discerned a triplet at δ 5.40 (1H, *t*, J_1= 7.1Hz, J_2= 13.2Hz) of H-24 indicating the presence of olefinic proton. Besides this, spectrum discerned a multiplet and singlet signals at δ 4.00 (H-26) and δ 3.28 (H-3) respectively revealed the presence of hydroxyl group in compound A.

The ^{13}C NMR in Figure 3.4 and Figure 3.5 showed the structure was composed of six methyls, twelve methylenes, six methines and six quaternary carbons. Peak at δ 127.1 (C-24) and δ 134.3 (C-25) represented the double bond in the compound A. The presence of hydroxyl groups were revealed by the peak at the position of δ 78.8 (C-3)

and δ 69.1 (C-26). The carbons of the six methyls gave signals at δ 18.2, δ 18.0, δ 13.6, δ 25.4, δ 19.4 and δ 19.3 for C-18, C-21, C-27, C-28, C-29 and C-30 respectively.

The HMBC correlation confirmed the methyl proton (H-27) has a correlation to C-24 and C-25 which were double bond. Besides, the proton (H-26) attached to a hydroxyl group correlated with C-24 and C-25. Moreover, the correlation of H-24, the olefinic proton to C-22, C-23, C-26, and C-27; H-26 correlated to C-27 confirmed that the assignation of side chain. The HMBC correlation showed the correlation of H-2 and H-1 with C-3 which is a hydroxyl carbon. In addition, the methylene proton H-19 was found having correlation with C-1, C-9, C-10 and C-11 which confirmed its position.

By comparing the NMR spectral data (Table 3.2) with the literature values[68], it was confirmed that compound A was cycloart-24-ene-3*β*,26-diol **199**.

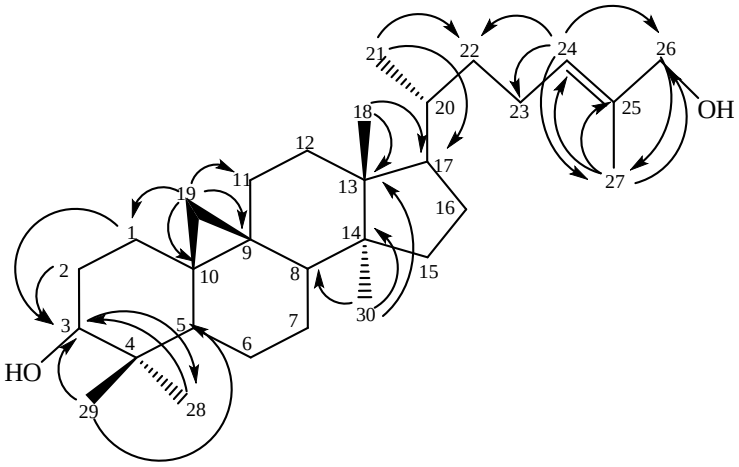


Figure 3.1: Selected HMBC Correlation of Compound A

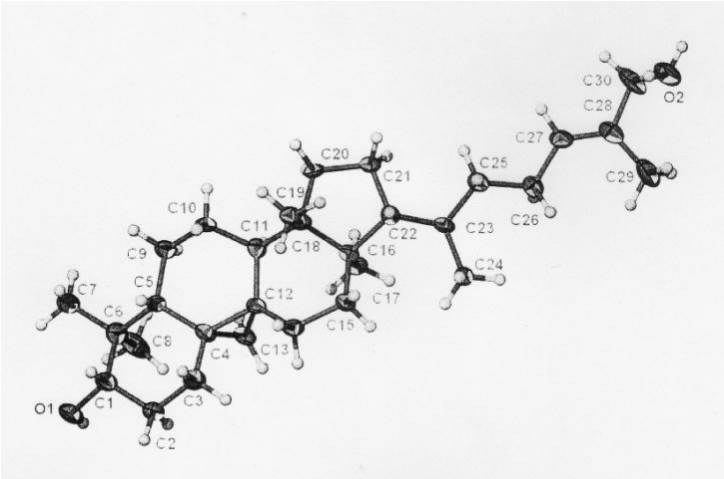


Figure 3.2: ORTEP diagram of Compound A

Table 3.2: ^{1}H NMR, ^{13}C NMR, HMBC Data of Compound A in $CDCl_3$

Position	δ_H (ppm)	δc (ppm)	HMBC (H→C)
1	1.26 (1H, *m*)	31.9	3
	1.51 (1H, *m*)		
2	1.44 (1H, *m*)	30.3	3
	1.74 (1H, *m*)		
3	3.28 (1H, *m*)	78.8	28
4	-	40.5	
5	1.29 (1H, *m*)	47.1	-
6	1.53 (1H, *m*)	21.1	-
	0.78 (1H, *m*)		
7	1.24 (1H, *m*)	28.1	-
	1.90 (1H, *m*)		
8	1.41 (1H, *m*)	48.0	-
9	-	20.0	-
10	-	26.1	-
11	1.06 (1H, *m*)	26.0	-
	1.29 (1H, *m*)		
12	1.04 (1H, *m*)	35.5	-
	1.36 (1H, *m*)		
13	-	45.3	-
14	-	48.8	-
15	1.55 (1H, *m*)	32.9	-
	1.34 (1H, *m*)		
16	1.58 (1H, *m*)	26.4	-
	1.96 (1H, *m*)		
17	1.54 (1H, *m*)	52.2	-
18	0.97 (3H, *s*)	18.2	13,15,17
19	0.33 (1H, *d*, *J*= 4.2 Hz)	29.9	1, 9, 10, 11
	0.55 (1H, *d*, *J*= 4.2 Hz)		
20	1.04 (1H, *m*)	36.0	-
21	0.89 (3H, *d*, *J*= 6.4 Hz)	18.0	17, 22
22	1.02 (1H, *m*)	35.9	-
	1.23 (1H, *m*)		
23	2.05 (1H, *m*)	24.5	-
	1.92 (1H, *m*)		
24	5.40 (1H, *t*, J_1= 7.1 Hz, J_2= 13.2 Hz)	127.1	22, 23, 26, 27
25	-	134.3	-
26	4.00 (2H, *s*)	69.1	24, 25, 27
27	1.59 (3H, *m*)	13.6	24, 25, 26
28	0.97 (3H, *s*)	25.4	3
29	0.81 (3H, *s*)	14.0	3,5
30	0.89 (3H, *s*)	19.3	8, 14, 13

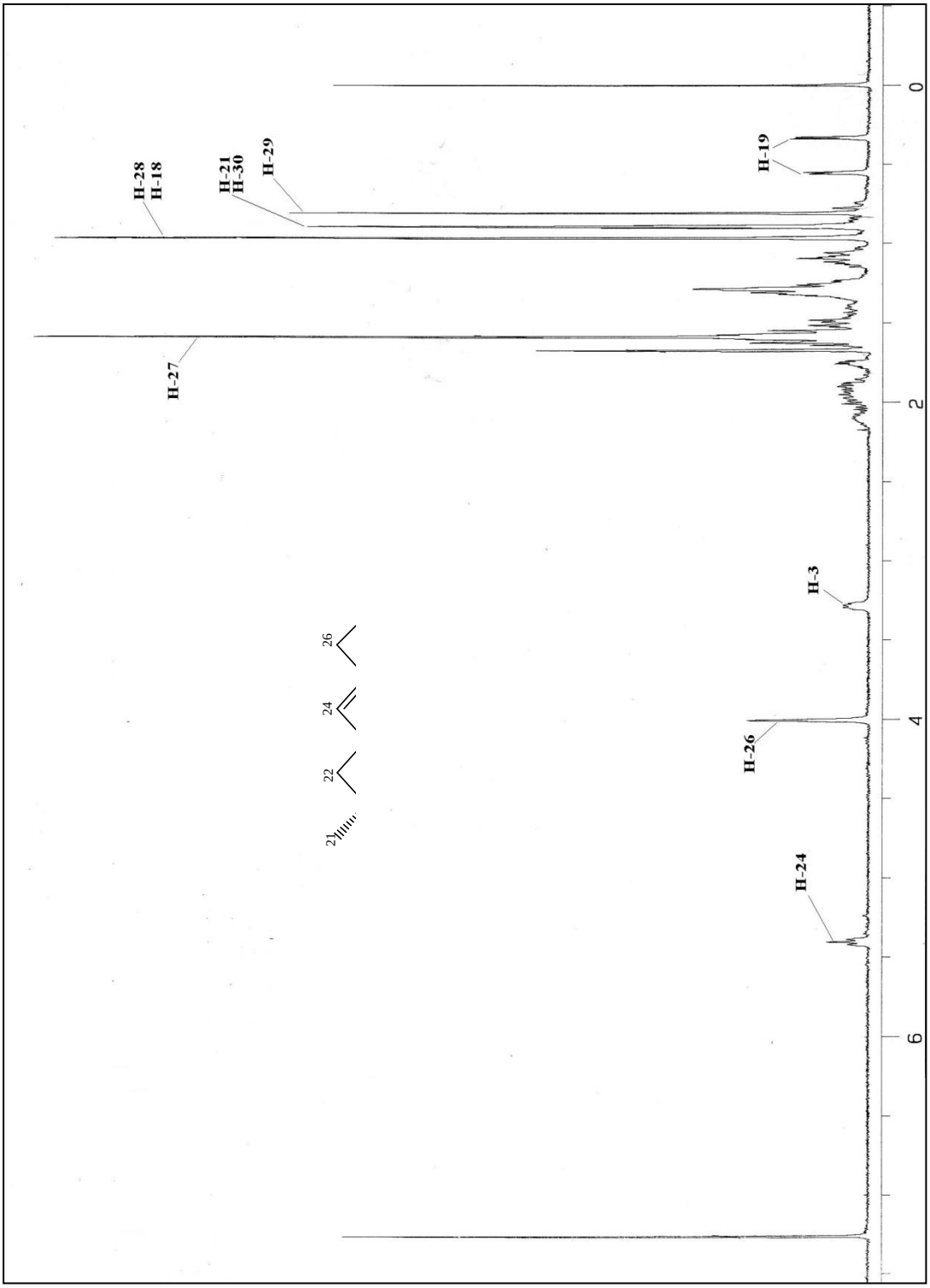


Figure 3.3: ^{1}H NMR spectrum of compound A in $CDCl_3$ (400 MHz)

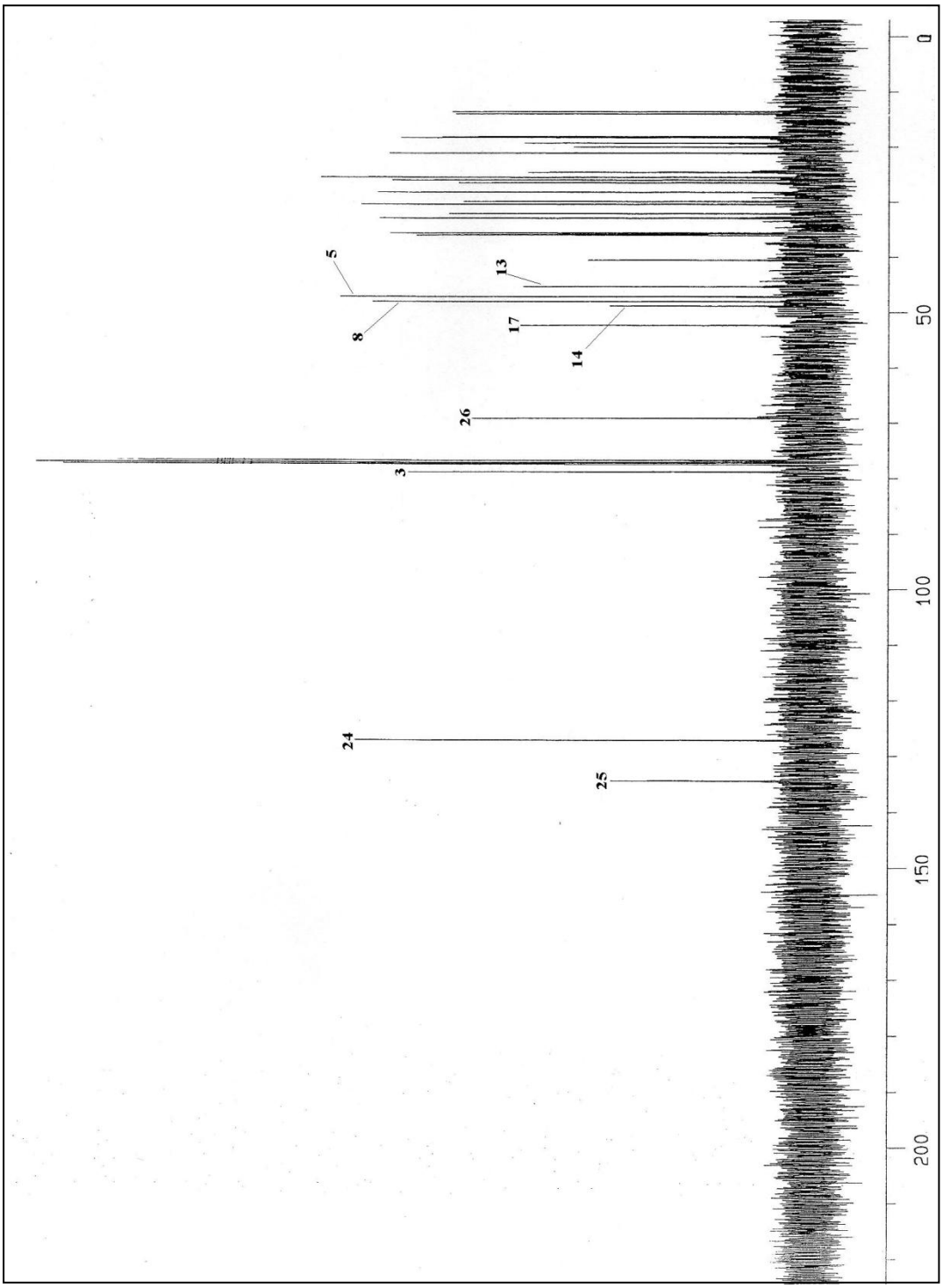


Figure 3.4: ^{13}C NMR spectrum of compound A in $CDCl_3$ (400 MHz)

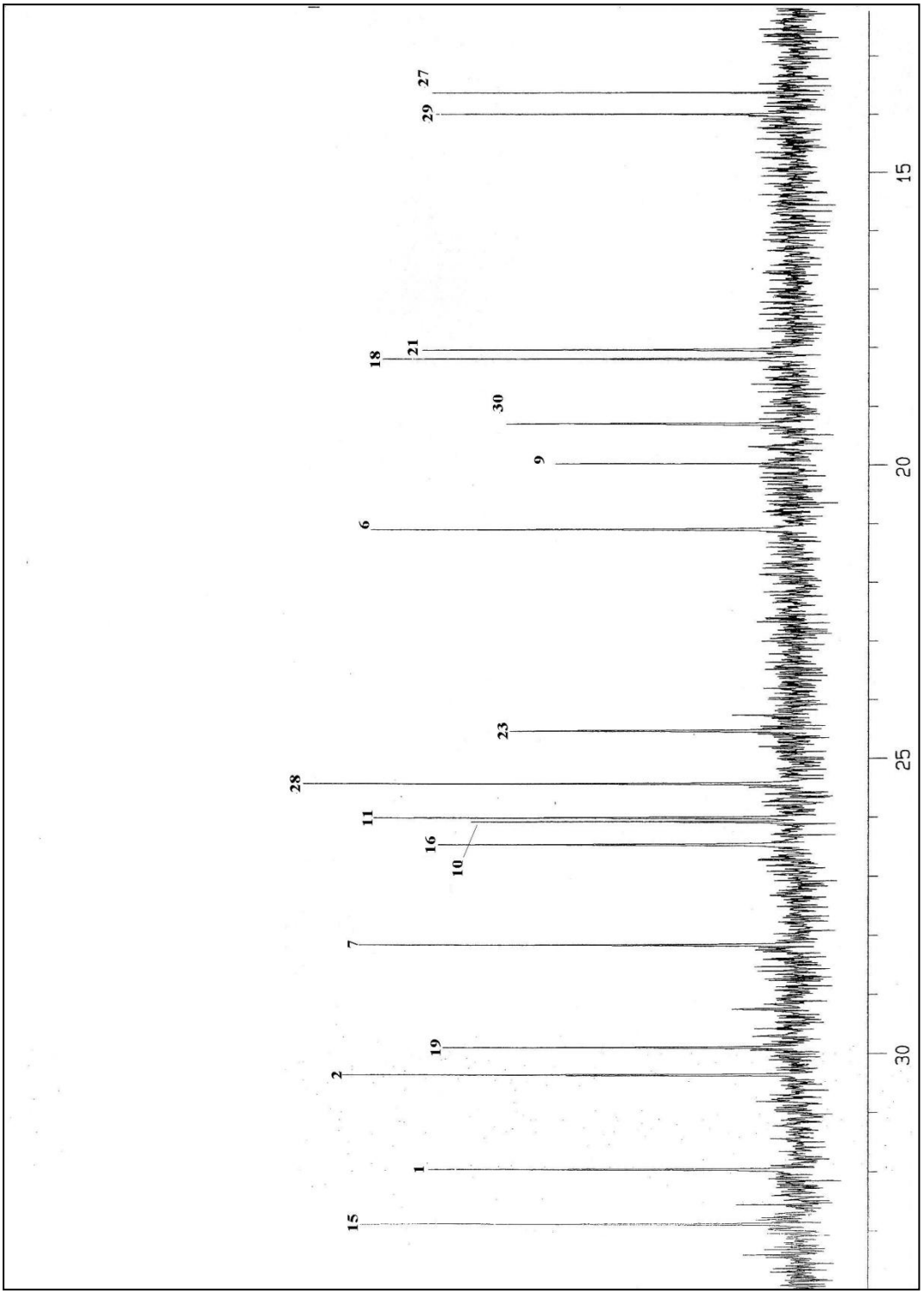
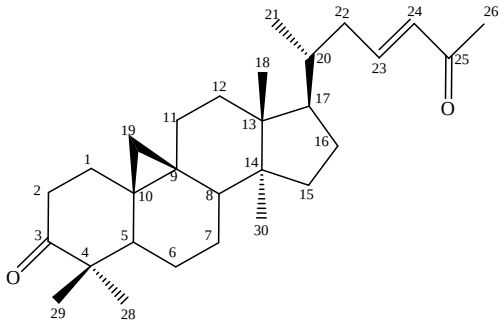


Figure 3.5: Expansion of ^{13}C NMR spectrum of compound A in $CDCl_3$ (δ10 – 40 ppm)

Compound B: Vaticinone **200**



Compound B was isolated as a white powder with a molecular formula of $C_{29}H_{44}O_2$ as a molecular ion peak at m/z 424 was shown in EI-MS. Its optical rotation, $[\propto]_D^{23.8}$ = +27.3^o (c 0.00011, CH_3OH). The UV spectrum revealed an absorption band at 221 nm and IR spectrum showed IR absorption at 2929, 1707 and 1621 cm^{-1} suggesting the presence of alkyl group, ketonic carbonyl and olefinic group respectively. It was then further confirmed by literature spectroscopic data[69].

The ^{1}H NMR spectrum in Figure 3.7 showed characteristic signals for six methyl groups at δ 0.91 (Me-30), δ 0.92 (Me-21), δ 1.02 (Me-18), δ 1.05 (Me-28), δ 1.10 (Me-29), δ 2.26 (Me-26). A pair of characteristic doublet signals at δ 0.56 (1H, *d*, *J*= 4.2 Hz) and δ 0.77 (1H, *d*, *J*= 4.2 Hz) were assigned for methylene group. These methylene signals at upfield region indicated compound B to be a cycloartane triterpenoid. The presence of a double bond can be shown by the existence of a doublet at δ 6.06 (1H, *d*, *J*= 15.9 Hz) and multiplet at δ 6.78 (1H, *ddd*, J_1= 6.1 Hz, J_2= 8.5 Hz, J_3= 15.6 Hz) which at the position H-24 and H-23 respectively.

The ^{13}C NMR and DEPT spectra in Figure 3.8 - Figure 3.11 further confirmed the existence of two olefinic carbon at δ 132.6 (C-24) and δ 147.7 (C-23) and two quaternary carbonyl carbon signals at δ 198.6 (C-25) and δ 216.7 (C-3) were revealed. The presence of six methyls in the structure of compound B were shown by the signals

at δ 18.2 (Me-18), δ 18.7 (Me-21), δ 27.0 (Me-26), δ 22.3 (Me-28), δ 20.8 (Me-29) and δ 19.4 (Me-30) respectively.

The connection and position of these structures was determined by the HMBC correlations (Figure 3.6 and 3.12). The vicinal proton of double bond (H-23) showed correlation to a quaternary carbonyl carbon C-25 (δ 198.6) and the quaternary carbonyl carbon correlated with methyl proton of H-26 (3H, *s*). This indicated that the carbonyl carbon is in the form of unsaturated ketone by conjugating with the double bond. A pair of methyl proton at H-28 (3H, *s*) and H-29 (3H, *s*) has correlation to the remaining carbonyl carbon C-3 (δ 216.7). Besides, the methylene proton H-19 was found to be correlated with C-11 (δ 26.7).

Compound B was identified by comparison of its spectral data with the literature values [67] thus confirming that compound B was a cycloartane triterpenoid named vaticinone **200**.

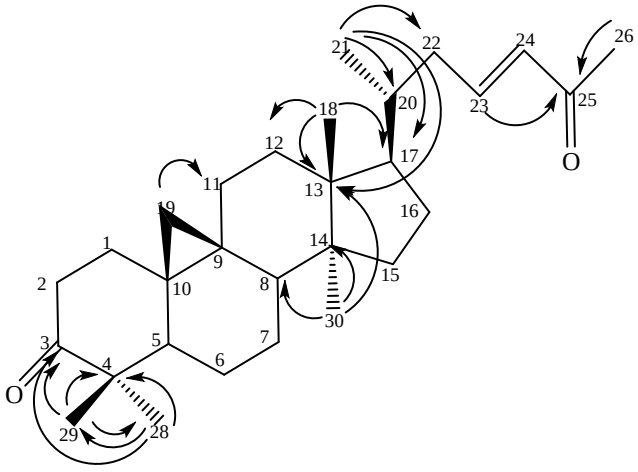


Figure 3.6: Key HMBC Correlation of Compound B

Table 3.3: ^{1}H NMR, ^{13}C NMR, HMBC Data of Compound B in $CDCl_3$

Position	δ_H (ppm)	δ_C (ppm)	HMBC (H→C)
1	1.82 (1H, *m*)	33.5	-
	1.52 (1H, *m*)		
2	2.26 (1H, *m*)	37.5	1, 3
	2.69 (1H, *td*, J_1= 7.3 Hz, J_2= 13.6 Hz)		
3	-	216.7	-
4	-	50.3	-
5	1.69 (1H, *m*)	48.5	-
6	1.54 (2H, *m*)	21.6	-
7	1.32 (1H, *m*)	25.9	-
	1.12 (1H, *m*)		
8	1.56 (1H, *m*)	48.0	-
9	-	21.1	-
10	-	26.1	-
11	2.02 (2H, *m*)	26.7	19
12	1.61 (2H, *m*)	32.7	11, 13, 14, 18
13	-	45.5	-
14	-	48.9	-
15	1.32 (2H, *m*)	35.6	16
16	1.90 (1H, *m*)	28.3	-
	1.32 (1H, *m*)		
17	1.62 (1H, *m*)	52.3	13, 18
18	1.02 (3H, *s*)	18.2	12, 13, 17
19	0.56 (1H, *d*, *J*= 4.2 Hz)	29.6	-
	0.77 (1H, *d*, *J*= 4.2 Hz)		
20	1.60 (1H, *m*)	36.2	13
21	0.92 (3H, *d*, *J*= 5.1 Hz)	18.7	17, 20, 22
22	2.34 (1H, *m*)	39.7	-
	1.94 (1H, *m*)		
23	6.78 (1H, *ddd*, J_1= 6.1 Hz, J_2= 8.5 Hz, J_3= 15.6 Hz)	147.7	25
24	6.06 (1H, *d*, *J*= 15.9 Hz)	132.6	-
25	-	198.6	-
26	2.26 (3H, *s*)	27.0	25
28	1.05 (3H, *s*)	22.3	3, 4, 29
29	1.10 (3H, *s*)	20.8	3, 4, 28
30	0.91 (3H, *s*)	19.4	8, 13, 14

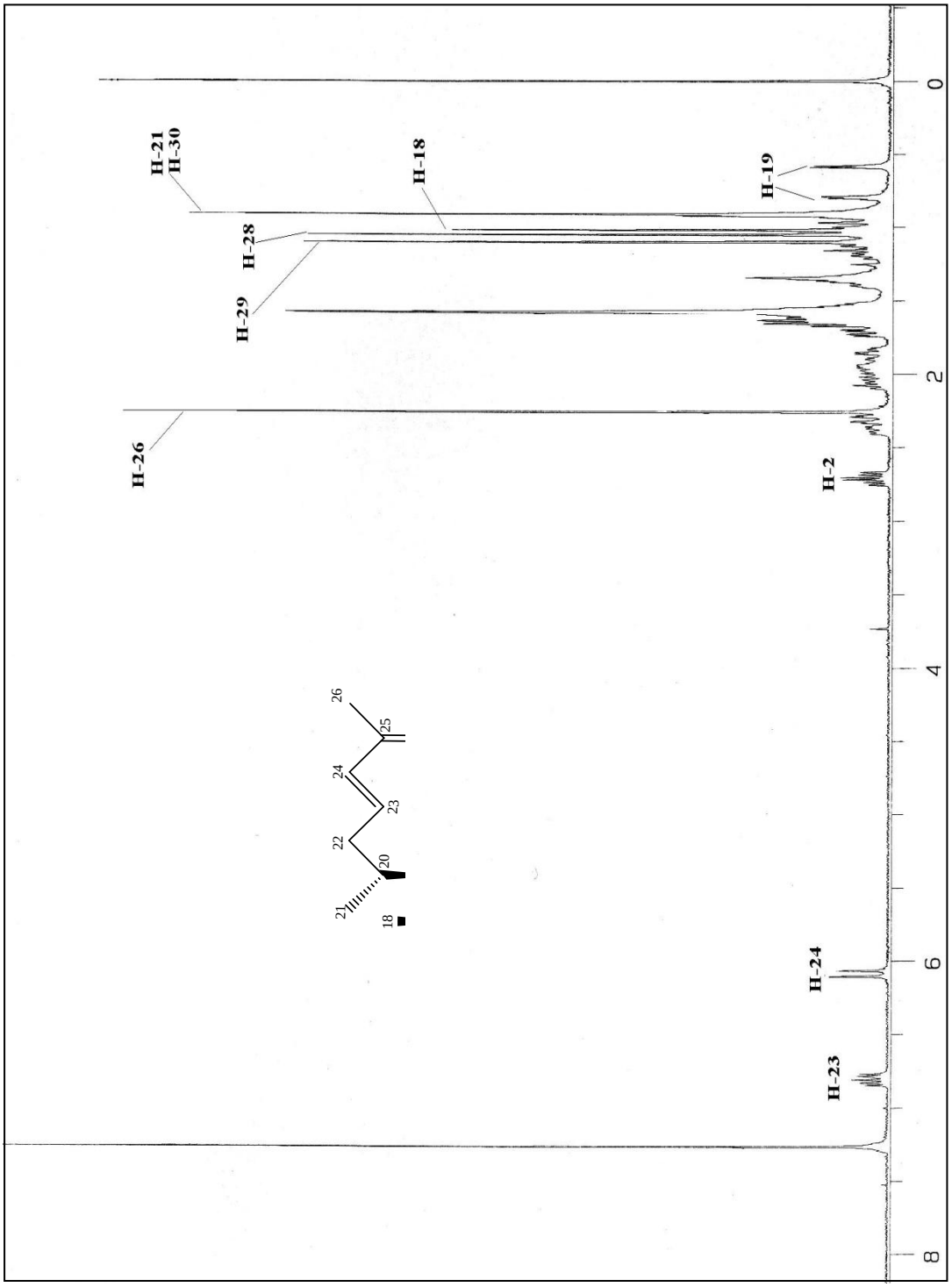


Figure 3.7: ^{1}H NMR spectrum of compound B in $CDCl_3$ (400 MHz)

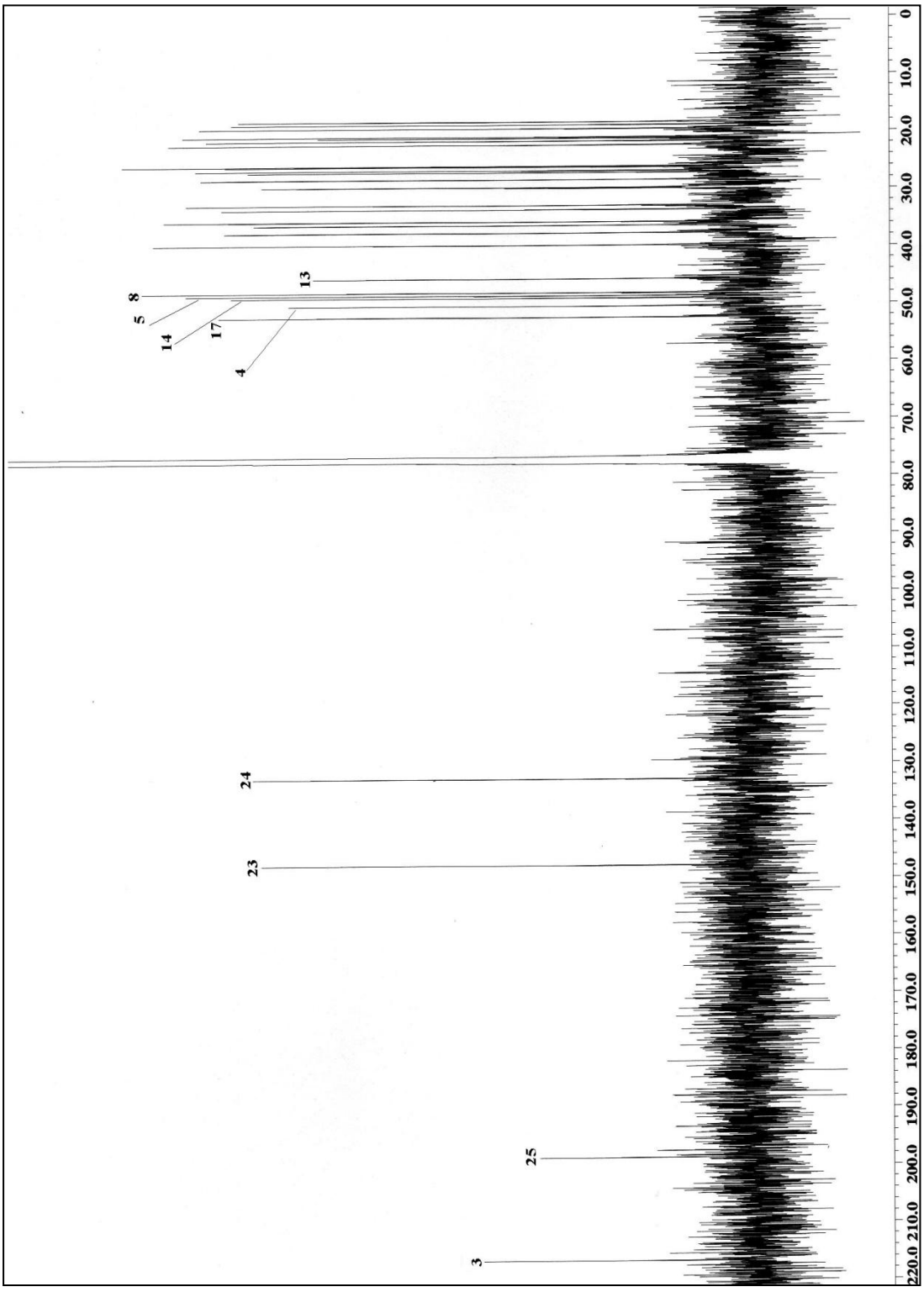


Figure 3.8: ^{13}C NMR spectrum of compound B in $CDCl_3$ (400 MHz)

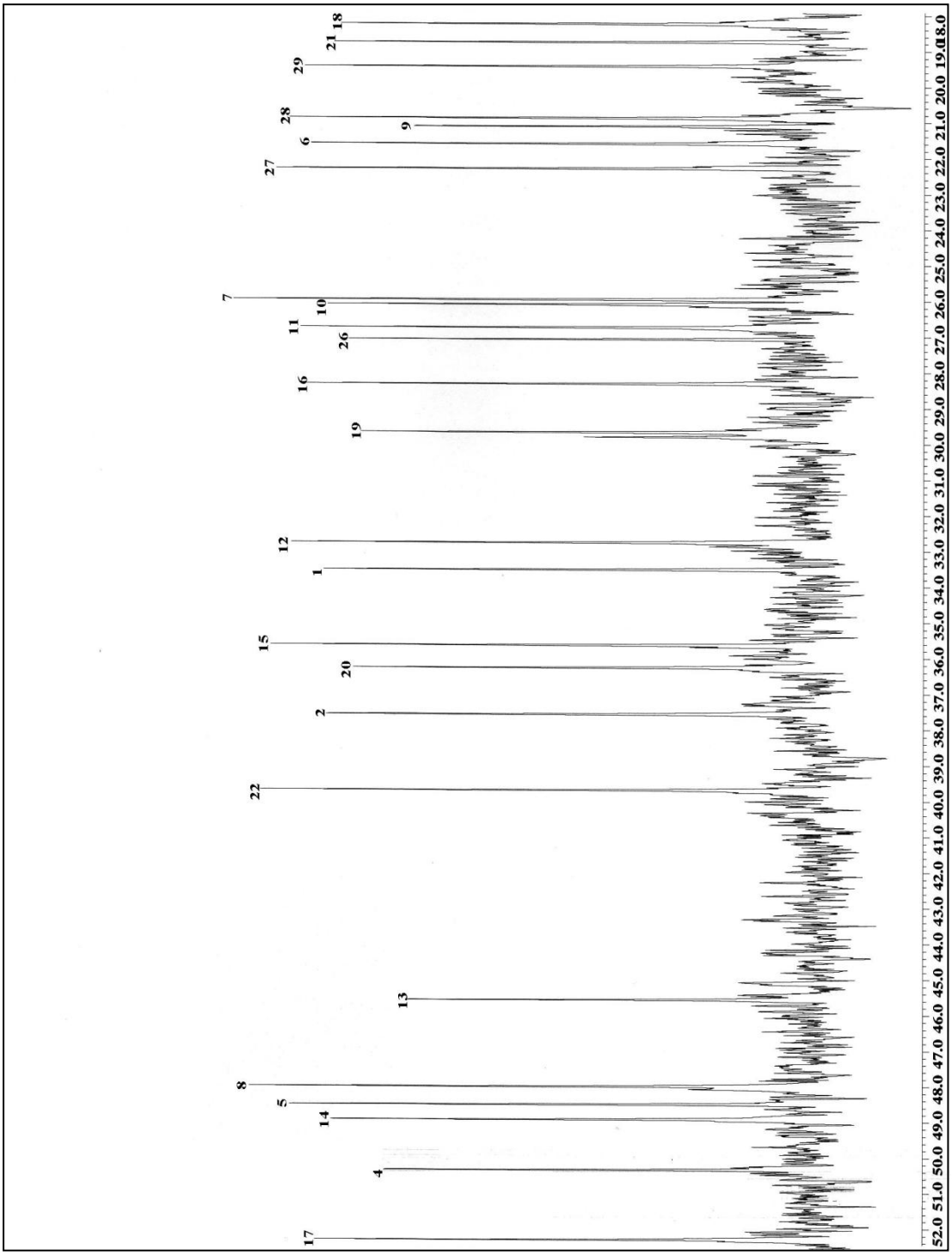


Figure 3.9: Expansion of ^{13}C NMR spectrum of compound B in $CDCl_3$ (δ18 -55 ppm)

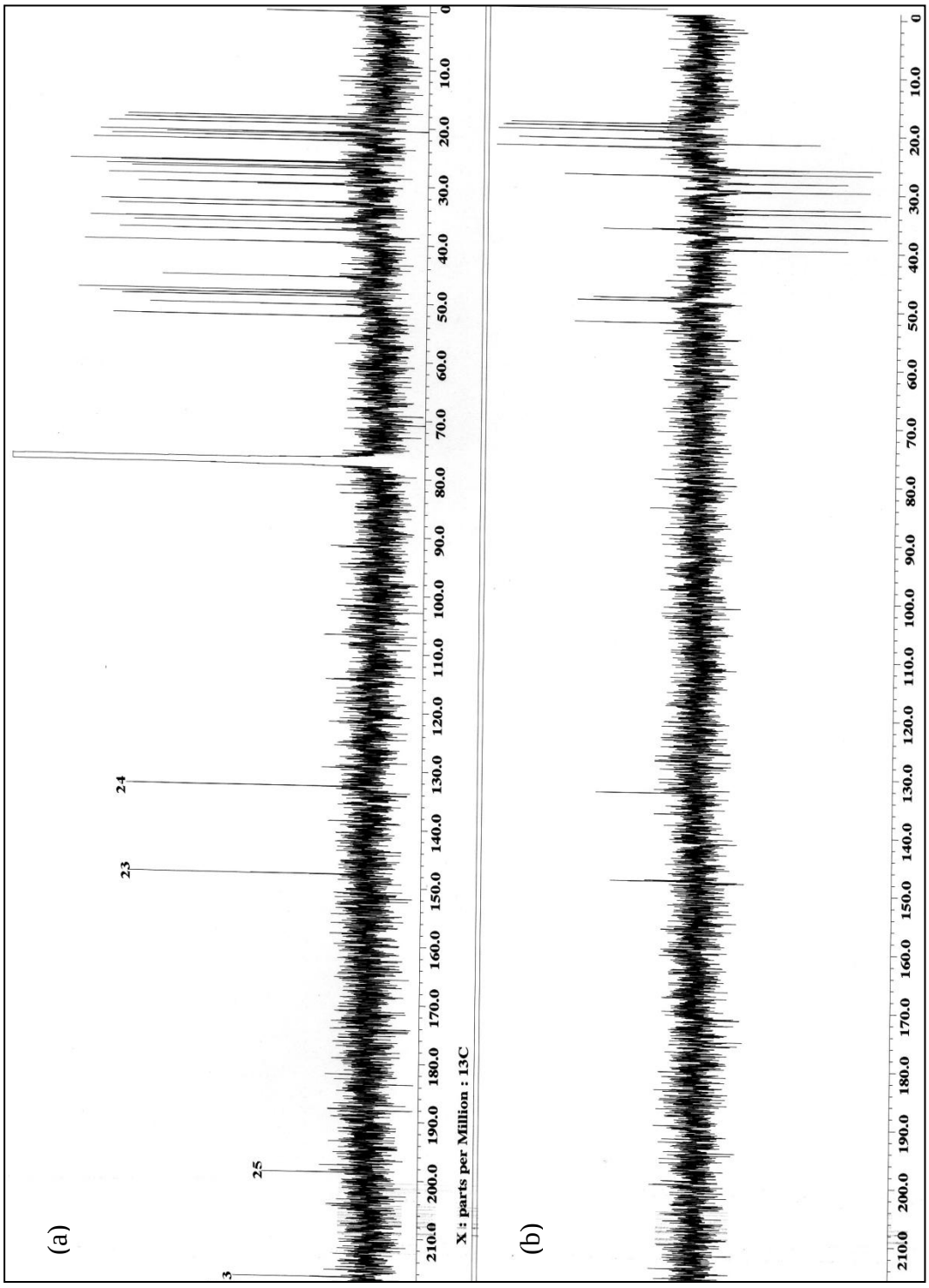


Figure 3.10: ^{13}C NMR (a) and DEPT-135 (b) spectra of compound B in $CDCl_3$ (400 MHz)

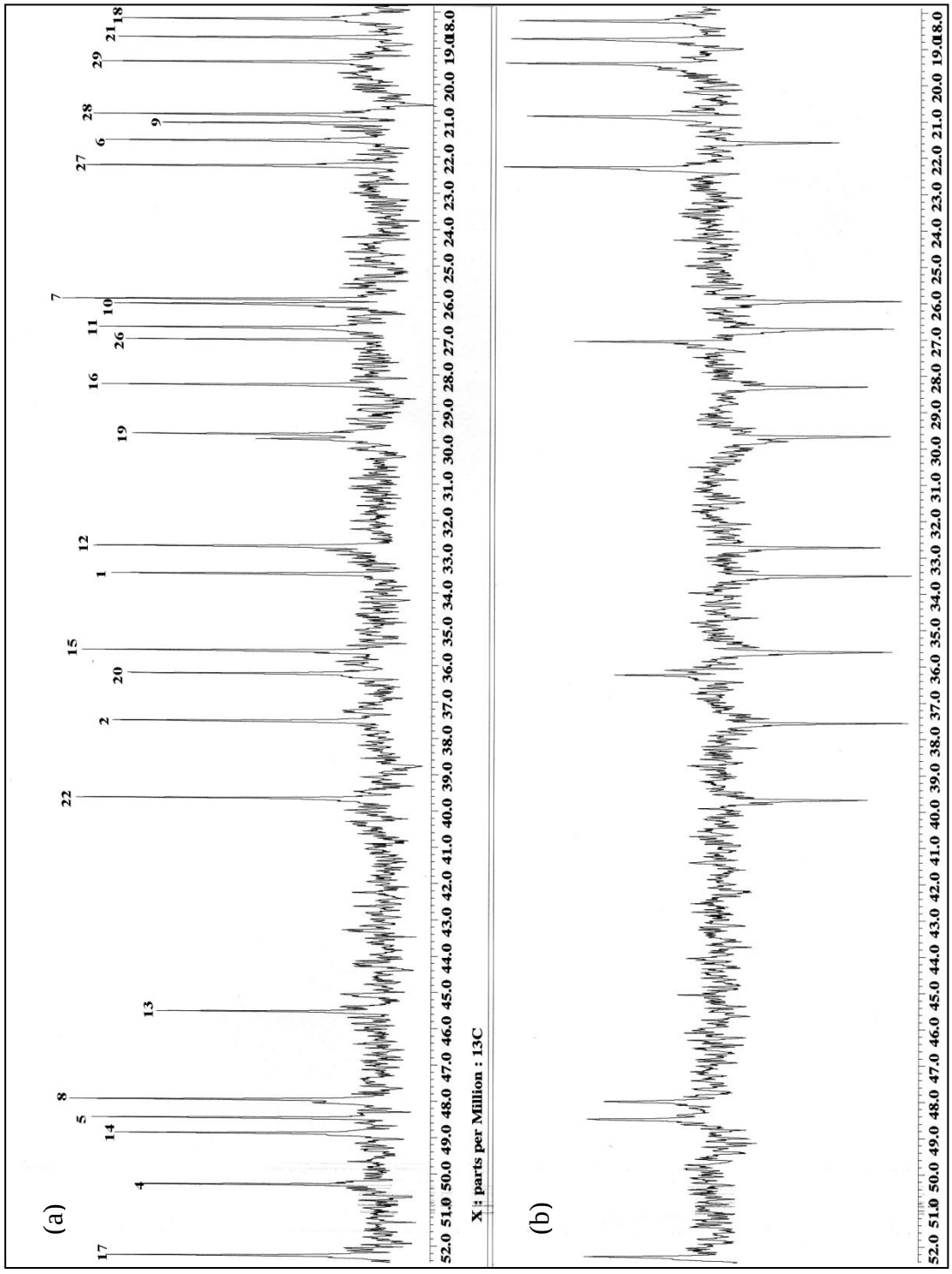
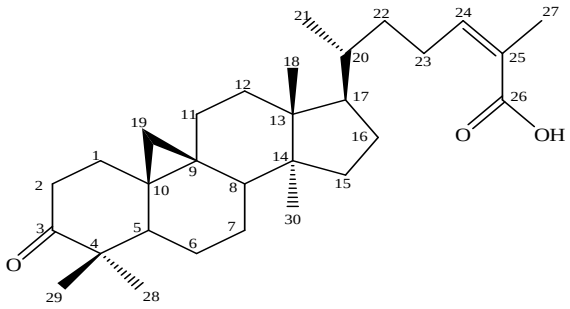


Figure 3.11: Expansion of ^{13}C NMR (a) and DEPT-135 (b) spectra of compound B in $CDCl_3$ (δ18 – 52 ppm)

Figure 3.12: HMBC spectrum of compound B in $CDCl_3$

Compound C: Schizandronic acid **201**



Compound C was isolated as colorless needle crystal with melting point 165-166^{o}C and $[\alpha]_D^{23.9}$ = +15.4^{o} (c 0.00052, CH_2Cl_2). The EI-MS spectral showed a molecular ion peak at m/z 454 corresponding to the molecular formula of $C_{30}H_{46}O_3$. UV spectrum showed an absorption band at 212 nm. IR spectrum showed absorption peak at 3393, 2934, 1707 cm^{-1} suggesting the presence of hydroxyl, alkyl and carbonyl group respectively.

The ^{1}H NMR spectral data in Figure 3.14 showed an upfield pair of doublet signal at δ 0.58 (1H, *d*, *J*= 5.0 Hz) and δ 0.79 (1H, *d*, *J*= 5.0 Hz) for geminal methylene group was characteristic of cycloartane triterpenes. Signals for six methyl group revealed at δ 0.91 (Me-30), δ 0.93 (Me-21), δ 1.00 (Me-18), δ 1.05 (Me- 29), δ 1.10 (Me-28), δ 1.85 (Me-27). A triplet at the downfield region of δ 6.90 (1H, *t*, J_1= 7.6 Hz, J_2= 7.6 Hz) discerned an olefinic proton (H-24). The methyl proton (H-27) neighboring with a double bond and carbonyl was shown by a singlet at δ 1.85 (3H, *s*).

The ^{13}C NMR spectrum in Figure 3.15 - 3.18 showed the presence of six methyl groups (C-18, C-21, C-27, C-28, C-29 and C-30), eleven methylenes (C-1, C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-19, C-22, C-23), five methines (C-5, C-8, C-17, C-20, C-24), eight quaternary carbon (C-3, C-4, C-9, C-10, C-13, C-14, C-25, C-26). Two out of

seven quaternary carbons (C-3 and C-26) were at the position of δ 216.9 and δ 173.1, indicating Compound C possesses two keto-groups.

In the HMBC spectrum (Figure 3.18 and 3.19), the downfield methyl proton H-27 (δ 1.85) has correlation to C-24 (δ 145.9), C-25 (δ 126.7), C-26 (δ 173.1). The olefinic proton H-24 (1H, *t*, J_1= 7.6 Hz, J_2= 7.6 Hz) was correlated to C-26. Hence, the position of double bond and keto-with a methyl group was confirmed through HMBC assignments. The carbonyl carbon (C-3) at the ring A was found to correlate with H-2 (2H, *m*) and H-28 (3H, *s*). The methyl proton H-18 (3H, *s*) at δ 1.00 correlated to C-12, C-13 and C-17 confirmed its position. The spectrum showed that another methyl proton H-30 (3H, *s*) which near to Me-18 has correlation with C-13, C-14 and C-15.

From the thorough analysis of the spectral data and comparison with the literature values[68], the identity of compound C was established as schizandronic acid **201**.

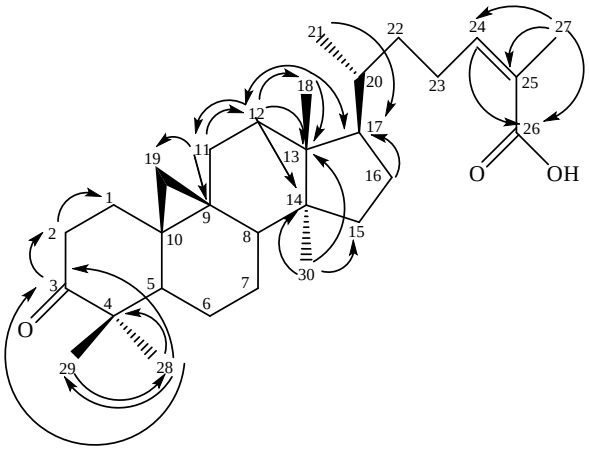


Figure 3.13: Selected HMBC Correlation of Compound C

Table 3.4: ^{1}H NMR, ^{13}C NMR, HMBC Data of Compound C in $CDCl_3$

Position	δ_H (ppm)	δc (ppm)	HMBC (H→C)
1	1.56 (1H, *m*)	33.5	-
	1.90 (1H, *m*)		
2	2.35 (1H, *m*)	37.6	1, 3
	2.70 (1H, *m*)		
3	-	216.9	-
4	-	50.3	-
5	1.66 (1H, *m*)	48.5	-
6	1.24 (1H, *m*)	28.2	-
	1.90 (1H, *m*)		
7	1.57 (1H, *m*)	21.6	-
	1.58 (1H, *m*)		
8	1.56 (1H, *m*)	48.0	-
9	-	21.1	-
10	-	26.0	-
11	1.18 (1H, *m*)	26.8	9,12,19
	2.08 (1H, *m*)		
12	1.64 (1H, *m*)	32.9	11, 13, 14, 18
	1.64 (1H, *m*)		
13	-	45.5	-
14	-	48.8	-
15	1.31 (1H, *m*)	35.6	-
	1.31 (1H, *m*)		
16	0.92 (1H, *m*)	29.7	17
	1.28 (1H, *m*)		
17	1.61 (1H, *m*)	52.3	21
18	1.00 (3H, *s*)	18.2	12, 13, 17
19	0.58 (1H, *d*, *J*= 5.0 Hz)	29.6	-
	0.79 (1H, *d*, *J*= 5.0 Hz)		
20	1.40 (1H, *m*)	36.0	-
21	0.93 (3H, *d*, *J*= 7.6 Hz)	18.2	17
22	1.54 (1H, *m*)	34.8	-
	1.18 (1H, *m*)		
23	2.10 (1H, *m*)	26.0	-
	2.22 (1H, *m*)		
24	6.90 (1H, *t*, J_1= 7.6 Hz, J_2= 7.6 Hz)	145.9	26
25	-	126.7	-
26	-	173.1	-
27	1.85 (3H, *s*)	12.1	24, 25, 26
28	1.10 (3H, *s*)	20.9	3, 4, 29
29	1.05 (3H, *s*)	22.3	4, 28
30	0.91 (3H, *s*)	19.4	13, 14, 15

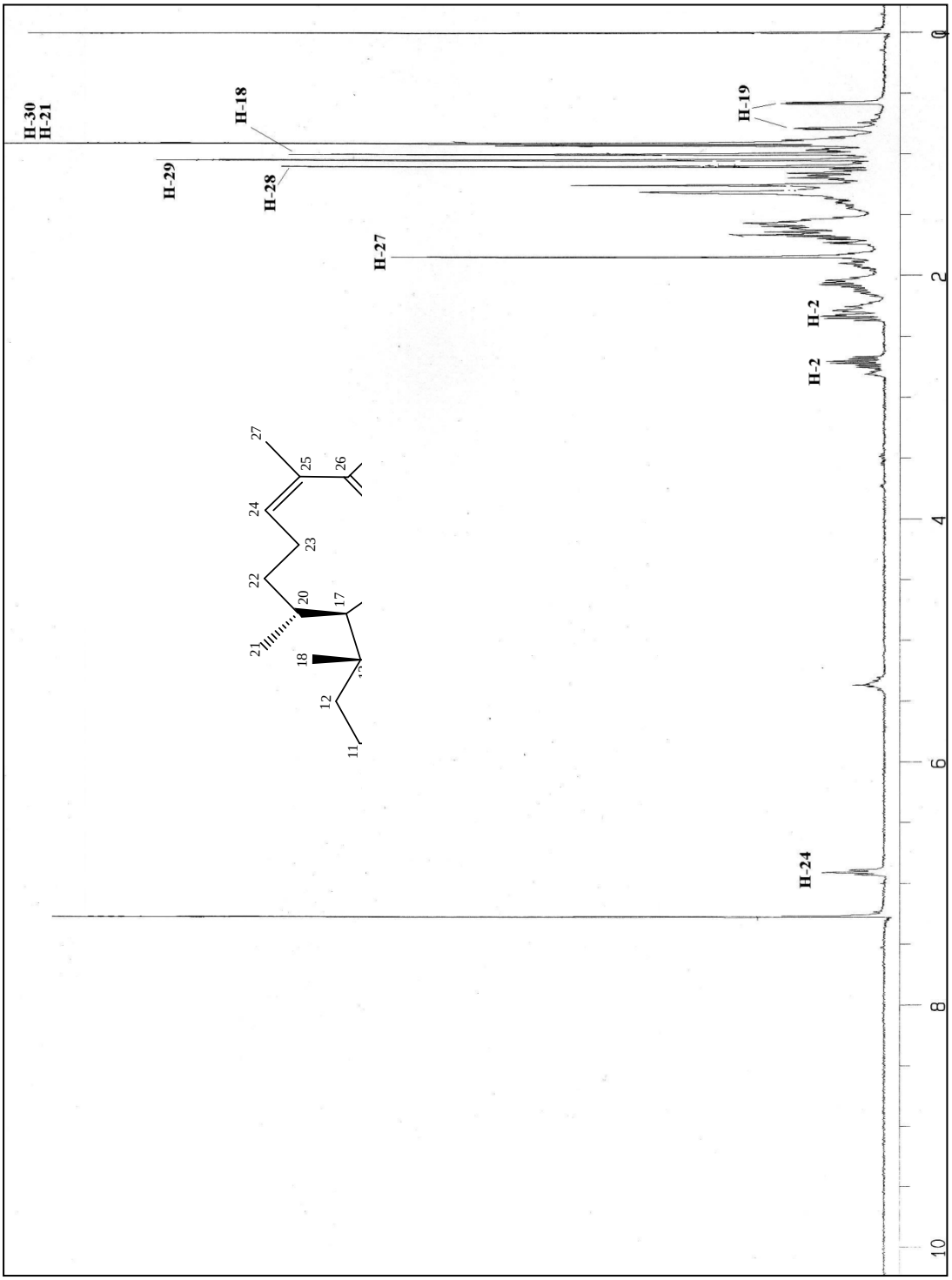


Figure 3.14: ^{1}H NMR spectrum of compound C in $CDCl_3$ (400 MHz)

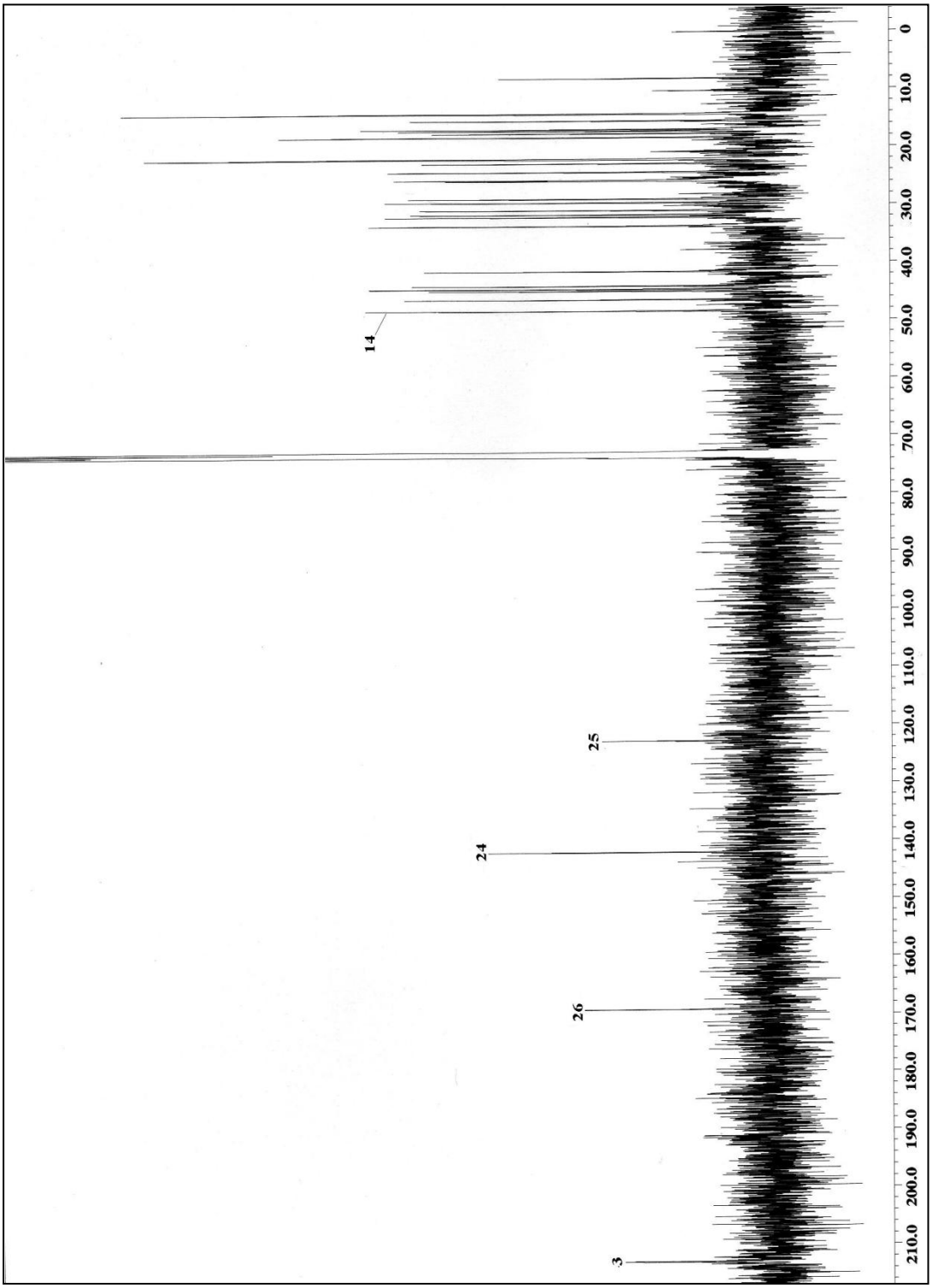


Figure 3.15: ^{13}C NMR spectrum of compound C in $CDCl_3$ (400MHz)

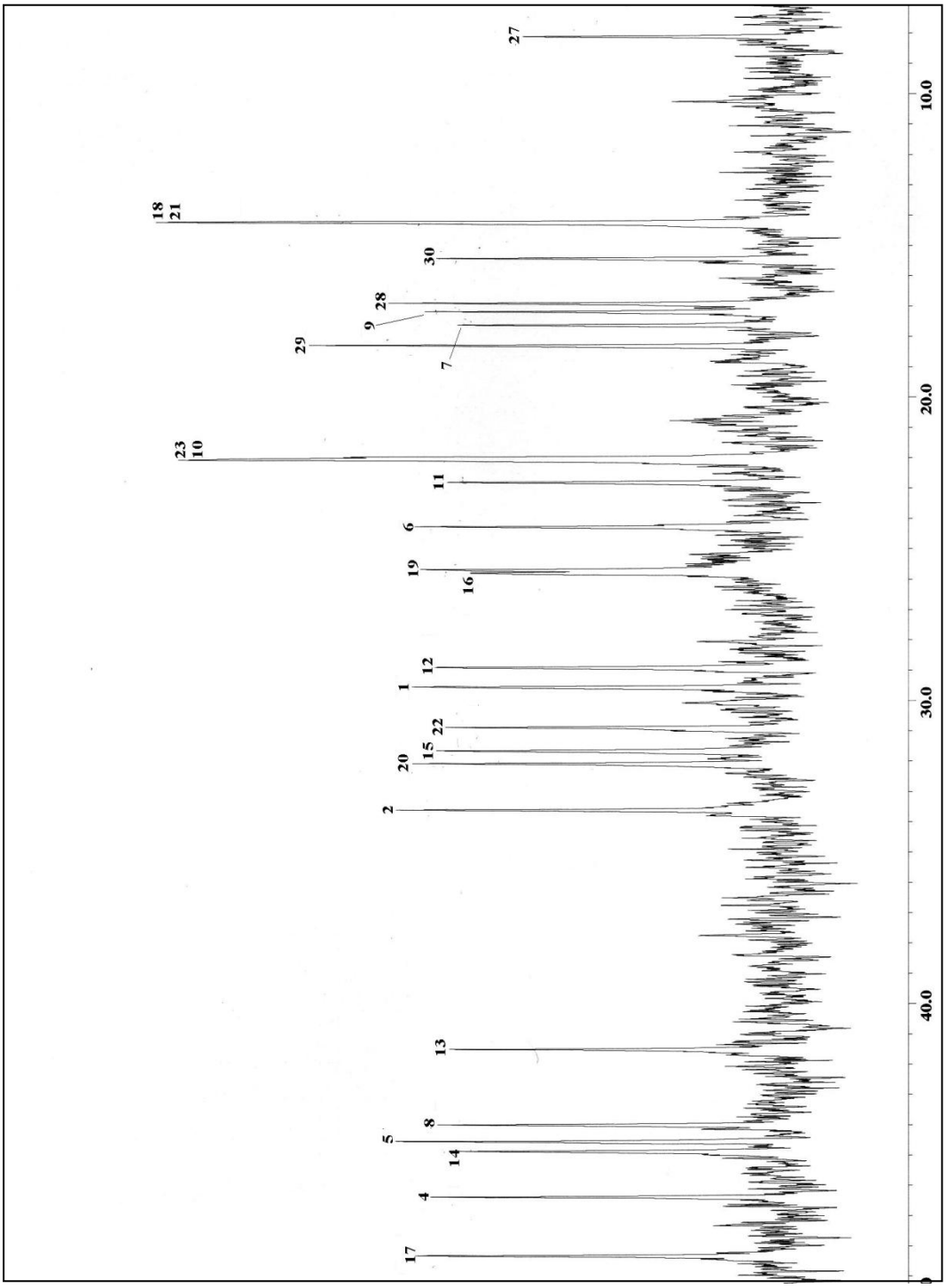


Figure 3.16: Expansion of ^{13}C NMR spectrum of compound C in $CDCl_3$ (δ5 – 50 ppm)

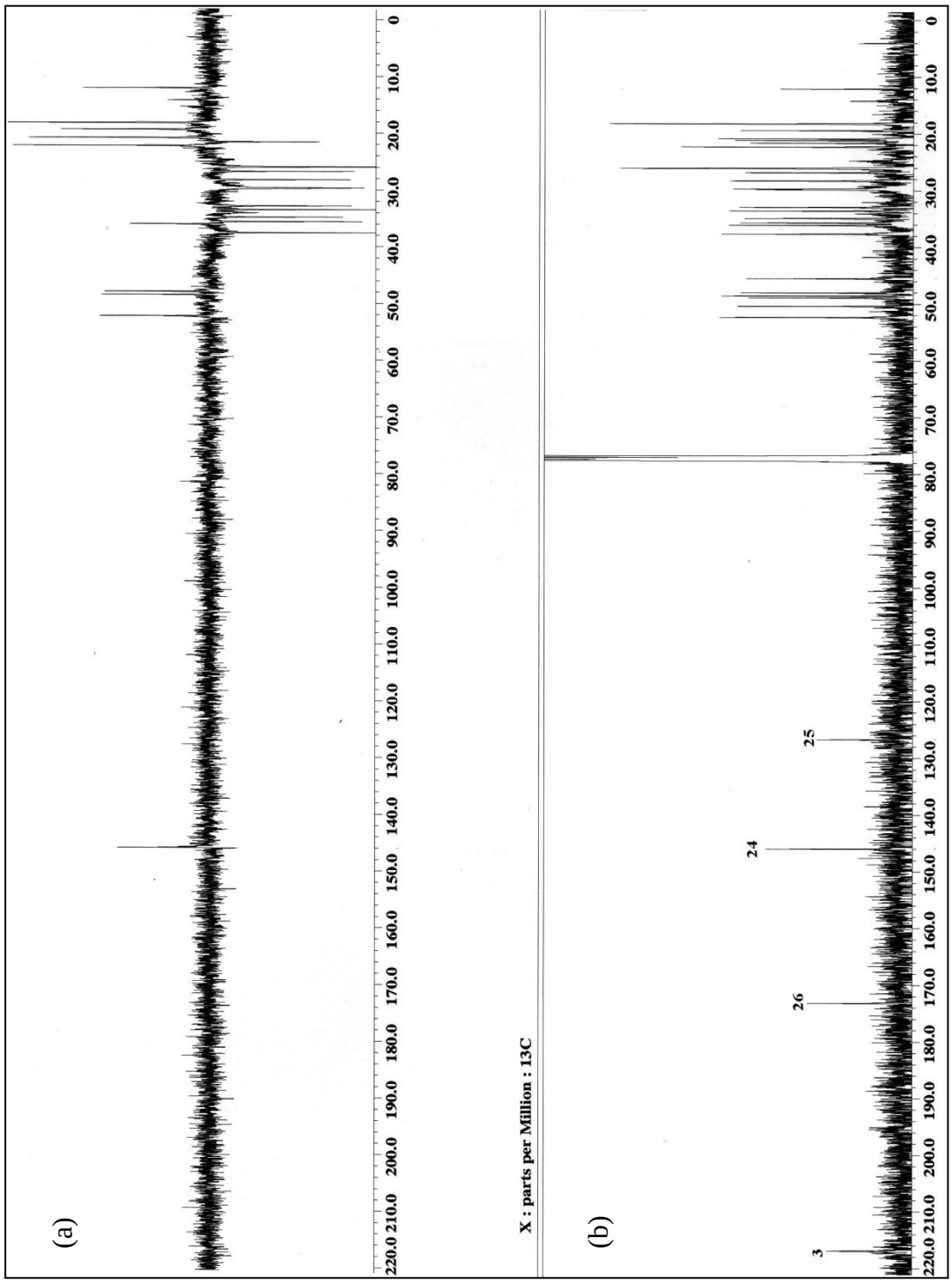


Figure 3.17: DEPT-135 (a) and ^{13}C NMR (b) spectra of compound C in $CDCl_3$

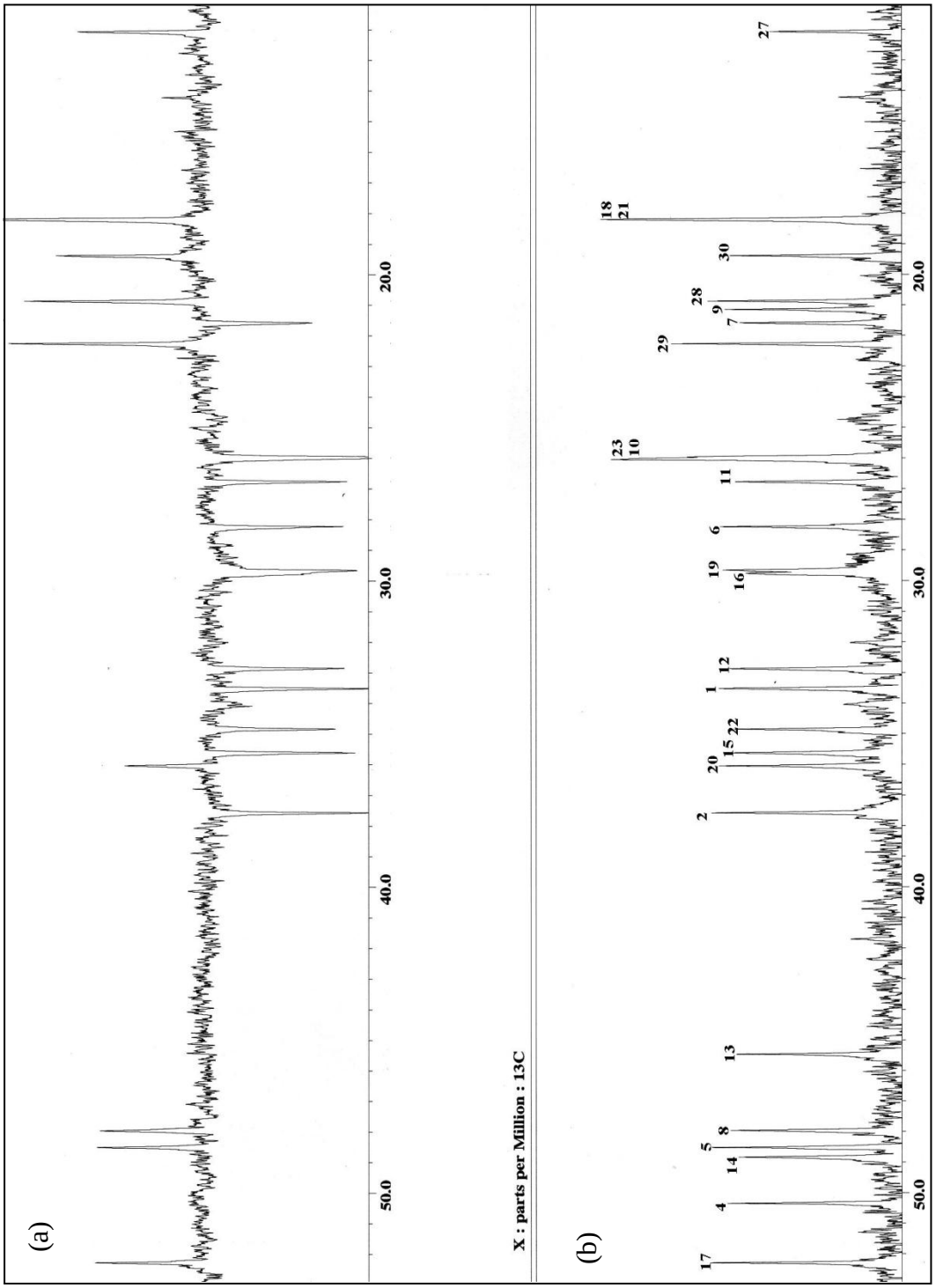


Figure 3.18: Expansion of DEPT-135 (a) and ^{13}C NMR (b) spectrum of compound C in $CDCl_3$ (δ11 – 53 ppm)

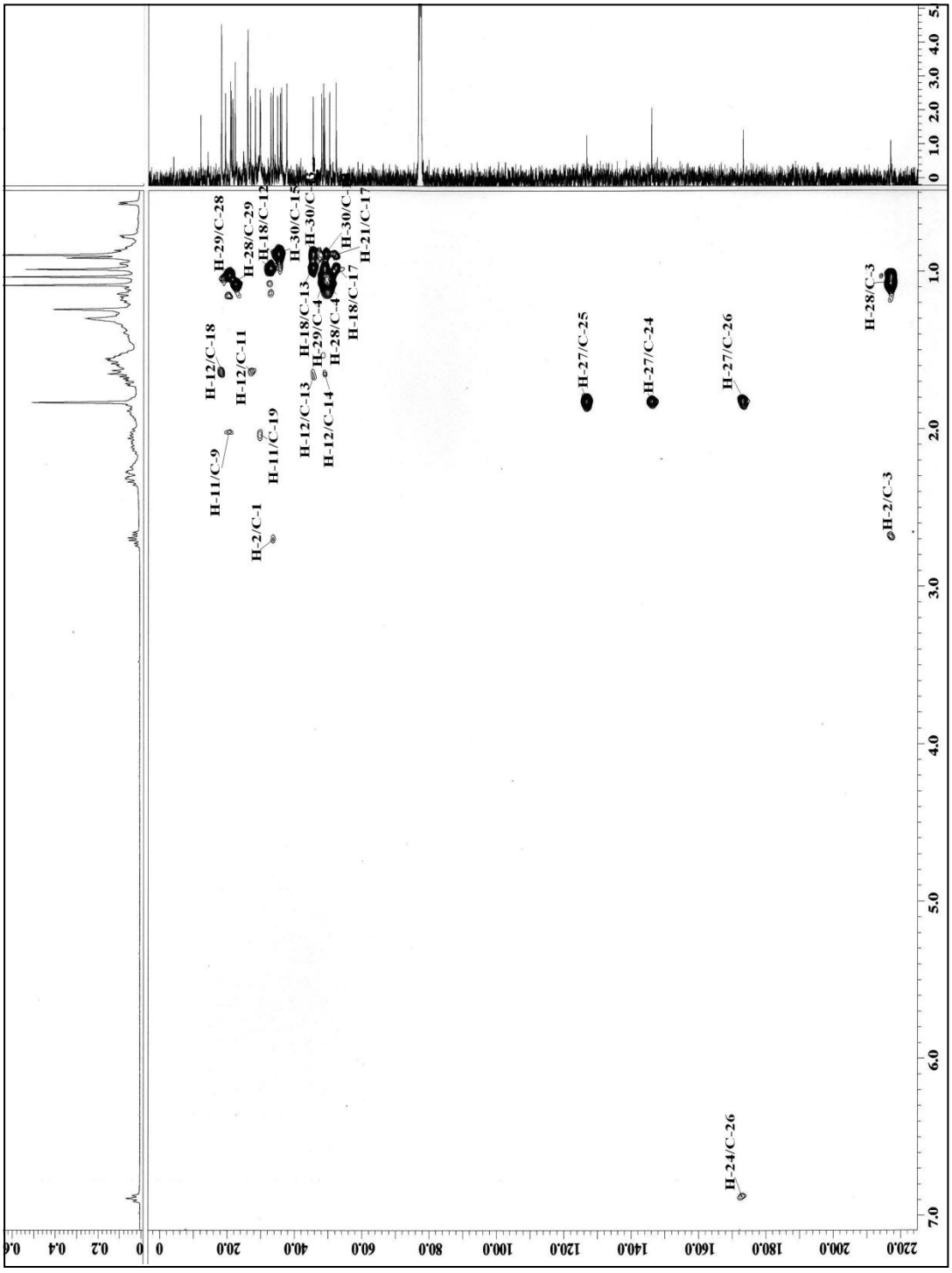
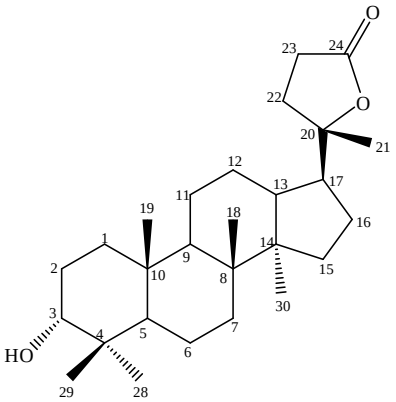


Figure 3.19: HMBC spectrum of compound C in $CDCl_3$

Compound D: Cabraleahydroxylactone **202**



Compound D was isolated as a colorless crystal with a molecular formula $C_{27}H_{44}O_3$ based on the molecular ion peak in EI-MS was showed at m/z 416. The optical rotation is $[\alpha]_D^{23.6}$ = +13.0^o (c 0.00023, CH_2Cl_2). Its melting point was 243-244^{o}C. The assignments of all carbons and protons were achieved by ^{1}H and ^{13}C NMR and comparison of the literature data[71]. It was then further confirmed by x-ray analysis. The IR spectrum revealed absorption peak at 3550, 2900, 1760 cm^{-1} suggesting the presence of hydroxyl, alkyl and carbonyl group respectively.

The ^{1}H NMR spectral data in Figure 3.21 revealed signals for six methyl groups at δ 0.83 (Me-29), δ 0.84 (Me-19), δ 0.89 (Me-30), δ 0.93 (Me-28), δ 0.95 (Me-18) and δ 1.35 (Me-21). The methyl signal δ 1.35 (3H, *s*) (Me-21) was at the most downfield compared to other methyl signals. This was due to the oxygen attached on the neighboring carbon (C-20). A deshielded broad singlet, most probably belonged to H-3, appeared at δ 3.39 (H-3).

The ^{13}C NMR spectrum in Figure 3.22 and 3.23 showed 27 carbon resonances comprises of six methyls, ten methylenes, five methines including one oxymethine and six quaternary carbons. The signals at δ 15.6, δ 16.1, δ 25.4, δ 28.4, δ 22.2 and δ 16.4 assigned for C-18, C-19, C-21, C-28, C-29 and C-30 respectively. Besides, the signal at

δ 177.0 indicated the carbonyl carbon, C-24 and the signal at δ 76.3 showed the existence of hydroxyl carbon, C-3.

Compound D was confirmed as a dammarane triterpenoid named cabraleahydroxylactone **202** by x- ray analysis and the comparison of aforementioned ^{1}H and ^{13}C spectroscopic data with the reported literature values[69].

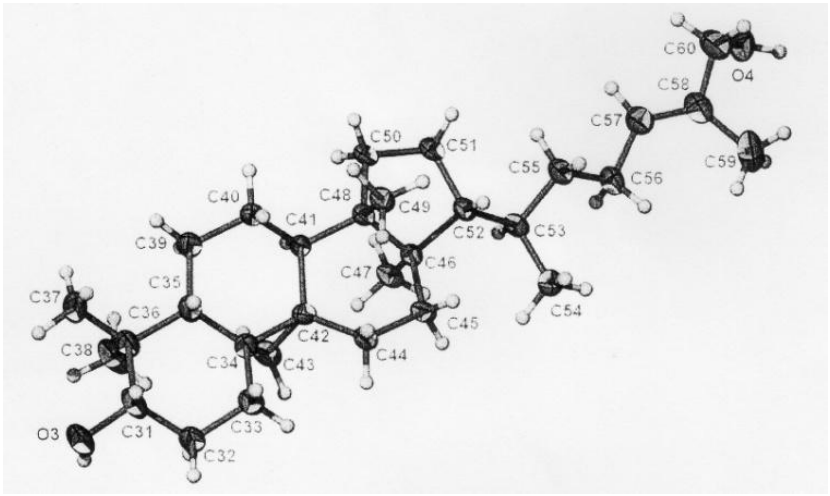


Figure 3.20: ORTEP Diagram of Compound D

Table 3.5: ^{1}H NMR and ^{13}C NMR Data of Compound D in $CDCl_3$

Position	δH (ppm)	δc (ppm)
1	1.26 (1H, *m*)	33.7
	1.35 (1H, *m*)	
2	1.55 (1H, *m*)	25.4
	1.91 (1H, *m*)	
3	3.39 (1H, *s*)	76.3
4	-	37.7
5	1.26 (1H, *m*)	49.4
6	1.41 (1H, *m*)	18.3
7	1.23 (1H, *m*)	35.2
	1.56 (1H, *m*)	
8	-	50.4
9	1.44 (1H, *m*)	50.4
10	-	37.3
11	1.19 (1H, *m*)	26.9
	1.72 (1H, *m*)	
12	1.20 (1H, *m*)	21.3
	1.55 (1H, *m*)	
13	1.71 (1H, *m*)	43.2
14	-	40.6
15	1.11 (1H, *m*)	31.2
	1.47 (1H, *m*)	
16	1.26 (1H, *m*)	25.1
	1.81 (1H, *m*)	
17	1.97 (1H, *m*)	49.6
18	0.95 (3H, *s*)	15.6
19	0.84 (3H, *s*)	16.1
20	-	90.4
21	1.35 (3H, *s*)	25.4
22	1.94 (1H, *m*)	31.2
	2.12 (1H, *m*)	
23	2.53 (1H, *ddd*, J_1= 4.6 Hz, J_2= 10.0 Hz, J_3= 17.2 Hz)	29.3
	2.62 (1H, *ddd*, J_1= 9.5 Hz, J_2= 10.3 Hz, J_3= 18.1 Hz)	
24	-	177.0
25	-	
26	-	
27	-	
28	0.93 (3H, *s*)	28.4
29	0.83 (3H, *s*)	22.2
30	0.89 (3H, *s*)	16.4

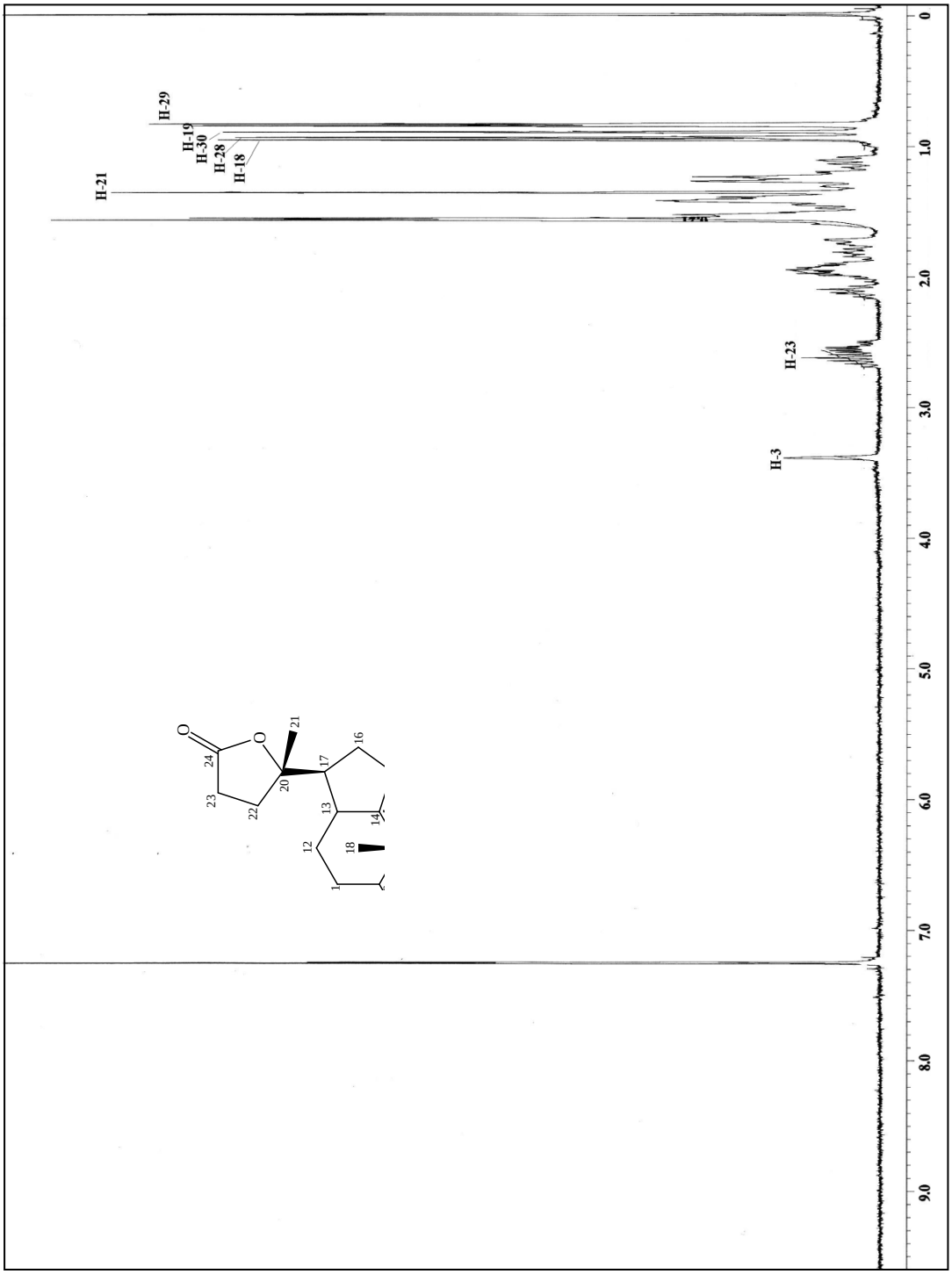


Figure 3.21: ^{1}H NMR spectrum of compound D in $CDCl_3$ (400 MHz)

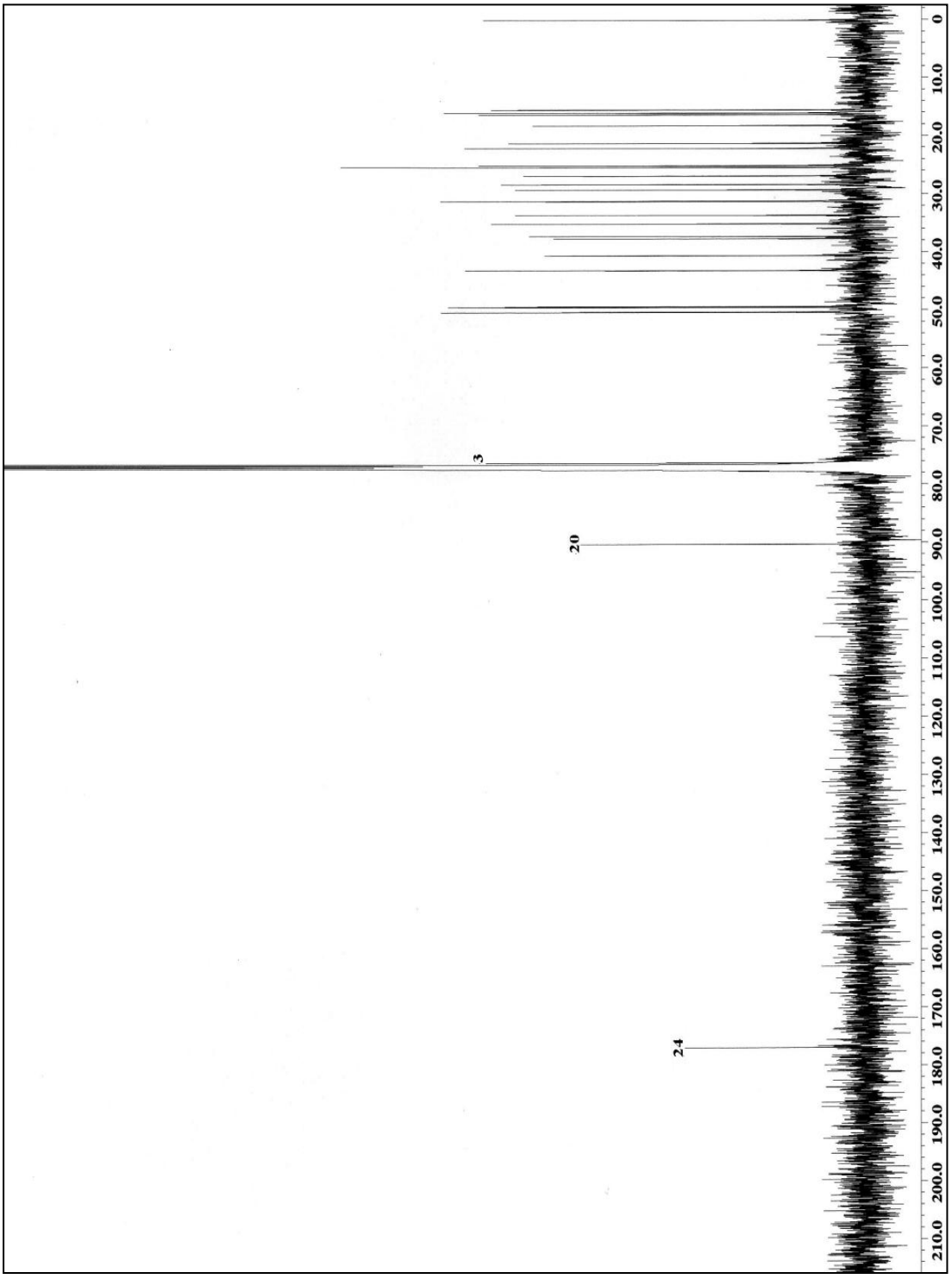


Figure 3.22: ^{13}C NMR spectrum of compound D in $CDCl_3$ (400 MHz)

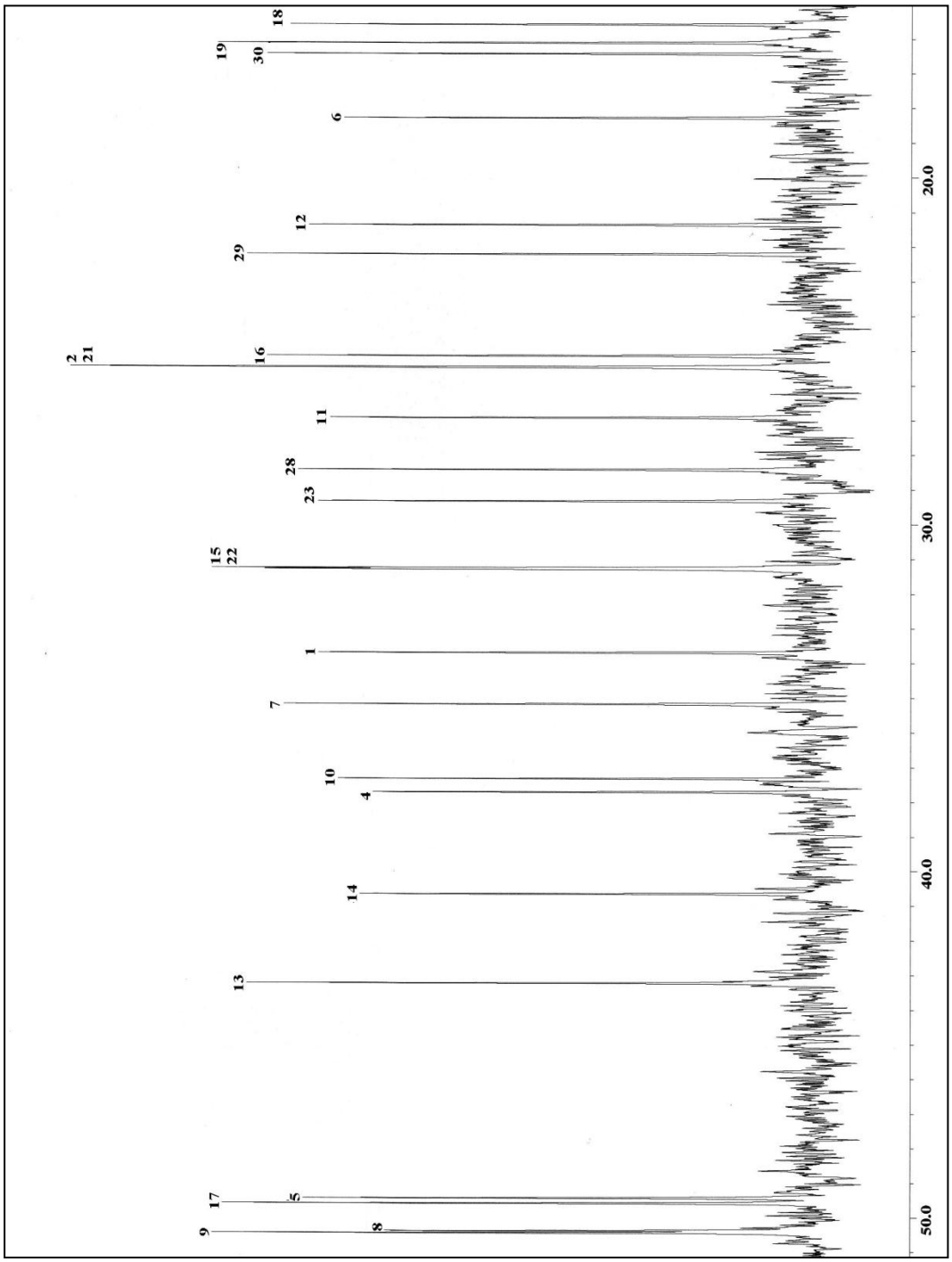
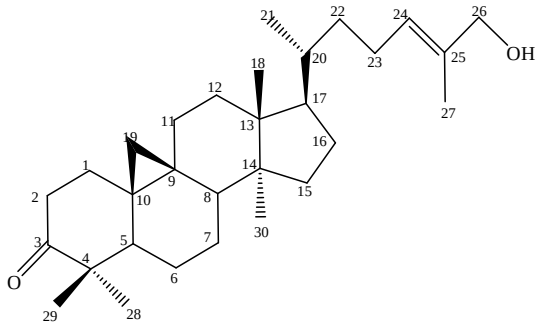


Figure 3.23: Expansion of ^{13}C NMR spectrum of compound D in $CDCl_3$ ($\delta 10 - 55$ ppm)

Compound E: 24(*E*)-cycloart-24-ene-26-ol-3-one **203**



Compound E, was obtained as colorless amorphous solid, $[\alpha]_D^{27.7}$ +29.7^o (c 0.00209, CH_2Cl_2). The ESI-TOF spectrum showed an $[M+Na]^+$ pseudomolecular ion peak at m/z 463.3813 (calcd 463.3678) which corresponded to the molecular formula of $C_{30}H_{48}O_2$. The IR spectrum showed absorption peaks at 3445, 2942, 1705cm^{-1} suggesting the presence of hydroxyl, alkyl and carbonyl groups respectively.

The cycloartane nature of compound E was deduced by the appearance of a pair of very shielded doublets at δ 0.58 and 0.79 (J = 4.4 Hz). The olefinic proton (H-24) resonated as a broad triplet at δ 5.36 (J= 7.1 Hz).). In addition, the oxymethylene protons which were attached to C-26 appeared as a broad singlet at δ 3.94. The ^{13}C NMR and DEPT spectra showed peaks corresponding to thirty carbons; six methyl, twelve methylene, five methine, and seven quaternary carbons. The peak at δ 216.7 is assignable to the ketonic carbonyl, C-3. In addition, the signals of the double bond carbons (C-24, C-25) appeared at δ 127.0 and δ 134.3 respectively. The methylene carbon C-26 of the side chain resonated downfield at δ 69.0 since it is attached to a hydroxyl group.

The HMBC spectrum showed correlations of H-24 with C-26 and C-27, H-26 with C-24, C-25 and C-27 thus confirming the position of the double bond at C-24 and C-25 respectively. Furthermore, the location of the hydroxyl group on C-26 was also

established by the HMBC correlations of H_2-26 with C-24, 25 and C-27. Thorough analysis of the DEPT, COSY, HSQC and HMBC spectra allowed the complete assignment of all protons and carbons (Table 1).

The NOESY experiment showed a correlation between H-24 and H_2-26 thus suggesting that the C-24-C-25 double bond assumes an E-configuration. Therefore, compound E was elucidated as 24(*E*)-cycloart-24-ene-26-ol-3-one **203**.

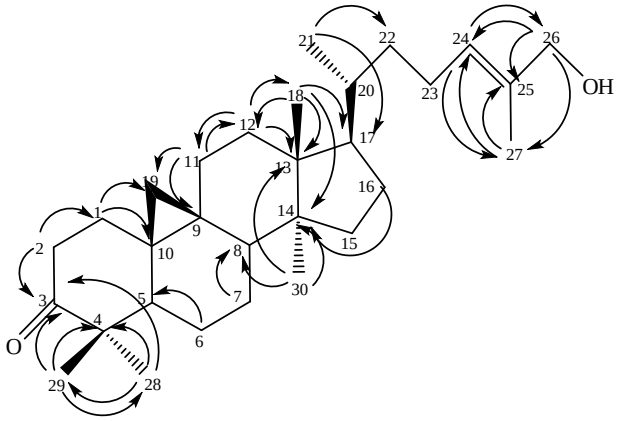


Figure 3.24: HMBC correlation of Compound E

Table 3.6: ^{1}H NMR, ^{13}C NMR and HMBC Data of Compound E in $CDCl_3$

Position	δ_H (ppm)	δ_C (ppm)	HMBC (H→C)
1	1.48 (1H, *m*)	33.4	10, 19
	1.78 (IH, *m*)		
2	2.30 (1H, *ddd*, J_1=4.1 Hz,	37.4	1, 3
	J_2 =8.2 Hz, J_3 = 14.4 Hz)		
	2.70 (1H, *dt*, J_1= 6.4 Hz,		
	J_2= 14.2 Hz)		
3	-	216.7	-
4	-	50.2	-
5	1.52 (1H, *m*)	48.4	-
6	1.50 (2H, *m*)	21.5	5
7	1.22 (1H, *m*)	28.1	8
	1.84 (1H, *m*)		
8	1.64 (1H, *m*)	47.8	-
9	-	21.1	-
10	-	25.9	-
11	1.10 (1H, *m*)	26.7	8, 9, 12, 13, 19
	1.98 (1H, *m*)		
12	1.60 (2H, *m*)	32.8	11, 13, 14, 17, 18,
13	-	45.3	-
14	-	48.7	-
15	1.02 (2H, *m*)	35.9	-
16	1.86 (1H, *m*)	24.5	14
	2.02 (1H, *m*)		
17	-	52.2	-
18	1.00 (3H, *s*)	18.1	12, 13, 14, 17
19	0.58 (1H, *d*, *J*= 4.26 Hz)	29.5	1, 9, 10
	0.79 (1H, *d*, *J*= 4.26 Hz)		
20	1.02 (1H, *m*)	35.9	-
21	0.91 (3H, *d*, *J*= 6.1Hz)	18.2	17, 22
22	1.24 (2H, *m*)	35.5	-
23	1.08 (1H, *m*)	25.8	-
	1.30 (1H, *m*)		
24	5.36 (1H, *t*, J_1= 7.1 Hz,	127.0	26, 27
	J_2= 14.1 Hz)		
25	-	134.3	-
26	3.94 (2H, *s*)	69.0	24, 25, 27
27	1.68 (3H, *s*)	13.6	24, 26
28	1.05 (3H, *s*)	22.2	3, 4
29	1.10 (3H, *s*)	20.7	3, 4
30	0.91 (3H, *s*)	19.3	8, 13, 14

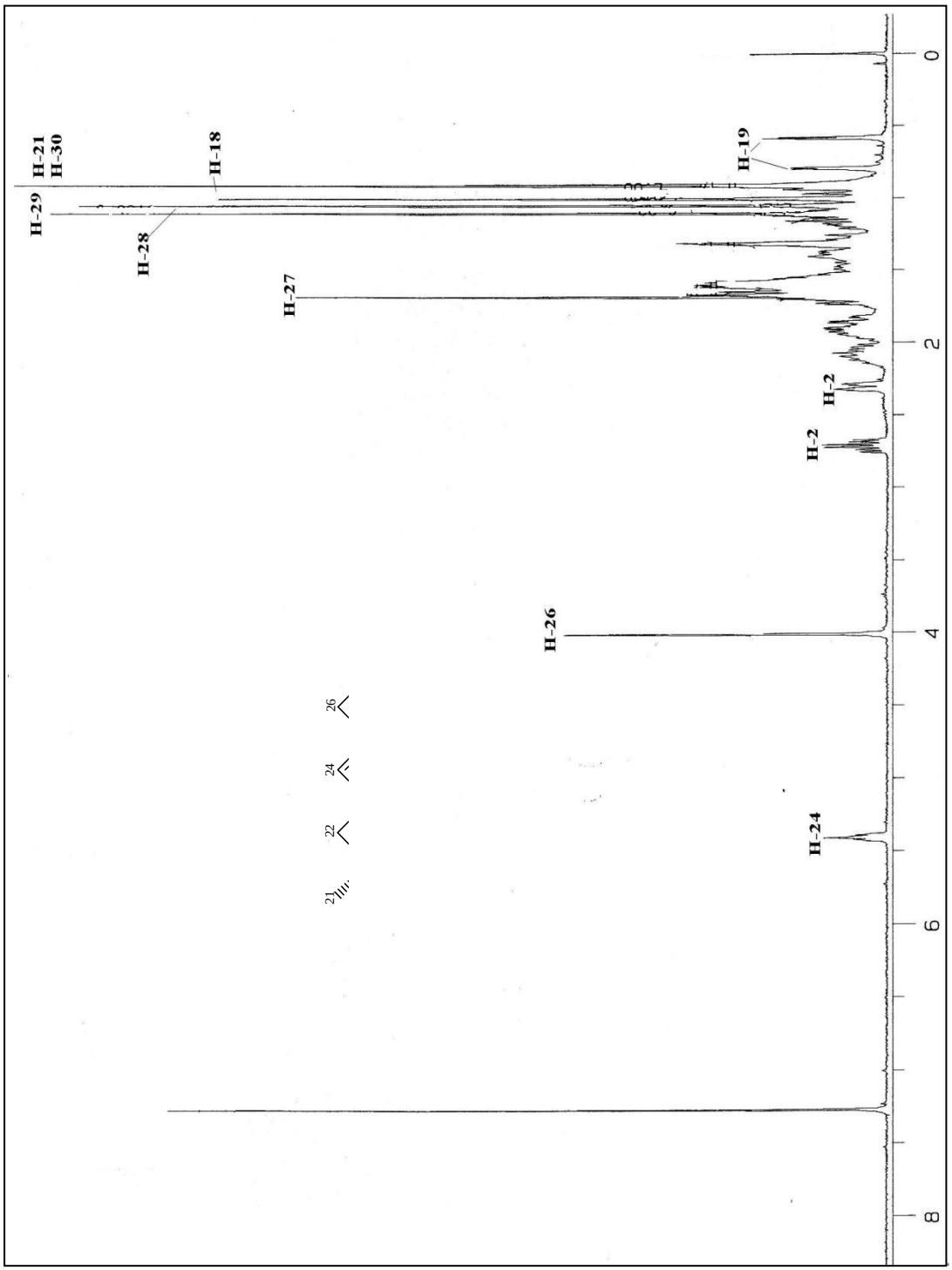


Figure 3.25: ^{1}H NMR spectrum of compound E in $CDCl_3$ (400 MHz)

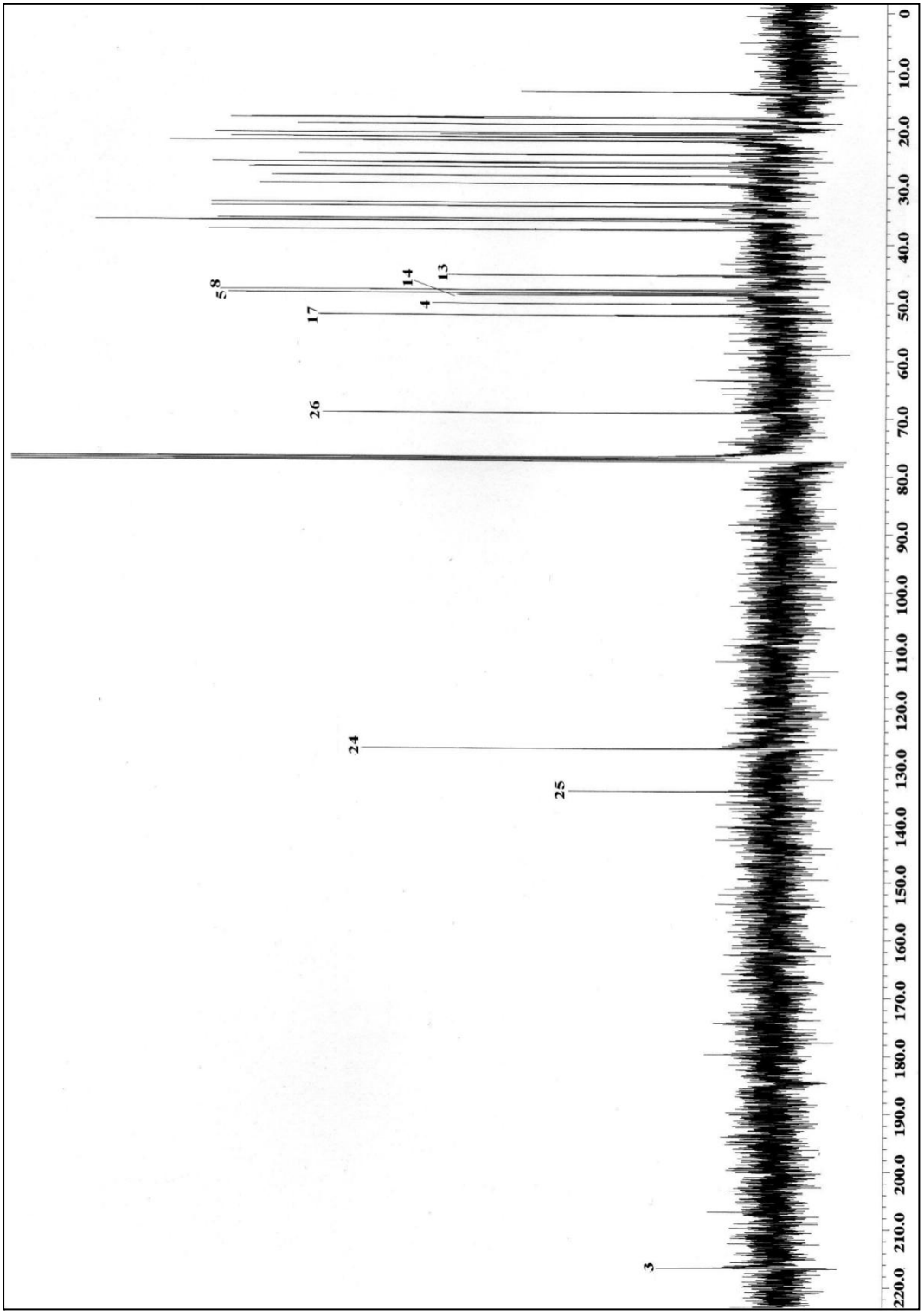


Figure 3.26: ^{13}C NMR spectrum of compound E in $CDCl_3$ (400 Hz)

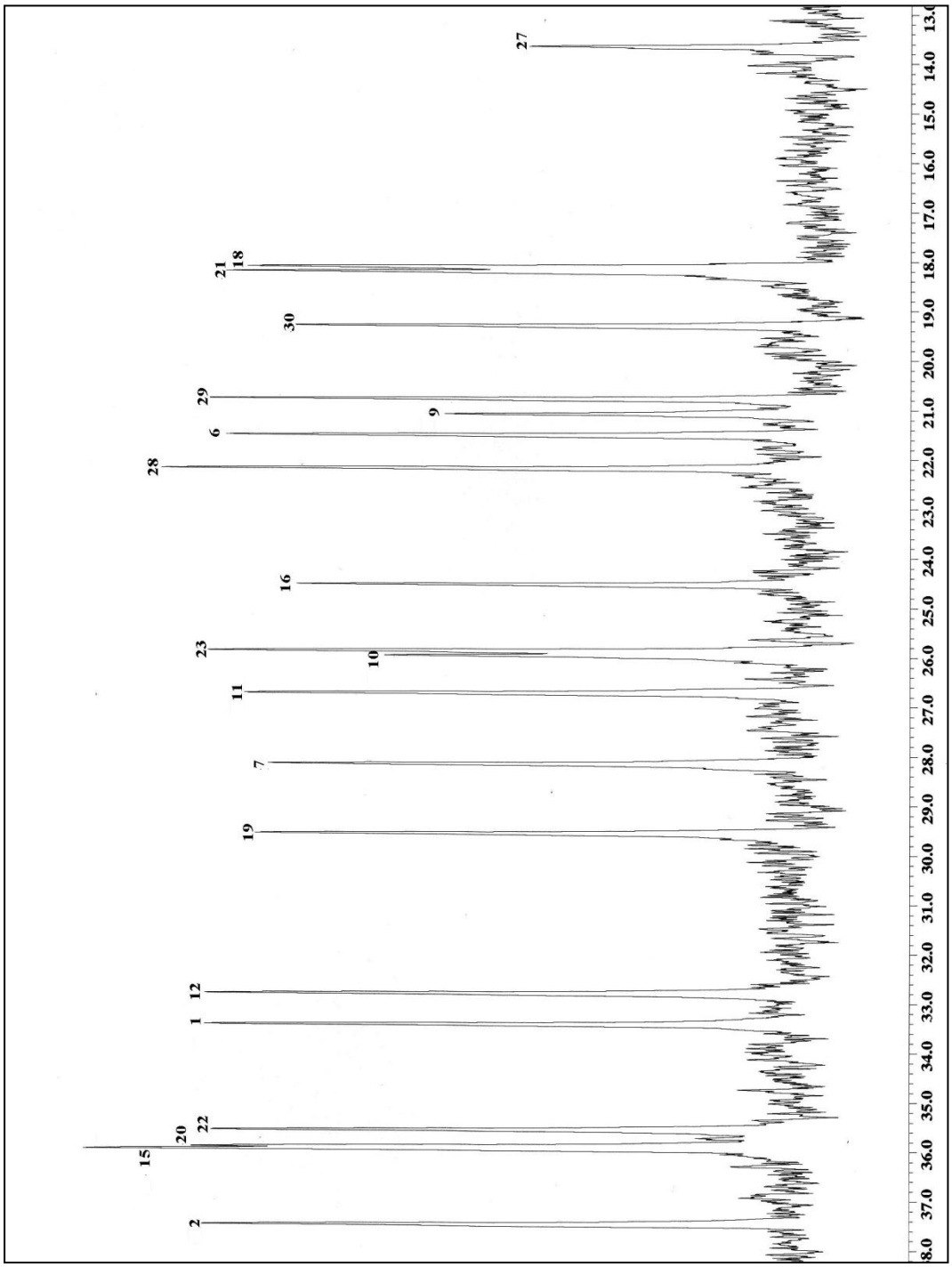


Figure 3.27: Expansion of ^{13}C NMR spectrum of compound E in $CDCl_3$ (δ 13 – 28 ppm)

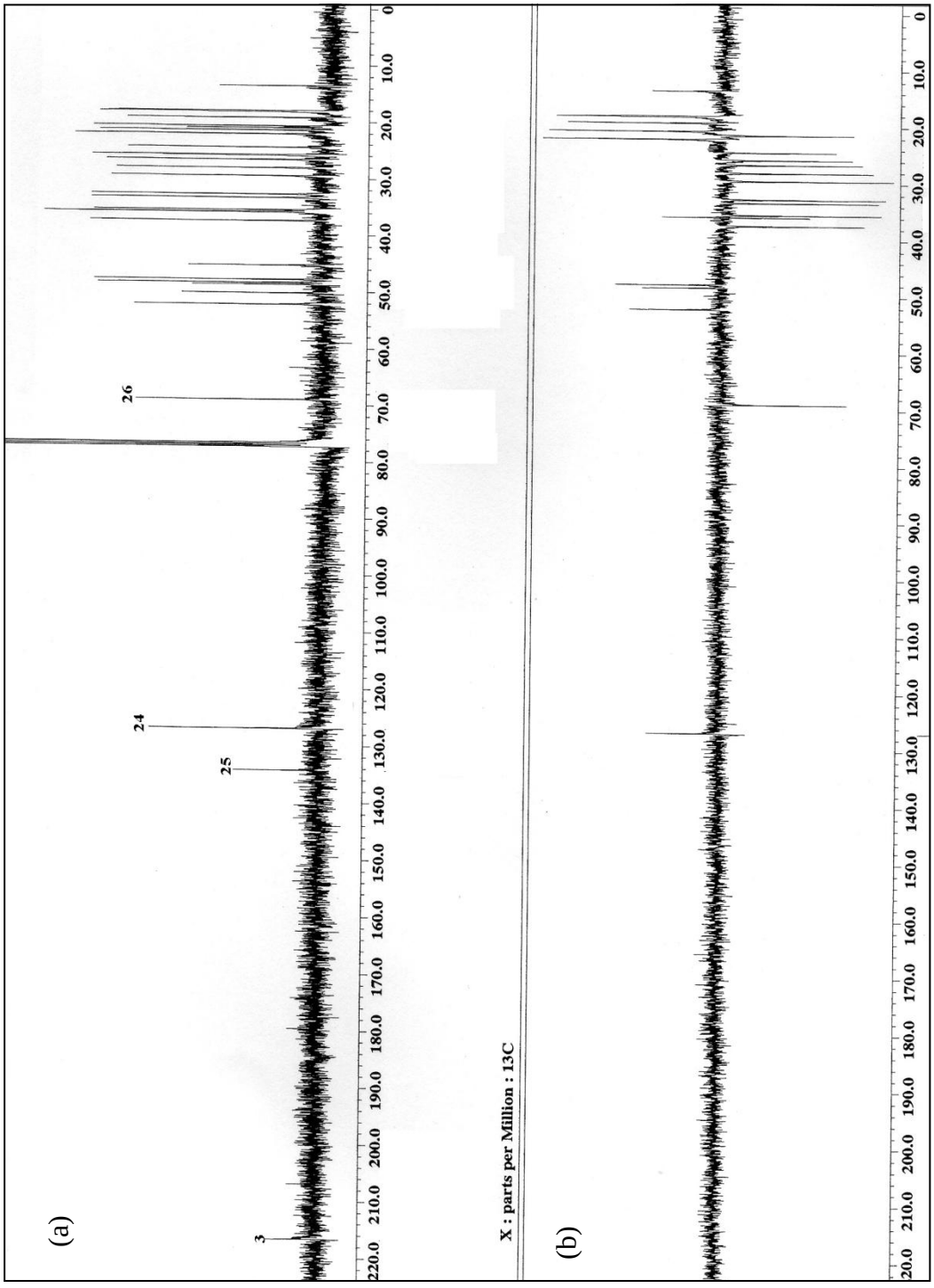


Figure 3.28: ^{13}C NMR (a) and DEPT-135 (b) spectra of compound E in $CDCl_3$ (400 Hz)

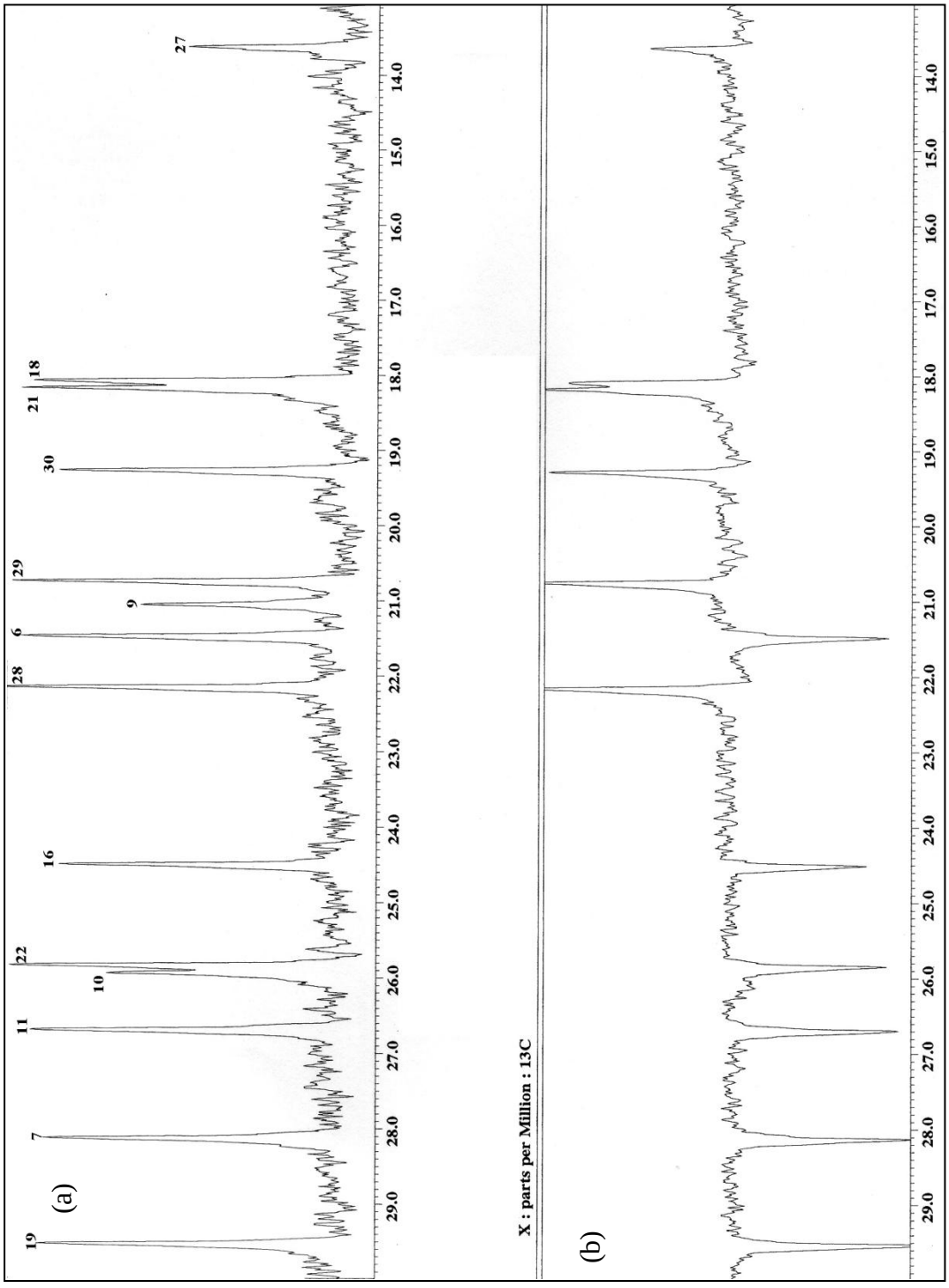


Figure 3.29: Expansion of ^{13}C NMR (a) and DEPT-135 (b) spectra of compound E in $CDCl_3$ (δ13 - 30 ppm)

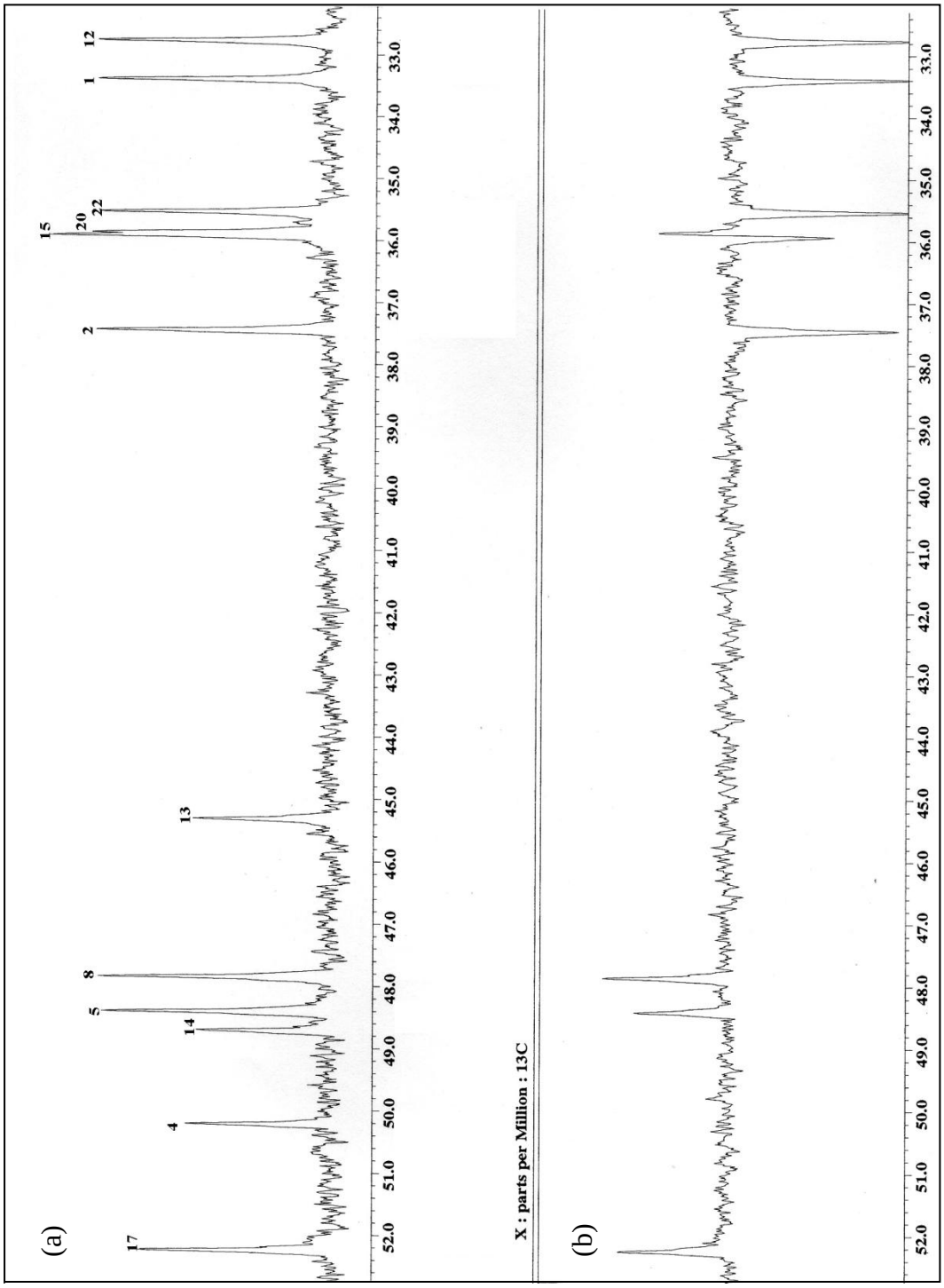


Figure 3.30: Expansion of ^{13}C NMR (a) and DEPT-135 (b) spectra of compound E in $CDCl_3$ (δ30 – 55 ppm)

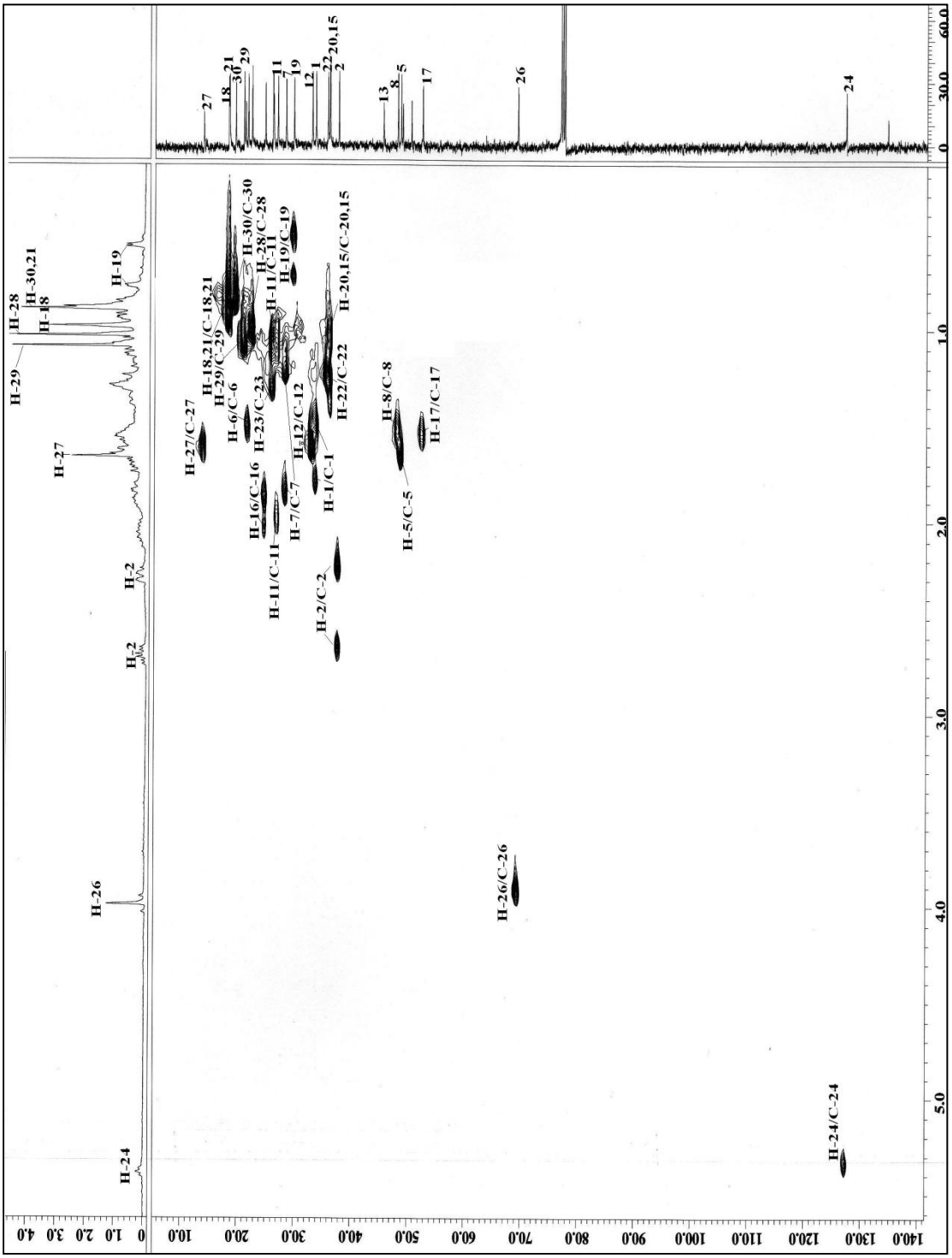


Figure 3.31: HSQC spectrum of compound E in $CDCl_3$

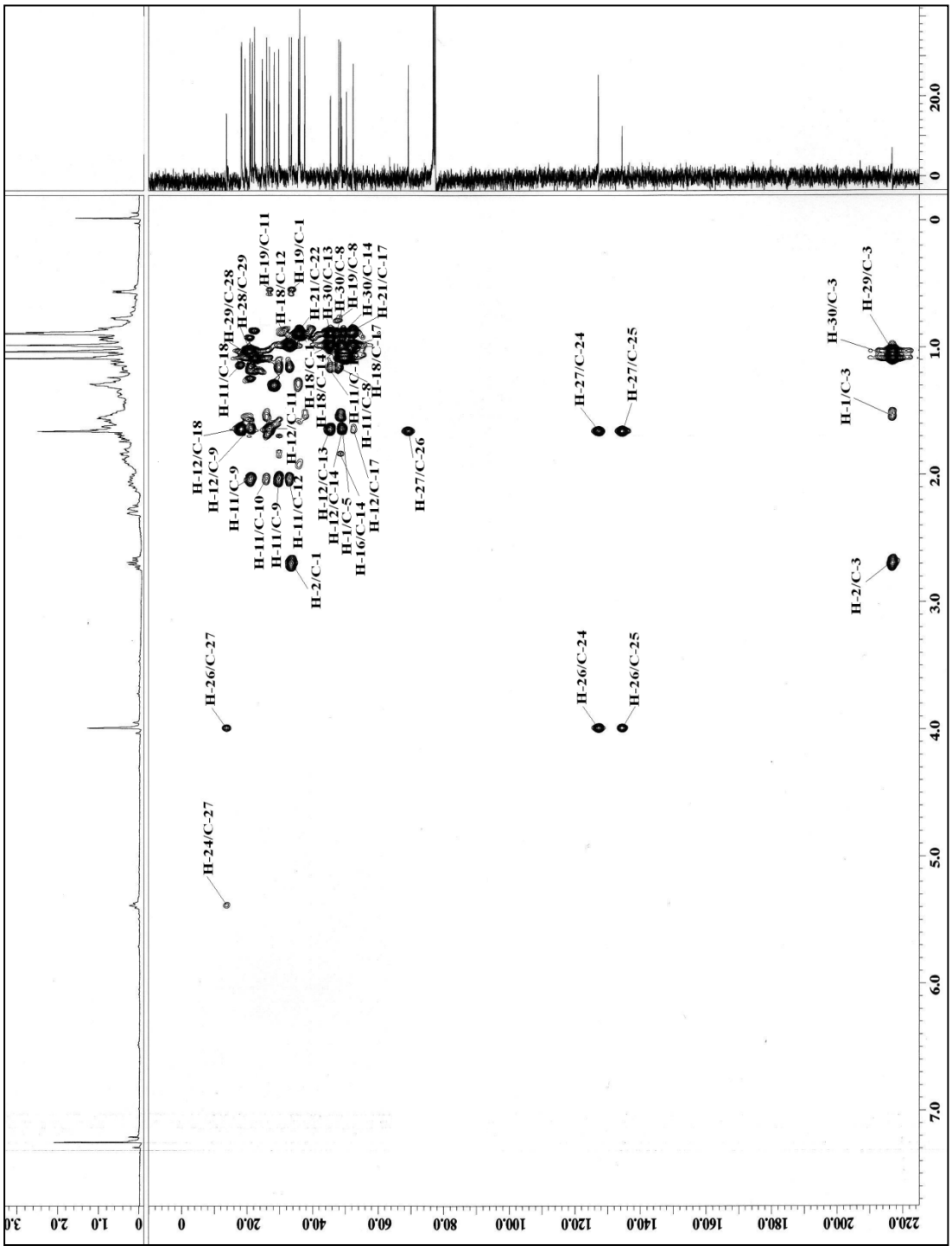
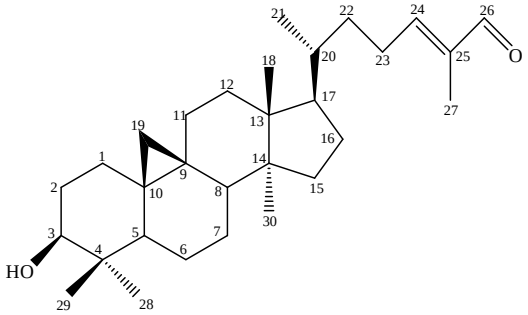


Figure 3.32: HMBC spectrum of compound E in $CDCl_3$

Compound F: 24(*E*)-3*β*-hydroxycycloart-24-ene-26-al **204**



Compound F was isolated as a pale yellow gel with molecular formula $C_{30}H_{48}O_2$ (EIMS) as the existence of a molecular peak at m/z 440 in EI-MS spectrum. The optical rotation is $[\alpha]_D^{24.3}$ = +33.1° (c 0.0016, CH_2Cl_2). An absorption band at 220 nm was shown in the UV spectrum. The IR spectrum showed absorption peaks at 3429, 2937, 1689 cm^{-1} suggesting the presence of hydroxyl, alkyl and carbonyl group respectively.

The ^{1}H NMR spectrum of compound F in Figure 3.34 showed the presence of six methyls; δ 0.81 (Me- 28), δ 0.90 (Me- 30), δ 0.93 (Me- 21), δ 0.97 (Me- 29), δ 0.97 (Me- 18) and δ 1.75 (Me- 27). The doublet at δ 0.93 (3H, *d*, *J*= 2.2 Hz) indicated it was a secondary methyl (Me- 21). Besides, a pair of doublets at the upfield region, δ 0.34 (1H, *d*, *J*= 4.2 Hz) and δ 0.56 (1H, *d*, *J*= 4.2 Hz) showed the characteristic of non-equivalent protons of a cyclopropyl methylene group (H-19)[70]. In addition, signals due to a methine proton geminal to a hydroxyl (δ 3.29, H-3), methine proton to a double bond (δ 6.50, H-24) and methine proton geminal to a carbonyl were observed. A singlet in the downfield region at δ 9.39 (1H, *s*) indicated the presence of an aldehyde proton,

The ^{13}C NMR and DEPT spectra (Figure 3.35- Figure 3.38) showed 30 signals consisting of six methyls (C-18, C-21, C-27, C-28, C-29, C-30), eleven methylenes (C-1, C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-19, C-22, C-23), seven methines (C-3, C-5, C-8, C-17, C-20, C-24, C-26), six quaternary (C-4, C-9, C-10, C-13, C-14, C-25) carbon

atoms. Signals at δ 18.2, δ 18.2, δ 9.3, δ 14.1, δ 25.5, δ 19.4 corresponded to the six methyls, C-18, C-21, C-27, C-28, C-29 and C-30 respectively. In the ^{13}C NMR spectrum, the signal of the C-26 carbonyl appeared at δ 195.6 and signal at δ 77.4 revealed the existence of the hydroxyl at C-3.

The HMBC correlations H-27/ C-24, C-25, C-26 and H-26/ C-24, C-25, C-27 indicated that the C-26 aldehyde is neighbouring to a methyl (H-27) and a double bond (H-24, H-25). In addition, the correlations H-2, H-28, H-29/ C-3 showed that the hydroxyl is located at the fused ring A.

Thorough study of the spectral data and comparison with the literature values[71,72] established that Compound F is the known cycloartane triterpenoid, 24(E)-3β-hydroxy-cycloart-24-ene-26-al **204**.

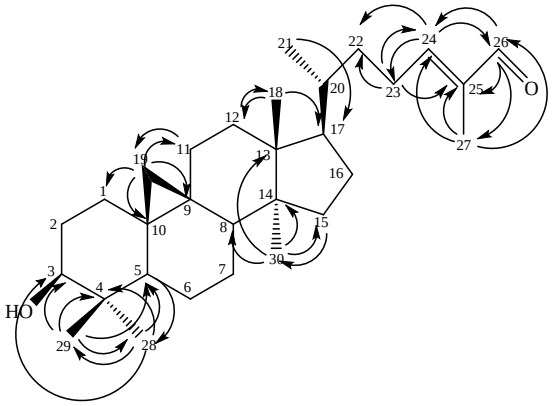


Figure 3.33: Selected HMBC correlation of Compound F

Table 3.7: ^{1}H NMR, ^{13}C NMR and HMBC Data of Compound F in $CDCl_3$

Position	δ_H (ppm)	δ_C (ppm)	HMBC (H→C)
1	1.22 (1H, *m*)	32.1	10
	1.24 (1H, *m*)		
2	1.53 (1H, *m*)	30.4	1, 3, 4, 10
	1.74 (1H, *m*)		
3	3.29 (1H, *m*)	77.4	-
4	-	40.6	-
5	1.30 (1H, *m*)	47.2	4, 28
6	1.54 (1H, *m*)	21.2	-
	1.60 (1H, *m*)		
7	1.06 (1H, *m*)	26.1	8
	1.34 (1H, *m*)		
8	1.51 (1H, *m*)	48.1	9, 10, 14, 15, 19
9	-	20.0	-
10	-	26.1	-
11	1.90 (1H, *m*)	26.5	8, 9, 12, 13, 19
	2.02 (1H, *m*)		
12	1.61 (1H, *m*)	33.0	13, 18
13	-	45.4	-
14	-	48.9	-
15	1.28 (2H, *m*)	35.6	16, 30
16	1.26 (1H, *m*)	28.3	-
	1.88 (1H, *m*)		
17	1.59 (1H, *m*)	52.3	12
18	0.97 (3H, *s*)	18.2	12, 17
19	0.56 (1H, *d*, *J*= 4.2 Hz)	30.0	1, 9, 10, 11
	0.34 (1H, *d*, *J*= 4.2 Hz)		
20	1.40 (1H, *m*)	36.1	-
21	0.93 (3H, *d*, *J*= 2.2 Hz)	18.2	17
22	-	34.8	-
23	2.24 (1H, *m*)	26.1	22, 24, 25
	2.40 (1H, *m*)		
24	6.50 (1H, *td*, J_1= 1.2 Hz, J_2= 7.8 Hz)	155.8	22, 23, 26
25	-	139.2	-
26	9.39 (1H, *s*)	195.6	24, 25, 27
27	1.75 (3H, *s*)	9.3	24, 25, 26
28	0.81 (3H, *s*)	14.1	3, 4, 5, 29
29	0.97 (3H, *s*)	25.5	3, 4, 5, 28
30	0.90 (3H, *s*)	19.4	8, 14, 15

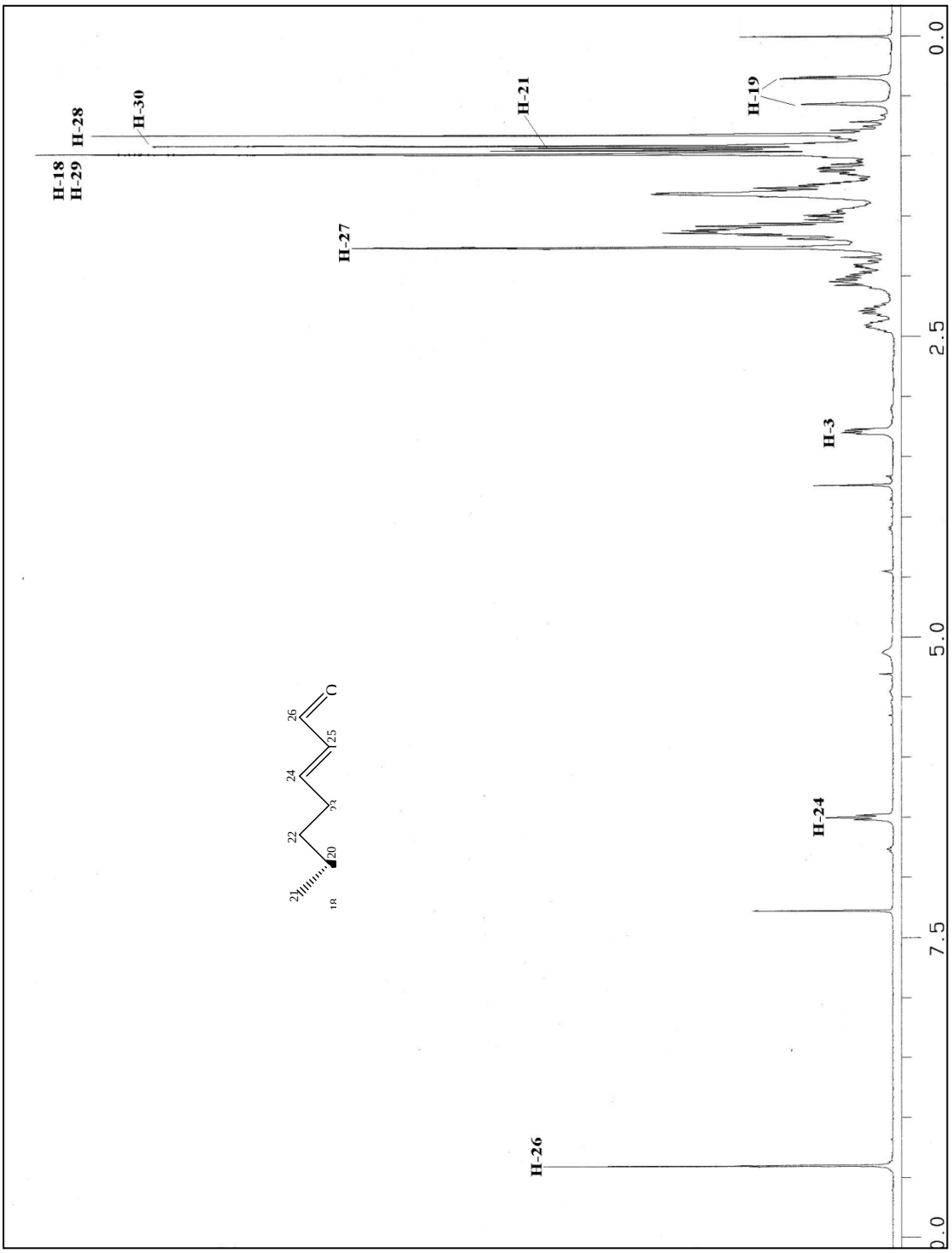


Figure 3.34: ^{1}H NMR spectrum of compound F in $CDCl_3$ (400 MHz)

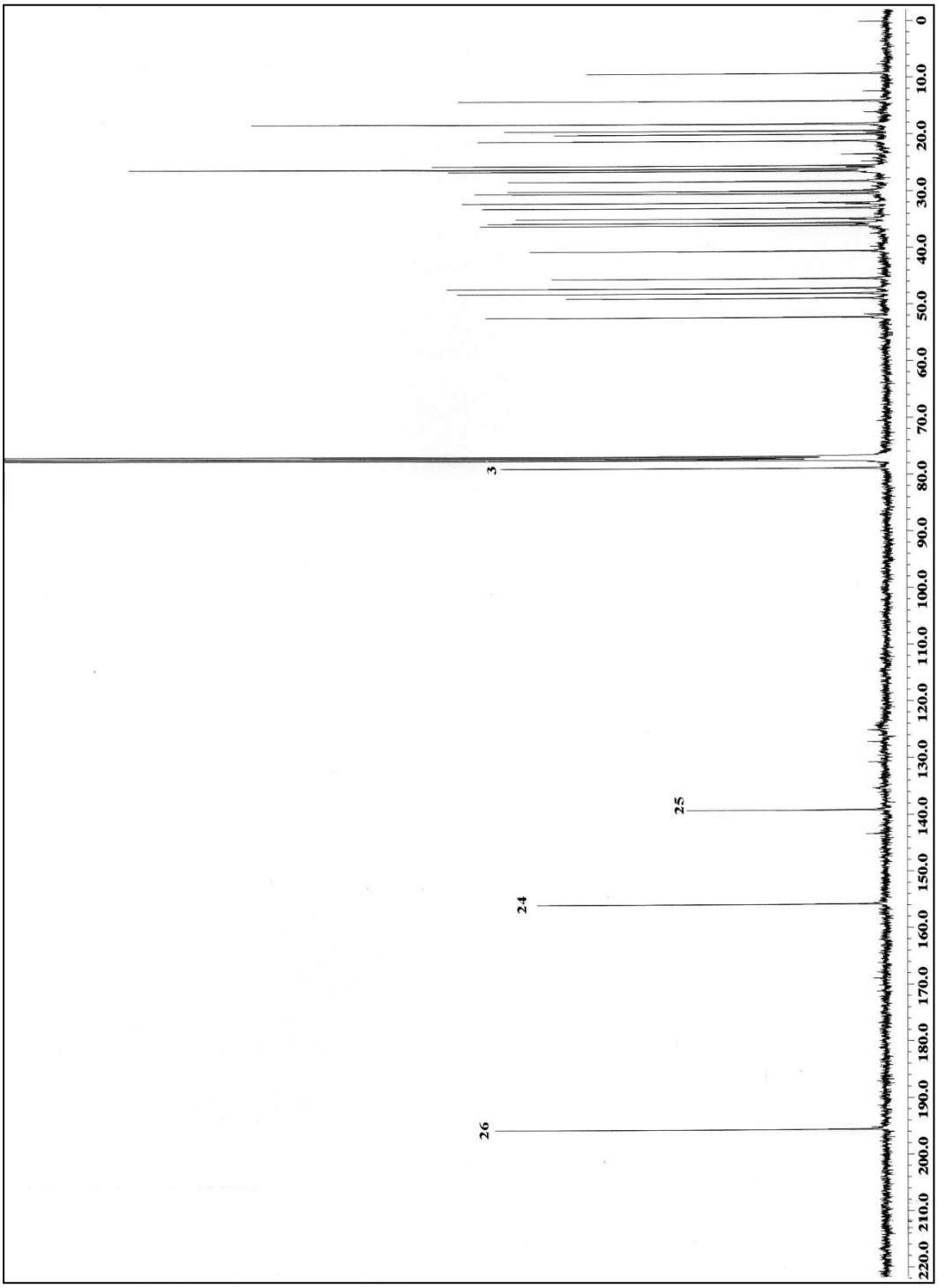


Figure 3.35: ^{13}C NMR spectrum of compound F in $CDCl_3$ (400 MHz)

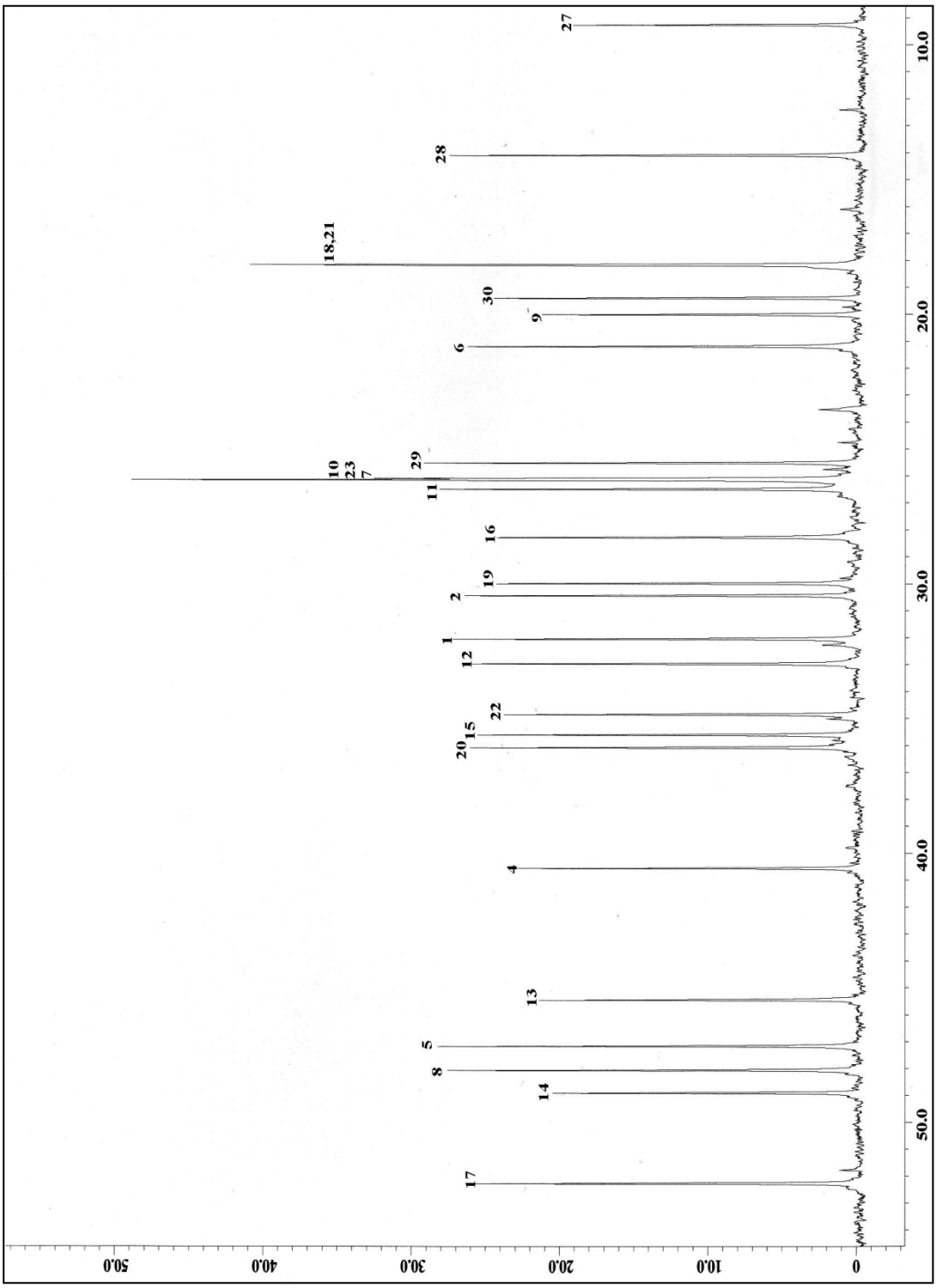


Figure 3.36: Expansion of ^{13}C NMR spectrum of compound F in $CDCl_3$ (δ 8 - 60 ppm)

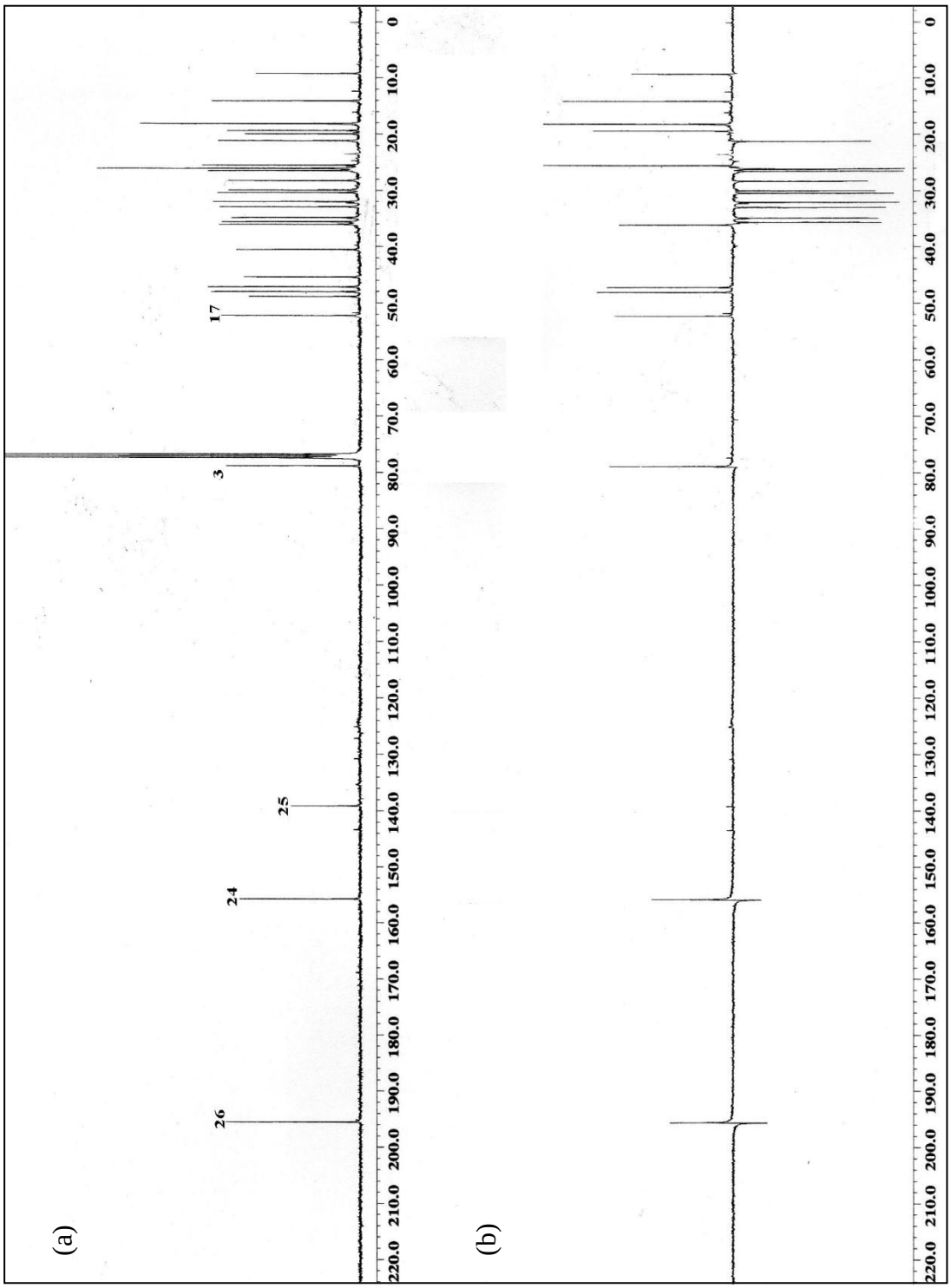


Figure 3.37: ^{13}C NMR (a) and DEPT-135 (b) spectra of compound F in $CDCl_3$ (400 MHz)

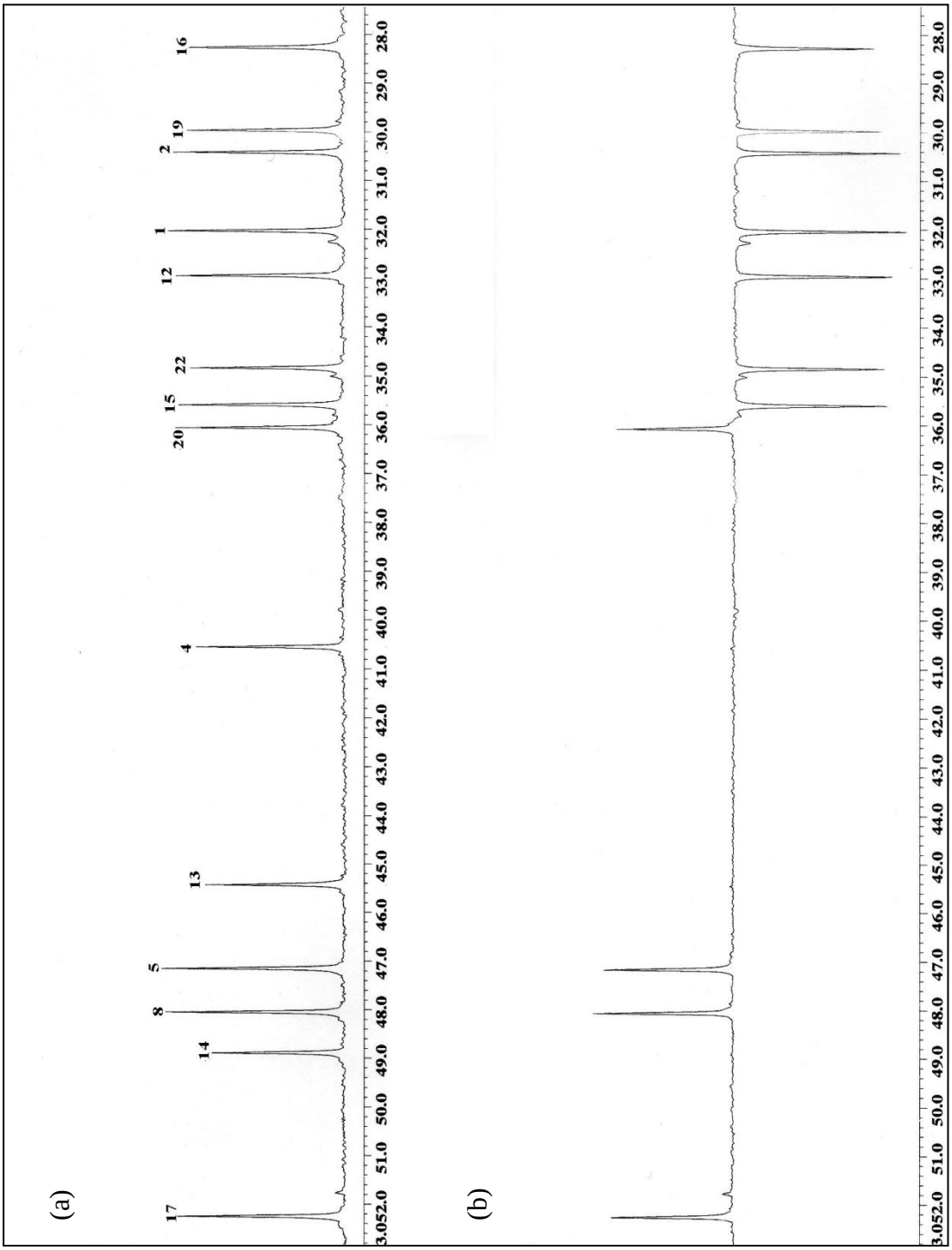


Figure 3.38: Expansion of ^{13}C NMR (a) and DEPT-135 (b) spectra of compound F in $CDCl_3$ (δ25 – 55 ppm)

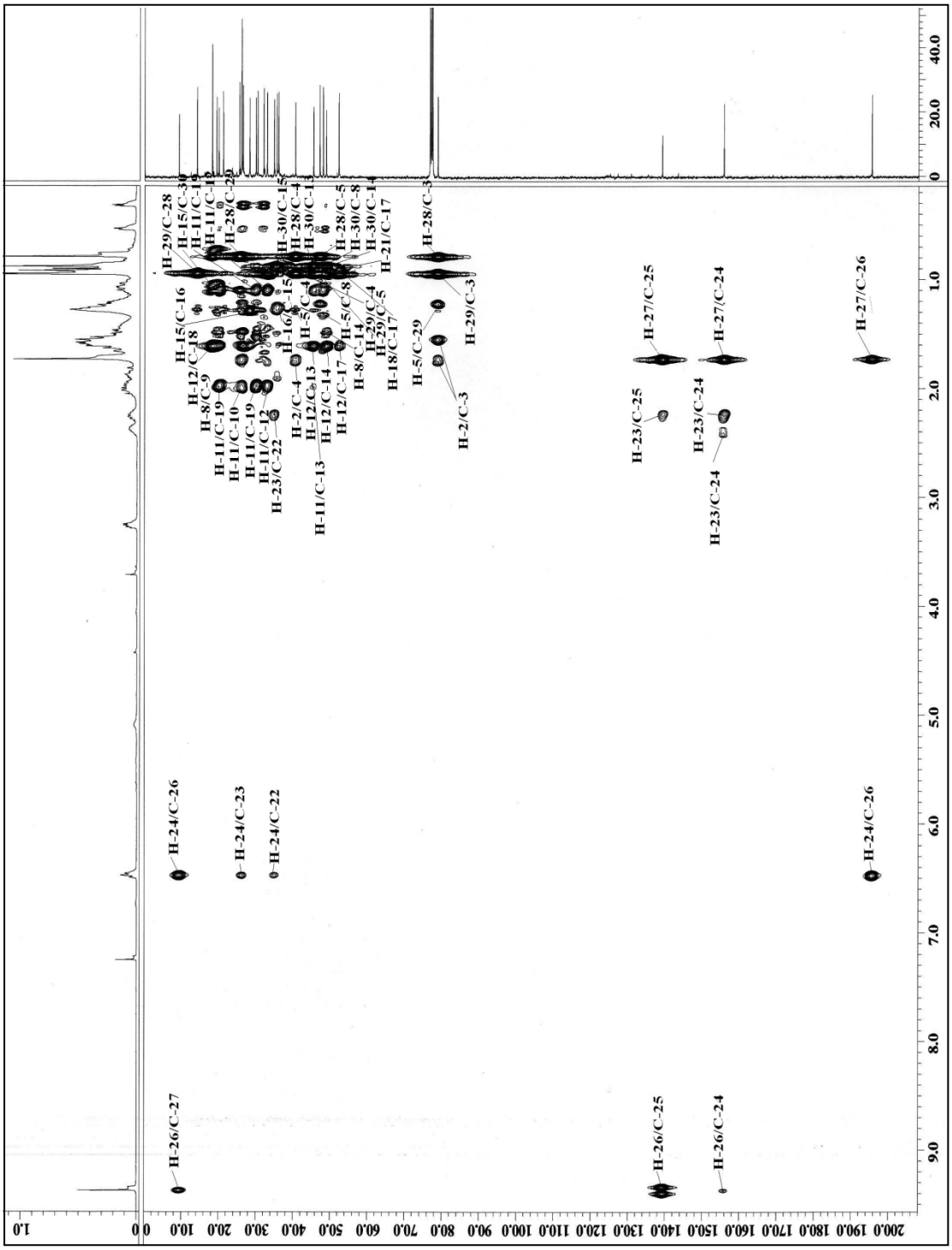
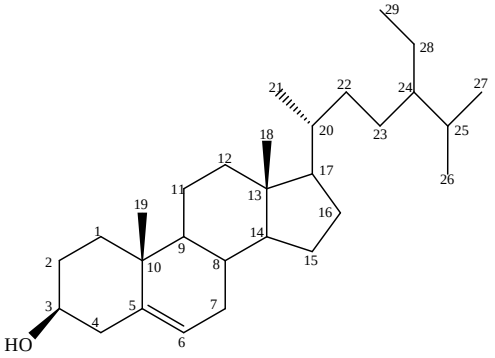


Figure 3.39: HMBC spectrum of compound F in $CDCl_3$

Compound G: *β*-Sitosterol **205**



Compound G was isolated as a colorless crystal with melting point 136-136^{o}C and the molecular ion peak at m/z 414 in EIMS spectrum suggested that the compound molecular formula was $C_{29}H_{50}O$. Its optical rotation, $[\propto]_D^{23.6}$ = -33.3^{o} (c 0.00209, CH_2Cl_2). An absorption band at 211 nm was revealed in the UV spectrum. The absorption peaks at 3418, 2936, 1644 and 1463 cm^{-1} displayed in IR spectrum showed the presence of hydroxyl, alkyl, carbonyl and olefinic group respectively.

The ^{1}H NMR spectrum of compound G in Figure 3.41 revealed the presence of six methyls at δ 0.68 (Me- 18), δ 0.79 (Me- 26), δ 0.85 (Me-27, Me- 29), δ 0.91 (Me-21), δ 1.00 (Me- 19). Among the signals, there was a doublet at δ 0.91 (3H, *d*, *J*= 8.1 Hz) assigned to Me-21. A multiplet at δ 3.48 (1H, *m*) due to a methine proton (H-3) geminal to hydroxyl was observed. The existence of a double bond is showed by a signal at downfield region, δ 5.34 (1H, *d*, *J*= 18.3 Hz).

The ^{13}C NMR in Figure 3.42 - 3.43 showed a total of 29 carbons consisting of six methyls (C-18, C-19, C-21, C-26, C-27, C-29), eleven methylenes (C-1, C-2, C-4, C-7,C-11, C-12, C-15, C-16, C-22, C-23, C-28), nine methines (C-3, C-6, C-8, C-9, C-14, C-17, C-20, C-24, C-25) and three quaternary (C-5, C-10, C-13) carbon atoms. Signal appeared at δ 121.8 and δ 140.8 revealed that compound G possessed a double bond in the compound.

The HMBC correlation H-6/ C-4, C-7, C-8, C-10 suggested that the position of the double bond in ring B. In addition, the correlations of H-1, H-2, H-4/ C-3 showed that the hydroxyl was located at C-3 of ring A.

Compound G was confirmed as *β*-Sitosterol **205** by the comparison of aforementioned ^{1}H and ^{13}C spectroscopic data with the reported literature values[73,74,75].

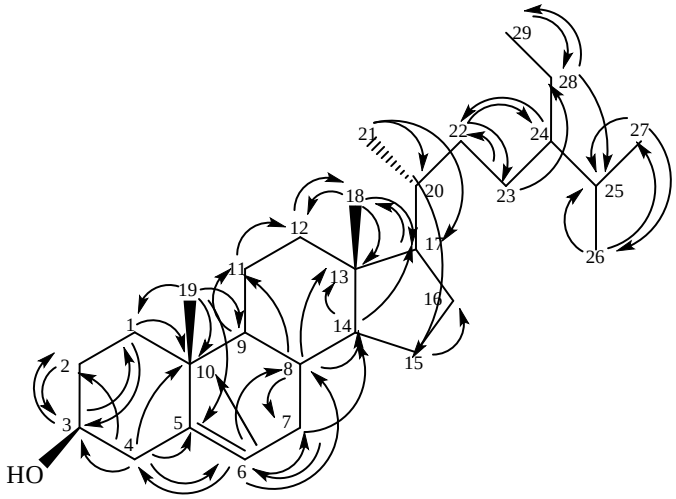


Figure 3.40: HMBC Correlation of Compound G

Table 3.8: ^{1}H NMR and ^{13}C NMR HMBC Data of Compound G in $CDCl_3$

Position	δ_H (ppm)	δ_C (ppm)	HMBC (H→C)
1	1.82 (1H, *m*)	37.4	3, 10
	1.06 (1H, *m*)		
2	1.80 (2H, *m*)	31.7	3
3	3.48 (1H, *m*)	71.9	1, 2
4	2.25 (1H, *m*)	42.4	2, 3, 5, 6, 10
5	-	140.8	-
6	5.34 (1H, *d*, *J*= 18.3 Hz)	121.8	4, 7, 8, 10
7	1.47 (1H, *m*)	32.0	5, 6, 14
	1.94 (1H, *m*)		
8	1.47 (1H, *m*)	32.0	7, 11, 13, 14
9	-	50.2	11
10	-	36.6	-
11	1.02 (1H, *m*)	21.2	12
	1.46 (1H, *m*)		
12	1.14 (1H, *m*)	28.3	18
	1.98 (1H, *m*)		
13	-	42.4	-
14	0.98 (1H, *m*)	56.9	13, 17
15	1.03 (1H, *m*)	24.4	8, 16
	1.56 (1H, *m*)		
16	1.24 (1H, *m*)	39.9	-
	1.80 (1H, *m*)		
17	1.10 (1H, *m*)	56.1	18
18	0.68 (3H, *s*)	11.9	13, 14, 17
19	1.00 (3H, *s*)	19.5	1, 5, 9, 10
20	1.32 (1H, *m*)	36.2	23
21	0.91 (3H, *d*, *J*=8.1 Hz)	18.9	17, 20
22	1.14 (2H, *m*)	26.2	23, 24
23	0.99 (1H, *m*)	34.0	22, 28
	1.30 (1H, *m*)		
24	0.91 (3H, *d*, *J*= 8.1 Hz)	45.9	22
25	1.64 (1H, *m*)	29.2	-
26	0.79 (3H, *s*)	19.1	25, 27
27	0.85 (3H, *s*)	19.9	25, 26
28	1.03 (1H, *m*)	23.2	25, 29
	1.22 (1H, *m*)		
29	0.85 (3H, *d*, *J*= 1.7 Hz)	12.1	28

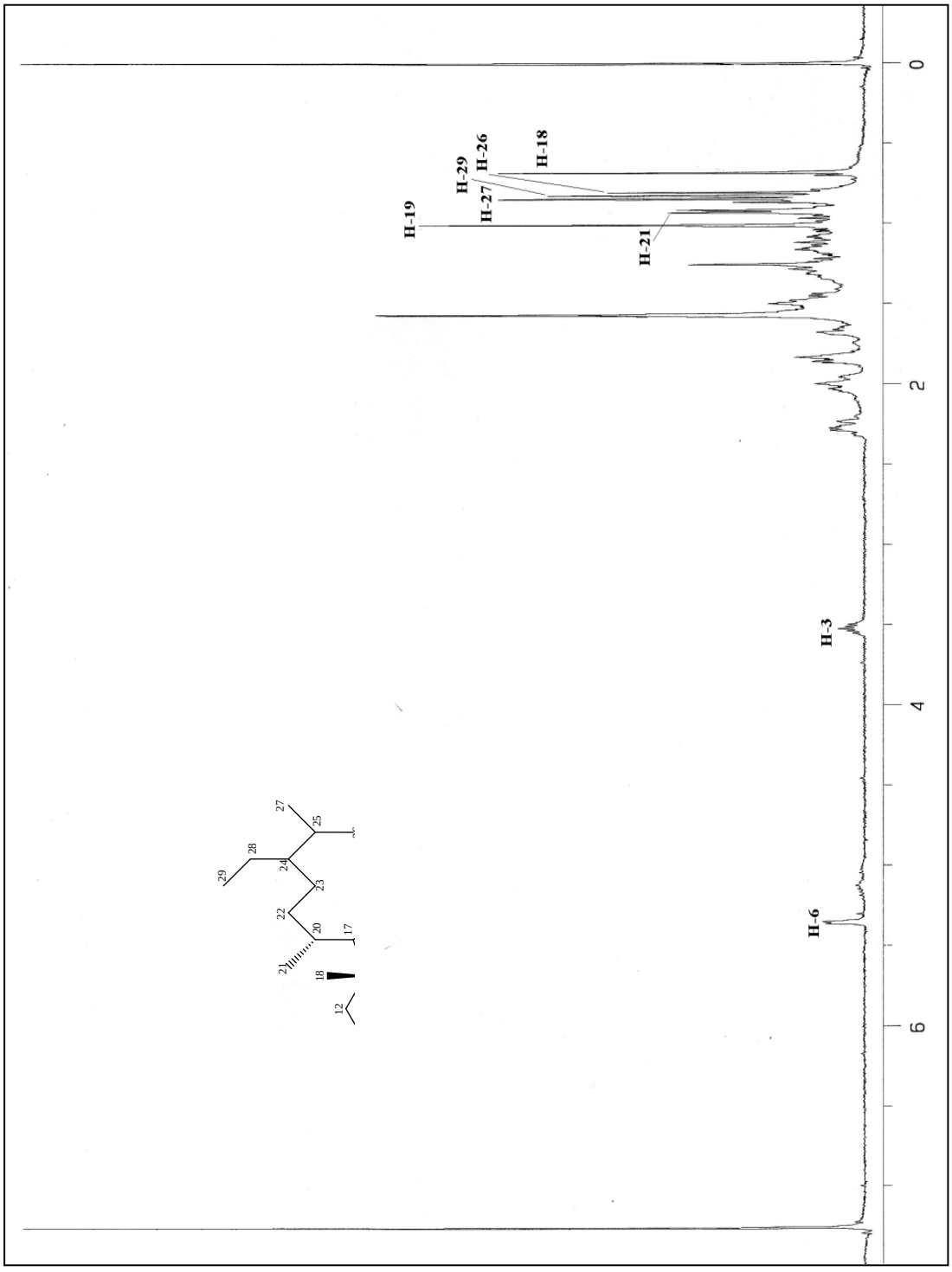


Figure 3.41: ^{1}H NMR spectrum of compound G in $CDCl_3$ (400 MHz)

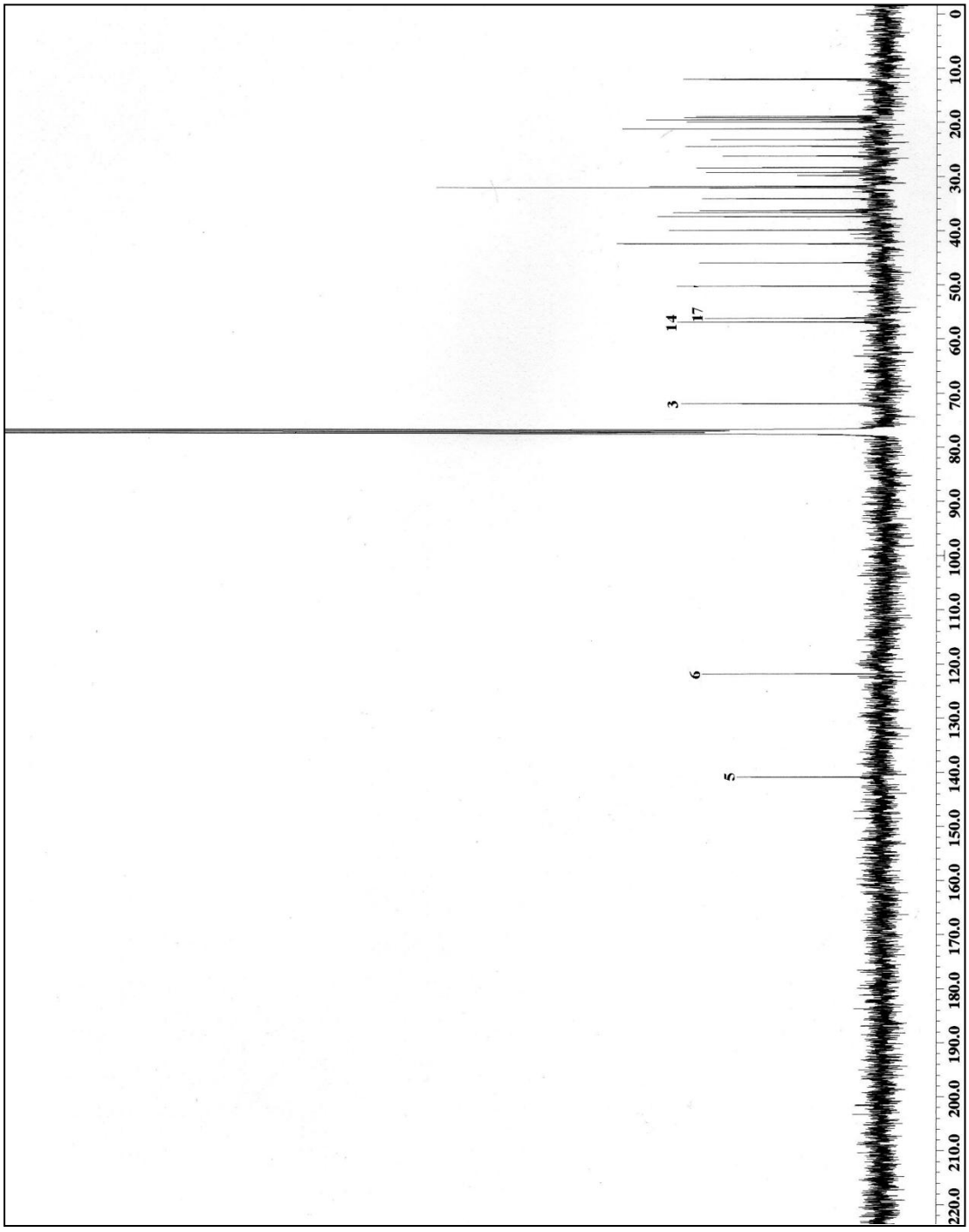


Figure 3.42: ^{13}C NMR spectrum of compound G in $CDCl_3$ (400 MHz)

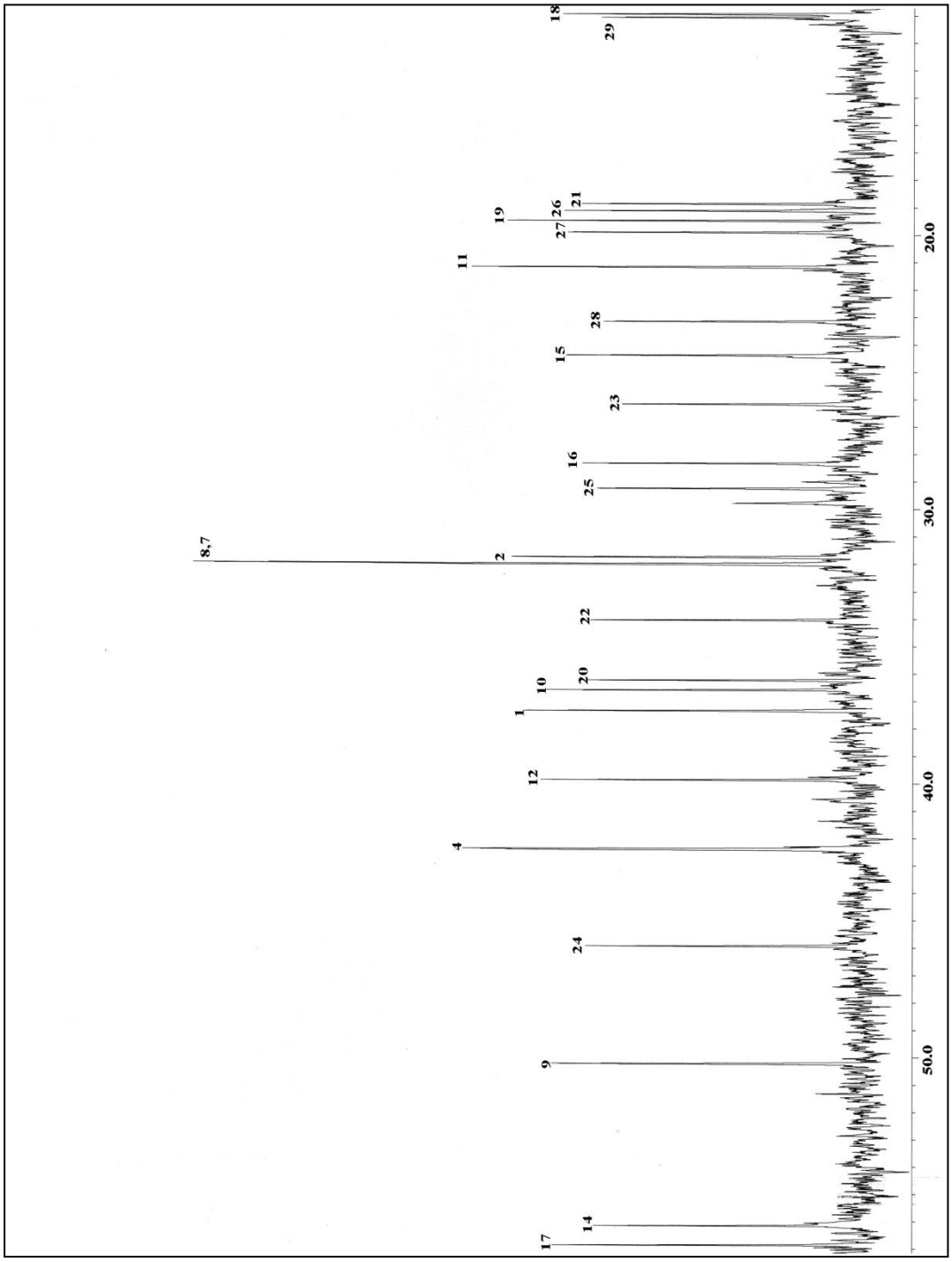


Figure 3.43: Expansiion of ^{13}C NMR spectrum of compound G in $CDCl_3$ (δ10- 60 ppm)

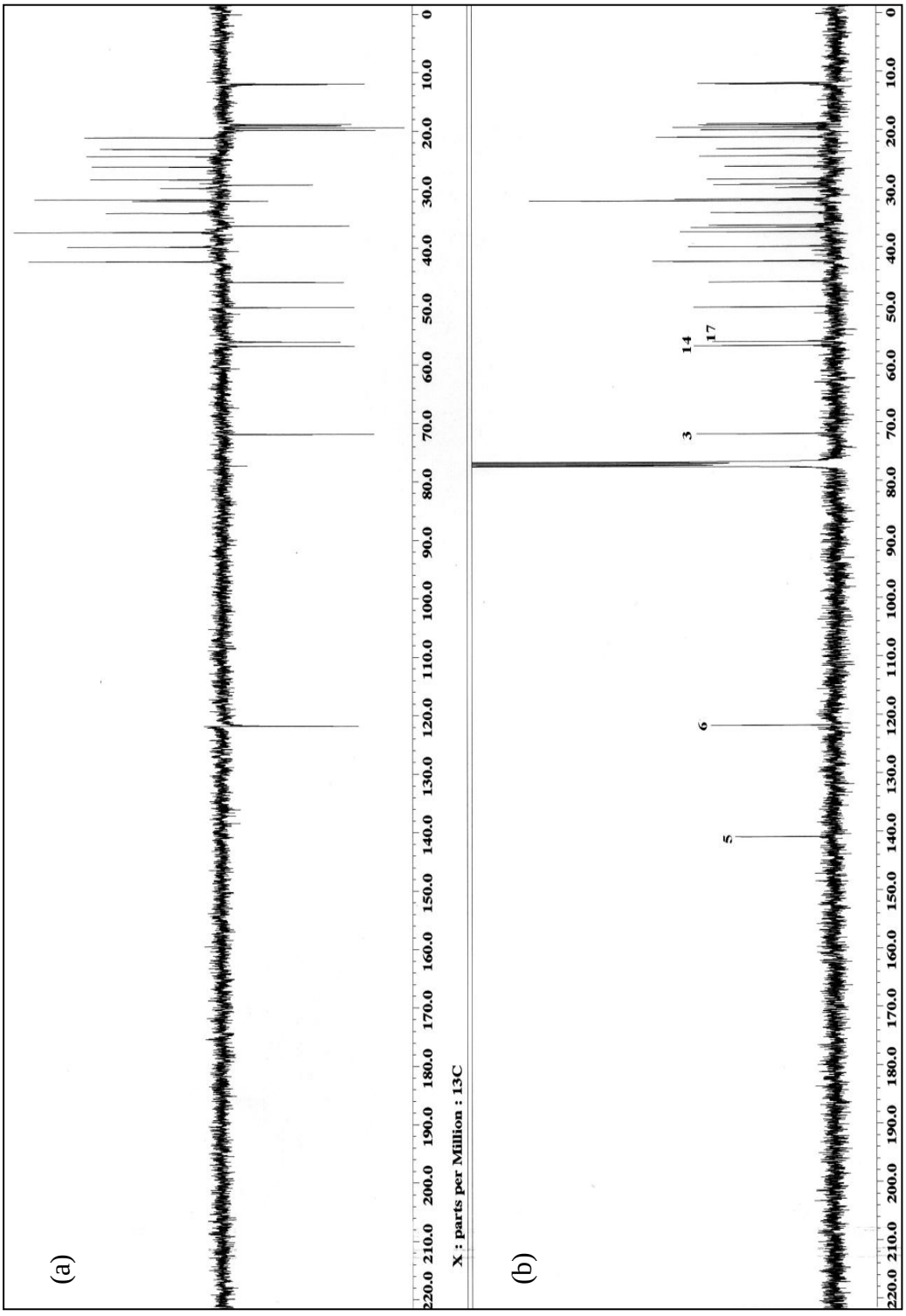


Figure 3.44: DEPT-135 (a) and ^{13}C NMR (b) spectrum of compound G in $CDCl_3$ (MHz)

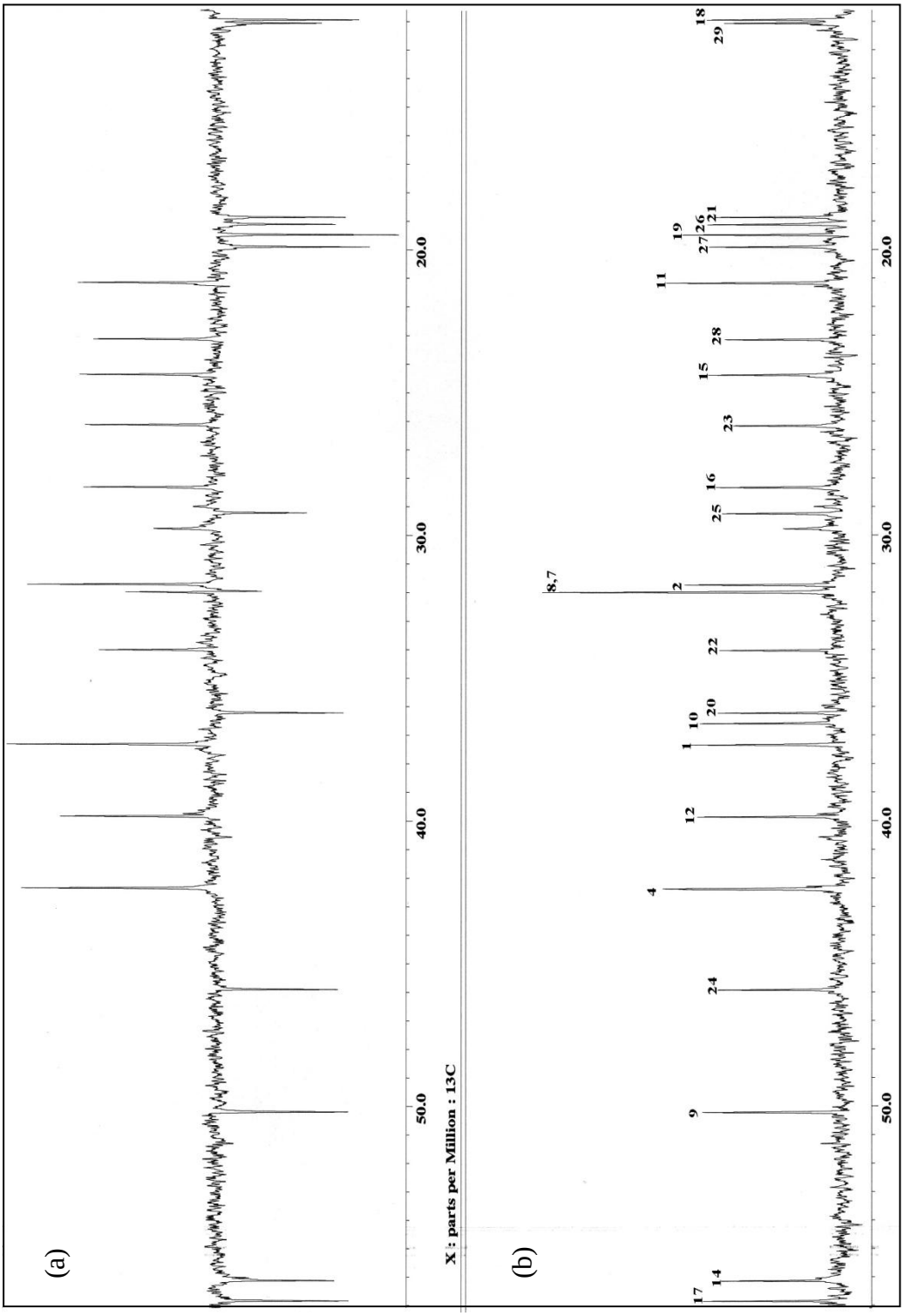
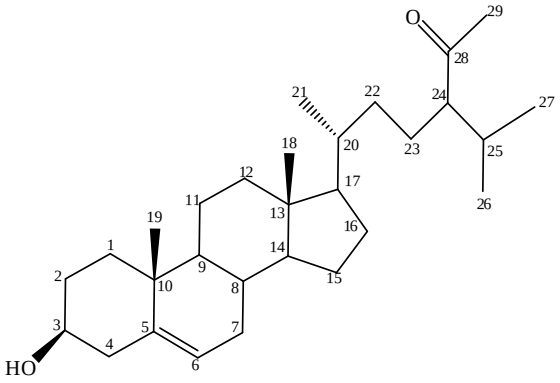


Figure 3.45: Expansion of DEPT-135 (a) and ^{13}C NMR (b) spectrum of compound G in $CDCl_3$ (δ 10-60 ppm)

Compound H: Stigmast-5-ene-28-one **206**



Compound H was isolated as a colorless crystal with melting point 147-148^{o}C and the EI-MS spectral showed a molecular ion peak at m/z 428 corresponding to the molecular formula of $C_{29}H_{48}O_{2.}$ In the UV spectrum, an absorption band at 208 nm was observed. Its IR spectrum showed IR absorption at 3419 cm^{-1} suggesting the presence of hydroxyl group and the absorption bands for acetyl appeared at 1713, 1456, 1374 cm^{-1}.

The ^{1}H NMR spectrum of compound H in Figure 3.48 showed the presence of six methyl signals at the position δ 0.70 (Me-18), δ 0.87 (Me-26), δ 0.89 (Me-27), δ 0.91 (Me-21), δ 1.00 (Me-19), δ 2.09 (Me-29). A doublet at δ 0.91 (3H, *d*, *J*= 2.0 Hz) showed the methyl proton (H-21) at the side chain. Besides, additional doublet at δ 0.89 (3H, *m*) indicated another methyl proton (H-27). A singlet at δ 2.09 (3H, *s*) indicated another methyl proton attached to a carbonyl carbon. A doublet at δ 5.32 (1H, *d*, *J*= 6.9 Hz) discerned a methine proton (H-6) geminal to a double bond. In addition, a multiplet at δ 3.50 (1H, *m*) of methine proton (H-3) attach to the hydroxyl at the ring.

The ^{13}C NMR data in Figure 3.49 - 3.50 revealed a total of 29 carbons comprises of six methyls (C-18, C-19, C-21, C-26, C-27, C-29), ten methylenes (C-1, C-2, C-4, C-7, C-11, C-12, C-15, C-16, C-22, C-23), nine methines (C-3, C-6, C-8, C-9, C-14, C-17, C-20, C-24, C-25) and four quaternary carbons (C-5, C-10, C-13, C-28). Signal

appeared at δ 213.5 showed that the existence of ketone moiety (C-28) and signal at δ 121.8 and δ 140.8 indicated that the compound possess a double bond (C-6, C-5).

The HMBC correlations H-29/ C-24, 28 and H-25/ C-24 confirmed that the position of the ketonic carbonyl at side chain. The HMBC correlation H-4/ C-3, 5, 6 confirmed the position of the double bond in ring B.

Compound H are being reported for the first time as naturally occurring compounds. Accordingly, Compound H was established as stigmast-5-ene-28-one **206** on the basis of spectroscopic methods and X-ray analysis (Figure 3.47).

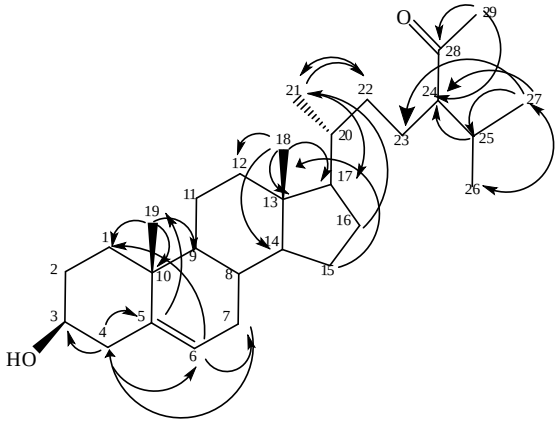


Figure 3.46: Selected HMBC correlations of Compound H

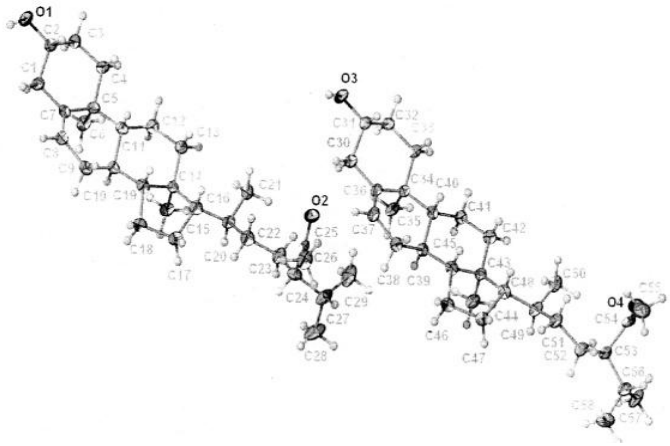


Figure 3.47: ORTEP Diagram of Compound H

Table 3.9: ^{1}H NMR, ^{13}C NMR and HMBC Data of Compound H in $CDCl_3$

Position	δ_H (ppm)	δ_C (ppm)	HMBC (H→C)
1	1.06 (1H, *m*)	37.3	-
	1.79 (1H, *m*)		
2	1.80 (2H, *m*)	31.7	-
3	3.50 (1H, *m*)	71.9	-
4	2.21 (1H, *m*)	42.4	3, 5, 6
5	-	140.8	-
6	5.32 (1H, *d*, *J*= 6.9 Hz)	121.8	4, 7
7	1.44 (1H, *m*)	32.0	-
	1.92 (1H, *m*)		
8	1.44 (1H, *m*)	32.0	-
9	0.89 (1H, *m*)	50.1	-
10	-	36.5	-
11	1.40 (1H, *m*)	21.1	-
	1.44 (1H, *m*)		
12	1.10 (1H, *m*)	39.8	-
	1.96 (1H, *m*)		
13	-	42.4	-
14	0.96 (1H, *m*)	55.9	-
15	0.98 (1H, *m*)	24.3	-
	1.52 (1H, *m*)		
16	1.14 (1H, *m*)	28.2	-
	1.74 (1H, *m*)		
17	1.04 (1H, *m*)	55.8	-
18	0.70 (3H, *s*)	11.9	12, 13, 14, 17
19	1.00 (3H, *s*)	20.1	1, 9, 10
20	1.32 (1H, *m*)	35.9	-
21	0.91 (3H, *d*, *J*= 2.0 Hz)	18.5	17, 22
22	0.88 (1H, *m*)	33.9	21
	1.20 (1H, *m*)		
23	1.32 (1H, *m*)	25.4	-
	1.54 (1H, *m*)		
24	2.11 (1H, *d*, *J*= 6.9 Hz)	60.6	-
25	1.78 (1H, *m*)	30.2	24
26	0.87 (3H, *d*, *J*= 2.2 Hz)	21.3	27
27	0.89 (3H, *d*, *J*= 2.2 Hz)	20.1	24, 25, 26
28	-	213.5	-
29	2.09 (3H,*s*)	30.0	24, 28

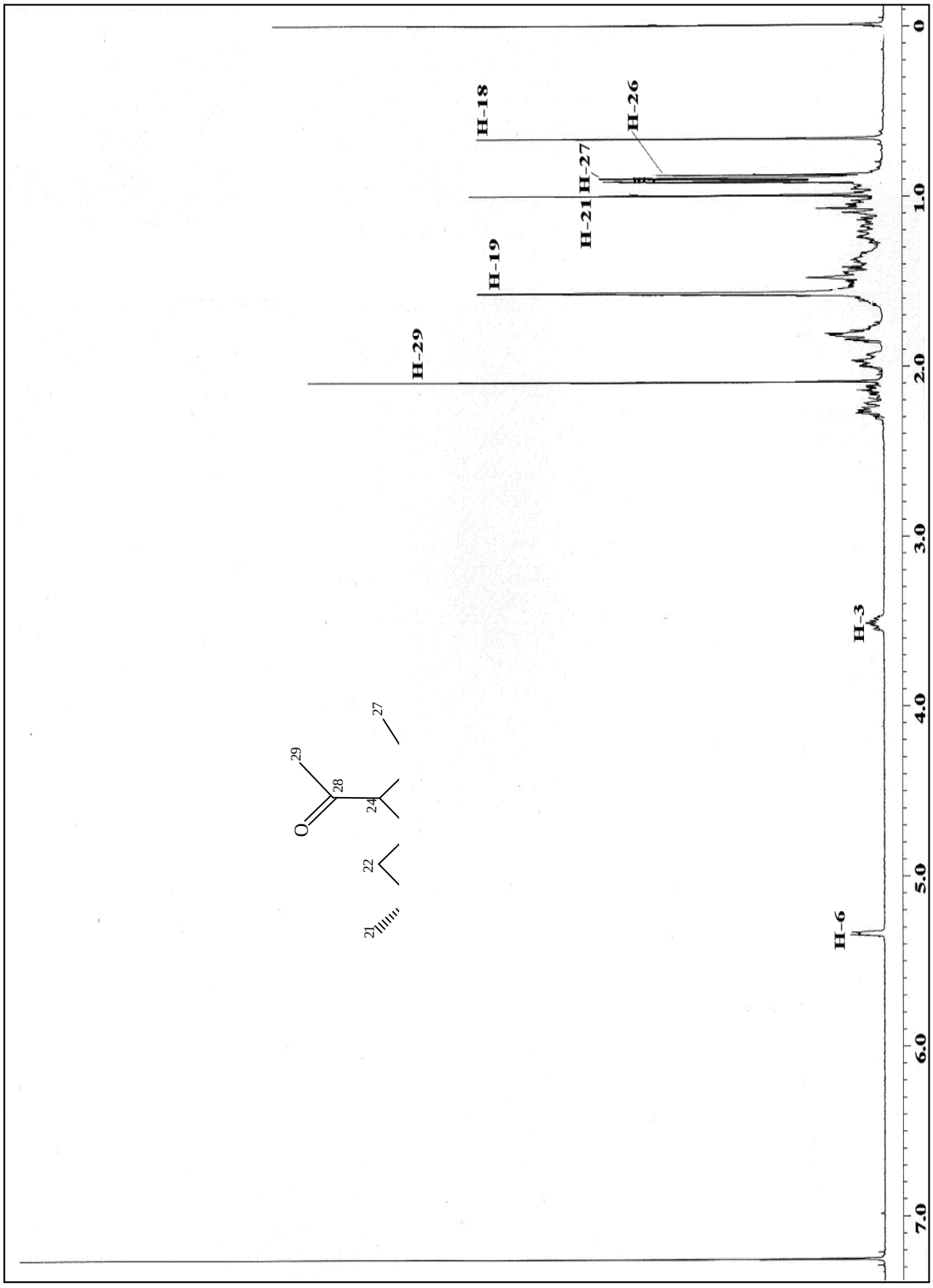


Figure 3.48: ^{1}H NMR spectrum of compound H in $CDCl_3$ (400 MHz)

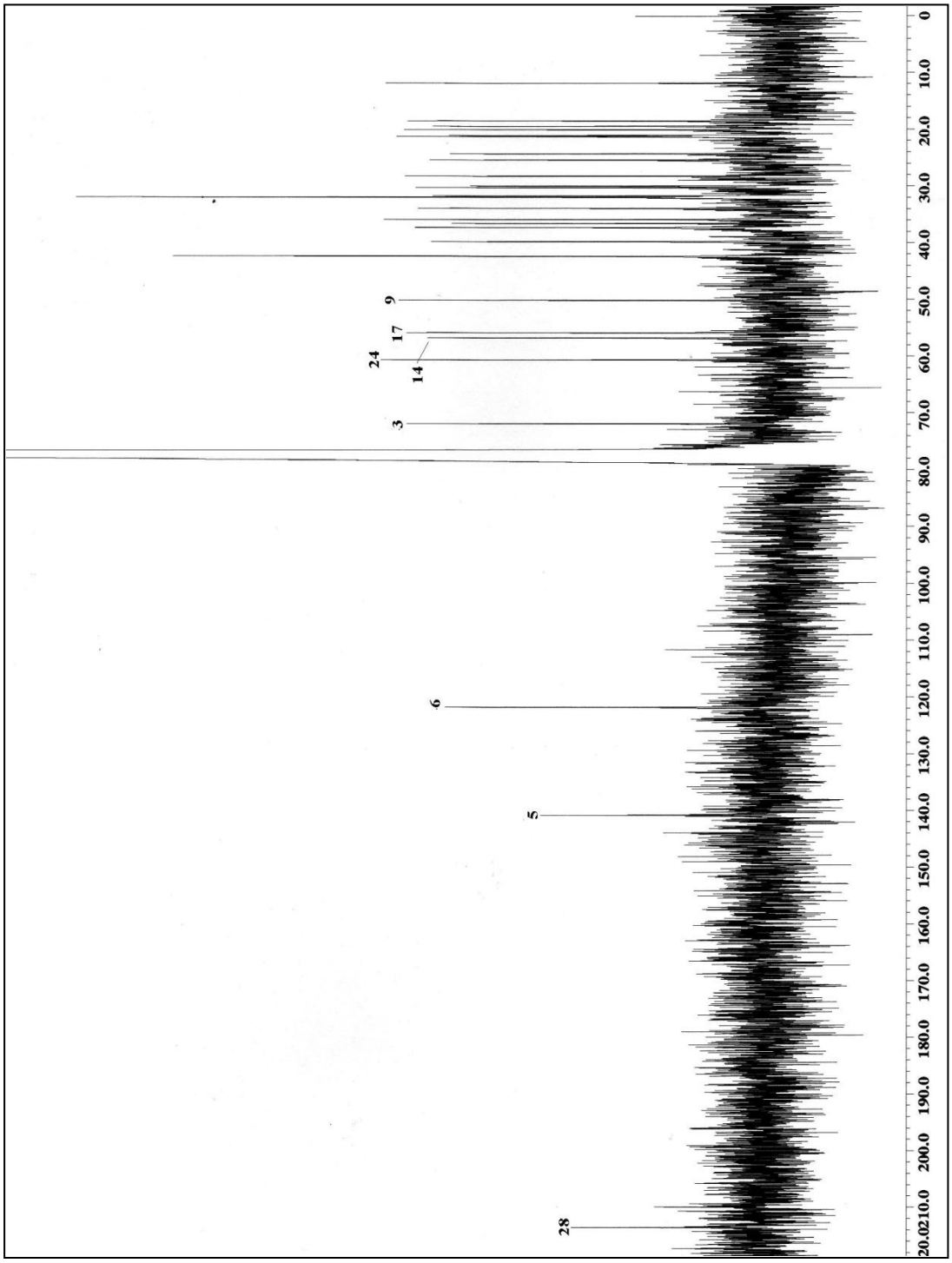


Figure 3.49: ^{13}C NMR spectrum of compound H in $CDCl_3$ (400 MHz)

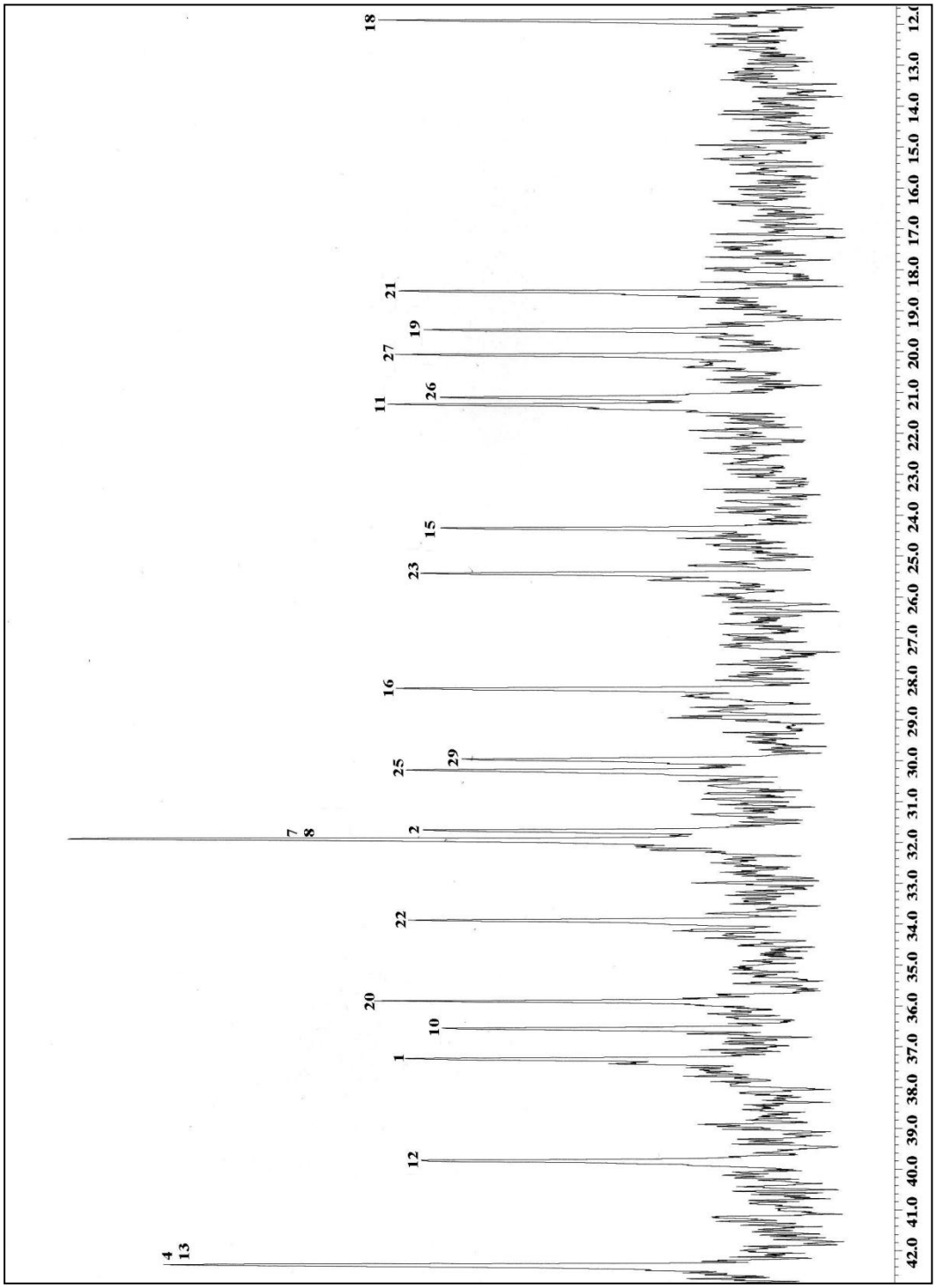


Figure 3.50: Expansion of ^{13}C NMR spectrum of compound H in $CDCl_3$ (δ10 – 45 ppm)

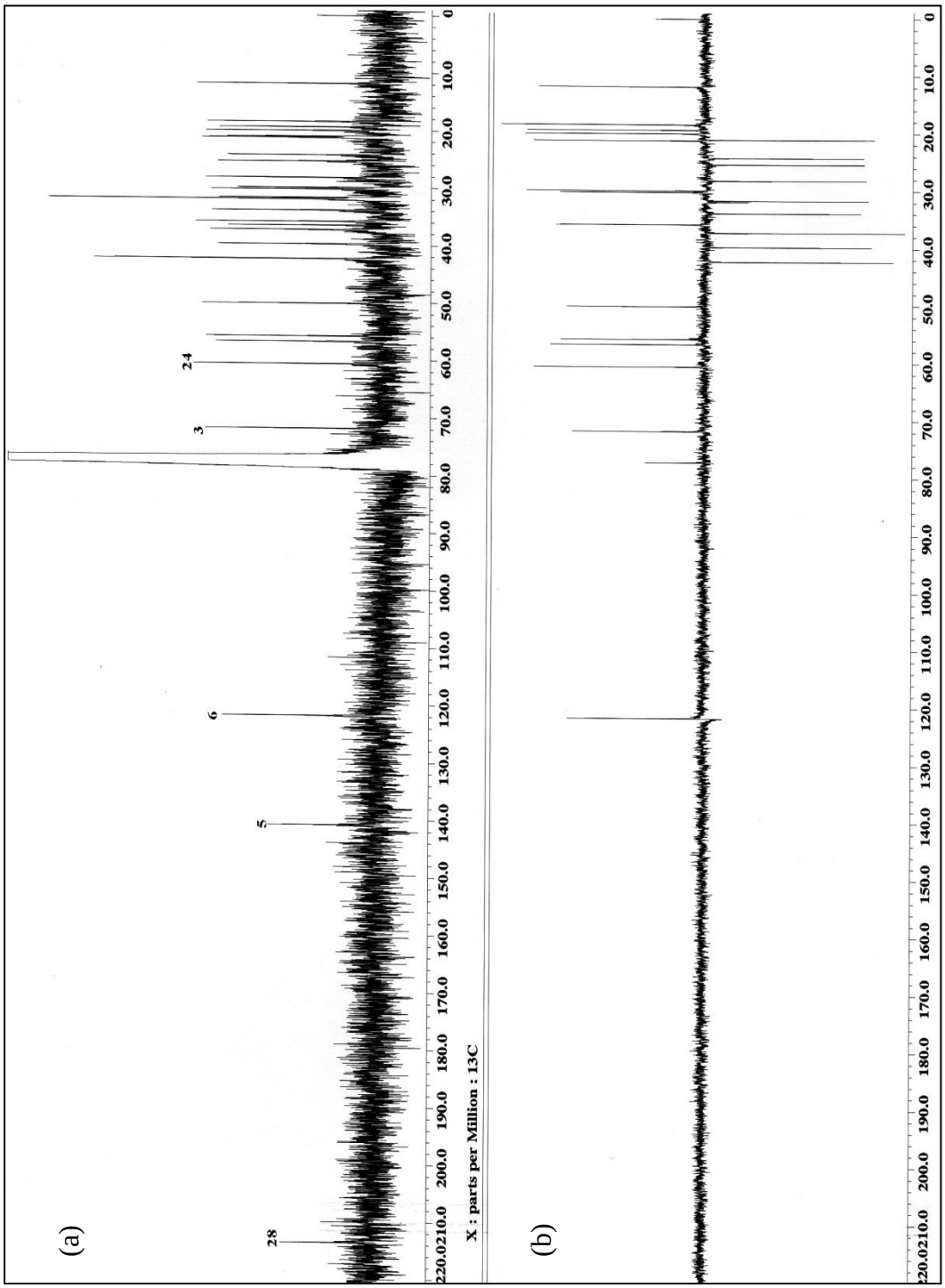


Figure 3.51: ^{13}C NMR (a) and DEPT-135 spectra of compound H in $CDCl_3$ (400 MHz)

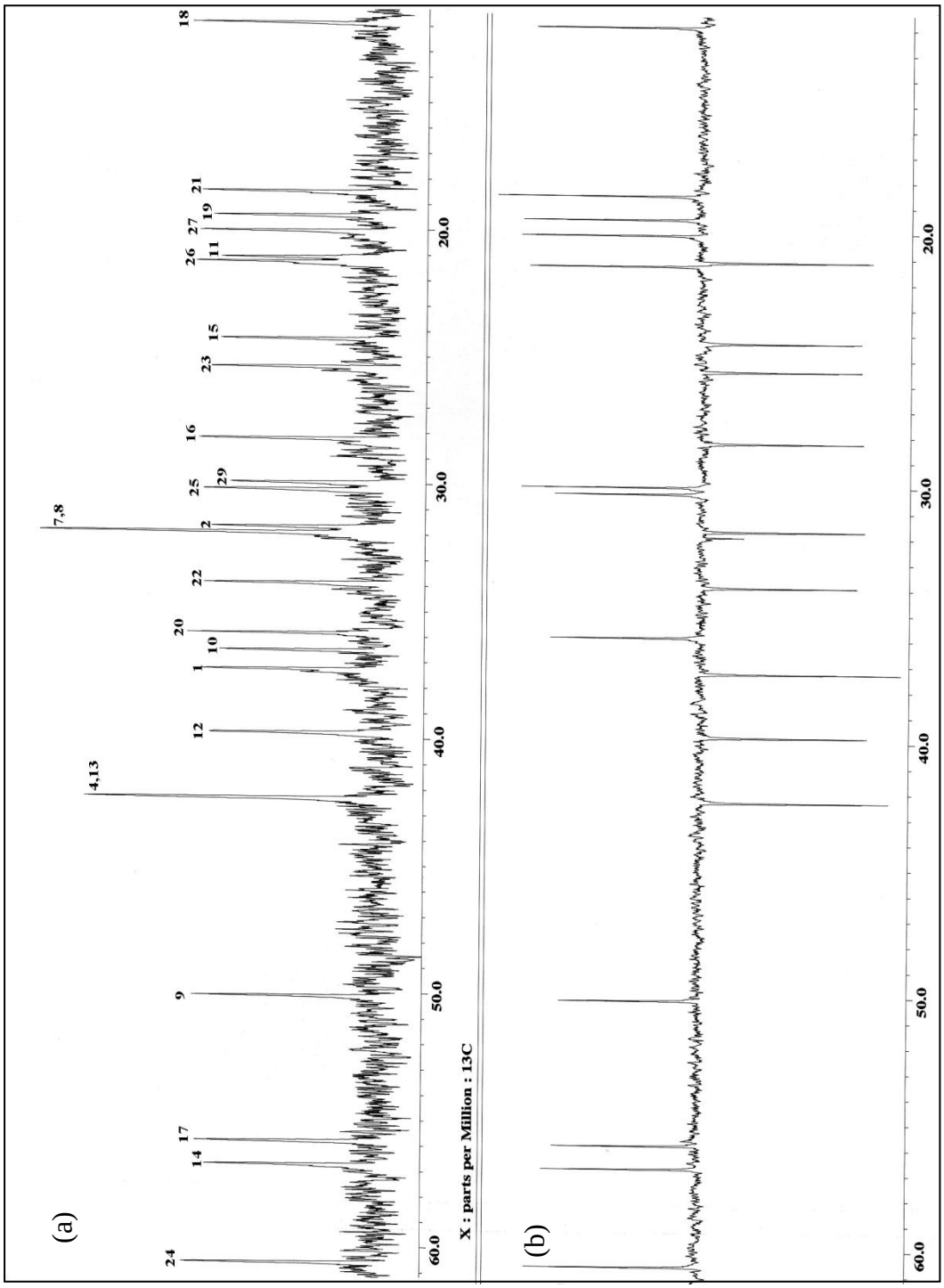


Figure 3.52: Expansion of ^{13}C NMR (a) and DEPT-135 spectra of compound H in $CDCl_3$ (δ 10 – 65 ppm)

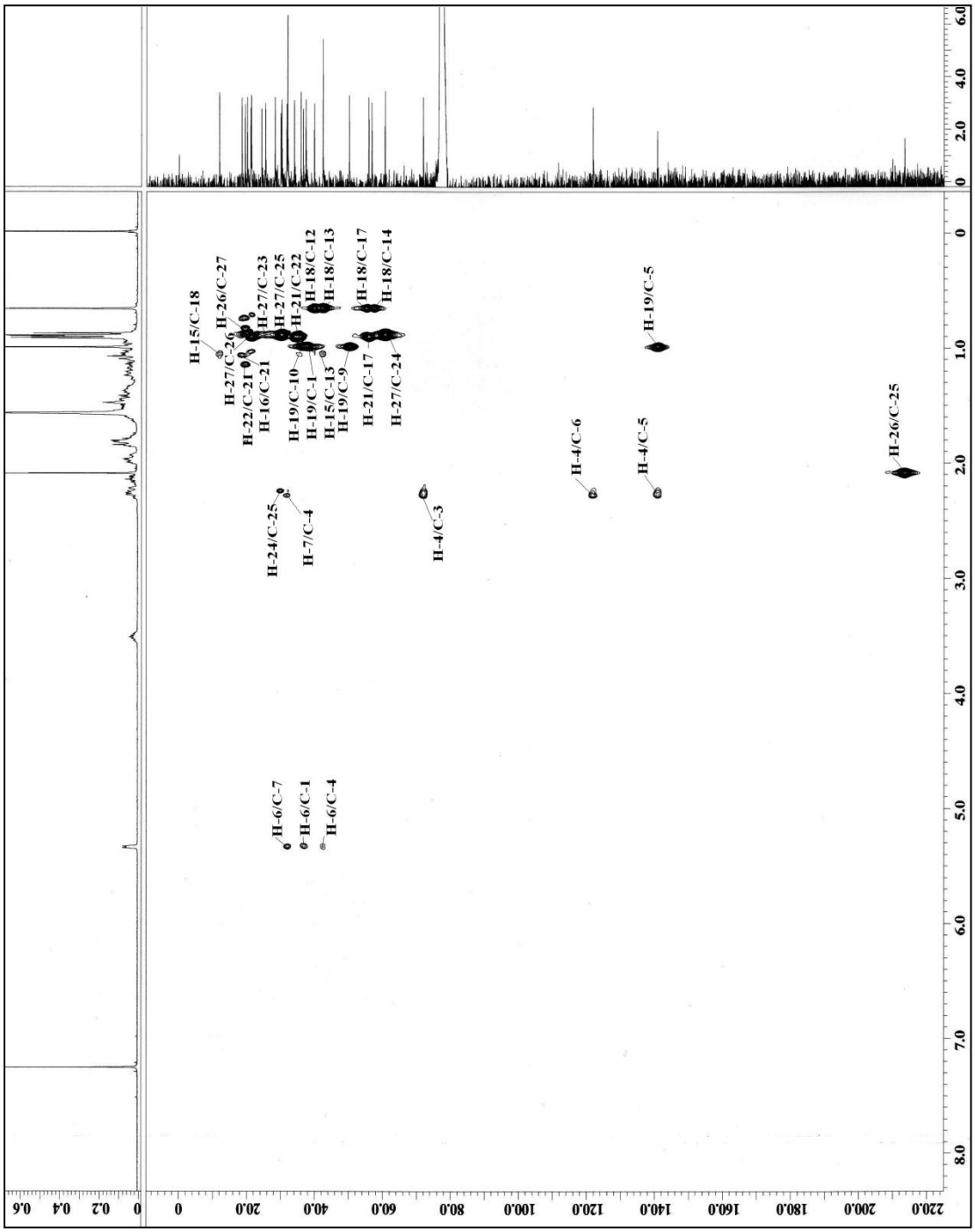


Figure 3.53: HMBC spectrum of compound H in $CDCl_3$

CHAPTER 4

CYTOTOXIC ACTIVITY

4.1 CYTOTOXIC ACTIVITY

Cytotoxic assay are widely used particulary in the natural products field. It measures the effect of a drug on the basic functions of cells which are common to all cells by assessing cellular damage. There are a few types of cytotoxic assay useful in monitoring the cyctotoxicity of natural products[76,77]. In this study, MTT or MTS colorimetric assay is used to measure the cytotoxicity of all compounds isolated from *Aglaia exima* against eight cell lines. This MTT assay is a quantitative test to measure the growth or death rate of cells. It is also a key tool for the development of natural products due to its reliable detection of anticancer[78]. Hereunder the materials used, procedures and results of the cytotoxic assay for the eight compounds.

Cytotoxicity of the compounds were evaluated against a panel of eight cancer cell lines; lung (A549), prostate (DU-145), skin (SK-MEL-5), pancreatic (BxPC-3), liver (Hep G2), colon (HT-29), breast (MCF-7) and (MDA-MB-231). These cancer cell lines were chosen from the National Cancer Institute (NCI) list of 60 cancer cell lines for drug screening and drug treatment conditions and were done according to the NCI recommendations (Boyd, 1995).

4.2 RESULTS OF CYTOTOXIC ACTIVITY

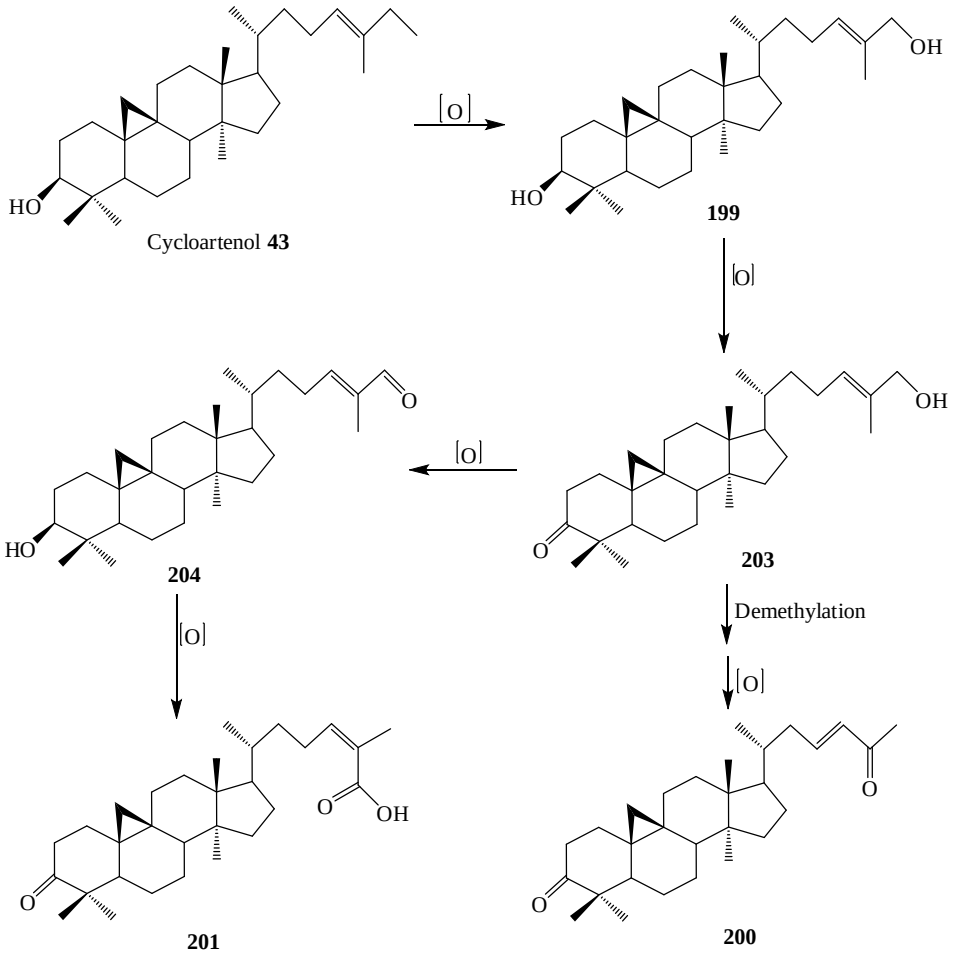
According to the results in Table 4.1, it was concluded that colon (HT-29) cancer cell lineS was found to be very susceptible towards 24(*E*)-cycloart-24-ene-26-ol-3-one **203** with IC_{50} values of 11.48 μM. Meanwhile, 24(*E*)-cycloart-24-ene-26-ol-3-one **203** revealed moderate activity to skin (SK-MEL-5) and breast (MCF-7) cell lines. Cycloart-24-ene-3*β*,26-diol **199** shows moderate effect against liver (Hep G2) and colon (HT-29), weak against lung (A549), skin (SK-MEL-5), breast (MCF-7) and (MDA-MB-231). Vaticinone **200** revealed moderate inhibitory effect towards colon (HT-29) and

weak towards skin (SK-MEL-5). At last, 24(*E*)-3*β*-hydroxycycloart-24-ene-26-al **204** has moderate effect against breast (MDA-MB-231) and weak against skin (SK-MEL-5). schizandronic acid **201**, cabraleahydroxylactone **202**, *β*-sitosterol **205** and stigmast-5-ene-28-one **206** exhibited no significant inhibitory effects with IC_{50} values over 200 μM.

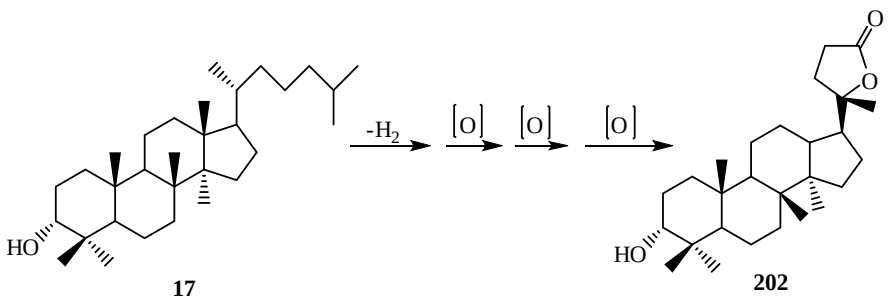
As comparing to the drug standard, Cisplatin, the two cycloartane-type triterpenoids, Cycloart-24-ene-3*β*,26-diol **199** and 24(*E*)-3*β*-hydroxycycloart-24-ene-26-al **204** have stronger inhibitory effect towards breast (MDA-MB-231). The new cycloartane-type triterpenoid, 24(*E*)-cycloart-24-ene-26-ol-3-one **203**, showed stronger affect than the drug standard, Cisplatin, against the colon (HT-29) and breast (MCF-7) cell lines.

The author has conducted a simple structure-activity relationship study on compounds cycloart-24-ene-3*β*,26-diol **199** and 24(*E*)-cycloart-24-ene-26-ol-3-one **203**. It was observed that the structure of these two compounds showed small difference where the hydroxyl group at position C-3 replaced by carbonyl group. However, the cytotoxic activity of these compounds revealed large difference against colon (HT-29) cell line with 99.31 μM and 11.48 μM. Thus, this indicated that the carbonyl group at the position C-3 may be responsible for the strong cytotoxic activity towards colon (HT-29) cell line.

In this study, the author has proposed the biosynthesis relationship between all the isolated cycloartane triterpenoid in scheme 4.1. Besides, the proposed relationship between the sterols were also depicted in scheme 4.2.



Scheme 4.1 Proposed biosynthesis relationship of compounds **199**, **200**, **201**, **203** and **204**



Scheme 4.2 Proposed biosynthesis pathway of compound **202**

This study has shown that *Aglaia exima* possess interesting compounds that may be subjected to further studies such as hemisynthesis and apoptotic studies.

Table 4.2: Cytotoxicity of Eight Compounds for Eight Cancel Cell Lines[a]

Name of Compounds	Lung (A549)	Prostate (DU-145)	Skin (SK-MEL-5)	Pancreatic (BxPC-3)	Liver (Hep G2)	Colon (HT-29)	Breast (MCF-7)	Breast (MDA-MB-231)
Cycloart-24-ene-3*β*,26-diol **199**	172.35	-	157.81	-	75.10	99.31	127.73	195.23
Vaticinone **200**	-	-	105.73	-	-	96.78	-	-
Schizandronic acid **201**	-	-	-	-	-	-	-	-
Cabraleahydroxylactone **202**	-	-	-	-	-	-	-	-
24(*E*)-cycloart-24-ene-26-ol-3-one **203**	-	-	96.58	-	-	11.48	86.20	-
24(*E*)-3*β*-hydroxycycloart-24-ene-26-al **204**	-	-	117.77	-	-	-	-	94.38
β- sitosterol **205**	-	-	-	-	-	-	-	-
Stigmast-5-ene-28-one **206**	-	-	-	-	-	-	-	-

[a] Results are expressed as IC_{50} values in μM . Blank indicates IC_{50} more than 200 μM

Table 4.2: Cytotoxicity of Drug Standards against Eight Cancel Cell Lines

(Mean ± SD, n=3)	**Drug standards**	
	Cisplatin	Vinblastine
Lung (A549)	36.17 ± 3.00 μM	29.01 ± 6.46 μM
Prostate (DU-145)	12.54 ± 0.50 μM	4.75 ± 1.13 μM
Skin (SK-MEL-5)	68.86 ± 1.13 μM	1.71 ± 0.24 μM
Pancreatic (BxPC-3)	22.10 ± 0.31 μM	2.03 ± 1.05 μM
Liver (Hep G2)	15.20 ± 1.04 μM	0.35 ± 0.41 μM
Colon (HT-29)	70.19 ± 2.21 μM	0.98 ± 0.33 μM
Breast (MCF-7)	90.11 ± 2.11 μM	28.11 ± 3.20 μM
Breast (MDA-MB-231)	306.73 ± 3.45 μM	35.32 ± 3.42 μM

CHAPTER 5

CONCLUSION

5.1 CONCLUSION

The chemical study on the hexane extract from the leaves of *Aglaia exima* was conducted and repeated chromatographic separation resulted in the isolation of one new compound; 24(*E*)-cycloart-24-ene-26-ol-3-one **203**, and seven known triterpenes and steroids; cycloart-24-ene-3*β*,26-diol **199**, vaticinone **200**, schizandronic acid **201**, cabraleahydroxylactone **202**, 24(*E*)-3*β*-hydroxy-cycloart-24-ene-26-al **204**, *β*-sitosterol **205** and stigmast-5-ene-28-one **206**. The elucidation of structures was confirmed through spectroscopic methods: NMR (nuclear magnetic resonance), MS (mass spectrometry), UV (ultraviolet), IR and X-ray analysis.

This particular *Aglaia* species (*Aglaia exima*) has not been studied before. However, there were chemical studies done on other *Aglaia* species and various types of triterpenoids were found in the plant of *Aglaia* species. Table 5.1 below shows the summary of the different types of triterpenoids found in *Aglaia* species. In this study, on *Aglaia exima,* three different types of triterpenoids (cycloartane, dammaranes and steroids) were found from the hexane crude of leaves. Of the eight pure compounds, four were obtained as colorless crystals which are cycloart-24-ene-3*β*,26-diol **199,** schizandronic acid **201**, cabraleahydroxylactone **202**, *β*-sitosterol **205** and stigmast-5-ene-28-one **206**.

From this study, the author has observed that the major compounds isolated from *Aglaia exima* were cycloartane-type triterpenoids which are similar to the findings from *A. forbesii, A. argentea, A. lawii, A. roxburghiana* and *A. harmsiana* (Table 5.1).

In addition, the majority of cycloartane-type triterpenoids isolated from the leaves of *Aglaia exima* were found to be cytotoxic against eight cancer cell lines; lung (A549), prostate (DU-145), skin (SK-MEL-5), pancreatic (BxPC-3), liver (Hep G2), colon (HT-29), breast (MCF-7) and (MDA-MB-231). The cycloart-24-ene-3-one-26-ol **203** was found to be very strongly active against colon (HT-29) cell line with IC_{50} values of 11.48 μM. No inhibitory effect was observed for the sterol and dammarane triterpenoids isolated from *Aglaia exima* (Chapter 4).

CHAPTER 6

EXPERIMENTAL

6.1 GENERAL

Solvents (hexane, dichloromethane, methanol) used for the bulk extraction are industrial grade and all of these solvents were distilled before use.

Shimazu UV-160A ultraviolet-visible spectrometer was used to obtain UV spectra with methanol as a solvent.

Infra- red spectra were obtained by using the Perkin Elmer 1600 Series FT-NMR, chloroform was used as solvent.

The ^{1}H-NMR spectra were taken by dissolving the sample in deuterated chloroform in the JEOL JNM-LA 400 FT-NMR system. Mostly of 13C, DEPT, HSQC and HMBC spectra were obtained from JEOL ECA 400. Chemical shifts is given in ppm on δ scale.

Mass spectra were carried out by Shimadzu Liquid Chromatograph Mass Spectrometer.

Silica gel 60 (0.063-0.200 mm) and silica gel 60 (0.040-0.063 mm) were used for column chromatography. Aluminium supported silica gel 60 F254 plates was used for TLC. The spots on the TLC were visualized under ultra-violet (UV) light (254 nm and 365 nm) followed with spraying by vanillin reagent. The vanilin reagent was prepared by diluting 10 mL of sulphuric acid with 90 mL of water in 100 mL ethanol and followed by adding 10 mg vanillin powder.

6.2 CYTOTOXIC ASSAY MATERIALS

Human cancer cell lines were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM), 100 mM non-essential amino acids, phosphate buffer solution (pH 7.2), 50 µg/ml gentamycin and 2.5 µg/ml amphotericin B were purchased from Invitrogen Corporation (Carlsbad, CA, USA). 200 mM L-glutamine, foetal bovine serum, 0.25% trypsin-EDTA, dimethyl sulphoxide (DMSO), cisplatin and vinblastine sulphate were purchased from Sigma–Aldrich (St. Louis, MO, USA). MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt] assay kit (CellTiter 96® AQ_{ueous} One Solution) was obtained from Promega (Madison, WI, USA).

6.3 PLANT MATERIAL

The leaves of *Aglaia exima* was collected from Hutan Simpanan Kg. Kepayang, Pahang, Malaysia on November 1997. Voucher specimen (KL 4762) has been deposited at the Herbarium of Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

6.4 EXTRACTION AND ISOLATION OF PLANT MATERIAL

Dried ground leaves (1 kg) of *Aglaia exima* were extracted exhaustively with hexane at room temperature for 4 days and then filter. The solution was decanted and then evaporated to leave a residue of 25 g hexane extracts. The extraction method depicted above was continued with dichloromethane and methanol. A yield of extracts for hexane (25 g), dichloromethane (64 g) and methanol (106 g) were obtained. The dichloromethane and methanol crude were kept for future use.

Of the 25 g of hexane crude, 15 g of the hexane crude was fractionated by using column chromatography over silica gel using a gradient mixture of hexane and ethyl acetate as eluent. Methanol was used at the end of separation in the purpose of flushing the column. Amount of silica used was in the ratio of 1 g mass of crude to 30 g silica gel (1:30). A total of 123 fractions were obtained from the rough separation of column chromatography. The fractions collected were then grouped into a series of fractions according to the R_f value and stains after monitoring with thin layer chromatography (TLC). Fraction tested on TLC with the same Rf value and color of spot was combined as a group. Each of the group were then separated and purified by repeated column chromatography (solvent mixture of hexane and ethyl acetate) and high pressure liquid chromatography (HPLC) until a single spot on TLC was obtained. From the 123 fractions, Fr. 54 was obtained as a colorless crystal **205** (34.2 mg). Meanwhile, further isolation of Fr. 67 (ethyl acetate-hexane 86:14, 0.72 g) in CC with silica gel (22 g) give **200** (6.5 mg), **203** (4.8 mg), **204** (25.6 mg) and **206** (5.1 mg). Besides, Fr. 94- Fr. 100 (ethyl acetate- hexane 60:40 50:50 20:80, 1.5398 g) were combined and further isolated by CC on silica gel (46 g) to furnish **201** (16.3 mg) and **202** (2.9 mg). Of 123 fractions, Fr. 92 was collected as a green crystal which was then recrystallized by ethyl acetate to obtain a colorless crystal, **199** (93.6 mg).

Table 6.1: Chromatographic Results and Yield of Compounds from the Leaves of *Aglaia exima*

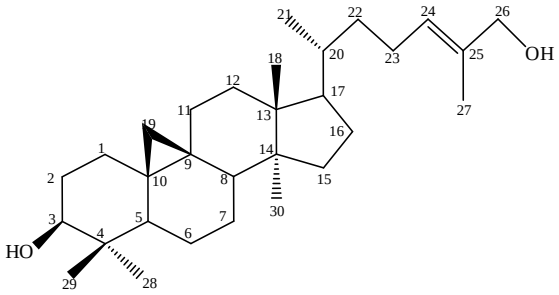
Solvent System for Column Chromatography (Hexane: Ethyl Acetate)	**Compounds**	**Yield (mg)**
90 : 10	*β*-Sitosterol **205**	34.2
86 : 14	Vaticinone **200**	6.5
	Cycloart-24-ene-3-one-26-ol **203**	19.1
	24(E)-3*β*-hydroxycycloart-24-ene-26-al **204**	25.6
	Stigmast-5-ene-28-one **206**	5.1
70 : 30	Cycloart-24-ene-3*β*,26-diol **199**	93.6
60 : 40	Schizandronic Acid **201**	16.3
50 : 50	Cabraleahydroxylactone **202**	2.9

6.5 METHOD OF CYTOTOXIC ASSAY

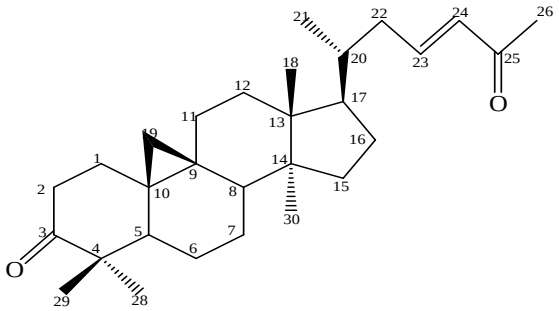
Cell lines were cultured in DMEM media supplemented with 2 mM L-glutamine, 10% foetal bovine serum, 50 µg/mL gentamycin and 2.5 µg/mL amphotericin B, maintained in a 37 ℃ humid atmosphere of 5% CO_2 cell incubator. Samples and drug standards (cisplatin and vinblastine sulphate) were dissolved in DMSO and immediately diluted with DMEM media to yield a final DMSO concentration of less than 0.5% v/v.

Cells were plated into 96-well microplates at 5,000–10,000 cells per well and maintained in the cell incubator for 24 hour. Then, 100 µL of samples were introduced in triplicates to a final concentration of 15–200 µM, with the exception of sample 5 that was further diluted down to 4 µM in BxPC-3 and HT-29 cell lines. Drug standards were also introduced to a final concentration of 0.03 - 2000 µM (cisplatin) and 0.002 - 100 µM (vinblastine sulphate). Cells were further incubated for 48 hours and then, cell viability was determined according to the manufacturer protocol of a commercial MTS assay kit (CellTiter 96 AQ_{ueous}® One Solution, Promega). Culture media were carefully refreshed with 100 µL of DMEM media, followed by 20 µL per well of MTS reagent. Microplates were returned to the incubator for 1 to 2 hours and absorbance of the formazan product was read on a microplate reader at 490nm with 690nm as the background wavelength (Infinite 200, Tecan, Männedorf, Swizerland). IC_{50} of samples and drug standards were determined using dose-response curves in Prism 5.02 software (GraphPad Software Inc., La Jolla, CA, USA).

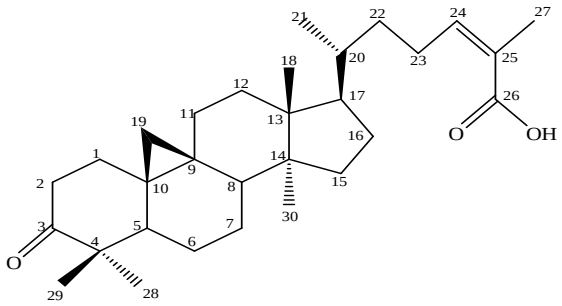
6.6 GENERAL SPECTRAL DATA OF ISOLATED COMPOUNDS



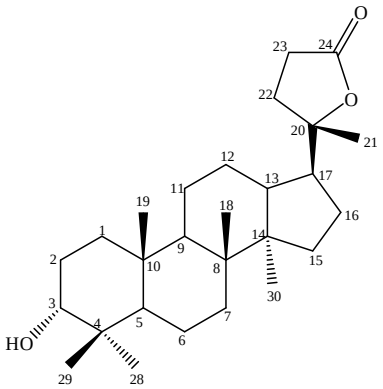
Cycloart-24-ene-3*β*,26-diol **199**	: Colorless Crystal
Molecular formula	: $C_{30}H_{50}O_2$
UV λ_{max} nm	: 210
IR υ_{max}	: 3400, 3042
Mass Spectrum *m/z*	: 442
^{1}H-NMR	: Refer Table 3.2
^{13}C-NMR	: Refer Table 3.2



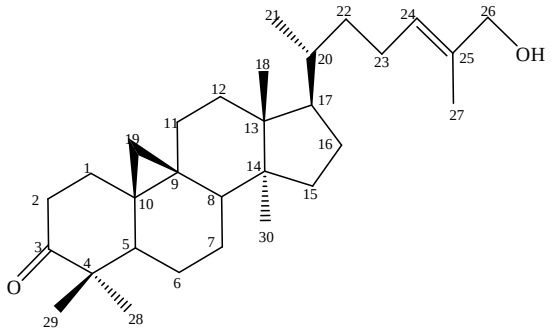
Vaticinone **200**	:White Powder
Molecular formula	: $C_{29}H_{44}O_2$
UV λ_{max} nm	: 221
IR υ_{max}	: 3437, 2929, 1707, 1621
Mass Spectrum *m/z*	: 424
^{1}H-NMR	: Refer Table 3.3
^{13}C-NMR	: Refer Table 3.3



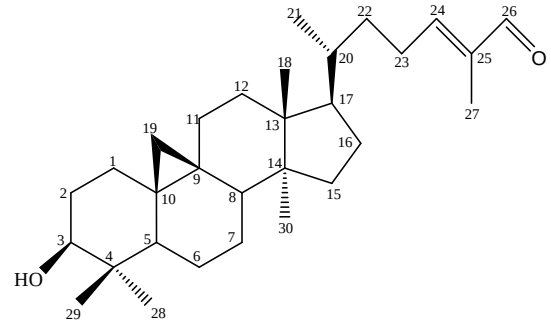
Schizandronic Acid **201** : Colorless Needle Crystal
Molecular formula : $C_{30}H_{46}O_3$
UV λ_{max} nm : 212
IR υ_{max} : 3393, 2934, 2869, 1705
Mass Spectrum *m/z* : 454
^{1}H-NMR : Refer Table 3.4
^{13}C-NMR : Refer Table 3.4



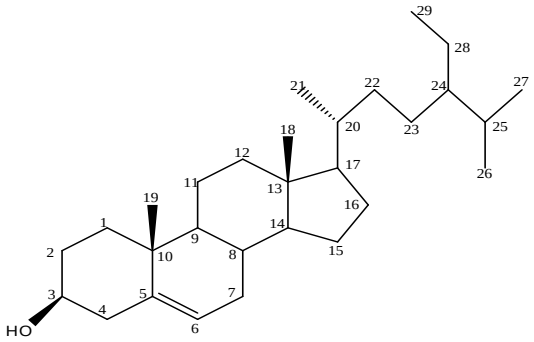
Cabraleahydroxylactone **202** : Colorless Crystal
Molecular formula : $C_{27}H_{44}O_3$
UV λ_{max} nm : -
IR υ_{max} : 3550, 2900, 2810, 1760
Mass Spectrum *m/z* : 416
^{1}H-NMR : Refer Table 3.5
^{13}C-NMR : Refer Table 3.5



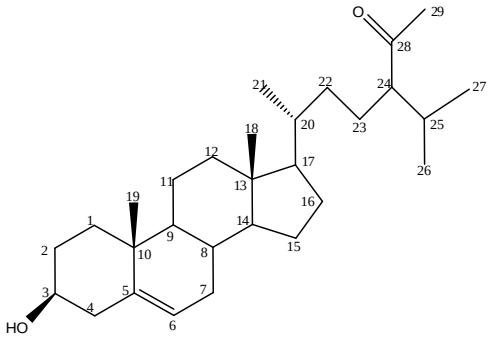
24(*E*)-cycloart-24-ene-26-ol-3-one **203**	: Colorless Amorphous Solid
Molecular formula	: $C_{30}H_{48}O_2$
UV λ_{max} nm	: 237
IR υ_{max}	: 3445, 2942, 2870, 1705
Mass Spectrum *m/z*	: 440
^{1}H-NMR	: Refer Table 3.6
^{13}C-NMR	: Refer Table 3.6



24(*E*)-3*β*-hydroxycycloart-24-ene-26-al **204**	: Pale Yellow Gel
Molecular formula	: $C_{30}H_{48}O_2$
UV λ_{max} nm	: 220
IR υ_{max}	: 3429, 2937, 2868, 1689
Mass Spectrum *m/z*	: 440
^{1}H-NMR	: Refer Table 3.7
^{13}C-NMR	: Refer Table 3.7



β-sitosterol **205**	: Colorless Crystal
Molecular formula	: $C_{29}H_{50}O$
UV λ_{max} nm	: 211
IR υ_{max}	: 3418, 2936, 1644, 1463
Mass Spectrum *m/z*	: 414
^{1}H-NMR	: Refer Table 3.8
^{13}C-NMR	: Refer Table 3.8



Stigmast-5-ene-28-one **206**	: Colorless Crystal
Molecular formula	: $C_{29}H_{48}O_2$
UV λ_{max} nm	: 208
IR υ_{max}	: 3419, 2934, 2852, 1713
Mass Spectrum *m/z*	: 412
^{1}H-NMR	: Refer Table 3.9
^{13}C-NMR	: Refer Table 3.9

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APPENDIX

Articles in Proceedings/Presented at Conferences/Seminar and Publication

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