Chemical Constituents from the Stem of *Punica granatum* and the Root of *Michelia alba*

Jintana Pongpuntaruk

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Organic Chemistry
Prince of Songkla University
2010

Thesis Title	Chemical Constituents from the Stem of □□□□□□□□□□□□□□□□ and the Root of □ □□□□□□□□□
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ชื่อวิทยานิพนธ์ องค์ประกอบทางเคมีจากลำต้นทับทิมและรากจำปี
ผู้เขียน นางสาวจินตนา พงศ์ภัณฑารักษ์
สาขาวิชา เคมีอินทรีย์
ปีการศึกษา 2552

บทคัดย่อ

ตอนที่ 1 องค์ประกอบทางเคมีจากลำต้นทับทิม

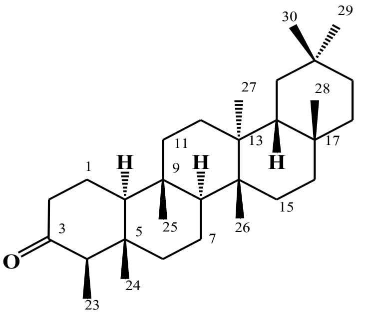
การศึกษาองค์ประกอบทางเคมีของส่วนสกัดหยาบเมทิลีนคลอไรด์ และ อะซีโตน จากลำต้นทับทิม สามารถแยกสารที่มีรายงานแล้วจำนวน 13 สาร ซึ่งเป็นสารประเภท triterpene 3 สาร คือ friedelin (**CMD1**), 5(6)-gluten-3 -ol (**CMD2**) และ betulinic acid (**CMD3**), สารประเภท steroids 7 สาร คือ สารผสมของ -sitosterol (**CMD4**) และ stigmasterol (**CMD5**), stigmast-4-en-3-one (**CMD6**), 6 -hydroxystigmast-4-en-3-one **(CMD7),** ergosterol peroxide **(CMD8),** 5 -cholest-7-en-3-one **(CMD9)** และ lophenol (**CMD10**), 5-methylmellein (**CMD11**), 3,4,3′-tri-O-methylellagic acid (**CMD12**), 5,7,3′,4′,5′-penta-O-methylgallocatechin (**CMD13**)

โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี

ตอนที่ 2 องค์ประกอบทางเคมีจากรากจำปี

การศึกษาองค์ประกอบทางเคมีของส่วนสกัด หยาบเมทิลีนคลอไรด์ จากรากจำปี สามารถแยกสารได้จำนวน 7 สาร ซึ่งเป็นสารประเภท sesquiterpene 6 สาร คือ costunolide (**JPD1**), parthenolide (**JPD2**), 9β-hydroxy-11βH-dihydroparthenolide (**JPD3**), reynosin (**JPD4**), T-cadinol (**JPD5**), สารใหม่ 1 สาร คือ -(3′,4′,5′-trihydroxy-3′-methylbutanoyloxy)-11βH-dihydroparthenolide (**JPD6**) และสารประเภท lignan 1 สาร คือ lariciresinol **(JPD7)**

โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี



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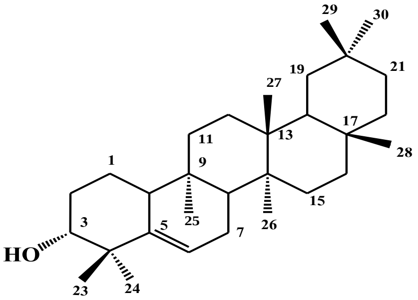

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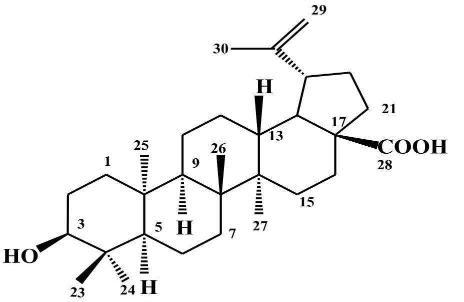

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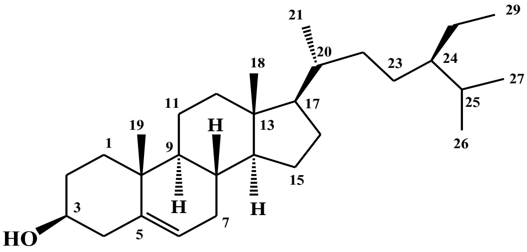

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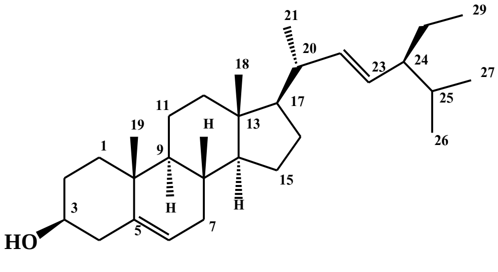

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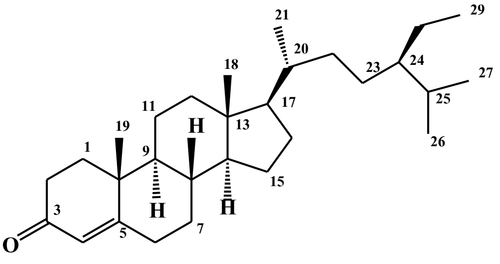

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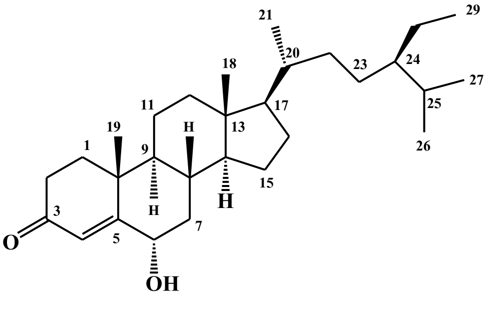

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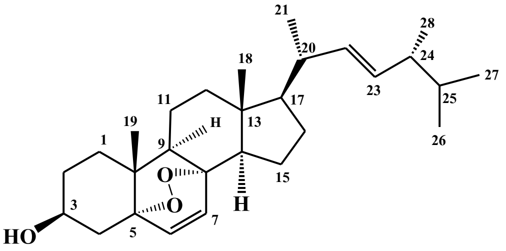

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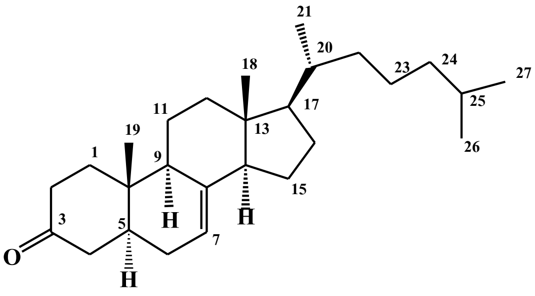

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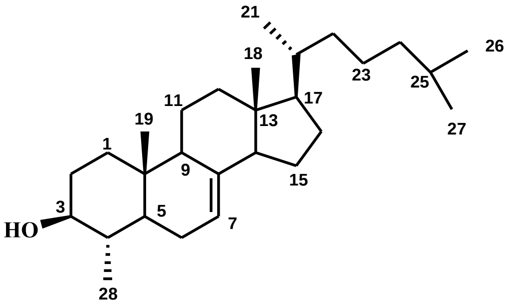

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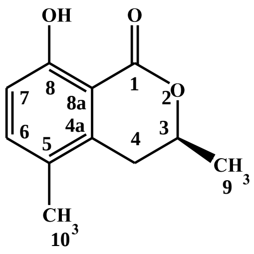

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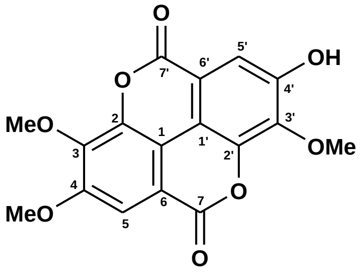

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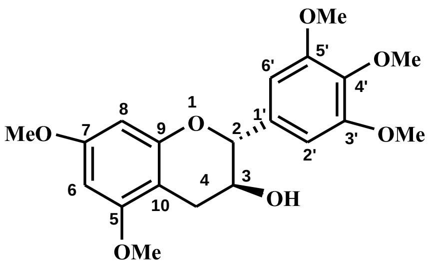

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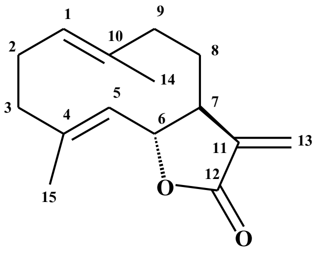

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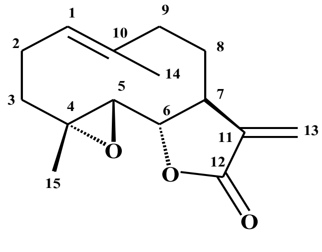

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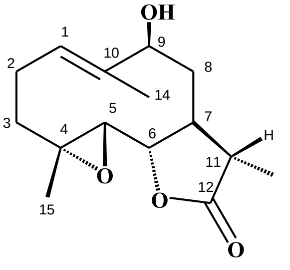

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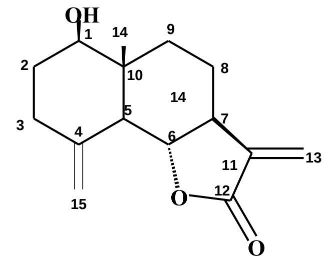

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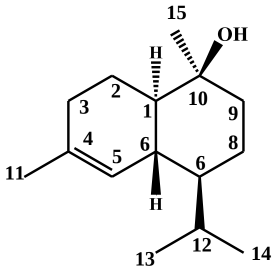

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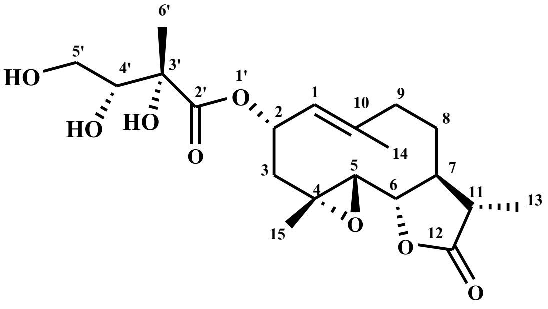

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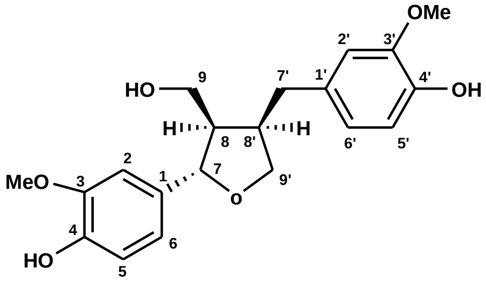


JPD7

Thesis Title	Chemical Constituents from the Stem of [illegible] and the Root of [illegible]
Author	Miss. Jintana Pongpuntaruk
Major Program	Organic Chemistry
Academic Year	2009

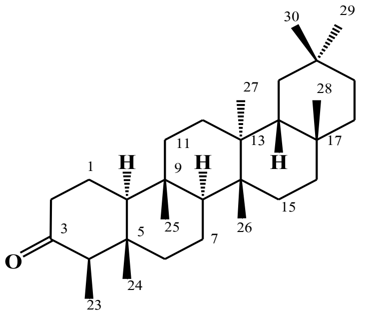
ABSTRACT

Part I Chemical Constituents from the Stem of Punica granatum

Investigation of the crude methylene chloride and acetone extracts of the stem of [illegible], yielded 13 known compounds; three triterpenes: friedelin (**CMD1**), 5(6)-gluten- -ol (**CMD2**) and betulinic acid (**CMD3**), seven steroids: a mixture of β-sitosterol (**CMD4**) and stigmasterol (**CMD5**), stigmast-4-en-3-one (**CMD6** -hydroxystigmast-4-en-3-one **(CMD7)**, ergosterol peroxide **(CMD8)**, -cholest-7-en-3-one **(CMD9)** and lophenol (**CMD10**), 5-methylmellein (**CMD11**), 3,4,3′-tri-O-methylellagic acid (**CMD12**) and 5,7,3′,4′,5′-penta-O-methylgallocatechin (**CMD13**). Their structures were elucidated by spectroscopic methods.

Part II Chemical Constituents from the root of Michelia alba

Investigation of the crude methylene chloride extract of the root of [illegible], yielded 7 compounds; six sesquiterpenes: costunolide (**JPD1**), parthenolide (**JPD2**), 9β-hydroxy-11βH-dihydroparthenolide (**JPD3**), reynosin (**JPD4**), T-cadinol (**JPD5**), a new compound -(3′,4′,5′-trihydroxy-3′-methylbutanoyloxy)-11βH-dihydroparthenolide (**JPD6**) and one lignan: lariciresinol **(JPD7)**. Their structures were elucidated by spectroscopic methods.



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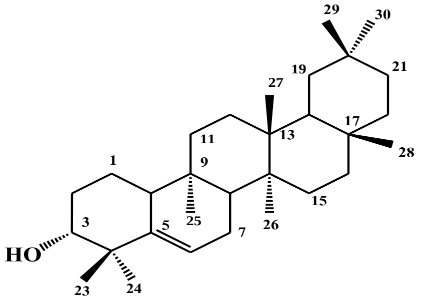

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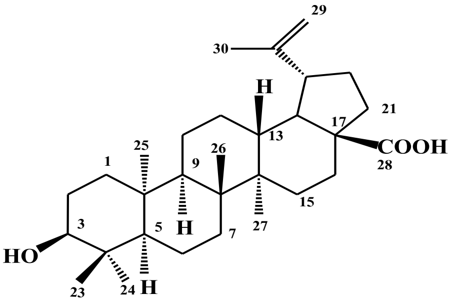

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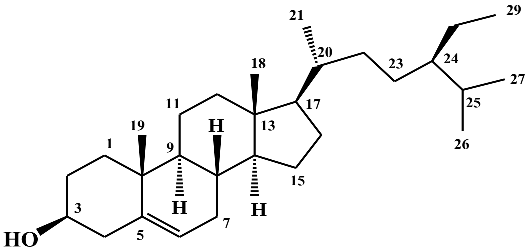

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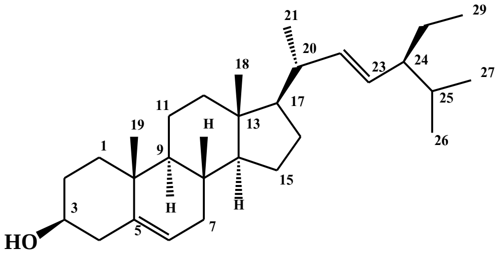

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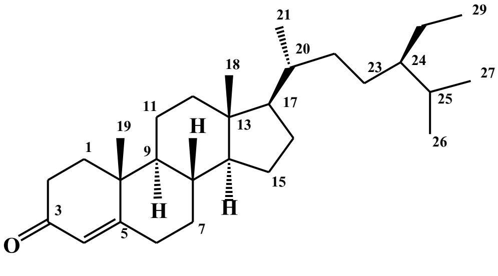

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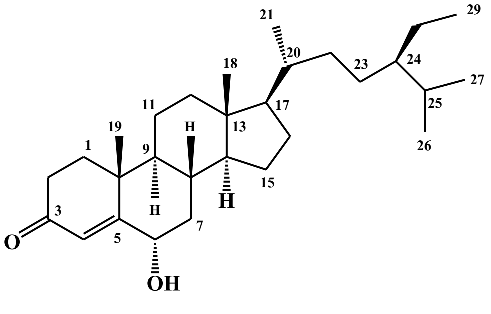

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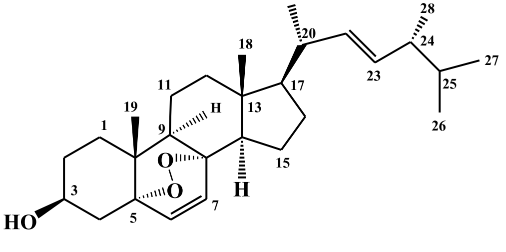

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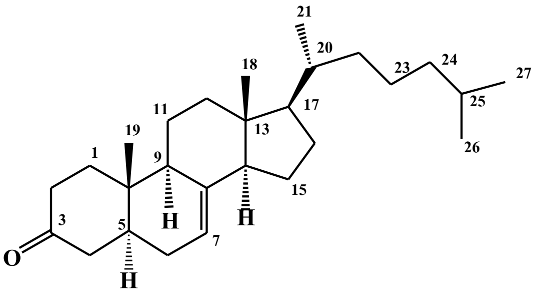

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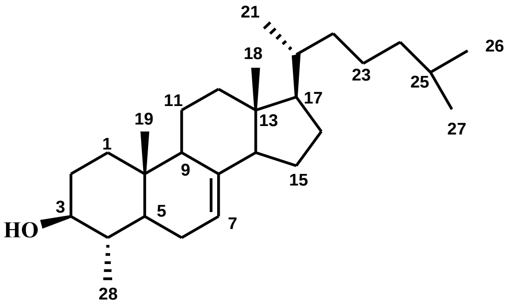

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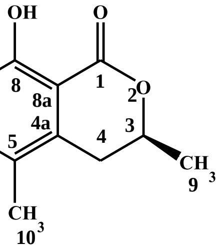

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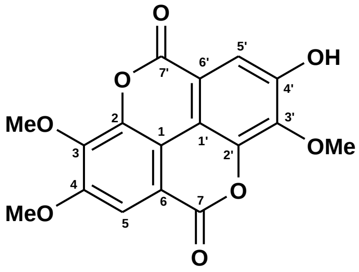

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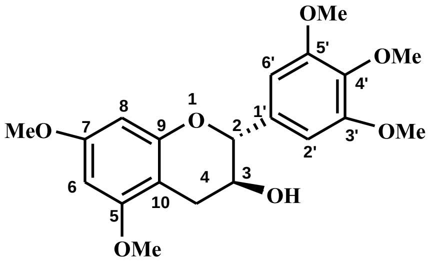

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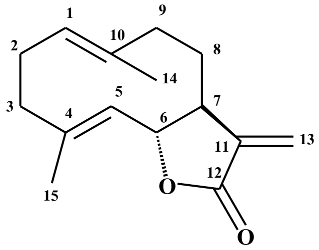

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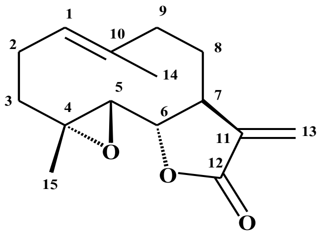

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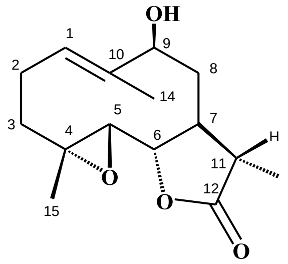

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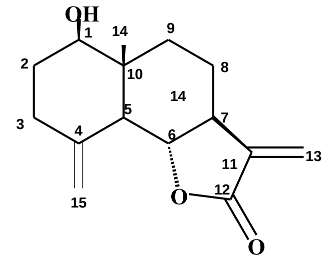

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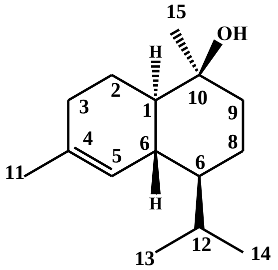

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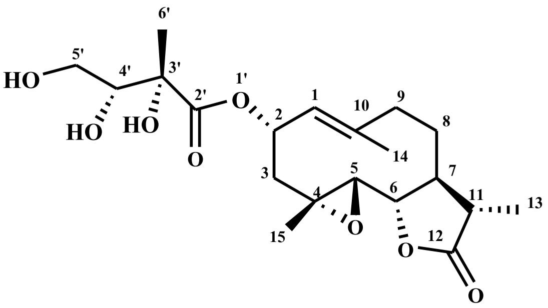

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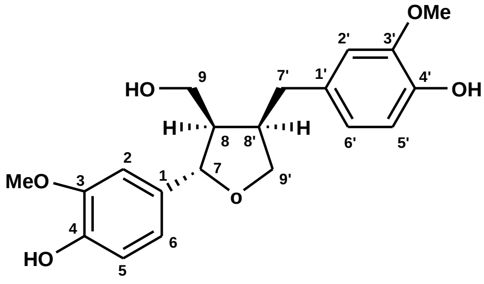

JPD7

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Jintana Pongpuntaruk

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this research is to investigate the chemical constituents from the stem of and the root of They are a part of the basic research on the Thai medicinal plants. Thirteen compounds and seven compounds have been isolated from the stem of and the root of respectively.

CONTENTS

PART 1 Chemical constituents from the stem of *Punica granatum*

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LIST OF ILLUSTATIONS (Continued)

LIST OF ILLUSTATIONS (Continued)

LIST OF ABBREVIATIONS AND SYMBOLS

□ = singlet

□ = doublet

□ = triplet

□ = quartet

□ = multiplet

□□ = doublet of doublet

□□□□ □□□□□□□□ □□□□□□□doublet of doublet of doublet □

□□ = doublet of triplet

□□□□ □□□□□□□□ □□□□□□□doublet of doublet of quartet □

□□□ = broad singlet

□□□ = broad doublet

g = gram

nm = nanometer

mp = melting point

cm^{-1} = reciprocal centimeter (wave number)

= chemical shift relative to TMS

□ = coupling constant

$_D$ = specific rotation

$_{max}$ = maximum wavelength

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

= absorption frequencies

= molar extinction coefficient

m/z = a value of mass divided by charge

°C = degree celcius

MHz = Megahertz

ppm = part per million

c = concentration

IR = Infrared

UV = Ultraviolet

MS = Mass Spectroscopy

EIMS = Electron Impact Mass Spectroscopy

FAB = Fast atom bombardment mass spectrometry

NMR = Nuclear Magnetic Resonance

1D NMR = One Dimensional Nuclear Magnetic Resonance

2D NMR = Two Dimensional Nuclear Magnetic Resonance

COSY = Correlation Spectroscopy

DEPT = Distortionless Enhancement by Polarization Transfer

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

HMBC = Heteronuclear Multiple Bond Correlation

HMQC = Heteronuclear Multiple Quantum Coherence

NOESY = Nuclear Overhauser Effect Spectrosopy

CC = Column Chromatography

QCC = Quick Column Chromatography

PLC = Preparative Thin Layer Chromatography

TMS = tetramethylsilane

$CDCl_3$ = deuterochloroform

CD_3OD = deuteromethanol

DMSO-d_6 = deuterodimethylsulfoxide

CHAPTER 1.1

Introduction

1.1.1 Introduction

Punica granatum LINN. (pomegranate in English), is widely distributed in Southeast Asia. It is an ancient and highly distinctive fruit, the predominant member of two species comprising the Punicaceae family, *granatum* and *protopunica*. The pomegranate fruit as a medicinal plant (Al-Maiman & Ahnad, 2002) is now supported by data obtained from modern science showing that the fruit contains anti-carcinogenic (e.g., Adhami & Mukhtar, 2006; Bell & Hawthorne, 2008), anti-microbial (Reddy, Gupta, Jacob, Khan, & Ferreira, 2007) and anti-viral compounds (Kotwal, 2007; (Shwartza *et al*., 2009). The methanolic extract from the flowers of *P. granatum* was found to inhibit a tumor necrosis factor-a (TNF-a)-induced cytotoxicity in L929 cells. (Xie *et al*., 2008).

P. granatum is a small–sized, shrubby tree, 12-16 feet tall, has spiny branches. The leaves are glossy and lanceshaped, and the bark of the tree turns gray as the tree ages. The flowers are large, red, white, or variegated and have a tubular calyx that eventually becomes the fruit. The ripe pomegranate fruit can be up to five inches wide with a deep red, leathery skin, is grenade-shaped, and crowned by the pointed calyx. The fruit contains many seeds separated by white, membranous pericarp, and each is surrounded by small amounts of tart red juice.

In Thailand, *P. granatum* has been found in every part of the country. It has many local Thai names : Thapthim (ทับทิม) Central; Phila (พิลา) Nong Khai; Phila Khao (พิลาขาว), Ma kong kaeo (มะก่องแก้ว) Nan; Ma Ko (มะเก๊าะ) Northern; Makchange (หมากจัง) Mee Hong Son (Smitinand, 2001).

a. trees **b.** stem

c. leaves **d.** fruits

e. seeds **f.** flowers

Figure 1 Different parts of *Punica granatum* LINN.

1.1.2 Review of Literatures

Chemical constituents isolated from *P. granatum* were summarized in **Table 1**. Information obtained from SciFinder Scholar copyright in 2009 will be presented and classified into groups: alkaloids, steroids, flavonoids, tannins, catechins, ellagic, coumarins, gallic acid, prenylpropanoid and triterpenoids.

Table 1 Compounds from plants of Punica genus.

a: tannins	b: flavonoids
c: steroids	d: triterpenes
e: alkaloids	f: ellagic acid
g: catechins	h: gallic acid
i: coumarins	j: phenylpropanoid

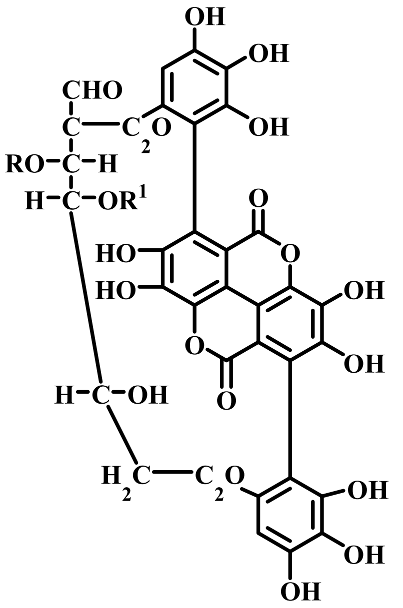
Scientific name	Part	Compounds	Bibliography
P. granatum	Fruit	punicalagin, **1a** punicalin, **2a**	Mayer *et al*., 1977
	Stem Bark	4,6-(*S,S*)-gallagyl-D-glucose, **3a** 2,3-(*S*)-hexahydroxydiphenoyl-4,6-(*S,S*)-gallagyl-D-glucose, **4a** 2-O-galloyl-4,6-(*S,S*)-gallagyl-D-glucose, **5a**	Tanaka *et al*., 1986
	Seeds	estrone, **3c** coumestrol, **1i** genistein, **1b** daidzein**, 2b** genistin, **3b** daidzin, **4b**	Moneam *et al*., 1988
	Root Bark	hygrine, **1e** sedridine, **2e** pseudopelletierine**, 3e** pelletierine**, 4e** norpseudopelletierine, **5e** *N*-methylpelletierine, **6e** norhygrine, **7e**	Neuhofer *et al*., 1993

Scientific name	Part	Compounds	Bibliography
	Head wood	3'-O-methyl-3,4-methylenedioxyellagic acid, **1f** methyl gallate, **1h** gallic acid, **2h** ellagic acid, **2f** 3,3'-di-O-methyl-ellagic acid, **2f** corilagin, **8a**	Tommy *et al.*, 2001
	Fruit	prodelphinidin B, **1g** prodelphinidin C**, 2g** catechin-(4-8)-gallocatechin, **3g** gallocatechin, **4g**	Plumb *et al.*, 2002
	Fruit	α-punicalagin, **6a** β-punicalagin**, 7a**	Machado *et al.*, 2002
	Seed	coniferyl 9-O-[β-D-apiofuranosyl(1β6)]-O-β-D-glucopyranoside, **1j** sinapyl 9-O-[β-D-apiofuranosyl(1β6)]-O-β-D-glucopyranoside, **2j** daucosterol, **1c** 3,3'-di-O-Methylellagic acid**, 3f** 3,3',4'-tri-O-Methylellagic acid, **4f**	Wang *et al.*, 2004
	Flower	pomegranatate, **5f** daucosterol, **1c** ellagic acid, **2f** maslinic acid, **1d** 3,3',4'-tri-O-Methylellagic acid, **4f** ethyl brevifolincarboxylate, **2i**	Wang *et al.*, 2006
Scientific	Part	Compounds	Bibliography

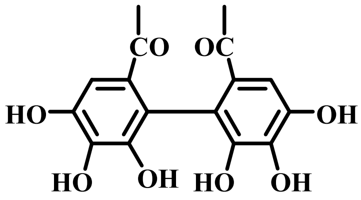
name			
	Flower	punicanolic acid, **2d** ursolic acid, **3d** β-sitosterol, **2c** asiatic acid, **2b** luteolin, **4d** tricetin , **6b** maslinic acid, **1d** 1,2,6-tri-O-Galloyl -β-D-glucopyranoside, **9a** 1,2-di-O-Galloyl-4,6-O-(*S*)-hexahydroxydiphenoyl -β-D-glucopyranoside, **10a**	Xie *et al.*,2008

structures

a: tannins



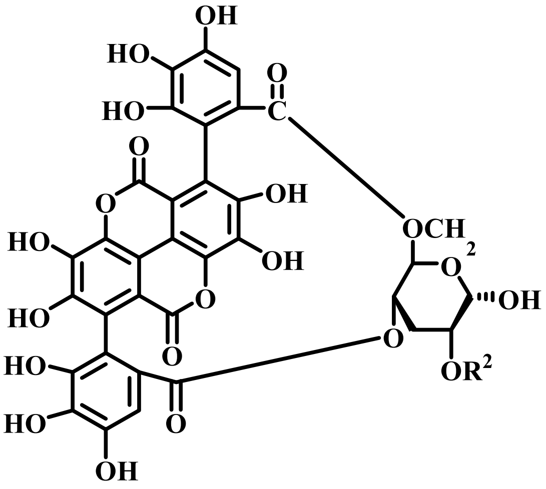
I, RR^1 =



II, R = R^1 = H

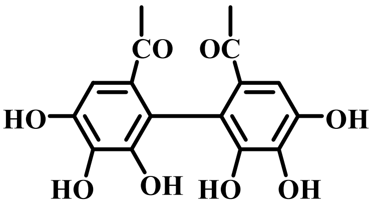
1a: punicalagin (I)

2a: punicalin (II)

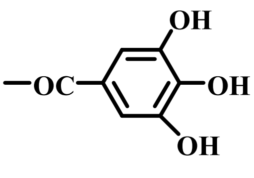


I, R = R^1 = H

II, R^{1}R^2 =



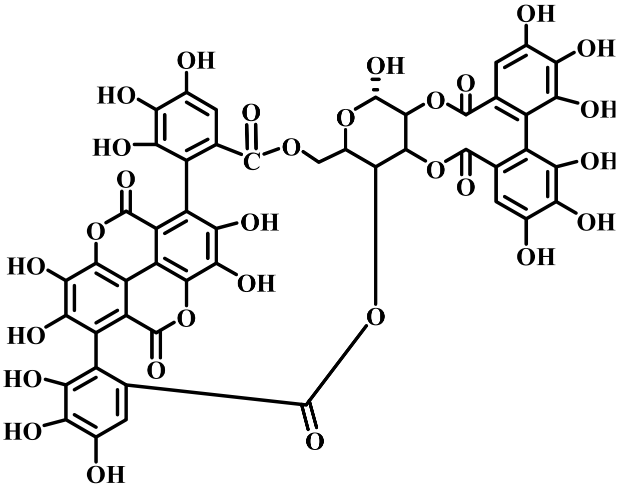
III, R^1 = H, R^2 =



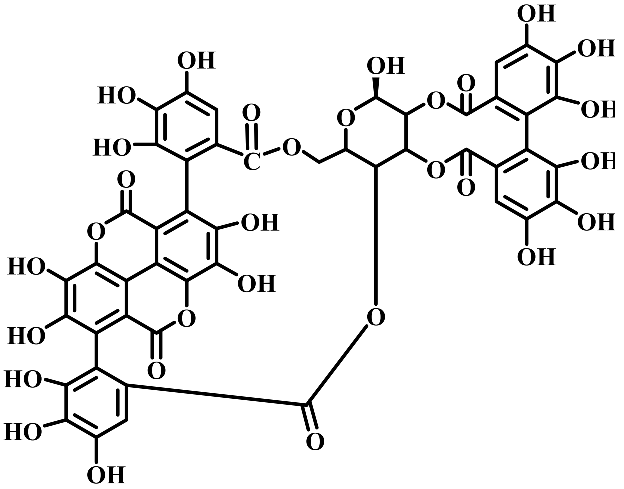
3a: 4,6-(*S,S*)-gallagyl-D-glucose

4a: 2,3-(*S*)-hexahydroxydiphenoyl-4,6(*S,S*)gallagyl-D-glucose

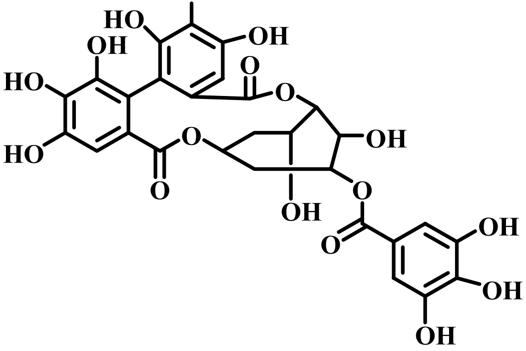
5a: 2-O-galloyl-4,6-(*S,S*)-gallagyl-glucose



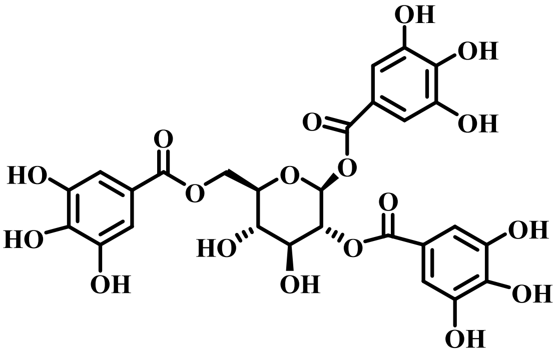
6a. α-punicalagin



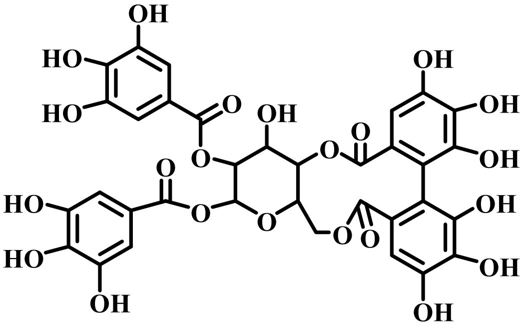
7a: β-punicalagin



8a: corilagin

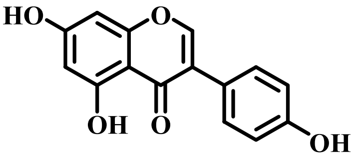


9a: 1,2,6-tri-O-galloyl -β-D-glucopyranoside

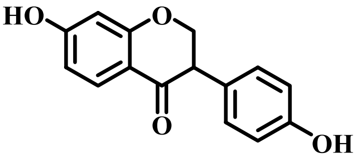


10a: 1,2-di-O-galloyl-4,6-O-(*S*)-hexahydroxydiphenoyl glucopyranoside

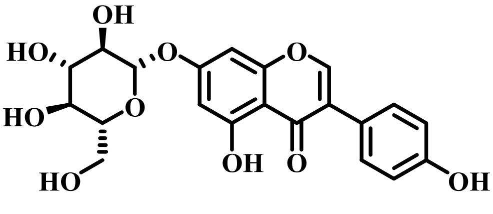
b: flavonoids



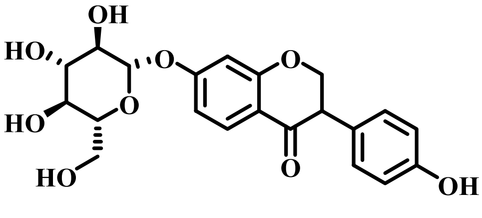
1b: genistein



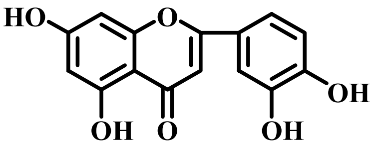
2b: daidzein



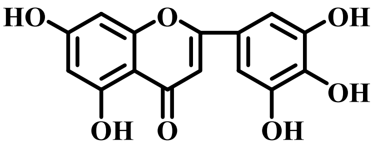
3b: genistin



4b: daidzin

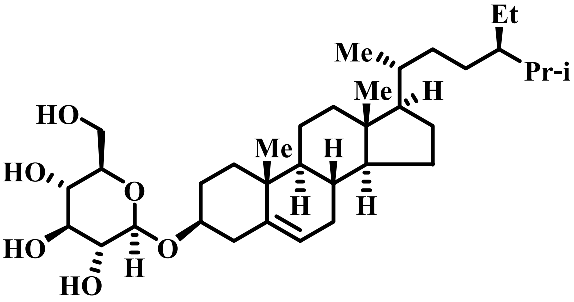


5b: asiatic acid

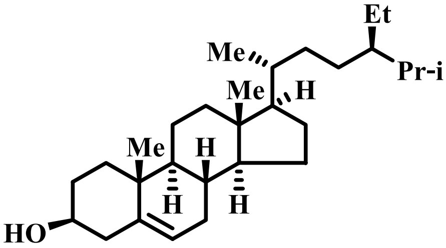


6b: tricetin

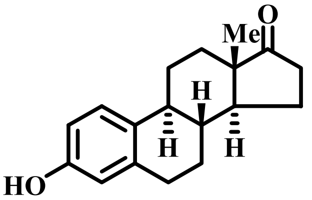
c: steroids



1c: daucosterol

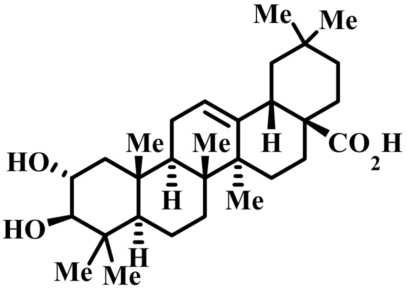


2c: β–sitosterol

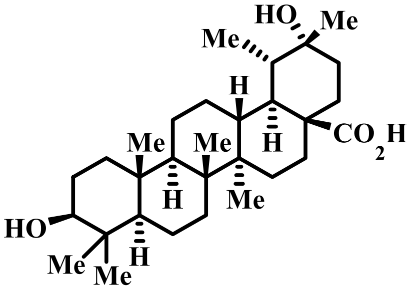


3c: estrone

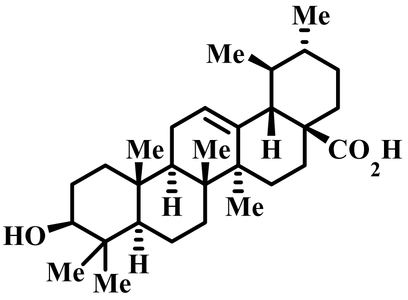
d: triterpenes



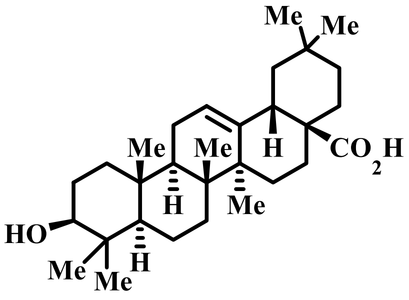
1d: maslinic acid



2d: punicanolic acid

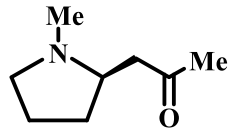


3d: ursolic acid

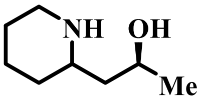


4d: luteolin

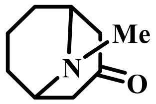
e: alkaloids



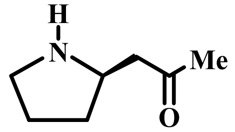
1e: hygrine



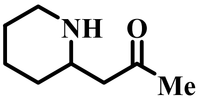
2e: sedridin



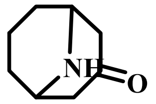
3e: pseudopelletierine



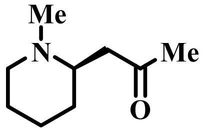
4e: pelletierine



5e: norpseudopelletierine

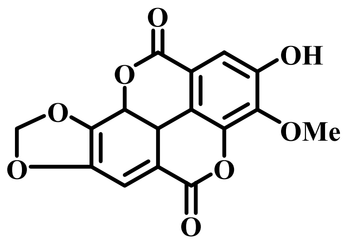


6e: *N*-methylpelletierine

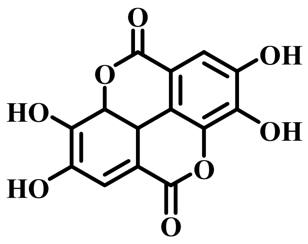


7e: norhygrine

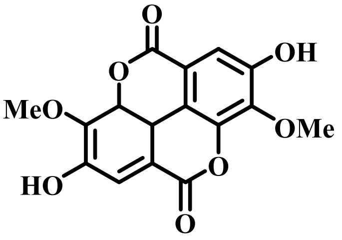
f: ellagic acid



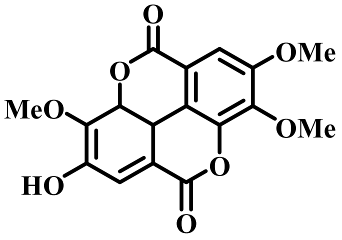
1f: 3'-O-methyl-3,4-methylenedioxyellagic acid



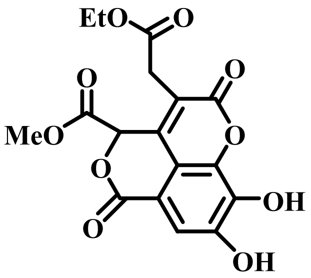
2f: ellagic acid



3f: 3,3'-di-O-methyl-ellagic acid

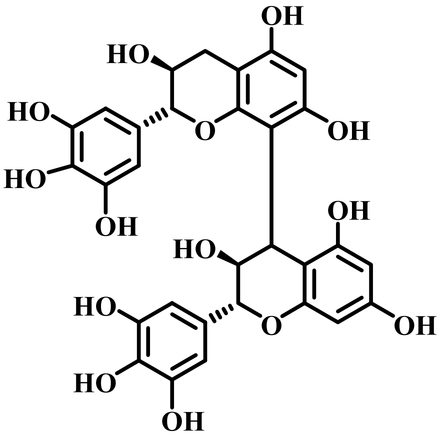


4f: 3,3',4'-tri-O-methylellagic acid

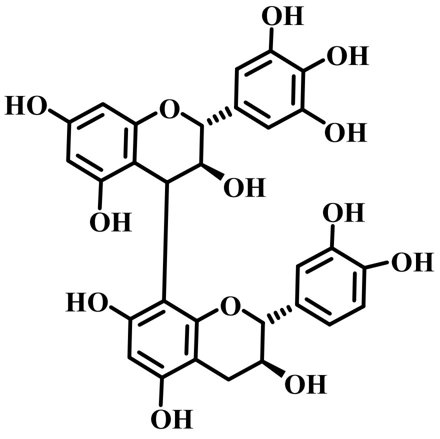


5f: pomegranatate

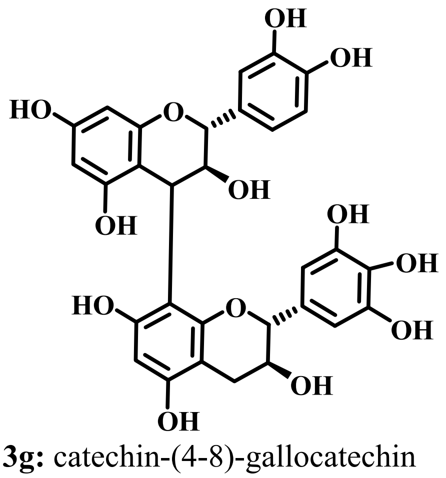
g: catechins



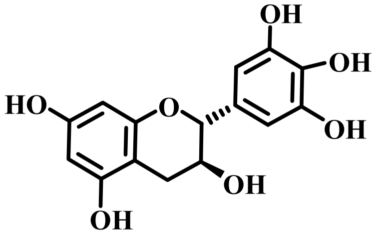
1g: prodelphinidin B



2g: prodelphinidin C

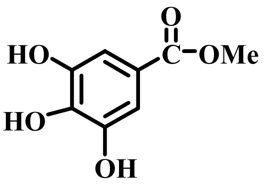


3g: catechin-(4-8)-gallocatechin

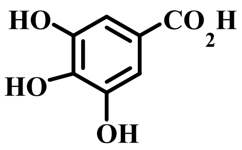


4g: gallocatechin

h: gallic acid

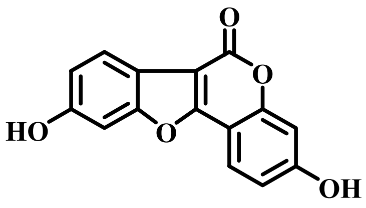


1h: methyl gallate

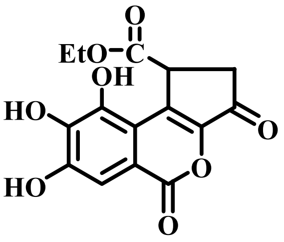


2h: gallic acid

i: coumarins

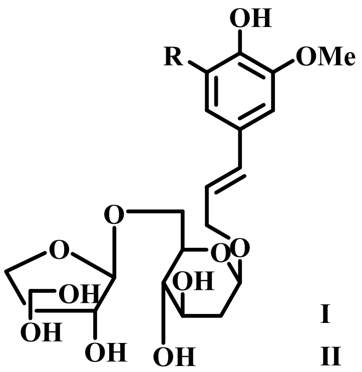


1i: coumestrol



2i: ethyl brevifolincarboxylate

j: phenylpropanoids



I **R = H**

II **R = OMe**

1j: coniferyl 9-O-[β-D-apiofuranosyl(1β6)]-O-β-D-glucopyranoside

2j: sinapyl 9-O-[β-D-apiofuranosyl(1β6)]-O-β-D-glucopyranoside

1.1.3 Objective

This part of research work involved isolation, purification and structure elucidation of chemical constituents from the stem of *Punica granatum*.

CHAPTER 1.2

EXPERIMENTAL

1.2.1 Instruments and Chemicals

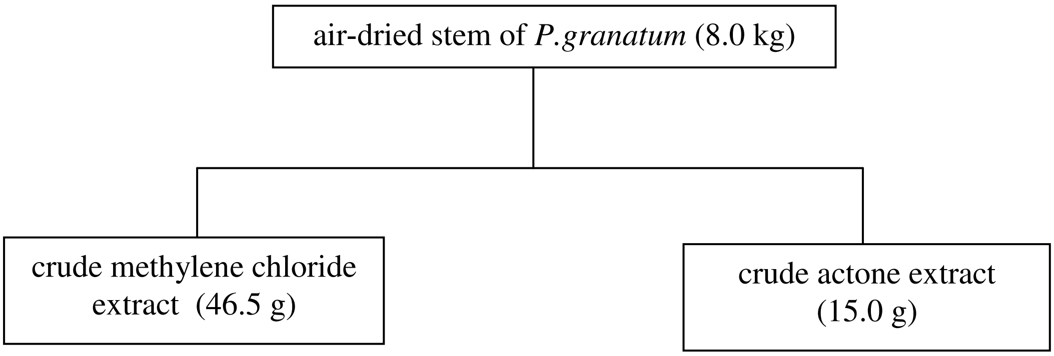
Melting points were determined on the Fisher-John melting point apparatus. The UV spectra were measured with a SPECORD S 100 (Analytikjena) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in MeOH solution. The optical rotation $[\alpha]_D$ was measured in chloroform and methanol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

1.2.2 Plant Material

The stem of *P. granatum* was collected from Chumphon province in the southern part of Thailand, in May 2008. Identification was made by Assoc. Prof. Dr. Kitichate Sridith and a specimen (No.0013591) deposited at PSU Herbarium, Department of Biology, Faculty of Science, Prince of Songkla University.

1.2.3 Extraction and Isolation

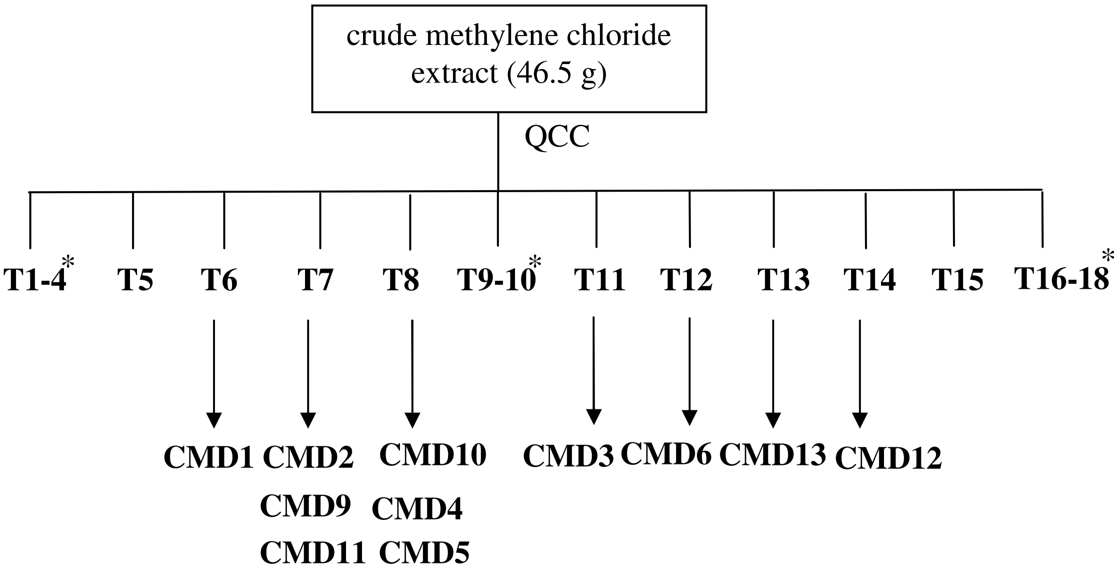
The chopped air-dried stem of *P. granatum* (8.0 kg) was successively extracted with methylene chloride and acetone (one week for each solvent) at room temperature. The solvent was evaporated under reduced pressure to give concentrated solution of methylene chloride extract as yellow viscous residue (46.5 g) and brownish acetone extract (15.0 g), respectively. The process of extraction was shown in **Scheme 1**



Scheme 1 Extraction of the stem of *P.granatum*

1.2.4 Isolation and Chemical Investigation

1.2.4.1 Investigation of the crude methylene chloride extract from the stem of *P. granatum*.



*No further investigation

Scheme 2 Isolation of compounds **CMD1- CMD6, CMD9- CMD13** from the methylene chloride extract

The crude methylene chloride extract as yellow viscous residue (46.5 g) was subjected to quick column chromatography over silica gel using solvent of increasing polarity from hexane through acetone. The eluates were collected and combined based on TLC characteristics to give eighteen fractions (T1-T18).

Fraction T6 (4.5 g) was filtered and washed with hexane to give **CMD1**: friedelin (1.2 g) as white crystal and the mother liquor as yellow viscous oil after evaporation of the solvent.

Fraction T7 (3.5 g) was purified by QCC with a gradient of acetone-hexane to afford twenty fractions (T7.1-T7.20).

Subfraction T7.15 (135.5 mg) was recrystallized from the methylene chloride to give **CMD9**: 5α-cholest-7-en-3-one (58.0 mg).

Subfraction T7.17 (56.7 mg) was purified by CC with 7% EtOAc/hexane to give **CMD2**: 5(6)-gluten-3α-ol (9.4 mg).

Subfraction T7.20 (30.2 mg) was purified by CC with 20% acetone/hexane to give **CMD11**: 5-methylmellein (4.5 mg).

Fraction 8 (6.7 g) was separated by CC with a gradient of acetone-hexane to afford twelve fractions (T8.1-T8.12).

Subfraction T8.7 (3.6 g) was filtered and washed with hexane to yield a mixture of **CMD4**: β-sitosterol and **CMD5**: stigmasterol (2.3 g) as a white solid and the mother liquor as yellow viscous oil after evaporation of the solvent.

Subfraction T8.10 (43.2 mg) was purified by CC with 15% acetone/hexane to give **CMD10**: lophenol (10.7 mg).

Fraction T11 (4.1g) was separated by CC with 30% EtOAc/hexane to give **CMD3**: betulinic acid (1.7 g).

Fraction T12 (1.2 g) was separated by CC with 30% acetone/hexane to give **CMD6**: stigmast-4-en-3-one (30 mg).

Fraction T13 (113.6 mg) was separated by CC with 30% acetone/hexane to give **CMD13**: 5,7,3′,4′,5′-penta-O-methylgallocatechin (8.2 mg).

Fraction T14 (221.7 mg) was separated by CC with 30% EtOAc/hexane to give **CMD12**: 3,4,3′-tri-O-methylellagic acid (9.9 mg).

Compound CMD1: friedelin, white solid, m.p. 245-247°C; $[\alpha]_D^{28}$: -28.2° (c = 0.63, $CHCl_3$); ref $[\alpha]_D^{28}$: -22.3° (c = 0.54, $CHCl_3$) (Ahad *et al*., 1991); IR (neat) ν_{max} 1715 (C=O stretching) cm^{-1}. For ^1H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 2**.

Compound CMD2: 5(6)-gluten-3α-ol, white solid, m.p. 210-212°C; $[\alpha]_D^{28}$: +61.6° (c = 0.7, $CHCl_3$); IR (neat) ν_{max} 3415 (O-H stretching) and 1618 (C=C stretching) cm^{-1}. For ^1H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 3**.

Compound CMD3: betulinic acid, white solid, m.p. 280-282°C; $[\alpha]_D^{28}$: +18.7° (c = 0.03, $CHCl_3$); ref $[\alpha]_D^{28}$: +17.7° (c = 0.03, $CHCl_3$) (Thongdeeying, 2005); IR (neat) ν_{max} 3413 (O-H stretching), 1686 (C=O stretching) and 1645 (C=C stretching) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 4**.

Compounds CMD4 and CMD5: a mixture of β-sitosterol and stigmasterol, white solid; IR (neat) ν_{max} 3425 (O-H stretching) and 1642 (C=C stretching) cm^{-1}.

Compound CMD6: stigmast-4-en-3-one, colorless viscous oil; $[\alpha]_D^{28}$: +67.7° (c = 0.47, $CHCl_3$); ref $[\alpha]_D^{28}$: +66.4° (c = 0.40, $CHCl_3$) (Della *et al.*, 1990); UV λ_{max} (MeOH) (log ε): 241 (4.21) nm; IR (neat) ν_{max} 1674 (C=O stretching) and 1616 (C=C stretching) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 5**.

Compound CMD9: 5α-cholest-7-en-3-one, white solid, m.p. 144-146 °C; $[\alpha]_D^{28}$: +12.1° (c = 0.05, $CHCl_3$); IR (neat) ν_{max} 3424 (O-H stretching) and 1616 (C=C stretching) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 8**.

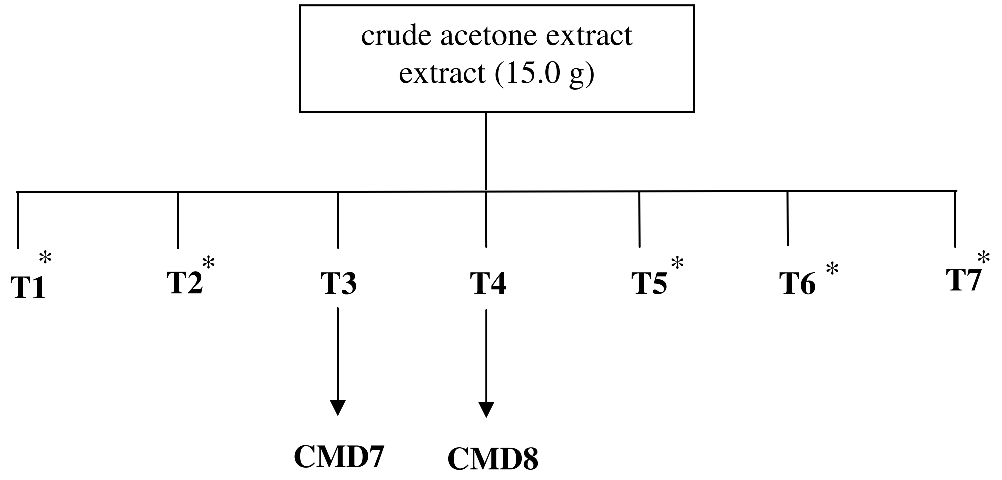
Compound CMD10: lophenol, white solid, m.p. 149-150°C; $[\alpha]_D^{28}$: +7.0° (c = 0.04, $CHCl_3$); ref $[\alpha]_D^{28}$: +5.0° (c = 0.03, $CHCl_3$) (Farines *et al.*, 1988); IR (neat) ν_{max} 3424 (O-H stretching) and 1618 (C=C stretching) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 9**.

Compound CMD11: 5-methylmellein, colorless viscous oil; $[\alpha]_D^{28}$: -122° (c = 0.8, $CHCl_3$); ref $[\alpha]_D^{28}$: -118° (c = 0.06, $CHCl_3$) (Cambie *et al.*, 1991); UV λ_{max} (MeOH) (log ε): 208 (3.32) nm; IR (neat) ν_{max} 3290 (O-H stretching), 1669 (C=O stretching) and 1610 (aromatic) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 10**.

Compound CMD12: 3,4,3′-tri-O-methylellagic acid, white solid; UV λ_{max} (MeOH) (log ε): 248 (3.55) and 371 (2.94) nm; IR (neat) ν_{max} 3400 (O-H stretching), 1744 (C=O stretching) and 1602 (aromatic) cm^{-1}. For ^1H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 11.**

Compound CMD13: 5,7,3′,4′,5′-penta-O-methylgallocatechin, colorless viscous oil; $[\alpha]_D^{28}$: -47.7° (c = 0.07, $CHCl_3$); UV λ_{max} (MeOH) (log ε): 207 (3.34) and 270 (2.59) nm; IR (neat) ν_{max} 3453 (O-H stretching) and 1602 (aromatic) cm^{-1}. For ^1H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 12.**

1.2.4.2 Investigation of the crude acetone extract from the stem of *P. granatum*



*No further investigation

Scheme 3 Isolation of compounds **CMD7** and **CMD8** from the acetone extract.

The brownish crude acetone extract of *P. granatum* (15.0 g) was subjected to quick column chromatography and eluted with hexane and acetone . The eluates were combined on the basis of TLC characteristic to give seven fractions (T1-T7).

Fraction T3 (1.4 g) was separated by CC with 2% methanol/CH_2Cl_2 to give **CMD7**: 6α-hydroxystigmast-4-en-3-one (4.1 mg).

Fraction T4 (1.7 g) was purified by CC with 30% acetone/hexane to afford seven fractions (T4.1-T4.7).

Subfraction T4.5 (35.6 g) was separated by CC with 2% methanol/CH_2Cl_2 to give **CMD8**: ergosterol peroxide (4.9 mg).

Compound CMD7: 6α-hydroxystigmast-4-en-3-one, colorless viscous oil; $[\alpha]_D^{28}$: +12.5° (c = 0.80, $CHCl_3$); ref $[\alpha]_D^{28}$: +10.7° (c = 0.63, $CHCl_3$) (Della Greca *et al*., 1990); UV λ_{max} (MeOH) (log ε): 241 (4.73); IR (neat) ν_{max} 3418 (O-H stretching), 1670 (C=O stretching) and 1645 (C=C stretching) cm^{-1}. For ^1H NMR

($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 6.**

Compound CMD8: ergosterol peroxide, colorless viscous oil; $[\alpha]_D^{28}$: -11.3° (c = 0.32, $CHCl_3$); ref $[\alpha]_D^{28}$: -12.8° (c = 0.42, $CHCl_3$) (Daengrot 2006); IR (neat) ν_{max} 3442 (O-H stretching), 1716 (C=O stretching). For ^1H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 7.**

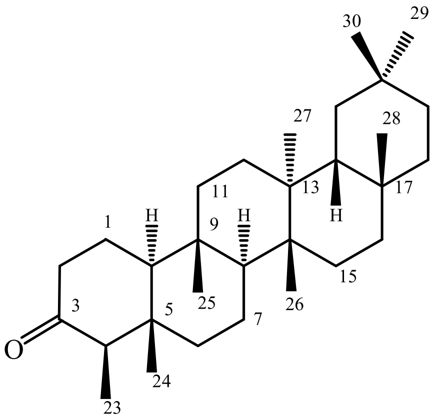
CHAPTER 1.3
RESULTS AND DISCUSSION

1.3.1 Structure elucidation of compounds from the stem of *P. granatum*

The crude methylene chloride and acetone extracts from the stem of *P. granatum* were subjected to repeated quick column and column chromatography over silica gel to furnish thirteen known compounds of three triterpenes: friedelin (**CMD1**), 5(6)-gluten-3α-ol (**CMD2**) and betulinic acid (**CMD3**), seven steroids: a mixture of *β*-sitosterol (**CMD4**) and stigmasterol (**CMD5**), stigmast-4-en-3-one (**CMD6**), 6α-hydroxystigmast-4-en-3-one **(CMD7),** ergosterol peroxide **(CMD8),** 5α-cholest-7-en-3-one **(CMD9)** and lophenol (**CMD10**), 5-methylmellein (**CMD11**), 3,4,3′-tri-O-methylellagic acid (**CMD12**), and 5,7,3′,4′,5′-penta-O-methylgallocatechin (**CMD13**).

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^{1}H, ^{13}C NMR, DEPT 135°, DEPT 90°, HMQC, HMBC, COSY and NOESY. The physical data of the known compounds were also compared with the reported values.

1.3.1.1 Compound CMD1



Compound CMD1 was obtained as a white solid, mp 245-247 °C, [α] D ^{28}: -28.2° (c = 0.63, $CHCl_3$). The IR spectrum showed absorption bands for carbonyl group at 1715 cm^{-1}. It gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^{13}C NMR spectral data recorded in $CDCl_3$ showed 30 signals for 30 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of eight methyl (δ 6.8, 14.7, 17.9, 18.7, 20.3, 31.8, 32.1 and 35.0), eleven methylene (δ 18.2, 22.3, 30.5, 32.4, 32.8, 35.3, 35.6, 36.0, 39.3, 41.3 and 41.5), four methine (δ 42.8, 53.1, 58.2 and 59.5) and seven quaternary carbons (δ 28.2, 30.0, 37.4, 38.3, 39.7, 42.2 and 213.3).

The ^{1}H NMR spectral data showed characteristic of friedelin as one methyl doublet at δ 0.89 (3H-23, *d*, *J* = 6.3 Hz) and seven methyl singlets at δ 0.72, 0.87, 0.95, 1.00, 1.01, 1.05 and 1.18.

The position of a methyl group 3H-23 was determined through an HMBC experiment in which the methyl protons at δ 0.89 (3H-23) showed correlations with C-3 (δ 213.3), C-4 (δ 58.2) and C-5 (δ 42.2). Thus on the basis of its spectroscopic data and comparison with the previously reported data of friedelin (Ahad *et al.*, 1991), compound CMD1 was therefore assigned as friedelin.

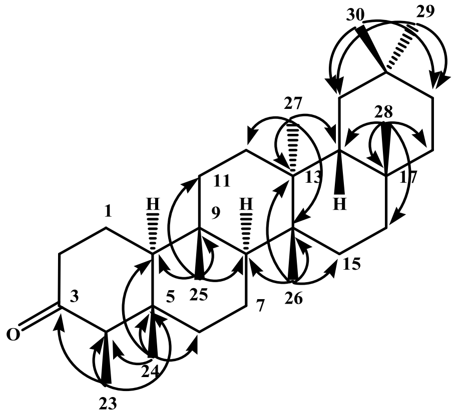


Figure 2 Selected HMBC correlations of **CMD1**

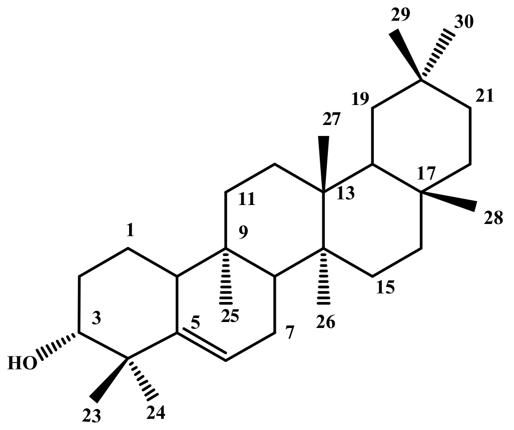
Table 2 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CMD1** ($CDCl_3$) and friedelin (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δH / ppm (multiplicity, J/Hz)	HMBC $^{1}H \rightarrow ^{13}C$
		CMD1	R	CMD1	
1	CH_2	22.3	22.3	1.64 (*m*), 1.69 (*m*)	-
2	CH_2	41.5	41.5	2.36 (*m*), 2.23 (*m*)	-
3	C	213.3	213.2	-	-
4	CH	58.2	58.2	2.24 (*m*)	-
5	C	42.2	42.2	-	-
6	CH_2	41.3	41.3	2.44 (*m*), 1.78 (*m*)	-
7	CH_2	18.2	18.2	1.52 (*m*), 1.39 (*m*)	-
8	CH	53.1	53.1	1.42 (*m*)	-
9	C	37.4	37.5	-	-
10	CH	59.5	59.5	1.56 (*m*)	-
11	CH_2	35.6	35.6	1.61 (*m*), 1.43 (*m*)	-
12	CH_2	30.5	30.5	1.46 (*m*), 1.34 (*m*)	-
13	C	39.7	39.7	-	-
14	C	38.3	38.3	-	-
15	CH_2	32.4	32.4	1.51 (*m*), 1.29 (*m*)	-
16	CH_2	36.0	36.0	1.61 (*m*), 1.36 (*m*)	-

Table 2 (Continued)

Position	Type of C	δc /ppm		δH / ppm (multiplicity, J/Hz)	HMBC $^{1}H \rightarrow {}^{13}C$
		CMD1	R	CMD1	
17	C	30.0	30.0	-	-
18	CH	42.8	42.8	1.53 (*m*)	-
19	CH$_2$	35.3	35.4	1.62 (*m*), 1.49 (*m*)	-
20	C	28.2	28.1	-	-
21	CH$_2$	39.3	39.3	1.48 (*m*), 0.93 (*m*)	-
22	CH$_2$	32.8	32.8	1.50 (*m*), 1.26 (*m*)	-
23	CH$_3$	6.8	6.8	0.89 (*d*, 6.3)	3, 4, 5
24	CH$_3$	14.7	14.7	0.72 (*s*)	4, 5, 6, 10
25	CH$_3$	17.9	18.0	0.87 (*s*)	8, 9, 10, 11
26	CH$_3$	18.7	18.7	1.01 (*s*)	8, 13, 14, 15
27	CH$_3$	20.3	20.3	1.05 (*s*)	12, 13, 14, 18
28	CH$_3$	32.1	32.1	1.18 (*s*)	16, 17, 18, 22
29	CH$_3$	31.8	31.8	1.00 (*s*)	19, 20, 21
30	CH$_3$	35.0	35.0	0.95 (*s*)	19, 20, 21

1.3.1.2 Compound CMD2



Compound CMD2 was obtained as a white solid, mp 210-212 °C, $[\alpha]_D^{28}$: +61.6° (c = 0.7, $CHCl_3$); The IR spectrum showed absorption band of a hydroxyl group at 3415 cm^{-1}.

The ^{13}C NMR spectral data recorded in $CDCl_3$ showed 30 signals for 30 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of eight methyl (δ 16.2, 18.4, 19.6, 25.4, 29.0, 32.0, 32.4 and 34.5), ten methylene (δ 18.2, 23.8, 27.9, 30.4, 32.1, 33.2, 34.6, 35.1, 36.1 and 39.0), five methine (δ 43.1, 47.7, 49.7, 76.4 and 122.1) and seven quaternary carbons (δ 28.3, 30.1, 34.9, 37.9, 39.3, 40.8 and 141.7).

The ^{1}H NMR spectral data showed eight methyl singlets at δ 0.85, 0.95, 0.99, 1.00, 1.04, 1.09, 1.14 and 1.16, a vinyl proton at δ 5.63 (1H, *d*, J = 6.0 Hz, H-6). The ^{13}C NMR spectrum confirmed the presence of a carbon-carbon double bond at δ 122.1 (C-6) and 141.7 (C-5). The broad singlet of H-3 indicated a (β) orientation of H-3.

On the basis of HMBC the vinyl proton H-6 at δ 5.63 showed correlations with C-4 (δ 40.8), C-5 (δ 141.7), C-7 (δ 23.8), C-8 (δ 47.7), and C-10 (49.7), suggesting the presence of a double bond between C-5 and C-6. Thus on the basis of its spectroscopic data and comparison with those reported in the literatures (Susidarti et al., 2006), compound CMD2 was therefore assigned as 5(6)-gluten-3α-ol.

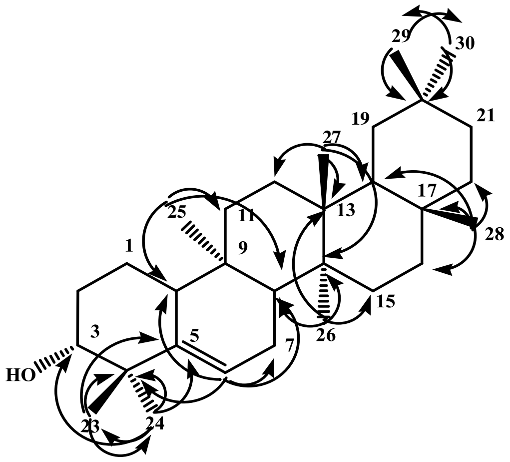


Figure 3 Selected HMBC correlations of **CMD2**

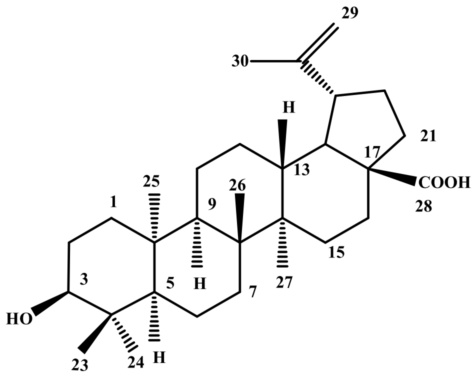
Table 3 ^1H, ^{13}C NMR and HMBC spectral data of compounds **CMD2** ($CDCl_3$) and 5(6)-gluten-3α-ol (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δ$_H$ / ppm (multiplicity, J/Hz)	HMBC $^1H \rightarrow {}^{13}C$
		CMD2	R	CMD3	
1	CH_2	18.2	18.3	1.00 (*m*), 1.60 (*m*)	-
2	CH_2	27.9	28.1	1.13 (*m*), 1.68 (*m*)	-
3	CH	76.4	76.6	3.47 (*br s*)	-
4	C	40.8	41.0	-	-
5	C	141.7	141.9	-	-
6	CH	122.1	122.3	5.63 (*d*, 6.0)	4, 5, 7, 8, 10
7	CH_2	23.8	23.9	1.68 (*m*), 2.01 (*m*)	-
8	CH	47.7	47.7	1.52 (*m*)	-
9	C	34.9	35.1	-	-
10	CH	49.7	49.9	1.98 (*m*)	-
11	CH_2	34.6	34.8	1.33 (*m*), 1.52 (*m*)	-
12	CH_2	30.4	30.6	1.38 (*m*), 1.15 (*m*)	-
13	C	37.9	38.1	-	-
14	C	39.3	39.5	-	-
15	CH_2	32.1	32.3	1.25 (*m*), 1.49 (*m*)	-
16	CH_2	39.0	39.2	0.92 (*m*), 1.57 (*m*)	-

Table 3 (Continued)

Position	Type of C	δc /ppm		δH / ppm (multiplicity, J/Hz)	HMBC $^1H \rightarrow ^{13}C$
		CMD2	R	CMD3	
17	C	30.1	30.3	-	-
18	CH	43.1	43.3	1.58 (*m*)	-
19	CH_2	33.2	33.4	1.25 (*m*), 1.50 (*m*)	-
20	C	28.3	28.5	-	-
21	CH_2	35.1	35.3	1.51 (*m*), 1.40 (*m*)	-
22	CH_2	36.1	36.3	1.53 (*m*), 1.42 (*m*)	-
23	CH_3	29.0	29.2	1.04 (*s*)	3, 5, 24
24	CH_3	25.4	25.7	1.14 (*s*)	3, 5, 23
25	CH_3	16.2	16.4	0.85 (*s*)	8, 10, 11
26	CH_3	18.4	18.6	1.00 (*s*)	8, 13, 14, 15
27	CH_3	19.6	19.8	1.09 (*s*)	13, 14, 18
28	CH_3	32.0	32.3	1.16 (s)	16, 17, 18, 22
29	CH_3	34.5	34.7	0.95 (s)	19, 21, 20, 30
30	CH_3	32.4	32.6	0.99 (*s*)	19, 21, 20, 29

1.3.1.3 Compound CMD3



Compound CMD3 was obtained as a white solid, mp. 280-282 °C, $[\alpha]_D^{28}$: +18.7° (c = 0.03, $CHCl_3$). It gave a purple vanillin-sulfuric acid test. The IR spectrum showed absorption band of a hydroxyl group at 3415 cm^{-1} and a carbonyl group at 1686 cm^{-1}.

The ^{13}C NMR spectral data recorded in $CDCl_3$ showed 30 signals for 30 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of six methyl (δ 14.5, 15.2, 15.6, 15.9, 19.1, and 27.6), eleven methylene (δ 18.2, 20.8, 25.4, 26.9, 29.6, 30.5, 32.2, 34.2, 37.1, 38.7 and 109.3), six methine (δ 38.2, 46.9, 49.1, 50.5, 55.3 and 78.7) and seven quaternary carbons (δ 37.1, 38.7, 40.6, 42.3, 56.1, 150.7 and 179.1).

The ^1H NMR spectral data showed characteristic of lupane triterpenes as one vinylic methyl at δ 1.69, two protons of an isopropenyl moiety at δ 4.61 (*br s*) and 4.74 (*br s*) and a typical lupine *β*H-19 proton at δ 3.01 (*m*). An oxymethine proton was shown at δ 3.19 (*dd*, J = 10.8, 5.4 Hz). The doublet of doublet splitting pattern together with a large coupling constant of H-3 with $J_{ax\text{-}ax}$ = 10.8 Hz and $J_{ax\text{-}eq}$ = 5.4 Hz indicated an axial (α) orientation of H-3. The ^{13}C NMR spectral data displayed a signal of carboxyl carbon at δ 179.1, thus suggesting a carboxylic functionality at C-28. The location of the carboxyl group was confirmed by HMBC experiment in which the methylene proton 2H-22 (δ 1.41 and 1.93) showed correlations with C-17 (δ 56.1), C-18 (δ 49.1) and C-28 (179.1). Thus on the basis of its spectroscopic data and comparison with those reported in the literatures (Macias *et al.*, 1994 and Thongdeeying, 2005), compound CMD3 was therefore assigned as betulinic acid.

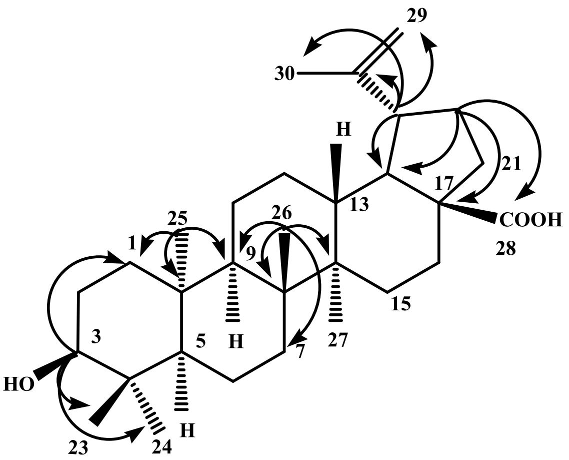


Figure 4 Selected HMBC correlations of **CMD3**

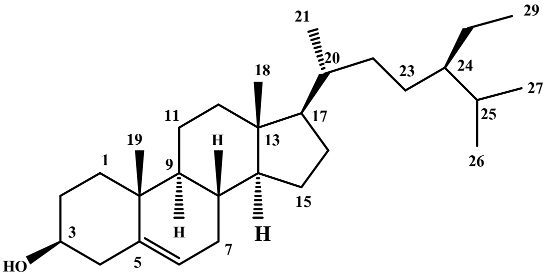
Table 4 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CMD3** ($CDCl_3$) and betulinic acid (**R**, $CDCl_3$)

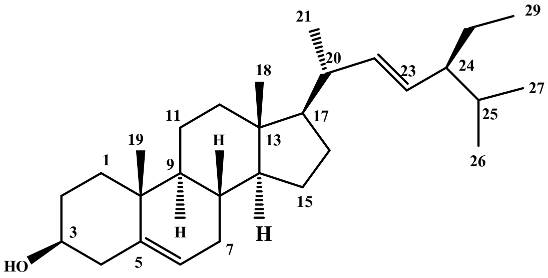
Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $^{1}H \rightarrow ^{13}C$
		CMD3	R	CMD3	R	
1	CH_2	38.7	38.5	0.88 (*m*), 1.65 (*m*)	0.95 (*m*), 1.70 (*m*)	-
2	CH_2	26.9	28.2	1.57 (*m*), 1.61 (*m*)	1.57 (*m*), 1.62 (*m*)	-
3	CH	78.7	78.1	3.19 (*dd,* 10.8, 5.4)	3.19 (*dd,* 10.8, 5.4)	1, 23, 24
4	C	38.7	39.4	-	-	-
5	CH	55.3	55.9	0.69 (*m*)	0.71 (*m*)	4, 6, 7, 9
6	CH_2	18.2	18.7	1.36 (*m*), 1.51 (*m*)	1.45 (*m*), 1.55 (*m*)	-
7	CH_2	34.2	34.7	1.38 (*m*)	1.42 (*m*)	-
8	C	40.6	41.0	-	-	-
9	CH	50.5	50.9	1.26 (*m*)	1.33 (*m*)	-
10	C	37.1	37.5	-	-	-
11	CH_2	20.8	21.1	1.23 (*m*), 1.43 (*m*)	1.25 (*m*), 1.45 (*m*)	-
12	CH_2	25.4	26.0	1.69 (*m*)	1.07 (*m*), 1.73 (*m*)	-
13	CH	38.2	39.2	2.22 (*m*)	2.30 (*m*)	-
14	C	42.3	42.8	-	-	-
15	CH_2	29.6	30.2	1.51 (*m*), 1.51 (*m*)	1.18 (*m*), 1.53 (*m*)	-
16	CH_2	32.2	32.8	1.40 (*m*), 2.25 (*m*)	1.43 (*m*), 2.23 (*m*)	-

Table 4 (Continued)

Position	Type of C	δc /ppm		δH / ppm (multiplicity, J/Hz)		HMBC $^1H \rightarrow ^{13}C$
		CMD3	R	CMD3	R	
17	C	56.1	56.6	-	-	-
18	CH	49.1	49.7	1.58 (*m*)	1.63 (*m*)	-
19	CH	46.9	47.7	3.01 (*m*)	3.02 (*m*)	18, 20, 21, 29, 30
20	C	150.7	151.4	-	-	-
21	CH_2	30.5	31.4	1.42 (*m*), 1.91 (*m*)	1.40 (*m*), 1.93(*m*)	-
22	CH_2	37.1	37.4	1.41 (*m*), 1.93 (*m*)	1.43 (*m*), 1.91(*m*)	17, 18, 28
23	CH_3	27.6	28.5	0.97 (*s*)	0.95 (*s*)	3,4,5, 24
24	CH_3	15.2	16.2	0.75 (*s*)	0.75 (*s*)	3, 4, 5, 23
25	CH_3	15.9	16.3	0.82 (*s*)	0.86 (*s*)	1, 5, 9, 10
26	CH_3	15.6	16.2	0.94 (*s*)	0.97 (*s*)	7, 8, 9, 14
27	CH_3	14.5	14.8	0.98 (*s*)	1.01 (*s*)	8, 13, 14, 15
28	C	179.1	179.0	-	-	-
29	CH_2	109.3	110.0	4.61 (*br s*) 4.74 (*br s*)	4.59 (*dd*, 2.2, 1.0) 4.71 (*d*, 2.2)	19, 30
30	CH_3	19.1	19.4	1.69 (*s*)	1.69 (*d*, 1.0)	19, 20, 29

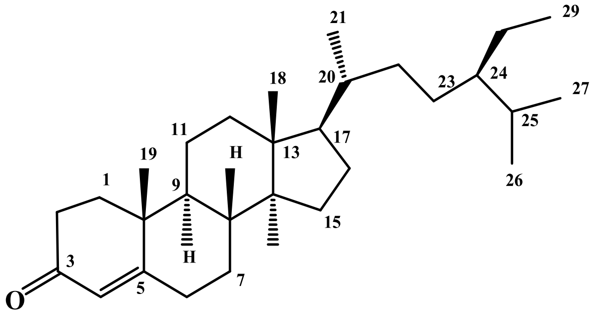
1.3.1.4 Compounds CMD4 and CMD5





The mixture of CMD4 and CMD5 was isolated as a white solid. Its IR spectrum showed absorption bands at 3425 (hydroxyl) and 1642 cm^{-1} (double bond). The ^{1}H NMR spectral data contained an oxymethine proton at δ 3.57-3.47 (*m*), three olefinic protons at δ 5.36 (*d, J* = 5.1 Hz), 5.16 (*dd, J* = 15.1, 8.4 Hz) and 5.01 (*dd, J* = 15.1, 8.4 Hz). The ^{1}H NMR (Cheenpracha, 2004) data was corresponded to a previous reported data of *β*-sitosterol and stigmasterol. Thus, this mixture was identified as *β*-sitosterol (CMD4) and stigmasterol (CMD5).

1.3.1.5 Compound CMD6



Compound CMD6 was isolated as colorless viscous oil; [α]$_D$^{28}: +67.7° (c = 0.47, $CHCl_3$). Its IR spectrum showed absorption bands for α,β-unsaturated carbonyl group at 1674 cm^{-1} and double bond at 1616 cm^{-1}. The UV absorption was shown at 241 nm.

The ^{13}C NMR spectral data recorded in $CDCl_3$ showed 29 signals for 29 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested a presence of six methyl (11.9, 12.0, 17.4, 18.7, 19.0 and 19.8), eleven methylene (21.0, 23.1, 24.2, 26.1, 28.2, 32.1, 32.9, 33.9, 34.0, 35.7 and 39.6), eight methine (29.2, 35.6, 36.1, 45.8, 53.8, 55.9, 56.1 and 123.7) and four quaternary carbons (38.6, 42.4, 171.6 and 199.6).

The ^{1}H NMR spectral data displayed a downfield vinyl proton at δ 5.72 (H-4). The ^{13}C NMR spectrum confirmed the presence of a carbon–carbon double bond at δ 123.7 (C-4) and 171.6 (C-5) and the downfield chemical shift of C-5 (δ 171.6) also indicated the presence of the conjugate carbonyl function. On the basis of HMBC the vinyl proton (δ 5.72) showed correlations with C-2 (δ 33.9), C-3 (δ 199.6), C-6 (δ 32.9) and C-10 (δ 38.6) suggesting the presence of a double bond between C-4 and C-5. On the basis of its spectroscopic data and comparison with previously reported data (Della *et al.*, 1990), Compound CMD6 was identified as stigmast-4-en-3-one.

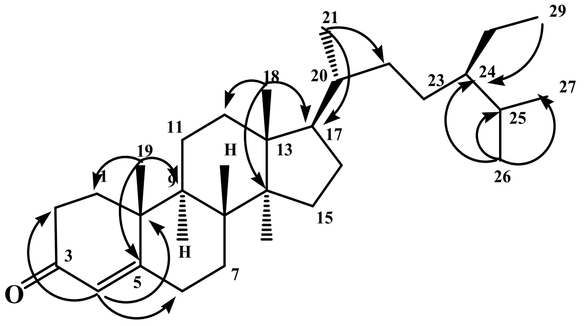


Figure 5 Selected HMBC correlations of **CMD6**

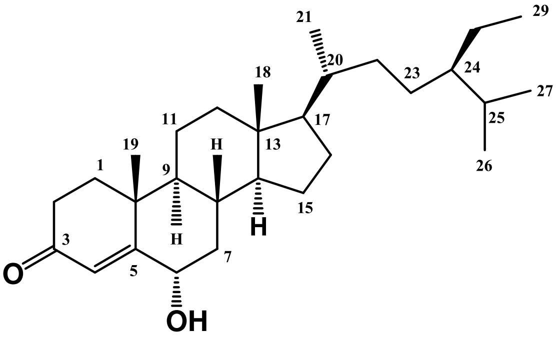
Table 5 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CMD6** (CDCl$_3$) and stigmast-4-en-3-one (**R**, CDCl$_3$)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		CMD6	R	CMD6	R	
1	CH$_2$	35.7	35.7	1.54 (*m*), 1.67 (*m*)	-	-
2	CH$_2$	33.9	33.9	2.28 (*m*), 2.50 (*m*)	-	-
3	C	199.6	198.9	-	-	-
4	CH	123.7	123.6	5.72 (*br s*)	5.74 (*d*, 2.2)	2, 3, 6, 10
5	C	171.6	171.0	-	-	-
6	CH$_2$	32.9	32.9	2.25 (*m*), 2.40 (*m*)	-	-
7	CH$_2$	32.1	32.1	1.10 (*m*), 1.85 (*m*)	-	-
8	CH	35.6	35.7	1.71 (*m*)	-	-
9	CH	53.8	53.8	0.92 (*m*)	-	-
10	C	38.6	38.6	-	-	-
11	CH$_2$	21.0	21.0	1.40 (*m*), 1.50 (*m*)	-	-
12	CH$_2$	39.6	39.5	1.15 (*m*), 2.04 (*m*)	-	-
13	C	42.4	42.4	-	-	-
14	CH	55.9	55.9	1.00 (*m*)	-	-
15	CH$_2$	24.2	24.1	1.23 (*m*), 1.29 (*m*)	-	-
16	CH$_2$	28.2	28.1	1.27 (*m*), 1.32 (*m*)	-	-

Table 5 (Continued)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		CMD6	R	CMD6	R	
17	CH	56.1	56.1	1.11 (*m*)	-	-
18	CH$_3$	12.0	12.0	0.71 (*s*)	0.72 (*s*)	12, 14, 17
19	CH$_3$	17.4	17.4	1.18 (*s*)	1.19 (*s*)	1, 5, 9, 10
20	CH	36.1	36.1	2.01 (*m*)	-	-
21	CH$_3$	18.7	18.7	0.92 (*d*, 6.3)	0.93 (*d*, 6.6)	17, 20, 22
22	CH$_2$	34.0	34.0	2.39 (*m*)	-	-
23	CH$_2$	26.1	26.0	1.17 (*m*)	-	-
24	CH	45.8	45.8	0.93 (*m*)	-	-
25	CH	29.2	29.1	1.26 (*m*)	-	-
26	CH$_3$	19.8	19.8	0.85 (*d*, 6.9)	0.84 (*d*, 6.8)	24, 25, 27
27	CH$_3$	19.0	19.2	0.84 (*d*, 6.6)	0.82 (*d*, 6.8.)	24, 25, 26
28	CH$_2$	23.1	23.1	1.29 (*m*)	-	-
29	CH$_3$	11.9	11.4	0.83 (*d*, 6.6)	0.85 (*d*, 7.2)	24, 28

1.3.1.6 Compound CMD7



Compound CMD7 was isolated as colorless viscous oil; [α] $_D$ ^{28}: +12.5° (c = 0.8, $CHCl_3$). The absorption bands for IR and UV spectral data were similar to compound CMD6 with additional IR hydroxyl absorption at 3446 cm^{-1}.

The ^1H and ^{13}C NMR spectral data of compounds CMD6 and CMD7 showed structural similarity, except for additional signal for an oxymethine proton at δ 4.33 (H-6) in CMD7. The multiplicity of the oxymethine proton signal as a doublet of doublet of doublet ($J_{ax\text{-}ax}$ = 17.7, $J_{ax\text{-}eq}$ = 5.7, $J_{allylic}$ = 1.2 Hz) from coupling with 2H-7 and H-4, indicated that H-6 was situated in an axial (*β*) position. The location of a hydroxyl group at C-6 was determined through an HMBC experiment in which the oxymethine proton signal at δ 4.33 (H-6) showed long-range correlations with C-3 (δ 198.5), C-4 (δ 118.7), C5 (δ 170.6), C-7 (δ 40.5), C-8 (δ 33.2) and C-10 (δ 38.0). Thus on the basis of its spectroscopic data and comparison with previously reported data (Della Greca *et al.*, 1990), compound CMD7 was assigned as 6α-hydroxystigmast-4-en-3-one.

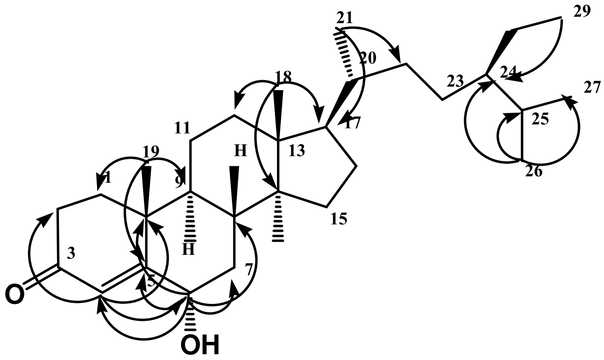


Figure 6 Selected HMBC correlations of **CMD7**

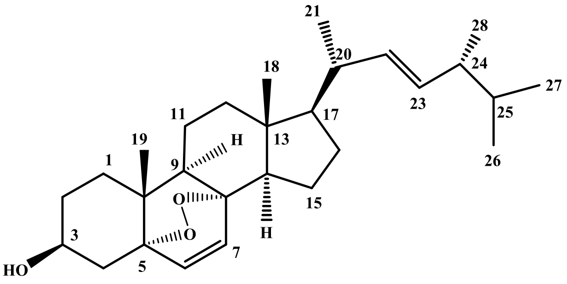
Table 6 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CMD7** ($CDCl_3$) and 6α-hydroxystigmast-4-en-3-one (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δH / ppm (multiplicity, J/Hz)	HMBC $^1H \rightarrow ^{13}C$
		CMD7	R	CMD7	
1	CH_2	35.3	36.3	1.74 (*m*), 1.79 (*m*)	-
2	CH_2	32.9	34.1	2.32 (*m*), 2.38 (*m*)	-
3	C	198.5	202.9	-	-
4	CH	118.7	119.4	6.17 (*d,* 1.2)	2, 3, 6, 10
5	C	170.6	171.0	-	-
6	CH	67.7	68.7	4.33 (*ddd*, 17.7, 5.7, 1.2)	4, 5, 7, 8, 10
7	CH_2	40.5	39.4	1.08 (*m*), 2.15 (*m*)	-
8	CH	33.2	33.8	1.63 (*m*)	-
9	CH	52.8	53.7	0.95 (*m*)	-
10	C	38.0	39.3	-	-
11	CH_2	20.0	21.0	1.51 (*m*), 1.55 (*m*)	-
12	CH_2	38.5	39.4	2.02 (*m*), 2.06 (*m*)	-
13	C	41.5	41.5	-	-
14	CH	54.7	55.5	1.12 (*m*)	-
15	CH_2	23.2	24.4	1.12 (*m*), 1.64 (*m*)	-
16	CH_2	28.7	28.1	1.28 (*m*), 1.71 (*m*)	-

Table 6 (Continued)

Position	Type of C	δc /ppm		δH / ppm (multiplicity, J/Hz)	HMBC $^1H \rightarrow ^{13}C$
		CMD7	R	CMD7	
17	CH	55.0	55.9	1.16 (*m*)	-
18	CH_3	10.9	11.9	0.71 (*s*)	12, 14, 17
19	CH_3	17.3	17.9	1.18 (*s*)	1, 5, 9, 10
20	CH	35.1	36.1	2.05 (*m*)	-
21	CH_3	17.7	18.7	0.92 (*d*, 6.3)	17, 20, 22
22	CH_2	32.8	33.9	2.48 (*m*)	-
23	CH_2	27.1	26.1	0.88 (*m*)	-
24	CH	44.8	45.8	0.97 (*m*)	-
25	CH	28.2	29.2	1.62 (*m*)	-
26	CH_3	18.8	19.7	0.84 (*d*, 6.9)	24, 25, 27
27	CH_3	18.0	19.0	0.81 (*d*, 6.6)	24, 25, 26
28	CH_2	22.1	23.1	1.18 (*m*)	-
29	CH_3	11.0	11.9	0.85 (*t*, 6.9)	24, 28

1.3.1.7 Compound CMD8



Compound CMD8 was isolated as colorless viscous oil; [α] $_D^{28}$: -11.3°(c = 0.33, $CHCl_3$). Its IR spectrum showed absorption bands for a hydroxyl group at 3414 cm^{-1}.

The ^{13}C NMR spectral data recorded in $CDCl_3$ showed 28 signals for 28 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested a presence of six methyl (δ 12.9, 17.6, 18.2, 19.6, 19.9 and 20.9), seven methylene (δ 20.6, 23.4, 28.6, 30.1, 34.7, 37.0 and 39.4), eleven methine (δ 33.1, 39.7, 42.8, 51.1, 51.7, 56.2, 66.5, 130.8, 132.3, 135.2 and 135.4) and four quaternary carbons (δ 36.9, 44.6, 79.4 and 82.2). Two quaternary carbon signals at δ 82.2 and δ 79.4 were, respectively, assignable to C-5 and C-8 bearing a 5α, 8α-peroxide bonds.

The ^1H NMR spectral data showed characteristic of ergostane-type sterol as four methyl doublets at δ 0.82 (3H, J = 6.6 Hz, Me-26), 0.83 (3H, J = 6.6 Hz, Me-27), 0.91 (3H, J = 6.9 Hz, Me-28) and 1.01 (3H, J = 6.6 Hz, Me-21) and two methyl singlets at δ 0.82 (Me-18) and 0.88 (Me-19). Two parts of olefinic proton signals at δ 6.27 (H-6) and 6.50 (H-7) (each 1H, *d*, J = 8.7 Hz) and 5.14 (H-22) and 5.23 (H-23) (each 1H, *dd*, J = 15.3, 7.8 Hz) were attributable to Δ^6 and Δ^{22} double bonds, respectively. The oxymethine proton signal at δ 3.97 (H-3, *m*) was assigned as H-3α due to the absence of NOESY cross peak with 3H-19 (δ 0.88).

The location of the peroxide bond was confirmed by HMBC experiment in which the olefinic proton H-6 (δ 6.27) showed correlations with C-4 (δ 39.4), C-5 (δ 82.2) and C-8 (δ 79.4). The olefinic proton H-7 (δ 6.50) showed long-range correlations with C-5 (δ 82.2), C-8 (δ 79.4), C-9 (δ 51.1) and C-14 (δ 51.7). Thus on the basis of its spectroscopic data and comparison with those reported in the literatures (Yue *et al.*, 2001, Rosecke *et al.*, 2000 and Daengrot 2006), compound CMD8 was, therefore, assigned as ergosterol peroxide.

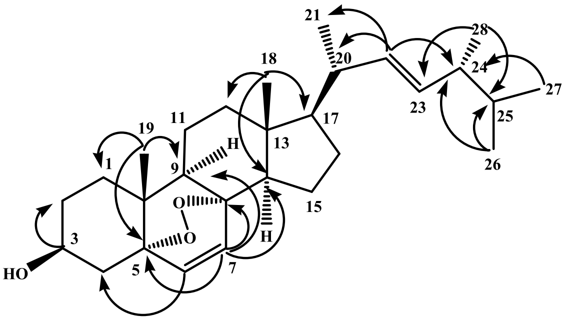


Figure 7 Selected HMBC correlations of **CMD8**

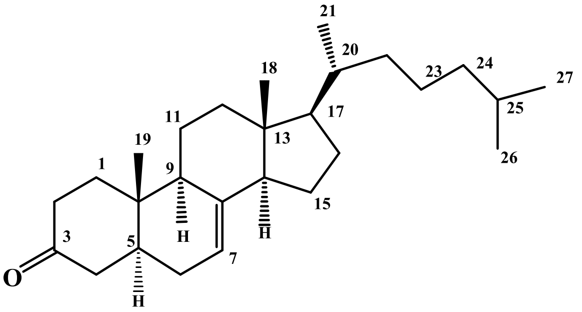
Table 7 ^1H, ^{13}C NMR and HMBC spectral data of compounds **CMD8** ($CDCl_3$) and ergosterol peroxide (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC (CMD) $H^1 \rightarrow {}^{13}C$
		CMD8	R	CMD8	R	
1	CH_2	30.1	30.2	1.56 (*m*), 1.85 (*m*)	-	-
2	CH_2	34.7	34.8	1.71 (*m*), 1.98 (*m*)	-	-
3	CH	66.5	66.5	3.97 (*m*)	3.97 (*m*)	2
4	CH_2	39.4	39.4	1.25 (*m*), 1.96 (*m*)	-	-
5	C	82.2	82.2	-	-	-
6	CH	135.4	135.2	6.27 (*d*, 8.7)	6.24 (*d*, 8.7)	4, 5, 8
7	CH	130.8	130.7	6.50 (*d*, 8.7)	6.51 (*d*, 8.7)	5, 8, 9, 14
8	C	79.4	79.4	-	-	-
9	CH	51.1	51.3	1.51 (*m*)	-	-
10	C	36.9	37.0	-	-	-
11	CH_2	20.6	20.7	1.42 (*m*), 1.61 (*m*)	-	-
12	CH_2	37.0	37.0	1.91 (*m*), 2.13 (*m*)	-	-
13	C	44.6	44.6	-	-	-

Table 7 (Continued)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC
		CMD8	R	CMD8	R	$H^1 \rightarrow {}^{13}C$
14	CH	51.7	51.7	1.61 (*m*)	-	-
15	CH_2	23.4	23.4	1.24 (*m*), 1.53 (*m*)	-	-
16	CH_2	28.6	28.6	1.40 (*m*), 1.76 (*m*)	-	-
17	CH	56.2	56.2	1.24 (*m*)	-	-
18	CH_3	12.9	12.9	0.82 (*s*)	0.82 (*s*)	12, 14, 17
19	CH_3	18.2	18.2	0.88 (*s*)	0.88 (*s*)	1, 5, 9
20	CH	39.7	39.7	2.04 (*m*)	-	-
21	CH_3	20.9	20.9	1.01 (*d*, 6.6)	0.91 (*d*, 6.6)	17, 20, 22
22	CH	135.2	135.2	5.14 (*dd*, 15.3, 7.8)	5.22 (*dd*, 15.3, 8.2)	20, 21, 24
23	CH	132.3	132.3	5.23 (*dd*, 15.3, 7.8)	5.14 (*dd*, 15.3, 7.6)	20, 24, 28
24	CH	42.8	42.8	1.87 (*m*)	-	-
25	CH	33.1	33.1	1.49 (*m*)	-	-
26	CH_3	19.6	19.6	0.82 (*d*, 6.6)	0.82 (*d*, 6.6)	24, 25, 27
27	CH_3	19.9	19.9	0.83 (*d*, 6.6)	0.82 (*d*, 6.6)	24, 25, 26
28	CH_2	17.6	17.6	0.91 (*d*, 6.9)	1.00 (*d*, 6.6)	23, 25

1.3.1.8 Compound CMD9



Compound CMD9 was isolated as a white solid. mp. 144-146 °C, $[\alpha]_D^{28}$: +12.1° (c = 0.05, $CHCl_3$). Its IR spectrum showed absorption bands for carbonyl group at 1708 cm^{-1} and double bond at 1630 cm^{-1}.

The ^{13}C NMR spectral data recorded in $CDCl_3$ showed 27 signals for 27 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested a presence of five methyl (δ 11.9, 12.4, 18.9, 22.6 and 22.8), eleven methylene (δ 21.7, 23.0, 24.0, 27.9, 30.1, 36.1, 38.1, 38.8, 39.5(x2) and 44.2), seven methine (δ 28.0, 36.5, 42.9, 48.9, 55.0, 56.2 and 117.0) and four quaternary carbons (δ 34.4, 43.4, 139.6 and 212.0).

The ^{1}H NMR spectral data displayed a downfield vinyl proton at δ 5.19 (H-7). The ^{13}C NMR spectrum confirmed the presence of a carbon–carbon double bond at δ 117.0 (C-7) and 139.6 (C-8). On the basis of HMBC the vinyl proton H-7 (δ 5.19) showed correlations with C-5 (δ 42.9), C-6 (δ 30.1), C-9 (δ 48.9) and C-14 (δ 55.0) suggesting the presence of a double bond between C-7 and C-8. On the basis of its spectroscopic data and comparison with previously reported data (Dolle *et al.*, 1991), Compound CMD9 was identified as 5α-cholest-7-en-3-one.

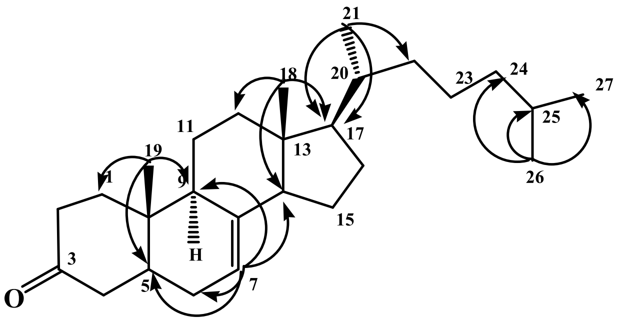


Figure 8 Selected HMBC correlations of **CMD9**

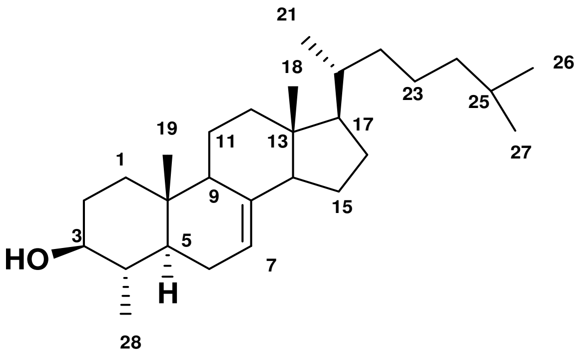
Table 8 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CMD9** ($CDCl_3$) and 5α-cholest-7-en-3-one (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)	HMBC $H^1 \rightarrow {}^{13}C$
		CMD9	R	CMD9	
1	CH_2	38.8	38.2	2.28 (*m*), 2.48 (*m*)	-
2	CH_2	39.5	39.6	1.22 (*m*), 2.22 (*m*)	-
3	C	212.0	211.8	-	-
4	CH_2	44.2	44.3	2.34 (*m*), 2.90 (*m*)	-
5	CH	42.9	43.0	1.83 (*m*)	-
6	CH_2	30.1	30.2	1.03 (*m*), 1.72 (*m*)	-
7	CH	117.0	117.0	5.19 (*br s*)	5, 6, 9, 14
8	C	139.6	139.6	-	-
9	CH	48.9	49.0	1.72 (*m*)	-
10	C	34.4	34.5	-	-
11	CH_2	21.7	21.8	1.57 (*m*), 2.10 (*m*)	-
12	CH_2	38.1	38.9	1.28 (*m*), 1.35 (*m*)	-
13	C	43.4	43.5	-	-
14	CH	55.0	55.1	1.82 (*m*)	-
15	CH_2	23.0	23.0	1.38 (*m*), 1.52 (*m*)	-
16	CH_2	27.9	28.0	1.23 (*m*), 1.91 (*m*)	-

Table 8 (Continued)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)	HMBC $H^1 \rightarrow {}^{13}C$
		CMD9	R	CMD9	
17	CH	56.2	56.3	1.22 (*m*)	-
18	CH_3	11.9	11.9	0.56 (*s*)	12, 13, 14, 17
19	CH_3	12.4	12.5	1.02 (*s*)	1, 5, 9, 10
20	CH	36.5	36.2	1.10 (*m*)	-
21	CH_3	18.9	18.9	0.92 (*d*, 6.6)	17, 20, 22
22	CH_2	36.1	36.2	1.38 (*m*)	-
23	CH_2	24.0	24.0	1.17 (*m*)	-
24	CH_2	39.5	39.6	2.10 (*m*)	-
25	CH	28.0	28.0	1.90 (*m*)	-
26	CH_3	22.6	22.6	0.87 (*d*, 6.6)	24, 25, 27
27	CH_3	22.8	22.8	0.87 (*d*, 6.6)	24, 25, 26

1.3.1.9 Compound CMD10



Compound CMD10 was obtained as a white solid. mp. 149-150 °C, [α] D ^{28}: +7.0° (c = 0.04, $CHCl_3$). The IR spectrum showed absorption band of a hydroxyl group at 3424 cm^{-1}.

The ^{13}C NMR spectral data recorded in $CDCl_3$ showed 28 signals for 28 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of six methyl (δ 11.9, 14.2, 15.2, 18.9, 22.6 and 22.8), ten methylene (δ 21.4, 22.9, 23.9, 26.7, 28.0, 31.0, 36.2, 37.0, 39.5 and 39.6), nine methine (δ 28.0, 36.2, 40.3, 46.7, 49.7, 55.0, 56.2, 76.3 and 117.5) and three quaternary carbons (δ 34.9, 43.4 and 139.2).

The ^{1}H NMR spectral data showed two methyl singlets at δ 0.52 and 0.83, four methyl doublets at δ 0.86, 0.87, 0.92 and 0.99 and a vinyl proton at δ 5.18 (1H, *dd*, *J* = 5.18, 1.5 Hz, H-7). The ^{13}C NMR spectral data confirmed the presence of a carbon-carbon double bond at δ 117.5 (C-7) and 139.2 (C-8). The doublet of doublet splitting pattern of H-3 at δ 3.12 (1H, *dd*, *J* = 10.5, 4.5 Hz) indicated its (α) orientation.

On the basis of HMBC the vinyl proton H-7 (δ 5.18) showed correlations with C-5 (δ 46.7), C-6 (δ 26.7), C-9 (δ 49.7) and C-14 (δ 55.0), suggesting the presence of a double bond between C-7 and C-8. Thus on the basis of its spectroscopic data and comparison with those reported in the literatures (Farines *et al.*, 1988), compound CMD10 was therefore assigned as lophenol.

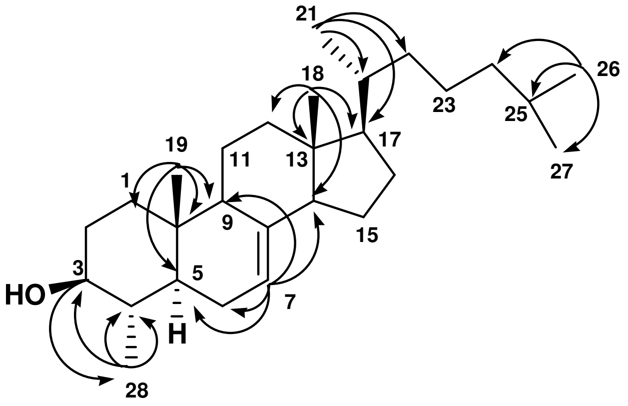


Figure 9 Selected HMBC correlations of **CMD10**

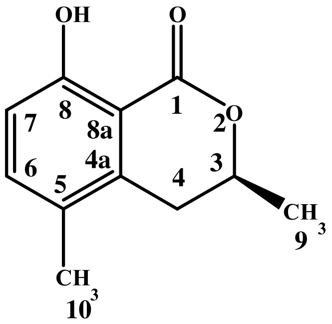
Table 9 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CMD10** ($CDCl_3$) and lophenol (**R**, $CDCl_3$)

Position	Type of C	δc /ppm	δ_H / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		CMD10	CMD10	R	
1	CH_2	37.0	1.83 (*m*), 1.13 (*m*)	-	-
2	CH_2	31.0	1.80 (*m*), 1.45 (*m*)	-	-
3	CH	76.3	3.12 (dd, 10.5, 4.5)	3.12 (*dd*, 10.6, 4.7)	28
4	CH	40.3	1.33 (*m*)	-	-
5	CH	46.7	1.12 (*m*)	-	-
6	CH_2	26.7	1.60 (*m*), 2.10 (*m*)	5.18 (*d*, 5.2)	-
7	CH	117.5	5.18 (dd, 5.8, 1.5)	-	5, 6, 9, 14
8	C	139.2	-	-	-
9	CH	49.7	1.62 (*m*)	-	-
10	C	34.9	-	-	-
11	CH_2	22.9	1.53 (*m*), 1.32 (*m*)	-	-
12	CH_2	39.5	1.12 (*m*), 1.35 (*m*)	-	-
13	C	43.4	-	-	-
14	CH	55.0	1.81 (*m*)	-	-
15	CH_2	23.9	1.15 (*m*), 1.52 (*m*)	-	-
16	CH_2	28.0	1.28 (*m*), 1.91 (*m*)	-	-

Table 9 (Continued)

Position	Type of C	δc /ppm	δ_H / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		CMD10	CMD10	R	
17	CH	56.2	1.20 (*m*)	-	-
18	CH_3	11.9	0.52 (*s*)	0.53 (s)	12, 13, 14, 17
19	CH_3	14.2	0.83 (*s*)	0.83 (s)	1, 5, 9, 10
20	CH	36.2	1.23 (*m*)	-	-
21	CH_3	18.9	0.92 (*d*, 6.3)	0.99 (*d*, 6.3)	17, 20, 22
22	CH_2	36.2	1.34 (*m*)	-	-
23	CH_2	39.6	1.21 (*m*)	-	-
24	CH_2	21.4	1.55 (*m*)	-	-
25	CH	28.0	1.85 (*m*)	-	-
26	CH_3	22.6	0.87 (*d*, 6.6)	0.87 (*d*, 6.5)	24, 25, 27
27	CH_3	22.8	0.86 (*d*, 6.6)	0.86 (*d*, 6.5)	24, 25, 26
28	CH_3	15.2	0.99 (d, 6.3)	0.92 (*d*, 5.8)	3, 4, 5

1.3.1.10 Compound CMD11



Compound CMD11 was obtained as a colorless viscous oil, $[\alpha]_D^{28}$: -122° (c = 0.03, $CHCl_3$)It exhibited UV absorption bands at 208, 248 and 323 nm for benzene chromophore. The IR spectrum showed absorption bands at 3290 and 1669 cm^{-1} indicating the presence of hydroxyl and chelated carbonyl groups, respectively.

The ^{13}C NMR spectral data displayed 15 signals for 15 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of two methyl (δ 18.1 and 20.9), one methylene (δ 31.9), three methine (δ 75.4, 115.7 and 137.4) and five quaternary carbons (δ 108.1, 134.9, 137.0, 160.6 and 170.3).

The ^1H NMR spectral data consisted of signals for two *ortho*-coupled aromatic protons of a 1,2,3,4-tetrasubstituted benzene at δ 6.82 (1H, *d, J* = 8.4 Hz, H-7) and 7.29 (1H, *d, J* = 8.4 Hz, H-6), one oxymethine proton at δ 4.68 (1H, *ddq, J* = 16.8, 11.4, 3.6 Hz, H-3), one methylene group at δ 2.72 (1H, *dd, J* = 16.8, 11.4 Hz, H-4) and 2.95 (1H, *dd, J* = 16.8, 3.6 Hz, H-4) and two methyl groups at δ 1.55 (3H, *d, J* = 6.0 Hz, Me-10) and 2.20 (3H, *s*, Me-9)

The locations of the two methyl groups (Me-9 and Me-10) at C-3 and C-5, respectively were deduced from HMBC correlations of Me-9 (δ 1.55) with C-3 (δ 75.4) and C-4 (δ 31.9) and of Me-10 (δ 2.20) with C-5 (δ 134.9), C-4a (δ 137.0) and C-6 (δ 137.4). On the basis of the above results and comparison with the reported data of 5-methylmellein [Cambie *et al.*, 1991], compound CMD11 was therefore assigned as 5-methylmellein.

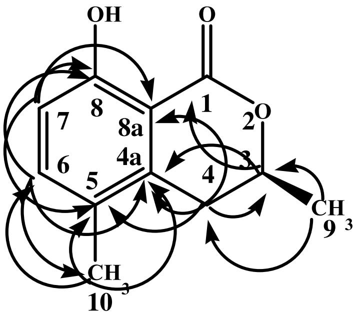
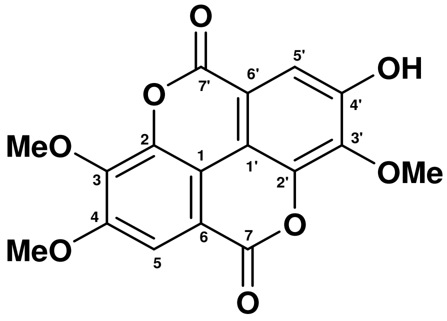


Figure 10 Selected HMBC correlations of **CMD11**

Table 10 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CMD11** ($CDCl_3$) and 5-methylmellein (**R**, $CDCl_3$)

Position	Type of C	δ_c /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $^{1}H \rightarrow {}^{13}C$
		CMD11	R	CMD11	R	
1	C	170.3	170.4	-	-	-
2	-	-	-	-	-	-
3	CH	75.4	75.4	4.68 (*ddq*, 16.8, 11.4, 3.6)	4.69 (*ddq*, 16.6, 11.4, 3.4)	1, 4a
4	CH_2	31.9	31.9	2.72 (*dd*, 16.8, 11.4), 2.95 (*dd*, 16.8, 3.6)	2.72 (*dd*, 11.6, 16.6), 2.95 (*dd*, 16.6, 3.4)	3, 4a, 5, 8a
4a	C	137.0	137.1	-	-	-
5	C	134.9	134.9	-	-	-
6	CH	137.4	137.9	7.29 (*d*, 8.4)	7.29 (*d*, 8.5)	4a, 8, 10
7	CH	115.7	115.7	6.82 (*d*, 8.4)	6.82 (*d*, 8.5)	5, 8, 8a
8	C	160.6	160.5	-	-	-
8a	C	108.1	108.1	-	-	-
9	CH_3	20.9	20.9	1.55 (*d*, 6.0)	1.55 (*d*, 6.3)	3, 4
10	CH_3	18.1	16.1	2.20 (*s*)	2.20 (*s*)	5, 4a, 6

1.3.1.11 Compound CMD12



Compound CMD12 was obtained as a white solid. It exhibited UV absorption bands at 248 and 371 nm for benzene chromophore. The IR spectrum showed absorption bands at 3400 and 1744 cm^{-1} indicating the presence of hydroxyl and carbonyl groups, respectively.

The ^{13}C NMR spectral data displayed 17 signals for 17 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of three methyl (δ 56.8, 61.5 and 61.8), two aromatic methine (δ 107.7 and 112.8) and 12 quaternary carbons (δ 111.7, 112.0, 112.7, 114.0, 140.7, 141.0, 141.4, 141.8, 152.8, 154.0, 158.7 and 159.1).

The ^{1}H NMR spectral data consisted of signals for two *singlets* aromatic protons at δ 7.68 (1H, *s*, H-5) and 7.64 (1H, *s*, H-5′), three methoxyl groups at δ 4.17 (3H, s, 3-OMe), 4.04 (3H, s, 4-OMe) and 4.19 (3H, s, 3′-OMe).

The locations of the two aromatic protons (H-5 and H-5′) were deduced from HMBC correlations of H-5 (δ 7.68) with C-3 (δ 141.8), C-4 (δ 154.0), C-6 (δ 114.0) and C-7 (δ 159.1) and of H-5′ (δ 7.64) with C-1′ (δ 111.7), C-3′ (δ 140.7), C-4′ (δ 152.8) and C-7′ (δ 158.7). On the basis of the above results and comparison with the reported data of 3,4,3′-tri-O-methylellagic acid [Bai *et al.*, 2008], compound CMD12 was assigned as 3,4,3′-tri-O-methylellagic acid.

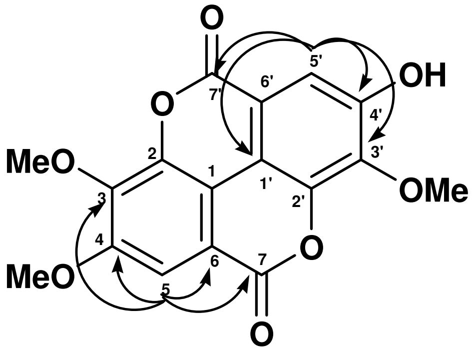
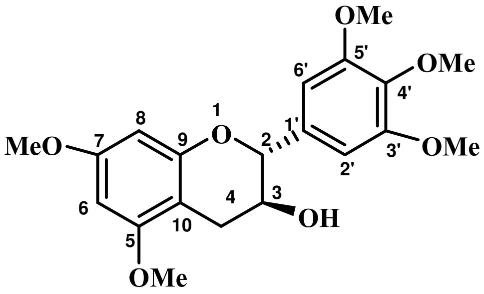


Figure 11 Selected HMBC correlations of **CMD12**

Table 11 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **CMD12** ($CDCl_3$+DMSO-d_6) and 3,4,3′-tri-O-methylellagic acid (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		CMD12	R	CMD12	R	
1	C	112.0	107.4	-	-	-
2	C	141.4	141.2	-	-	-
3	C	141.8	141.1	-	-	-
4	C	154.0	153.7	-	-	-
5	CH	107.7	107.4	7.68 (*s*)	7.51 (*s*)	3, 4, 6, 7
6	C	114.0	113.5	-	-	-
7	C	159.1	158.6	-	-	-
1′	C	111.7	107.4	-	-	-
2′	C	141.0	140.6	-	-	-
3′	C	140.7	140.3	-	-	-
4′	C	152.8	153.2	-	-	-
5′	CH	112.8	111.8	7.64 (*s*)	7.60 (*s*)	1′, 3′ ,4′ ,7′
6′	C	112.7	112.5	-	-	-
7′	C	158.7	153.7	-	-	-
3-OMe	CH_3	61.8	61.3	4.17 (*s*)	4.03 (*s*)	3
4-OMe	CH_3	56.8	56.7	4.04 (*s*)	3.99 (*s*)	4
3′-OMe	CH_3	61.5	60.9	4.19 (*s*)	4.05 (*s*)	3′

1.3.1.12 Compound CMD13



Compound CMD13 was obtained as a colorless viscous oil, $[\alpha]_D^{28}$: -47.7° (c = 0.o7, $CHCl_3$)The IR spectrum showed absorption band for a hydroxyl at 3453 cm^{-1}. The UV spectrum showed absorption maxima at 207 and 270 nm.

The ^{13}C NMR spectral data displayed 20 signals for 20 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of four aromatic methine carbons (δ 92.0, 93.0 and 104.0 (x2)), two oxymethine carbons (δ 68.4 and 82.2), a methylene carbon (δ 22.8), eight quaternary aromatic carbons (δ 101.1, 133.4, 138.2, 153.6 (x2), 155.2, 158.8 and 159.8) and five methoxyl carbons (δ 55.4, 55.5, 56.2 and 60.8 (x2)).

The ^{1}H NMR spectral data suggested the presence of four aromatic protons (δ 6.12, 6.15 and 6.68 (x2)), two methine protons (δ 4.08 and 4.63), two methylene protons (δ 2.60 and 3.10) and five methoxyl groups at δ 3.76, 3.81, 3.86 (x2) and 3.88 (each 3H, *s*, OCH_3). Two doublet resonances at δ 6.12 and 6.15 with the coupling constant of 2.1 Hz corresponded to the resonances of meta protons H-6 and H-8, respectively. A singlet at δ 6.68 were assigned for the resonances of H-2′ and H-6′. The spectra further showed the resonances of H-2 (δ 4.63, *d*, *J* = 8.4 Hz), H-3 (m) and 2H-4 (δ 2.60, *dd*, *J* = 16.3, 9.0 Hz and 3.10, *dd*, *J* = 16.3, 6.0 Hz).

The downfield chemical shift of H-2 (δ 4.63) and H-3 (δ 4.08) indicated that these two protons were next to oxygen-bearing carbons. From NOESY experiment, the methine proton at δ 4.63 (H-2) showed no cross peak with H-3 supporting that H-2 and H-3 were *trans*. From comparison of the reported data of gallocatechin (Foo *et al.*, 2000), compound CMD13 was therefore assigned as 5,7,3′,4′,5′-penta-O-methylgallocatechin.

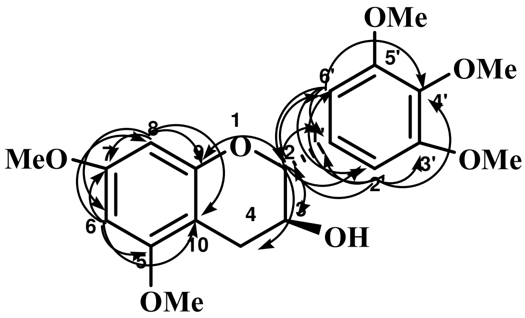


Figure 12 Selected HMBC correlations of **CMD13**

Table 12 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **CMD13** ($CDCl_3$) and comparison with ^{1}H NMR of gallocatechin.

Position	Type of C	δc /ppm	δ$_H$ / ppm multiplicity, J/Hz)		HMBC $H^1 \rightarrow ^{13}C$
		CMD13	CMD13	Gallocatechin	
1	-	-	-	-	-
2	CH	82.2	4.63 (*d*, 8.4)	4.55 (*d*, 7.2)	3, 4, 9, 1′, 2′, (6′)
3	CH	68.4	4.08 (*m*)	3.97 (*m*)	1′, 10
4	CH_2	22.8	2.60 (*dd*, 16.3, 9.0) 3.10 (*dd*, 16.3, 6.0)	2.4-2.9 (*m*)	2, 5, 9, 10
5	C	158.8	-		-
6	CH	92.0	6.12 (*d*, 2.1)	5.94 (*dd*, 2.2)	5, 7, 8, 10
7	C	159.8	-		-
8	CH	93.0	6.15 (*d*, 2.1)	5.88 (*d*, 2.2)	6, 7, 9, 10
9	C	155.2	-		-
10	C	101.1	-		-
1′	C	133.4	-		-
2′, 6′	CH	104.0	6.68 (*s*)	6.4 (*s*)	2, 1′, 3′, 4′
3′, 5′	C	153.6	-	-	-
4′	C	138.2	-	-	-
5-OMe	CH_3	55.5	3.81 (*s*)	-	5
7-OMe	CH_3	55.4	3.76 (*s*)	-	7
3′, 5′-OMe	CH_3	60.8	3.88 (*s*)	-	3′, 5′
4′-OMe	CH_3	56.2	3.86 (*s*)	-	4′

CHAPTER 2.1
Introduction

2.1.1 Introduction

Michelia alba DC. (*M. longifolia* B.) is a member of the Magnoliaceae family and called “champee” in Thailand (Smitinand, 2001). The genus *Michelia* contains about 50 species. *Michelia* species have been used for the treatment of cancer, for example *M. champaca* has been used in India for the treatment of abdominal tumors whereas *M. hypoleuca* and *M. officinalis* for carcinomatous sores and leukemia, respectively (Chen *et al.*, 2008). In the previous report, parthenolide and costunolide have been isolated from the chloroform extract of the fresh bark of *Michelia longifolia* Blume. Parthenolide displayed significant activity against the human laryngeal epidermoid carcinoma (ED_{50} = 0.76) and the 9KB cell culture system (ED_{50} = 0.45). Costunolide showed reproducible inhibitory activity against the KB cell culture of a human carcinoma of the nasopharynx (Likhitwitayawuid *et al.*, 1998).

M. alba is an evergreen tropical tree from Southeast Asia, 10-12 m tall. The bark is distinct ridges and brown color. Leaves are single arrange alternate oval, length 20 cm, width 8 cm. The flowers are fragrant white and have 8-12 petals.

a.trees **b.** stem

c. leaves **f.** flowers

Figure 13 Different parts of *Michelia alba* DC.

2.1.2 Review of Literatures

Chemical constituents isolated from the ten species of this genus were summarized in **Table 13**. Information obtained from SciFinder Scholar copyright in 2009 will be presented and classified into groups: monoterpenoids, sesquiterpenoids, triterpenoids, alkaloids, steroids, amide, lignin, benzenoids and aliphatic.

Table 13 Compounds from plants of *Michelia* genus

a: aliphatic
b: steroids
c: amide
d: triterpenoids
e: sesquiterpenoids
f: monoterpenoids
g: lignin
h: alkaloids
i: benzenoids

Scientific name	Part	Compounds	Bibliography
M. alba	Not specified	oxoushinsunin, **1h**	Yang *et al.*, 1962
		ushinsunin, **2h**	
		norushinsunin, **3h**	
	Not specified	dehydrolinalool oxide, **1f**	
		costunolide, **1e**	Asaruddin *et al.*, 2003
		caryophyllene oxide, **2e**	
		dihydrocostunolide, **3e**	
		dihydroparthenolide, **4e**	
		parthenolide, **5e**	
	Leaves	(-)-anonaine, **4h**	
		(-)-norushinsunine, **5h**	Chen *et al.*, 2008
		(-)-ushinsunine, **6h**	
		(-)-*N*-acetylanonaine, **7h**	
		liriodenine, **8h**	
		oxoxylopine, **9h**	
		michelenolide, **6e**	
		costunolide, **1e**	
		11,13-dehydrolanuginolide, **7e**	
		N-trans-feruloyltyramine, **1c**	
		(+)-syringaresinol, **1g**	

Scientific name	Part	Compounds	Bibliography
M. alba	Leaves	4-hydroxybenzaldehyde, **1i**	Chen *et al*., 2008
		4-hydroxybenzoic acid, **2i**	
		methylparaben, **3i**	
		β-sitosterol, **1b**	
		stigmasterol, **2b**	
		palmitic acid, **1a**	
		stearic acid, **2a**	
		linoleic acid, **3a**	
	Flower	eugenol methyl ether, **4i**	Hung *et al*., 2009
		camphene, **2f**	
		α-pinene, **3f**	
		caryophyllene, **8e**	
		germacrene D, **9e**	
		estragole, **5i**	
		spathulenol, **10e**	
		α-humulene, **11e**	
		eucalyptol, **4f**	
M. fuscata	Not specified	deacetyllanuginolide, **12e**	Iida *et al*., 1982
		michefuscalide, **13e**	
		azuleno[4,5-b]furan-2(3H)-one, **15e**	
		michefuscalide, **13e**	
		lipiferolide, **17e**	
		(-)-syringaresinol, **2g**	
	Not specified	tribenzylmagnolamine, **10h**	Tanaka *et al*., 1981
		tri-o-ethylmagnolamine, **11h**	
		coclaurine, **12h**	
		reticuline, **13h**	
		magnolamine, **14h**	

Scientific name	Part	Compounds	Bibliography
M. fuscata	Leaves	thalictrine picrate, **15h** D-(-)-2,2-dimethylcoclaurinium picrate, **16h** (-)-magnocurarine, **17h** α-magnoflorine, **18h**	Yakugaku *et al.*, 1959
	Not specified	magnolamine, **14h** (+)-armepavine, **19h** tri-o-methylmagnolamine, **20h** o-methylcodamine, **21h**	Ito *et al.*, 1959
		magnolamine, **14h** evoeuropine, **22h** magnolin, **23h**	Aleshinskaya *et al*, 1957
M. hydyosperma	Not specified	β-pinene, **5f** α-terpineol, **6f** safrole, **6i**	Liu *et al.*, 2007
	Fruit	methyl eugenol ether, **7i** epi-α-Selinene, **18e** β-sesquiphellandrene, **19e** α-cubebene, **20e** α-bergamotene, **21e** eudesma-4(14),11-diene, **22e** α-muurolene, **23e** α-caryophyllene, **24e** copaene , **25e** β-phellandrene, **16f** β-elemene, **8f** β-bisabolene, **26e** δ-cadinene, **27e**	

Scientific name	Part	Compounds	Bibliography
M. hydyosperma	Fruit	eucalyptol, **9f**	Liu *et al*., 2007
	Not specified	(+)-limonene, **10f**	
		safrole, **6i**	Wu *et al*., 1981
		methyl eugenol ether, **7i**	
M. lacei	Branches	(+)-alloaromadendrane-4α,10β-diol, **28e**	Chen *et al*., 2002
		D-aromadendrane-4β,10α-diol, **29e**	
		parthenolide, **5e**	
		spathulenol, **30e**	
		syringin, 8i	
M. lanuginose	Bark	(-)-parthenolide, **5e**	Talapatra *et al*., 1978
		11βH,13-dihydroparthenolide, **4e**	
	Bark	michelanugine, **24h**	Talapatra *et al*., 1975
		N,O- diacetylmichelanugine, **25h**	
		oxoushinsunine, **1h**	
		oxoxylopine, **9h**	
M. maudiae	Leaves	(±)-γ-cadinene, **31e**	Cao *et al*., 2007
		γ-murolene, **32e**	
		4-carene, **11f**	
		l-alloaromadendrene, **33e**	
		l-terpinen-4-ol, **12f**	
		β-cubebene, **34e**	

Scientific name	Part	Compounds	Bibliography
M. maudiae	Leaves	(±)-3-carene, **13f** (R)-(+)-α-pinene, **3f** α-caryophyllene, **24e** espatulenol, **35e** (+)-limonene, **10f** α-copaene, **25e** elixene, **14f** β-caryophyllene oxide, **36e** δ-terpinene, **15f** (+)-ledol, **37e** β-phellandrene, **16f** (-)-β-cadinene, **38e** β-elemene, **39e** 2-borneol, **17f** α-gurjunene, **40e** (+)-aromadendrene, **41e** β-selinenol, **42e** eucalyptol, **4f** β-pinene, **5f** γ-caryophyllen, **43e** γ-terpinene, **18f** α-terpineol, **6f** 3,3-dimethyl-2-methylenenorbornane, **7f**	Cao *et al.*, 2007

Scientific name	Part	Compounds	Bibliography
M. maudiae	Leaves	β-caryophyllene, **44e**	Cao *et al*., 2007
M. montana	Bark	safrole, **6i**	Dutta *et al*., 1987
		sarisan, **9i**	
	Leaves	asaricin, **10i**	Van Genderren *et al*., 1999
		α-asaron, **11i**	
		myristicin, **12i**	
		safrole, **6i**	
		eugenyl methyl ether, **13i**	
M. nilagirica	Root Bark	parthenolide, **5e**	Kumar *et al*., 1995
		costunolide, **1e**	
M. rajaniana	Bark	(-)-parthenolide, **5e**	Ruangrungsi *et al*., 1988
		oxoushinsunine, **1h**	
M. szechuanica	Aerial part	sphaelactone A, **45e**	Lin *et al*., 1999
		3,4-divanilyltetrahydrofuran, **3g**	
		(-)-syringaresinol, **2g**	
		sinapaldehyde, **14i**	
		syringaldehyde, **15i**	
M. yunnanennsis	Flower	(+)-methylxanthoxylol, **4g**	Xiong *et al*., 2008
		horsfieldin, **5g**	
		(-)-sesamin, **6g**	
		(-)-eudesmin, **7g**	
	Not specified	1β-hydroxyarbusculin A**, 46e**	Hong *et al*., 1998
		reinosin**, 47e**	
		(-)-parthenolide**, 5e**	
		oleanolic acid**, 1d**	
		syringaldehyde**, 15i**	
	Not specified	12,13-di-acetoxy-1,4,6,11-eudesmanetetrol**, 48e**	Hong *et al*., 1998

Structures

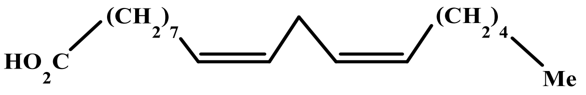
a: aliphatic

$HC_2O—(CH_2)_4—Me$

1a: palmitic acid

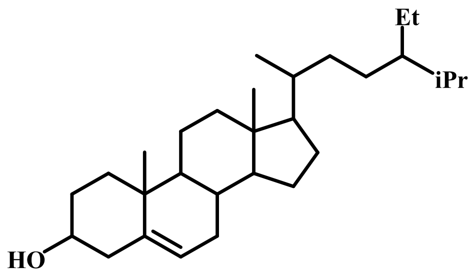
$HO_2C—(CH_2)_{16}—Me$

2a: stearic acid

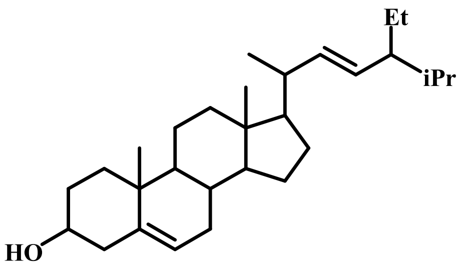


3a: linoleic acid

b: steroids

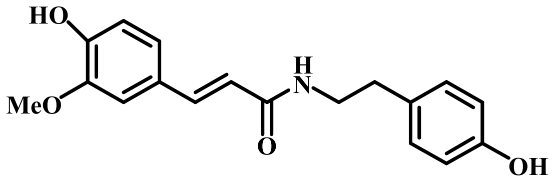


1b: β-sitosterol



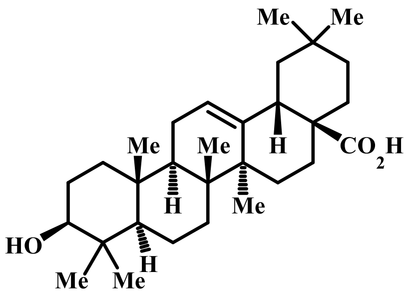
2b: stigmasterol

c: amide



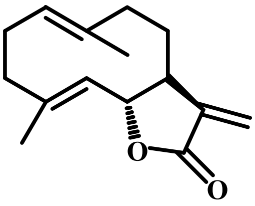
1c: *N-trans*-feruloyltyramine

d: triterpenoids

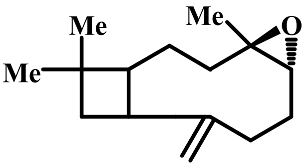


1d: oleanolic acid

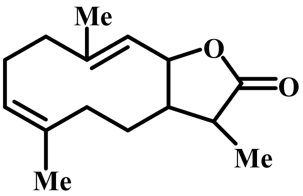
e: sesquiterpenoids



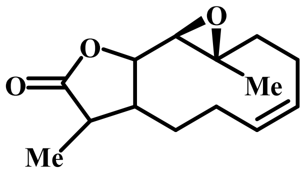
1e: costunolide



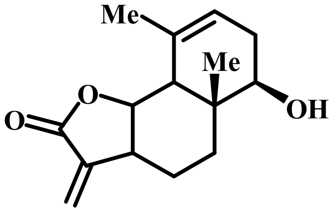
2e: caryophyllene oxide



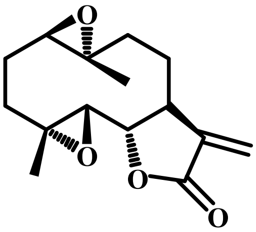
3e: dihydrocostunolide



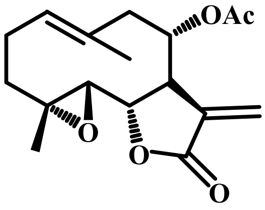
4e: dihydroparthenolide



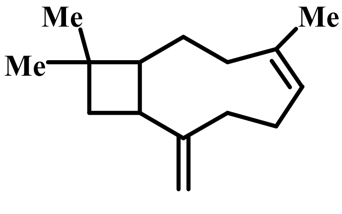
5e: parthenolide



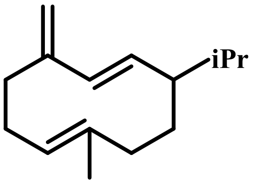
6e: michelenolide



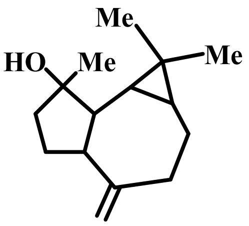
7e: 11,13-dehydrolanuginolide



8e: caryophyllene

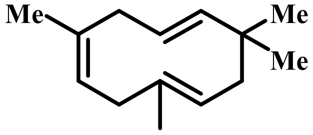


9e: germacrene D

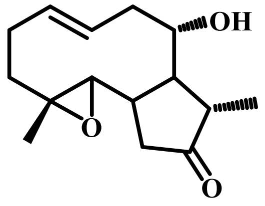


10e: spathulenol

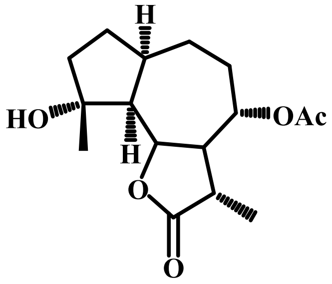
e: sesquiterpenoids



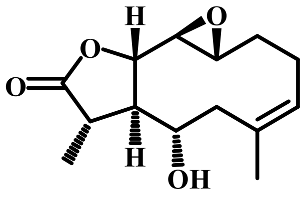
11e: α-humulene



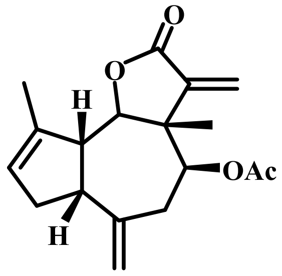
12e: deacetyllanuginolide



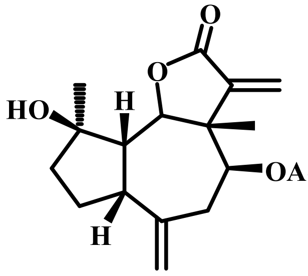
13e: michefuscalide



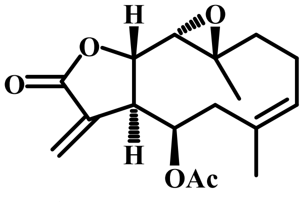
14e: 11,13-dihydrostizolin



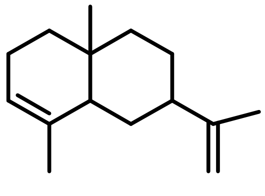
15e: azuleno[4,5-b]furan-2(3H)-one



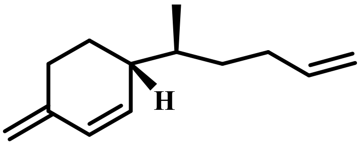
16e: β-cyclolipiferolide



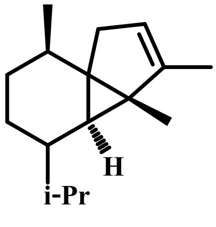
17e: lipiferolide



18e: epi-α-selinene

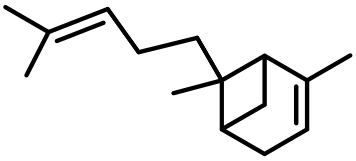


19e: β-sesquiphellandrene

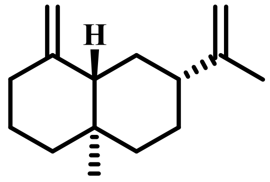


20e: α-cubebene

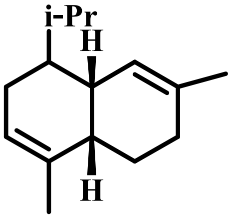
e: sesquiterpenoids



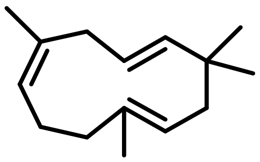
21e: α-bergamotene



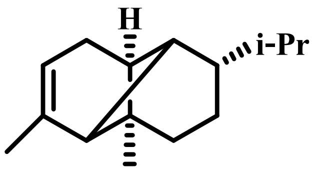
22e: eudesma-4(14),11-diene



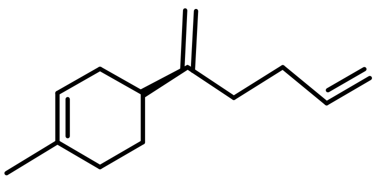
23e: α-muurolene



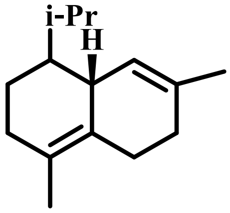
24e: α-caryophyllene



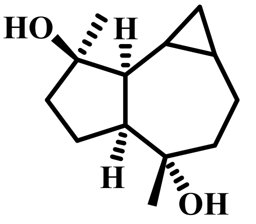
25e: copaene



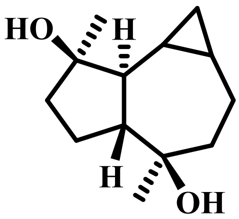
26e: β-bisabolene



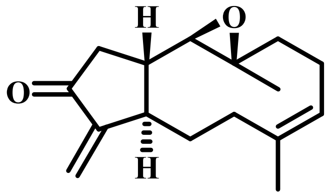
27e: δ-cadinene



28e: (+)-alloaromadendrane-4α,10β-diol

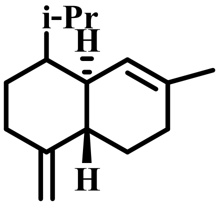


29e: D-aromadendrane-4β,10α-diol

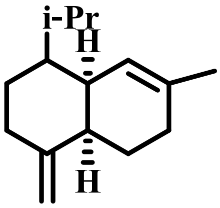


30e: spathulenol

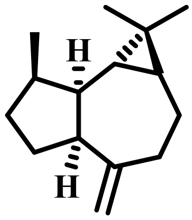
e: sesquiterpenoids



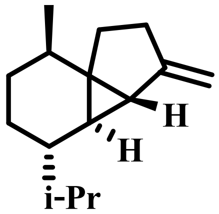
31e: (±)-γ-cadinene



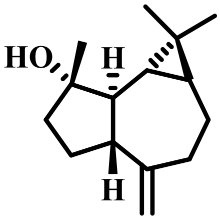
32e: γ-murolene



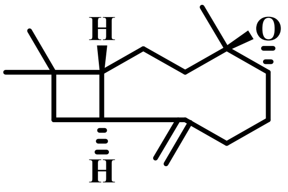
33e: l-alloaromadendrene



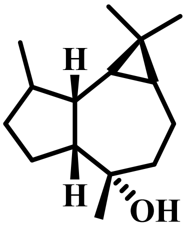
34e: β-cubebene



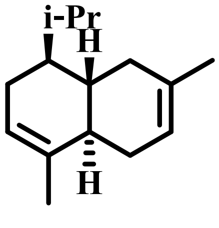
35e: espatulenol



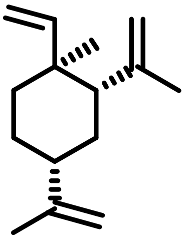
36e: β-caryophyllene oxide



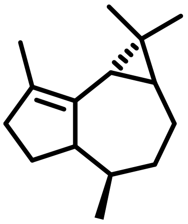
37e: (+)-ledol



38e: (-)-β-cadinene

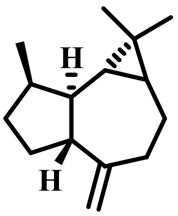


39e: β-elemene

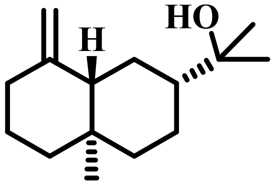


40e: α-gurjunene

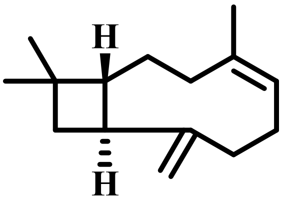
e: sesquiterpenoids



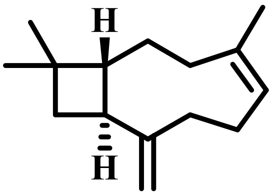
41e: (+)-aromadendrene



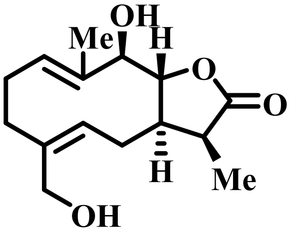
42e: β-selinenol



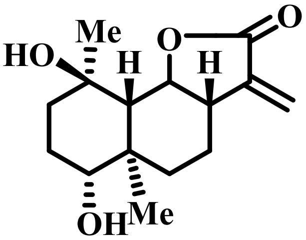
43e: γ-caryophyllen



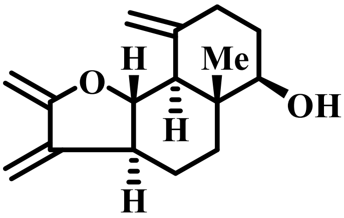
44e: β-caryophyllene



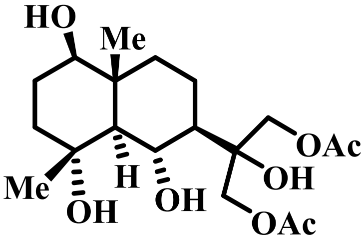
45e: sphaelactone A



46e: 1β-hydroxyarbusculin A

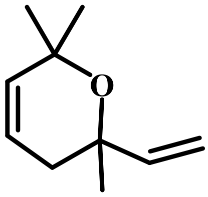


47e: reinosin

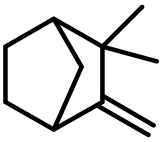


48e: 12,13-di-acetoxy-1,4,6,11-eudesmanetetrol

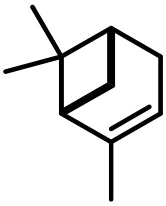
f: monoterpenoids



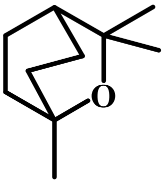
1f: dehydrolinalool oxide



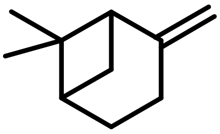
2f: camphene



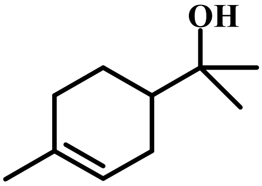
3f: α-pinene



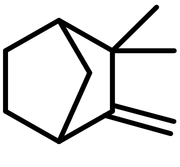
4f: eucalyptol



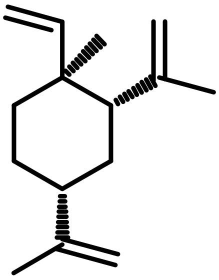
5f: β-pinene



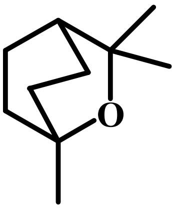
6f: α-terpineol



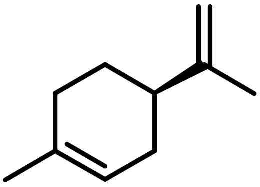
7f: 3,3-dimethyl-2-methylenenorbornane



8f: β-elemene

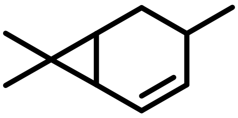


9f: eucalyptol

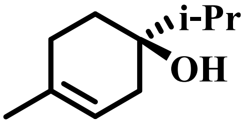


10f: (+)-limonene

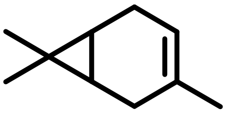
f: monoterpenoids



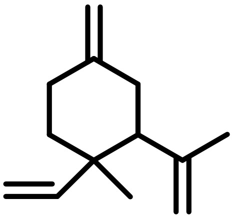
11f: 4-carene



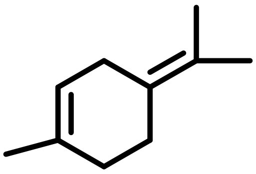
12f: l-terpinen-4-ol



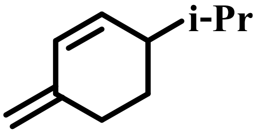
13f: 3-carene



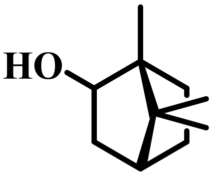
14f: elixene



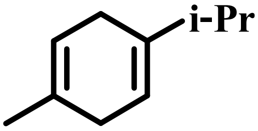
15f: δ-terpinene



16f: β-phellandrene

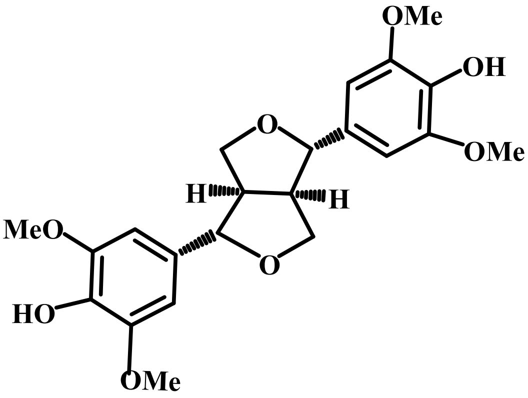


17f: 2-borneol

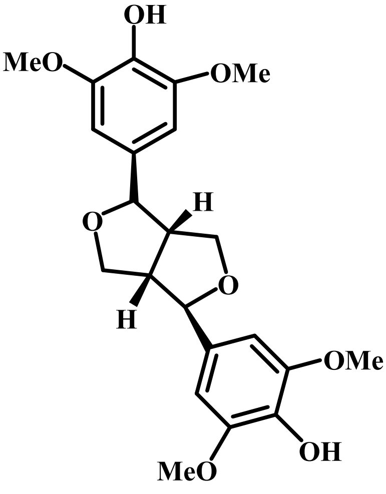


18f: γ-terpinene

g: lignin

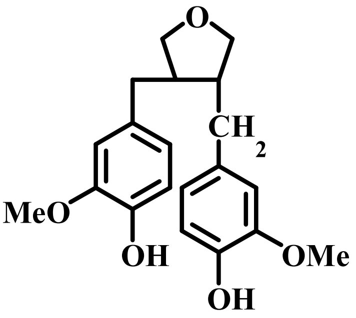


1g: syringaresinol

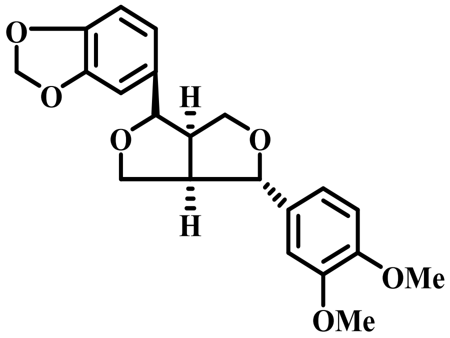


2g: (-)-syringaresinol

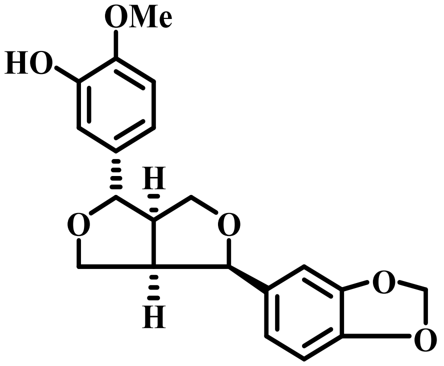
g: lignin



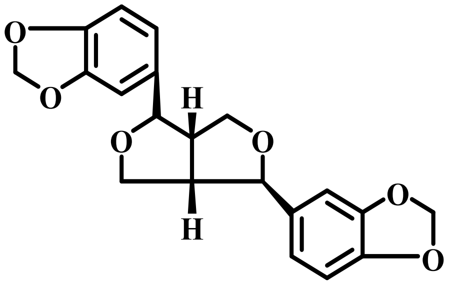
3g: 3,4-divanilyltetrahydrofuran



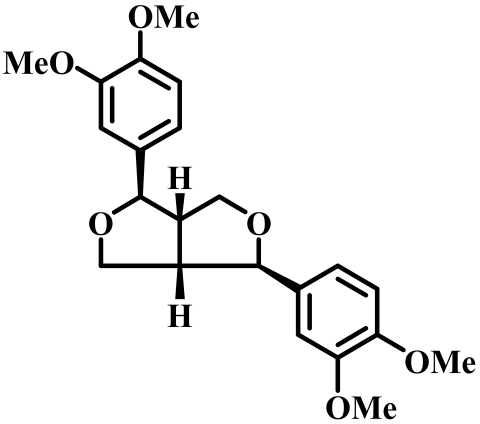
4g: (+)-methylxanthoxylol



5g: horsfieldin

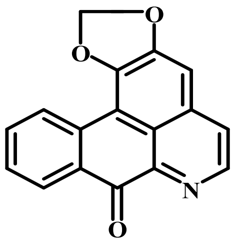


6g: (-)-sesamin

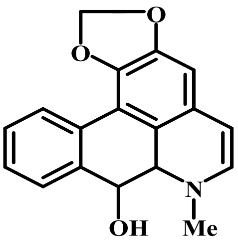


7g: (-)-eudesmin

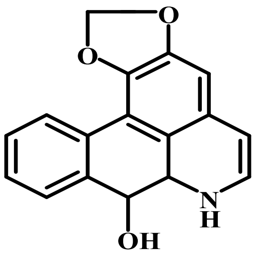
h: alkaloids



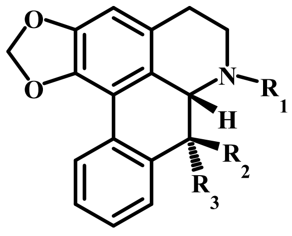
1h: oxoushinsunin



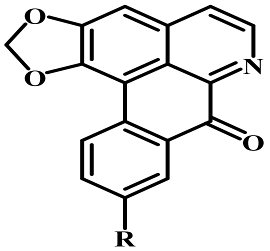
2h: ushinsunin



3h: norushinsunin

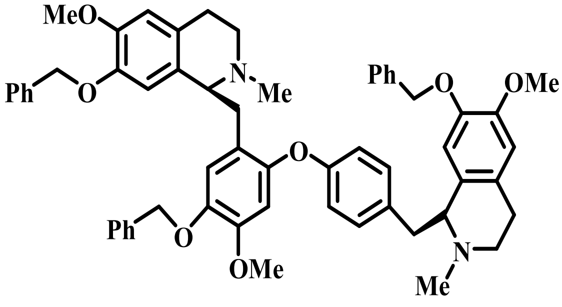


4h: $R_1 = R_2 = R_3 = H$	**4h**: (-)-anonaine
5h: $R_1 = R_2 = H$, $R_3 = OH$	**5h**: (-)-norushinsunine
6h: $R_1 = CH_3$, $R_2 = H$, $R = OH$	**6h**: (-)-ushinsunine
7h: $R = COCH_3$, $R_2 = R_3 = H$	**7h**: (-)-*N*-acetylanonaine

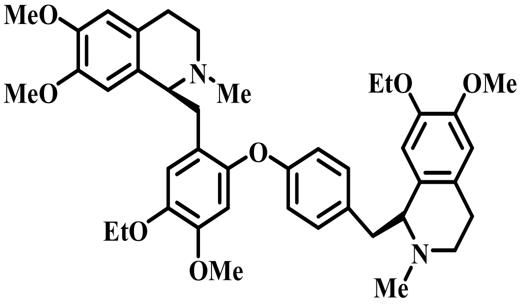


8h: $R = H$	**8h**: liriodenine
9h: $R = OH$	**9h**: oxoxylopine

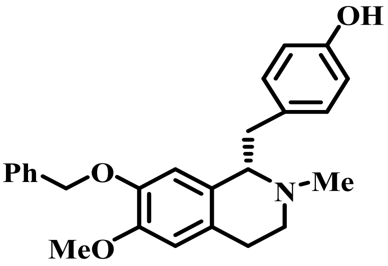
h: alkaloids



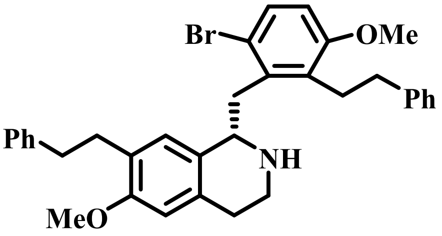
10h: tribenzylmagnolamine



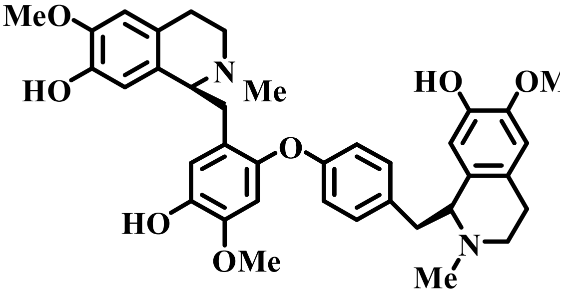
11h: tri-O-ethylmagnolamine



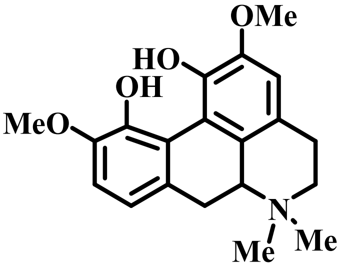
12h: coclaurine



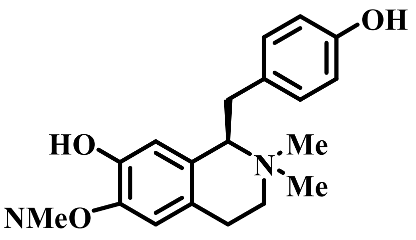
13h: reticuline



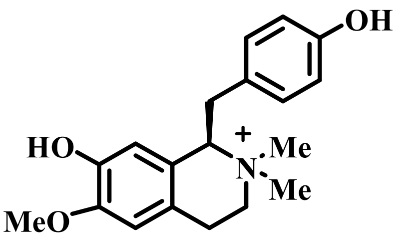
14h: magnolamine



15h: thalictrine picrate

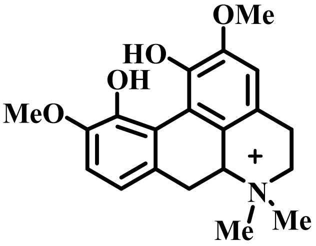


16h: D-(-)-2,2-dimethylcoclaurinium
picrate

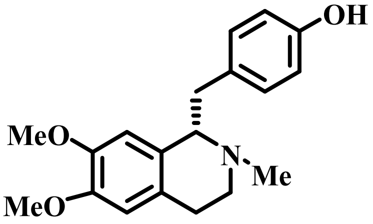


17h: (-)-magnocurarine

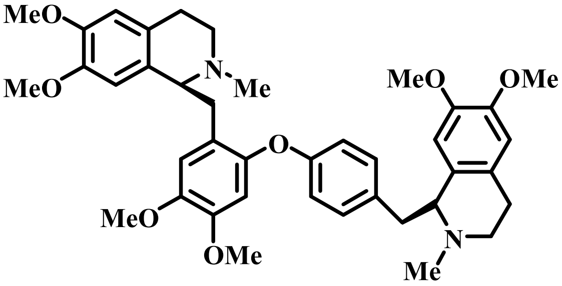
h: alkaloids



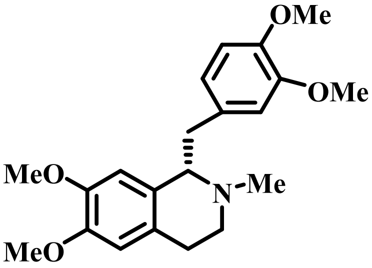
18h: α-magnoflorine



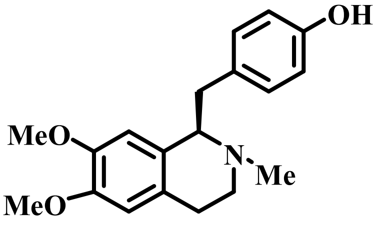
19h: (+)-armepavine



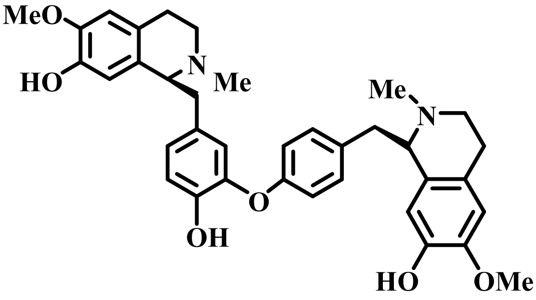
20h: tri-O-methylmagnolamine



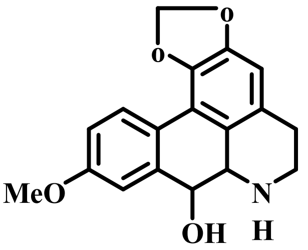
21h: O-methylcodamine



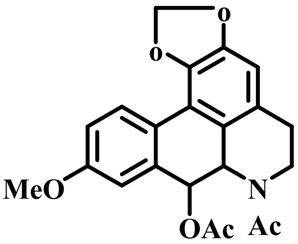
22h: evoeuropine



23h: magnolin

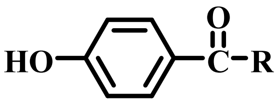


24h: michelanugine

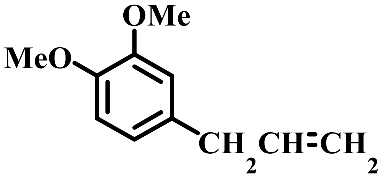


25h: N,O-diacetylmichelanugine

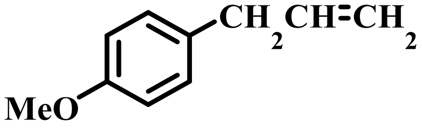
i: benzenoids



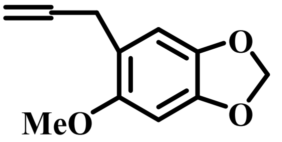
1i: R = H	**1i**: 4-hydroxybenzaldehyde
2i: R = OH	**2i**: 4-hydroxybenzoic acid
3i: R = OCH_3	**3i**: methylparaben



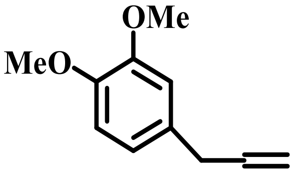
4i: eugenol methyl ether



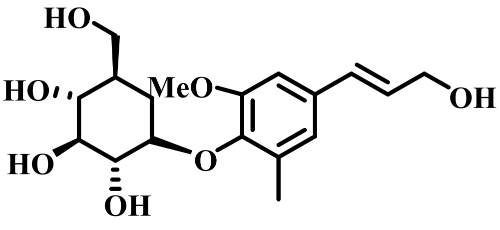
5i: estragole



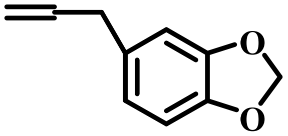
6i: safrole



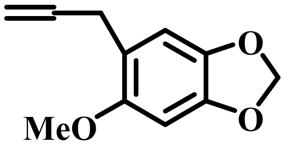
7i: methyl eugenol ether



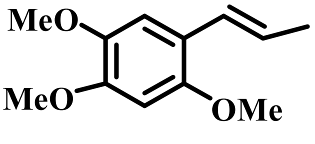
8i: syringing



9i: sarisan

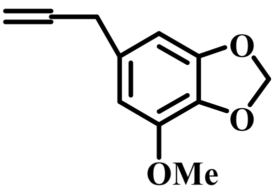


10i: asaricin

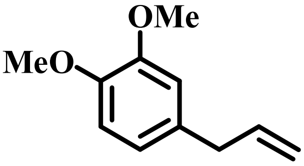


11i: α-asaron

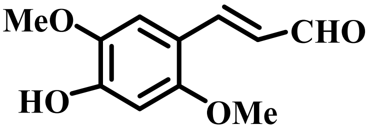
i: benzenoids



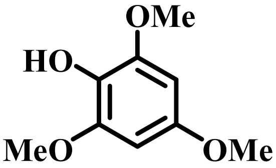
12i: myristicin



13i: eugenyl methyl ether



14i: sinapaldehyde



15i: syringaldehyde

2.1.3 Objective

This part of research work involved isolation, purification and structure elucidation of chemical constituents from the root of *Michelia alba*.

CHAPTER 2.2

EXPERIMENTAL

2.2.1 Instruments and Chemicals

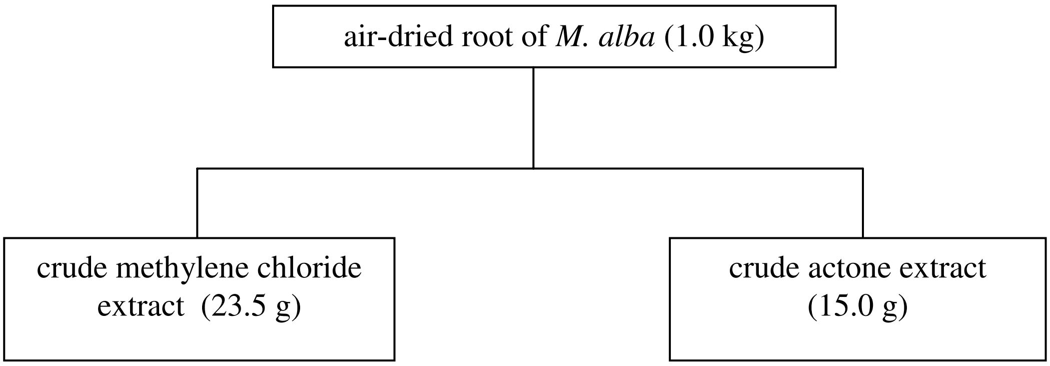
Melting points were determined on the Fisher-John melting point apparatus. The UV spectra were measured with a SPECORD S 100 (Analytikjena) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in MeOH solution. The optical rotation $[\alpha]_D$ was measured in chloroform and methanol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

2.2.2 Plant Material

The root of *M. alba* was collected from Chumphon province in the southern part of Thailand, in May 2008. Identification was made by Assoc. Prof. Dr. Kitichate Sridith and a specimen (No. 0013594) deposited at PSU Herbarium, Department of Biology, Faculty of Science, Prince of Songkla University.

2.2.3 Extraction and Isolation

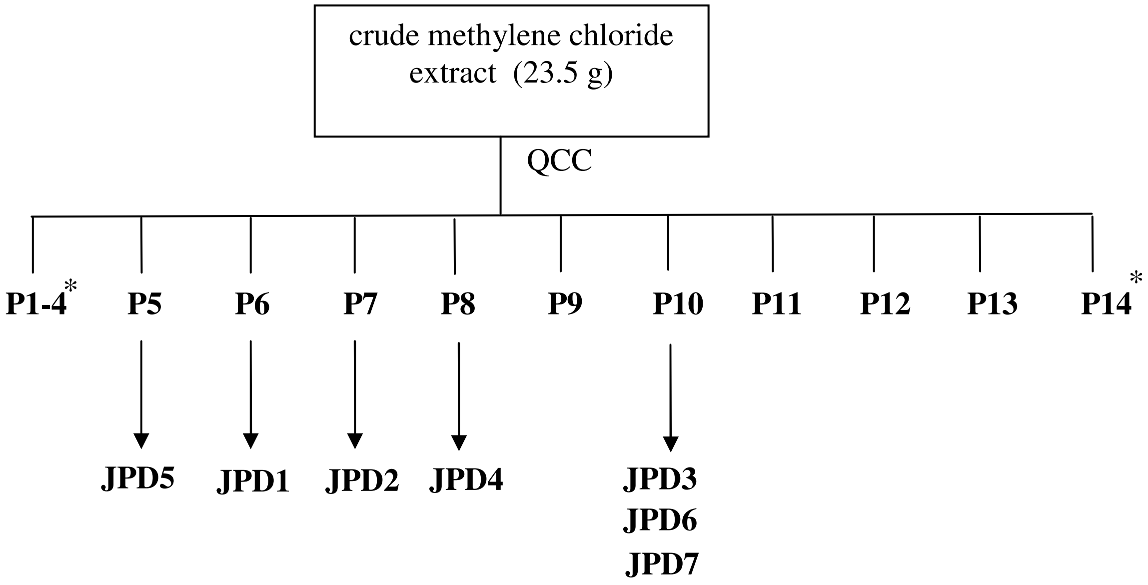
The chopped air-dried root of *M. alba* (1.0 kg) was successively extracted with methylene chloride and acetone (one week for each solvent) at room temperature. The solvent was evaporated under reduced pressure to give crude methylene chloride extract as green viscous residue (23.5 g) and crude acetone extract (15.0 g), respectively. The process of extraction was shown in **Scheme 4.**



Scheme 4. Extraction of the root of *M. alba*

2.2.4 Isolation and Chemical Investigation

2.2.4.1 Investigation of the crude methylene chloride extract from the root of *M. alba*



*No further investigation

Scheme 5 Isolation of compounds **JPD1- JPD7** from the methylene chloride extract

The crude methylene chloride extract as green viscous residue (23.5 g) was subjected to quick column chromatography over silica gel using solvent of increasing polarity from hexane through EtOAc. The eluates were collected and combined based on TLC characteristics to give fourteen fractions (P1-P14).

Fraction P5 (235.0 mg) was purified by CC with 10% acetone/hexane to give **JPD5**: T-cadinol (55.0 mg).

Fraction P6 (2.3 g) was filtered and washed with hexane to give **JPD1**: costunolide (1.21 g) as white crystal and the mother liquor as violet viscous oil after evaporation of the solvent.

Fraction P7 (2.8 g) was filtered and washed with hexane to give **JPD2**: parthenolide (1.71 g) as white crystal and the mother liquor as green viscous oil after evaporation of the solvent.

Fraction P8 (115.7 mg) was separated by CC with 30% EtOAc/hexane to give **JPD4**: reynosin (10.7 mg).

Fraction P10 (111.8 mg) was separated by CC with 30% acetone/hexane to give **JPD3**: 9*β*-hydroxy-11*β*H-dihydroparthenolide (6.7 mg), **JPD6**: 2-(3′,4′,5′-trihydroxy-3′-methylbutanoyloxy)-11*β*H-dihydroparthenolide (14.0 mg) and **JPD7**: lariciresinol (8.8 mg).

Compound JPD1: costunolide, white solid, m.p. 103-105 °C; $[\alpha]_D^{28}$: +132° (c = 0.30, $CHCl_3$);ref $[\alpha]_D^{28}$: +131° (c = 0.30, $CHCl_3$) (Ming *et al.*, 1989); UV λ_{max} (MeOH) (log ε): 207 (3.56) nm; IR (neat) ν_{max} 1763 (C=O stretching) and 1663 (C=C stretching) cm^{-1}. For ^1H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 14.**

Compound JPD2: parthenolide, white solid, m.p. 113-115 °C; $[\alpha]_D^{28}$: -50° (c = 0.49, $CHCl_3$); ref $[\alpha]_D^{28}$: -26° (c = 0.03, $CHCl_3$) (Galal *et al.*, 1999); UV λ_{max} (MeOH) (log ε): 205 (3.59) nm; IR (neat) ν_{max} 1769 (C=O stretching) and 1680 (C=C stretching) cm^{-1} . For ^1H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 15.**

Compound JPD3: 9*β*-hydroxy-11*β*H-dihydroparthenolide, white solid, m.p. 143-145°C; $[\alpha]_D^{28}$: -49.3° (c = 1.45, $CHCl_3$). UV λ_{max} (MeOH) (log ε): 205 (3.62) nm; IR (neat) ν_{max} 3444 (O-H stretching), 1769 (>C=O stretching) and 1669 (C=C stretching) cm^{-1} . For ^1H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 16.**

Compound JPD4: reynosin, white solid, m.p. 133-135 °C; $[\alpha]_D^{28}$: +95.6 (c = 0.06, $CHCl_3$); ref $[\alpha]_D^{28}$: +137° (c = 0.11, $CHCl_3$) (Abegaz *et al.*, 1991); UV λ_{max} (MeOH) (log ε): 205 (3.63) nm; IR (neat) ν_{max} 3467 (O-H stretching), 1766

(C=O stretching) and 1654 (C=C stretching) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 17.**

Compound JPD5: T-cadinol, white solid, m.p. 44-46 °C; $[\alpha]_D^{28}$: +5° (c = 0.9), $CHCl_3$); ref $[\alpha]_D^{28}$: +3° (c = 1.2, $CHCl_3$) (Claeson *et al.*, 1991); IR (neat) ν_{max} 3450 (O-H stretching) and 1668 (C=C stretching) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 18.**

Compound JPD6: 2α-(3′,4′,5′-trihydroxy-3′-methylbutanoyloxy)-11*β*H-dihydroparthenolide, colorless viscous oil; $[\alpha]_D^{28}$: -43° (c = 0.7) , $CHCl_3$). UV λ_{max} (MeOH) (log ε): 206 (3.76) nm; IR (neat) ν_{max} 3437 (O-H stretching), 1770 (>C=O stretching) and 1639 (C=C stretching) cm^{-1}. HRFAB: *m/z* $[M+H]^+$ 399.2015 (calcd for $C_{20}H_{31}O_8$, 3992019); For ^{1}H NMR ($CDCl_3$, 300 MHz) spectral data and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 19.**

Compound JPD7: lariciresinol, yellow viscous oil; $[\alpha]_D^{28}$: +35° (c = 1.3), $CHCl_3$); ref $[\alpha]_D^{28}$: +30° (c = 0.10, $CHCl_3$) (xie *et al.*, 2003); UV λ_{max} (MeOH) (log ε): 205 (3.76), 228 (3.24) and 281 (2.87) nm; IR (neat) ν_{max} 3419 (O-H stretching) and 1604 (C=C stretching) cm^{-1}. For ^{1}H NMR ($CDCl_3$, 300 MHz) ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data see **Table 20.**

CHAPTER 2.3

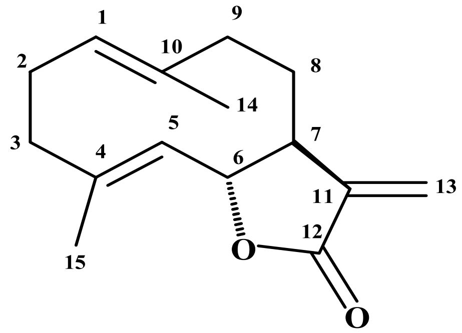
RESULTS AND DISCUSSION

2.3.1 Structure elucidation of compounds from the root of *M. alba*

The crude methylene chloride extract from the root of *M. alba* were subjected to repeated quick column and column chromatography over silica gel to furnish one new sesquiterpene: 2α-(3′,4′,5′-trihydroxy-3′-methylbutanoyloxy)-11*β*H-dihydroparthenolide (**JPD6**) together with five known sesquiterpenes: costunolide (**JPD1**), parthenolide (**JPD2**), 9*β*-hydroxy-11*β*H-dihydroparthenolide (**JPD3**), reynosin (**JPD4**) and T-cadinol (**JPD5**), and one known lignan: lariciresinol **(JPD7)**.

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^{1}H, ^{13}C NMR, DEPT 135°, DEPT 90°, HMQC, HMBC, COSY and NOESY. The physical data of the known compounds were also compared with the reported values. Mass spectra were determined for the new sesquiterpene: 2α-(3′,4′,5′-trihydroxy-3′-methylbutanoyloxy)-11*β*H-dihydroparthenolide (**JPD6**).

2.3.1.1 Compound JPD1



Compound JPD1 was obtained as a white solid, mp 103-105 °C, $[\alpha]_D^{28}$: +132° (c = 0.30, $CHCl_3$). The IR spectrum showed absorption bands at 1763 cm^{-1} indicating the presence of an α,β-unsaturated γ-lactone.

The ^{13}C NMR spectral data displayed 15 signals for 15 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of two methyl (δ 16.0 and 17.6), five methylene (δ 26.1, 27.9, 39.3, 40.9 and 119.8), four methine (δ 50.7, 82.0, 127.0 and 127.3) and four quaternary carbons (δ 136.8, 140.0, 141.3 and 170.3).The ^1H NMR spectral data showed signals assignable to two tertiary methyls at δ 1.42 (*s*, Me-14) and δ 1.70 (*s*, Me-15), a methine at δ 2.57 (m, H-7), the downfield exocyclic methylenes at δ 5.53 (1H, *d*, *J* = 3.6 Hz, H-13) and 6.25 (1H, *d, J* = 3.6 Hz, H-13), a methine bearing the oxygen function at δ 4.57 (*t, J* = 9.9 Hz, H-6), and two olefins at δ 4.84 (1H, *brdd, J* = 10.5, 3.9 Hz, H-1) and 4.74 (1H, *brd, J* = 9.9 Hz, H-5) together with four methylene protons.

The locations of the two methyl groups (Me-14 and Me-15) at C-10 and C-4, respectively were deduced from HMBC correlations of Me-14 (δ 1.42) with C-9 (δ 40.9), C-10 (δ 136.8) and C-1 (δ 127.0) and of Me-15 (δ 1.70) with C-3 (δ 39.3), C-4 (δ 140.0) and C-5 (δ 127.3). The stereochemistry at C-6 and C-7 in compound JPD1 was assigned from NOESY experiments. Since no cross peak was observed between H-6 and H-7, compound JPD1 should contain a *trans*-fused lactone ring. The lack of NOESY cross peaks between H-1 and Me-14 and between H-5 and Me-15 suggested *E*-configurations of both double bonds. On the basis of the above results and comparison with the reported data of costunolide [Ming *et al.*, 1989], compound JPD1 was assigned as costunolide.

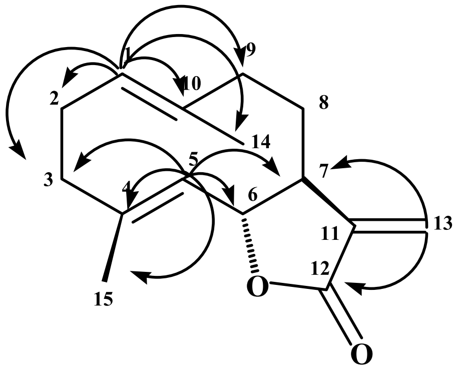


Figure 14 Selected HMBC correlations of **JPD1**

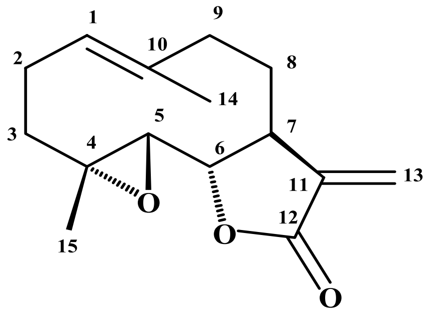
Table 14 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **JPD1** ($CDCl_3$) and costunolide (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $H^{1}\rightarrow{}^{13}C$
		JPD1	R	JPD1	R	
1	CH	127.0	127.0	4.84 (*brdd*, 10.5, 3.9)	4.84 (*brdd*, 12.3, 4.0)	2, 3, 9, 14
2	CH_2	26.1	28.2	2.0-2.4 (*m*)	1.67 (*m*), 2.0-2.4 (*m*)	1, 3, 4, 10
3	CH_2	39.3	41.1	2.4-2.0 (*m*)	2.4-2.0 (*m*)	6, 7, 12
4	C	140.0	140.0	-	-	-
5	CH	127.3	127.2	4.74 (*brd*, 9.9)	4.73 (*brd*, 10.5)	3, 6, 7, 11, 15
6	CH	82.0	82.0	4.57 (*t*, 9.9)	4.57 (*t*, 9.5)	4, 5, 7, 8, 11
7	CH	50.7	50.5	2.57 (*m*)	2.56 (*m*)	6, 9, 11, 12, 13
8	CH_2	27.9	26.3	1.67 (*m*), 2.0-2.4 (*m*)	2.0-2.4 (*m*)	6, 7, 9, 10
9	CH_2	40.9	39.7	2.0-2.4 (*m*)	2.0-2.4 (*m*)	1, 7, 8, 10
10	C	136.8	136.9	-	-	-
11	C	141.3	141.4	-	-	-
12	C	170.3	170.4	-	-	-

Table 14 (Continued)

Position	Type of C	δc /ppm		δH / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		JPD1	R	JPD1	R	
13	CH_2	119.8	119.7	5.53 (*d*, 3.6), 6.25 (*d*, 3.6)	5.51 (*d*, 3.5) 6.25 (*d*, 3.5)	6, 7, 12
14	CH_3	16.0	16.3	1.42 (*s*)	1.40 (*s*)	1, 2, 8, 9, 10
15	CH_3	17.6	17.5	1.70 (*s*)	1.70 (*s*)	3, 4, 5, 6

2.3.1.2 Compound JPD2



Compound JPD2 was obtained as a white solid, mp 113-115 °C, $[\alpha]_D^{28}$: -50° (c = 0.49, $CHCl_3$). The IR spectrum showed absorption bands of an α,β-unsaturated γ-lactone at 1769 cm^{-1}.

The ^{13}C NMR spectral data showed 15 signals for 15 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of two methyl (δ 17.0 and 17.3), five methylene (δ 24.2, 31.7, 36.4, 41.2 and 121.3), four methine (δ 47.7, 66.4, 82.5 and 125.3) and four quaternary carbons (δ 61.6, 134.7, 139.3 and 169.3). The ^{1}H NMR spectral data displayed the signals for exocyclic methylene protons conjugated with the γ-lactone ring system at δ 5.63 (H-13, *d, J* = 3.6 Hz) and 6.35 (H-13, *d, J* = 3.6 Hz), a lactone proton signal at δ 3.86 (H-6, *t, J* = 8.7 Hz), an oxymethine proton at δ 2.79 (1H, *d, J* = 8.7 Hz, H-5), two methyl signals at δ 1.71 (Me-14, *s*) and 1.30 (Me-15, *s*). The ^{1}H and ^{13}C NMR spectral data of compound JPD2 were closely related to those of compound JPD1 suggesting the same sesquiterpene lactone skeleton. The differences were shown at positions 4 and 5 in which an olefinic methine proton H-5 at δ_H 4.74 in JPD1 was replaced by an oxymethine proton at δ_H 2.79 (*d*, *J* = 8.7 Hz) in JPD2 and the chemical shifts of C-4 (δ 140.0) and C-5 (δ 127.3) which were those of sp^2 carbons in JPD1 were replaced by those of C-4 (δ 61.6) and C-5 (δ 66.4) in JPD2 whose values suggested an epoxide functionality.

The stereochemistry at C-4, C-5, C-6 and C-7 was deduced by NOESY experiment. Cross peaks were observed between H-5/H-7, H-6/Me-15, with the absence of cross peaks between H-6/H-7 and H-5/Me-15. These results indicated the *trans*-fused lactone ring and also the orientation of the epoxy group to be *trans* to Me-

15 and to H-5. Thus on the basis of its spectroscopic data and comparison with the previously reported data of parthenolide (Galal *et al*., 1999), compound JPD2 was therefore, assigned as parthenolide.

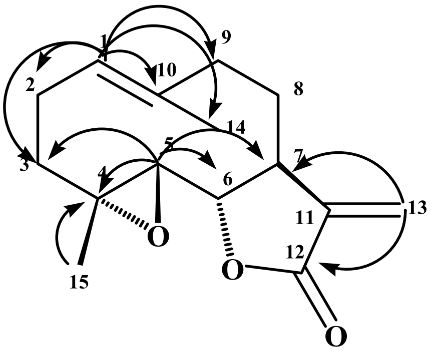


Figure 15 Selected HMBC correlations of **JPD2**

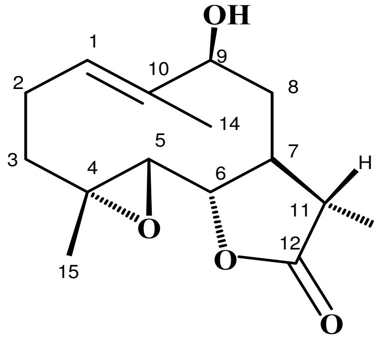
Table 15 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **JPD2** ($CDCl_3$) and parthenolide (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δ$_H$ / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow ^{13}C$
		JPD2	R	JPD2	R	
1	CH	125.3	125.2	5.21 (*br d*, 11.7)	5.21 (*dd*, 12.2, 4.0)	2, 3, 8, 9, 14
2	CH_2	24.2	24.1	2.10-2.25 (*m*), 2.46 (*m*)	2.09-2.24 (*m*), 2.46 (*ddd*, 13.8, 12.2, 12.5)	1, 3, 4, 10
3	CH_2	36.4	36.3	1.25 (*m*), 2.10-2.25 (*m*)	1.25 (*m*), 2.09-2.24 (*m*)	1, 2, 4, 5, 15
4	C	61.6	61.5	-	-	-
5	CH	66.4	66.3	2.79 (*d*, 8.7)	2.79 (*d*, 8.9)	3, 4, 6, 7, 15
6	CH	82.5	82.4	3.86 (*t*, 8.7)	3.86 (*dd*, 8.9, 8.3)	4, 7, 8, 11, 12
7	CH	47.7	47.6	2.78 (*m*)	2.78 (*m*)	5, 9, 11, 12, 13
8	CH_2	31.7	30.6	2.10-2.25 (*m*) 1.72 (*m*)	2.09-2.24 (*m*) 1.73 (*m*)	6, 7, 9, 10
9	CH_2	41.2	41.1	2.10-2.25 (*m*) 2.38 (*m*)	2.09-2.24 (*m*) 2.38 (*m*)	1, 7, 8, 10, 14

Table 15 (Continued)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		JPD2	R	JPD2	R	
10	C	134.7	134.6	-	-	-
11	C	139.3	139.2	-	-	-
12	C	169.3	169.2	-	-	-
13	CH_2	121.3	121.1	5.63 (*d*, 3.6), 6.35 (*d*, 3.6)	5.63 (*d*, 3.6), 6.35 (*d*, 3.6)	7, 11, 12
14	CH_3	17.0	16.5	1.71 (*s*)	1.72 (*s*)	1, 9, 10
15	CH_3	17.3	17.2	1.30 (*s*)	1.31 (*s*)	3, 4, 5

2.3.1.3 Compound JPD3



Compound JPD3 was obtained as a white solid , mp 143-145 °C, $[\alpha]_D^{28}$: -49.3° (c = 1.45, $CHCl_3$). The IR spectrum showed absorption bands at 3444 and 1769 cm^{-1} indicating the presence of hydroxyl and γ-lactone functionalities, respectively.

The ^1H and ^{13}C NMR spectral data of compound JPD3 were comparable to those of compound JPD2. The major differences between compound JPD3 and compound JPD2 were that compound JPD3 did not show the two downfield doublets at δ_H 5.63 and 6.35 due to the exocyclic methylene protons as in compound JPD2. Instead, in compound JPD3 a new methyl signal at δ_H 1.30 (*d*, J = 7.2 Hz) appeared together with a multiplet signal of a methine proton at δ_H 2.30. A new oxymethine proton was also evidenced at δ_H 4.15 (m) whose position at C-9 was determined through an HMBC experiment which showed correlations with C-1 (δ 125.8), C-7 (δ 48.3), C-8 (δ 37.8) and C-14 (δ 10.8). The new methyl protons at δ 1.30 (Me-13) was attached to the ring at C-11 due to its HMBC correlations with C-7 (δ 48.3), C-11 (δ 42.0) and C-12 (δ 177.2). NOESY experiment displayed cross peaks of H-7/Me-13/H-9 and H-6/H-11 suggesting 9*β*OH and 11*β*H. Thus on the basis of its spectroscopic data and comparison with the previous report [Galal et al., 1999], compound JPD3 was assigned as 9*β*-hydroxy-11*β*H-dihydroparthenolide.

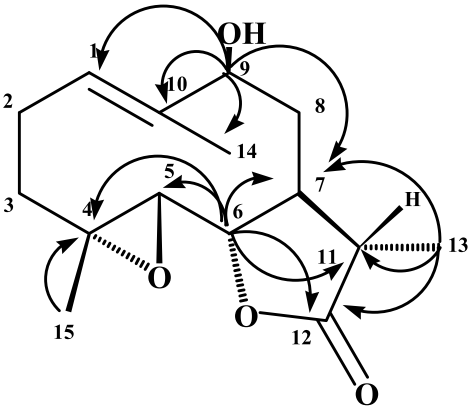


Figure 16 Selected HMBC correlations of **JPD3**

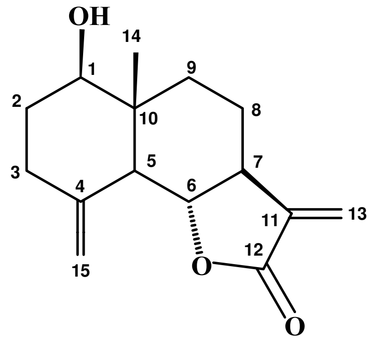
Table 16 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **JPD3** ($CDCl_3$) and 9*β*-hydroxy-11*β*H-dihydroparthenolide (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δ$_H$ / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		JPD3	R	JPD3	R	
1	CH	125.8	126.6	5.36 (*dd*, 12.0, 2.7)	5.37 (*dd*, 12.3, 1.2)	2, 3, 9, 14
2	CH_2	23.6	24.2	2.16 (*m*), 2.46 (*m*)	2.16 (m), 2.46 (*dddd*, 13.4, 12.2, 5.4, 4.5)	1, 3, 4, 10
3	CH_2	36.3	36.8	1.12 (*m*), 2.13 (*m*)	1.12 (*ddd*, 13.0, 5.6, 5.5), 2.14 (*m*)	1, 5, 15
4	C	61.4	61.8	-	-	-
5	CH	66.0	66.5	2.61 (*d*, 8.7)	2.6 (*d*, 8.9)	3, 4, 6, 7
6	CH	81.3	81.7	3.81 (*t*, 8.7)	3.8 (*t*, 8.6)	4, 5, 7, 8, 11
7	CH	48.3	48.9	1.96 (*m*)	1.96 (*m*)	5, 9, 11, 13
8	CH_2	37.8	38.2	1.96 (*m*), 1.89 (*m*)	1.96 (*m*), 1.86 (*m*)	6, 9, 11
9	CH	80.0	80.0	4.15 (*m*)	4.16 (*m*)	1, 7, 10 ,14
10	C	136.6	136.9	-	-	-
11	CH	42.0	42.5	2.30 (*m*)	2.29 (*m*)	6, 8, 12, 13
12	C	177.2	177.4	-	-	-

Table 16 (Continued)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		JPD3	R	JPD3	R	
13	CH_3	13.6	13.6	1.30 (d, 7.2)	1.30 (d, 7.0)	7, 11, 12
14	CH_3	10.8	11.3	1.73 (s)	1.73 (s)	1, 9, 10
15	CH_3	17.2	17.7	1.31 (s)	1.31 (s)	3, 4, 5

2.3.1.4 Compound JPD4



Compound JPD4 was obtained as a white solid, mp 133-135 °C, $[\alpha]_D^{28}$: +95.6 (c = 0.26, $CHCl_3$). The IR spectrum showed absorption bands at 3467 and 1766 cm^{-1} indicating the presence of hydroxyl and γ-lactone functionalities, respectively.

The ^{13}C NMR and DEPT spectral data exhibited 15 carbons, attributable to one methyl (δ 11.6), six methylene (δ 21.4, 31.3, 33.5, 35.7, 110.0 and 117.0), four methine (δ 49.6, 53.0, 78.2 and 79.6) and four quaternary carbons (δ 43.0, 139.2, 142.4 and 170.7). The ^1H NMR spectral data displayed signals assignable to a tertiary methyl at δ 0.81 (Me-14), an oxymethine at δ 3.55 (1H, *dd, J* = 11.4, 4.5 Hz, H-1) and two sets of exocyclic methylene protons at δ 4.85 (1H, *br s,* H-15), 5.00 (1H, *br s*, H-15) and 5.43 (1H, *d, J = 3.6,* H-13), 6.10 (1H, *d, J = 3.6* Hz, H-13).

The locations of the two sets of exocyclic methylene protons at C-13 and C-15 were confirmed by HMBC correlations of 2H-13 at δ 5.43 and 6.10 with the carbons at C-11 (δ 139.2), C-12 (δ 170.7) and C-7 (δ 49.6), and of 2H-15 at δ 4.85 and 5.00 with C-3 (δ 33.5), C-4 (δ 142.4) and C-5 (δ 53.0). In addition an oxymethine proton at δ 3.55 showed correlations with C-2 (δ 31.3), C-3 (δ 33.5), C-10 (δ 43.0), C-5 (δ 53.0) and C-14 (δ 11.6) suggesting a hydroxyl group at C-1. NOESY experiment displayed cross peak between H-1/H-5, H-5/H-7, H-6/Me-14 and no cross peaks between H-6/H-7 suggesting that Me-14 and H-6 were on the same side whereas those of H-1, H-5 and H-7 were on the same side but opposite to Me-14 and H-6 and the lactone ring was *trans*-fused as in compounds JPD1 and JPD2. On the basis of the above analysis and comparison with the literatures, the structure of JPD4 was identified as reynosin (Abegaz *et al.*, 1991).

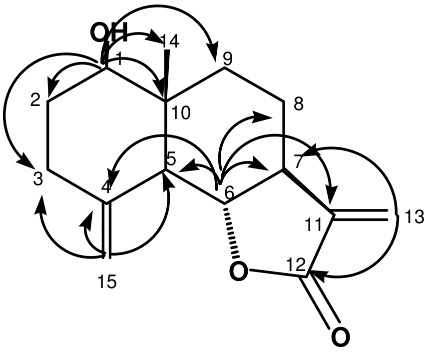
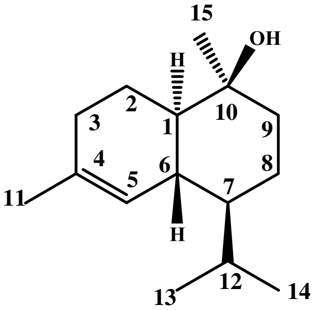


Figure 17 Selected HMBC correlations of **JPD4**

Table 17 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **JPD4** ($CDCl_3$) and reynosin (**R**, $CDCl_3$)

Position	Type of C	δc /ppm	δ$_H$ / ppm (multiplicity, J/Hz)		HMBC $^{1}H \rightarrow ^{13}C$
		JPD4	JPD4	R	
1	CH	78.2	3.55 (*dd*, 11.4, 4.5)	3.55 (*dd*, 12.0, 6.0)	2, 3, 5, 10, 14
2	CH_2	31.3	-	-	-
3	CH_2	33.5	1.60 (*m*), 1.80 (*m*)	-	-
4	C	142.4	-	-	-
5	CH	53.0	2.19 (*d*, 10.8)	-	1, 3, 7, 9
6	CH	79.6	4.02 (*t,* 10.8)	4.02 (*t*, 11.0)	4, 5, 8, 10, 11, 12
7	CH	49.6	2.55 (*td*, 11.5, 3.0)	-	5, 6, 8, 11, 13
8	CH_2	21.4	1.60 (*m*), 2.10 (*m*)	-	-
9	CH_2	35.7	1.30 (*m*), 2.15 (*m*)	-	-
10	C	43.0	-	-	-
11	C	139.2	-	-	-
12	C	170.7	-	-	-
13	CH_2	117.0	5.43 (*d*, 3.6), 6.10 (*d*, 3.6)	5.43 (*d*, 3.6), 6.10 (*d*, 3.6)	7, 11, 12
14	CH_3	11.6	0.81 (*s*)	0.80 (*s*)	1, 5, 9, 10
15	CH_2	110.0	4.85 (*br s*) 5.00 (*br s*)	4.85 (*br s*) 5.00 (*br s*)	3, 4, 5

2.3.1.5 Compound JPD5



Compound JPD5 was obtained as a white solid, m.p. 44-46 °C, $[\alpha]_D^{28}$: +5° (c = 0.9), $CHCl_3$). The IR spectrum showed absorption bands of hydroxyl group at 3450 $cm^{-1}$and double bond at 1668 cm^{-1}.

The ^{13}C NMR spectral data showed 15 signals for 15 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of four methyl (δ 15.2, 21.2, 26.1 and 28.2), four methylene (δ 19.8, 22.6, 30.9 and 40.3), five methine (δ 23.7, 37.7, 46.6, 47.9 and 122.6) and two quaternary carbons (δ 70.7, and 134.3).

The ^1H NMR spectral data displayed the signals for an isopropyl group at δ 2.18 (1H, *m*, H-12), 0.79 (3H, *d*, *J* = 6.9 Hz, Me-14) and 0.91 (3H, *d*, *J* = 6.9 Hz, Me-13), a three-proton singlet at δ 1.22 for a methyl attached to a quaternary carbon bearing a hydroxyl group, a trisubstituted olefinic proton at δ 5.55 (1H, *brs*, H-5) and a methyl group at δ 1.67 (*br s*).

The stereochemistry at C-1, C-6, C-7 and C-10 was deduced by NOESY experiment. Cross peaks were observed between H-1/H-7, H-1/Me-15, with the absence of cross peaks between H-1/H-6. These results indicated the *trans*-fused ring of JPD5. Thus on the basis of its spectroscopic data and comparison with the previously reported data of T-cadinol (Claeson *et al.*, 1991), compound JPD5 was therefore, assigned as T-cadinol.

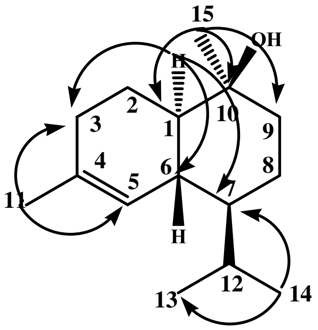
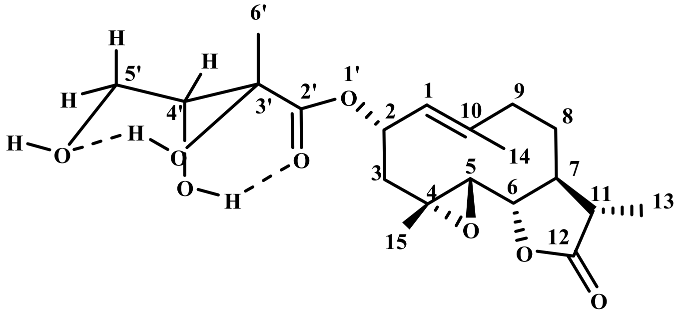


Figure 18 Selected HMBC correlations of **JPD5**

Table 18 ^1H, ^{13}C NMR and HMBC spectral data of compounds **JPD5** ($CDCl_3$) and T-cadinol (**R**, $CDCl_3$)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		JPD5	R	JPD5	R	
1	CH	47.9	47.9	1.10 (*dd*, 10.2, 2.1)	1.09 (*ddd*, 12.3, 10.2, 1.9)	3, 6, 7
2	CH_2	22.6	22.6	1.93 (*m*), 1.35 (*m*)	1.92 (*m*), 1.35 (*m*)	-
3	CH_2	30.9	30.9	1.89-2.20 (*m*)	1.92-2.08 (*m*)	-
4	C	134.3	134.3	-	-	-
5	CH	122.6	122.6	5.55 (*brs*)	5.55 (*brs*)	1, 3, 6, 7, 11
6	CH	37.7	37.7	1.97 (*brs*)	1.95 (*brs*)	2, 4, 5, 10, 12
7	CH	46.6	46.6	1.00 (*tt*, 11.1, 2.1)	1.00 (*tt*, 11.3, 3.2)	1, 6, 8, 9, 12, 13
8	CH_2	19.8	19.8	1.45 (*m*), 1.33 (*m*)	1.47(*m*), 1.32 (*m*)	-
9	CH_2	40.3	40.3	1.40 (*m*), 1.72 (*m*)	1.41(*m*), 1.74 (*m*)	-
10	C	70.7	70.6	-	-	-
11	CH	23.7	23.8	1.67 (*brs*)	1.67 (*brs*)	3, 4, 5
12	CH_3	26.1	26.2	2.18 (*hept d*, 3.3)	2.18 (*hept d*, 3.2)	6, 7, 8, 13, 14
13	CH_3	21.2	21.4	0.91 (*d*, 6.9)	0.91 (*d*, 6.9)	7, 12, 14
14	CH_3	15.2	15.2	0.79 (*d*, 6.9)	0.79 (*d*, 7.0)	7, 12, 13
15	CH_3	28.2	28.5	1.22 (*s*)	1.22 (*s*)	1, 9, 10

2.3.1.6 Compound JPD6



Compound JPD6 was obtained as a colorless gum, [α] $_D$ ^{28}: -43° (c = 0.7) , CHCl$_3$). It was assigned a molecular formula $C_{20}H_{31}O_8$ $[M+H]^+$ on the basis of a molecular ion at *m/z* 399.2015 by HRFABMS. The IR spectrum showed absorption bands of an α,β-unsaturated γ-lactone at 1770 cm^{-1}and hydroxyl at 3437 cm^{-1}.

The ^{13}C NMR spectral data showed 20 signals for 20 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of four methyl (δ 17.0, 17.6, 18.2 and 21.4), four methylene (δ 29.4, 41.2, 45.4 and 72.0), seven methine (δ 42.4, 51.8, 66.3, 66.4, 73.2, 81.8 and 128.9) and five quaternary carbons (δ 60.7, 73.5, 136.2, 177.2 and 178.2).

The ^{1}H and ^{13}C NMR spectral data of compound JPD6 were closely related to those of compound JPD3 suggesting the same sesquiterpene skeleton. The differences were shown in the main skeleton at C-9, of which that of JPD3 was an oxymethine carbon (δ 80.0) whereas that of JPD6 was a methylene carbon (δ 41.2). Another difference was shown as an additional ester side chain signals of JPD6 at δ 4.14 (1H, *dd*, *J* = 3.6, 1.0 Hz, H-4′), 4.37 (1H, *dd*, *J* = 10.7, 3.6 Hz, H$_a$-5′), 4.31 (1H, *dd*, *J* = 10.7, 1.0 Hz, H$_b$-5′) and 1.47 (3H, *s*, Me-6′). The oxymethine H-4′ (δ 4.14) showed COSY cross peak with an oxymethine H-5′ (δ 4.37) and also showed HMBC correlations with C-2′ (δ 178.2), C-3′ (δ 73.5), C-5′ (δ 72.0) and C-6′ (δ 21.4). The methyl protons Me-6′ (δ 1.47) showed HMBC correlations with C-2′ (δ 178.2), C-3′ (δ 73.5) and C-4′ (δ 73.2). These informations suggested a 2,3,4-trihydroxy-2-methylbutanoyloxy side chain whose attachment at C-2 of a sesquiterpene skeleton was determined through an HMBC experiment in which the oxymethine proton signal at δ 4.66 (1H, dt, *J* = 10.5, 5.7 Hz, H-2) showed correlations with C-1 (δ 128.9), C-3 (δ 45.4) and C-10 (δ 136.2). The multiplicity of the oxymethine proton H-2 signal as a doublet of triplet ($J_{ax\text{-}ax}$ = 10.5, $J_{ax\text{-}eq}$ = 5.7 Hz) from coupling with H-1 and 2H-3, indicated that H-2 was situated in an axial (*β*) position. NOESY experiment displayed cross peaks of H-1/H-5/H-7, H-6/H-11/Me-15 and H-2/Me-14/Me15/H-3*β*. suggesting α-orientation of 2,3,4-trihydroxy-2-methylbutanoyloxy side chain. Compound JPD6 was therefore suggested as 2α-(3′,4′,5′-trihydroxy-3′-methylbutanoyloxy)-11*β*H-dihydro parthenolide, a new compound.

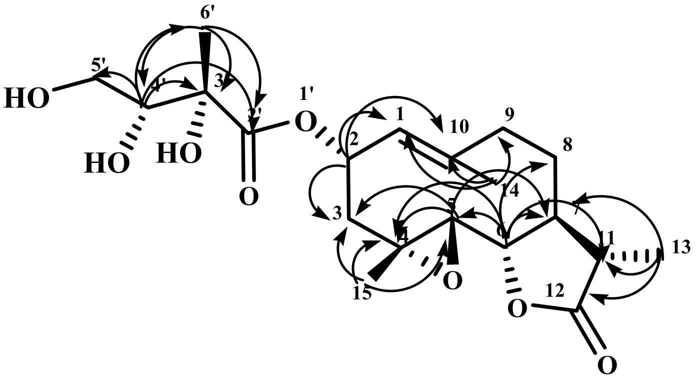


Figure 19 Selected HMBC correlations of **JPD6**

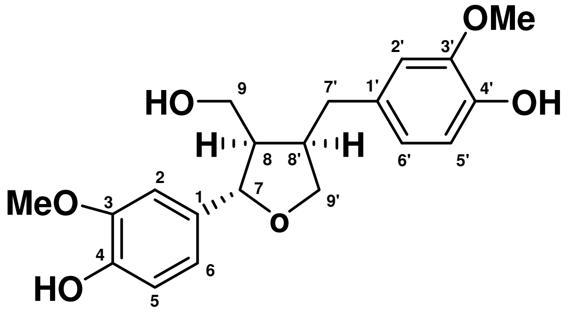
Table 19 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **JPD6** ($CDCl_3$) and comparison of ^{13}C NMR with **JPD3**

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)	HMBC $H^1 \rightarrow {}^{13}C$	COSY
		JPD6	JPD3	JPD6		
1	CH	128.9	125.8	5.25 (*brd*, 10.5)	3, 8, 9, 14	2
2	CH	66.4	23.6	4.66 (*dt*, 10.5, 5.7)	1, 3, 10	1, 3
3	CH_2	45.4	36.3	2.55 (*dd*, 12.0, 5.7), 1.22 (*dd*, 12.0, 10.5)	1, 2, 4, 5, 6 -	2
4	C	60.7	61.4	-	-	-
5	CH	66.3	66.5	2.79 (*d*, 9.3)	3, 4, 7	6

Table 19 (Continued)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)	HMBC $H^1 \rightarrow {}^{13}C$	COSY
		JPD6	JPD3	JPD6		
6	CH	81.8	81.3	3.80 (*t*, 9.3)	4, 5, 7, 8, 11	5, 7
7	CH	51.8	48.3	1.88 (*m*)	5, 6, 8, 9, 11, 13	6, 8, 11
8	CH_2	29.4	37.8	1.95 (*m*), 1.65 (m)	-	-
9	CH_2	41.2	80.0	2.10 (*m*), 2.30 (m)	1, 7, 8, 10	-
10	C	136.2	136.6	-	-	-
11	CH	42.4	42.0	2.30 (*m*)	7, 8, 12, 13	7, 13
12	C	177.2	177.2	-	-	-
13	CH_3	13.2	13.6	1.29 (*d*, 6.9)	7, 11, 12	11
14	CH_3	17.6	10.8	1.77 (*s*)	1, 8, 9, 10	-
15	CH_3	18.2	17.2	1.30 (*s*)	3, 4, 5	-
2′	C	178.2	-	-	-	-
3′	C	73.5	-	-	-	-
4′	CH	73.2	-	4.14 (*dd*, 3.6, 1.0)	2′, 3′, 5′, 6′	5′
5′	CH_2	72.0	-	4.37 (*dd*, 10.7, 3.6) 4.31 (*dd*, 10.7, 1.0)	2′, 3′, 4′ ,6′	4′
6′	CH_3	21.4	-	1.47 (*s*)	2′, 3′, 4′	-

2.3.1.7 Compound JPD7



Compound JPD7 was isolated as a colorless viscous oil, $[\alpha]_D^{28}$: +35° (c = 1.3), $CHCl_3$). Ihe IR spectrum showed absorption bands due to hydroxyl at 3419 cm^{-1} and double bond at 1604 cm^{-1}. The UV absorption was shown at 205, 228 and 281nm.

The ^{13}C NMR spectral data recorded in $CDCl_3$ showed 20 signals for 20 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested a presence of four oxygenated olefinic quaternary carbons at δ 144.0, 145.0, 146.5 and 146.6, two olefinic quaternary carbons at δ 132.3 and 134.8, six aromatic carbons at δ 108.3, 111.2, 114.2, 114.4, 118.8 and 121.9, an oxygenated methine carbon at δ 82.8, two methine carbons at δ 42.4 and 52.6, two oxygenated methylene carbons at δ 60.9 and 72.9 and two methoxyl carbons at δ 55.9x2.

The ^{1}H NMR spectral data showed signals at δ 6.88 (1H, *d*, *J* = 1.8, H-2), 6.82 (1H, *d*, *J* = 8.4, H-5), 6.79 (1H, *dd*, *J* = 8.4, 1.8, H-6), 6.68 (1H, *d*, *J* = 1.8, H-2′), 6.85 (1H, *d*, *J* = 8.4, H-5′) and 6.69 (1H, *dd*, *J* = 8.4, 1.8, H-6′) indicating two 1,3,4-trisubstituted benzene rings. An oxygenated methine signal at δ 4.78 (1H, *d*, *J* = 6.6 Hz, H-7), two methine signals at δ 2.40 (1H, *m*, H-8) and 2.73 (1H, *m*, H-8′) and two methoxyl signals at δ 3.86 (3H, *s*, 3-OMe) and δ 3.88 (3H, *s*, 3′-OMe) were observed.

On the basis of HMBC the oxygenated methine proton H-7 at δ 4.78 showed correlations with C-1 (δ 134.8), C-8 (δ 52.6), C-9 (δ 60.9), C-8′ (δ 42.4) and C-9′ (δ 72.9), a methine proton H-8 at δ 2.40 showed correlations with C-1 (δ 134.8), C-9 (δ 60.9), C-7′ (δ 33.3), C-8′ (δ 42.4) and C-9′ (δ 72.9) and that of H-8′ at δ 2.73 showed correlations with C-7 (δ 82.8), C-8 (δ 52.6), C-9 (δ 60.9), C-1′ (δ 132.3), C-7′ (δ 33.3) and C-9′ (δ 72.9).

The stereochemistry at C-7, C-8 and C-8′ was deduced by NOESY experiment. Cross peaks were observed between H-8/H-8′, with the absence of cross peaks between H-8/H-7. These results indicated that H-8 and H-8′ were *cis* and H-8 and H-7 were *trans*. On the basis of its spectroscopic data and comparison with previously reported data (xie *et al*., 2003). Compound JPD7 was identified as lariciresinol.

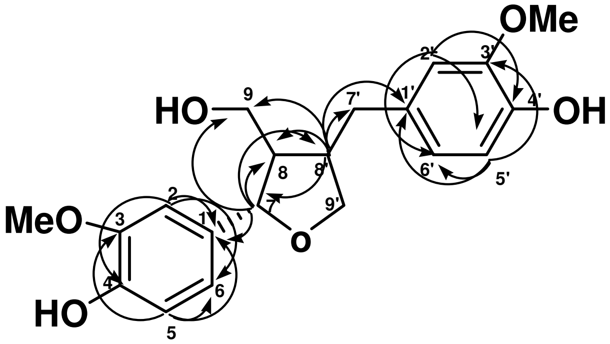


Figure 20 Selected HMBC correlations of **JPD7**

Table 20 ^{1}H, ^{13}C NMR and HMBC spectral data of compounds **JPD7** (CDCl$_3$) and lariciresinol (**R**, MeOD)

Position	Type of C	δc /ppm		δ$_H$ / ppm (multiplicity, J/Hz)		HMBC $H^1 \rightarrow {}^{13}C$
		JPD7	R	JPD7	R	
1	C	134.8	135.8	-	-	-
2	CH	108.3	110.7	6.88 (*d*, 1.8)	6.90 (*d*, 1.8)	1, 4,6
3	C	146.5	149.0	-	-	-
4	C	145.0	147.1	-	-	-
5	CH	114.2	116.0	6.82 (*d,* 8.4)	6.76 (*m*)	1, 3, 6
6	CH	118.8	119.8	6.79 (*dd*, 8.4, 1.8)	6.75 (*m*)	1, 2, 4, 5
7	CH	82.8	84.1	4.78 (*d*, 6.6)	4.74 (*d*, 7.0)	1, 8, 9, 8′, 9′
8	CH	52.6	54.0	2.40 (*m*)	2.37 (*m*)	1, 9, 7′, 8′, 9′
9	CH$_2$	60.9	60.5	3.74 (*dd*, 8.4, 6.6) 3.90 (*dd*, 8.4, 7.2)	3.62 (*dd*, 10.9, 6.5) 3.83 (*dd*, 10.9, 8.0)	—

Table 20 (Continued)

Position	Type of C	δc /ppm		δ_H / ppm (multiplicity, J/Hz)		HMBC $H^1 \Rightarrow {}^{13}C$
		JPD7	R	JPD7	R	
1′	C	132.3	133.6	-	-	-
2′	CH	111.2	113.5	6.68 (*d*, 1.8)	6.79 (*d*, 1.9)	4′, 5′, 6′
3′	C	146.6	149.0	-	-	-
4′	C	144.0	145.8	-	-	-
5′	CH	114.4	116.2	6.85 (*d*, 8.4)	6.71 (*d*, 8.0)	1′, 3′, 6′
6′	CH	121.9	122.2	6.69 (*dd*, 8.4, 1.8)	6.64 (*dd*, 8.0, 1.9)	2′, 4′, 7′
7′	CH_2	33.3	33.7	2.54 (*dd*, 13.2, 10.8) 2.92 (*dd*, 13.2, 5.1)	2.48 (*dd*, 13.4, 11.1) 2.92 (*dd*, 13.4, 4.8)	
8′	CH	42.4	43.9	2.73 (*m*)	2.73 (*m*)	7, 8, 9, 1′, 7′, 9′
9′	CH_2	72.9	73.5	3.77 (*dd*, 8.4, 5.7) 4.05 (*dd*, 8.4, 6.6)	3.72 (*dd*, 8.4, 5.8) 3.97 (*dd*, 8.4, 6.5)	7, 8, 7′
3-OMe	CH_3	55.9	56.4	3.86 (*s*)	3.82 (*s*)	3
3′-OMe	CH_3	55.9	56.4	3.88 (*s*)	3.84 (*s*)	3′

CHAPTER 4
CONCLUSION

Thirteen known compounds; three triterpenes: friedelin (**CMD1**), 5(6)-gluten-3α-ol (**CMD2**) and betulinic acid (**CMD3**), seven steroids: a mixture of *β*-sitosterol (**CMD4**) and stigmasterol (**CMD5**), stigmast-4-en-3-one (**CMD6**), 6α-hydroxystigmast-4-en-3-one **(CMD7)**, ergosterol peroxide **(CMD8)**, 5α-cholest-7-en-3-one **(CMD9)** and lophenol (**CMD10**), 5-methylmellein (**CMD11**), 3,4,3′-tri-O-methylellagic acid (**CMD12**) and 5,7,3′,4′,5′-penta-O-methylgallocatechin (**CMD13**) were isolated from the stem of *Punica granatum*. Their structures were elucidated by spectroscopic methods. A mixture of **CMD4** and **CMD5** (2.3 g) and **CMD1** (1.2 g) were major components.

One new sesquiterpene, 2α-(3′,4′,5′-trihydroxy-3′-methylbutanoyloxy)-11*β*H-dihydroparthenolide (**JPD6**), and six known compounds, five sesquiterpenes: costunolide (**JPD1**), parthenolide (**JPD2**), 9*β*-hydroxy-11*β*H-dihydroparthenolide (**JPD3**), reynosin (**JPD4**) and T-cadinol (**JPD5**), one lignan: lariciresinol **(JPD7)** were isolated from the root of *Michelia alba*. Their structures were elucidated by spectroscopic methods. Compounds **JPD1** (1.21 g) and **JPD2** (1.71 g) were major components.

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APPENDIX

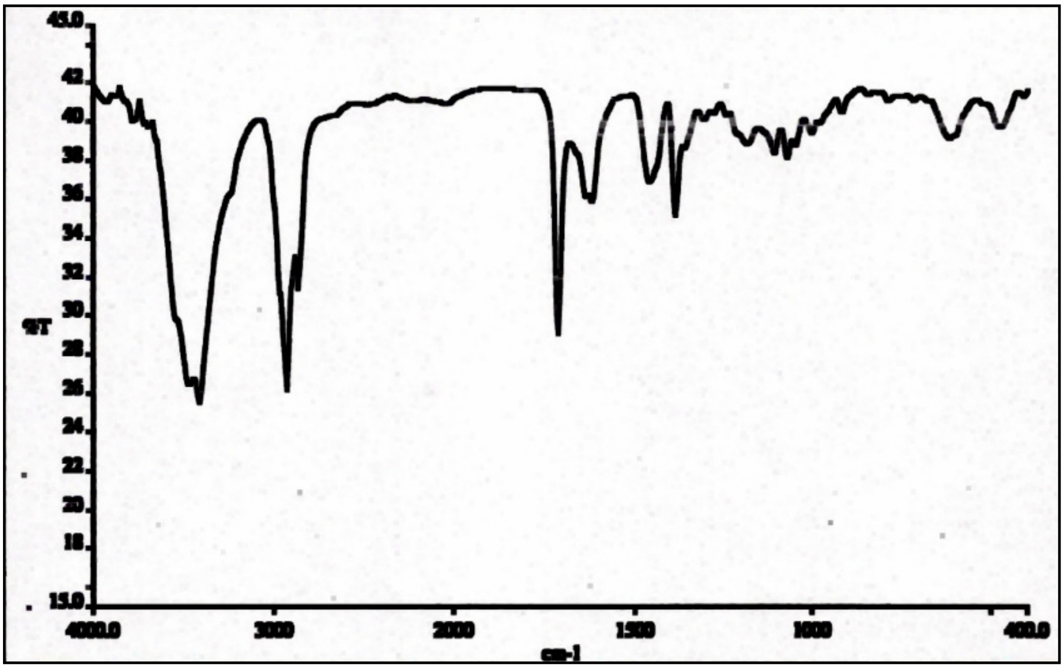

Figure 21 IR (neat) spectrum of compound **CMD1**

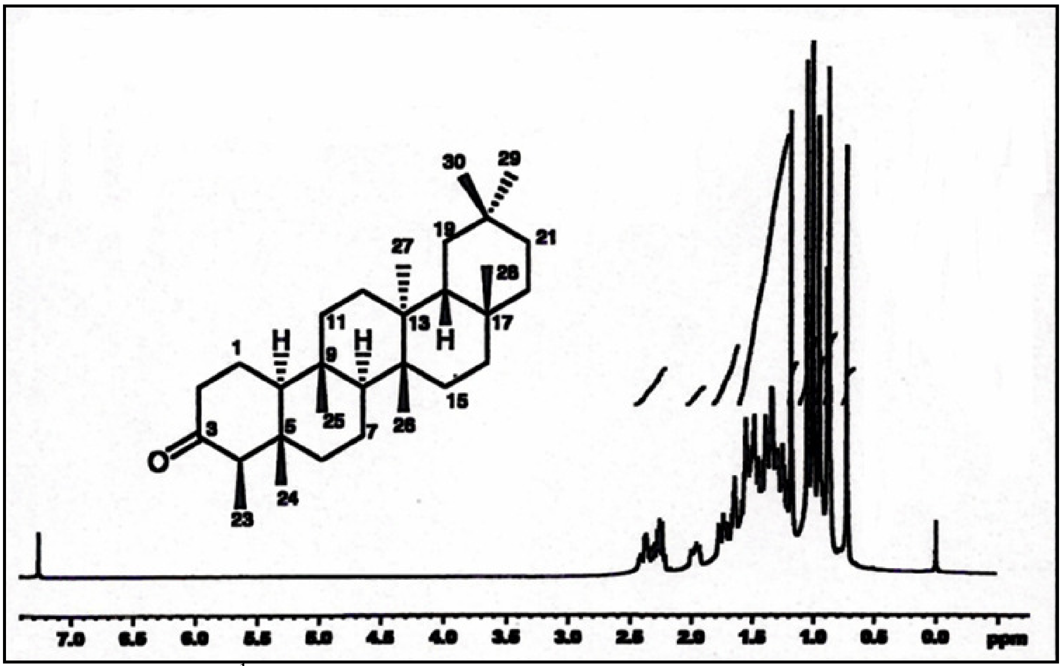

Figure 22 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **CMD1**

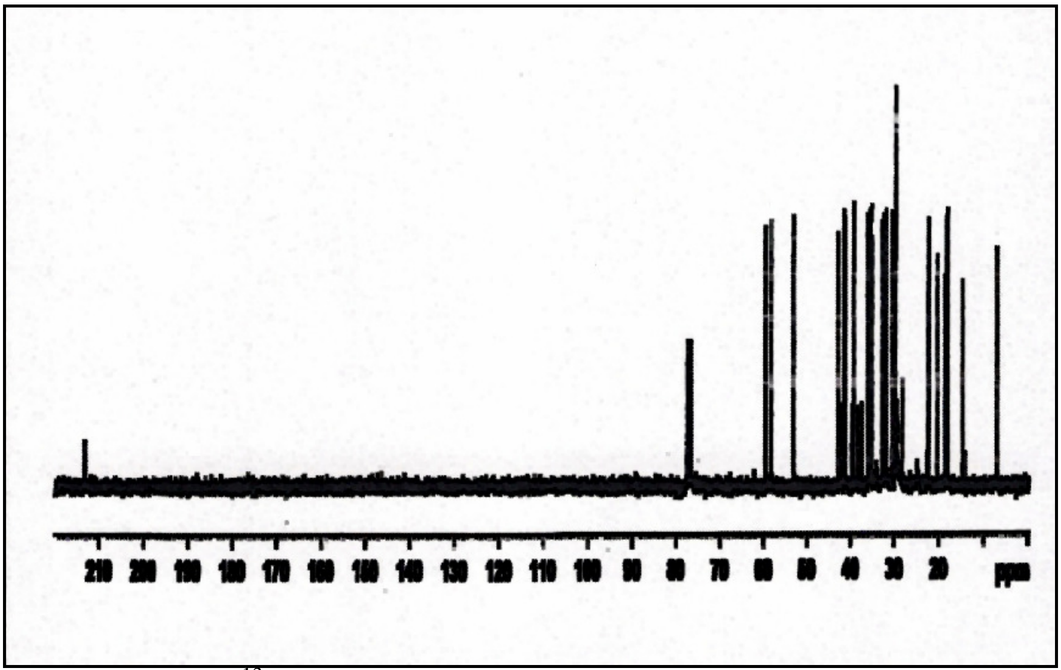

Figure 23 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **CMD1**

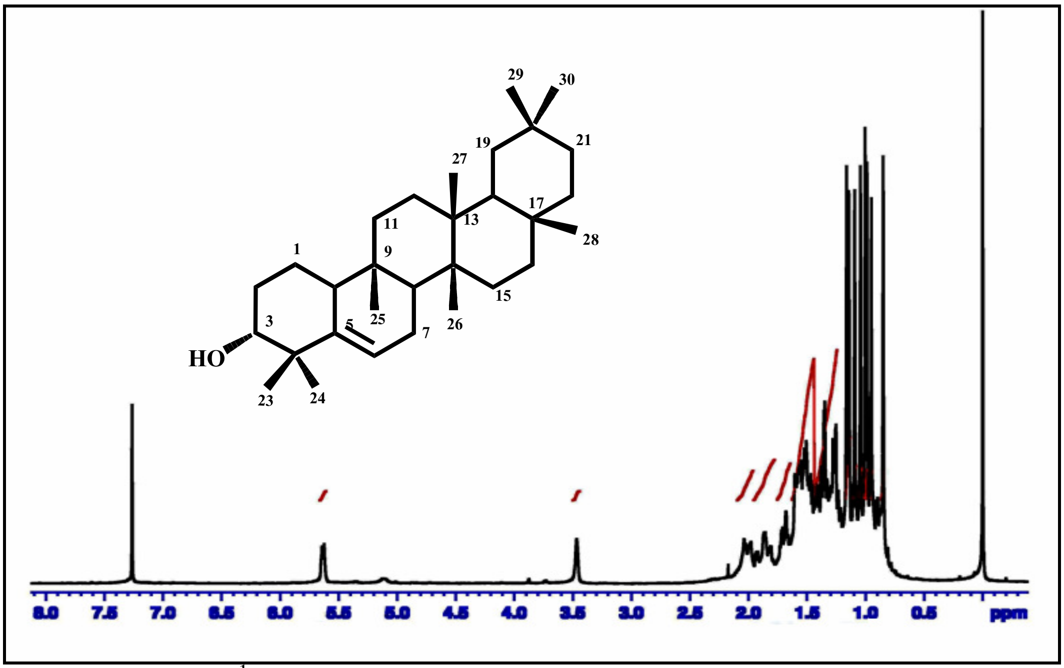

Figure 24 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **CMD2**

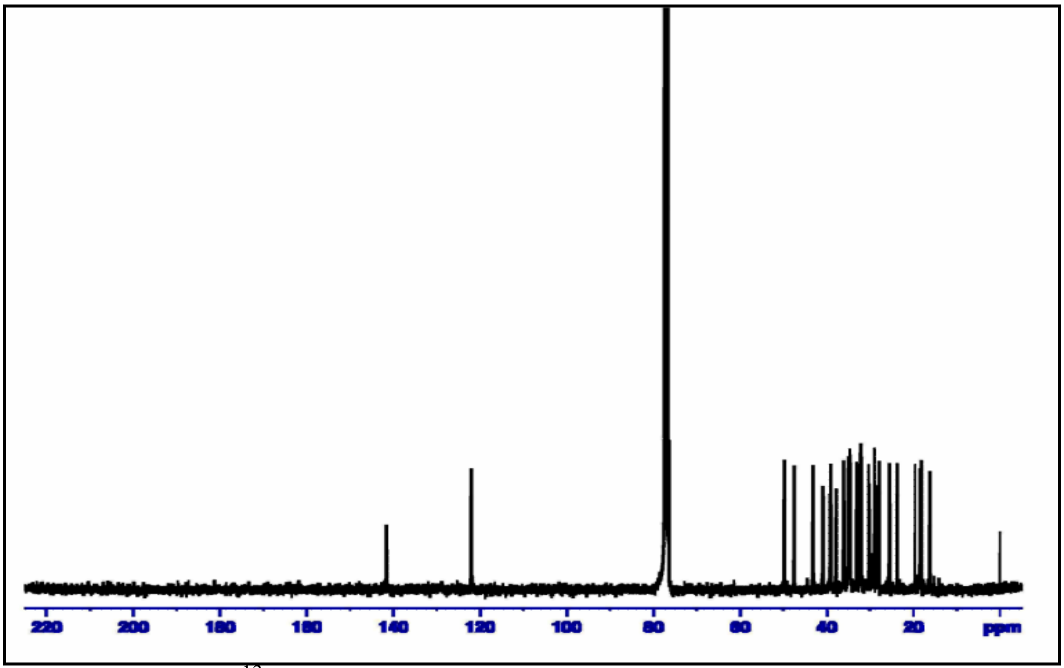

Figure 25 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **CMD2**

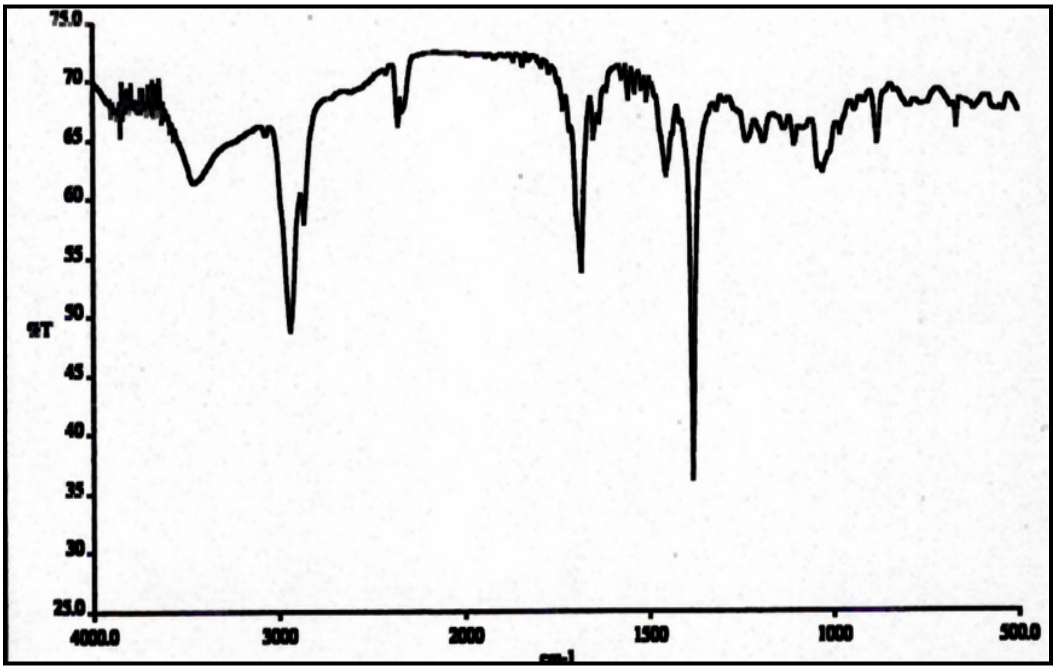

Figure 26 IR (neat) spectrum of compound **CMD3**

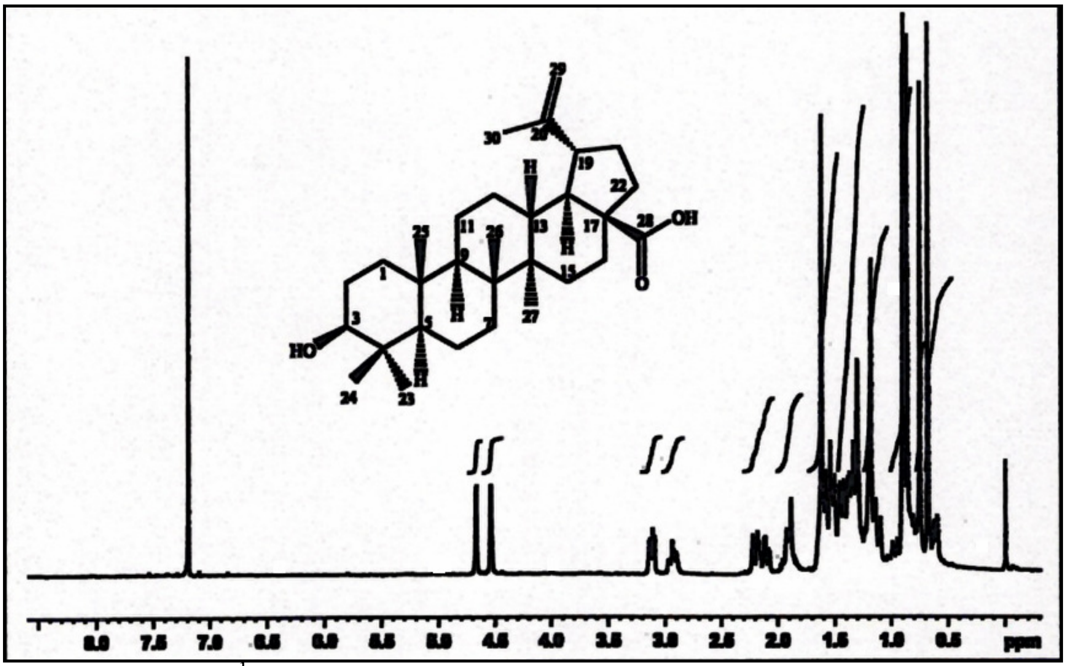

Figure 27 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CMD3**

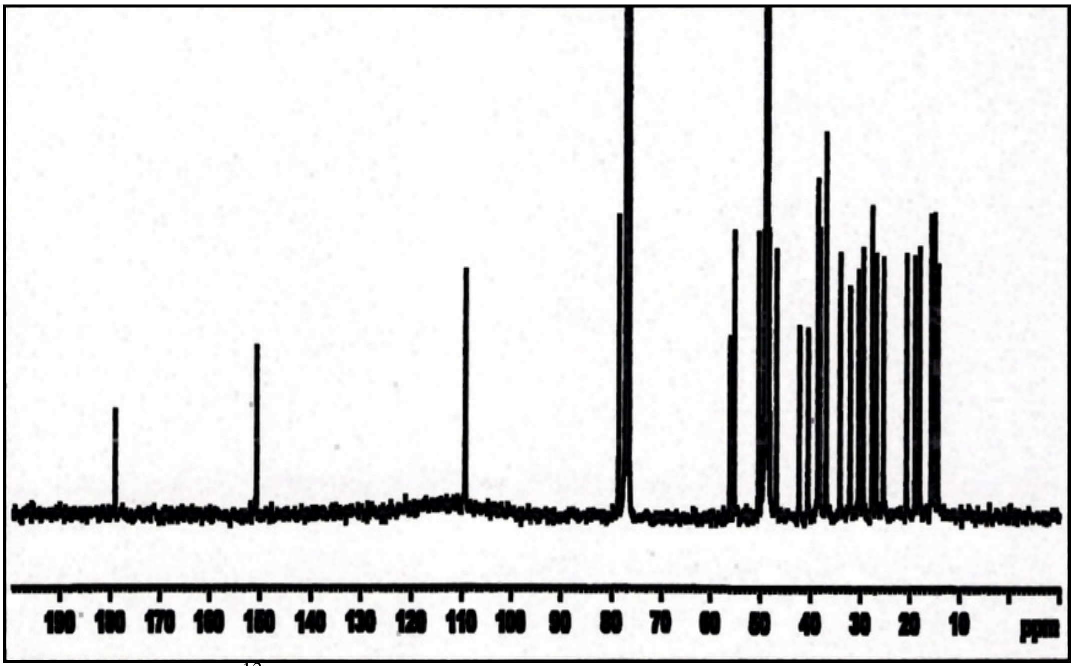

Figure 28 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CMD3**

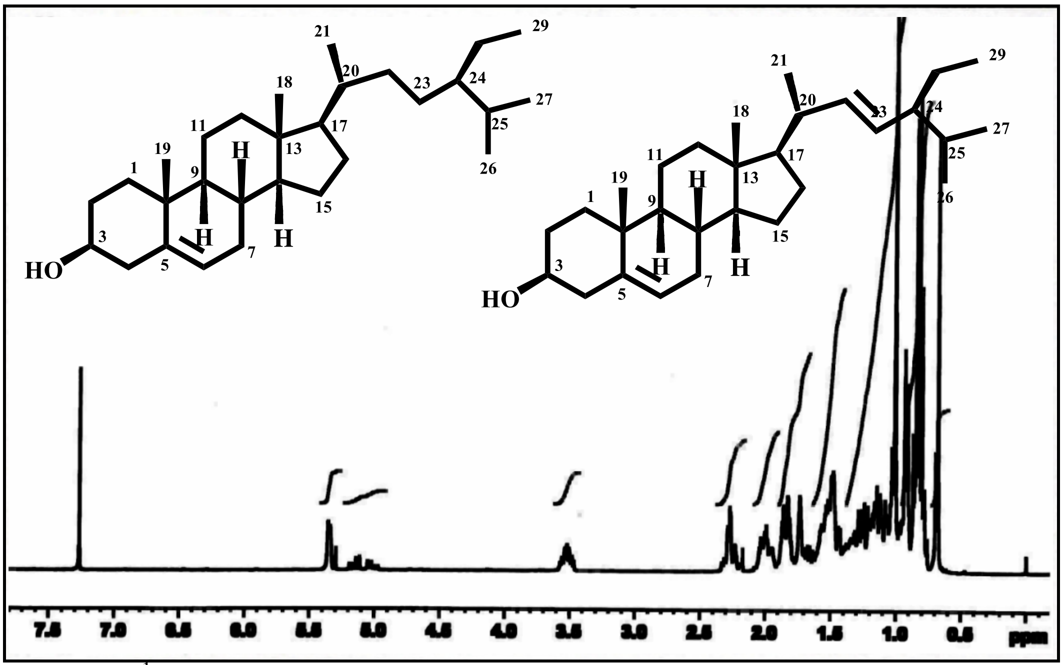

Figure 29 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compounds **CMD4+CMD5**

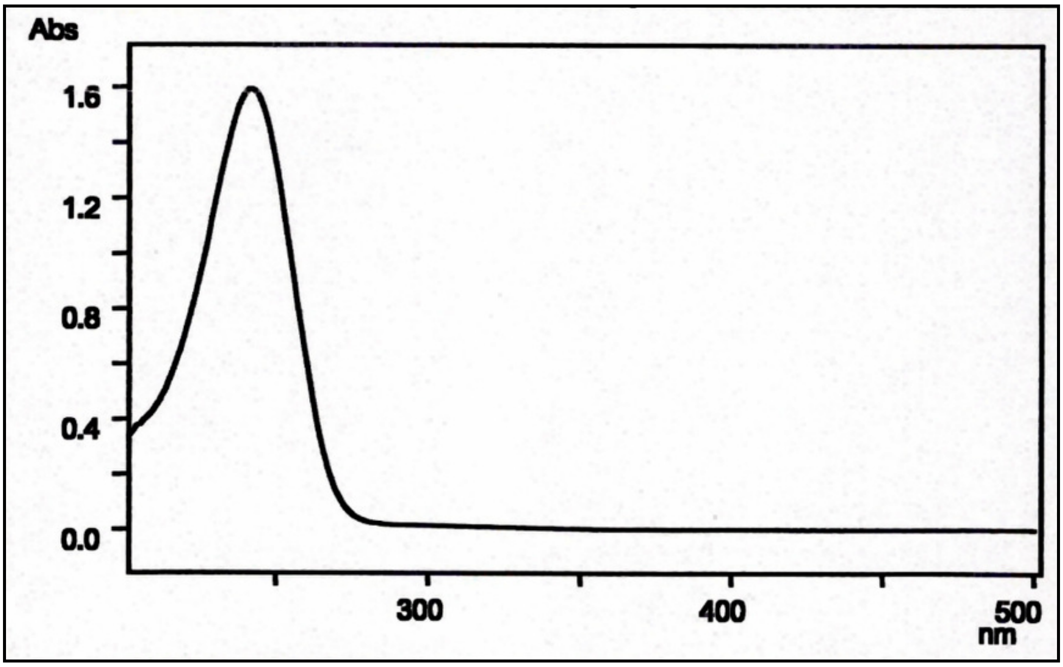

Figure 30 UV (MeOH) spectrum of compound **CMD6**

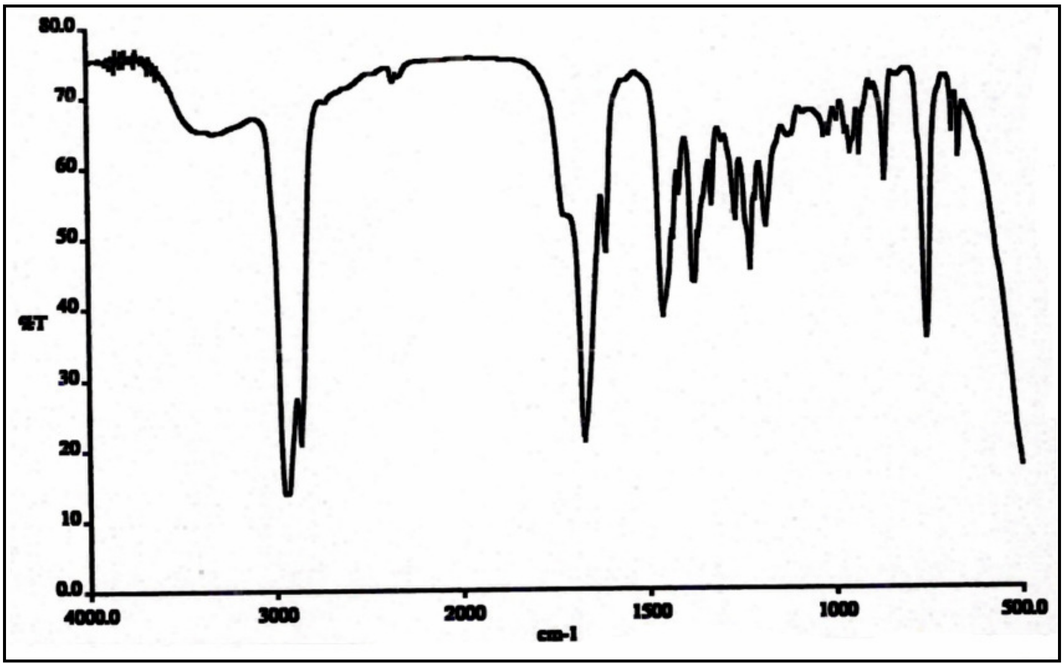

Figure 31 IR (neat) spectrum of compound **CMD6**

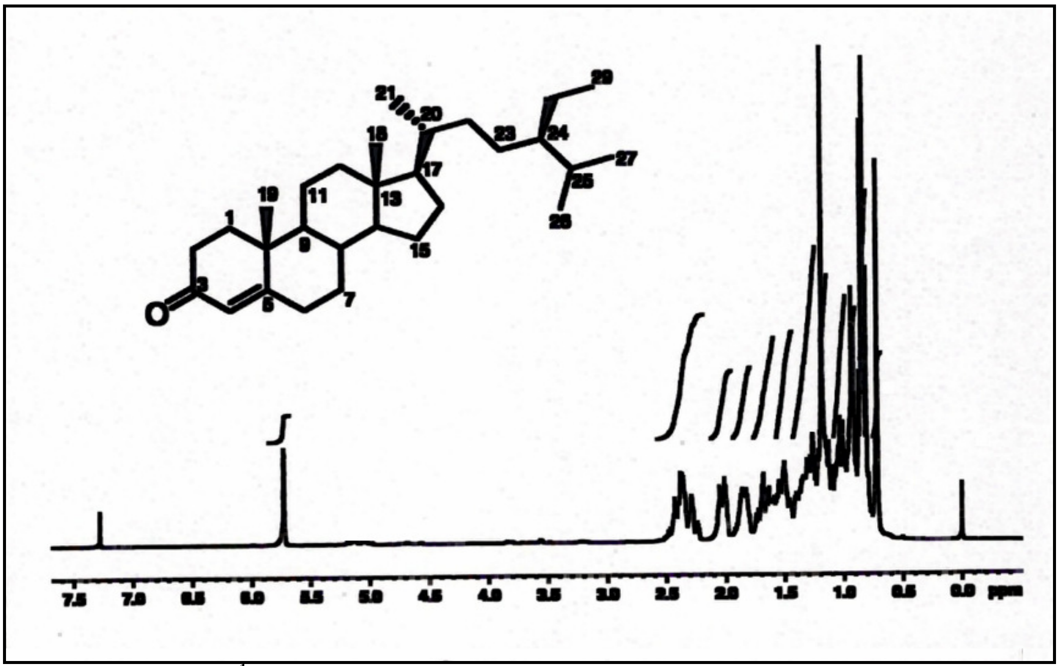

Figure 32 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CMD6**

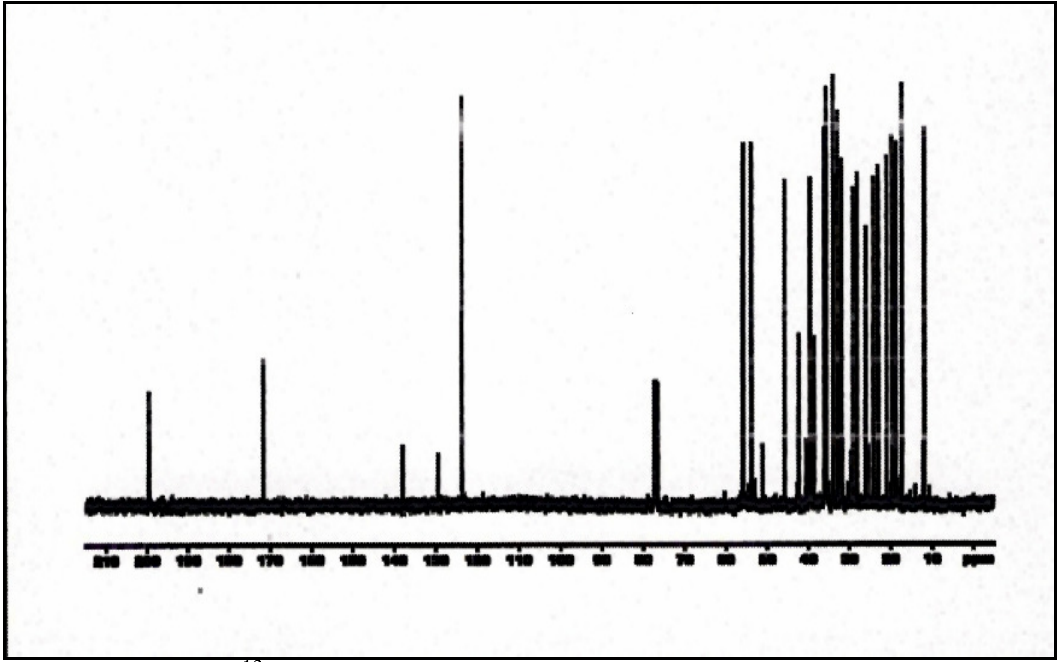

Figure 33 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **CMD6**

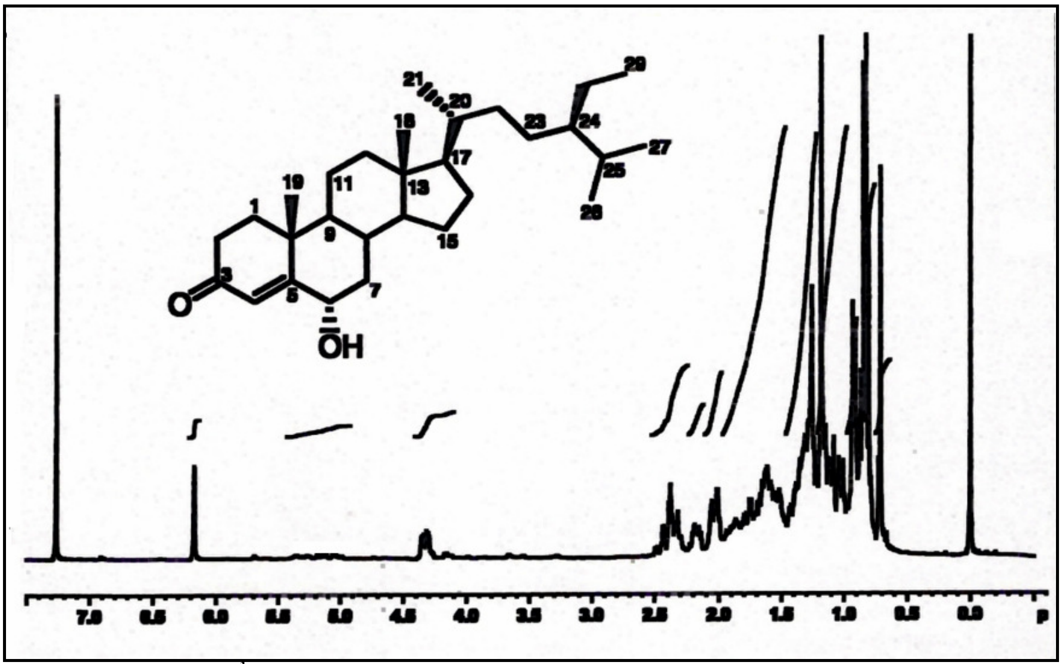

Figure 34 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **CMD7**

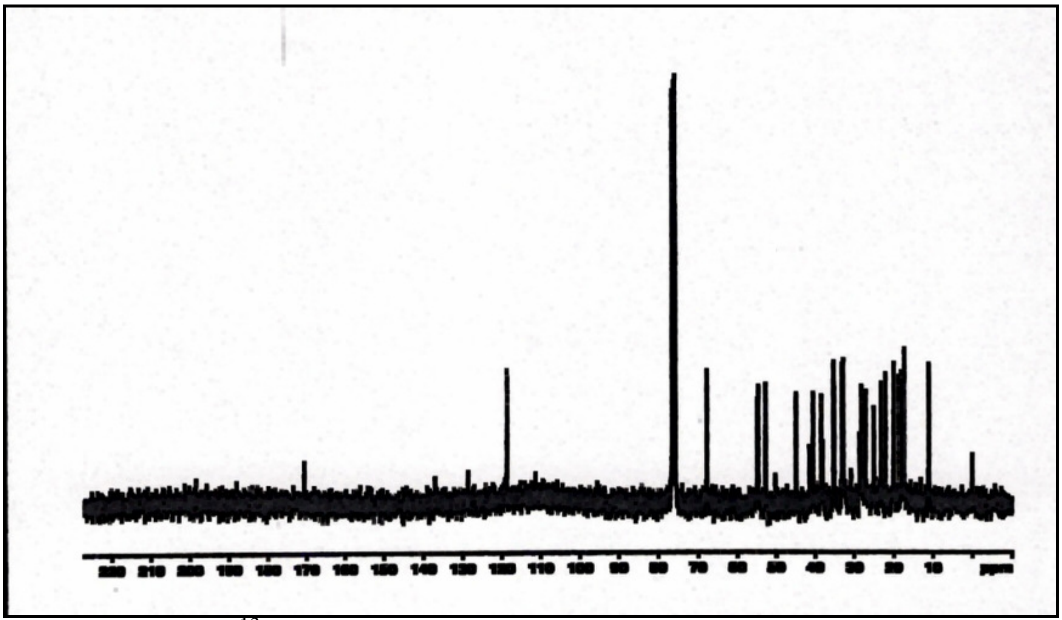

Figure 35 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **CMD7**

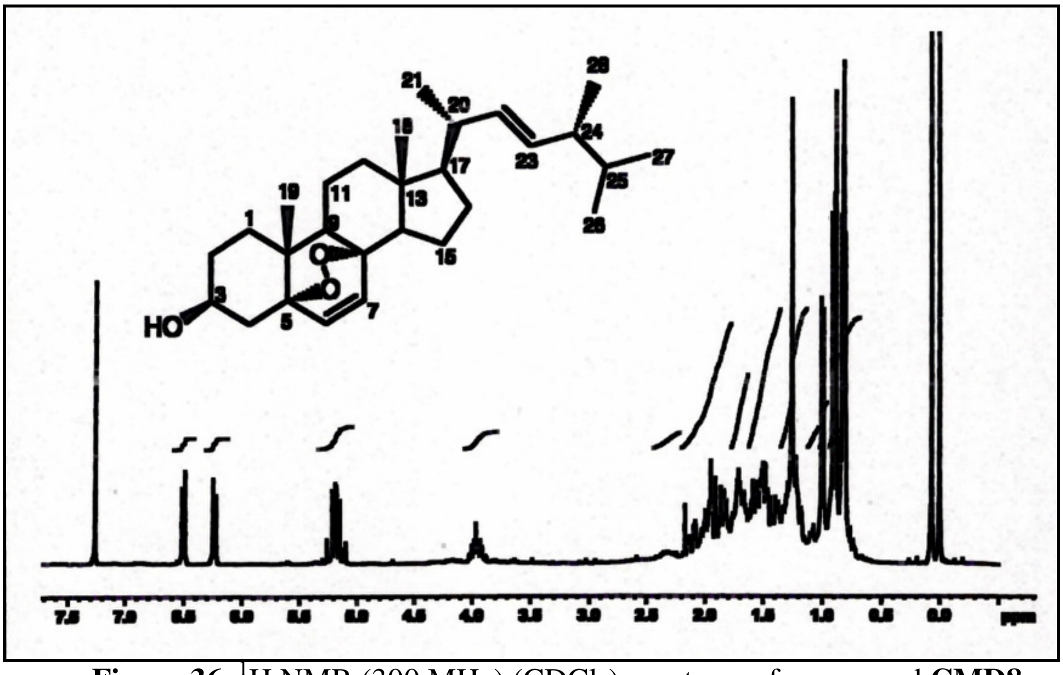

Figure 36 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **CMD8**

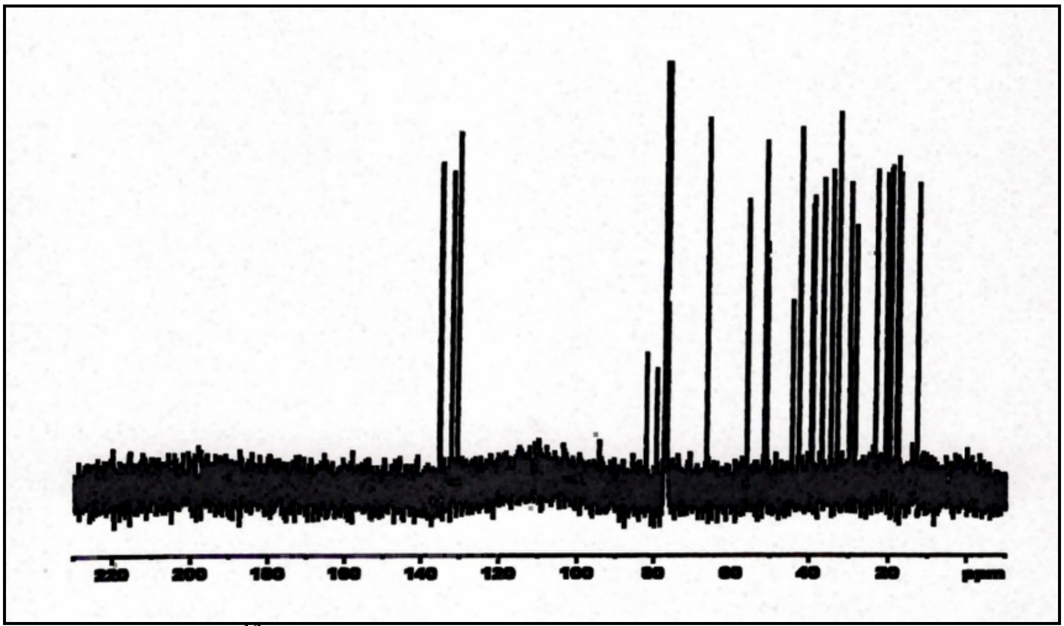

Figure 37 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **CMD8**

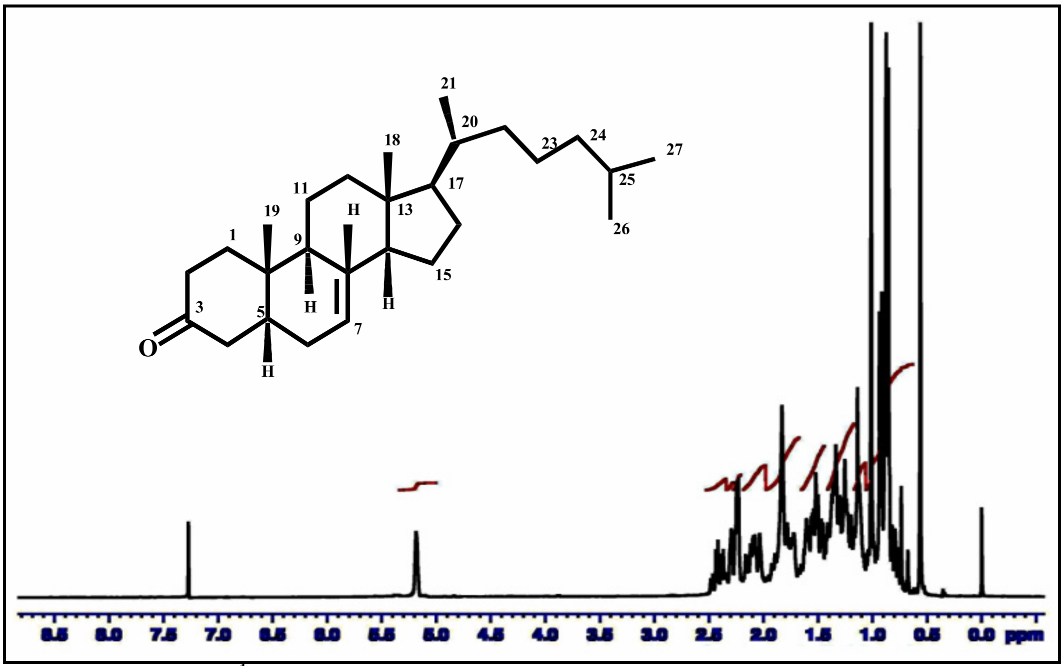

Figure 38 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **CMD9**

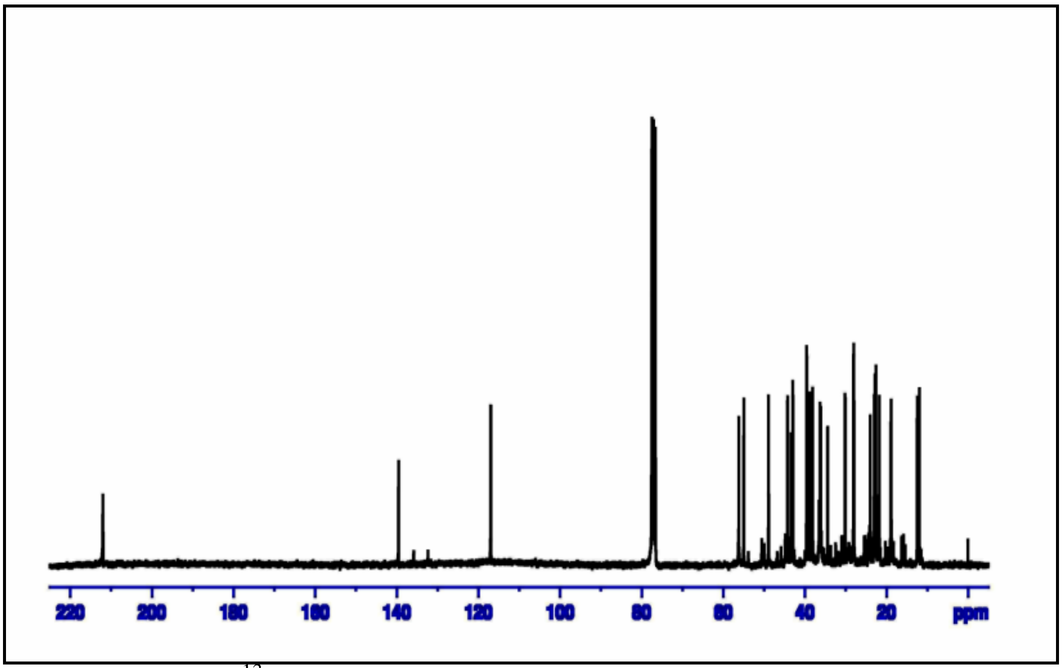

Figure 39 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **CMD9**

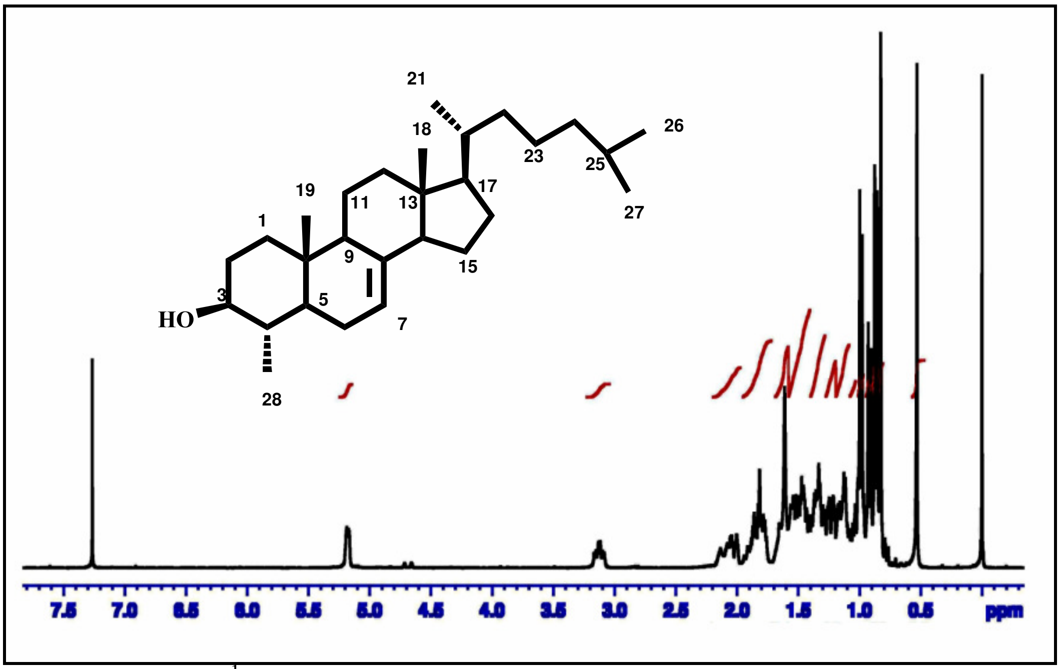

Figure 40 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **CMD10**

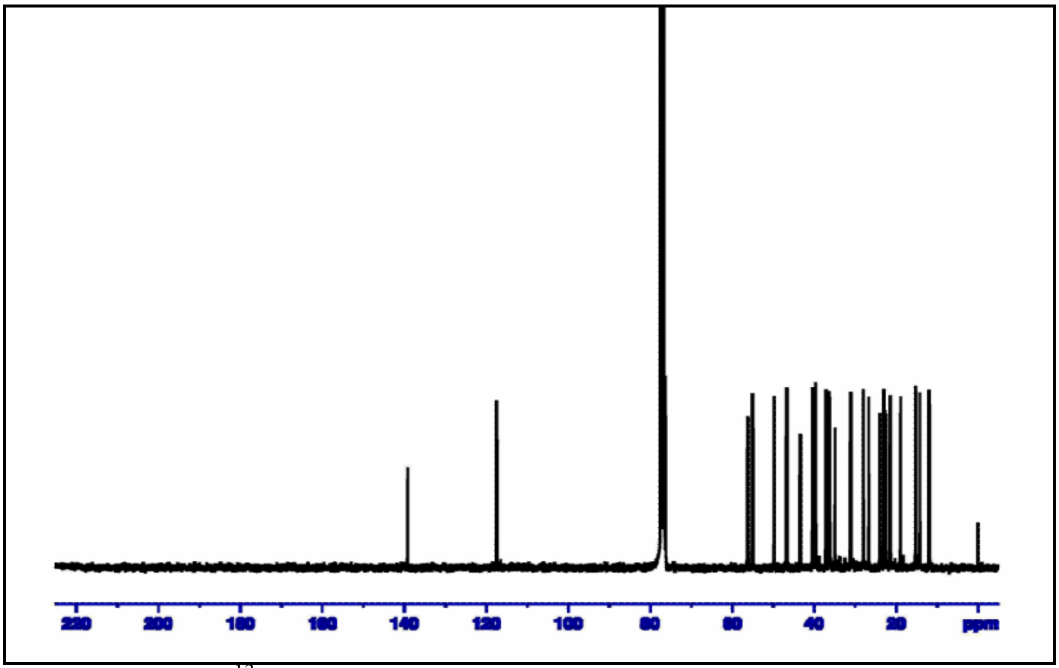

Figure 41 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CMD10**

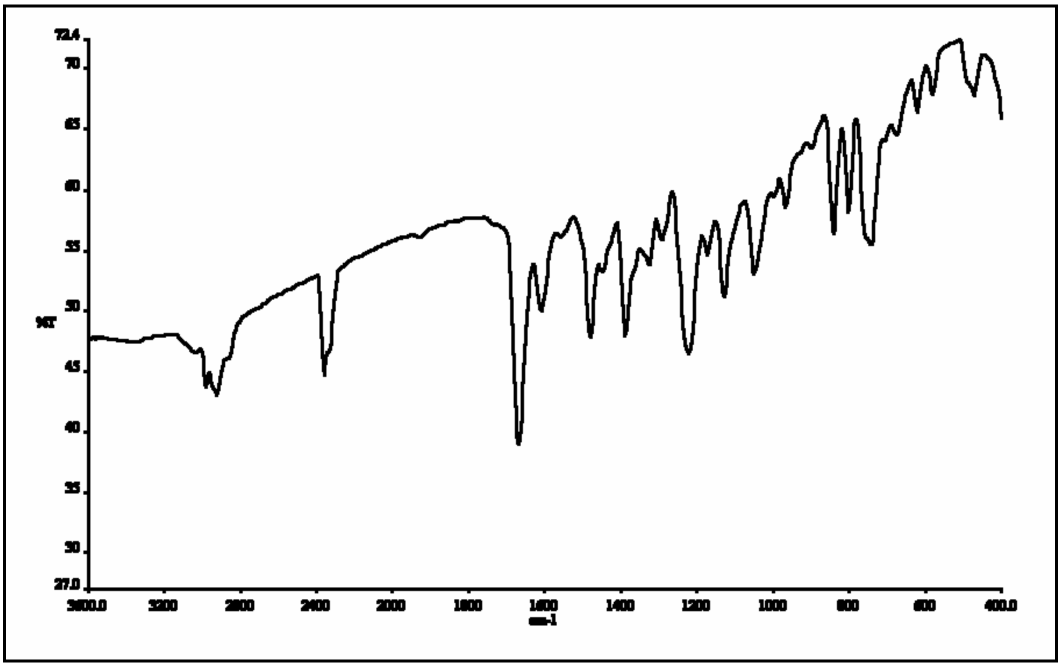

Figure 42 IR (neat) spectrum of compound **CMD11**

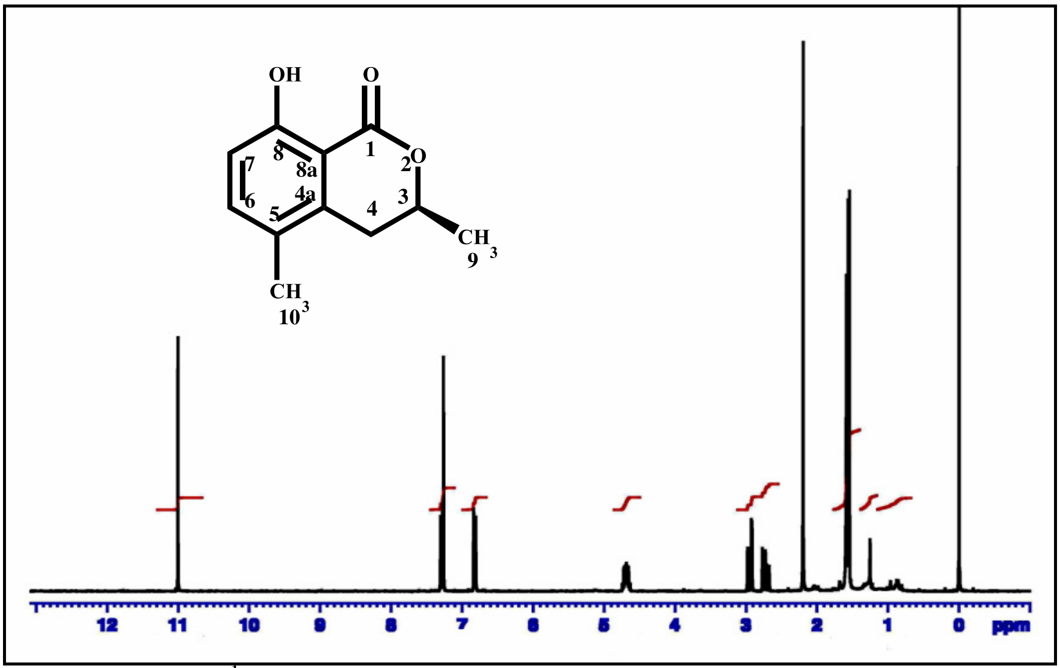

Figure 43 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **CMD11**

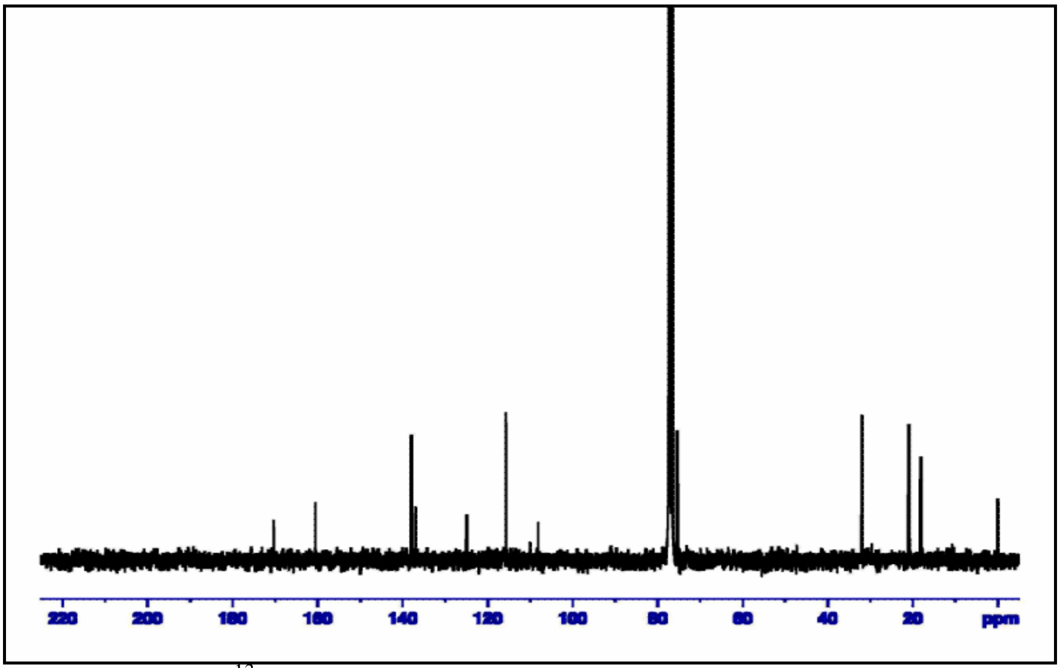

Figure 44 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **CMD11**

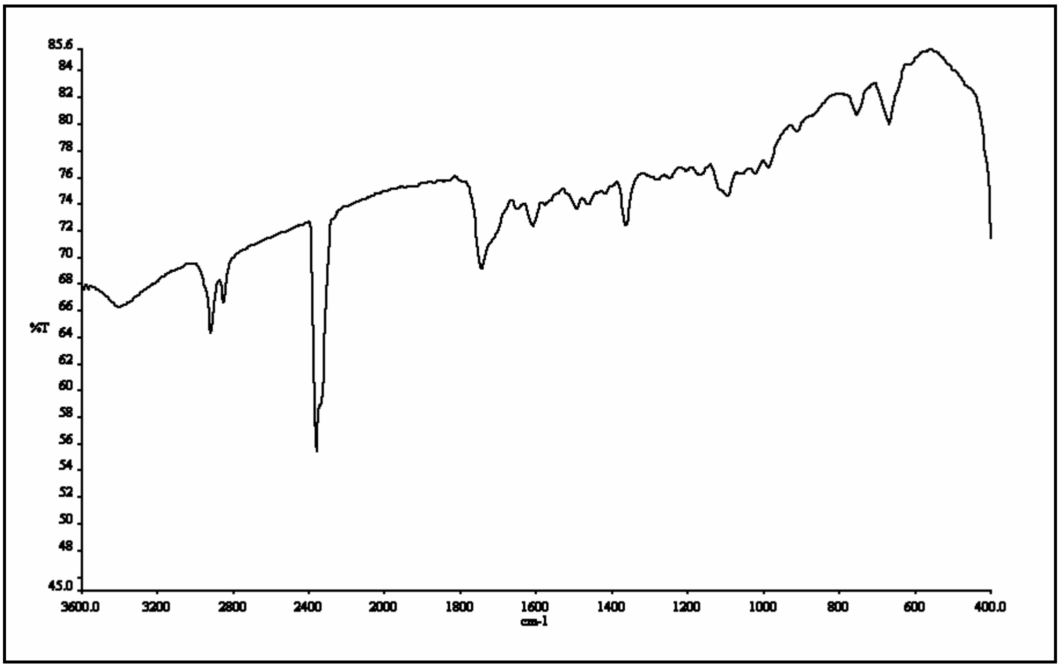

Figure 45 IR (neat) spectrum of compound **CMD12**

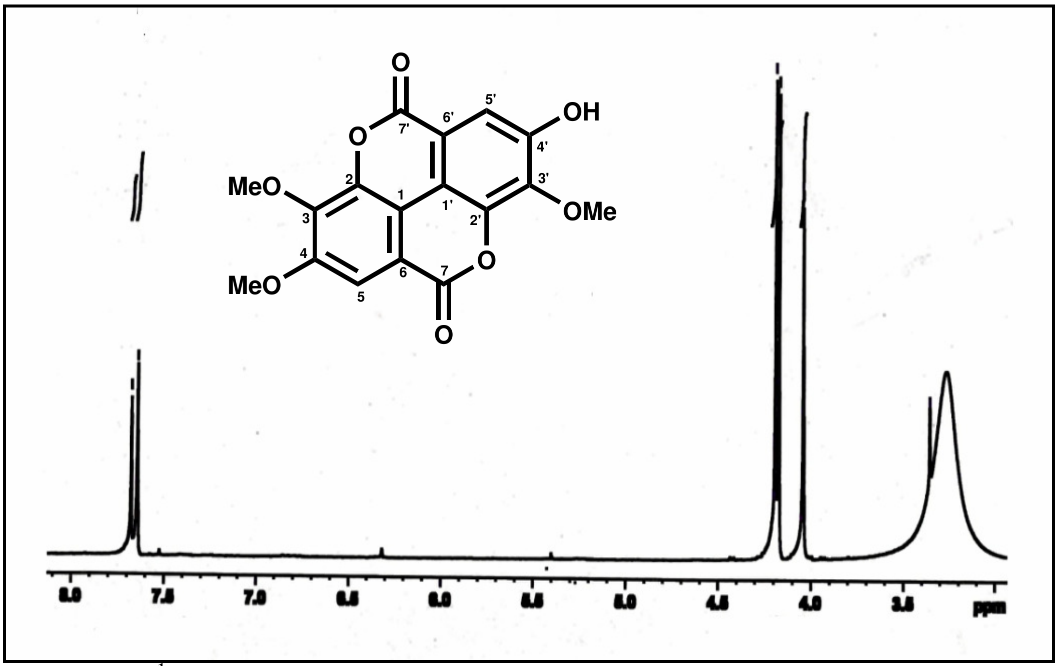

Figure 46 ^{1}H NMR (300 MHz) (DMSO-d_6+$CDCl_3$) spectrum of compound **CMD12**

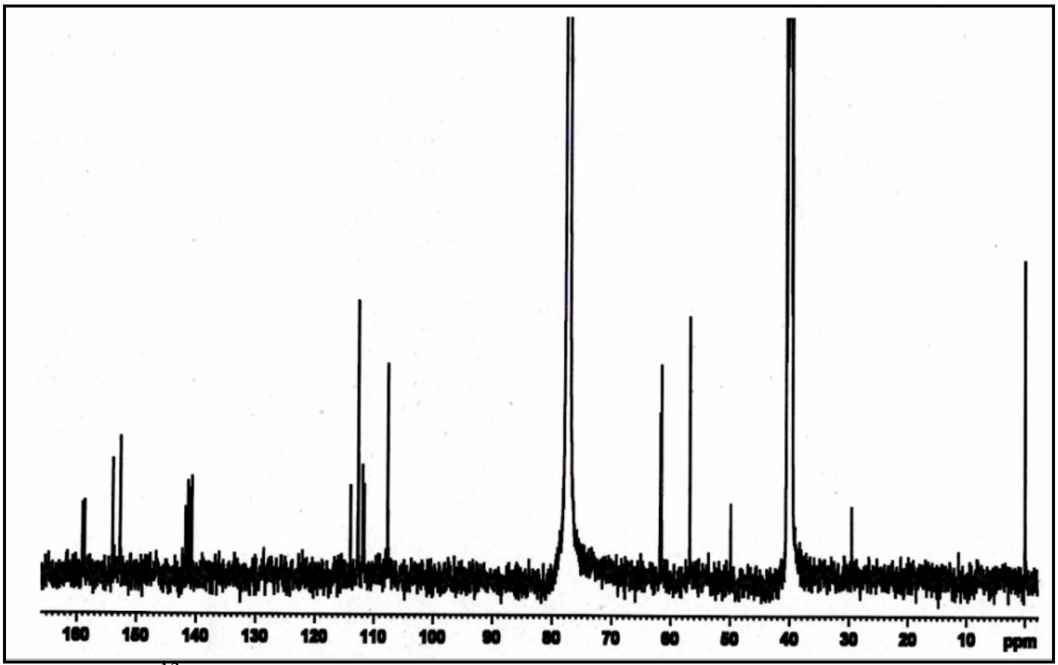

Figure 47 ^{13}C NMR (75 MHz) (DMSO-d_6+$CDCl_3$) spectrum of compound **CMD12**

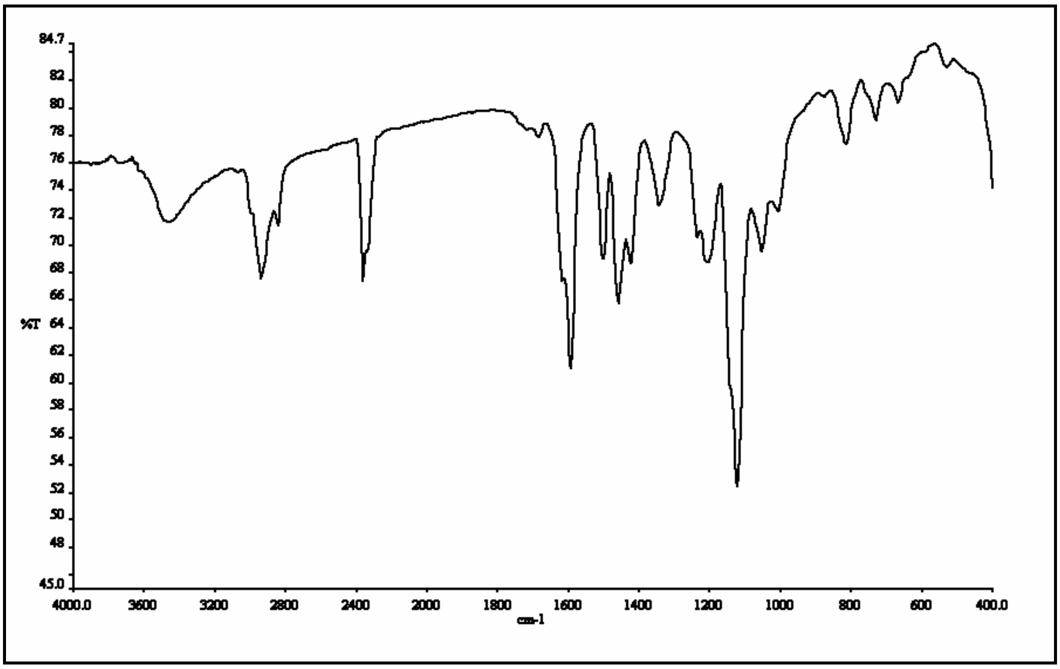

Figure 48 IR (neat) spectrum of compound **CMD13**

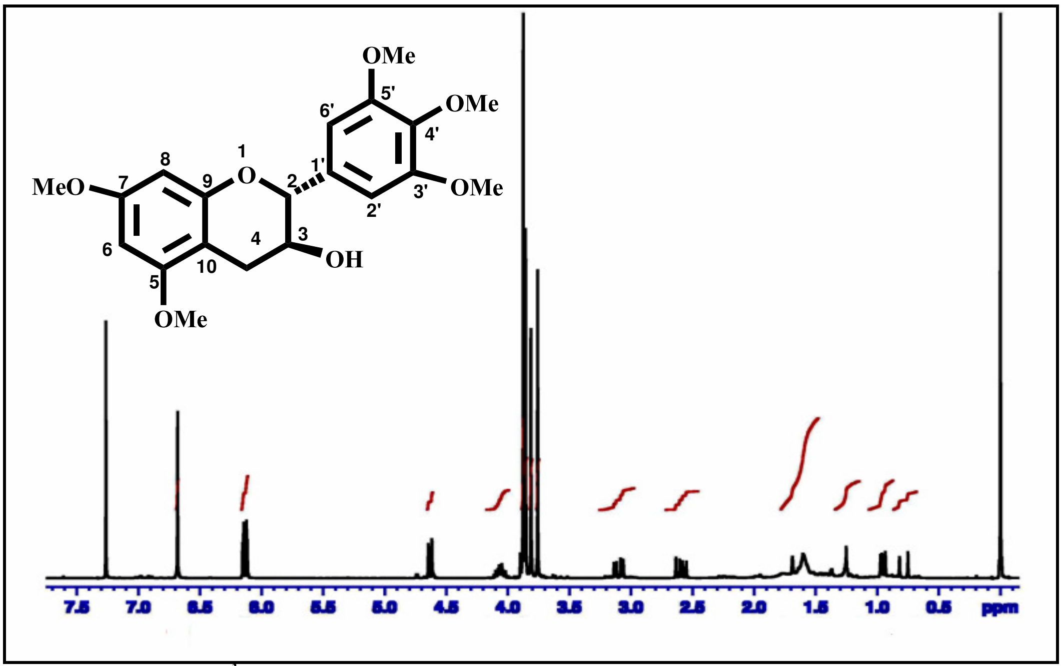

Figure 49 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **CMD13**

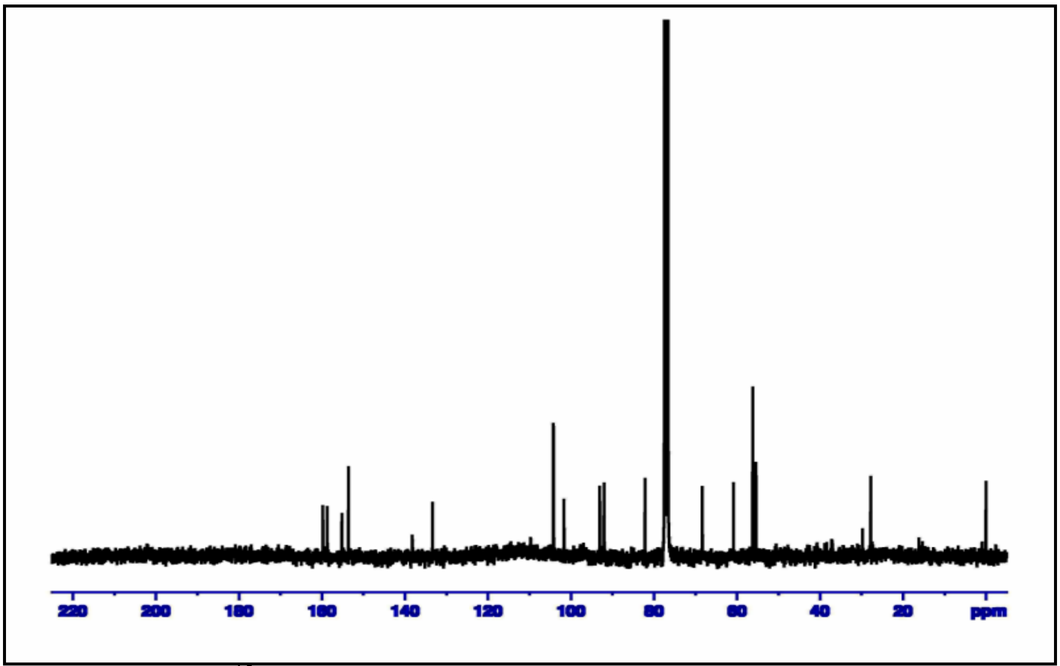

Figure 50 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **CMD13**

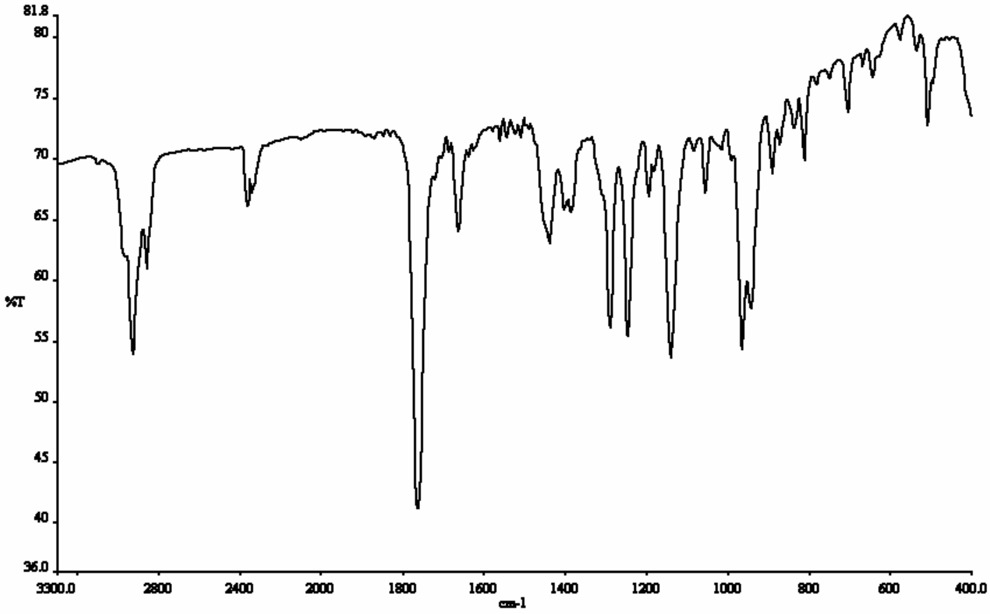

Figure 51 IR (neat) spectrum of compound **JPD1**

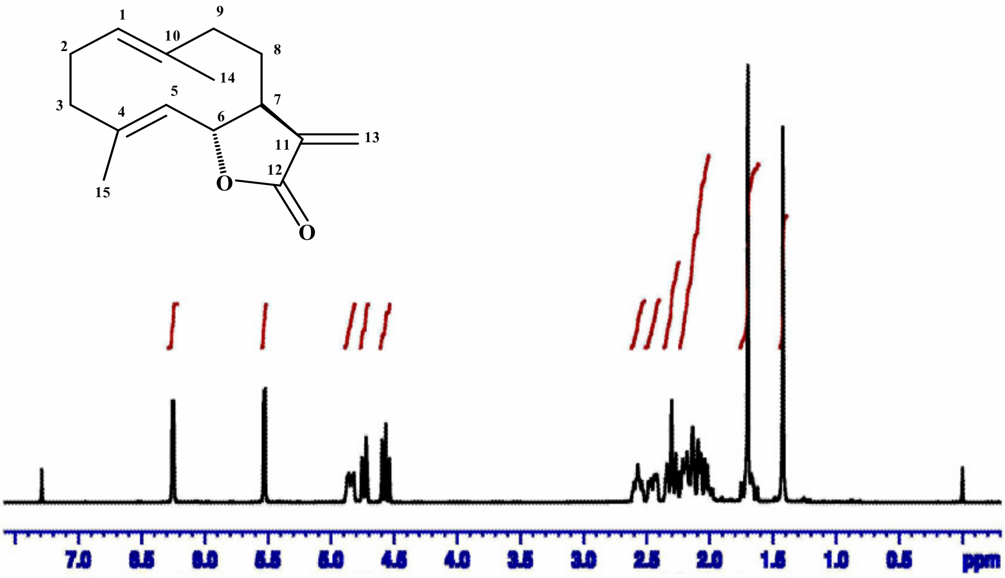

Figure 52 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **JPD1**

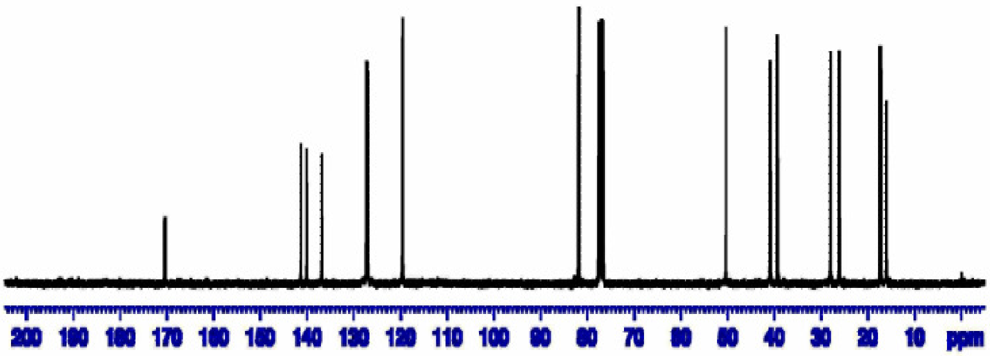

Figure 53 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **JPD1**

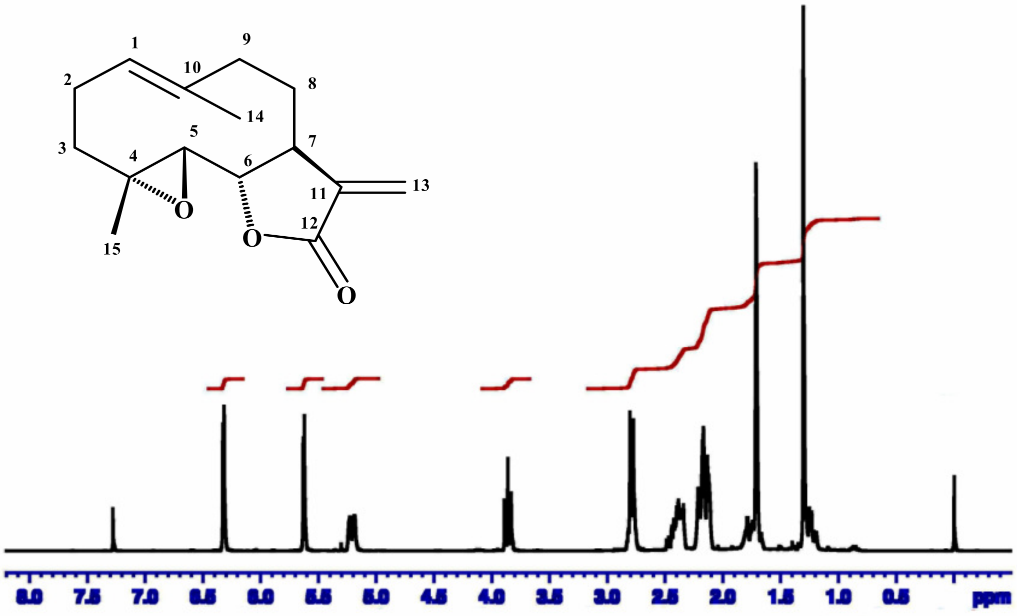

Figure 54 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **JPD2**

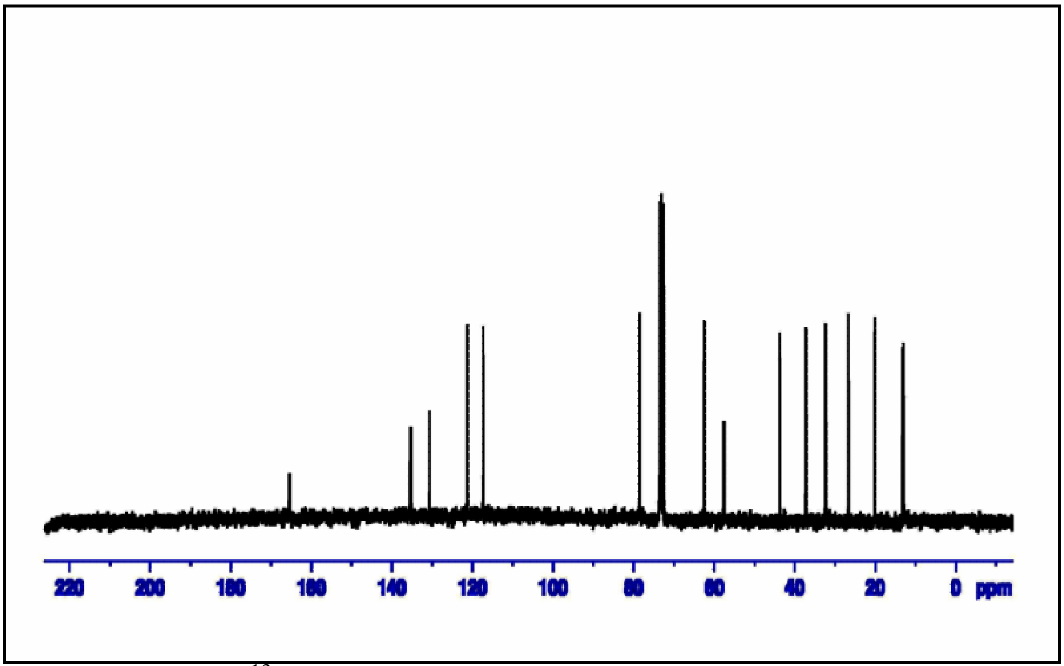

Figure 55 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **JPD2**

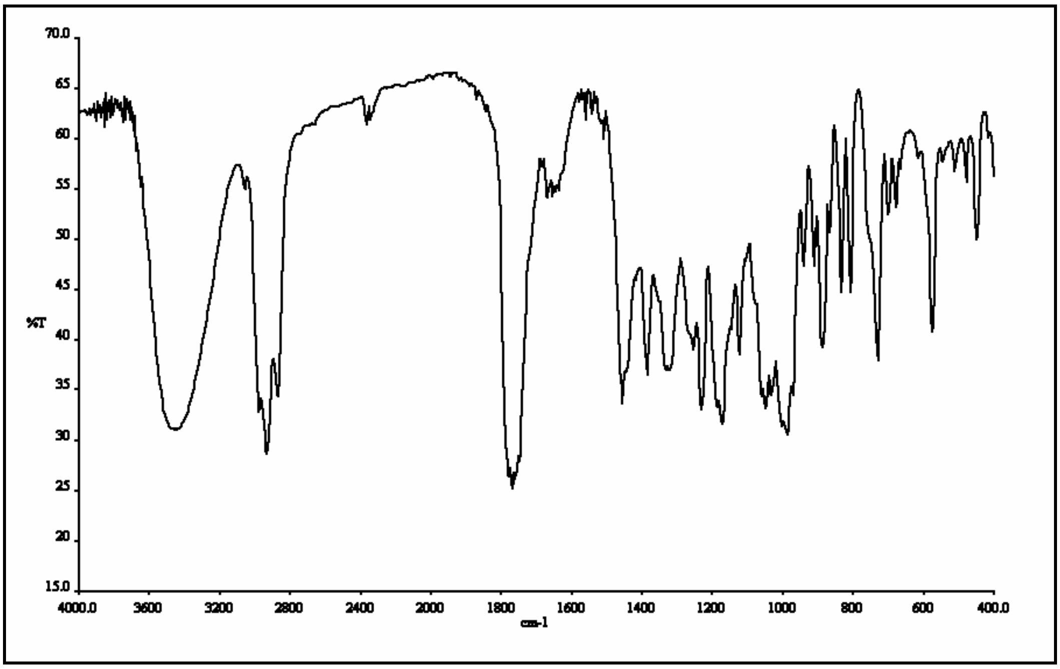

Figure 56 IR (neat) spectrum of compound **JPD3**

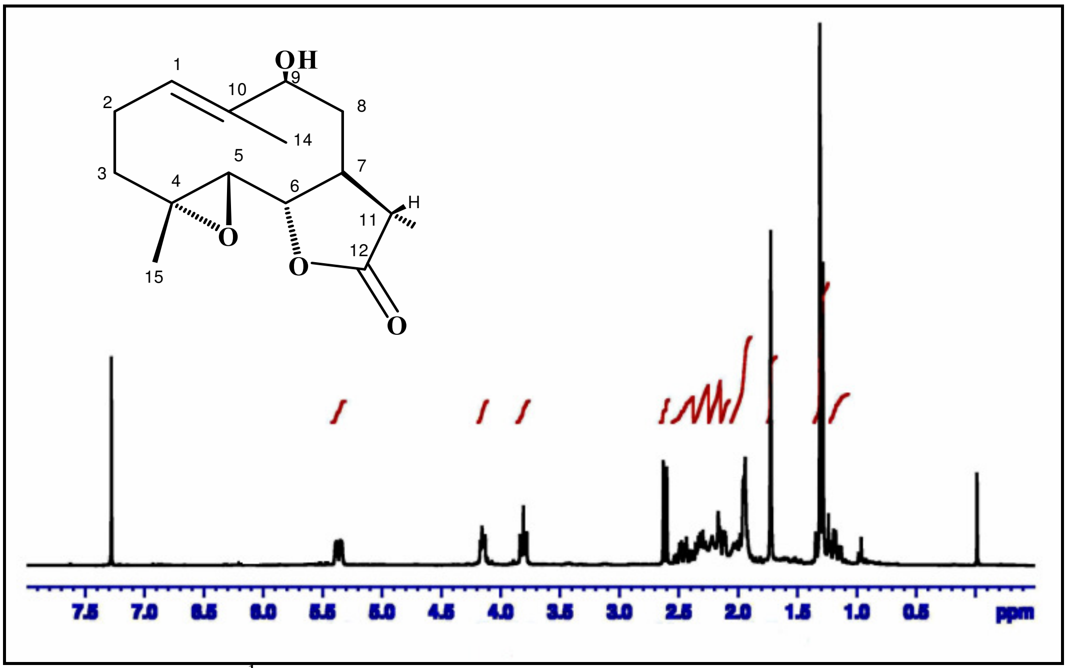

Figure 57 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **JPD3**

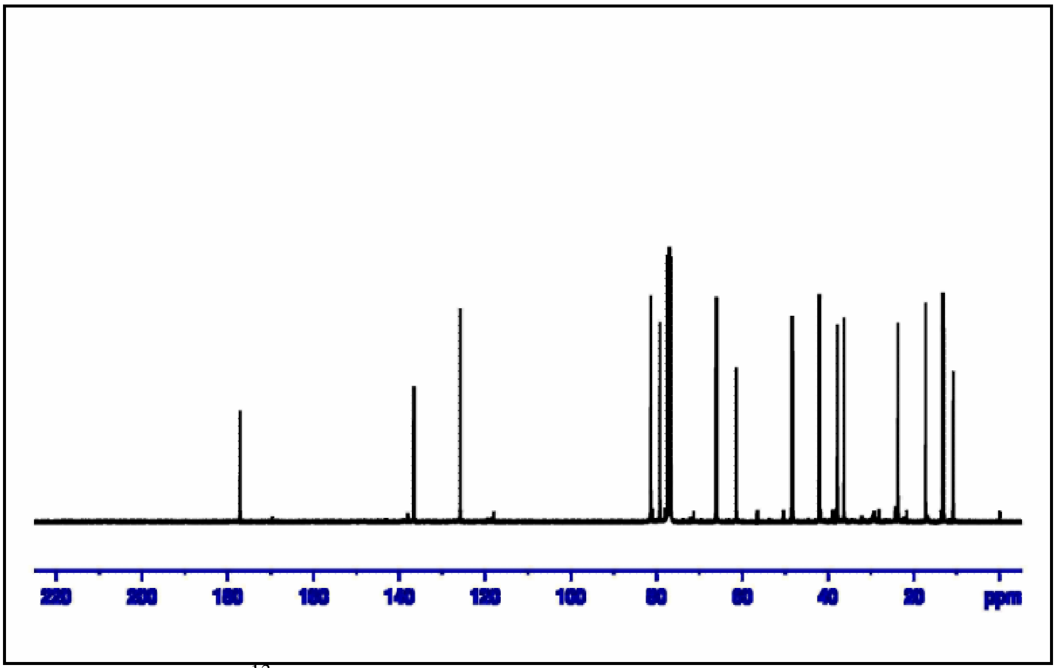

Figure 58 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **JPD3**

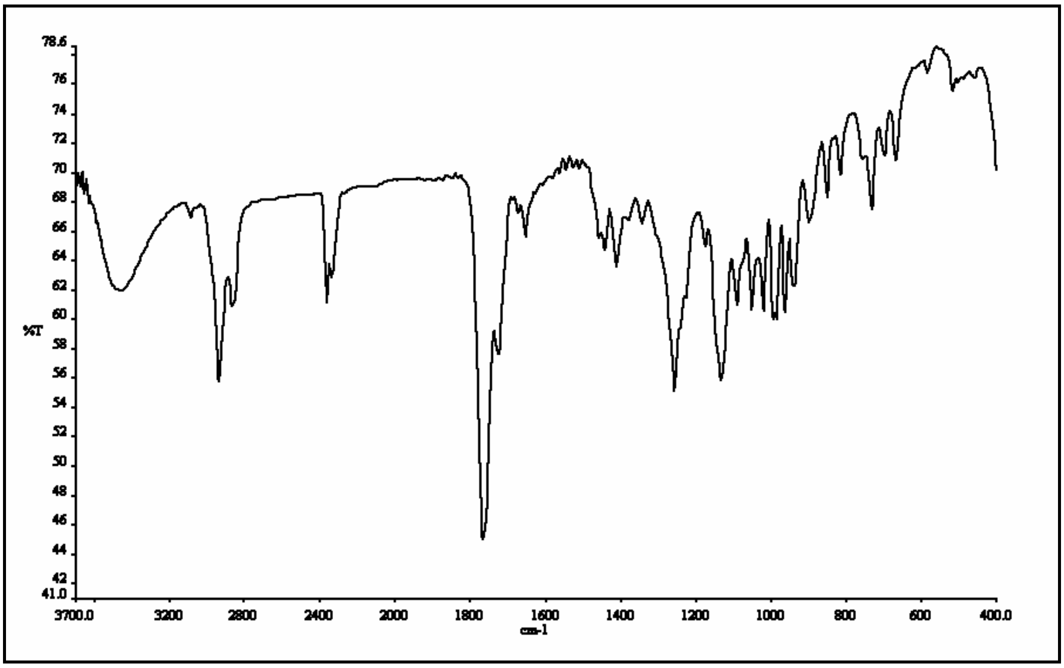

Figure 59 IR (neat) spectrum of compound **JPD4**

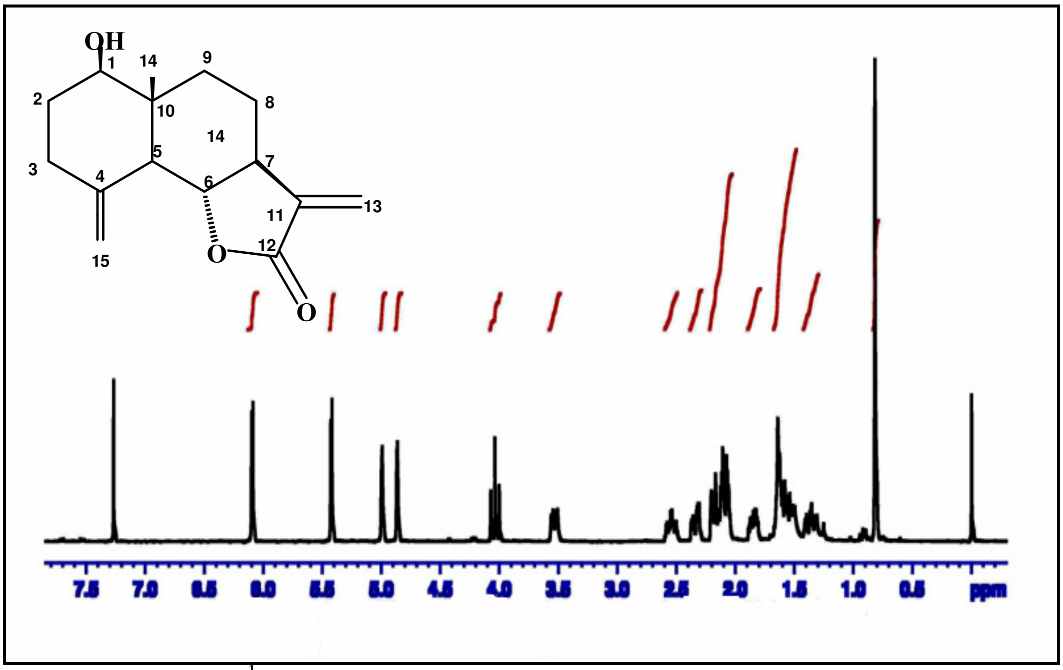

Figure 60 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **JPD4**

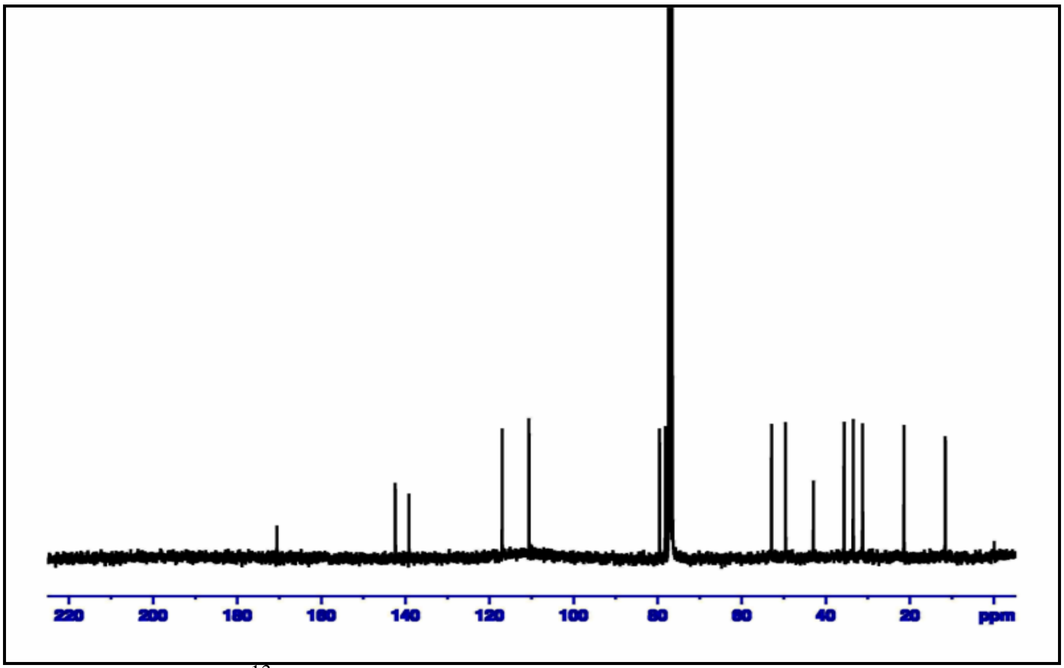

Figure 61 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **JPD4**

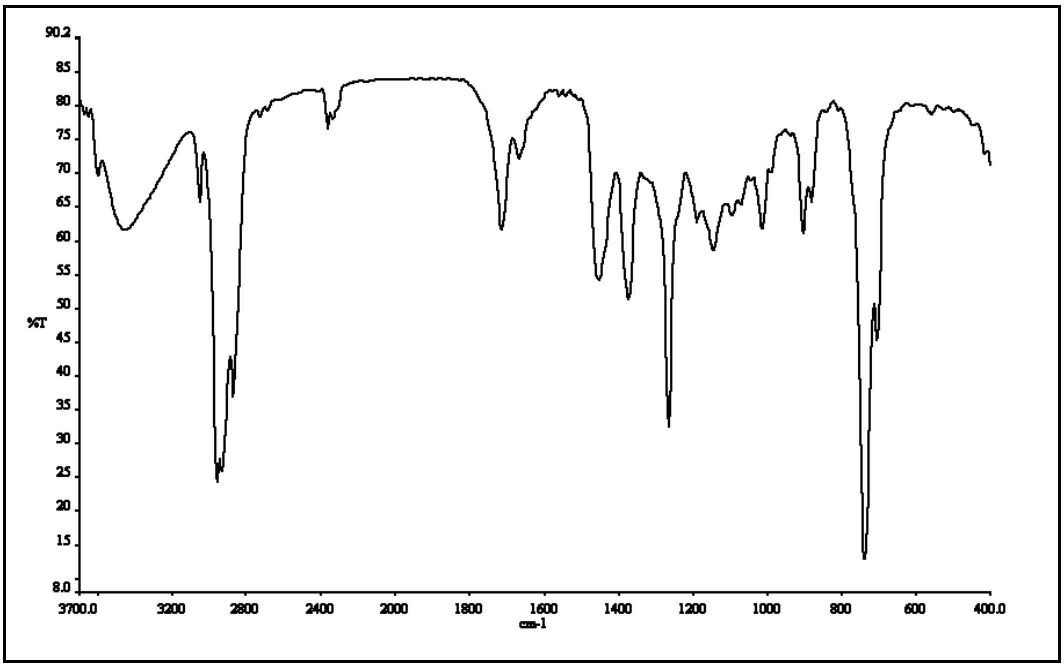

Figure 62 IR (neat) spectrum of compound **JPD5**

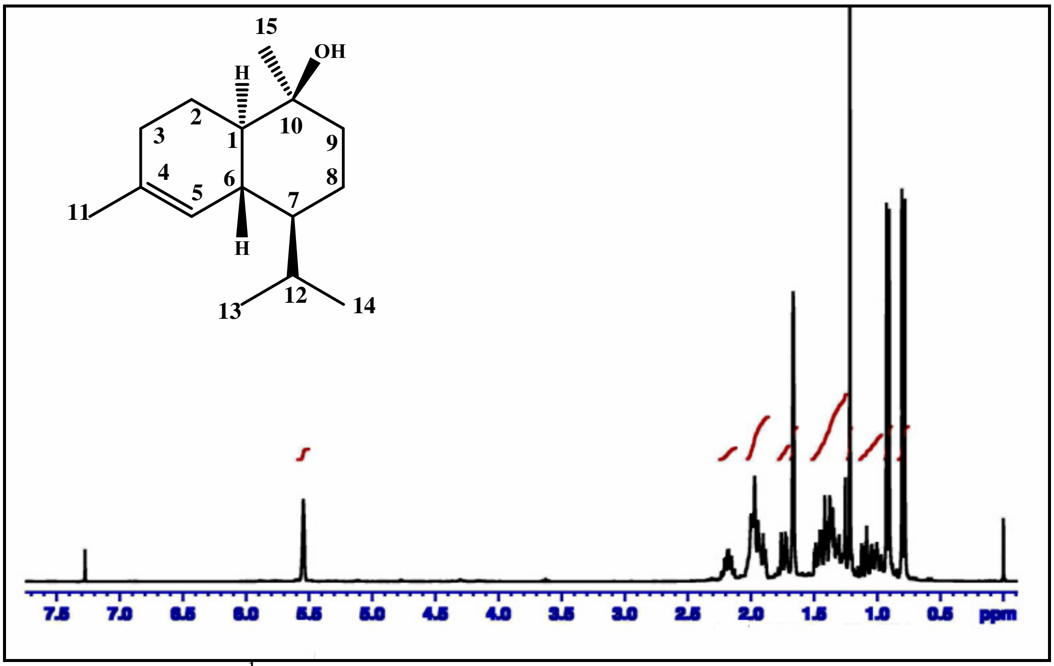

Figure 63 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **JPD5**

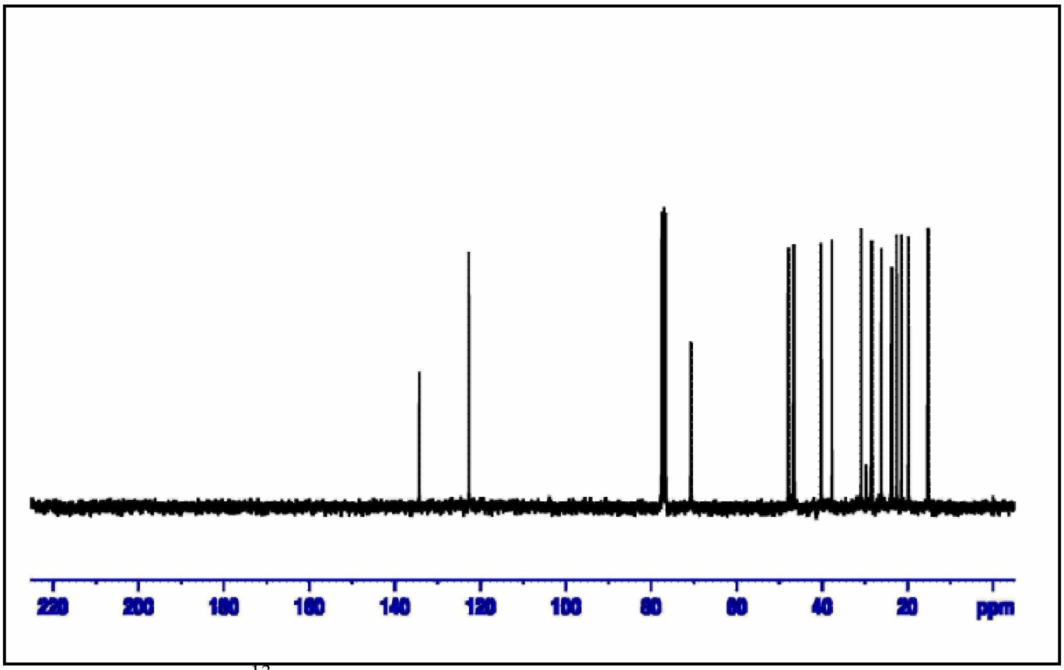

Figure 64 ^{13}C NMR (75 MHz) (CDCl$_3$) spectrum of compound **JPD5**

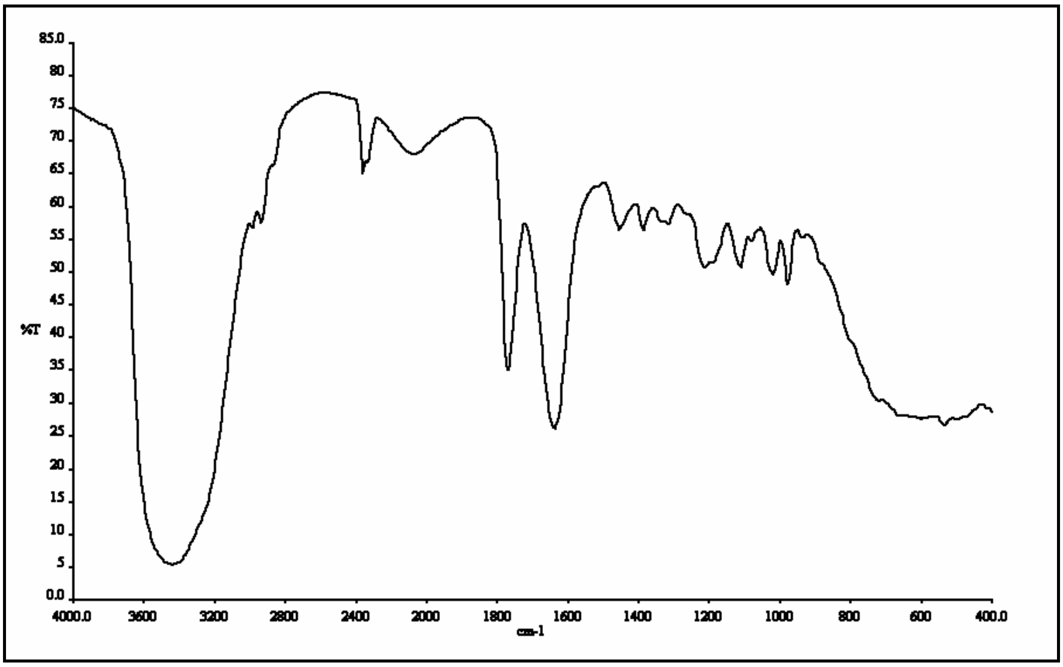

Figure 65 IR (neat) spectrum of compound **JPD6**

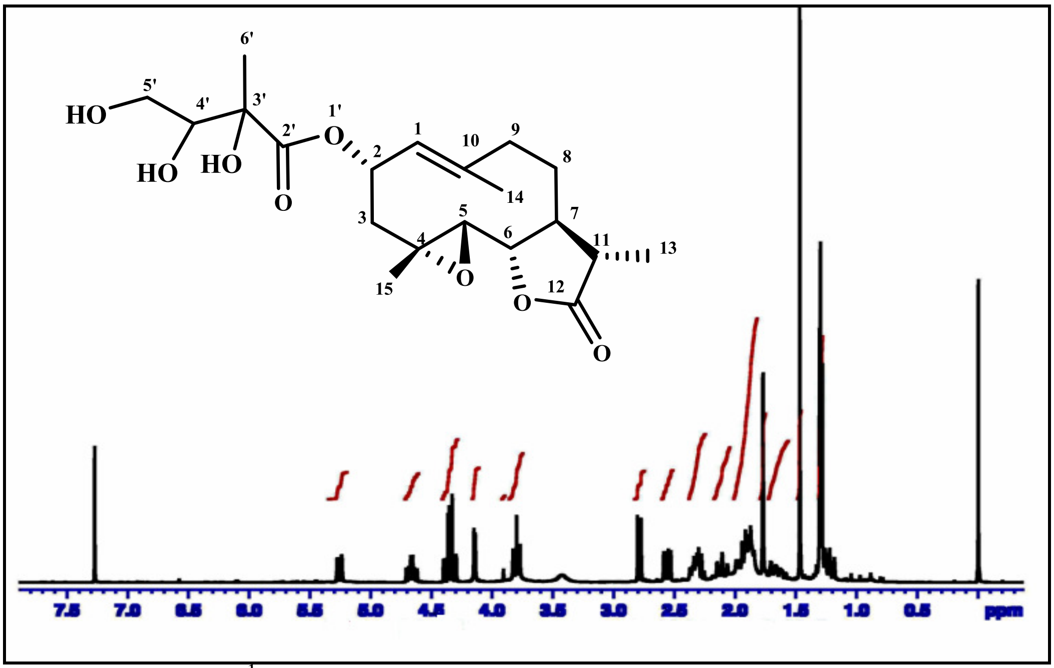

Figure 66 ^{1}H NMR (300 MHz) (CDCl$_3$) spectrum of compound **JPD6**

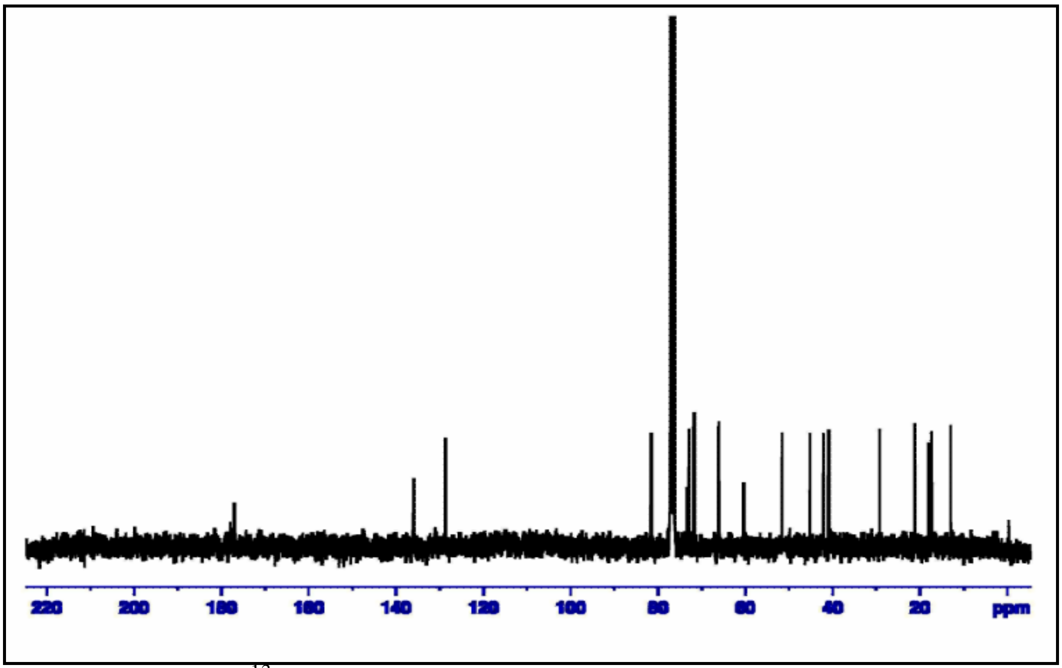

Figure 67 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **JPD6**

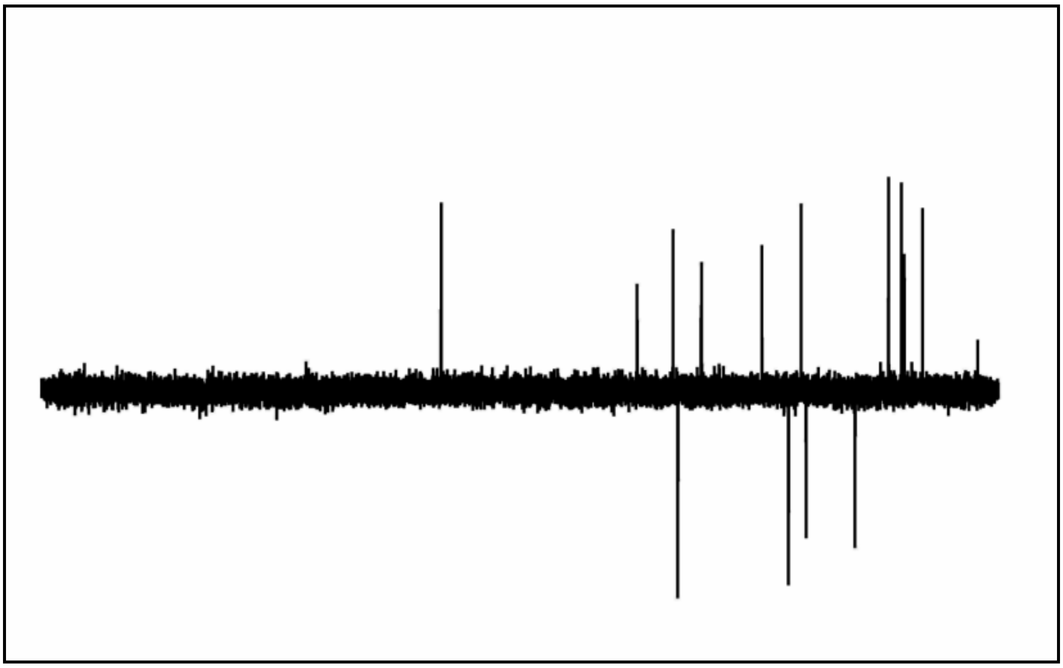

Figure 68 DEPT 135° ($CDCl_3$) spectrum of compound **JPD6**

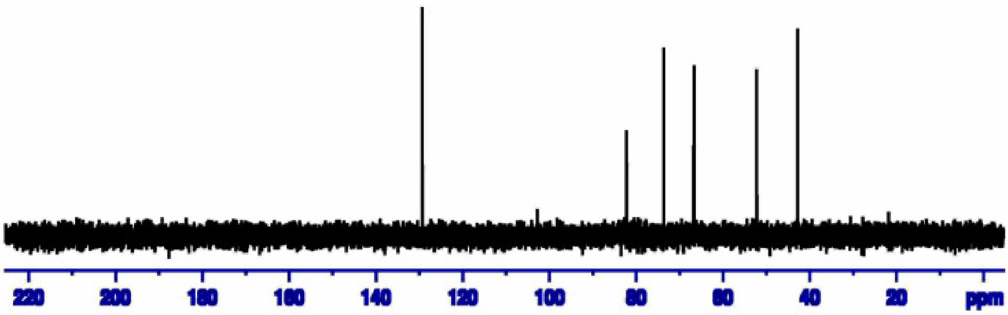

Figure 69 DEPT 90° ($CDCl_3$) spectrum of compound **JPD6**

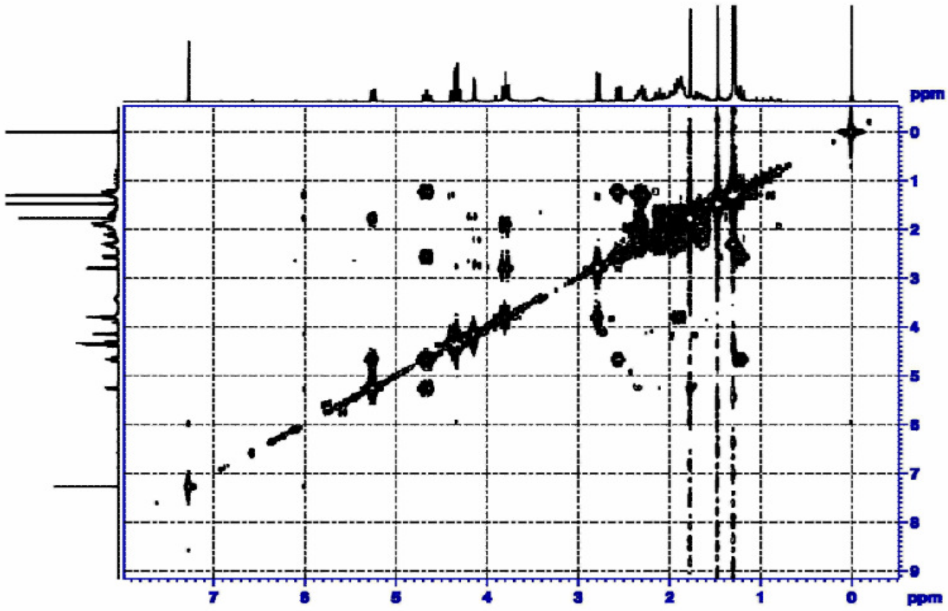

Figure 70 2D COSY ($CDCl_3$) spectrum of compound **JPD6**

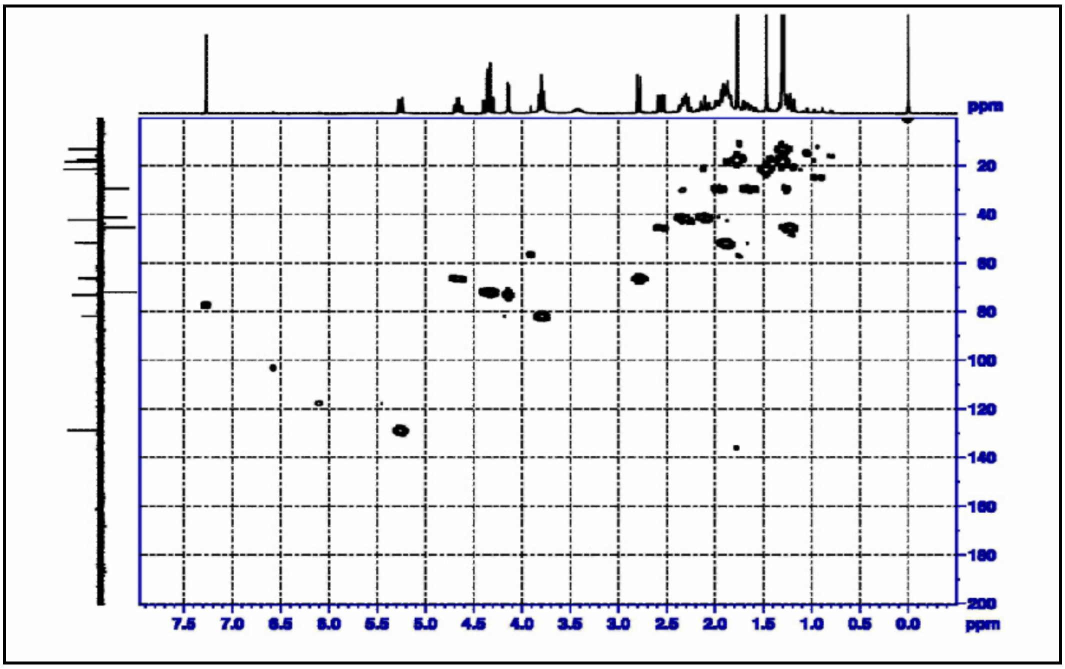

Figure 71 2D HMQC ($CDCl_3$) spectrum of compound **JPD6**

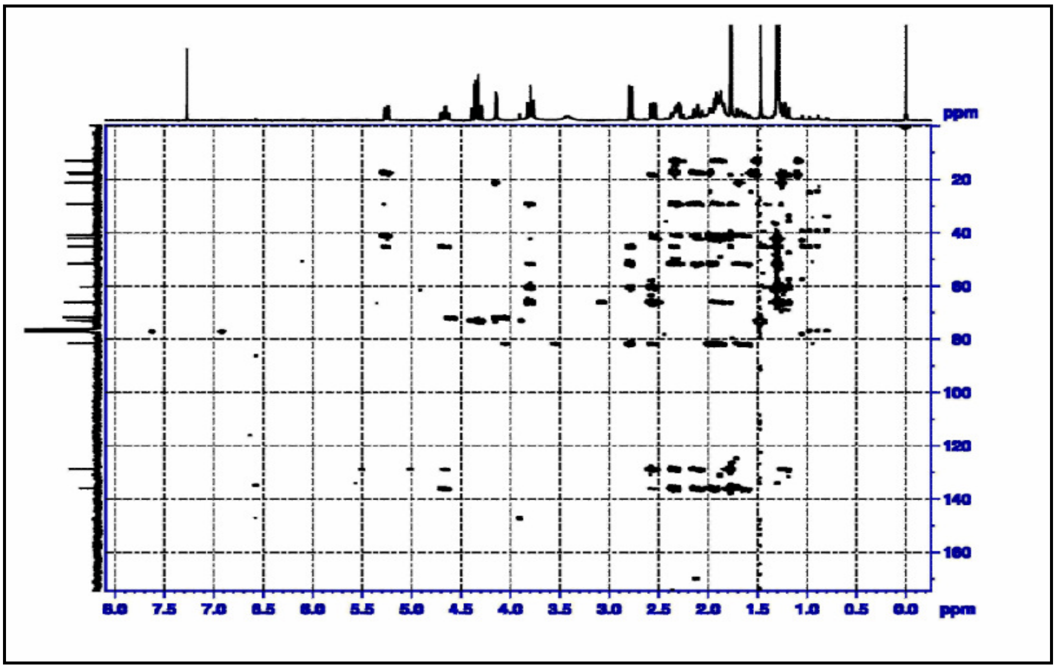

Figure 72 2D HMBC ($CDCl_3$) spectrum of compound **JPD6**

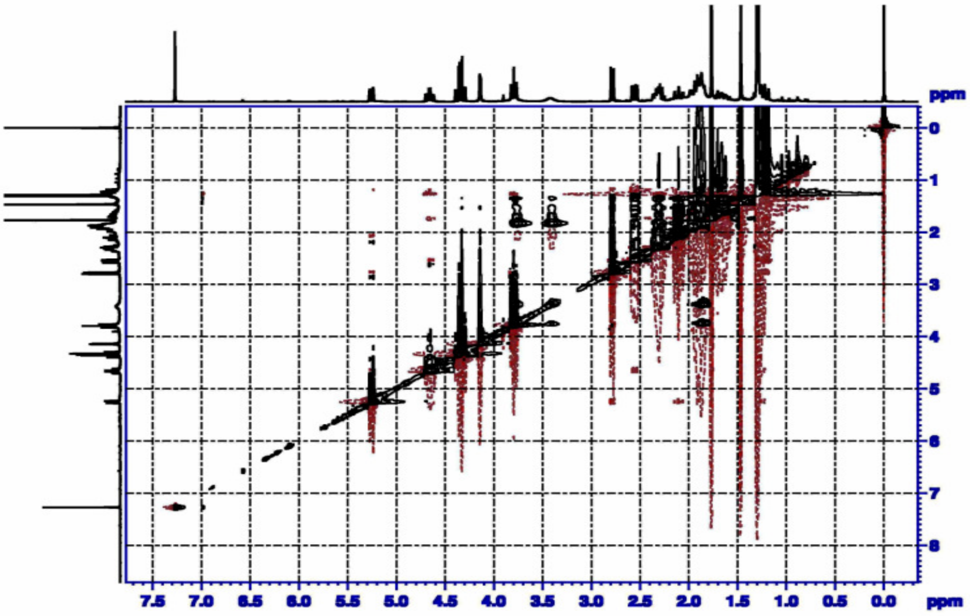

Figure 73 2D NOESY ($CDCl_3$) spectrum of compound **JPD6**

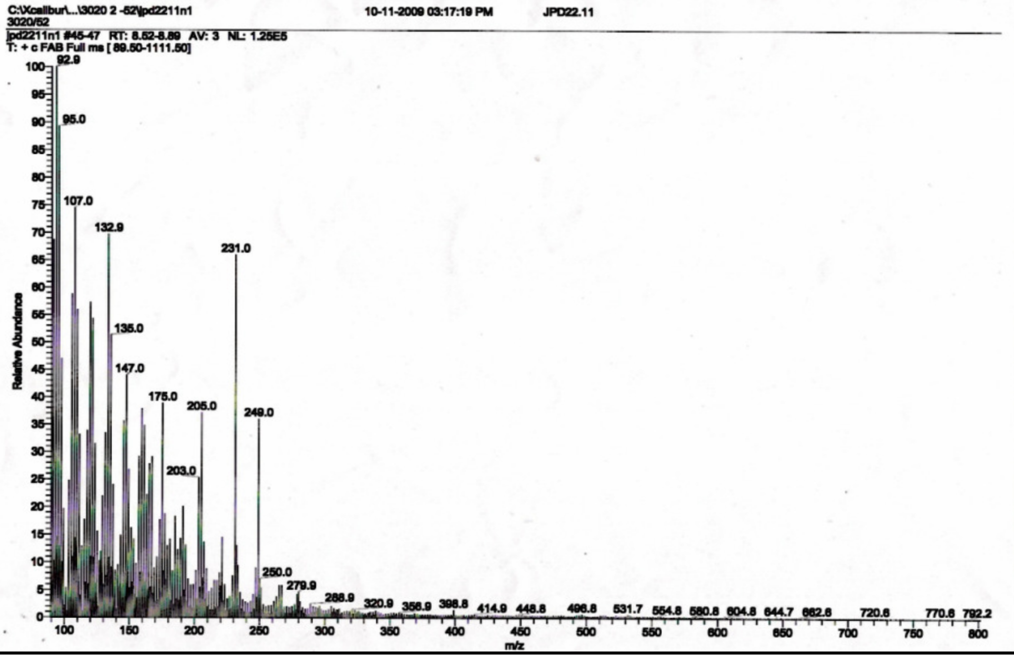

Figure 74 EIMS spectrum of compound **JPD6**

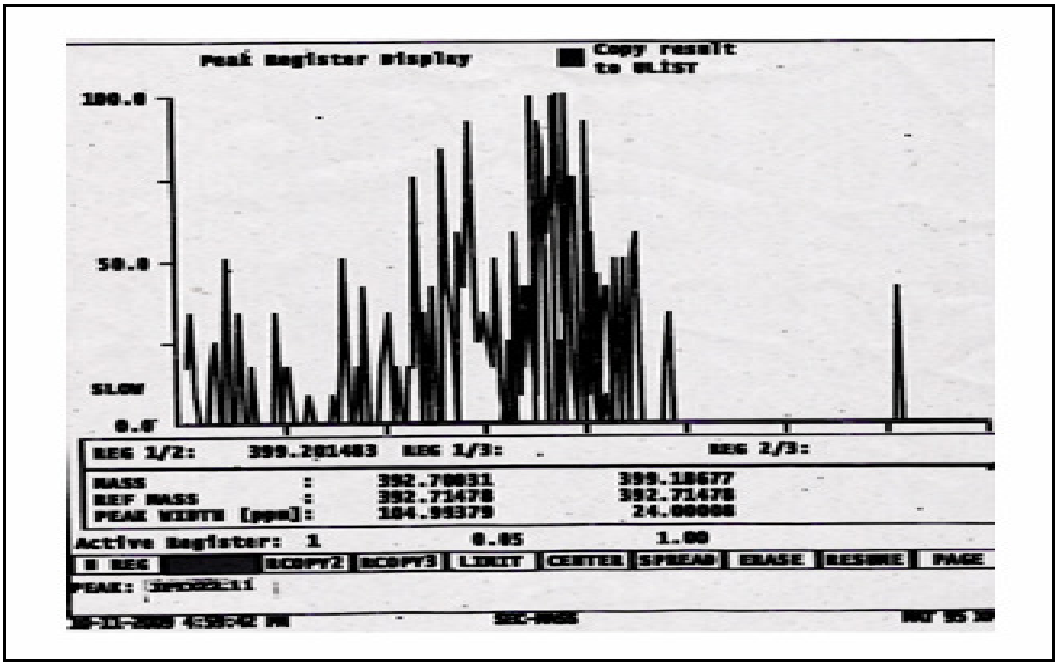

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Figure 75 HRFAB spectrum of compound **JPD6**

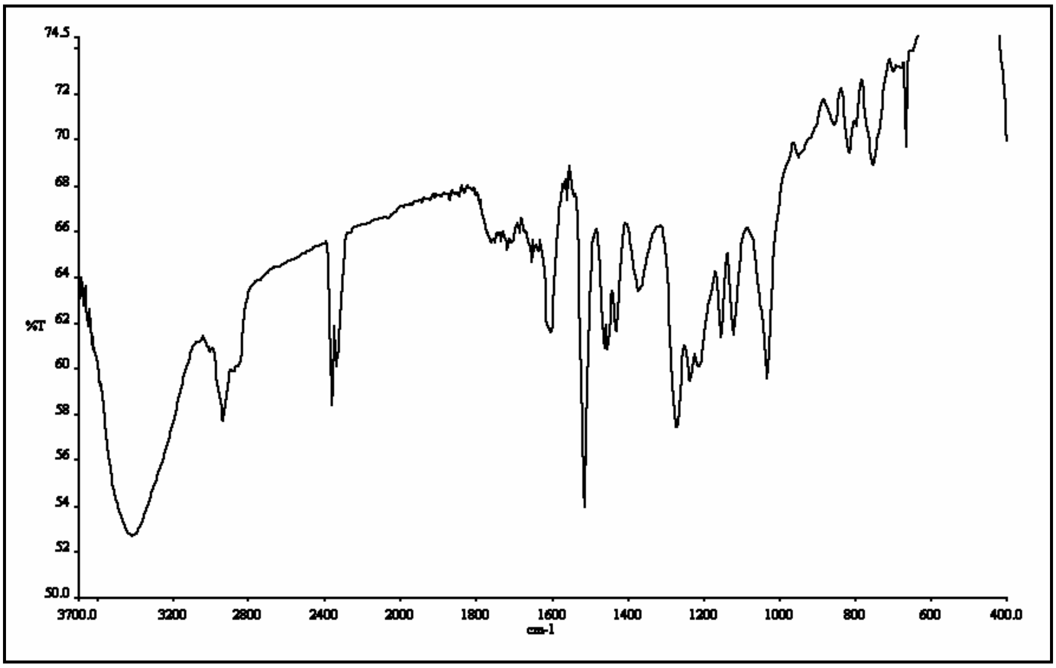

Figure 76 IR (neat) spectrum of compound **JPD7**

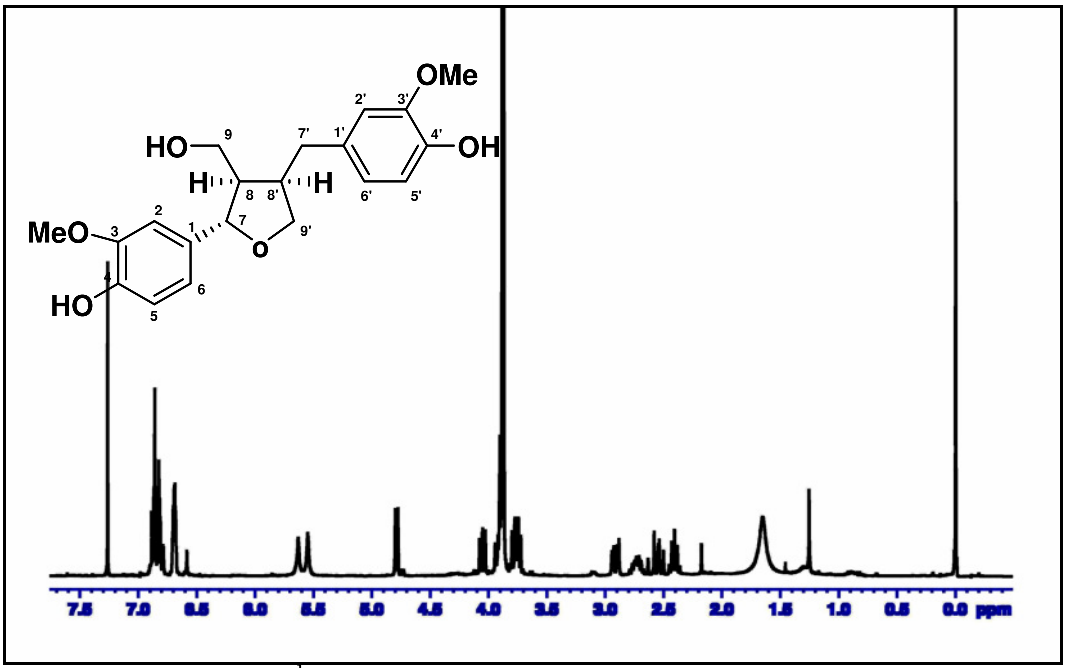

Figure 77 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **JPD7**

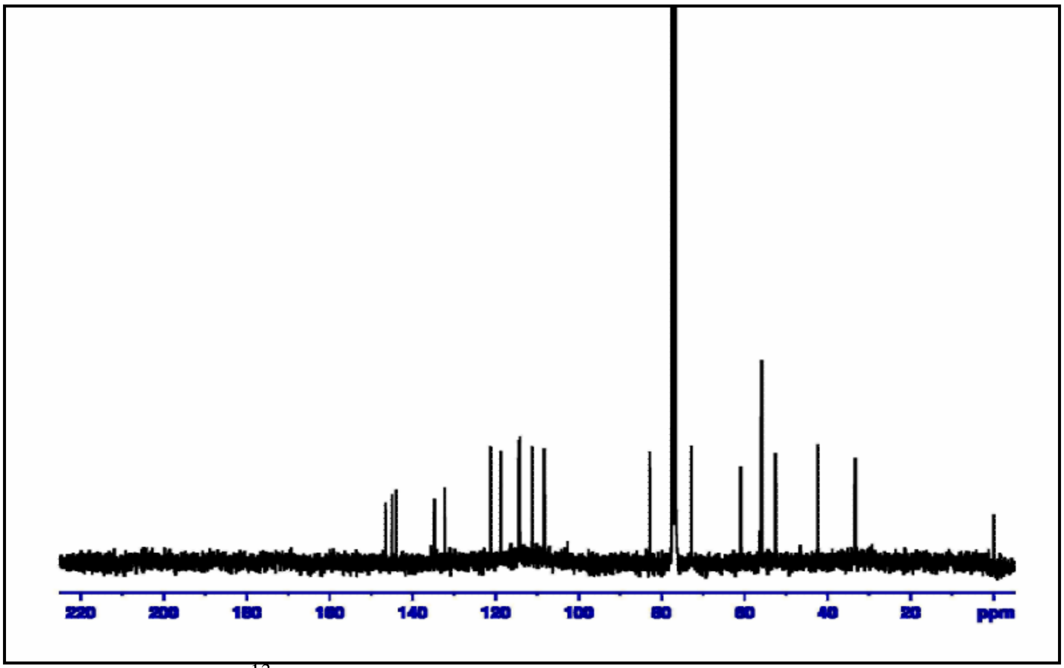

Figure 78 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **JPD7**

VITAE

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Educational Attainment

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Scholarship Awards during Enrolment

The Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC)

List of Publication and Proceedings

Proceedings

Pongpuntaruk, J., Ponglimanont, C. and Karalai, C. 2010. Sesquiterpene Lactones from the Root of *Michelia alba* DC. 16th National Graduate Research Conference, Maejo University, Chiang Mai, Thailand, March 11-12, 2010 pp. 13 (Poster presentation)