Chemical Constituents from the Roots and Fruits of *Diospyros wallichii* and the Roots and Twigs of *Premna obtusifolia* and Their Biological Activities

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A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Organic Chemistry

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Thesis Title Chemical Constituents from the Roots and Fruits of *Diospyros wallichii* and the Roots and Twigs of *Premna obtusifolia* and Their Biological Activities

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I hereby certify that this work has not already been accepted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

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ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมีจากราก และ ผล ต้นตำตะโก และ ราก และ กิ่ง ต้นอัคคีทวารทะเล และ ฤทธิ์ทางชีวภาพ
ผู้เขียน	นายอับดุลวาหาบ สาแล๊ะ
สาขาวิชา	เคมีอินทรีย์
ปีการศึกษา	2555

บทคัดย่อ

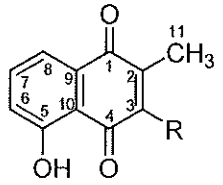
ตอนที่ 1 องค์ประกอบทางเคมีจากรากและผลต้นตำตะโก

การศึกษาองค์ประกอบทางเคมีจากส่วนสกัด เฮกเซน และไดคลอโรมีเทน จากส่วนราก และ ผล ของต้นตำตะโก สามารถแยกสารใหม่ได้ 3 สาร ซึ่งประกอบด้วยสารประเภทอนุพันธ์แนพโทควิโนน 1 สาร (**DW9**), อนุพันธ์แนพทาลีน 2 สาร (**DW11** และ **DW12**) และยังสามารถแยกสารประกอบที่มีการรายงานแล้ว 18 สาร คือ สารประเภทอนุพันธ์แนพโทควิโนน 9 สาร (**DW1-DW7, DW8** และ **DW10**), อนุพันธ์คูมาริน 1 สาร (**DW13**), ไตรเทอร์พีนอยด์ 6 สาร (**DW14-DW19**) และ สารผสมสเตอรอยด์ (**DW20** และ **DW21**) โครงสร้างของสารประกอบเหล่านี้สามารถวิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี และเปรียบเทียบกับสารที่มีรายงานวิจัยแล้ว นอกจากนี้ยังมีการทดสอบฤทธิ์ต้านเชื้อแบคทีเรีย และ ต้านมะเร็ง พบว่าสารประกอบ **DW8** และ **DW9** มีฤทธิ์ต้านเซลล์มะเร็งเต้านม ที่ค่า IC_{50} = 0.06 และ 0.09 μM ตามลำดับ

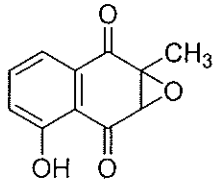
ตอนที่ 2 องค์ประกอบทางเคมีจากรากและกิ่งต้นอัคคีทวารทะเล

การศึกษาองค์ประกอบทางเคมีจากส่วนสกัด เฮกเซน และไดคลอโรมีเทน จากส่วน ราก และ กิ่ง ต้นอัคคีทวารทะเล สามารถแยกสารใหม่ได้ 11 สาร ประกอบด้วยสารประกอบ ไอโซพิมาเรนไดเทอร์พีนอยด์ 2 สาร (**PO1** และ **PO2**), โรเซนไดเทอร์พีนอยด์ 1 สาร (**PO3**), อะบีเทนไดเทอร์พีนอยด์ 4 สาร (**PO4, PO5, PO18** และ **PO19**), ไอทีเซนไดเทอร์พีนอยด์ 4 สาร (**PO23-PO26**) และยังสามารถแยกสารประกอบที่มีการรายงานแล้ว 18 สาร (**PO6, PO7, PO8-PO15, PO16, PO17, PO20-PO22** และ **PO27-PO29**) โครงสร้างของสารประกอบเหล่านี้สามารถวิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี และเปรียบเทียบกับสารที่มีรายงานวิจัยแล้ว และสำหรับสารประกอบ **PO10, PO11, PO13, PO20, PO22** และ **PO25** ใช้ข้อมูลทางเอกซ์เรย์ประกอบการพิสูจน์โครงสร้างอีกด้วย

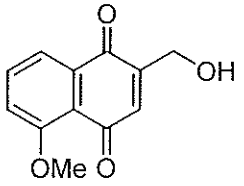
นอกจากนี้ยังมีการทดสอบฤทธิ์ต้านเชื้อแบคทีเรีย และ ต้านการอักเสบ พบว่า สารประกอบ **PO24** มีฤทธิ์ต้านการอักเสบ ที่ค่า IC_{50} = 1.7 μM.



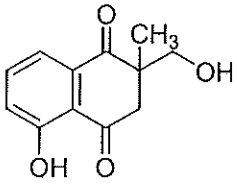
R= H, **DW1**: Plumbagin
R= OH, **DW2**: Droserone



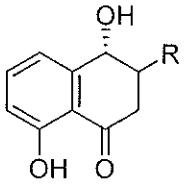
DW3: 2,3-Epoxyplumbagin



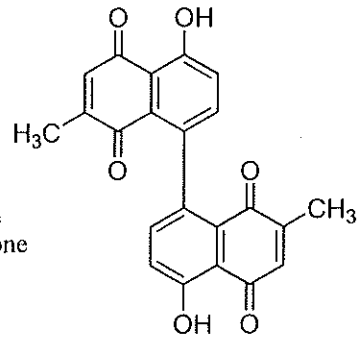
DW4: 2-Hydroxymethyl-5-methoxy-1,4-napthoquinone



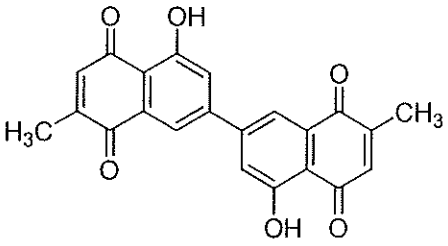
DW5: Diomuscinone



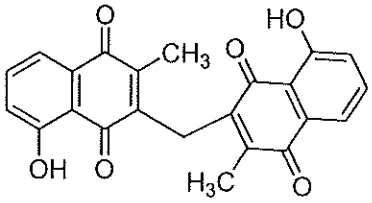
R= ····CH$_3$, **DW6**: *iso*-Shinanolone
R= ▬CH$_3$, **DW7**: *epi*-Isoshinanolone



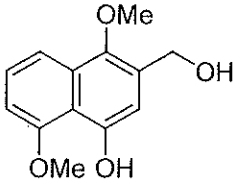
DW8: Maritinone



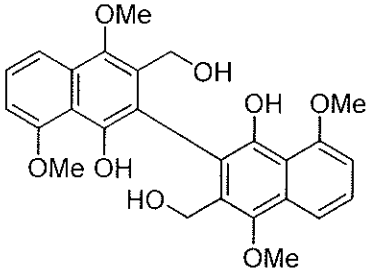
DW9: 5,5'-Dihydroxy-2,2'-dimethyl-7,7'-binaphthalen-1,1',4,4'-tetraone



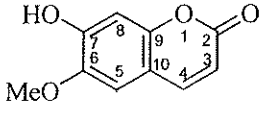
DW10: Methylene-3,3'-biplumbagin



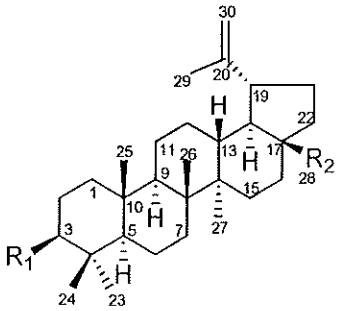
DW11: 2-Hydroxy-methyl-1, 5-dimethoxynaphthalen-4-ol



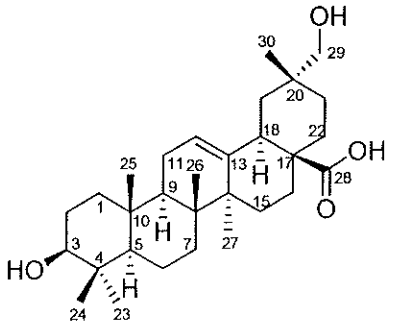
DW12: 2,2'-*bis*-Hydroxymethyl-1,1',5,5'-tetramethoxy-3,3'-binaphthalen-4,4'-diol



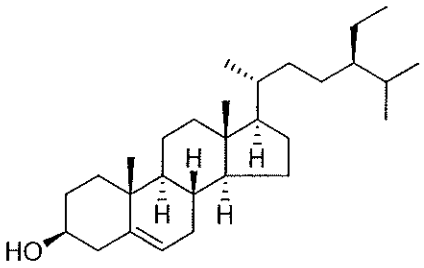
DW13: Scopoletin



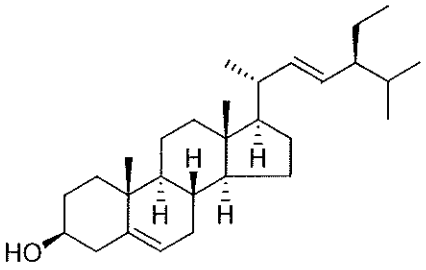
R_1 = OH, R_2 = CH_3, **DW14**: Lupeol
R_1 = =O, R_2 = CH_3, **DW15**: Lupenone
R_1 = OH, R_2 = CH_2OH, **DW16**: Betulin
R_1 = OH, R_2 = CHO, **DW17**: Betulinaldehyde
R_1 = OH, R_2 = CO_2H, **DW18**: Betulinic acid



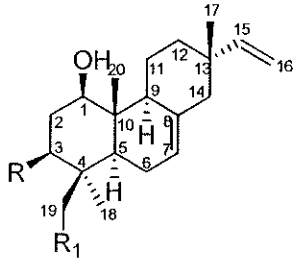
DW19: 3,29-Dihydroxyolean-12-en-28-oic acid



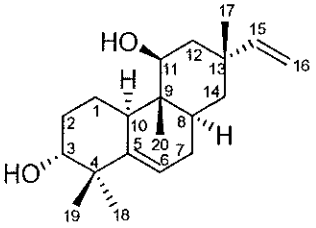
DW20: β-Sitosterol



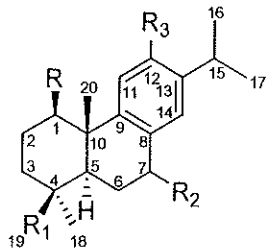
DW21: Stigmasterol



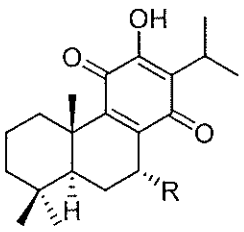
R= OH, R_1= H, **PO1**: Isopimara-7,15-dien-1β,3β-diol
R= H, R_1= OH, **PO2**: Isopimara-7,15-dien-1β,19-diol



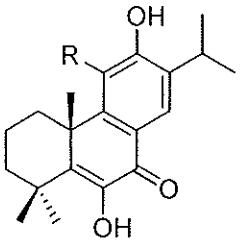
PO3: 13-*epi*-5,15-Rosadien-3α,11β-diol



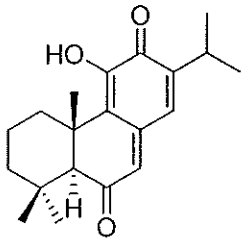
R = OH, R_1, R_2, R_3 = H, **PO4**: Abietatrien-1β-ol
R,R_3 = OH, R_1,R_2 = H, **PO5**: Abietatrien-1β,12-diol
R, R_1, R_2 = H, R_3 = OH, **PO6**: Ferruginol
R, R_1, R_2 = H, R_3 = OCH_3, **PO7**: *O*-Methyl ferruginol
R,R_2 = H, R_1 = CO_2H, R_3 = OH, **PO8**: Lambertic acid
R,R_1,R_3 = H, R_2 = =O, **PO9**: Sugiol



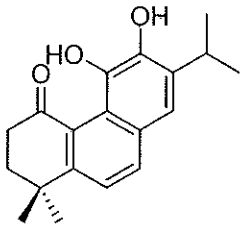
R = H, **PO10**: Royleanone
R = OH, **PO11**: Horminone



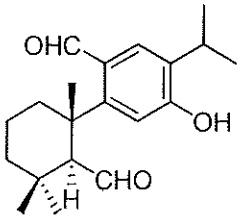
R = H, **PO12**: Montbretrol
R = OH, **PO13**: 14-Deoxycoleon



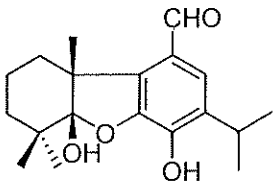
PO14: Taxodion



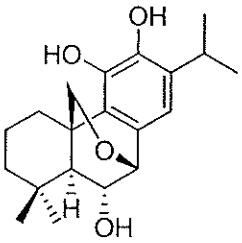
PO15: Arucadiol



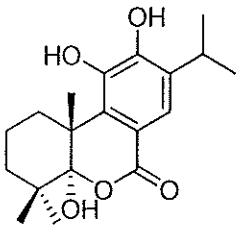
PO16:12-Hydroxy-6,7-seco abieta-8,11,13-triene-6,7-dial



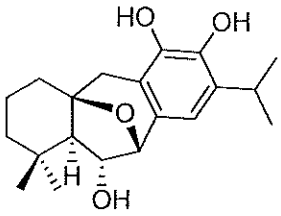
PO17: Salvicanaraldehyde



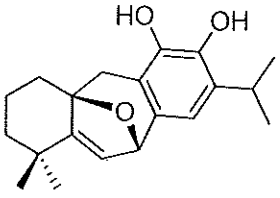
PO18: 6α,11,12-Trihydroxy-7β, 20-epoxy-8,11,13-abietatriene



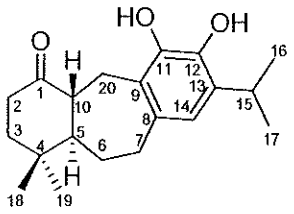
PO19: 5α,11,12-Trihydroxy-6-oxa-abieta-8,11,13-trien-7-one



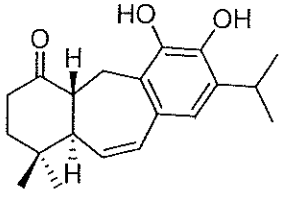
PO20: 5,6-Dihydro-6α-hydroxysalviasperanol



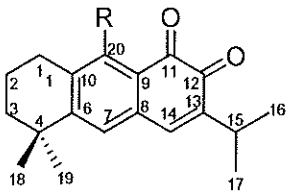
PO21: Salviasperanol



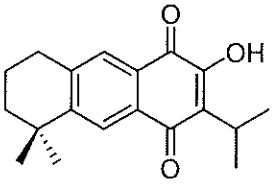
PO22: 11,12-Dihydroxy-8, 11,13-icetexatrien-1-one



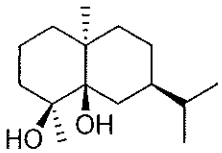
PO23: 11,12-Dihydroxy-6,8, 11,13-icetexatatraen-1-one



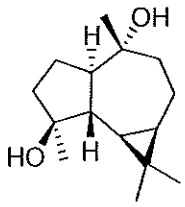
R = OH, **PO24**: Obtusinone A
R = H, **PO25**: Obtusinone B



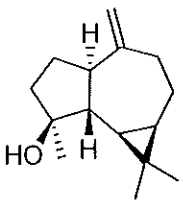
PO26: Obtusinone C



PO27: 4β,5β-Dihydroxy-10-*epi*-eudesmane



PO28: 4β,10β-Dihydroxy aromadendrane



PO29: Spathulenol

Thesis Title	Chemical Constituents from the roots and fruits of *Diospyros wallichii* and the roots and twigs of *Premna obtusifolia* and their Biological activities.
Author	Mr. Abdulwahab Salae
Major Program	Organic Chemistry
Academic Year	2012

ABSTRACT

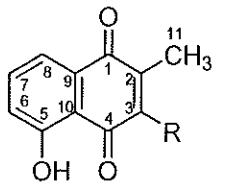
Part I Chemical constituents from the roots and fruits of Diospyros wallichii

Phytochemical investigation of the hexane and CH_2Cl_2 extracts of the roots and fruits of *D. wallichii*, afforded three new compounds as one binaphthoquinone (**DW9**) and two naphthalene derivatives (**DW11** and **DW12**), together with eighteen known compounds including nine naphthoquinones (**DW1-DW7**, **DW8** and **DW10**), one coumarin (**DW13**), six triterpenoids (**DW14-DW19**) and a mixture of steroids (**DW20-DW21**). Their structures were elucidated by spectroscopic methods and comparison with those reported in the literature. The antibacterial and cytotoxic activities of the isolates were also evaluated. Compound **DW8** and **DW9** showed significant cytotoxic activity against human cancer cell line MCF-7 with IC_{50} values of 0.06 and 0.09 μM, respectively.

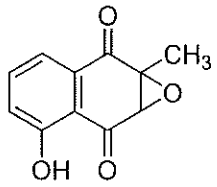
Part II Chemical constituents from the roots and twigs of Premna obtusifolia

Phytochemical investigation of the hexane and CH_2Cl_2 extracts of the roots and twigs of *P. obtusifolia* led to the isolation and characterization of eleven new compounds as two isopimarane diterpenoids (**PO1** and **PO2**), one rosane diterpenoid (**PO3**), four abietane diterpenoids (**PO4**, **PO5**, **PO18** and **PO19**), four icetexane diterpenoids (**PO23-PO26**), along with eighteen known compounds (**PO6**, **PO7**, **PO8-PO15**, **PO16**, **PO17**, **PO20- PO22** and **PO27- PO29**). Their structures were elucidated by spectroscopic methods and comparison with those reported in the literature. The structures of **PO10**, **PO11**, **PO13**, **PO20**, **PO22** and **PO25** were additionally confirmed by X-ray diffraction analysis. The antibacterial and anti-

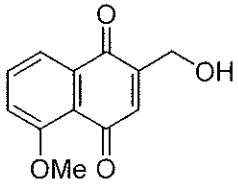
inflammatory activities of the isolates were also evaluated. Compound **PO24** exhibited potential anti-inflammatory activity with the IC_{50} value of 1.7 μM.



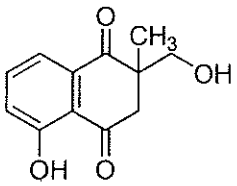
R= H, **DW1**: Plumbagin
R= OH, **DW2**: Droserone



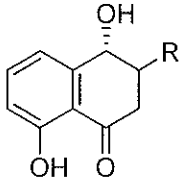
DW3: 2,3-Epoxyplumbagin



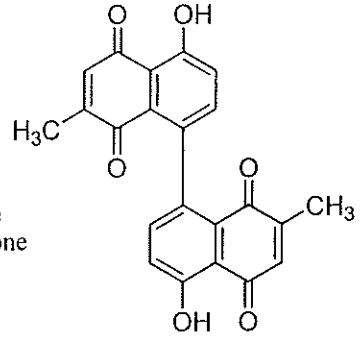
DW4: 2-Hydroxymethyl-5-methoxy-1,4-napthoquinone



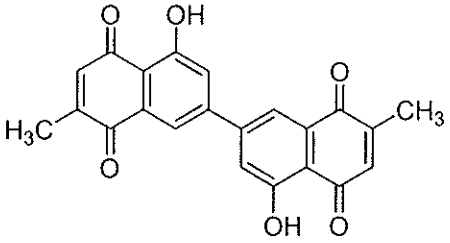
DW5: Diomuscinone



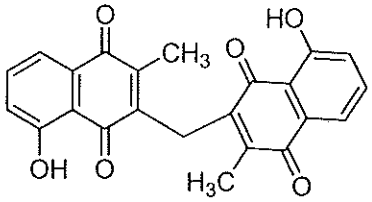
R= ····CH_3 , **DW6**: *iso*-Shinanolone
R= —CH_3 , **DW7**: *epi*-Isoshinanolone



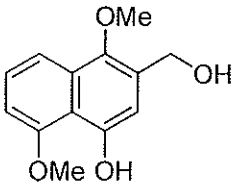
DW8: Maritinone



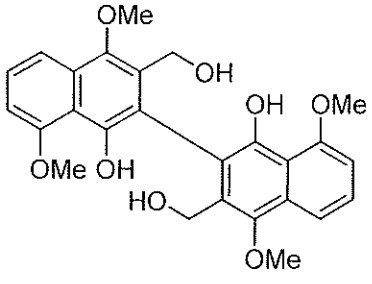
DW9: 5,5'-Dihydroxy-2,2'-dimethyl-7,7'-binaphthalen-1,1',4,4'-tetraone



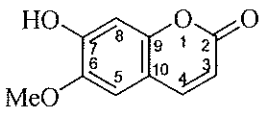
DW10: Methylene-3,3'-biplumbagin



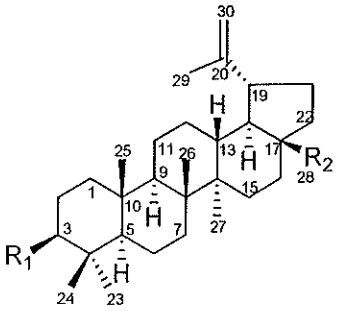
DW11: 2-Hydroxy-methyl-1, 5-dimethoxynaphthalen-4-ol



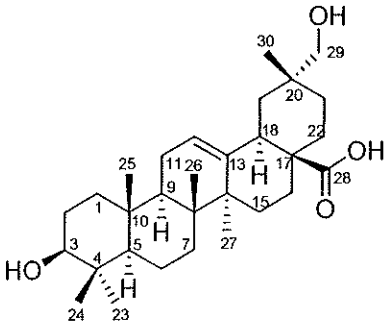
DW12: 2,2'-*bis*-Hydroxymethyl-1,1',5,5'-tetramethoxy-3,3'-binaphthalen-4,4'-diol



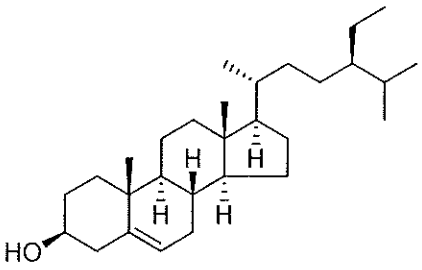
DW13: Scopoletin



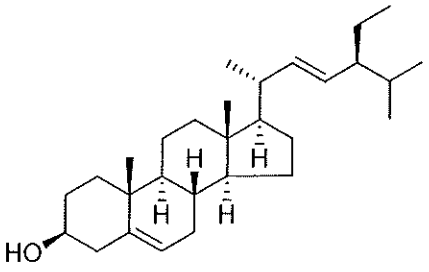
R_1 = OH, R_2 = CH_3, **DW14**: Lupeol
R_1 = =O, R_2 = CH_3, **DW15**: Lupenone
R_1 = OH, R_2 = CH_2OH, **DW16**: Betulin
R_1 = OH, R_2 = CHO, **DW17**: Betulinaldehyde
R_1 = OH, R_2 = CO_2H, **DW18**: Betulinic acid



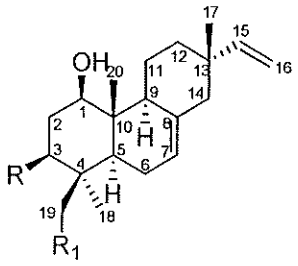
DW19: 3,29-Dihydroxyolean-12-en-28-oic acid



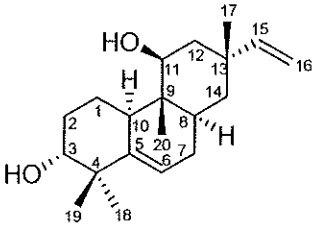
DW20: β-Sitosterol



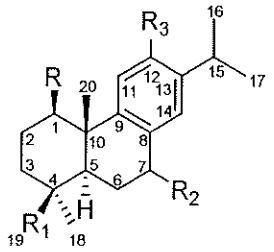
DW21: Stigmasterol



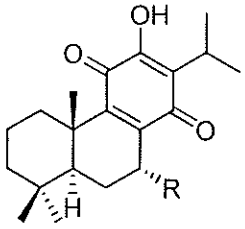
R= OH, R_1= H, **PO1**: Isopimara-7,15-dien-1β,3β-diol
R= H, R_1= OH, **PO2**: Isopimara-7,15-dien-1β,19-diol



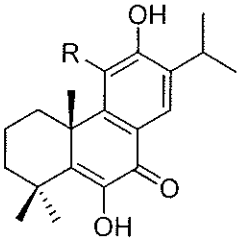
PO3: 13-*epi*-5,15-Rosadien-3α,11β-diol



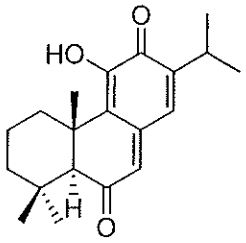
R = OH, R_1, R_2, R_3 = H, **PO4**: Abietatrien-1β-ol
R,R_3 = OH, R_1,R_2 = H, **PO5**: Abietatrien-1β,12-diol
R, R_1, R_2 = H, R_3 = OH, **PO6**: Ferruginol
R, R_1, R_2 = H, R_3 = OCH_3, **PO7**: *O*-Methyl ferruginol
R,R_2 = H, R_1 = CO_2H, R_3 = OH, **PO8**: Lambertic acid
R,R_1,R_3 = H, R_2 = =O, **PO9**: Sugiol



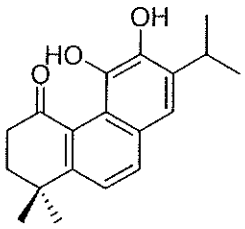
R = H, **PO10**: Royleanone
R = OH, **PO11**: Horminone



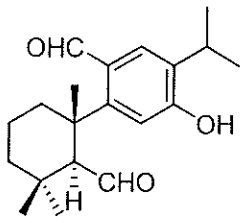
R = H, **PO12**: Montbretrol
R = OH, **PO13**: 14-Deoxycoleon



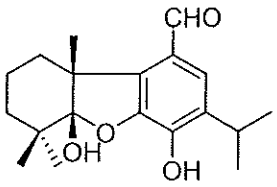
PO14: Taxodion



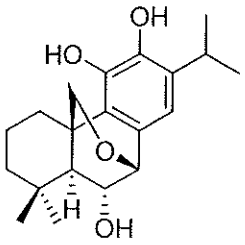
PO15: Arucadiol



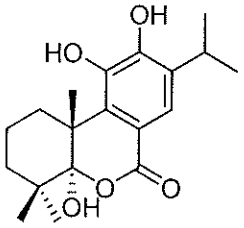
PO16:12-Hydroxy-6,7-seco abieta-8,11,13-triene-6,7-dial



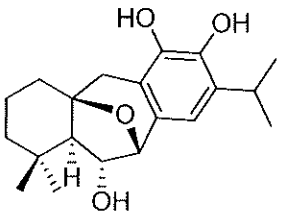
PO17: Salvicanaraldehyde



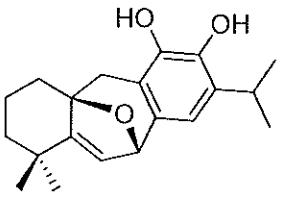
PO18: 6α,11,12-Trihydroxy-7β, 20-epoxy-8,11,13-abietatriene



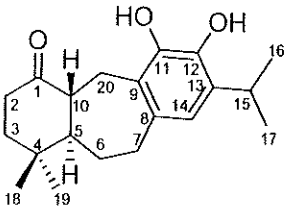
PO19: 5α,11,12-Trihydroxy-6-oxa-abieta-8,11,13-trien-7-one



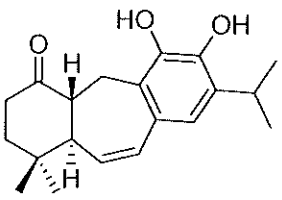
PO20: 5,6-Dihydro-6α-hydroxysalviasperanol



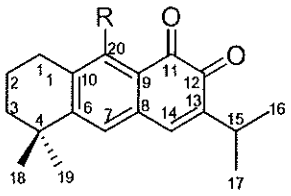
PO21: Salviasperanol



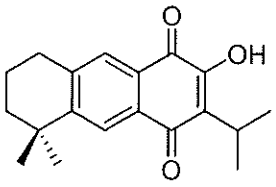
PO22: 11,12-Dihydroxy-8, 11,13-icetexatrien-1-one



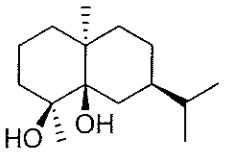
PO23: 11,12-Dihydroxy-6,8, 11,13-icetexatatraen-1-one



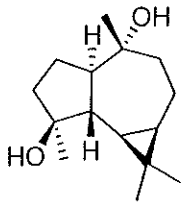
R = OH, **PO24**: Obtusinone A
R = H, **PO25**: Obtusinone B



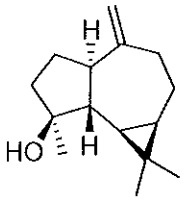
PO26: Obtusinone C



PO27: 4β,5β-Dihydroxy-10-*epi*-eudesmane



PO28: 4β,10β-Dihydroxy aromadendrane



PO29: Spathulenol

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Abdulwahab Salae

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this research is to investigate the chemical constituents of *Diospyros wallichii* and *Premna obtusiflia*. They are parts of the basic research on the utilization of the Thai medicinal plants. Chemical investigation of constituents from the roots and fruits of *D. wallichii* and the roots and twigs of *P. obtusiflia* led to isolation of fourteen new compounds (**DW9**, **DtW11**, **DW12**, **PO1-PO5**, **PO18**, **PO19**, and **PO23-PO26**), together with thirty-six known compounds. Some of the compounds exhibited significant cytotoxic activity against MCF-7 cell line (Human breast adenocarcinoma), antibacterial and anti-inflammatory activities. Hence they have potential to be developed into drug.

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LIST OF ABBREVIATIONS AND SYMBOLS

s	=	singlet
d	=	doublet
t	=	triplet
q	=	quartet
m	=	multiplet
dd	=	doublet of doublet
dt	=	doublet of triplet
br s	=	broad singlet
br d	=	broad doublet
qd	=	quartet of doublet
g	=	Gram
nm	=	Nanometer
m.p.	=	Melting point
cm^{-1}	=	Reciprocol centimeter (wave number)
δ	=	Chemical shift relative to TMS
J	=	Coupling constant
$[\alpha]_D$	=	Specific rotation
λ_{max}	=	Maximum wavelength
ν	=	Absorption frequencies
ε	=	Molar extinction coefficient
m/z	=	A value of mass divided by charge
°C	=	Degree celcius
MHz	=	Megahertz
ppm	=	Part per million
C	=	Concentration
FT-IR	=	Fourier Transfrom Infrared
UV-Vis	=	Ultraviolet-Visible

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

HREIMS	=	High Resolution Chemical Impact Mass Spectrometry
EIMS	=	Electron Impact Mass Spectrometry
HREIMS	=	High Resolution Electron Impact 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
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
NOESY	=	Nuclear Overhauser Effect Correlation Spectroscopy
CC	=	Column Chromatography
VLC	=	Vacuum liquid Chromatography
DCM	=	Dichloromethane
TMS	=	Tetramethylsilane
$CDCl_3$	=	Deuterochloroform
CD_3OD	=	Deuteromethanol
CO_2	=	Carbon dioxide
HCl	=	Hydrochloric acid
DMSO	=	Dimethyl sulfoxide
CAPE	=	Caffeic acid phenethylester
MCF-7	=	Human breast adenocarcinoma
µg/ml	=	Microgram per milliliter
IC_{50}	=	The half maximal inhibitory concentration
NO	=	Nitric oxide

CHAPTER 1
INTRODUCTION

1.1 Chemical constituents from *Diospyros wallichii* King&Gamble

1.1.1 Introduction

The genus *Diospyros* (Family Ebenaceae), includes about 400 species. In Thailand, 60 *Diospyros* species have been described by Phengklai. Many of them are distributed in the forest throughout the country. Their fine and hard wood is of high value, and the leaves, bark and fruit of some species have been used as medicinal herbs, such as the fruit of *D. Montana* is used to treat hiccups, ulcers, urinary diseases and dysentery. The fruit of *D. decandra* and *D. malabarica* is used as an emmenagogue in Vietnam and leukorrhea in India, respectively (Utsunomiya *et al.*, 1998). *Diospyros wallichii* is known in Thailand as "damtako (ดำตะโก)". This plant is grown to about 10-20 m in height. It has small white flower. The round fruits are poisonous and used for fishing.

Figure 1 Parts of *Diospyros wallichii*

1.1.2 Review of literatures

Chemical constituents isolated from seventeen species of *Diospyros* genus were summarized in **Table 1**. Information from the Scifinder Scholar copyright in 2012 will be presented and they can be classified into groups as follows: alkaloid, benzenoids, betulide, carotenoids, coumarins, diterpenoids, fatty acids, flavonoids, hydrocarbons, lignans, naphthalenes, phenolics, quinones, sesquiterpenoid, steroids, sugars, triterpenoids, miscellaneous terpenoids and vitamin

Table 1 Compounds from plants of *Diospyros* genus.

a:	alkaloid	**h:**	flavonoids	**o:**	sterroids
b:	benzenoids	**i:**	hydrocabons	**p:**	sugars
c:	betenolide	**j:**	lignans	**q:**	triterpenoids
d:	carotenoids	**k:**	naphthalenes	**r:**	terpenoids
e:	coumarins	**l:**	phenolics	**s:**	vitamin
f:	diterpenoids	**m:**	quinones		
g:	fatty acids	**n:**	sesquiterpenoid		

Plant	**part**	**Compound**	**Bibliography**
D. angustifolia	Stem barks	Diospyrosonaphthoside, **99k** Diospyrososide, **105l** Lupeol, **215q** Betulinic acid, **219q** β-Amyrin, **246q** 3β-Hydroxy-28,19β-oleanolide, **303q** Diospyrosooleanolide, **310q** Friedelin, **315q**	Pathak *et al.*, 2004

Table 1 (continued)

Plant	part	Compound	Bibliography
D. assimilis	Roots	5-Hydroxy-4-methoxy-2-naphthaldehyde, **93k**	Ganapaty *et al.*, 2006
		4-Hydroxy-3,5-dimethoxy-2-naphthaldehyde, **94k** 4-Hydroxy-5-methoxy-2-naphthaldehyde, **95k** Plumbagin, **112m** Diospyrin, **145m** 8'-Hydroxyisodiospyrin, **150m** Hydroxyisodiospyrin, **162m**	
D. batocana	Root barks	7-Methyljuglone, **113m** 2-Methylnaphthazarin, **115m** Diospyrin, **145m** Isodiospyrin, **152m** Rotundiquinone, **164m** Biramentaceone, **165m** Mamegakinone, **167m** Diosquinone, **170m** Batocanone, **171m**	Alves *et al.*, 1983
D. blancoi	Leaves	Isoarborinol methyl ether, **224q** α-Amyrin palmitate, **250q** α-Amyrin palmitoleate, **252q** β-Amyrin palmitate, **293q** β-Amyrin palmitoleate, **294q**	Ragasa *et al.*, 2009
D. buxifolia	Wood	Diosindigo A, **173m**	Musgrave *et al.*, 1970
D. canaliculata	Stem barks	Gerberinol, **33e**	Tangmouo *et al.*, 2005

Table 1 (continued)

Plant	part	Compound	Bibliography
D. canaliculata	Stem barks	Plumbagin, **112m** Canaliculatin, **179m** Diospyrone, **190m** Betulinic acid, **219q** Lupenone, **221q**	Tangmouo *et al.*, 2005
D. cauliflora	Roots	Tetralone, **132m**	Auamcharoen *et al.*, 2009
D. celebica	Heartwoods	Macassar II, **77k** Macassar III, **78k** Dihydrodiosindigo B, **98k** 8-Methoxy-6-methyl-1,2-naphthoquinone, **111m** Diomelquinone, **124m** Diosindigo B, **174m** Diosindigo B_1, **175m** Diosindigo B_2, **176m** Celebaquinone, **177m** Isocelebaquinone, **178m** Betulinic acid, **219q**	Maiti *et al.*, 1986, 1990
D. chamaethamnus	Root barks	2-Methyl naphthazarin, **115m** 7-Methyljuglone, **113m** Biramentaceone, **165m** Mamegakinone, **177m** Xylospyrin, **191m** Diospyrin, **145m** Diosquinone, **170m** Isodiospyrin, **152m**	Costa *et al.*, 1998

Table 1 (continued)

Plant	part	Compound	Bibliography
D. chloroxylon	Roots	2-Methyl-3,6-dihydroxy-4,5-dimethoxynaphthalene, **79k** 2-Methyl-3,4,5,6-tetrametho xynaphthalene, **80k**	Sidhu *et al.*, 1967
D. costata	Fruits	Lycopene, **22d** α-Carotene, **23d** β-Carotene, **24d** Cryptoxanthin, **25d** Zeaxanthin, **26d** Violaxanthin, **27d**	Karl, 1935
D. crassiflora	Stem barks	Gerberinol, **33e** Plumbagin, **112m** Crassiflorone, **184m** Cyclocanaliculatin, **185m** Lupeol, **215q** Betulinic acid, **219q** Luponone, **220q**	Tangmouo *et al.*, 2006
	Leaves	Diosfeboside A, **36e** Diosfeboside B, **37e** Kaempferol 3-O-α-L-rhamno pyranosyl-(1→2)-β-D-gluco pyranoside, **65h** Stigmasterol , **200o** Stigmasterol 3-O-β-D-glucopyranoside, **206o** Betulinic acid, **219q** Ursolic acid, **247q**	Akak *et al.*, 2010

Table 1 (continued)

Plant	part	Compound	Bibliography
D. decandra	Stem barks	Diospyric acid A, **258q** Diospyric acid B, **259q** Diospyric acid C, **260q** Diospyric acid D, **261q** Diospyric acid E, **262q**	Nareeboon *et al.*, 2006
	Barks	Vismiaefolic acid, **273q** Dimethyl-2α,3β-di-*O*-acetyl-19-*nor*-20-dimethyl-11-oxo urs-12-en-24,28-dioate, **276q** Dimethyl-2α,3β-di-*O*-acetyl-18,19-*seco*-19-oxours-11,13 (18)-dien-24,28-dioate, **277q** Dimethyl-2α,3β-di-*O*-acetyl-19α-hydroxy-11-oxours-12-en-24,28-dioate, **278q** 2α,3β,2′,3′,4′,6′-Hexa-*O*-acetyl-24-methyl-28-1′-β-D-glucopyranosyl-19α-hydroxy urs-12-en-24,28-dioate, **283q** 24-Methyl-2α,3β,2′,3′,4′,2″,3″,4″,6″-nona-*O*-acetyl-28-1′-β-D-[glucopyranosyl-(1″→6′)-glucopyranosyl]-19α-hydroxy urs-12-en-24,28-dioate, **284q**	Sutthivaiyakit *et al.*, 2012
D. dendo	Leaves	3β-*O*-*cis*-*p*-Coumaroyl-20β-hydroxy-12-ursen-28-oic acid, **311q**	Hu *et al.*, 2006

Table 1 (continued)

Plant	part	Compound	Bibliography
D. dendo	Leaves	3β-*O-trans-p*-Coumaroyl-2α - hydroxy-12-ursen-28-oic acid, **312q** 3β-*O-cis-p*-Coumaroyl-2α-hydroxy-12-ursen-28-oic acid, **313q** 3β-*O-trans*-Feruloyl-2α-hydroxy-12-ursen-28-oic acid, **314q** Ursolic acid, **247q**	Hu *et al.*, 2006
D. discolor	Stem barks	Aglycone, **196m** 1,3,5-Trihydroxy-6-methoxy-2-methylanthraquinone-8-*O*-β-D-glucopyranoside, **197m**	Srivastava *et al.*, 1985
	Roots	Diospyrin, **145m** 8′-Hydroxyisodiospyrin, **163m** Habibone, **168m**	Ganapaty *et al.*, 2005
	Twigs	Betulinaldehyde, **218q** Betulinic acid methyl ester, **219q** Ursaldehyde, **289q** 24-Ethyl-3β-methoxylanost-9(11)-en-25-ol, **317q** 3β-Methoxy-24-methylenelanost-9(11)-en-25-ol, **318q** 3β-Methoxy-25-methyl-24-methylenelanost-9(11)-en-21-ol, **319q**	Chen *et al.*, 2007

Table 1 (continued)

Plant	part	Compound	Bibliography
D. discolor	Twigs	3β-Methoxy-24-methylanost-9(11),25-dien-24-ol, **320q**	Chen *et al.*, 2007
	Fruits	D-Galactose, **207d** D-Galacturonic acid, **208d** D-Glucose, **209p** L-Arabinose, **210p** D-Xylose, **211p** L-Rhamnose, **212p** β-D-Glucopyranose pentaacetate, **213p** D-Fructose, **214p**	Haq *et al.*, 1984
D. ebenum	Stem barks	Elliptinone, **136m** Ebenone, **186m** Ellagic acid, **35e** 6-Hydroxy-4,5-dimethoxy-2-naphthoic acid, **83k** β-Sitosterol, **199o** Stigmasterol, **200o** Betulin, **216q** Betulinic acid, **219q** α-Amyrin, **246q** Ursolic acid, **247q** α-Amyrenone, **251q** Baurenol, **254q**	Sankaram *et al.*, 1984 Sharma *et al.*, 1985
	Heartwoods	6-Hydroxy-4,5-dimethoxy-2-naphthaldehyde, **81k** 4,5,6-Trimethoxy-2-naphthaldehyde, **82k**	Brown *et al.*, 1965

Table 1 (continued)

Plant	part	Compound	Bibliography
D. ebenum	Heartwoods	Betulinic acid, **223q** 6-Hydroxy-4,5-dimethoxy-2-naphthoic acid, **83k**	Brown *et al.*, 1965 Gupta *et al.*, 1969
D. ehretioides	Wood	Ehretione, **164m**	Lillie *et al.*, 1976
	Fruits	Palmarumycins JC, **101k** Palmarumycins JC2, **102k** Isodiospyrin, **151m** Isodiospyrol A, **172m**	Prajoubklang *et al.*, 2005
D. eriantha	bark	(+)-Syringaresinol, **75j** β-Sitosterol, **199o** Lupeol, **215q** Betulin, **216q** Betulinaldehyde, **218q** Betulinic acid, **219q** 3β-Acetoxyurs-11-en-28,13-olide, **274q** 3β-Acetoxyoleanolic acid, **292q** Friedelin, **315q**	Chen *et al.*, 1992
	Heartwoods	Syringic acid, **7b** 3,4,5-Trimethoxyphenol, **103l** 2,6-Dimethoxy-1,4-benzoquinone, **109m** 2-Methoxy-7-methyljuglone, **127m**	Chen *et al.*, 1994

Table 1 (continued)

Plant	**part**	**Compound**	**Bibliography**
D. eriantha	Heartwoods	3-Methoxy-7-methyljuglone, **128m** Stigmast-4-en-3-one, **201o** Stigmast-4-ene-3,6-dione, **203o** 3β-Hydroxystigmast-5-en-7-one, **204o** Lupeol, **215q** Betulin, **216q** Betulinaldehyde, **218q** Betulinic acid, **219q** Friedelin, **315q**	Chen *et al.*, 1994
D. ferrea	Fruits	Isodiospyrin, **152m** 8'-Hydroxyisodiospyrin, **163q** β-Sitosterol, **199o** Stigmasterol, **200o** Betulin, **216q** Lupenone, **221q** Lupeol, **215q** Lup-20(29)-en-3β,30-diol, **226q** Friedelin, **315q** β-Friedelinol, **316q**	Kau *et al.*, 1997
D. glandulosa	Stems	Lupeol, **215q** Betulin, **216q** Ursolic acid, **247q** β-Amyrin, **285q** Oleanolic acid, **290q**	Thanakijcharoenpath *et al.*, 2005

Table 1 (continued)

Plant	part	Compound	Bibliography
D. greeniwayi	Stem barks	Plumbagin, **112m** 7-Methyljuglone, **113m** 3,8'-Biplumbagin, **134m** Isodiospyrin, **152m** Ehretione, **164m** Habibone, **168m** Neodiospyrin, **169m**	Khan *et al.*, 1998
D. hallierii	Root bark, Stem barks, and Leaves	Isoxylopyrin, **192m** 7-Methyljuglone, **113m** Lupeol, **215q**	Khan *et al.*, 1999
D. ismailii	Root bark	Canaliculatin, **179m** Ismailin, **189m**	Jeffreys *et al.*, 1983
	Wood	4-Hydroxy-5-methyl coumarin, **28e**	Zakaria,1989
D. japonica	Roots	7-Methyljuglone, **113m** Shinanolone, **129m** Isodiospyrin, **152m** Bisisodiospyrin, **188m** Lupeol, **215q** Betulin, **216q** Betulinic acid, **219q** Taraxerol, **305q**	Kuroyanagi *et al.*, 1971
D. kaki	Roots	Gerberinol, **33e** 11-Methylgerberinol, **34e**	Paknikar *et al.*, 1996
	Leaves	Tatarine C, **1a** Kakispyrone, **20b** Kampferol, **47h**	Chen *et al.*, 2005, 2007

Table 1 (continued)

Plant	part	Compound	Bibliography
D. kaki	Leaves	Quercetin, **48h**	Chen *et al.*,
		Myricetin, **49h**	2005, 2007
		Annulatin, **50h**	
		Astragalin, **52h**	
		Trifolin, **53h**	
		Hyperin, **55h**	
		Isorhamnetin-3-*O*-β-D-glucopyranoside, **56h**	
		Isoquercetin, **57h**	
		Vitexin, **62h**	
		2″-*O*-Rhamnosylvitexin, **63h**	
		Kakispyrol, **106l**	
		4,4′-Dihydroxy-α-truxillic acid, **107l**	
		Pomolic acid, **256q**	
		24-Hydroxyursolic acid, **264q**	
		Rotungenic acid, **265q**	Thuong *et al.*,
		Coussaric acid, **267q**	2008
		Barbinervic acid, **268q**	
		3α, 19α-Dihydroxyurs-12,20 (30)-dien-24,28-dioic acid, **270q**	
		3α,19α-Dihydroxyurs-12-en-24,28-dioic acid, **271q**	
		Kakisaponin A, **279q**	
		Ursolic acid, **247q**	Thuong *et al.*,
		24-Hydroxy-3-*epi*-ursolic acid, **263q**	2008

Table 1 (continued)

Plant	part	Compound	Bibliography
D. kaki	Leaves	19,24-Dihydroxyurs-12-en-3-on-28-oic acid, **266q** Oleanolic acid, **290q** 24-Hydroxy-3-*epi*-oleanolic acid, **299q** Spathodic acid, **300q** Kaempferol-3-*O*-α-L-rhamno pyranoside, **54h** Myricetin-3-*O*-α-L-rhamno pyranoside, **58h** Myricetin-3-*O*-β-D-gluco pyranoside, **59h** 2″-*O*-rhamnosylvitexin, **63h** 8-C-[α-L-rhamnopyranosyl-(1→4)]-α-D-glucopyranosyl apigenin, **66h** Kakidiol, **269q** Kakisaponin B, **280q** Kakisaponin C, **281q** Rosamultin, **282q** Byzantionoside B, **321r** Blumeol C glucoside, **322r**	Thuong *et al.*, 2008 Chen *et al.*, 2009
	Fruits	α-Carotene, **23d** β-Carotene, **24d** Gallic acid, **6b** α-Cryptoxanthin, **25d** Zeaxanthin, **26d** Neolutein, **205o**	Daood *et al.*, 1992

Table 1 (continued)

Plant	part	Compound	Bibliography
D. kaki	Fruits	D-Glucose, **209p** D-Fructose, **214p**	Daood *et al.*, 1992
D. leucomelas	Leaves	Betulin, **216q** Betulinic acid, **219q** Ursolic acid, **247q**	Recio *et al.*, 1995
D. lotus	Roots	7-Methyljuglone, **113m** Isodiospyrin, **152m** Memegakinone, **167m** Bisisodiospyrin, **188m** Betulinic acid, **219q** Oxyallobetulin, **302q** Tetraxerol, **304q**	Yoshihira *et al.*, 1971
	Leaves	Gallic acid, **6b** Methyl gallate, **9b** Ellagic acid, **35e** Kaempferol, **47h** Quercetin, **48h** Myricetin, **49h** Myricetin 3-*O*-α-rhamnoside, **60h** Myricetin 3-*O*-β-glucuronide, **61h**	Said *et al.*, 2009
	Fruits	Gallic acid, **6b** Methyl gallate, **9b** Ellagic acid, **35e** Kaempferol, **47h** Quercetin, **48h** Myricetin, **49h**	Loizzo *et al.*, 2009

Table 1 (continued)

Plant	part	Compound	Bibliography
D. lotus	Fruits	Myricetin 3-*O*-β-glucuronide, **59h** Myricetin 3-*O*-α-rhamnoside, **60h**	Loizzo *et al.*, 2009
D. lycioides	Twigs	1',2-Binaphthalen-4-one-2',3-dimethyl-1,8'-epoxy-1,4',5,5',8,8'-hexahydroxy-8-O-β-glucopyranosyl-5'-O-β-xylopyranosyl -(1→6)-β-glucopyranoside, **194m**	Li *et al.*, 1998
D. mafeinsis	Stem barks	Isodiospyrin, **152m** Diosquinone, **170m** 6",8'-Bisdiosquinone, **193m**	Khan *et al.*, 1999
D. malanonilau	Heartwood	*n*-Hexacosanol, **71i** *n*-Nonacosane, **72i** β-Sitosterol, **199o** Betulin , **216q** Betulic acid, **219q** Ursolic acid, **247q** Oleanolic acid, **290q** Morolic acid, **298q**	Singh *et al.*, 1988
D. maritima	Stems	3-Ethoxy-1-(4-hydoxy-3-methoxyphenyl)-1-propanone , **10b** Maritolide, **21c** Plumbagin, **112m** Isodiospyrin , **152m**	Kuo *et al.*, 1997, 1998

Table 1 (continued)

Plant	part	Compound	Bibliography
D. maritima	Stems	8′-Hydroxyisodiospyrin, **172m** β-Sitosterol, **199o** Stigmasterol, **200o** Stigmast-4-en-3-one, **201o** Ergosta-4,6,8(14),22-tetraen-3-one, **202o** Lupeol, **215q** Betulin, **216q** Lupenone, **221q** 3-(*E*)-coumaroyllupeol, **228q** 3-(*E*)-Feruloylbetulin, **232q** 28-Acetyl-3-(*E*)-coumaroyl betulin, **236q** Diospyrolide, **244q** Erythrodiol, **285q** Taraxerol, **305q** Taraxerone, **308q** 4-Hydroxybenzaldehyde, **2b** 4-Hydroxy-3,5-dimethoxy-Benzaldehyde, **3b** Acetovanillone, **4b** Vanillin, **5b** 1-(4,6-Dihydroxy-2-methyl phenyl) ethanone, **11b** Ethyl 2,4-dihydroxy-6-methyl-benzoate, **12b** *trans*-Coniferylaldehyde, **13b**	Kuo *et al.*, 1997, 1998 Chang *et al.*, 1998, 1999, 2007, 2009

Table 1 (continued)

Plant	part	Compound	Bibliography
D. maritima	Stems	(*E*)-3-(4-Acetyloxy-3,5-di methoxyphenyl)-2-propenal, **14b** 5-Hydroxy-2-methyl chromanone, **31e** 5,7-Dihydroxy-2-methyl chomanone, **32e** Butylmethyl succinate, **73i** 4-Ketopinoresinol, **76j** *bis* (6-Hydroxy-2,3,4-tri methoxylphen-1-yl)methane, **104l** Diomelquinone, **124m** *epi*-Isoshinanolone, **131m** 2-Ethoxy-8′-hydroxyisodios pyrin, **160m** 3-Ethoxy-8′-hydroxyisodios pyrin, **161m** Bisisodiospyrin, **188m** Lupeol, **215q** Betulin, **216q** Betulin-28-acetate, **217q** Betulinaldehyde, **218q** Betulinic acid, **219q** 3-Oxo-20(29)-lupen-28-oic acid, **222q** Betulic acid acetate, **227q** 3-(*E*)-Coumaroyllupeol, **228q**	Chang *et al.*, 1998, 1999, 2007, 2009

Table 1 (continued)

Plant	part	Compound	Bibliography
D. maritima	Stems	3-(*Z*)-Coumaroyllupeol, **229q** 3-(*E*)-Coumaroyl betulinal dehyde, **230q** Lupeol caffeate, **233q** 3-*O*-Betulinic acid *p*-coumarate, **234q** (*E*)-Betulin-3β-*p*-coumarate, **235q** 3-(*E*)-Coumaroyl-28-palmi toylbetulin, **240q** (*Z*)-Betulin-3β-*p*-coumarate, **241q** 3-(*Z*)-Coumaroyl-28-palmi toylbetulin, **242q** 3-(*E*)-Feruloyl-28-palmitoyl betulin, **243q** 3β-Acetoxy- urs-12-en-28-oic Acid, **249q** 3β-Hydroxyurs-12-en-28,13-olide, **273q** 3-*O*-Palmitoylerythrodiol, **287q** 28-*O*-Acetylerythrodiol, **288q** 3β-Acetoxyoleanolic acid, **292q** 3β-Hydroxytaraxastan-28,20β - olide, **309q** α-Tocopherol, **323s**	Chang *et al.*, 1998, 1999, 2007, 2009
D. maritima	Barks	7,8-Dimethoxy-6-hydroxy coumarin, **30e**	Gu *et al.*, 2004

Table 1 (continued)

Plant	part	Compound	Bibliography
D. maritima	Barks	Plumbagin, **112m** 7-Methyljuglone, **113m** 2-Methoxy-7-methyljuglone, **127m** 3-Methoxy-7-methyljuglone, **128m** Shinanolone, **129m** Maritinone, **135m** Chitranone, **139m** Zeylanone, **138m**	Gu *et al.*, 2004
	Heartwood	3-(*E*)-Coumaroylbetulin-28-yl ethylsuccinate, **237q** 3-(*E*)-Coumaroylbetulin-28-yl ethyl (2R)-2-hydroxy succinate, **238q** 3-(*E*)-Coumaroylbetulin-28-yl ethyl nonanedioate, **239q** Diospyrolide, **244q** Diospyrolidone, **245q**	Kou *et al.*, 2000
	Fresh fruits	Vanillin, **5b** Scopoletin, **29e** Abbeokutone, **38f** 3α,16α,17-Trihydroxy kaurane, **39f** Plumbagin, **112m** 2,3-Epoxyplumbagin, **116m** 3-Bromoplumbagin, **117m** 3-Chloroplumbagin, **118m**	Higa *et al.*, 1987, 1988, 1998, 2002

Table 1 (continued)

Plant	**part**	**Compound**	**Bibliography**
D. maritima	Fresh fruits	Droserone, **119m** 3-Methylplumbagin, **120m** 3-(2-hydroxyethyl) plumbagin, **121m** 6-(1-Ethoxyethyl) plumbagin, **122m** 3,3'-Biplumbagin, **133m** 3,8'-Biplumbagin, **134m** Maritinone, **135m** Elliptinone, **136m** Isozeylanone, **138m** Chitranone, **139m** Methylene-3,3'-biplumbagin, **141m** Ethylidene-3,3'-biplumbagin, **142m** Ethylidene-3,6'-biplumbagin, **143m** Ethyllidene-6,6′-biplumbagin, **144m** Lupeol, **215q** Betulin, **216q** Betulinic acid, **219q** Lupenone, **221q** β-Amyrin, **285q** Oleanolic acid, **290q** Glutinol, **304q** Friedelin, **315q**	Higa *et al.*, 1987, 1988, 1998, 2002

Table 1 (continued)

Plant	part	Compound	Bibliography
D. melanoxylon	Heartwoods	3-Methyl-1,8-dimethoxy naphthalene, **84k** 3-Methyl-2,8-dimethoxy-1-naphthol, **85k** 3-Methyl-8-methoxy-1-naphthol, **86k** 3-Methyl-4,8-dimethoxy-1-naphthol, **87k** 3-Methyl-8-methoxy-1,2-naphthoquinone, **110m** 2-methyl-5-methoxy-1,4-naphthoquinone, **123m** Methyl-3-hydroxy-5-methoxy naphthoquinone, **125m** 2-Methyl-5,6-dimethoxy-1,4-naphthoquinone, **126m** 2-3,3′-Dimer-6-hydroxy-5-methoxy-2-methylnaphthoquinone, **140m** Biramentaceone, **165m** Diosindigo A, **173m** Diosindigo B, **174m** Violet quinone, **182m**	Sidhu *et al.*, 1968, 1973 Sankaram *et al.*, 1981
	Leaves	Ursolic acid, **247q** Corsolic acid, **255q** Pomolic acid methyl ester, **257q**	Mallavadhani *et al.*, 1998, 2001, 2005

Table 1 (continued)

Plant	part	Compound	Bibliography
D. melanoxylon	Leaves	Jacoumaric acid methyl ester, **275q** Maslinic acid methyl ester, **297q** Gallic acid, **6b** Piotoaitechuic acid methyl ester, **8b** 5,7-Dihydroxy-3-*O*-β-D-glucopy ranosyl-1‴→6" glucopyranno side-2-{4-hydroxyphenyl}-4*H*-benzopyran-4-one, **67h** 4,6-Dihydroxy-2-(4-hydroxy phenyl) hydroxymethylene-3(2*H*)-benzofuranone, **68h** Selin-4(15)-en-lβ,11-diol, **198n** β-Sitosterol, **199o** α-Amyrin, **246q** Ursolic acid, **247q** Uvaol, **248q** Corsolic acid, **255q** Pomolic acid methyl ester, **257q** Jacoumaric acid methyl ester, **275q** β-Amyrin, **285q**	Mallavadhani *et al.*, 1998, 2001, 2005

Table 1 (continued)

Plant	part	Compound	Bibliography
D. melanoxylon	Leaves	Oleanolic acid, **290q** Maslinic acid methyl ester, **297q**	Mallavadhhani *et al.*, 1998, 2001, 2005
D. mespiliformis	Seed	Myristic acid, **40g** Palmitic acid, **41g** Stearic acid, **42g** Arachidic acid, **43g** Myristoleic acid, **44g** Palmitoleic acid, **45g** Linoleic acid, **46g**	Chivandi *et al.*, 2009
D. mollis	leaves	3-Methyl-naphthalene-1,8-diol, **89k** Diospyrol , **97k** Diospyrol-8,8′-di-*O*-(6-β-D-apio furanosyl-β-D-glucopyranoside), **100k**	Mongkolsuk *et al.*, 1965 Paphassarang *et al.*, 1984
D. montana	Bark	Tetrahydrodiospyrin, **151m**	Pardhasaradhi *et al.*, 1979
	Seeds	8-Hydroxyoctadec-10(Z)-enoic acid, **70i**	Rauf et al., 1987
D. montana	Heartwood Stem bark, Bark	7-Methyljuglone, **113m** Diospyrin, **145m** 2′-Chlorodiospyrin, **147m** 3′-Chlorodiospyrin, **148m** 3′-Chloro-2′-hydroxy diospyrin, **149m** Isodiospyrin, **152m** 8′-Hydroxydiospyrin, **163m**	Lillie *et al.*, 1976

Table 1 (continued)

Plant	part	Compound	Bibliography
D. montana	Heartwood Stem bark, Bark	Biramentacene, **165m** Mamegakinone, **167m** Diosquinone, **170m** Cyclodiospyrin, **178m** Chromenone acid, **180m** Chromenone ester, **181m** β-Sitosterol, **199o** Lupeol , **215q** Betulin, **216q** Betulinic acid, **219q** Allobetulin, **301q** Oxyallobetulin , **302q**	Lillie *et al.*, 1976
D. morrisiana	Stems	Isodiospyrin, **152m** β-Amyrin, **285q** Olean-12-en-3-one, **295q** β-Amyrin acetate, **291q**	Yan *et al.*, 1989
	Heartwoods	3-Methoxyjuglone, **114m** Shinanolone, **129m** Isodiospyrin, **152m** 3'-Methoxy isodiospyrin, **154m** 3,3'-Dimethoxy isodiospyrin, **157m** β-Sitosterol, **199o** Sitosterol 3-*O*-β-D-glucopyrano side, **206o** Lupeol , **215q** Betulinic acid, **219q**	Chen *et al.*, 1987

Table 1 (continued)

Plant	part	Compound	Bibliography
D. morrisiana	Heartwoods	2'-Methoxy isodiospyrin, **153m** 2,2'-Dimethoxy isodiospyrin, **154m** 2,3'-Dimethoxy isodiospyrin, **156m** 8′-Hydroxy-3-methoxy isodios pyrin, **158m** 2'-Chloro-3,3'-dimethoxy isodios pyrin, **159m** β-D-Dlucopyranose pentaacetate, **213p**	Chen *et al.*, 1987
D. nigra	Aerial parts	Diospyrodin, **74i**	Dinda *et al.*, 2006
D. peregrina	Roots	5,7,3,5′-Tetrahydroxy-3′-methoxy flavanone, 4′-*O*-α-L-rhamnopyranoside, **64h**	Chauhan *et al.*, 1979
	Stems	Nonadecan-7-ol-2-one, **69i**	Chauhan *et al.*, 1980
	Fruits	Peregrinol, **223q** Ferano-(2″,3″,7,8)-3′,5′-dimetho xy-5-hydroxyflavone, **51h**	Jain *et al.*, 1994 , 1997
		Marsformosanone, **253q**	Bhaumik *et al.*, 1981
D. quaesita	Leves	Betulinic acid 3-caffeate, **231q** Diospyrosin , **108l**	Ma *et al.*, 2008
D. quiloensis	Heartwoods	5-Hydroxy-4,6,8-trimethoxy naphthaldehyde, **71k**	Harper *et al.*, 1970

Table 1 (continued)

Plant	part	Compound	Bibliography
D. quiloensis	Heartwoods	4,5,6,8-Tetramethoxy naphthalene, **79k** 4,5-Dimethoxy naphthaldehyde, **91k** 4,5,6-Trimethoxy naphthaldehyde, **92k** 5-Hydroxy-4-methoxy-2-naphthaldehyde, **93k**	Harper *et al.*, 1970
D. rhodocalyx	Barks	Tβ-Sitosterol, **199o** Lupeol, **215q** Betulin, **216q** Stigma-4-en-3-one, **201o** Betulinic acid, **219q** Lupenone, **221q** Taraxerol, **305q** Taraxeryl acetate, **307q** Araxerone, **308q**	Sutthivaiyakit *et al.*, 1995
D. samoensis	leaves	Plumbagin, **112m** *iso*-Shinanolone, **130m** Maritinone, **135m** Elliptinone, **136m**	Richomme *et al.*, 1991
D. sanza-minika	Stem barks	Norbergenin, **15b** 11-*O*-*p*-Hydroxybenzoyl norbergenin, **16b** 4-*O*-Galloylnorbergenin, **17b** 4-*O*-(3′-Methylgalloyl) norbergenin, **18b**	Tangmouo *et al.*, 2009

Table 1 (continued)

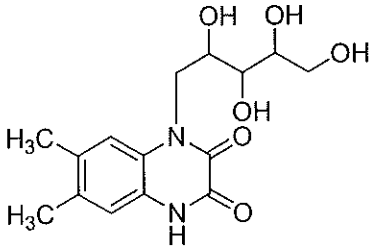
Plant	part	Compound	Bibliography
D. sanza-minika	Stem barks	4-*O*-Syringoylnorbergenin, **19b**	Tangmouo *et al.*, 2009
D. sylvatica	Roots	Plumbagin, **112m** Diospyrin, **145m** Isodiospyrin, **152m** Diosindigo A, **173m** Microphyllone, **187m** 2-Methyl-anthraquinone, **195m**	Ganapaty *et al.*, 2004
D. tricolor	Roots	Diosquinone, **170m**	Alake, 1994
D. usambarensis	Stem barks	Diosindigo A, **173m** Diosindigo B, **174m** 7-Methyljuglone, **113m** Mamegakinone, **167m** Bisisodiospyrin, **188m**	Khan, *et al.*, 1989
D. verrucosa	Root barks	7-Methyljuglone, **113m** Isodiospyrin, **152m** Diosquinone, **170m** Diosindigo A, **173m** Betulin , **216q** Betulinic acid, **219q**	Khan, *et al.*, 1987
D. verginiana	Roots	4-Hydroxy-5,6-dimethoxy naphthalene-2-carbaldehyde, **96k** 7-Methyljuglone, **113m** Shinanolone, **129m** Diospyrin, **145m**	Wang *et al.*, 2011

Table 1 (continued)

Plant	**part**	**Compound**	**Bibliography**
D. verginiana	Roots	Isodiospyrin, **152m** Lupeol, **215q** Betulin, **216q** Betulinaldehyde, **218q** Betulinic acid, **219q** 12,13-Dehydro-20,29-dihydro betulin, **225q** Ursolic acid, **247q**	Wang *et al.*, 2011
D. villosiuscula	Roots and Stem barks	Diosindigo A, **173m** Diosindigo B, **174m** Taraxerol methyl ether, **306q**	Khan, *et al.*, 1999

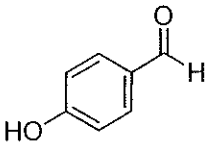
Structures

a: alkaloid

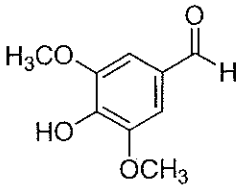


1a: Tatarine C

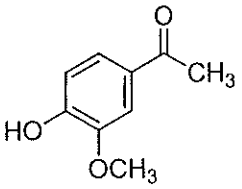
b: benzenoids



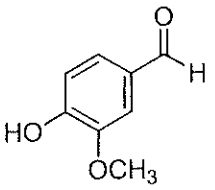
2b: 4-Hydroxybenzaldehyde



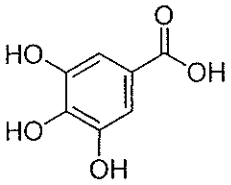
3b: 4-Hydroxy-3,5-dimethoxy-benzaldehyde



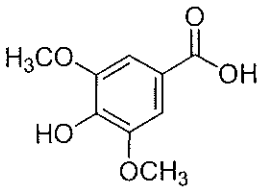
4b: Acetovanillone



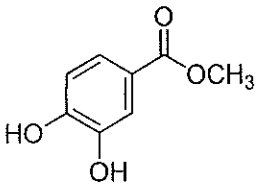
5b: Vanillin



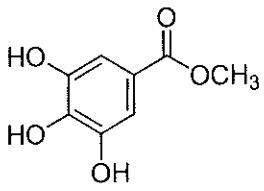
6b: Gallic acid



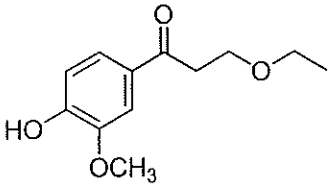
7b: Syringic acid



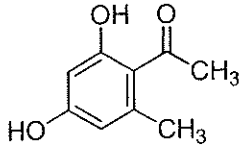
8b: Piotoaitechuic acid methyl ester



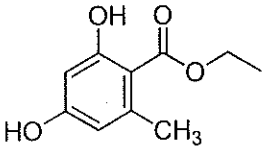
9b: Methyl gallate



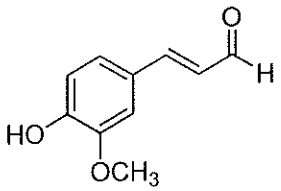
10b: 3-Ethoxy -1- (4-hydoxy-3-methoxyphenyl)-1-propanone



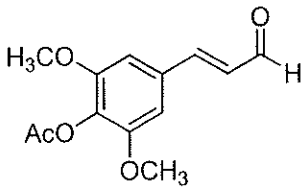
11b: 1-(4,6-Dihydroxy-2-methylphenyl) ethanone



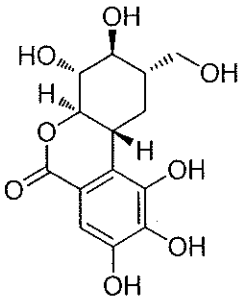
12b: Ethyl 2,4-dihydroxy-6- methyl benzoate



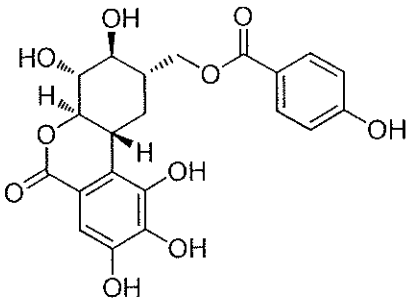
13b: *trans*-Coniferylaldehyde



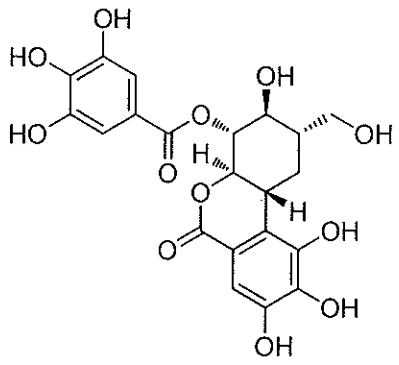
14b: (*E*)-3-(4-Acetyloxy-3,5-dimethoxy phenyl)-2-propenal



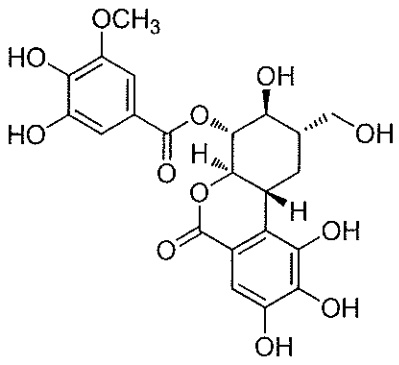
15b: Norbergenin



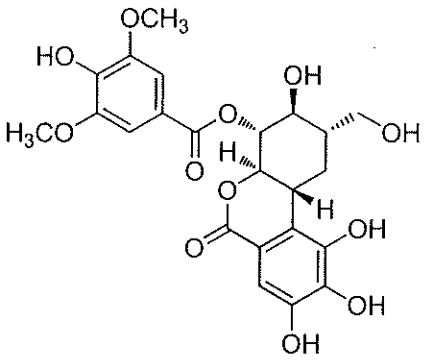
16b: 11-*O*-*p*-Hydroxybenzoyl norbergenin



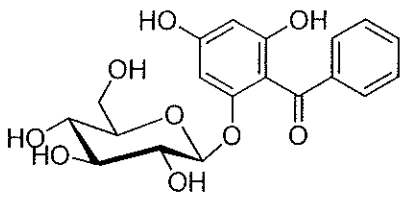
17b: 4-*O*-Galloylnorbergenin



18b: 4-*O*-(3′-Methylgalloyl) norbergenin

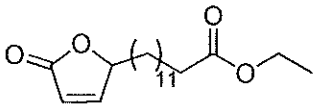


19b: 4-*O*-Syringoylnorbergenin



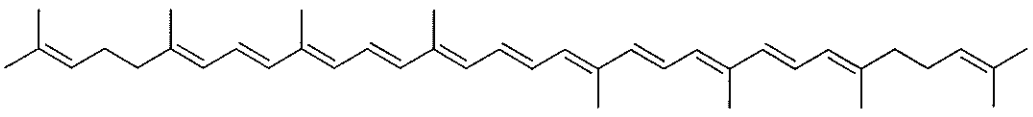
20b: Kakispyrone

c: betenolide



21c: Maritolide

d: carotinoids



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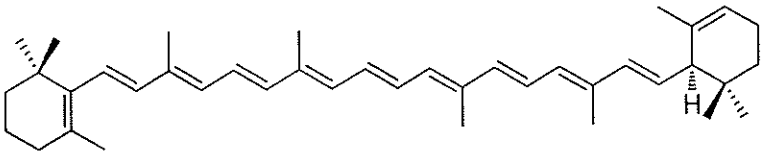
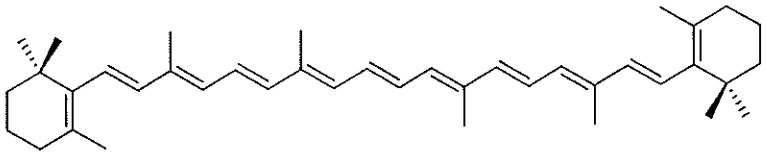
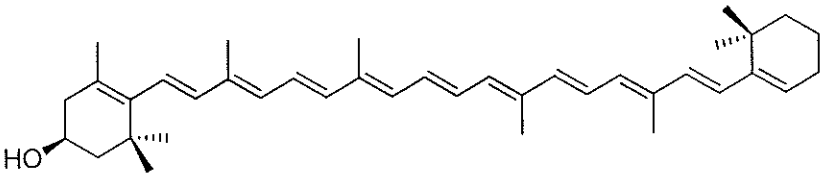


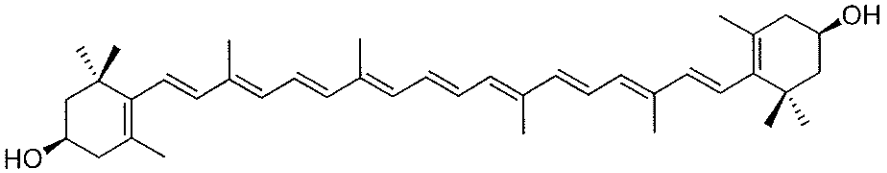
23d: α-Carotene



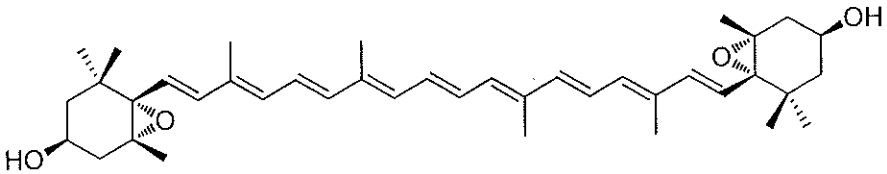
24d: β-Carotene



25d: Cryptoxanthin

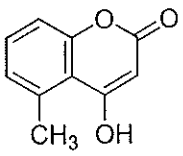


26d: Zeaxanthin

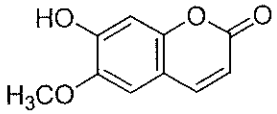


27d: Violaxanthin

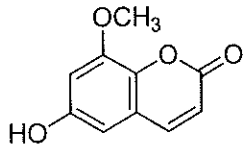
e: coumarins



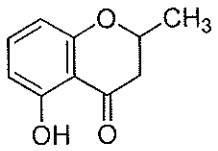
28e: 4-Hydroxy-5-methylcoumarin



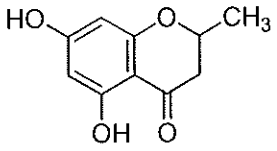
29e: Scopoletin



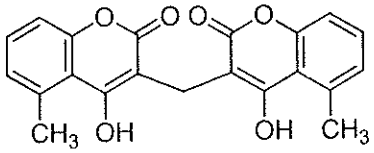
30e: 7,8-Dimethoxy-6-hydroxycoumarin



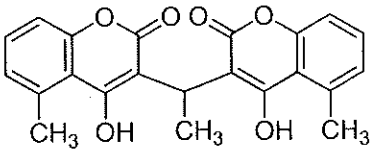
31e: 5-Hydroxy-2-methylchromanone



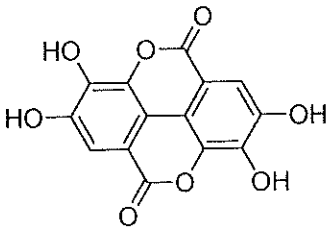
32e: 5,7- Dihydroxy-2-methyl chromanone



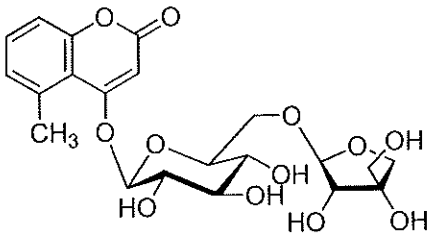
33e: Gerberinol



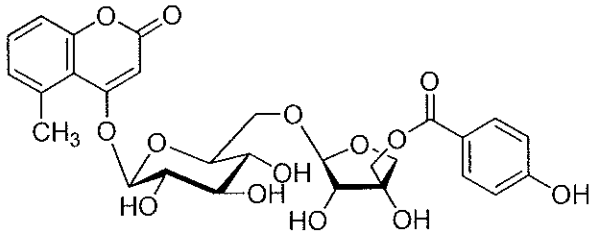
34e: 11-Methylgerberinol



35e: Ellagic acid

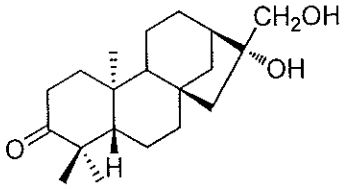


36e: Diosfeboside A

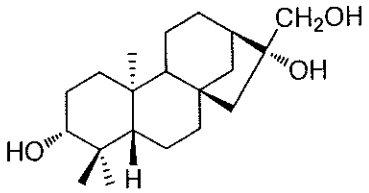


37e: Diosfeboside B

f: diterpenoids

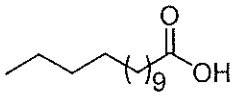


38f: Abbeokutone

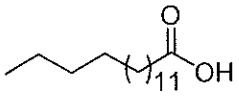


39f: 3α,16α,17-Trihydroxykaurane

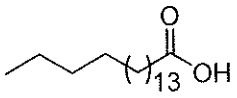
g: fatty acids



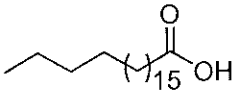
40g: Myristic acid



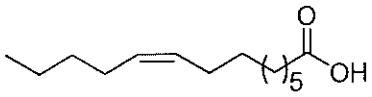
41g: Palmitic acid



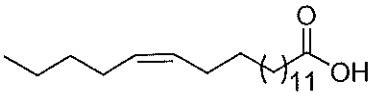
42g: Stearic acid



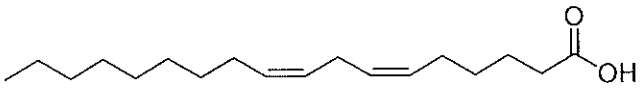
43g: Arachidic acid



44g: Myristoleic acid

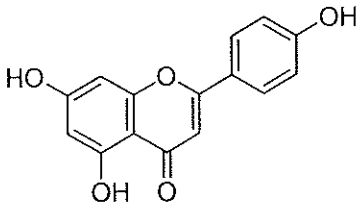


45g: Palmitoleic acid

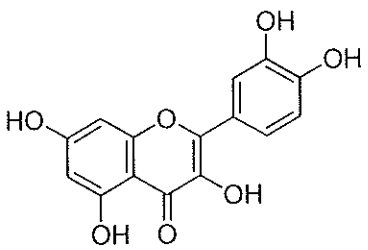


46g: Linoleic acid

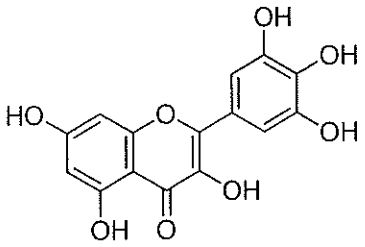
h: flavonoids



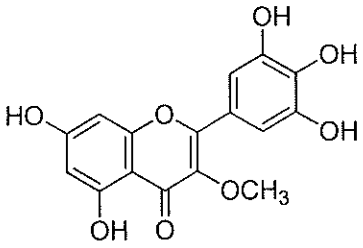
47h: Kaempferol



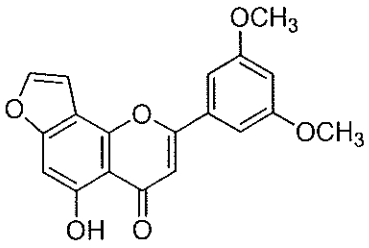
48h: Quercetin



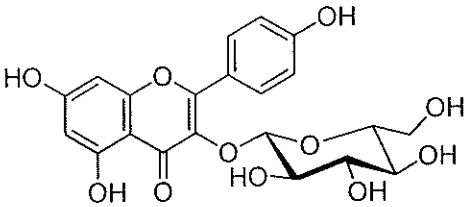
49h: Myricetin



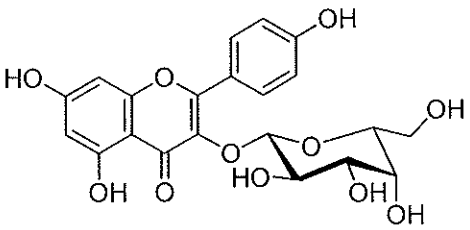
50h: Annulatin



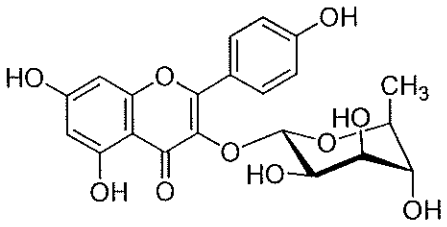
51h: Ferano-(2″,3″,7,8)-3′,5′-dimethoxy-5-hydroxyflavone



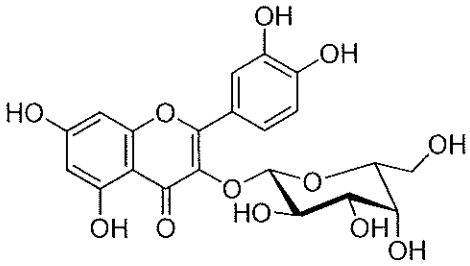
52h: Astragalin



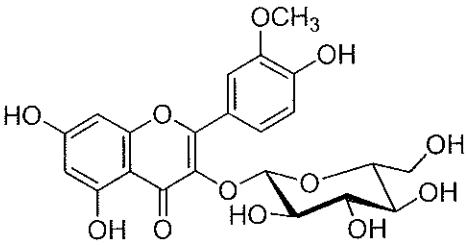
53h: Trifolin



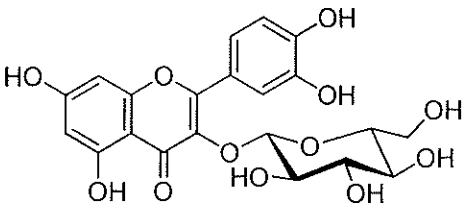
54h: Kampferol-3-*O*-α-L-rhamnopyranoside



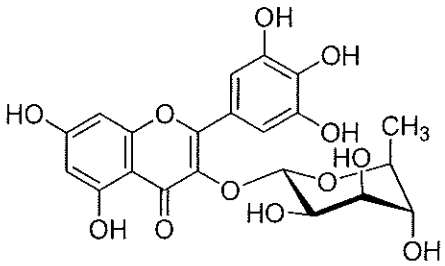
55h: Hyperin



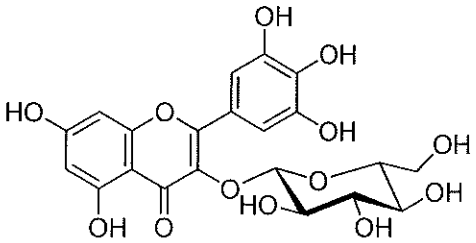
56h: Isorhamnetin-3-*O*-β-D-glucopyranoside



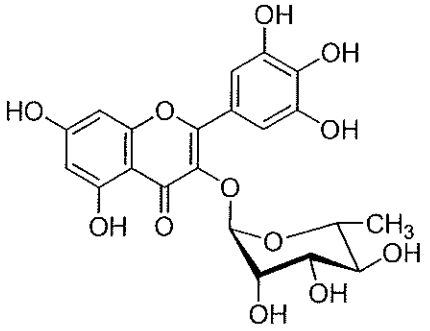
57h: Isoquercetin



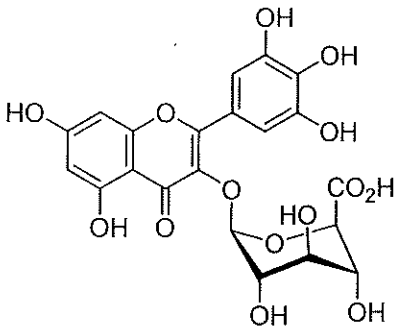
58h: Myricetin-3-*O*-α-L-rhamnopyra noside



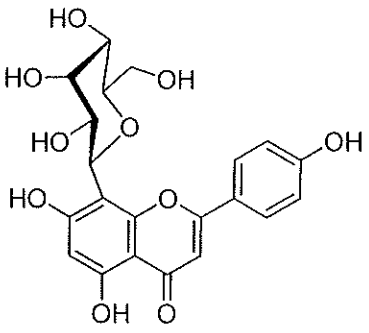
59h: Myricetin-3-*O*-β-D-glucopyranoside



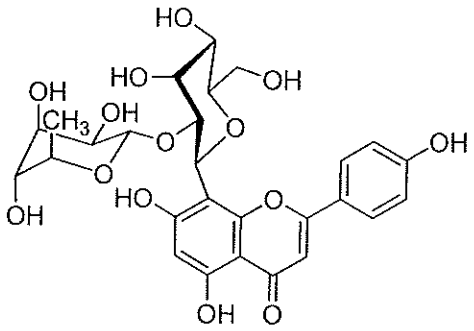
60h: 56h: Myricetin 3-*O*-α-rhamnoside



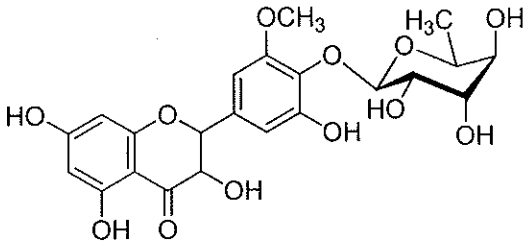
61h: Myricetin 3-*O*-β-glucuronide



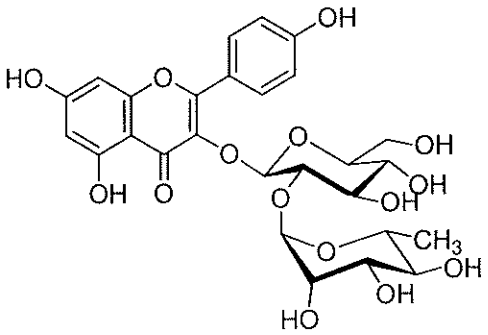
62h: Vitexin



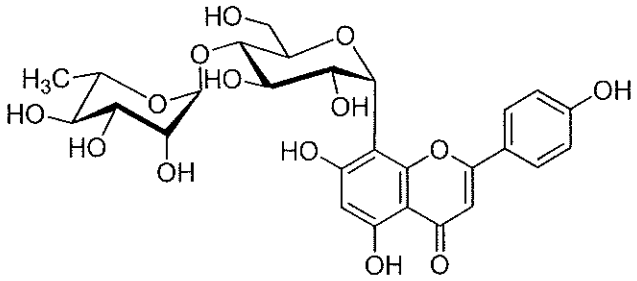
63h: 2″-*O*-Rhamnosyl vitexin



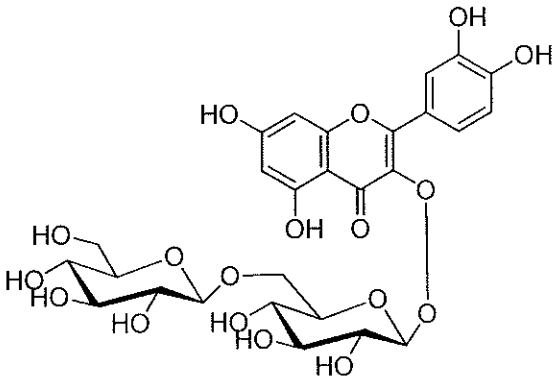
64h: 5,7,3,5′-Tetrahydroxy-3′-methoxy flavanone, 4′-*O*-α-L-rhamnopyranoside



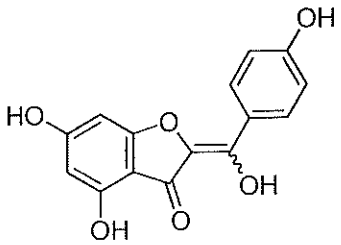
65h: kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside



66h: 8-C-[α-L-Rhamnopyranosyl-(1→4)]-α-D-glucopyranosyl apigenin

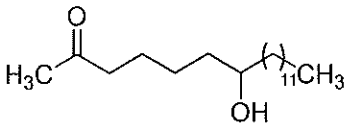


67h: 5,7-Dihydroxy-3-*O*-β-D-glucopyranosyl -1‴→6"glucopyrannside-2-{4-hydroxy phenyl}-4H-benzopyran-4-one

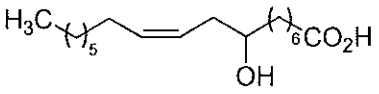


68h: 4,6-Dihydroxy-2-(4-hydroxy phenyl)hydroxymethylene-3(2*H*)-benzofuranone

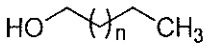
i: hydrocabons



69i: Nonadecan-7-ol-2-one

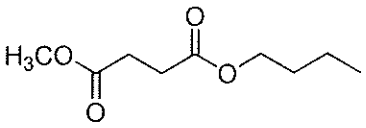


70i: 8-Hydroxyoctadec-10(Z)-enoic acid

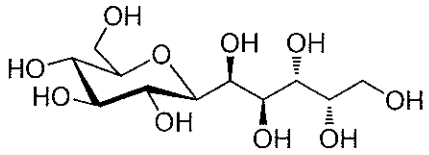


71i: n=22; *n*-Hexacosanol

72i: n= 24; *n*-Nonacosane

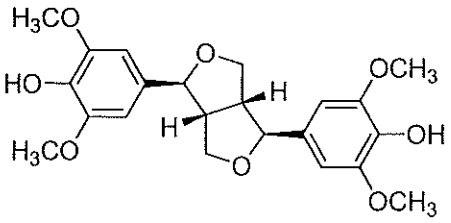


73i: Butylmethyl succinate

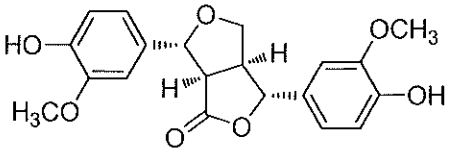


74i: Diospyrodin

j: lignans

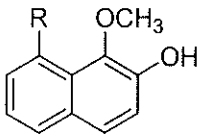


75j: (+)-Syringaresinol



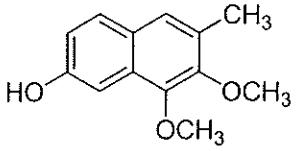
76j: 4-Ketopinoresinol

k: naphthalenes

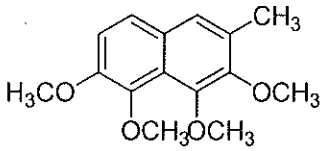


77k: R= OH; Macassar II

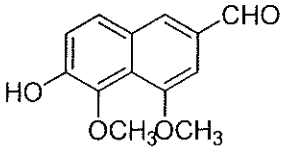
78k: R= OCH_3; Macassar III



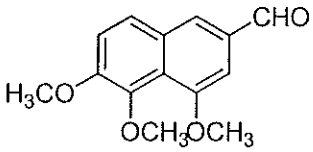
79k: 2-Methyl-3,6-dihydroxy-4,5-dimethoxynaphthalene



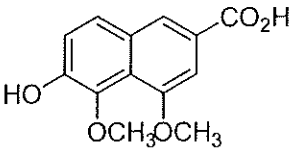
80k: 2-Methyl-3,4,5,6-tetramethoxy naphthalene



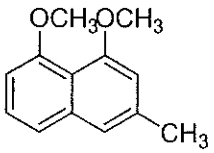
81k: 6-Hydroxy-4,5-dimethoxy-2-naphthaldehyde



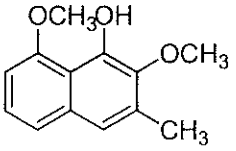
82k: 4,5,6-Trimethoxy-2-naphthadehyde



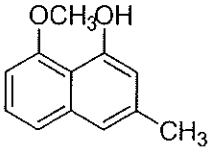
83k: 6-Hydroxy-4,5-dimethoxy-2-naphthoic acid



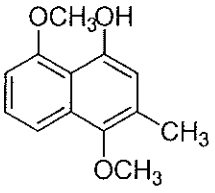
84k: 3-Methyl-1,8-dimethoxynaphthalene



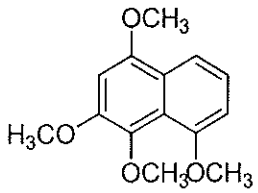
85k: 3-Methyl-2,8-dimethoxy-1-naphthol



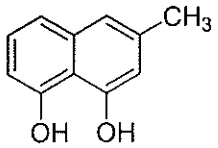
86k: 3-Methyl-8-methoxy-1-naphthol



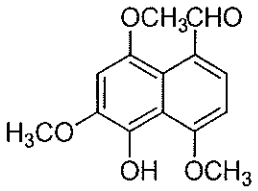
87k: 3-Methyl-4,8-dimethoxy-1-naphthol



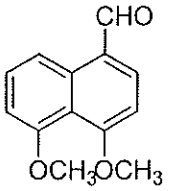
88k: 4,5,6,8-Tetramethoxy naphthalene



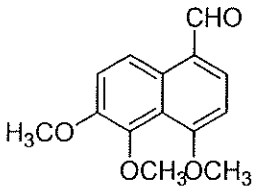
89k: 3-Methyl-naphthalene-1,8-diol



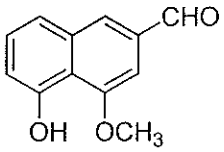
90k: 5-Hydroxy-4,6,8-trimethoxynaph thaldehyde



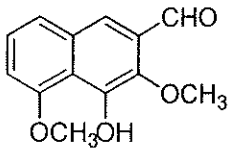
91k: 4,5-Dimethoxy naphthaldehyde



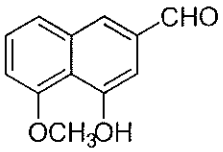
92k: 4,5,6-Trimethoxynaphthalehyde



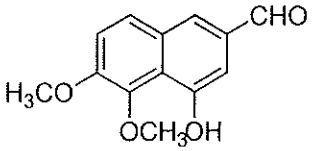
93k: 5-Hydroxy-4-methoxy-2-naphthaldehyde



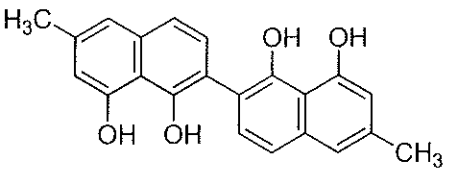
94k: 4-Hydroxy-3,5-dimethoxy-2-naphthaldehyde



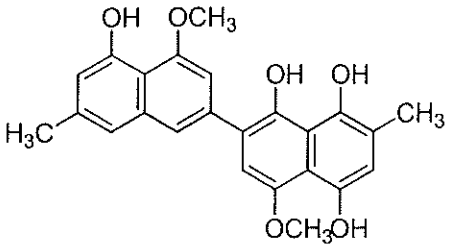
95k: 4-Hydroxy-5-methoxy-2-naphthaldehye



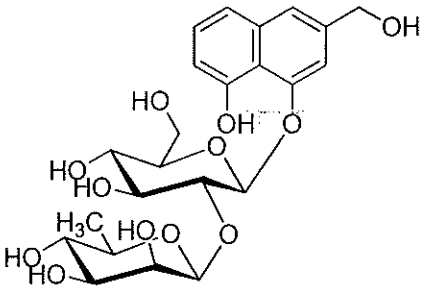
96k: 4-Hydroxy-5,6-dimethoxy naphthalene-2-carbaldehyde



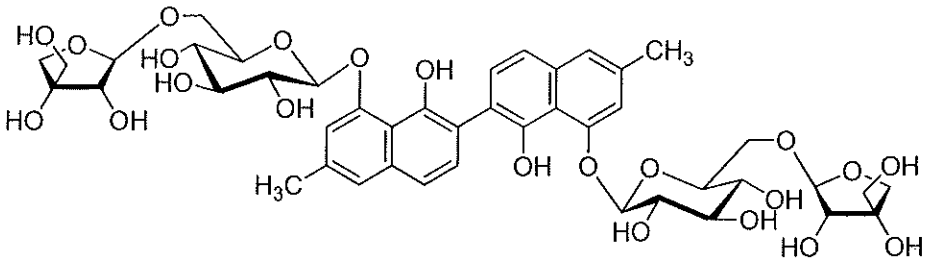
97k: Diospyrol



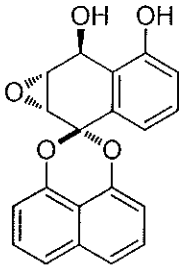
98k: Dihydrodiosindigo B



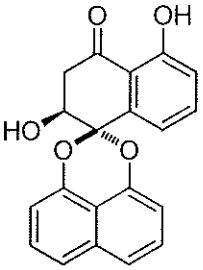
99k: Diospyrosonaphthoside



100k: Diospyrol-8,8′-di-*O*-(6-β-D-apiofuranosyl-β-D-glucopyranoside)

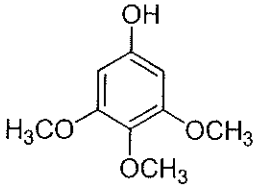


101k: Palmarumycins JC1

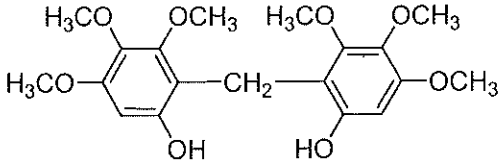


102k: Palmarumycins JC2

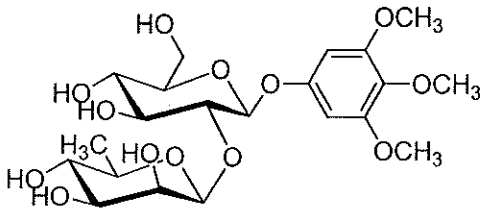
l: phenolics



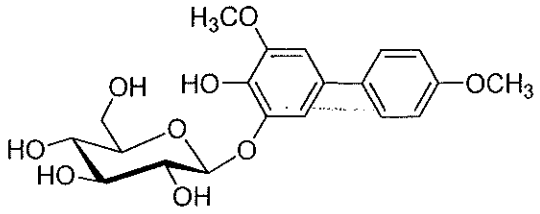
103l: 3,4,5-Trimethoxyphenol



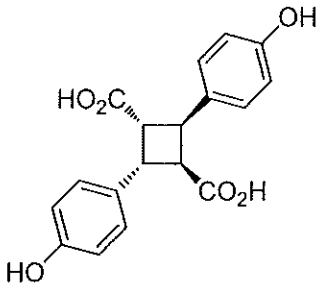
104l: *bis* (6-Hydroxy-2,3,4-trimethoxylphen-1-yl)methane



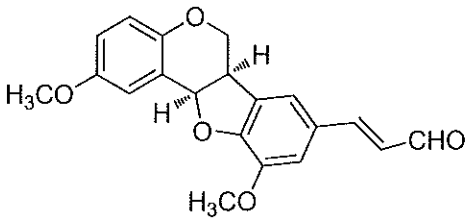
105l: Diospyrososide



106l: Kakispyrol

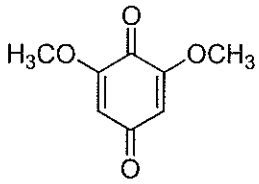


107l: 4,4′-Dihydroxy-α-truxillic acid

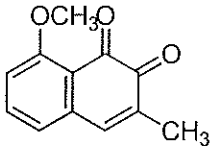


108l: Diospyrosin

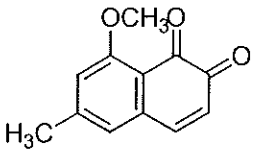
m: quinones



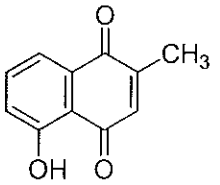
109m: 2,6-Dimethoxy-1,4-benzoquinone



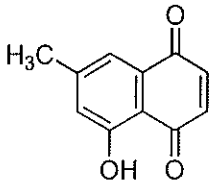
110m: 3-Methyl-8-methoxy-1,2-naphthoquinone



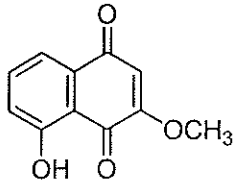
111m: 8-Methoxy-6-methyl-1,2-naphthoquinone



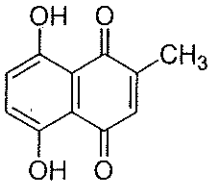
112m: Plumbagin



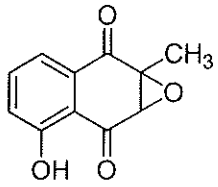
113m: 7-Methyljuglone



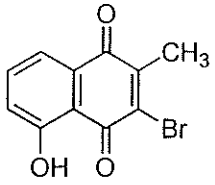
114m: 3-Methoxyjuglone



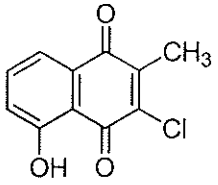
115m: 2-Methyl naphthazarine



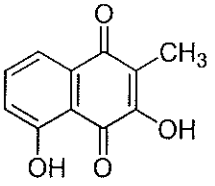
116m: 2,3-Epoxyplumbagin



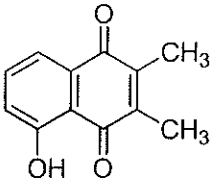
117m: 3-Bromoplumbagin



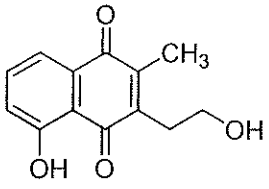
118m: 3-Chloroplumbagin



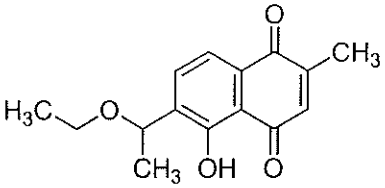
119m: Droserone



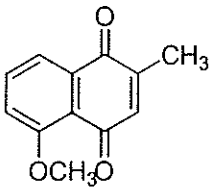
120m: 3-Methylplumbagin



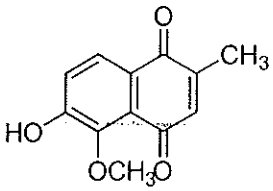
121m: 3-(2-Hydroxyethyl) plumbagin



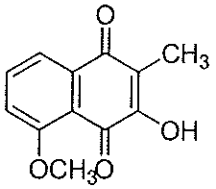
122m: 6-(1-Ethoxyethyl) plumbagin



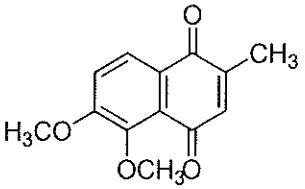
123m: 2-Methyl-5-methoxy-1,4-naphthoquinone



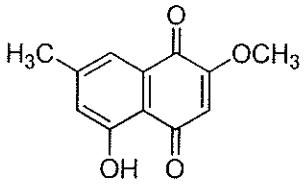
124m: Diomelquinone



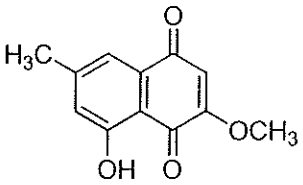
125m: 2-Methyl-3-hydroxy-5-methoxy-1,4-naphthoquinone



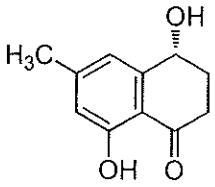
126m: 2-Methyl-5,6-dimethoxy-1,4-naphthoquinone



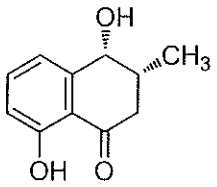
127m: 2-Methoxy-7- methyljuglone



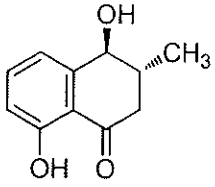
128m: 3-Methoxy-7- methyljuglone



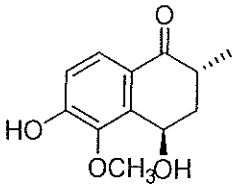
129m: Shinanolone



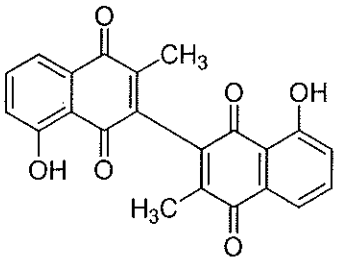
130m: *iso*-Shinanolone



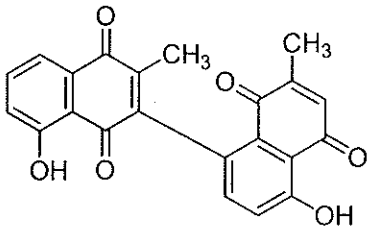
131m: *epi*-Isoshinanolone



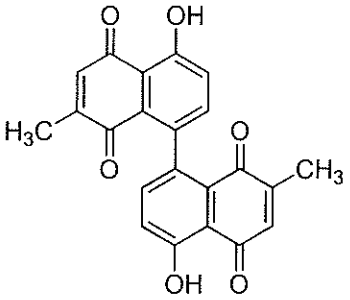
132m: Tetralone



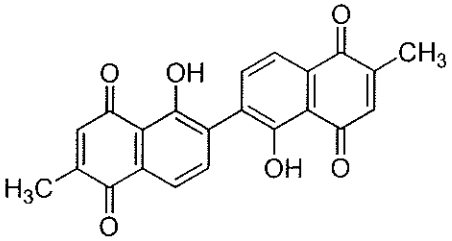
133m: 3,3'-Biplumbagin



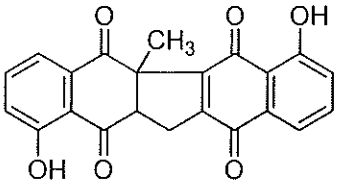
134m: 3,8'-Biplumbagin



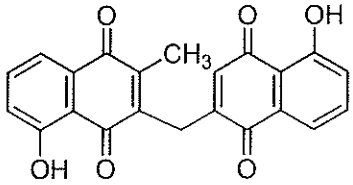
135m: Maritinone



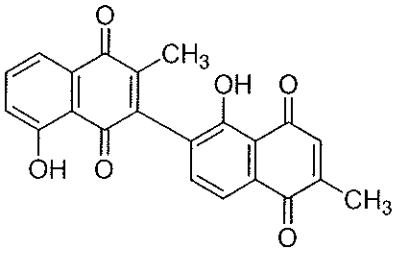
136m: Elliptinone



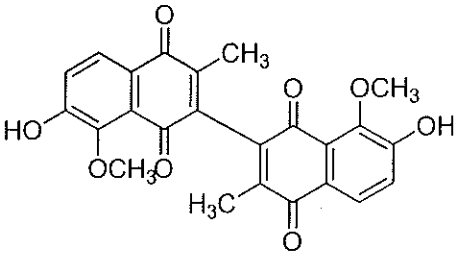
137m: Zeylanone



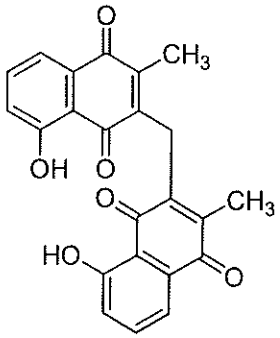
138m: Isozeylanone



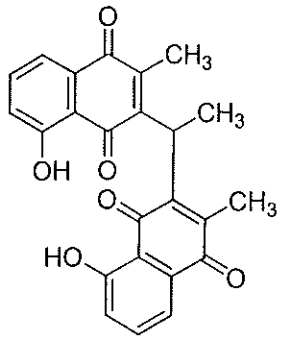
139: Chitranone



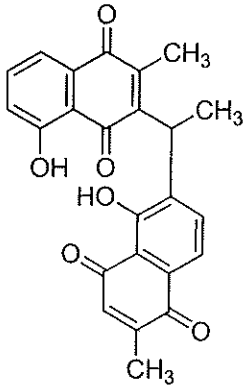
140m: 3,3′-Dimer-6-hydroxy-5-methoxy-2-methyl naphthoquinone



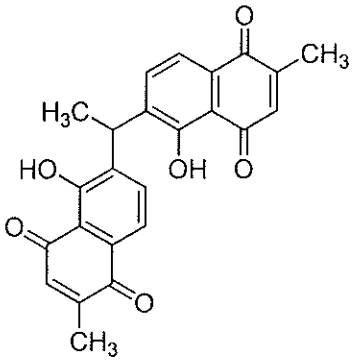
141m: Methylene-3,3'-biplumbagin



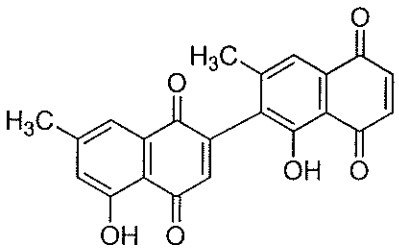
142m: Ethylidene-3,3'-biplumbagin



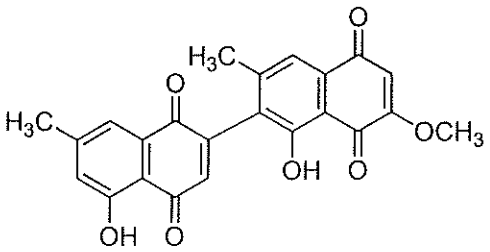
143m: Ethylidene-3,6'-biplumbagin



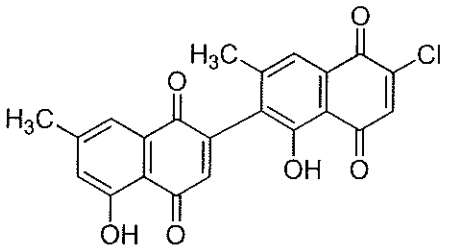
144m: Ethyllidene-6,6′-biplumbagin



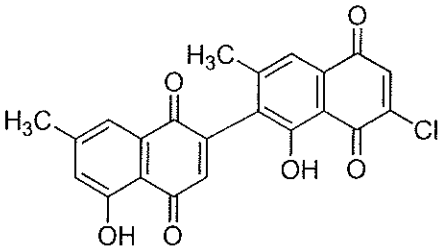
145m: Diospyrin



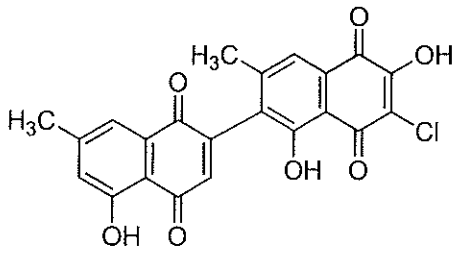
146m: 3′-Methoxydiospyrin



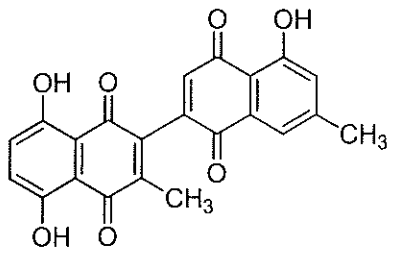
147m: 2′-Chlorodiospyrin



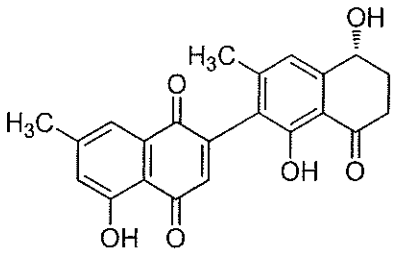
148m: 3′-Chlorodiospyrin



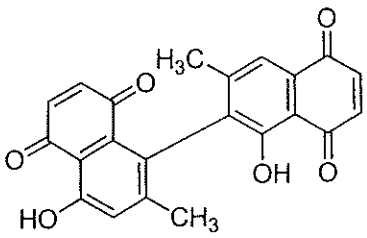
149m: 3′-Chloro-2′-hydroxydiospyrin



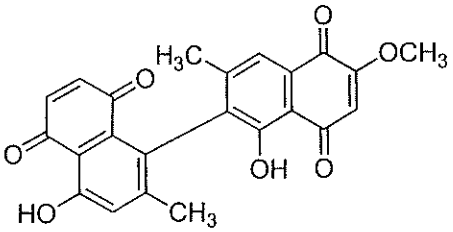
150m: 8′-Hydroxydiospyrin



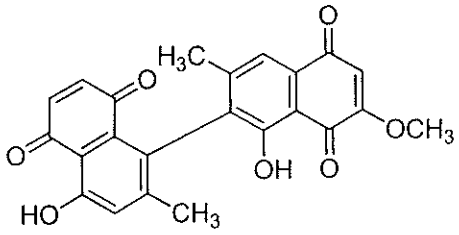
151m: Tetrahydrodiospyrin



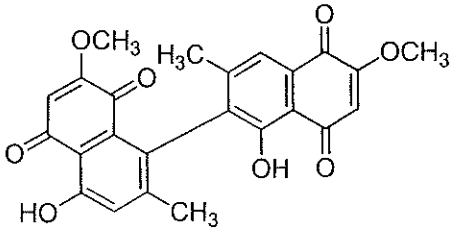
152m: Isodiospyrin



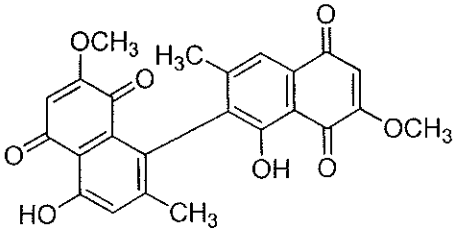
153m: 2′-Methoxy isodiospyrin



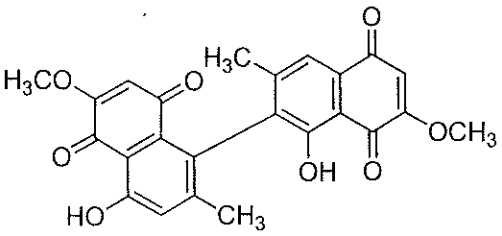
154m: 3′-Methoxy isodiospyrin



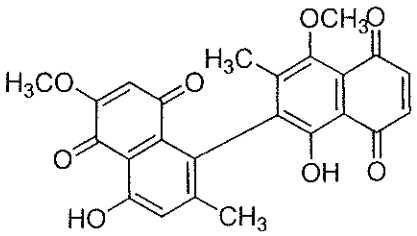
155m: 2,2′-Dimethoxy isodiospyrin



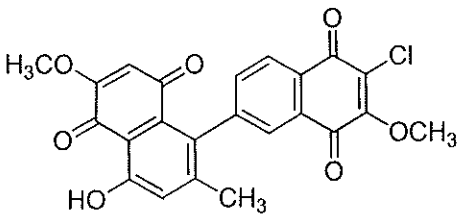
156m: 2,3′-Dimethoxy isodiospyrin



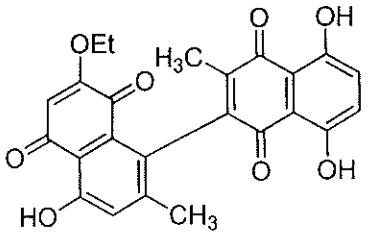
157m: 3,3′-Dimethoxy isodiospyrin



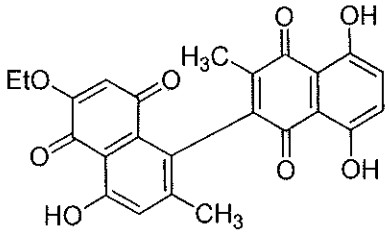
158m: 8′-Hydroxy-3methoxy isodiospyrin



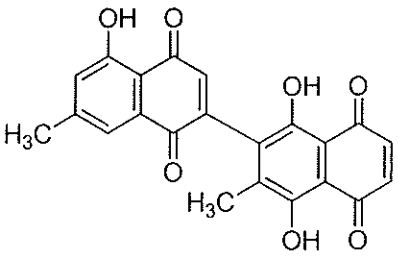
159m: 2'-Chloro-3,3'-dimethoxyisodiospyrin



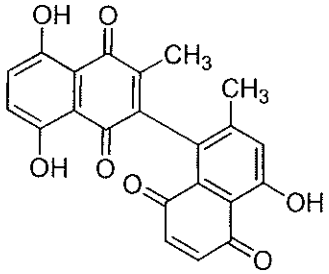
160m: 2-Ethoxy-8′-hydroxyisodiospyrin



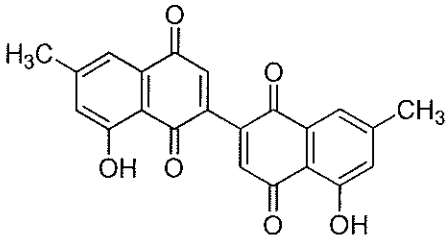
161m: 3-Ethoxy-8′-hydroxyisodiospyrin



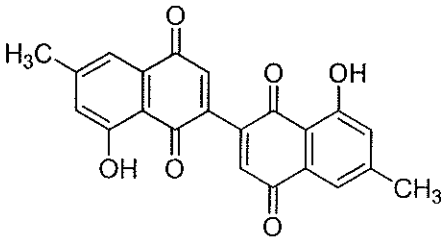
162m: Hydroxyisodiospyrin



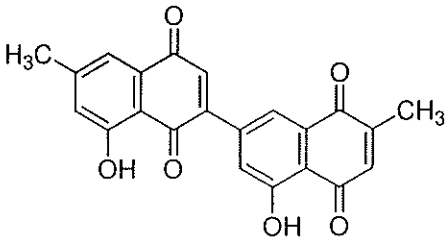
163m: 8'-Hydroxyisodiospyrin



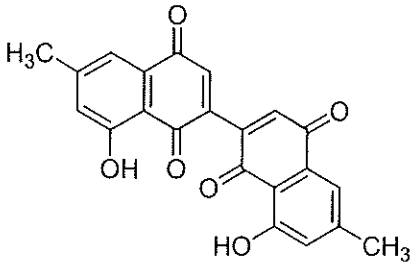
164m: Rotundiquinone



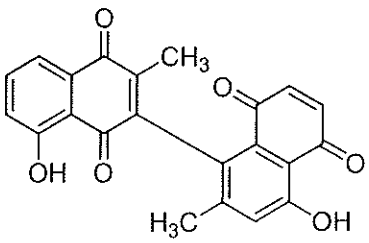
165m: Biramentaceone



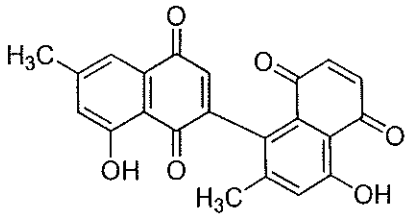
166m: Ehretione



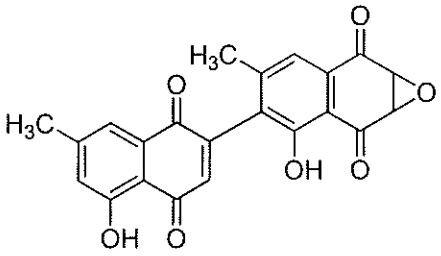
167m: Mamegakinone



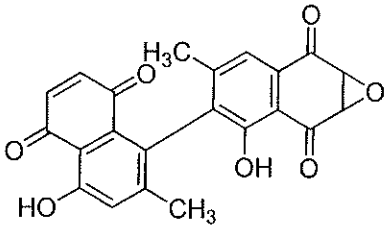
168m: Habibone



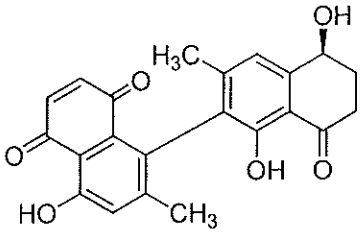
169m: Neodiospyrin



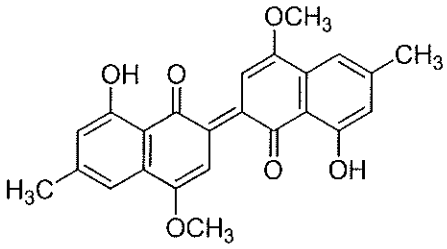
170m: Diosquinone



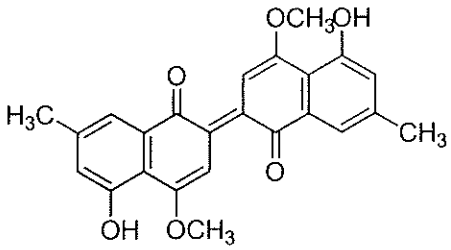
171m: Batacanone



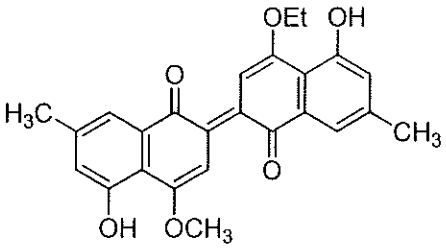
172m: Isodiospyrol A



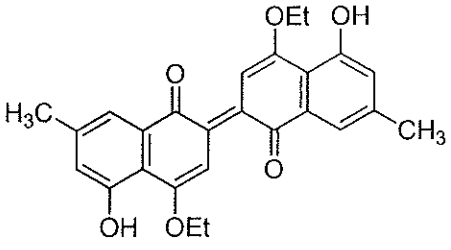
173m: Diosindigo A



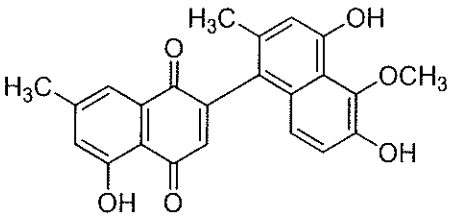
174m: Diosindigo B



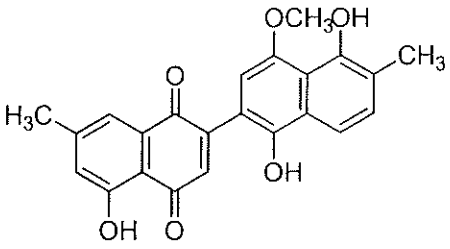
175m: Diosindigo B_1



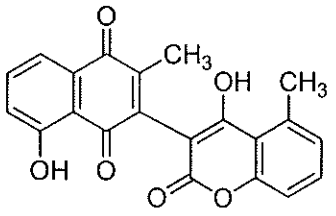
176m: Diosindigo B_2



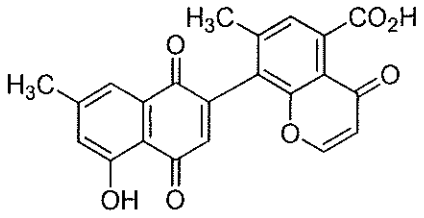
177m: Celebaquinone



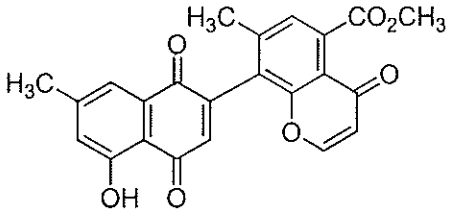
178m: Isocelebaquinone



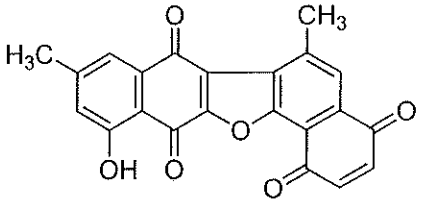
179m: Canaliculatin



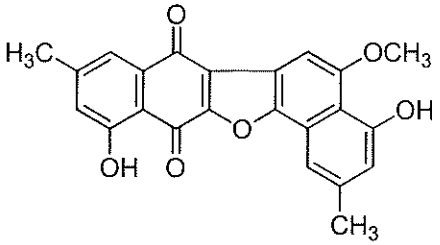
180m: Chromenone acid



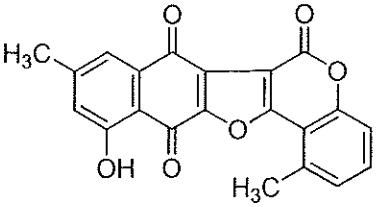
181m: Chromenone ester



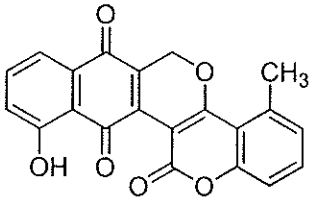
182m: Violet quinone



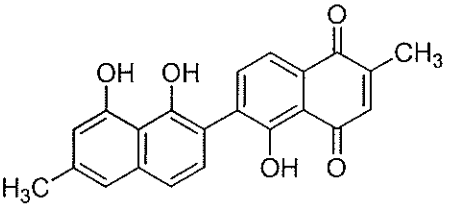
183m: Cyclodiospyrin



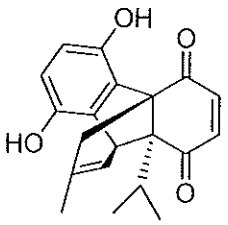
184m: Crassiflorone



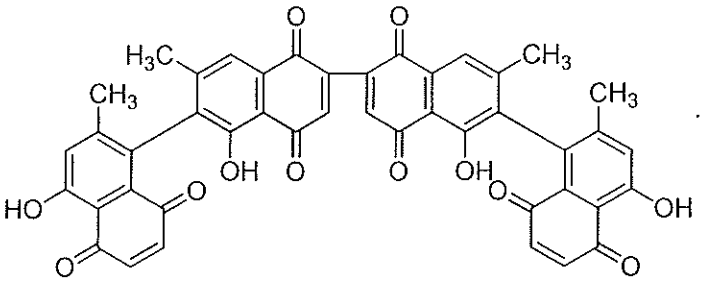
185m: Cyclocanaliculatin



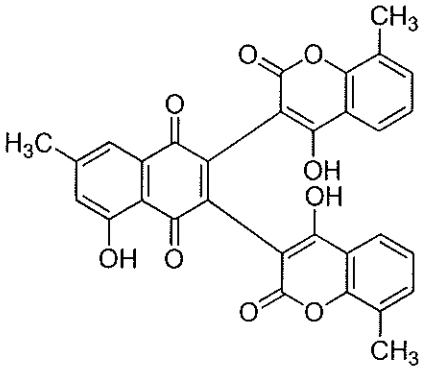
186m: Ebenone



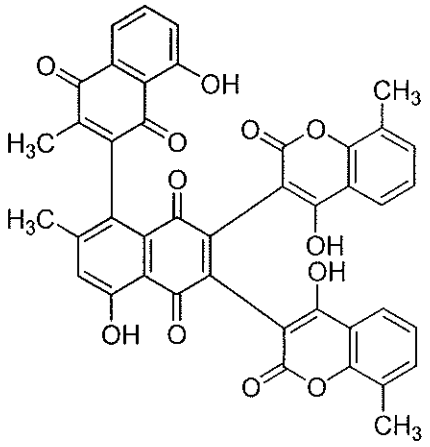
187m: Microphyllone



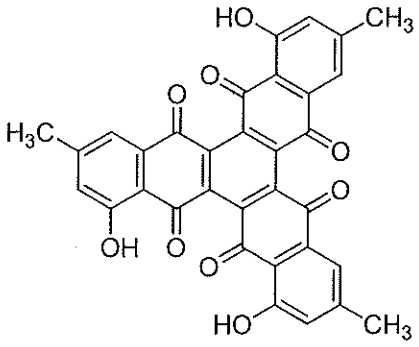
188m: Bisisodiopyrin



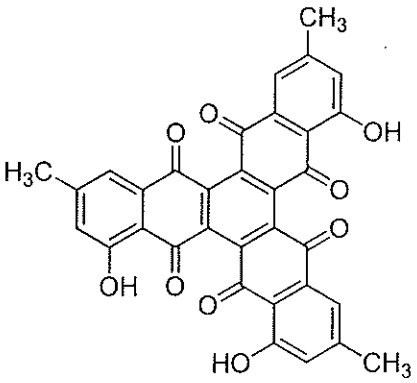
189m: Ismailin



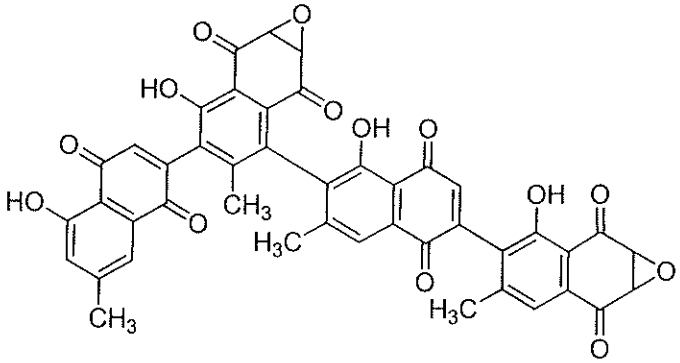
190m: Diospyrone



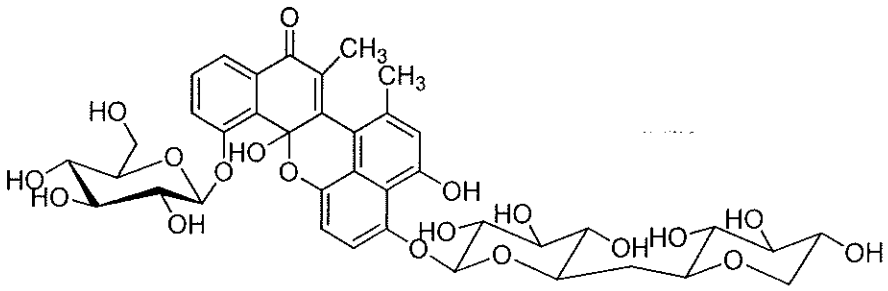
191m: Xylospyrin



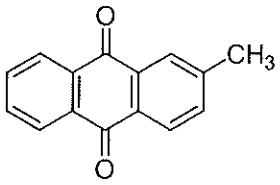
192m: Isoxylospyrin



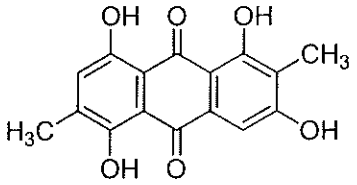
193m: 6",8'-Bisdiosquinone



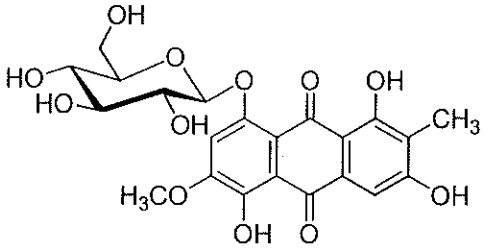
194m: 1',2-Binaphthalen-4-one-2',3-dimethyl-1,8'-epoxy-1,4',5,5',8,8'-hexahydroxy-8-*O*-β-glucopyranosyl-5'-*O*-α-xylopyranosyl(1→6)-β-glucopyranoside



195m: 2-Methyl anthraquinone

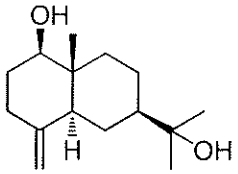


196m: Aglycone



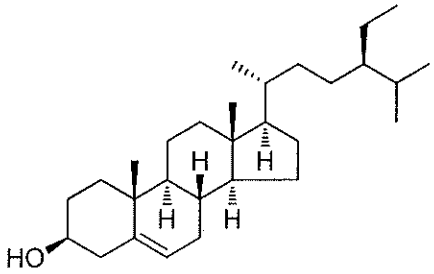
197m: 1,3,5-Trihydroxy-6-methoxy-2-methylanthraquinone 8-*O*-β-D-glucopyranoside

n: sesquiterpenoid

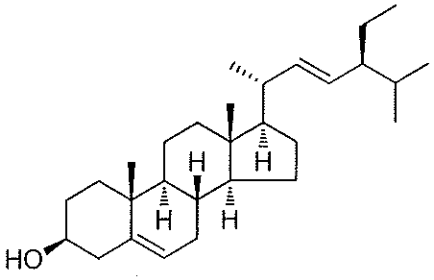


198n: Selin-4(15)-en-1β,11-diol

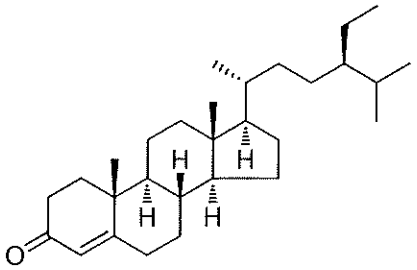
o: steroids



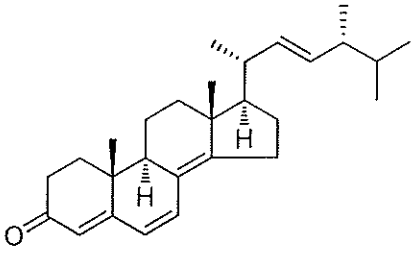
199o: β-Sitosterol



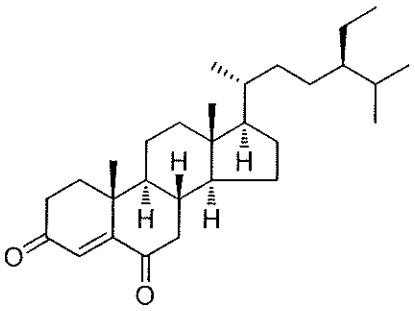
200o: Stigmasterol



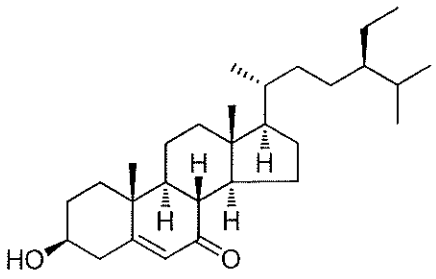
201o: Stigmasta-4-en-3-one



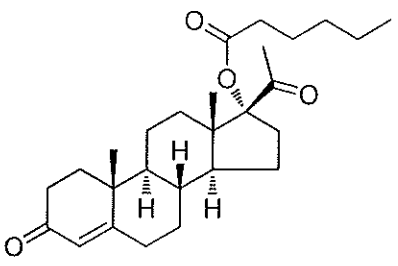
202o: Ergosta-4,6,8(14),22-tetraen-3-one



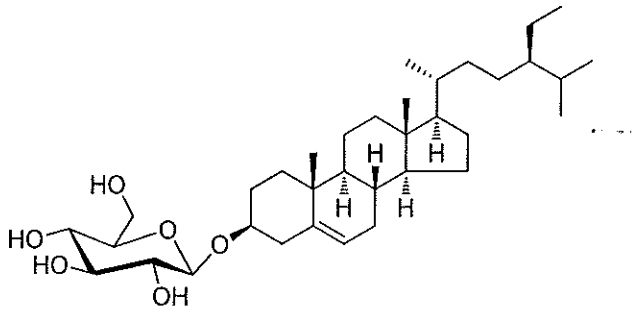
203o: Stigmast-4-ene-3,6-dione



204o: 3β-Hydroxystigmast-5-en-7-one

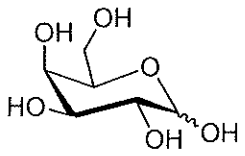


205o: Neolutein

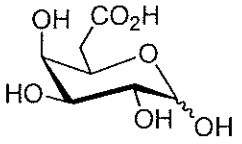


206o: Sitosterol 3-*O*-β-D-glucopyranoside

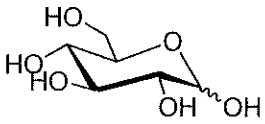
p: sugars



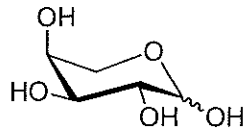
207p: D-Galactose



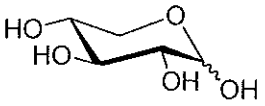
208p: D-Galacturonic acid



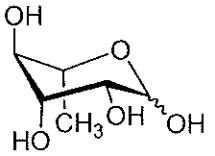
209p: D-Glucose



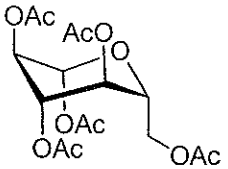
210p: L-Arabinose



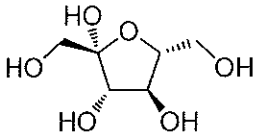
211p: D-Xylose



212p: L-Rhamnose

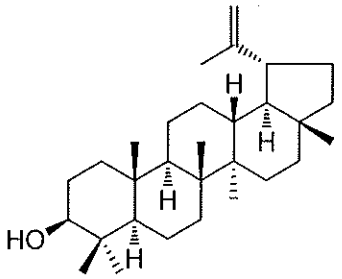


213p: β-D-Glucopyranose pentaacetate

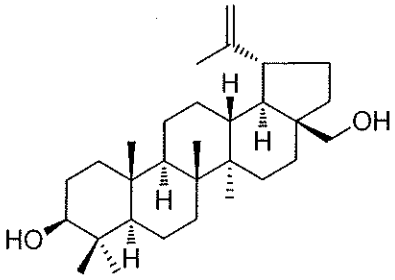


214p: D-Fructose

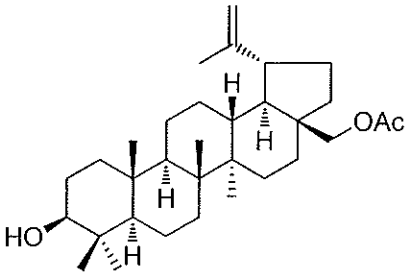
q: triterpenoids



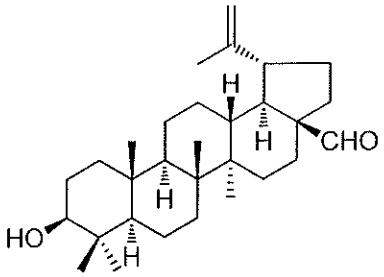
215q: Lupeol



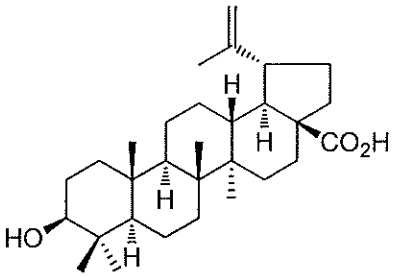
216q: Betulin



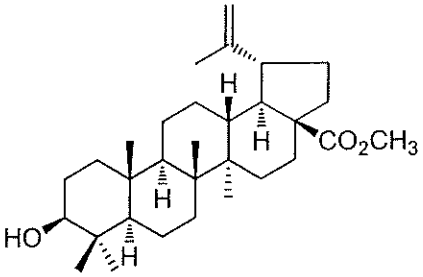
217q: Betulin-28-acetate



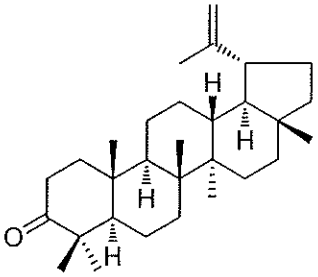
218q: Betulinaldehyde



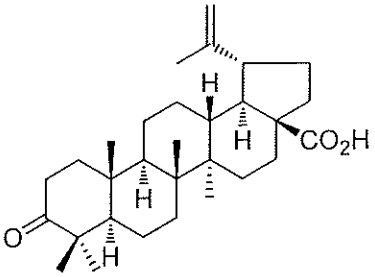
219q: Betulinic acid



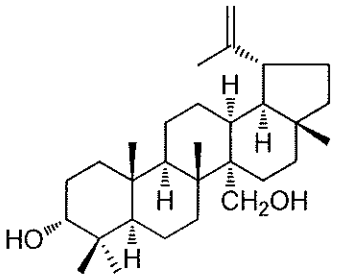
220q: Betulinic acid methyl ester



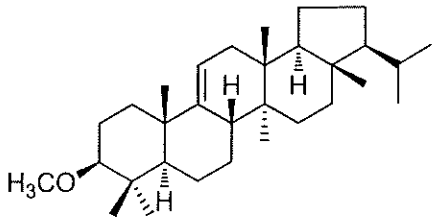
221q: Lupenone



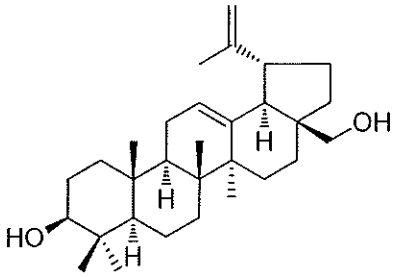
222q: 3-Oxo-20(29)-lupen-28-oic acid



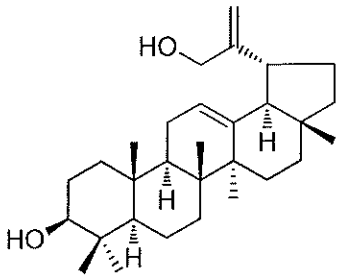
223q: Peregrinol



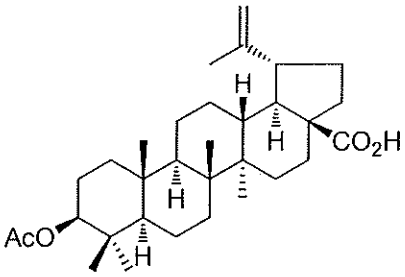
224q: Isoarborinol methyl ether



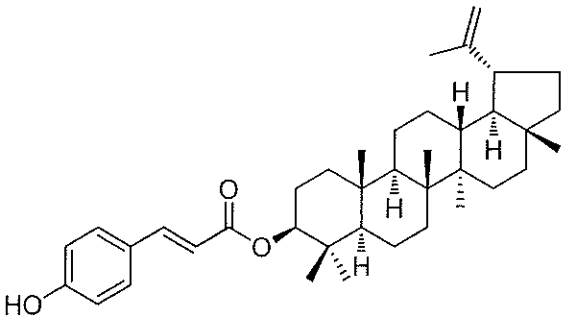
225q: 12,13-Dehydro-20,29-dihydrobetulin



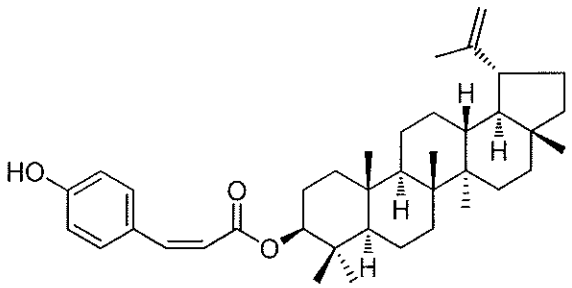
226q: Lup-20(29)-en-3β,30-diol



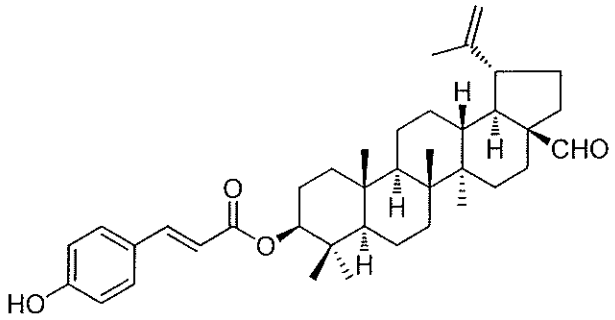
227q: Betulic acid acetate



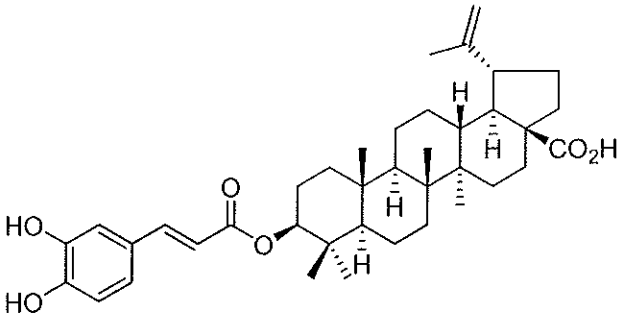
228q: 3-(*E*)-Coumaroyllupeol



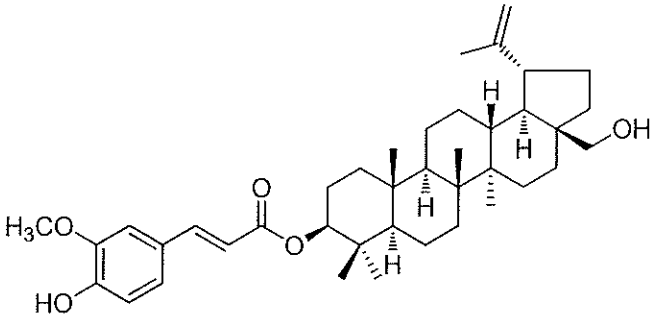
229q: 3-(*Z*)-Coumaroyllupeol



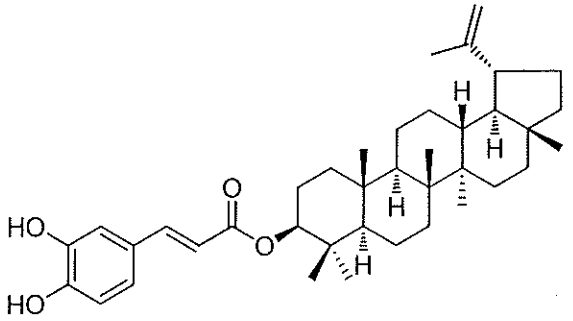
230q: 3-(*E*)-Coumaroylbetulinaldehyde



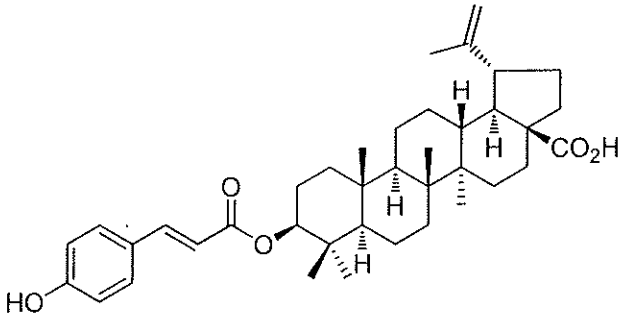
231q: Betulinic acid 3-caffeate



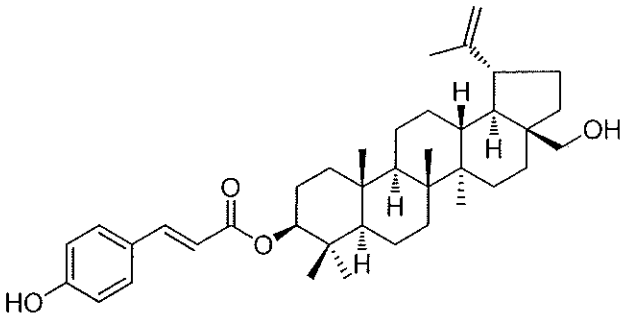
232q: 3-(*E*)-Feruloylbetulin



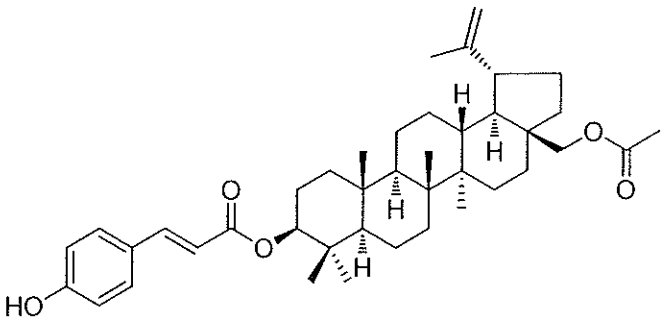
233q: Lupeol caffeate



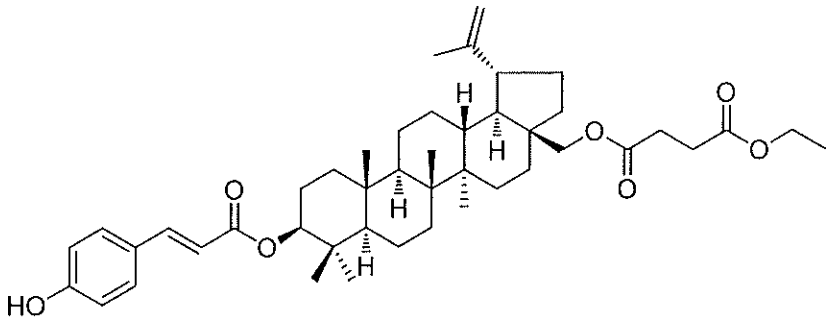
234q: 3-*O*-Betulinic acid *p*-coumarate



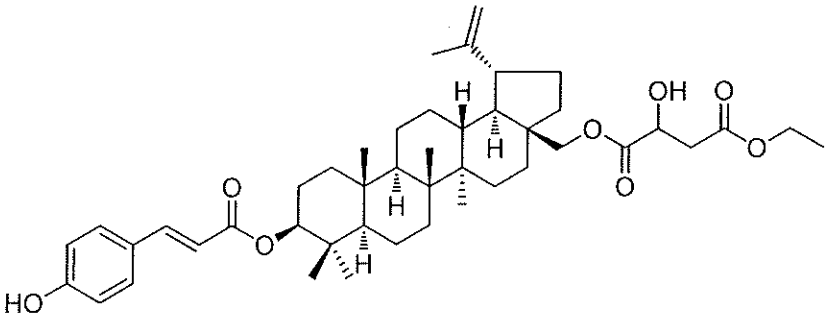
235q: (*E*)-Betulin-3β-*p*-coumarate



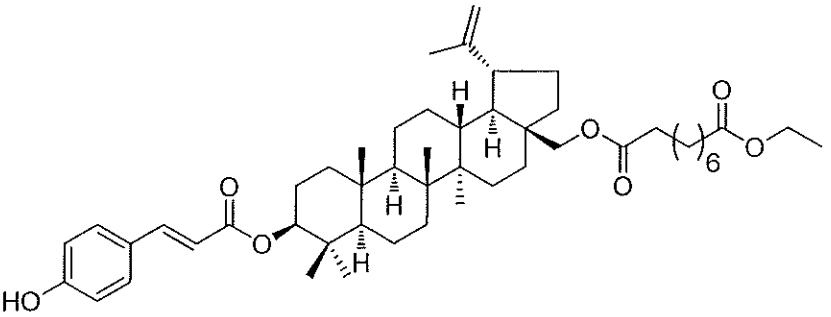
236q: 28-Acetyl-3-(*E*)-coumaroylbetulin



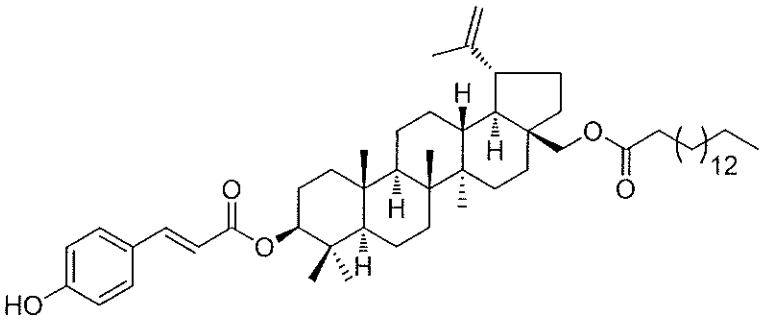
237q: 3-(*E*)-Coumaroylbetulin-28-yl ethyl succinate



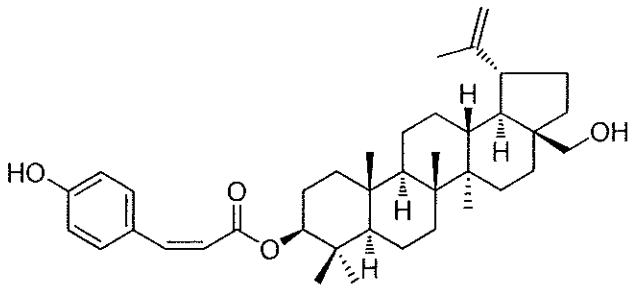
238q: 3-(*E*)-Coumaroylbetulin-28-yl ethyl (2*R*)-2-hydroxysuccinate



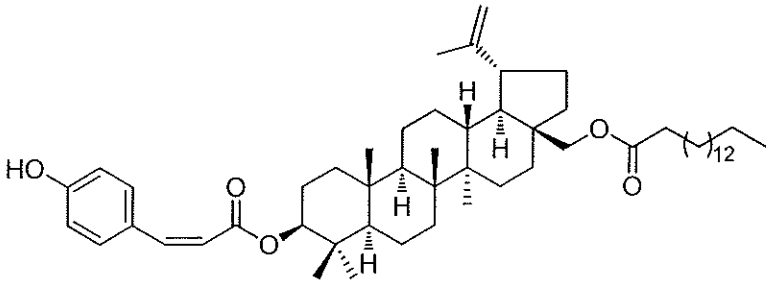
239q: 3-(*E*)-Coumaroylbetulin-28-yl ethyl nonanedioate



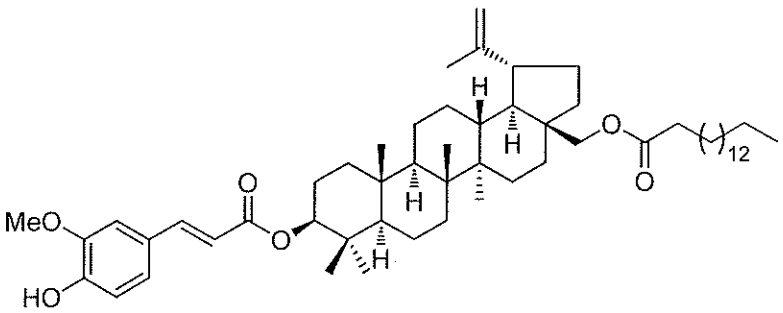
240q: 3-(*E*)-Coumaroyl-28-palmitoylbetulin



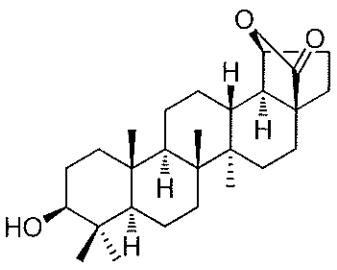
241q: (*Z*)-Betulin-3β-*p*-coumarate



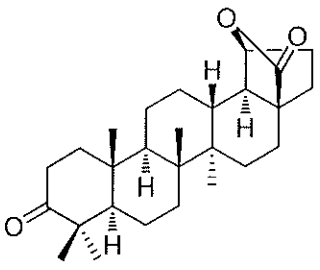
242q: 3-(*Z*)-Coumaroyl-28-palmitoylbetulin



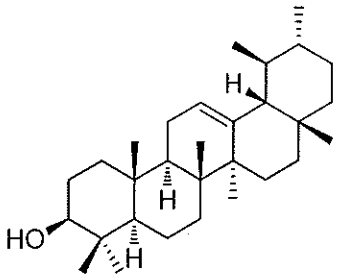
243q: 3-(*E*)-Feruloyl-28-palmitoylbetulin



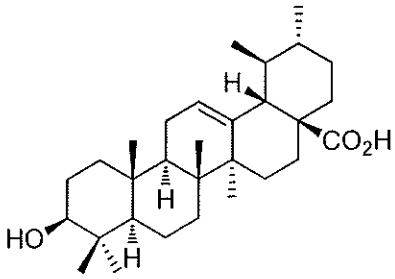
244q: Diospyrolide



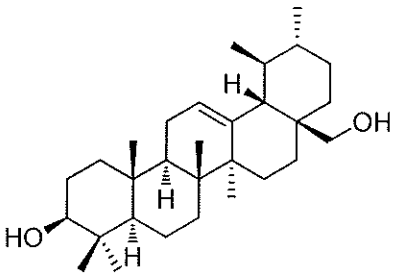
245q: Diospyrolidone



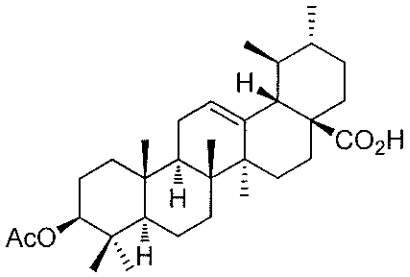
246q: α-Amyrin



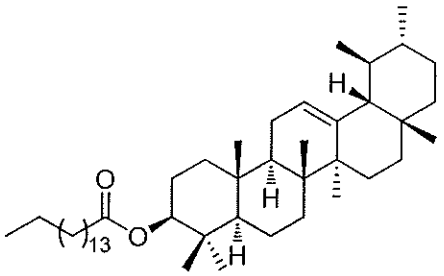
247q: Ursolic acid



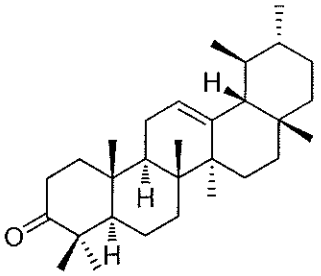
248q: Uvaol



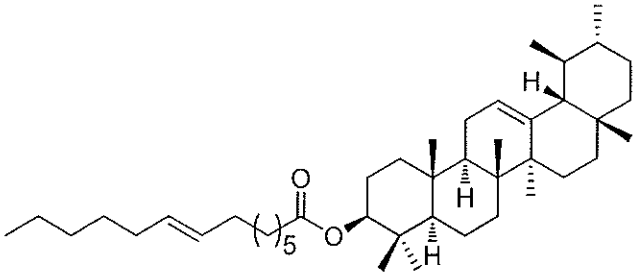
249q: 3β-Acetoxyurs-12-en-28-oic acid



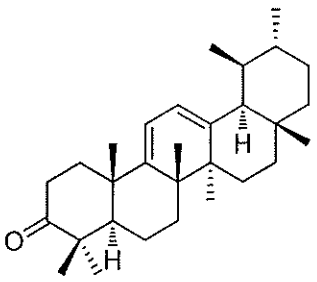
250q: α-Amyrin palmitate



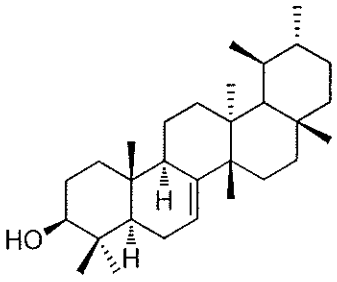
251q: α-Amyrenone



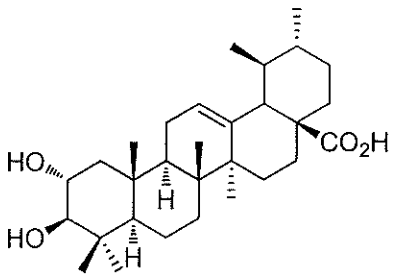
252q: α-Amyrin palmitoleate



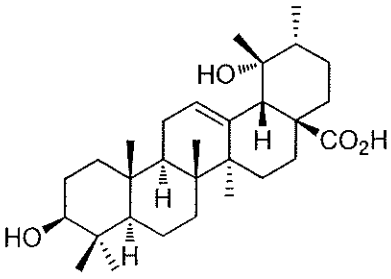
253q: Marsfomosanone



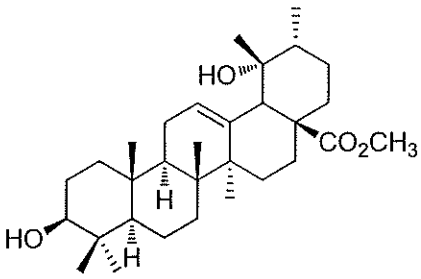
254q: Baurenol



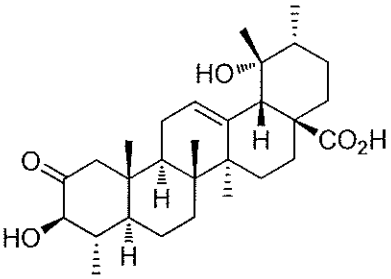
255q: Corsolic acid



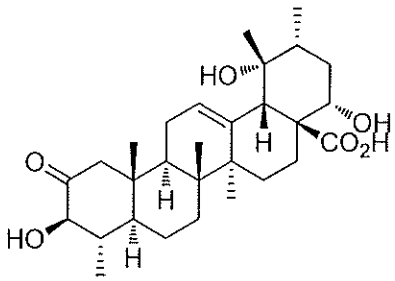
256q: Pomolic acid



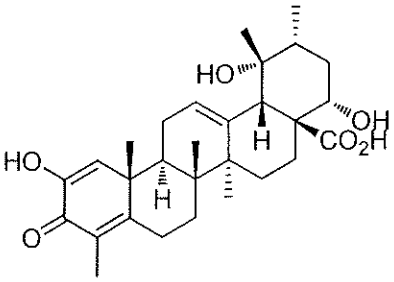
257q: Pomolic acid methyl ester



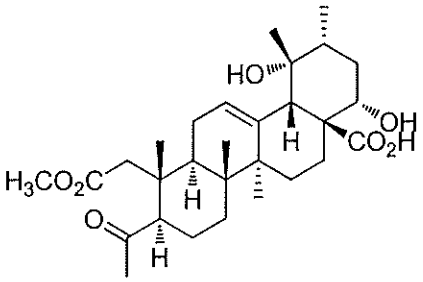
258q: Diospyric acid A



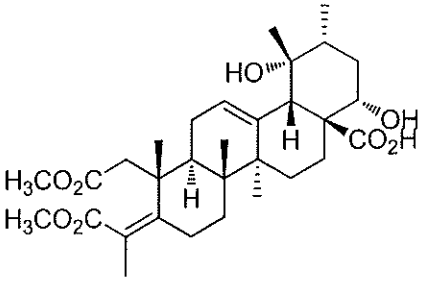
259q: Diospyric acid B



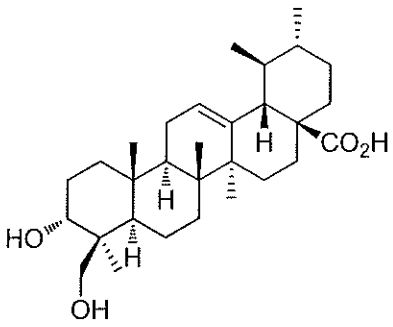
260q: Diospyric acid C



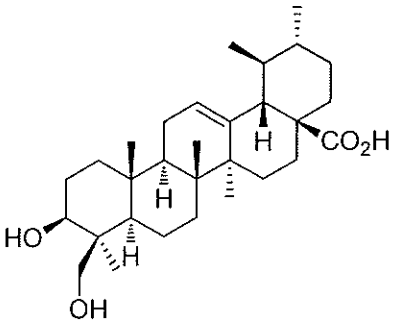
261q: Diospyric acid D



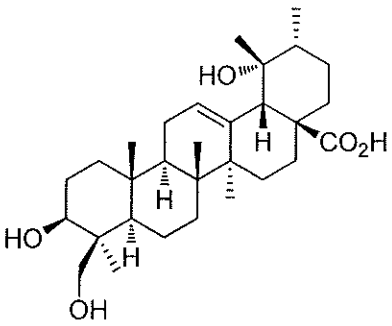
262q: Diospyric acid E



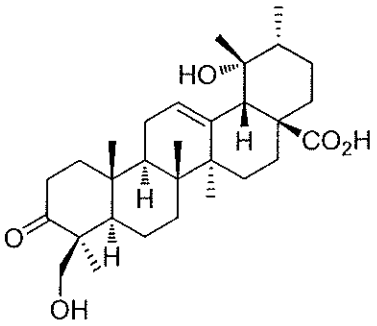
263q: 24-Hydroxy-3-*epi*-ursolic acid



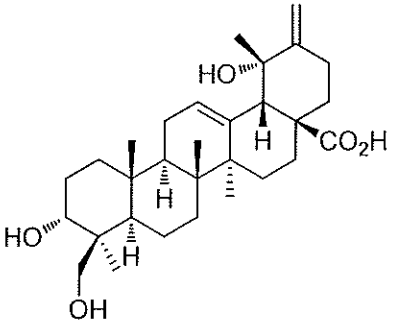
264q: 24-Hydroxyursolic acid



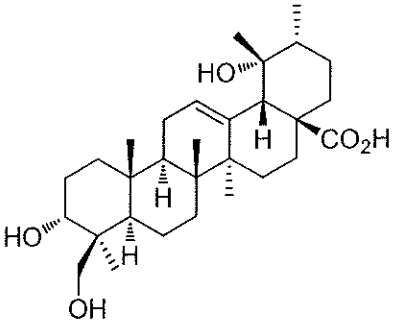
265q: Rotungenic acid



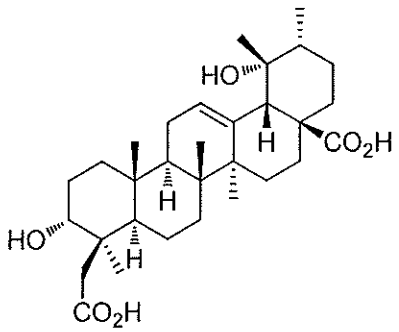
266q:19,24-Dihydroxyurs-12-en-3-on-28-oic acid



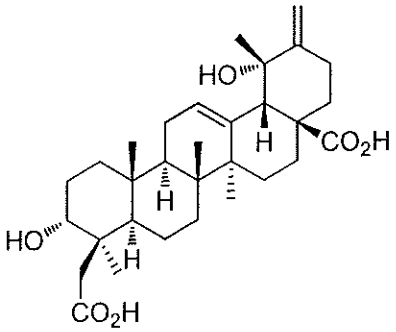
267q: Coussaric acid



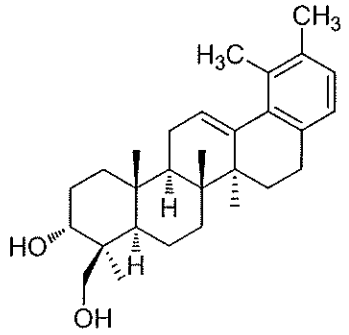
268q: Barbinervic acid



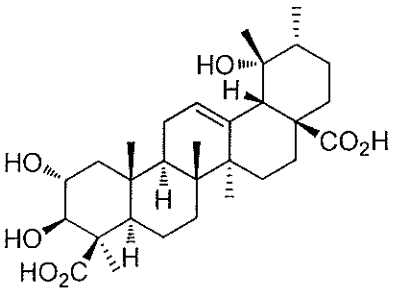
269q: Kakidiol



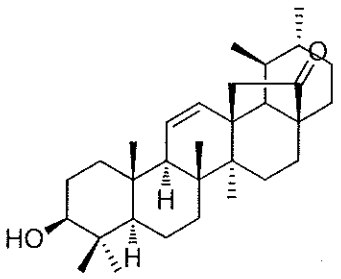
270q: 3α,19α-Dihydroxyurs-12,20(30)-dien-24,28-dioicacid



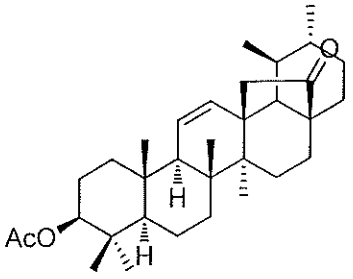
271q: 3α,19α-Dihydroxyurs-12-en-24,28-dioic acid



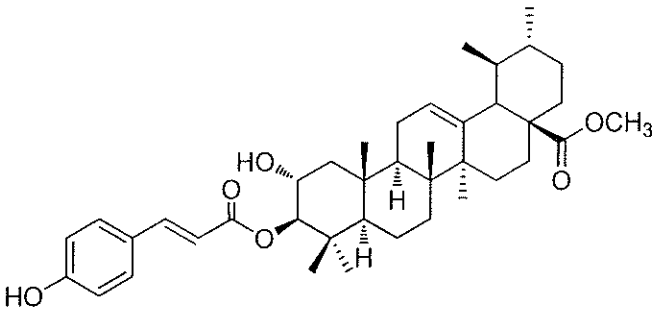
272q: Vismiaefolic acid



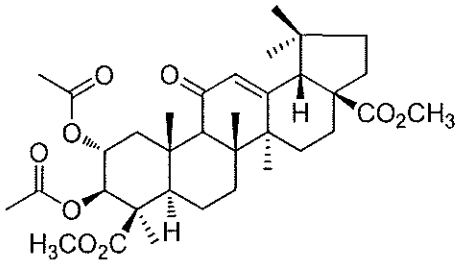
273q: 3β-Hydroxyurs-12-en-28,13-olide



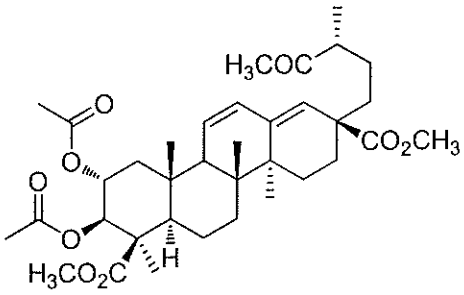
274q: 3β-Acetoxy-urs-11-ene-28,13-olide



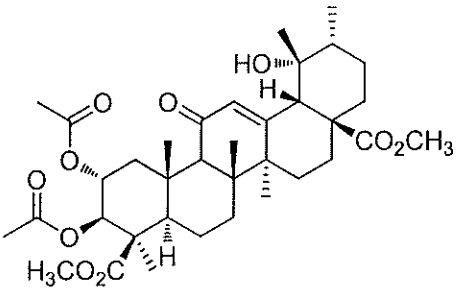
275q: Jacoumaric acid methyl ester



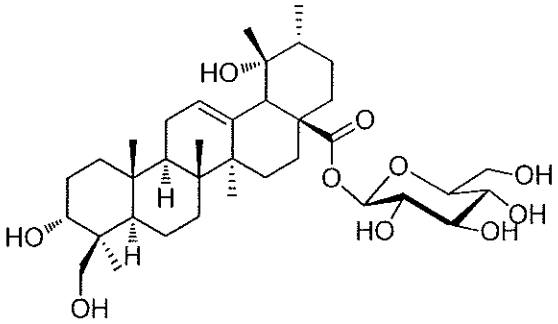
276q: Dimethyl-2α,3β-di-*O*-acetyl-19-*nor*-11-oxoolean-12-en-24,28- dioate



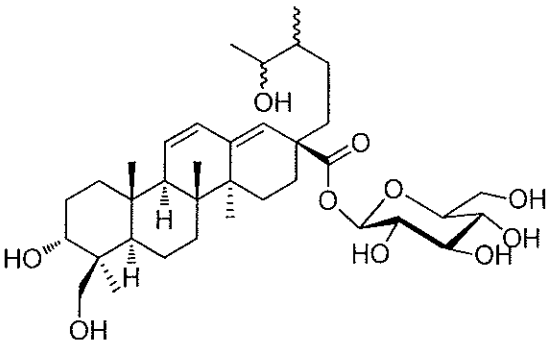
277q: Dimethyl-2α,3β-di-*O*-acetyl-18,19-*seco*-19-oxours-11,13(18) -dien-24,28-dioate



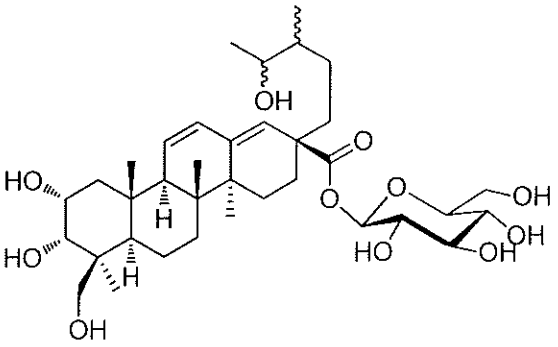
278q: Dimethyl-2α,3β-di-*O*-acetyl-19α-hydroxy-11-oxours-12-en-24,28-dioate



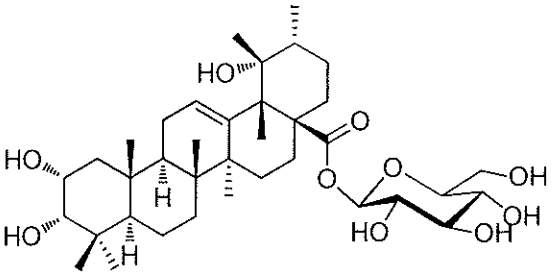
279q: Kakisaponin A



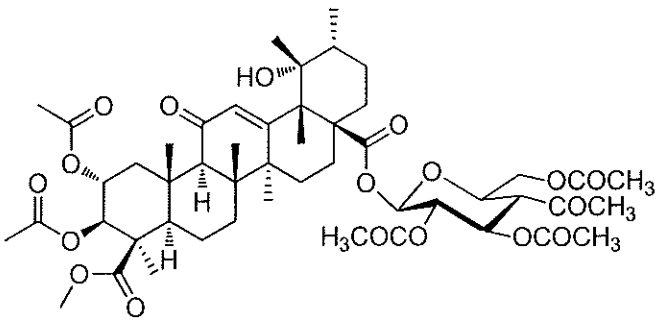
280q: Kakisaponin B



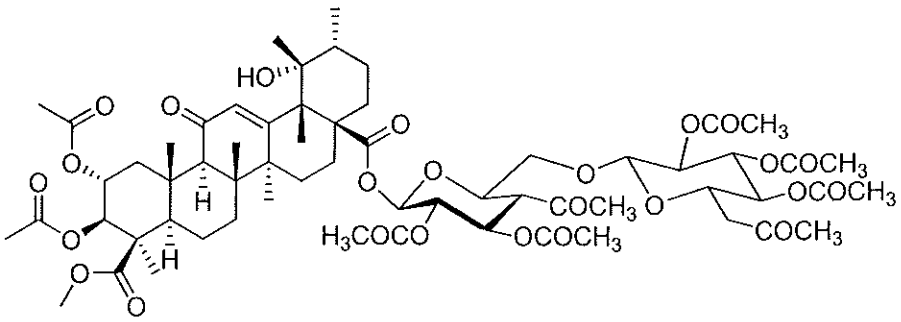
281q: Kakisaponin C



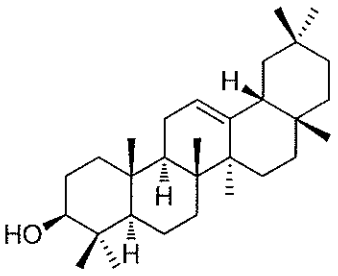
282q: Rosamultin



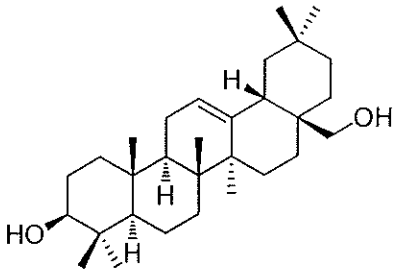
283q: 2α,3β,2',3',4',6'-Hexa-*O*-acetyl-24-methyl-28-10-β-D-glucopyranosyl-19α-hydroxyurs-12-en-24,28-dioate



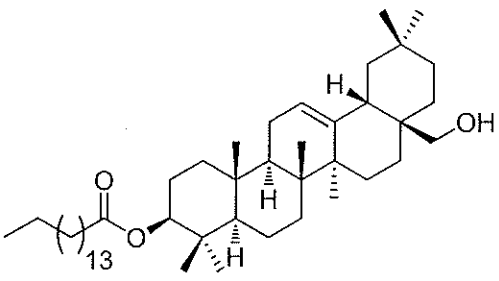
284q: 24-Methyl-2α,3β,2',3',4',2",3",4",6"-nona-*O*-acetyl-28-1'-β-D--[glucopyranosyl-(1"→6')-glucopyranosyl]-19α-hydroxyurs-12-en-24,28-dioate



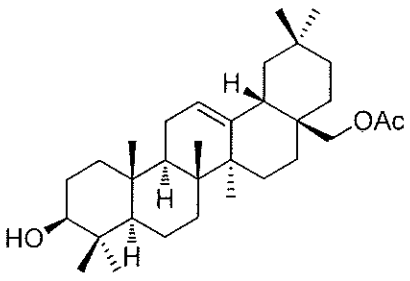
285q: β-Amyrin



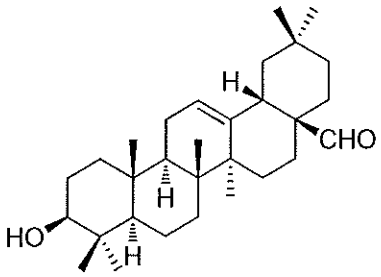
286q: Erythrodiol



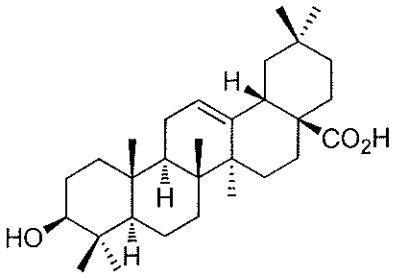
287q: 3-*O*-Palmitoylerythrodiol



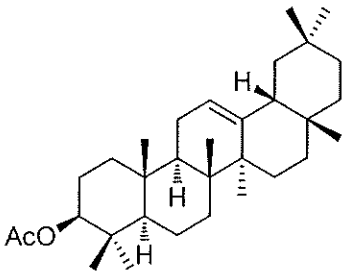
288q: 28-*O*-Acetylerythrodiol



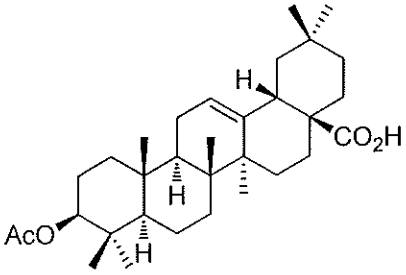
289q: Ursaldehyde



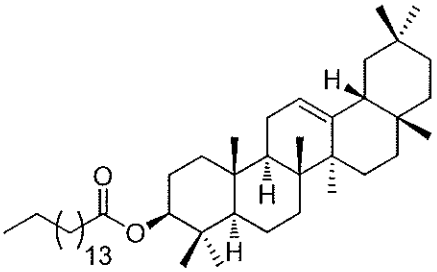
290q: Oleanolic acid



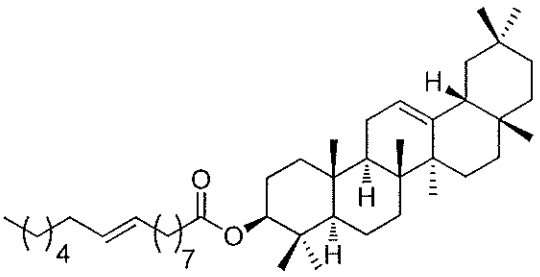
291q: β-Amyrin acetate



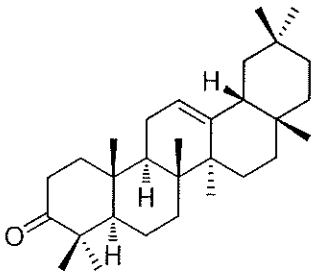
292q: 3β-Acetoxyoleanolic acid



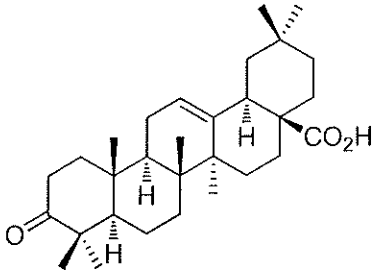
293q: β-Amyrin palmitate



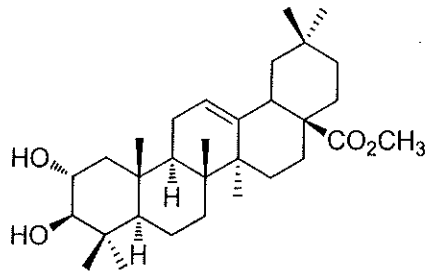
294q: β-Amyrin palmitoleate



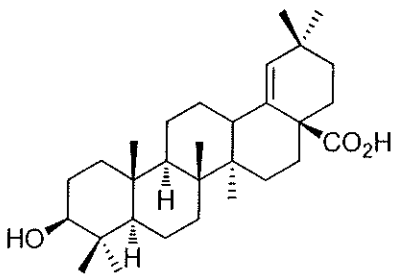
295q: Olean-12-en-3-one



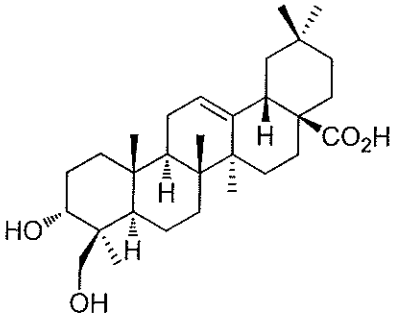
296q: Oleanolic acid



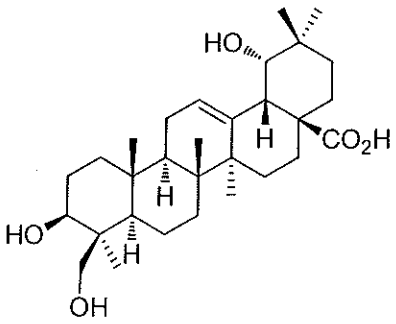
297q: Maslinic acid methyl ester



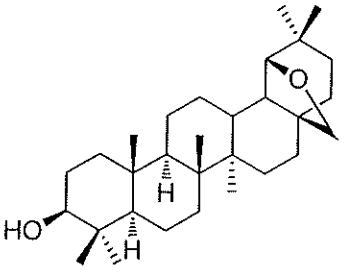
298q: Morolic acid



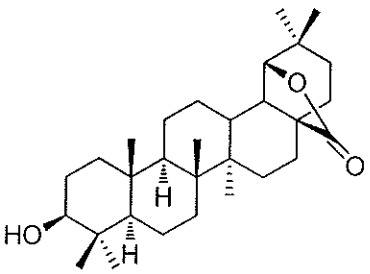
299q:24-Hydroxy-3-*epi*-oleanolic acid



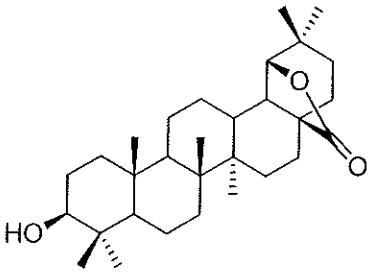
300q: Spathodic acid



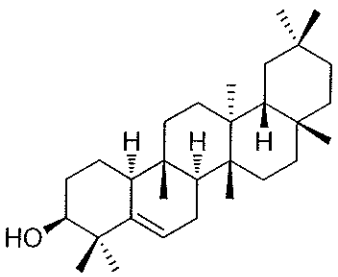
301q: Allobetulin



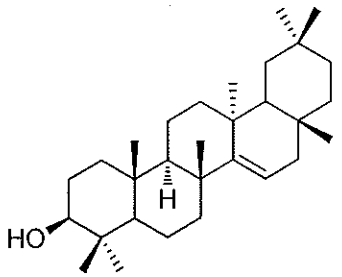
302q: Oxyallobetulin



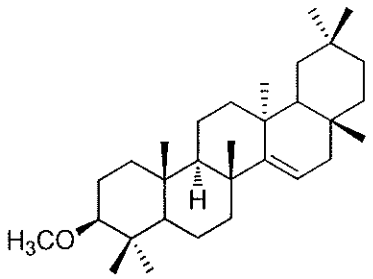
303q: 3β-Hydroxy-28,19β-oleanolide



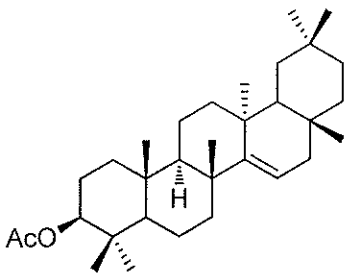
304q: Glutinol



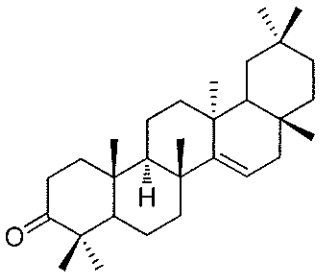
305q: Taraxerol



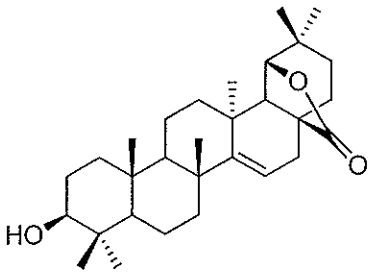
306q: Taraxerol methyl ether



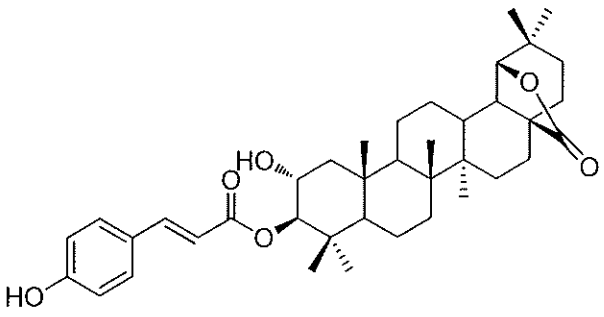
307q: Taraxeryl acetate



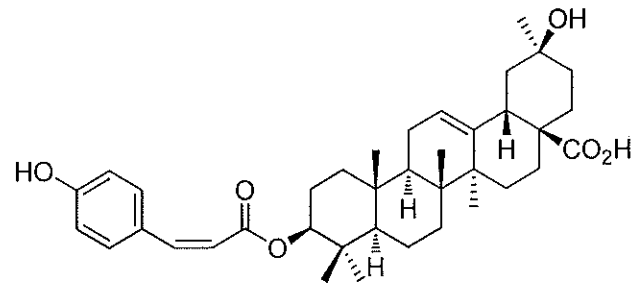
308q: Taraxerone



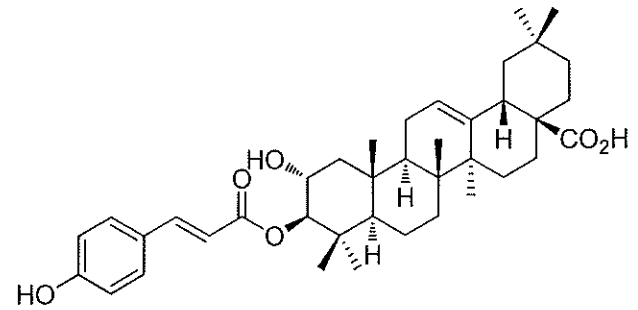
309q: 3β-Hydroxytaraxastan-28, 20β-olide



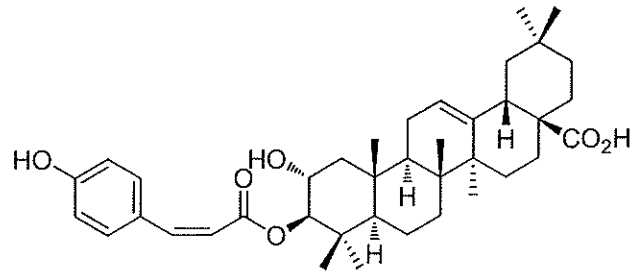
310q: Diospyrosooleanolide



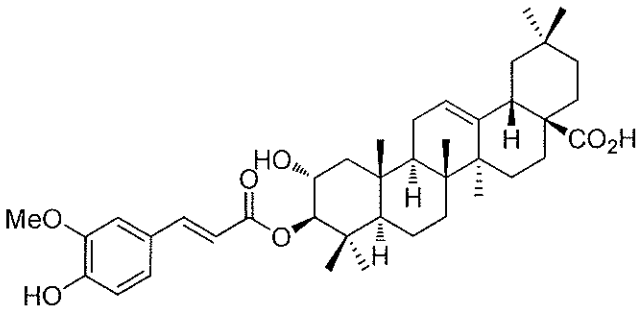
311q: 3β-*O-cis-p*-Coumaroyl-20β-hydroxy-12-ursen-28-oic acid



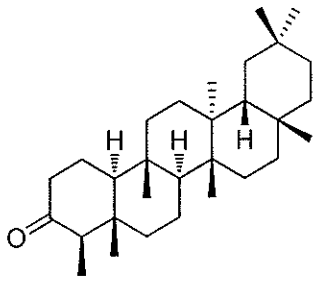
312q: 3β-*O-trans-p*-Coumaroyl-2α-hydroxy-12-ursen-28-oic acid



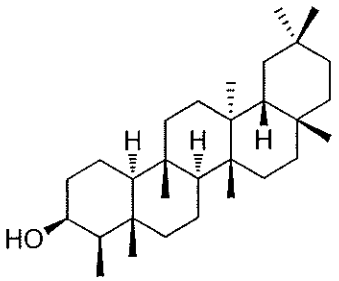
313q: 3β-*O-cis-p*-Coumaroyl-2α-hydroxy-12-ursen-28-oic acid



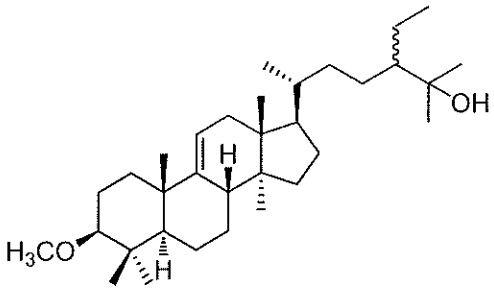
314q: 3β-*O-trans*-Feruloyl-2α-hydroxy-12-ursen-28-oic acid



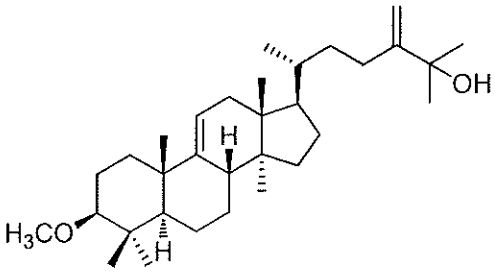
315q: Friedelin



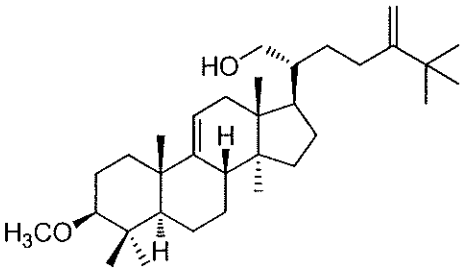
316q: β-Friedelinol



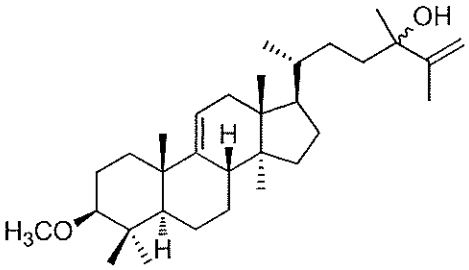
317q: 24-Ethyl-3β -methoxylanost-9(11)-en-25-ol



318q: 3β-Methoxy-24-methylenelanost-9(11)-en-25-ol

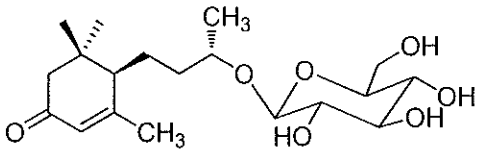


319q: 3β-Methoxy-25-methyl-24-methylenelanost-9(11)-en-21-ol

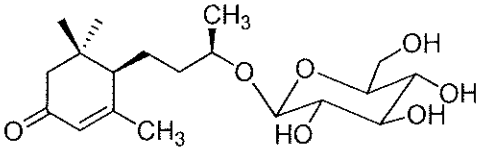


320q: 3β-Methoxy-24-methyllanosta-9(11),25-dien-24-ol

r: terpenoids

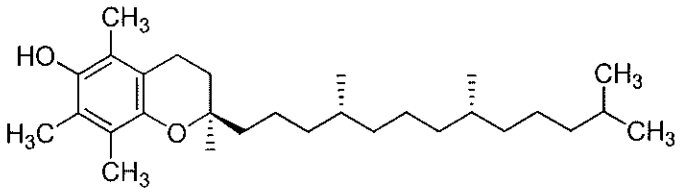


321r: Byzantionoside B



322r: Blumeol C glucoside

s: vitamin



323q: α-Tocopherol

1.2 Chemical constituents from *Premna obtusifolia*

1.2.1 Introduction

The genus Premna L. belonging to the family Verbenaceae was established by Linnaeus based on *P. serratifolia*. The genus now contains about 200 species worldwide which are mainly distributed in tropical and subtropical Asia, Africa, Australia and the Pacific Islands. A preliminary study of the genus in Thailand was first undertaken by Fletcher, enumerating 30 species. *Premna obtusifolia*, a plant widely distributed in the southern part of Thailand and locally known as "akkhi thawan thale (อัคคีทวารทะเล)", has been used in folk medicine to treat fever, headaches, coughs, skin rash, diarrhea, migraine, pneumonia, asthma, and ear-aches (Chopra, 1969; Nandwani et al., 2008).

P. obtusifolia is usually found in the higher parts of the mangrove-occupied areas, often above mean high tide levels. The substratum here is mud or muddy sand that is often harder and drier than in the lower tidal areas. The tree produces serrate yellow green leaves which release a powerful odor upon breaking. The flowers are small and white and organized in clusters. The fruit is a small dark drupe.

Figure 2 Parts of *Premna obtusifolia*

1.2.2 Review of literatures

Chemical constituents isolated from sixteen species of *Premna* genus were summarized in **Table 2**. Information from the Scifinder Scholar copyright in 2012 will be presented and they can be classified into groups as follows: alkaloids, benzenoids, coumarin, diterpenoids, fatty acids, flavonoids, hydrocarbons, iridoids, lignans, phenylethanoids, sesquiterpenoids, steroids, terpenoids, triterpenoids and xanthones

Table 2 Compounds from plants of *Premna* genus.

a:	alkaloids	**f:**	flavonoids	**k:**	sesquitepenoid
b:	benzenoids	**g:**	hydrocabons	**l:**	steroids
c:	coumarin	**h:**	iridoids	**m:**	terpenoids
d:	diterpenoids	**i:**	lignans	**n:**	triterpenoids
e:	fatty acids	**j:**	phenylethanoids	**o:**	xanthones

Plant	**part**	**Compound**	**Bibliography**
P. corymbosa	Stems	Premnoside C, **97h** Saccatoside, **98h** Premnoside D, **100h** 6-α-L-(4"-*O*-Feruloyl)-rhamnopyranosylcatalpol, **109h** 4-*epi*-Gummadiol-*O*-β-D-glucopyranoside, **137i** Premnafolioside, **140j** Verbascoside, **141j** Martynoside, **142j** (-)-Olivile, **145j** Plucheoside D1, **148j**	Yuasa *et al.*, 1993

Table 2 (continued)

Plant	part	Compound	Bibliography
P. corymbosa	Stems	Lyoniresinol 9'-β-D-glucopyranoside, **146j** *eryrhro*-(4-Hydroxy-3-methoxyphenyl)- 2-{4-[2-formyl-(*E*)-vinyl]-2-methoxyphenoxy}-propan-l,3-diol, **149j** *thero*-(4-Hydroxy-3-methoxyphenyl)- 2-{4-[2-formyl-(*E*)-vinyl]-2-methoxyphenoxy}-propan-l,3-diol, **150j**	Yuasa *et al.*, 1993
	Stem barks	Rutin, **83f** Scutellarioside II, **119h** Leonuriside A, **147j**	Nguyen *et al.*, 2008
	Flowers	10-*O*-*trans*-*p*-Methoxy cinnamoylcatalpol, **110h** Verbascoside, **141j**	Pham *et al.*, 2008
P. crassa	Stem	Stearic acid, **64e** Sitosterol, **160l** Friedelin, **175n** β-Friedelanol, **176n** β-	Wei *et al.*, 1990
P. flavescens	Aerial parts	Quercetin 3-*O*-α-L-rhamnopyranoside, **80f** Kaempferol 3-*O*-β-D-glucopyranoside, **81f** Afzelin, **82f**	Trinh *et al.*, 1998

Table 2 (continued)

Plant	part	Compound	Bibliography
P. fulva	Stem bark	Vanillic acid, **7b** Naringenin, **66f** β-Sitosterol, **160l** β-Daucosterol, **162l** Lupenone, **168n** Friedelin, **175n** β-Friedelanol, **176n**	Zeng *et al.*, 1989, 1990
		p-Hydroxybenzoic acid, **6b** Vanillic acid, **7b** Naringenin, **66f** Apigenin, **67f** Vitexin, **85f** 4'-Hydroxy-8,3'- dimethoxy-6-acroleinylflavan-3,4-diol, **92f** Syringaresinol, **136i**	Chen *et al.*, 2010
	Stems	Naringenin, **66f** Apigenin, **67f** Vicenin 2, **86f**	Huang *et al.*, 2009
P. hainanensis	Root	*p*-Hydroxybenzoic acid, **6b** Hexacosoic acid, **65e** β-Sitosterol, **160l** β-Daucosterol, **162l** Lupeol, **166n** Friedelin, **175n**	Dai *et al.*, 1989
P. herbacea	Roots	Pygmaeoherin, **15c** Sugiol, **27d** Pygmaeocins B, **43d**	Meng *et al.*, 1988, 1990,

Table 2 (continued)

Plant	part	Compound	Bibliography
P. herbacea	Root nodules	Pygmaeocins C, **44d** Pygmaeocin A, **61d** 5,6- Didehydropygmaeocin A, **62d** Bharangin, **55d**	Shihari *et al.*, 2011
	Roots	11-Hydroxy-5,7,9,13-abietatetraene-2,12-dione, **28d** Isobharangin, **29d** Fuerstione, **30d** 15-Deoxyfuerstione, **31d** 3β-Acetoxyfuerstione, **32d** Neobharangin, **54d** Bharangin , **55**	Satish *et al.*, 2011
P. integrifolia	Root bark	Sandaracopimaradiene, **22d** 7α-Hydroxysandaraco pimar-8(14),15-diene, **23d** 11β-Hydroxy-7-ketosanda racopimar-8(14),15-diene, **24d** Sandaracopimar-8(14),15-diene,caryophyllen-3-one, **25d** Premnenol, **26d** 11,14-Dihydroxy-12,16-epoxyabieta-5,8,11,13-tetra ene-7-one, **41d** 11,12,16- Trihydroxyabieta-5,8,11,13-tetraen-7-one, **42d** Premnaspirodiene, **157k**	Rao *et al.*, 1985, 1987

Table 2 (continued)

Plant	part	Compound	Bibliography
P. integrifolia	Root bark	Buddledin C, **158k** 5-Hydroxy-4,5-dihydro caryophyllen-3-one, **159k**	Rao *et al.*, 1985, 1987
	Stem bark	Quercetin 3-rutinoside, **84f** Scutellarioside II, **119h** Premnacorymboside A, **132h** Leonuriside A, **147j** Aphelandrine, **4a** Luteolin, **69f**	Nguyen *et al.*,2008 Dasgupta *et al.*, 1984
	Leaves	4-Hydroxybenzaldehyde, **5b** 4-Hydroxy-2-methoxybenzal dehyde, **8b** Hexyl glucoside, **94j** Scutellarioside II, **119h** Premnaodoroside A, **125h** 1-*O-trans-p*-Coumaroyl-α-L-rhamnopyranoside, **154j** 10-*O-trans-p*-Methoxy cinnamoylcatalpol, **110h** Premnaodoroside A, **132h** Premnazole, **1a** 1β,3α,8β-trihydroxy-pimara-15-ene, **20d** 2α,19-dihydroxy-pimara-7,15-diene, **21d**	Nguyen *et al.*, 2008, 2009 barik *et al.*, 1992 Yadav *et al.*, 2010

Table 2 (continued)

Plant	part	Compound	Bibliography
P. integrifolia	Leaves	6α,11,12,16-tetrahydroxy-7-oxo- abieta-8,11,13- triene, **40d**	Yadav *et al.*, 2010
	Flowers	Verbascoside, **141** 10-*O-trans-p*-Methoxy cinnamoylcatalpol, **110h** Premnacorymboside A, **132h**	Nguyen *et al.*, 2008
P. japonica	Leaves	6-*O*-α-L-(2″-*O*-Isoferuloyl, 4″-*O*-acetyl)rhamnopyrano sylcatalpol, **99h** 6-O-α-L-(3″-*O*-Isoferuloyl, 4″-*O*-acetyl)rhamnopyrano syl catalpol, **101h** 6-*O*-α-L-(4″-*O-trans- p*-Coumaroyl)rhamnopyranosylc atapols, **102h** 6-*O*-α-L-(2″-*O*-Caffeoyl) rhamnopyranosylcatapol, **103h** 6-*O*-α-L-(3″-*O*-Caffeoyl) rhamnopyranosylcatalpol, **104h** 6-*O*-α-L-(2″-*O-p*-Methoxy cinnamoyl)rhamnopyranosylca talpol, **105h** 6-*O*-α-L-(3″-*O-p*-Methoxy cinnamoyl)rhamnopyranosylca talpol, **106h**	Otsuka *et al.*, 1990, 1991

Table 2 (continued)

Plant	part	Compound	Bibliography
P. japonica	Leaves	Saccatoside, **98h** 6-*O*-α-L-(2″-*O*-*p*-Methoxy cinnamoyl-4-*O*-acetyl)rham nopyrano sylcatalpol, **107h** 6-*O*-α-L-(3″-*O*-*p*-Methoxy cinnamoyl-4″-*O*-acetyl) rhamnopyra nosylcatalpol, **108h**	Otsuka *et al.*, 1990, 1991
P. latifolia	Leaves	Saropeptate, **2a** Aurantiamide, **3a** Premnalatin, **13b** 1,3-Benzodioxole-5-carbo xylic acid,1,2-ethanediyl ester, **14b** Vicenin 2, **86f** Triacontanol, **93g** (+)-Sesamin, **134i** β-Daucosterol, **162l**	Rao *et al.*, 1986
	Root bark	Sandaracopimar-15-en-8β-ol, **16d** 11-Ketosandaracopimar-15-en-18β-ol, **17d** Sandaracopimar-15-en-1β,8β-diol, **18d** 15-Sandaracopimaran-1β,8β,12β-triol, **19d** Nellionol, **33d** Anhydronellionol, **34d** Dehydronellionol, **35d** Ferruginol, **36d**	Rao *et al.*, 1978, 1982, 1984

Table 2 (continued)

Plant	part	Compound	Bibliography
P. latifolia	Root bark	Taxodione, **37d** Premnolal, **38d** Dehydropremnolal, **39d** Premnaspiral, **156k** Premnaspirodiene, **157k**	Rao *et al.*, 1978, 1982, 1984
	Stem bark	Latifolionol, **56d** Dihydrolatifolionol, **57d** Premnalatifolin A, **63d** Glucoside-B, **95h** Glucoside-C, **96h**	Suresh *et al.*, 2011
P. microphylla	Roots	1-Hydroxy-2,3-methylenedioxy-6-methoxycarbonyl-7-acetylxanthone, **177o** 1,3-Dihydroxy-2-methoxy-6-methoxycarbonyl-7-acetyl xanthone, **178o** 6,3′-Dihydroxy-7-methoxy-4′,5′-methylenedioxy isoflavone, **88f** 6,3′-Dihydroxy-7-methoxy-4′,5′-methylenedioxy isoflavone 6-*O*-β-D-glucopyranoside, **89f** 6-3′-Dihydroxy-7-methoxy-4′,5′-methylenedioxy isoflavone 6-*O*-β-D-xylo pyranosyl (1→6)-β-D-glucopyranoside, **90f** 6,3′-Dihydroxy-7-methoxy-4′,5′-methylenedioxy isoflavone 6-*O*-α-L-rhamno pyranoside, **91f**	Wang *et al.*, 2003 Zhong *et al.*, 2003

Table 2 (continued)

Plant	part	Compound	Bibliography
P. microphylla	Leaves	28-*O*-α-L-Rhamnopyrano syl(l→2)-β-D-glucopyrano side tormentic acid ester, **172n** Arjunolic acid, **173n** Hyptatic acid A, **174n**	Zhan *et al.*, 2009
P. oligotricha	Aerial parts	(5*R*,8*R*,9*S*,10*R*)-12-Oxo-*ent*-3,13 (16)-clerodien-15-oic acid, **58d** *ent*-12-Oxolabda-8,13(16)-dien-15-oic acid, **59d** *ent*-8β,12α-Epidioxy-12β-hydroxylabda-9(11),13-dien-15-oic acid, **60d** 7α-Hydroxy-6,11-cyclo farnes-3(15)-en-2-one, **155k**	Habtemariam *et al.*, 1991, 1993
P. recinosa	Leaves	(+)-1-Hydroxypinoresinol, **135i** (+)-Lariciresinol, **138i** (-)-*seco*-Isolariciresinol, **139i**	Habtemariam, 1995
P. schimperi	Leaves	(5*R*,8*R*,9*S*,10*R*)-12-Oxo-*ent*-3,13 (16)-clerodien-15-oic acid, **58d**	Habtemariam *et al.*, 1990
P. subscanden	Leaves	10-*O-trans-p*-Methoxy cinnamoylcatalpol, **110** 10-*O-cis-p*-Methoxy cimmamoylcatalpol, **111h** 10-*O-trans*-Caffeoylcatalpol, **112h** 10-*O-trans*-Isoferuloylcatalpol, **113h**	Sudo *et al.*, 1997, 1998, 1999

Table 2 (continued)

Plant	**part**	**Compound**	**Bibliography**
P. subscanden	Leaves	10-*O-trans-p*-Methoxy cinnamoyl asystasioside E, **114h** 10-*O-cis-p*-Methoxy cinnamoyl asystasioside E, **115h** 10-*O-trans-p*-Coumaroyl asystasioside E, **116h** 10-*O-cis-p*-Coumaroyl asystasioside E, **117h** 10-*O-cis-p*-Coumaroyl catalpol, **118h** Scutellarioside II, **119h** 4″-Methoxy-*E*-globularinin, **120h** 4″-Methoxy-Z-globularinin, **121h** 4″-Hydroxy-Z-globularinin, **122h** 4″-Methoxy-*E*-globularimin, **123h** 4″-Methoxy-Z-globularimin, **124h** Premnaodoroside A, **125h** Premnaodoroside B, **126h** Premnaodoroside C, **127h** Premnaodoroside D, **128h** Premnaodoroside E, **129h** Premnaodoroside F, **130h**	Sudo *et al.*, 1997, 1998, 1999

Table 2 (continued)

Plant	part	Compound	Bibliography
P. subscanden	Leaves	Premnaodoroside G, **131h** Premcorymboside A, **132h** 4,4′-Dimethoxy-β-truxinic acid catalpol diester, **133h** Verbascoside, **141j** Premnethanoside A, **143j** Premnethanoside B, **144j** Decaffeoylacteoside, **151j** Benzyl alcohol β-D-(2′-*O*-β-D-xylopyranosyl)glucopyranoside, **152j** Phenethyl alcohol β-D-(2′-*O*-β-D-glucopyranosyl) glucopyranoside, **153j** 7-(3,5-Dihydroxy-1,1,5-trimethylcyclohexylidene)-9-methylprop-8-enyl 9-*O*-β-D-glucopyranoside, **163m** 3-Hydroxy-5,6-epoxy-β-ionol 9-*O*-β-D-glucopyra noside, **164m** Premnaionoside, **165m**	Sudo *et al.*, 1997, 1998, 1999
P. szemaoensis		Linarigenin, **68f** 5,4′-Dihydroxy-7-methoxy flavonol, **70f** 5,3′-Dihydroxy-7,4′-di methoxyflavonol, **71f** 3′,4′,5-Trihydroxy-3,7-di methoxyflavone, **72f**	Li *et al.*, 2008

Table 2 (continued)

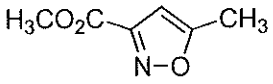
Plant	part	Compound	Bibliography
P. szemaoensis	Leaves	5-Hydroxy-3′,4′,6,7-tetra methyoxyflavone, **73f** 3,7,3′-Trimethylquercetin, **75f** 5-Hydroxy-7,3′,4′-tri methyoxy flavonol, **79f**	Li *et al.*, 2008
P. tomentosa	Heart wood	Vicenin 3, **87f**	Jyotsna *et al.*, 1984
	Leaves	Myricetin-3′,4′,7-trimethyl ether, **77f** Premnones A, **45d** Premnones B, **46d** Premnones C, **47d** 5,3′-Dihydroxy-3,7,4′,5′-tetra methoxyflavone, **74f** 3,7,3′-Trimethylquercetin, **75f** 5,3′-Dihydroxy-3,6,7,4′,5′ pentamethoxyflavone, **76f** Chrysosplenetin, **78f** 2α-Hydroxyursolic acid, **170n** Ursolic acid, **169n** 3-*epi*-Corosolic acid lactone, **171n**	Balakrishna *et al.*, 2003 Chin *et al.*, 2006
	Roots	4-(4-Methoxyphenyl)-2-butanone, **9b** Syranzaldehyde, **10b** Acetoxy syranzaldehyde, **11b**	Ayinampudi *et al.* 2012

Table 2 (continued)

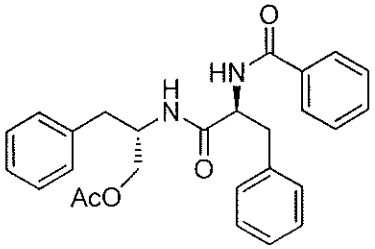
Plant	**part**	**Compound**	**Bibliography**
P. tomentosa	Roots	Coniferaldehyde, **12b** Icetexatriene-1, **52d** Icetexatriene-2, **53d** Lupeol, **166n** Betulin, **167n**	Ayinampudi *et al.* 2012
	Stem bark	4-(4-Methoxyphenyl)-2-butanone, **9b** Syringaldehyde, **10b** Coniferaldehyde, **12b** Icetexane-1, **48d** Icetexane-2, **49d** Icetexatriene-3, **50d** Icetexane-4, **51d** Lupeol, **166n** Betulin, **167n**	Hymavathi *et al.*, 2009

Structures

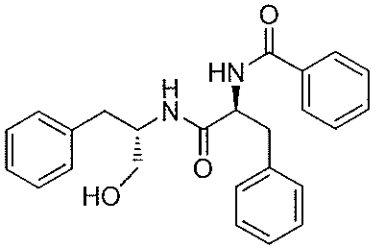
a: alkaloids



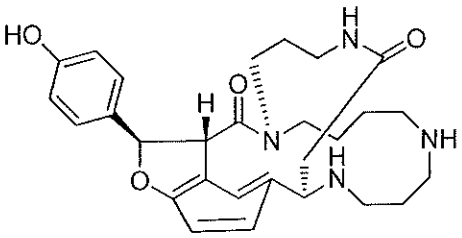
1a: Premnazole



2a: Saropeptate

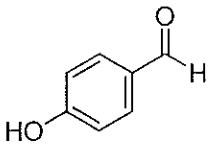


3a: Aurantiamide

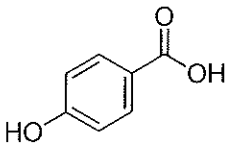


4a: Aphelandrine

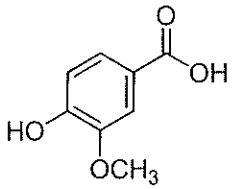
b: benzenoids



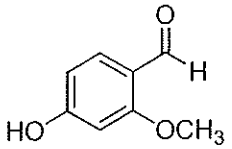
5b: 4-Hydroxybenzaldehyde



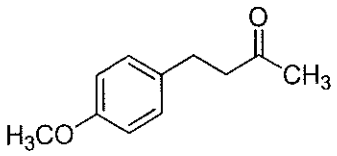
6b: *p*-Hydroxybenzoic acid



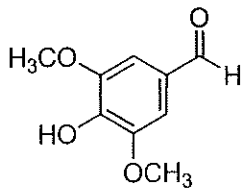
7b: Vanillic acid



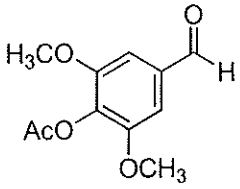
8b: 4-Hydroxy-2-methoxybenzaldehyde



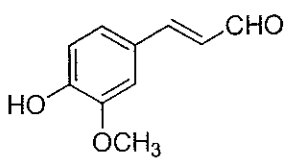
9b: 2-(4-Methoxyphenyl)-2-butanone



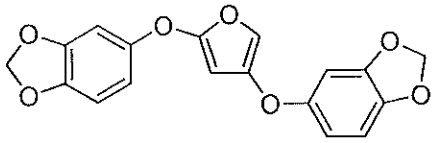
10b: Syrangaldehyde



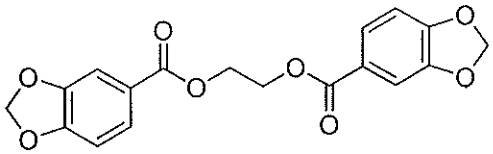
11b: Acetoxy syrangaldehyde



12b: Coniferaldehyde

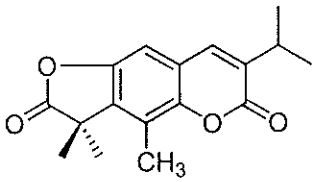


13b: Premnalatin



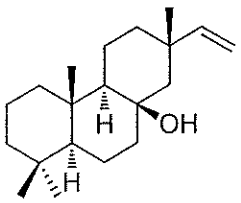
14b: 1,3-Benzodioxole-5-carboxylic acid, 1,2-ethanediyl ester

c: coumarins

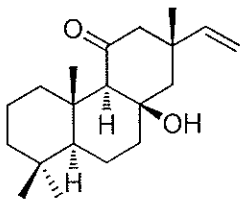


15c: Pygmaeoherin

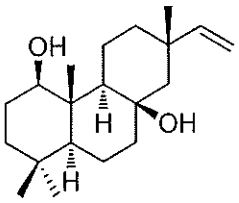
d: diterpenoids



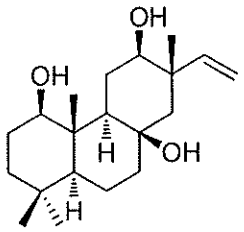
16d: Sandaracopimar-15-en-8β-ol



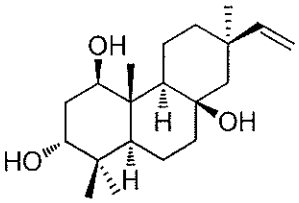
17d: 11-Ketosandaracopimar-15-en-18β-ol



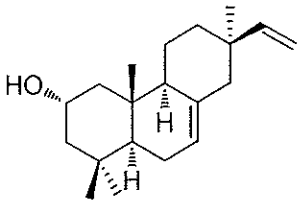
18d: Sandaracopimar-15-en-1β,8β-diol



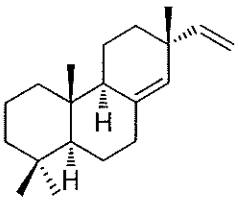
19d: Sandaracopimaran-1β,8β,12β-triol



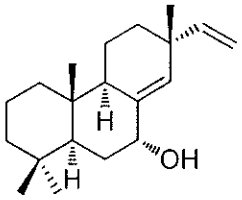
20d: 1β,3α,8β-Trihydroxy-pimara-15-ene



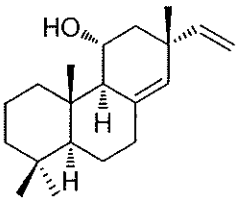
21d: 2α,19-Dihydroxy-pimara-7,15-diene



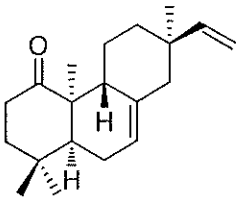
22d: Sandaracopimaradiene



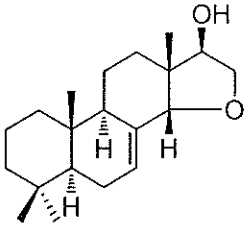
23d: 7α-Hydroxysandaracopimar-8(14),15-diene



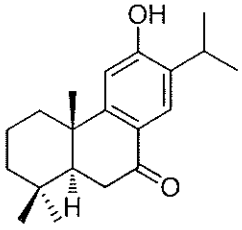
24d: 11β-Hydroxy-7-ketosanda racopimar-8(14),15-diene



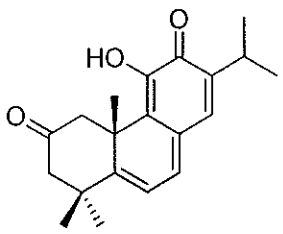
25d: Sandaracopimar-8(14),15-diene, caryophyllen-3-one



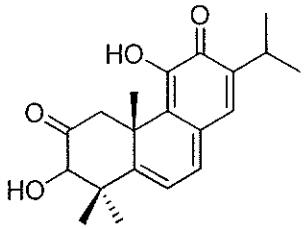
26d: Premnenol



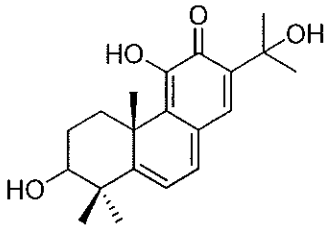
27d: Sugiol



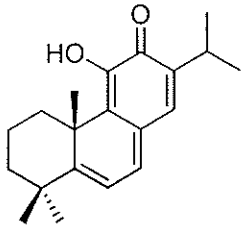
28d: 11-Hydroxy-5,7,9,13-abietatetraene-2,12-dione



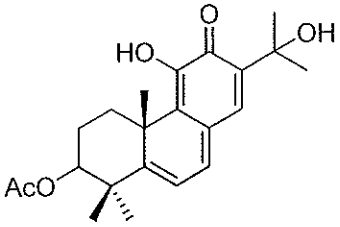
29d: Isobharangin



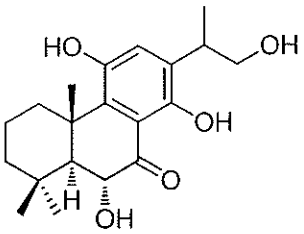
30d: Fuerstione



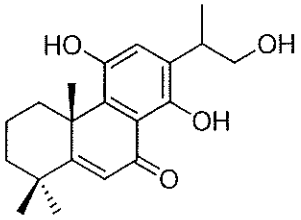
31d: 15-Deoxyfuerstione



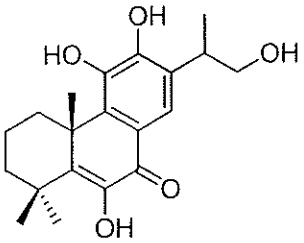
32d: 3β-Acetoxyfuerstione



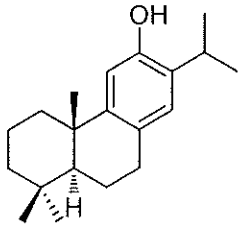
33d: Nellionol



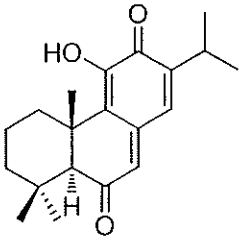
34d: Anhydronelliono



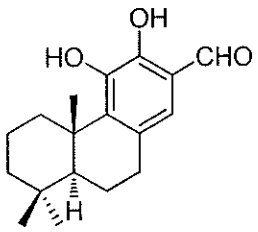
35d: 15-Dehydronellionol



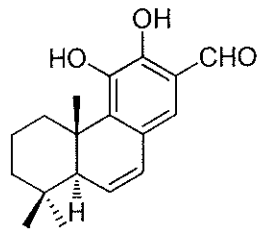
36d: Ferruginol



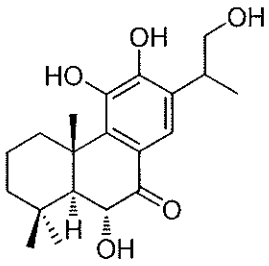
37d: Taxodione



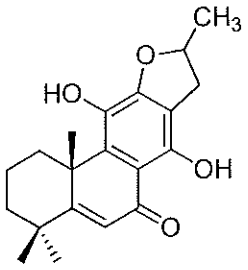
38d: Premnolal



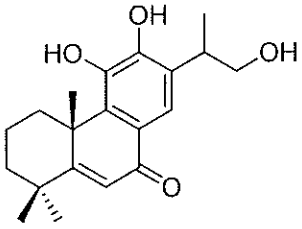
39d: Dehydropremnolal



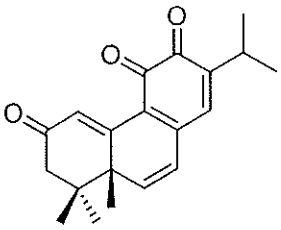
40d: 6α,11,12,16-Tetrahydroxy-7-oxo-abieta-8,11,13-triene



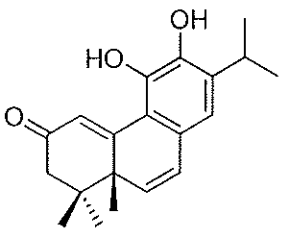
41d: 11,14-Dihydroxy-12,16-epoxyabieta-5,8,11,13-tetraene-7-one



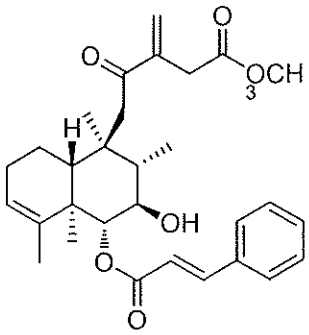
42d: 11,12,16-Trihydroxyabieta-5,8,11,13-tetraen-7-one



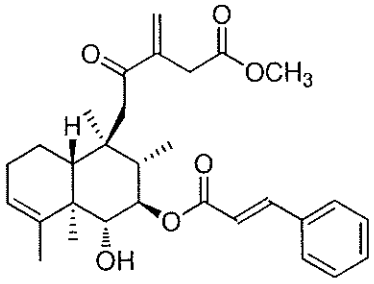
43d: Pygmaeocins B



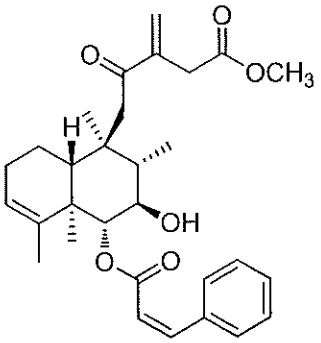
44d: Pygmaeocins C



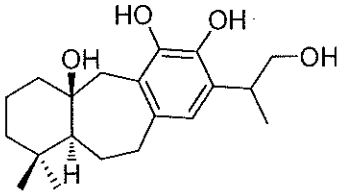
45d: Premnone A



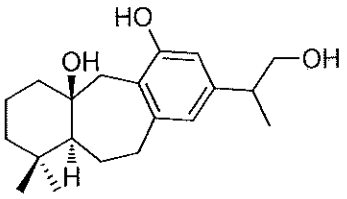
46d: Premnone B



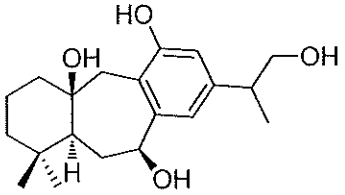
47d: Premnone C



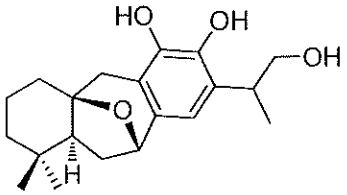
48d: Icetexane-1



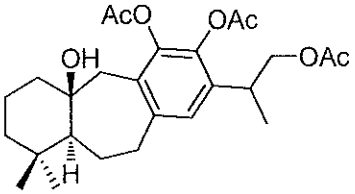
49d: Icetexane-2



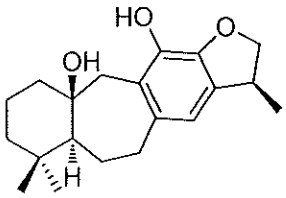
50d: Icetexatriene-3



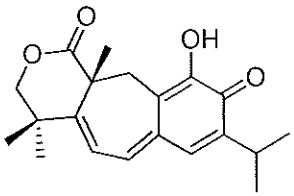
51d: Icetexane-4



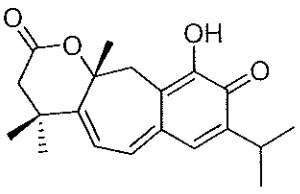
52d: Icetexatriene-1



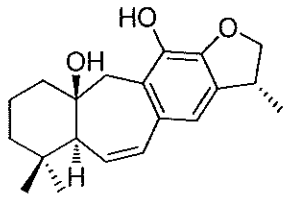
53d: Icetexatriene-2



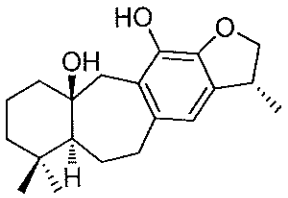
54d: Neobharangin



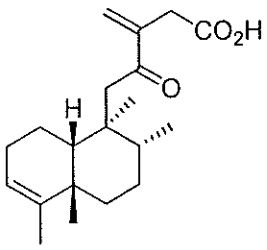
55d: Bharangin



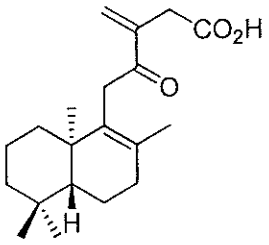
56d: Latifolionol



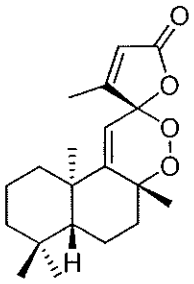
57d: Dihydrolatifolionol



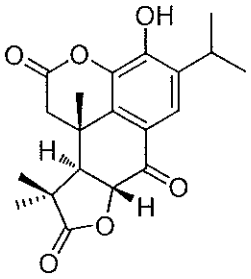
58d: (5*R*,8*R*,9*S*,10*R*)-12-Oxo-*ent*-3, 13(16)-clerodien-15-oic acid



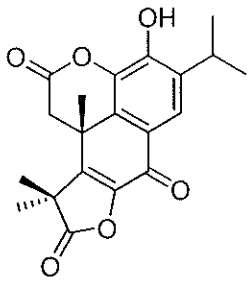
59d: *ent*-12-Oxolabda-8,13(16)-dien-15-oic acid



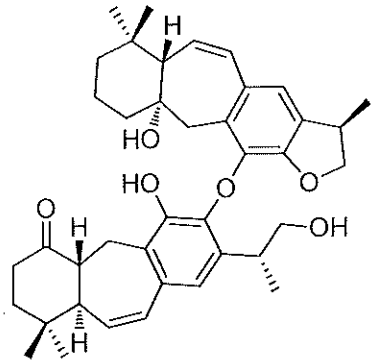
60d: *ent*-8β,12α-Epoxy-12β-hydroperoxy labda-9(11),13-dien-15-oic acid



61d: Pygmaeocin A

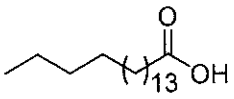


62d: 5,6-Didehydropygmaeocin A

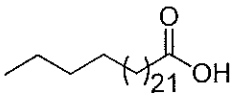


63d: Premnalatifolin A

e: fatty acids

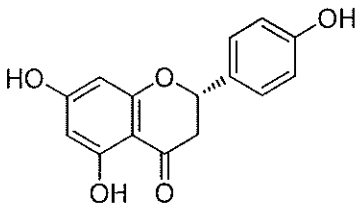


64e: Stearic acid

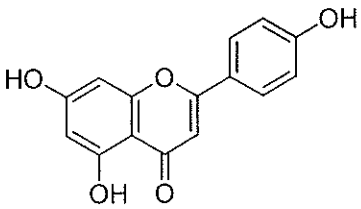


65e: Hexacosanoic acid

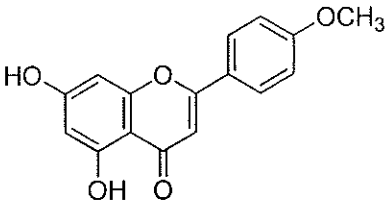
f: flavonoids



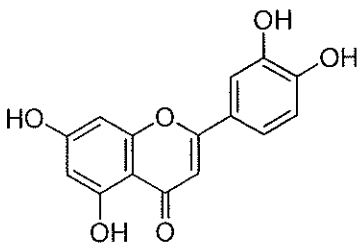
66f: Naringenin



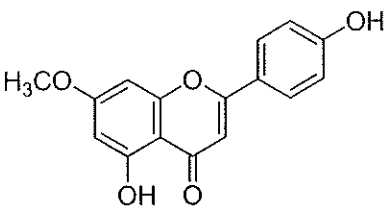
67f: Apigenin



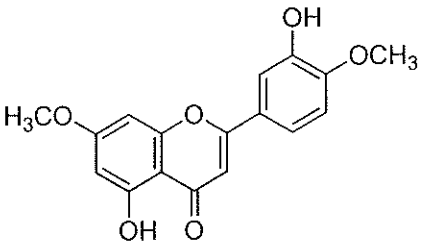
68f: Linarigenin



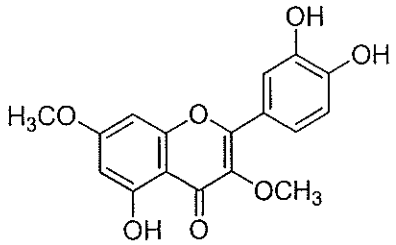
69f: Luteolin



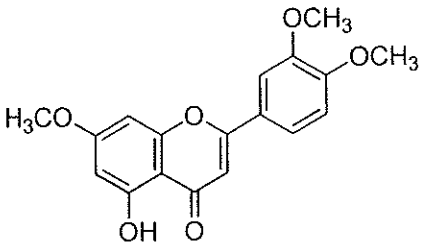
70f: 5,4′-Dihydroxy-7- methoxyflavonol



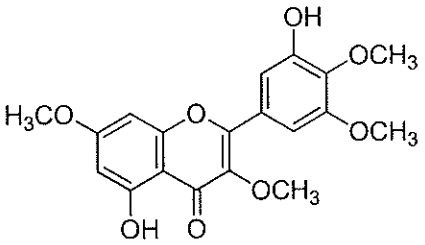
71f: 5,3′-Dihydroxy-7,4′-dimethoxyflavonol



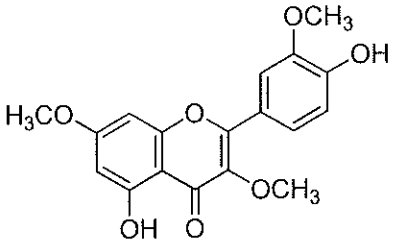
72f: 3′,4′,5-Trihydroxy-3,7-dimethoxyflavone



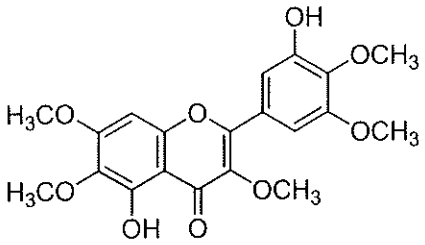
73f: 5-Hydroxy-7,3′,4′-trimethyoxyflavonol



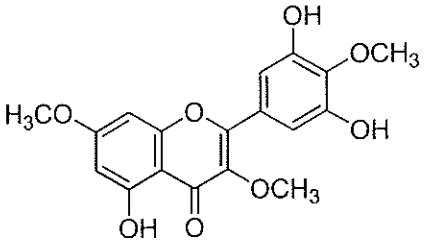
74f: 5,3′-Dihydroxy-3,7,4′,5′-tetramethoxyflavone



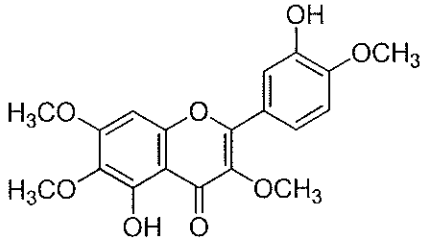
75f: 3,7,3′-Trimethylquercetin



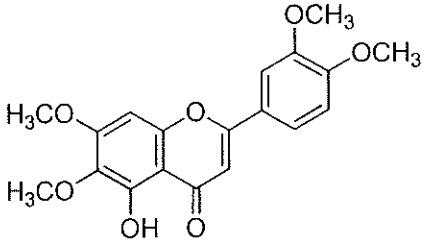
76f: 5,3′-Dihydroxy-3,6,7,4′,5′-pentamethoxyflavone



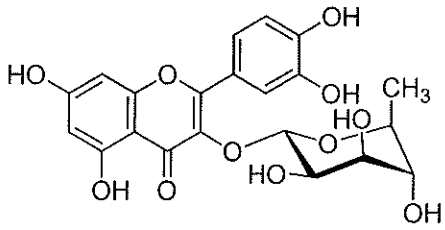
77f: Myricetin-3′,4′,7-trimethyl ether



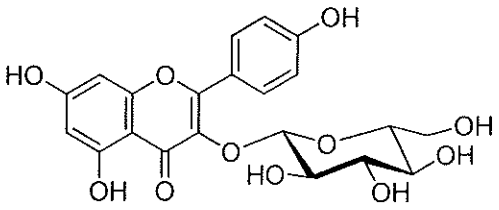
78f: Chrysosplenetin



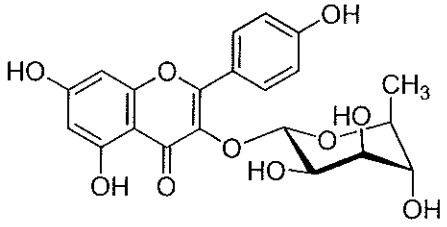
79f: 5-Hydroxy-3′,4′,6,7-tetramethoxyflavone



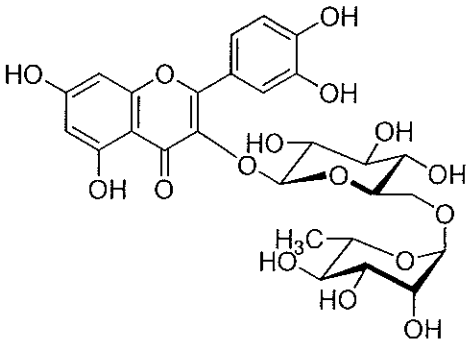
80f: Quercetin 3-*O*-α-L-rhamnopyranoside



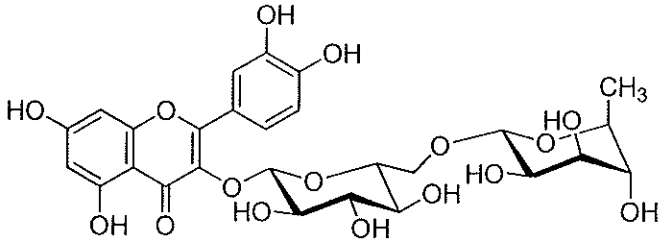
81f: Kaempferol 3-*O*-β-D-glucopyranoside



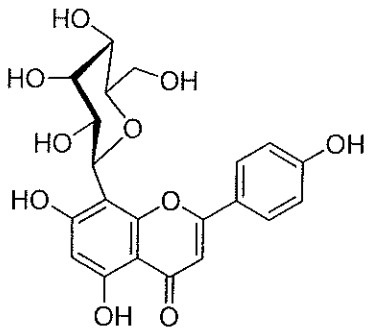
82f: Afzelin



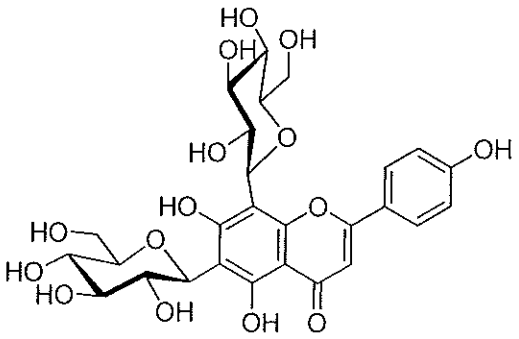
83f: Rutin



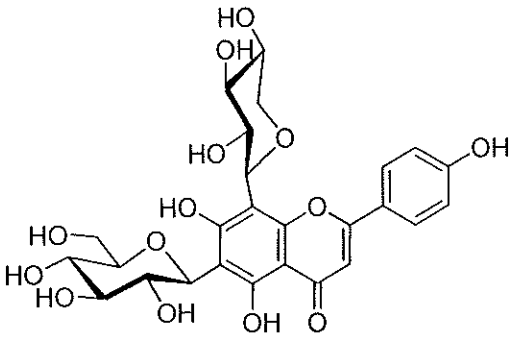
84f: Quercetin 3-rutinoside



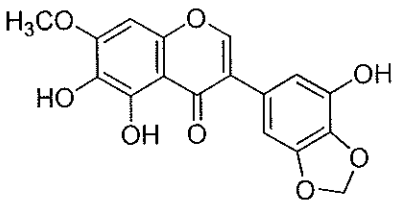
85f: Vitexin



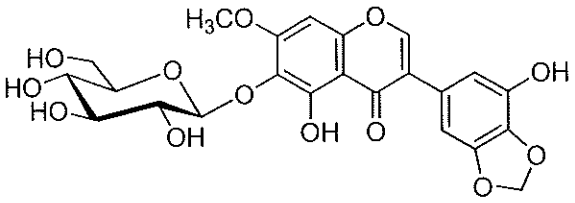
86f: Vicenin 2



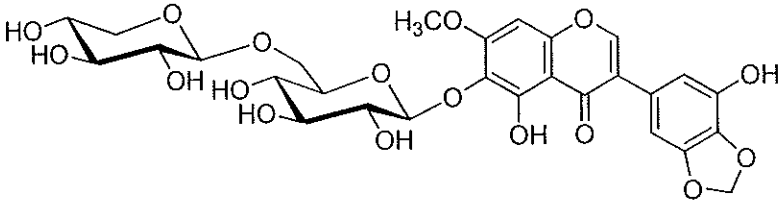
87f: Vicenin 3



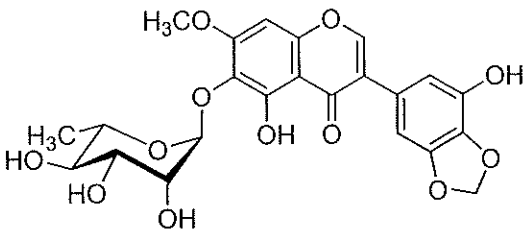
88f: 6,3′-Dihydroxy-7-methoxy-4′,5′-methylenedioxyisoflavone



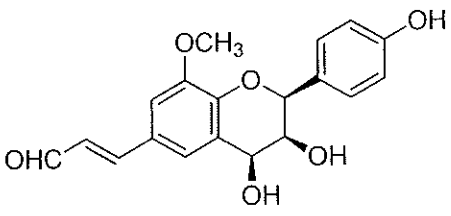
89f: 6,3′-Dihydroxy-7-methoxy-4′,5′-methylenedioxyisoflavone 6-*O*-β-D-glucopyranoside



90f: 6-3′-Dihydroxy-7-methoxy-4′,5′-methylenedioxyisoflavone 6-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranoside

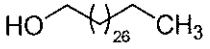


91f: 6,3′-Dihydroxy-7-methoxy-4′,5′-methylenedioxyisoflavone 6-*O*-α-L-rhamnopyranoside

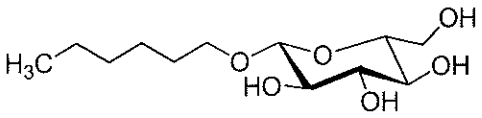


92f: 4'-Hydroxy-8,3'-dimethoxy-6-acroleinylflavan-3,4-diol

g: hydrocabons

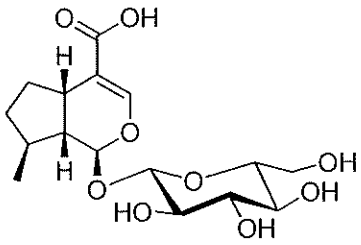


93g: Triacontanol

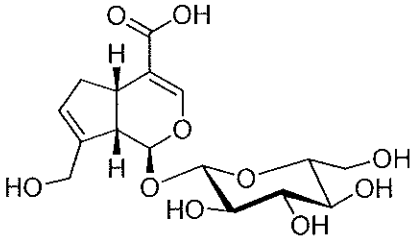


94g: Hexyl glucoside

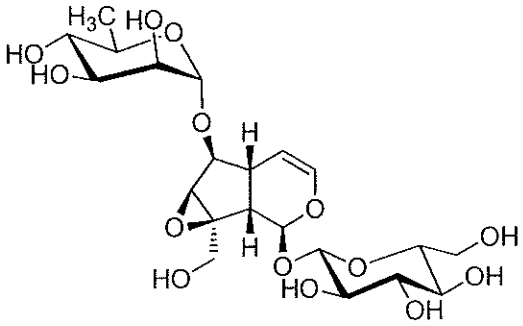
h: iridoids



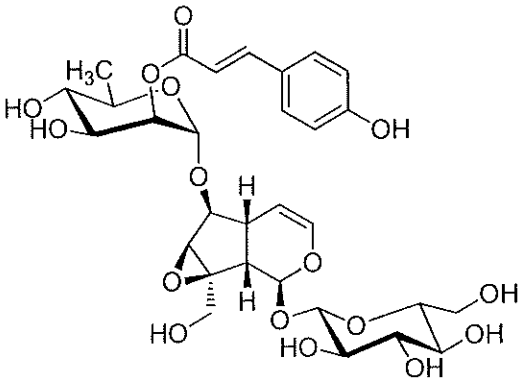
95h: Glucoside-B



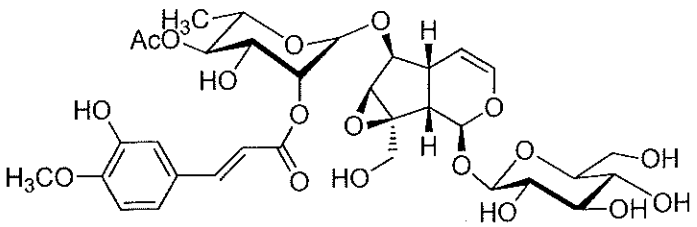
96h: Glucoside-C



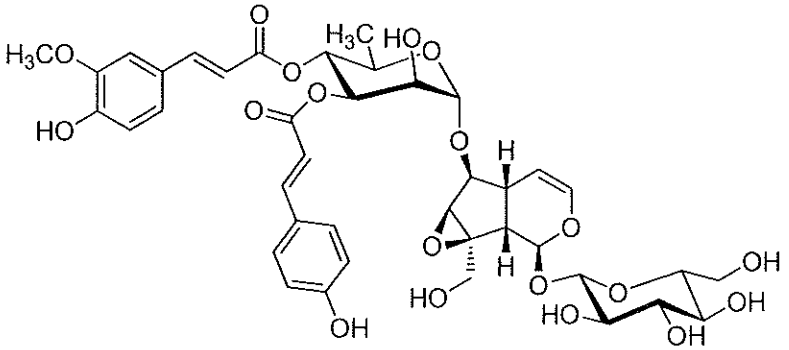
97h: Premnoside C



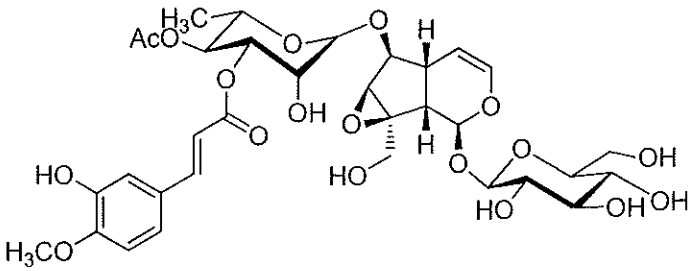
98h: Saccatoside



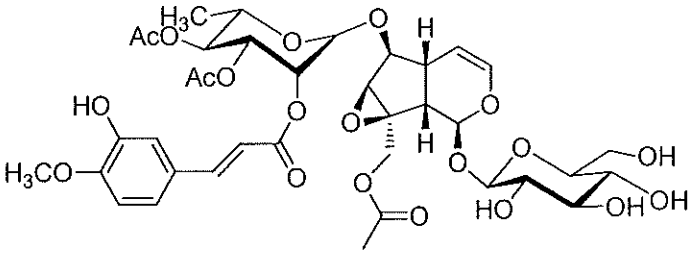
99h: 6-*O*-α-L-(2″-*O*-Isoferuloyl, 4″-*O*-acetyl)rhamnopyranosylcatalpol



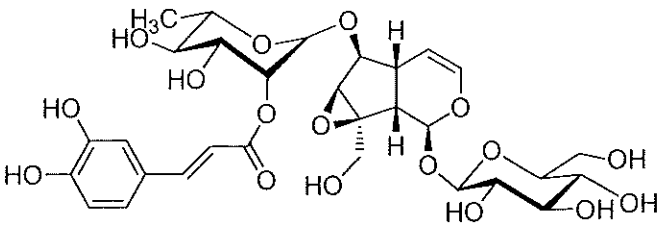
100h: Premnoside D



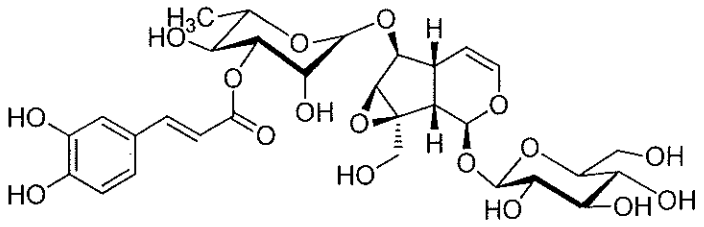
101h: 6-*O*-α-L-(3″-*O*-Isoferuloyl, 4″-*O*-acetyl)rhamnopyranosylcatalpol



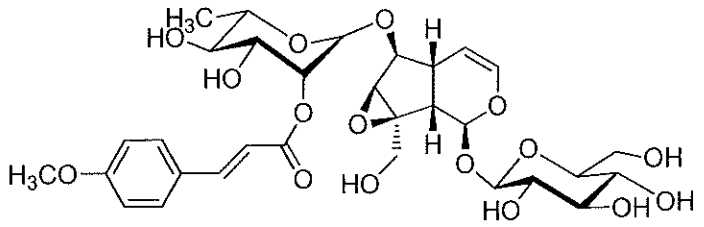
102h: 6-*O*-α-L-(4″-*O*-*trans*-*p*-Coumaroyl)rhamnopyranosylcatapols



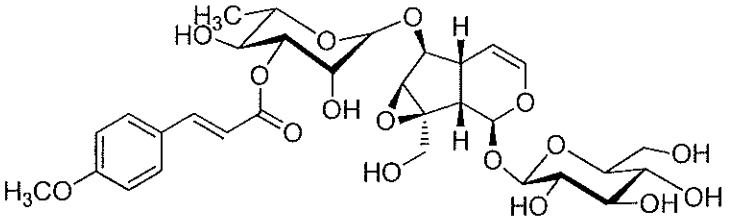
103h: 6-*O*-α-L-(2″-*O*-Caffeoyl)rhamnopyranosylcatapol



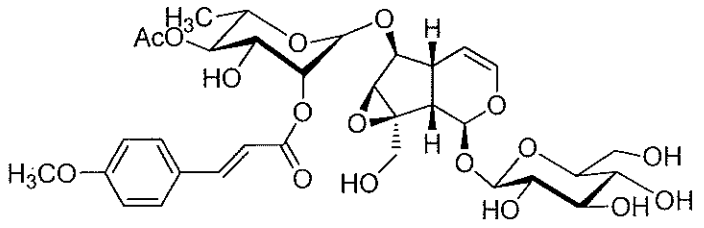
104h: 6-*O*-α-L-(3″-*O*-Caffeoyl)rhamnopyranosylcatalpol



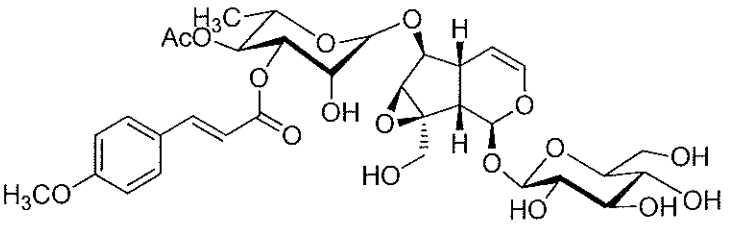
105h: 6-*O*-α-L-(2″-*O*-*p*-Methoxycinnamoyl)rhamnopyranosylcatalpol



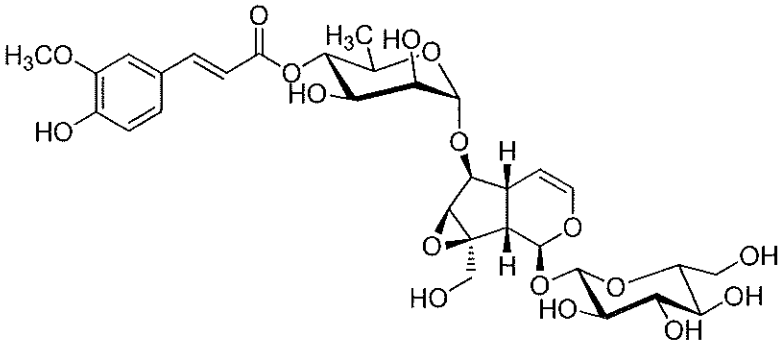
106h: 6-*O*-α-L-(3″-*O*-*p*-Methoxycinnamoyl)rhamnopyranosylcatalpol



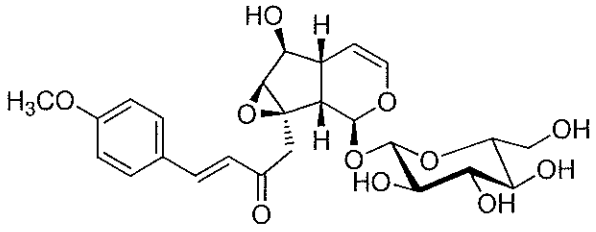
107h: 6-*O*-α-L-(2″-*O*-*p*-Methoxycinnamoyl-4-*O*-acetyl)rhamnopyranosylcatalpol



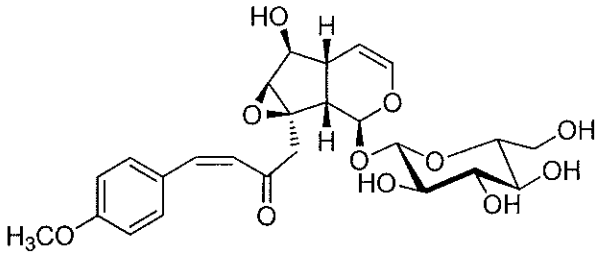
108h: 6-*O*-α-L-(3″-*O*-*p*-Methoxycinnamoyl-4″-*O*-acetyl)rhamnopyranosylcatalpol



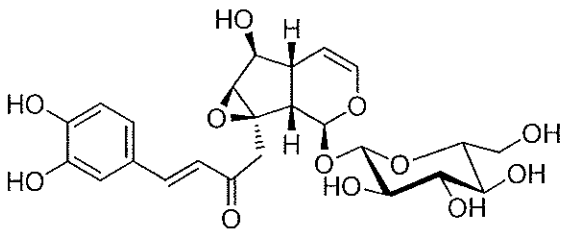
109h: 6-α-L-(4"-*O*-Feruloyl)-rhamnopyranosylcatalpol



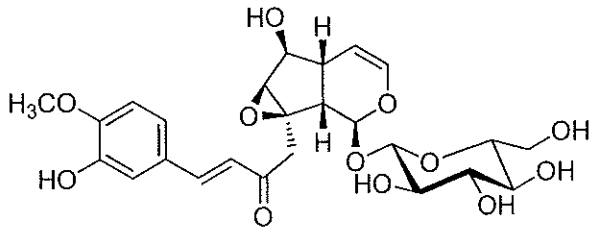
110h: 10-*O*-*trans*-*p*-Methoxycinnamoylcatalpol



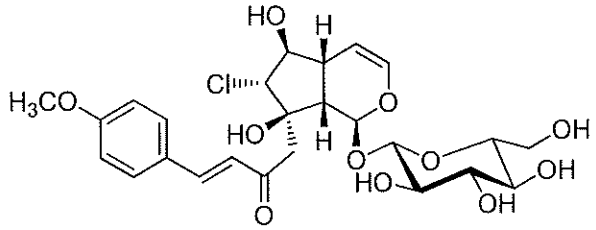
111h: 10-*O*-*cis*-*p*-Methoxycinnamoylcatalpol



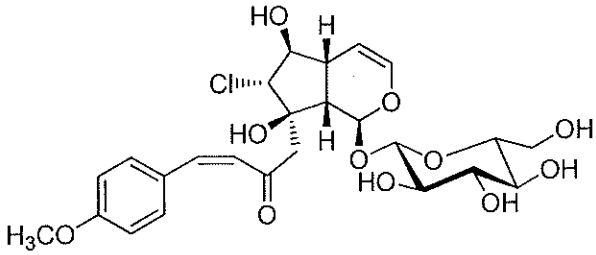
112h: 10-*O*-*trans*-Caffeoylcatalpolcatalpol



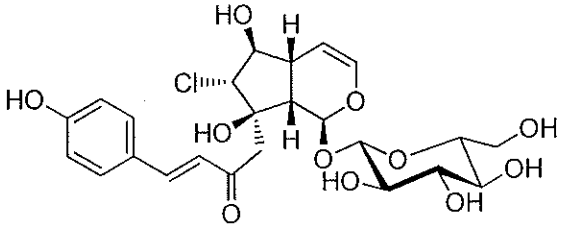
113h: 10-*O-trans*-Isoferuloylcatalpol



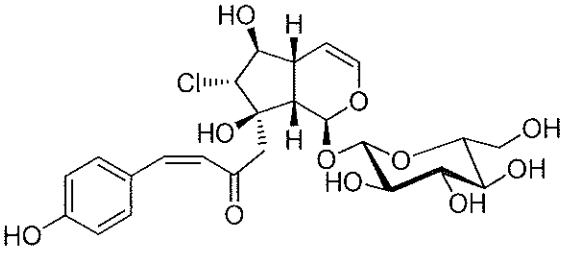
114h: 10-*O-trans-p*-Methoxycinnamoyl asystasioside E



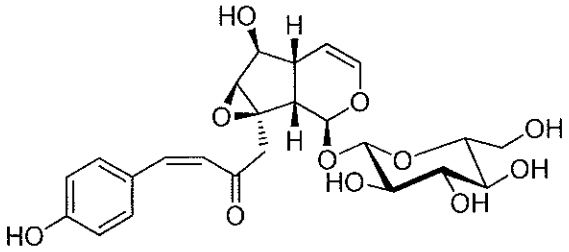
115h: 10-*O-cis-p*-Methoxycinnamoyl asystasioside E



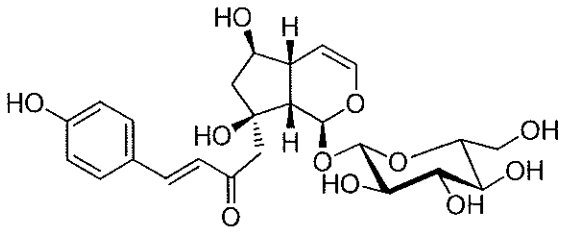
116h: 10-*O-trans-p*-Coumaroyl asystasioside E



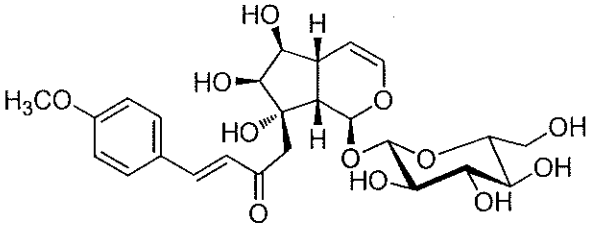
117h: 10-*O-sis-p*-Coumaroyl asystasioside E



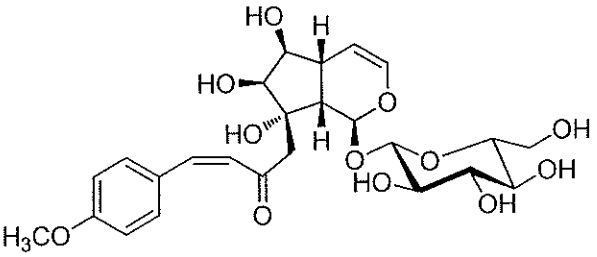
118h: 10-*O*-*cis*-*p*-Coumaroylcatalpol



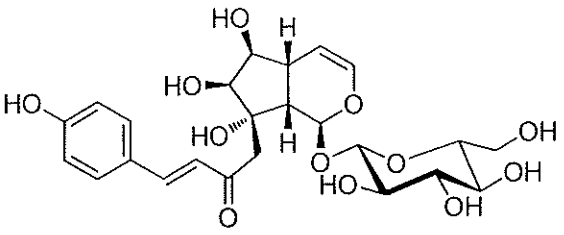
119h: Scutellarioside II



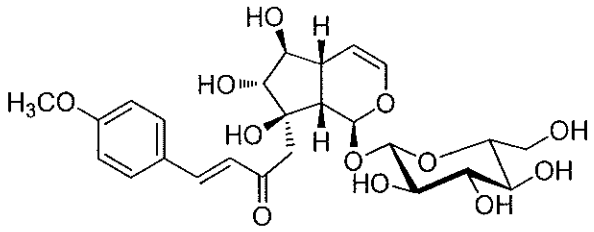
120h: 4″-Methoxy-*E*-globularinin



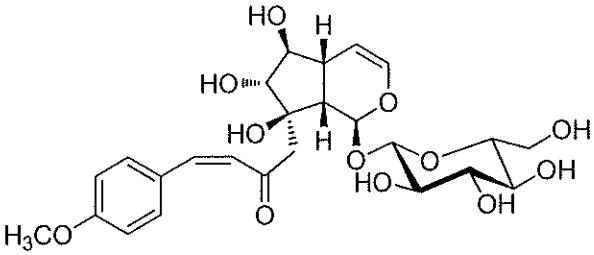
121h: 4″-Methoxy-*Z*-globularinin



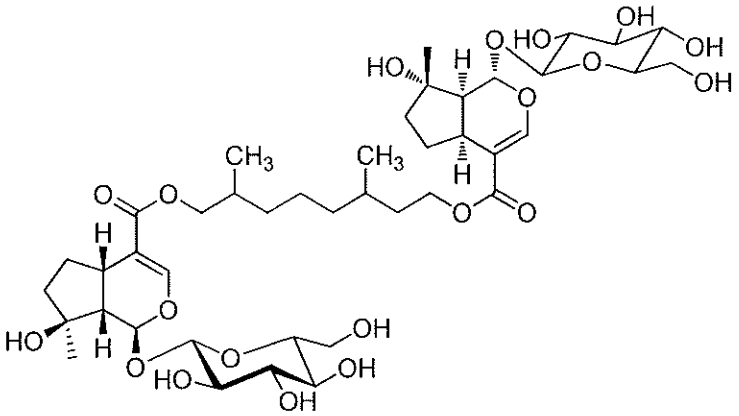
122h: 4″-Hydroxy-*E*-globularinin



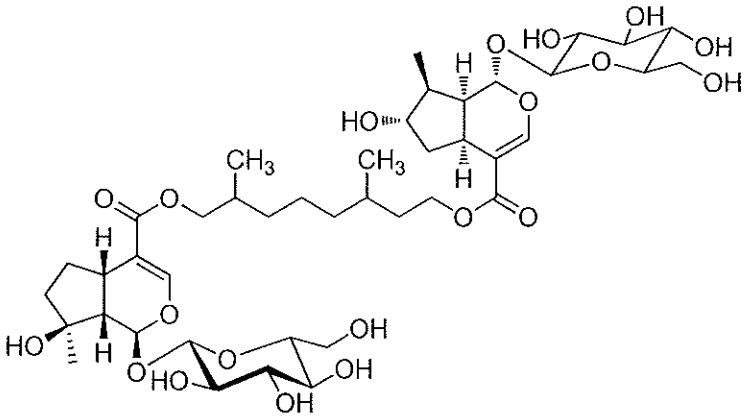
123h: 4″-Methoxy-*E*-globularimin



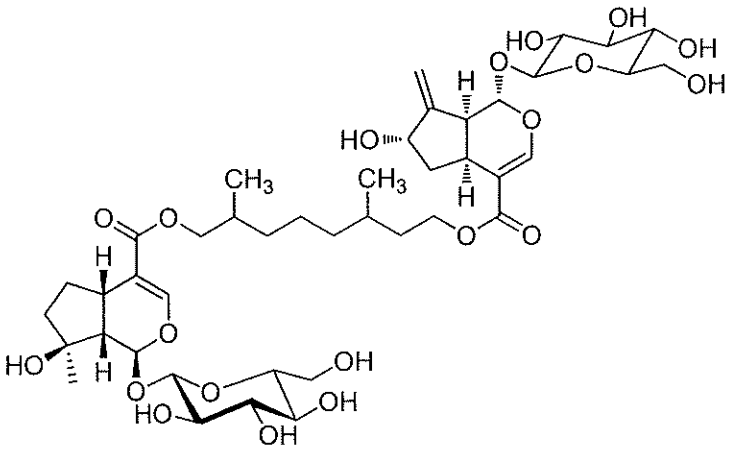
124h: 4″-Methoxy-*Z*-globularimin



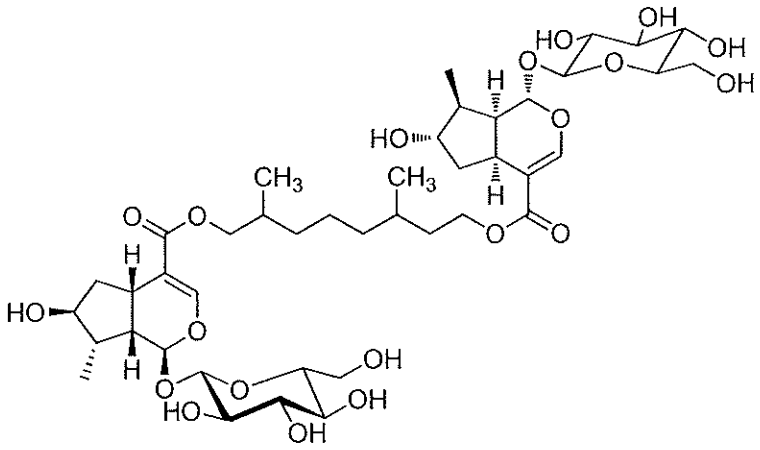
125h: Premnaodoroside A



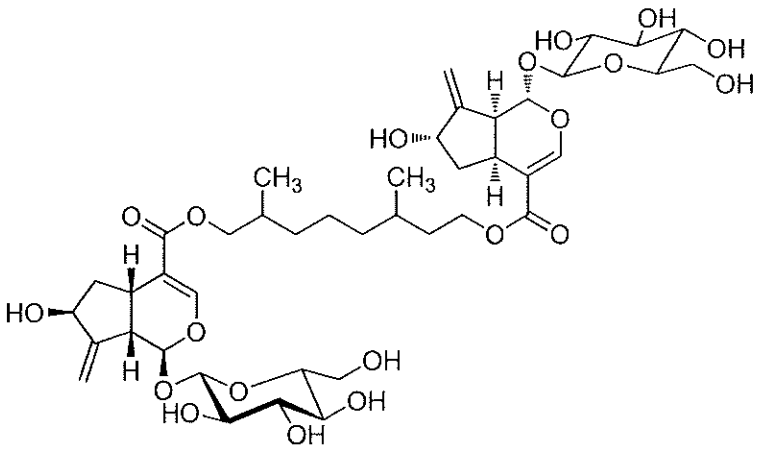
126h: Premnaodoroside B



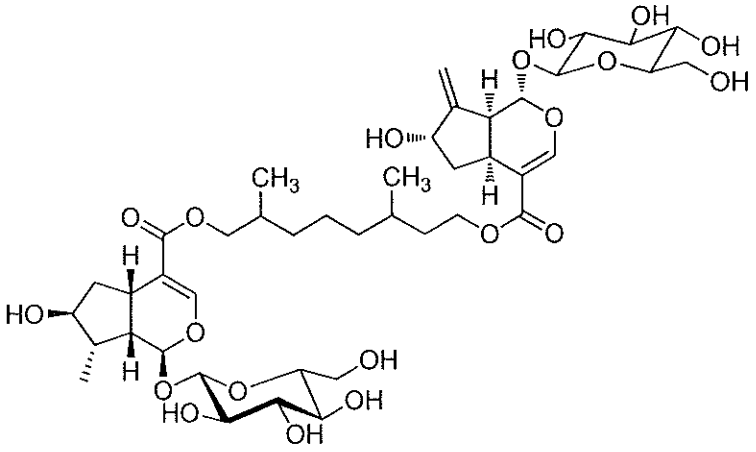
127h: Premnaodoroside C



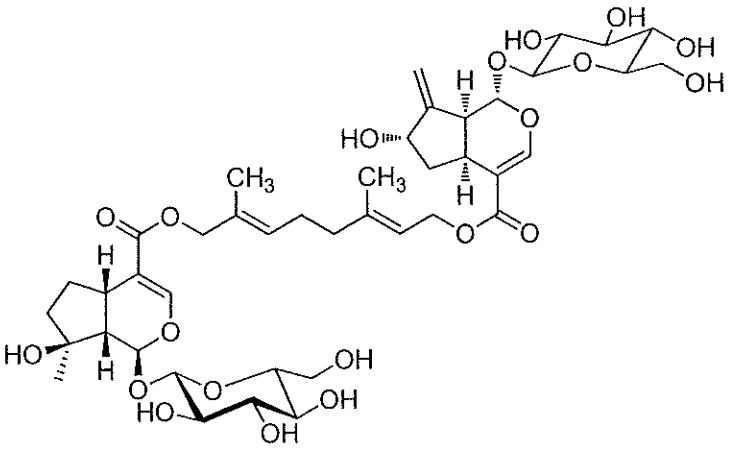
128h: Premnaodoroside D



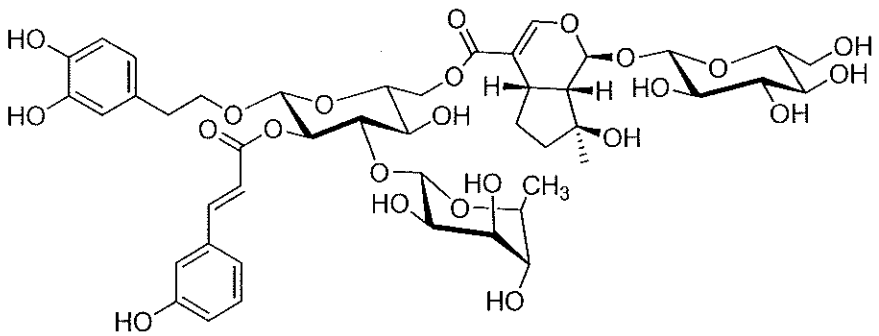
129h: Premnaodoroside E



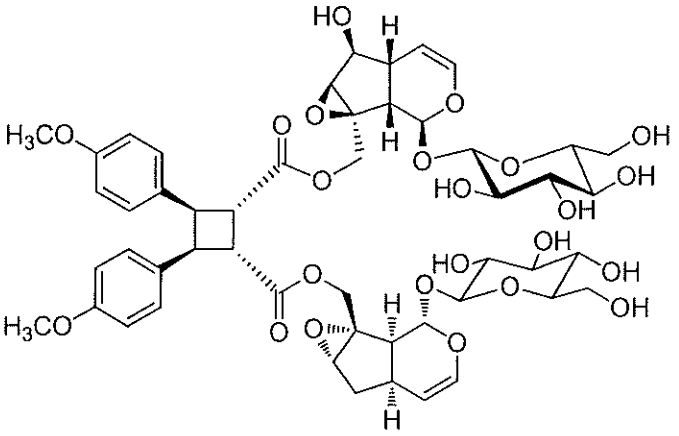
130h: Premnaodoroside F



131h: Premnaodoroside G

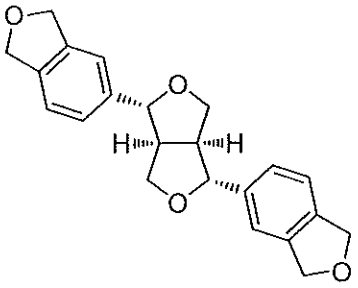


132h: Premcorymboside A

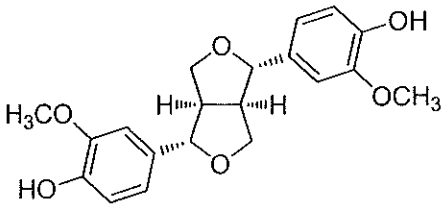


133h: 4,4′-Dimethoxy-β-truxinic acid catalpol diester

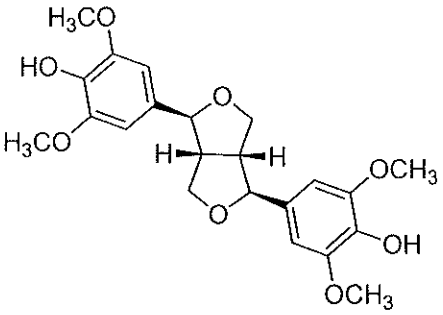
i: lignans



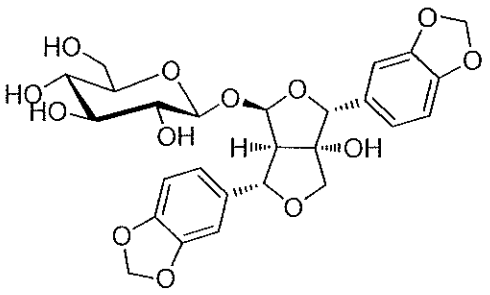
134i: (+)-Sesamin



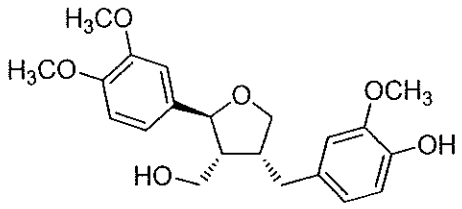
135i: (+)-1-Hydroxypinoresinol



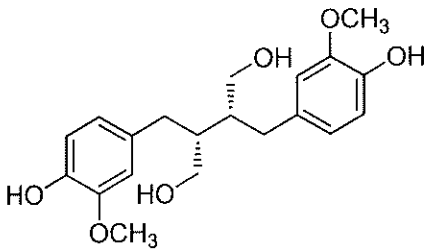
136i: Syringaresinol



137i: 4-*epi*-Gummadiol-*O*-β-D-glucopyranoside

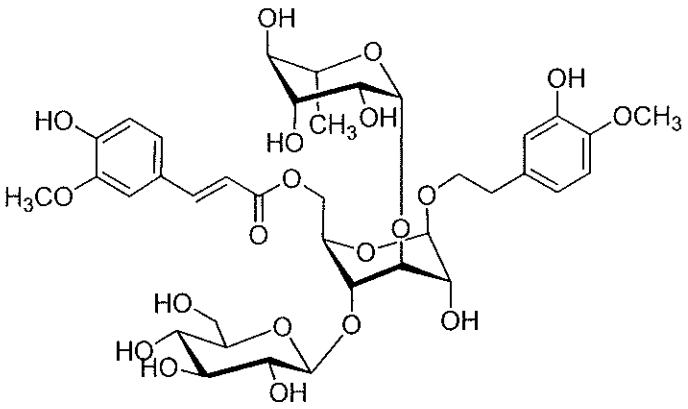


138i: (+)-Lariciresinol

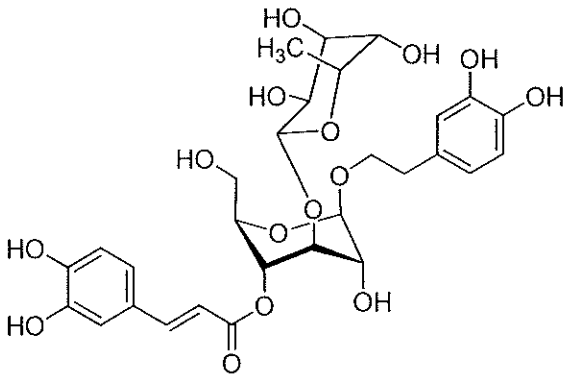


139i: (-)-*seco*-Isolariciresinol

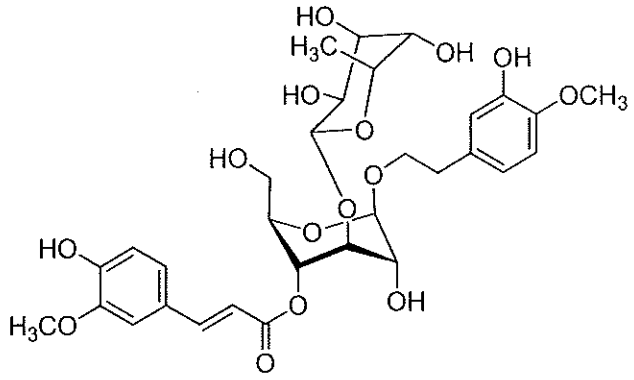
j: phenylethanoids



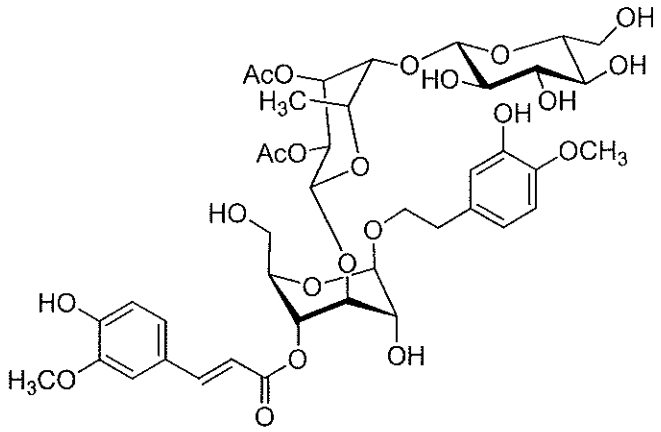
140j: Premnafolioside



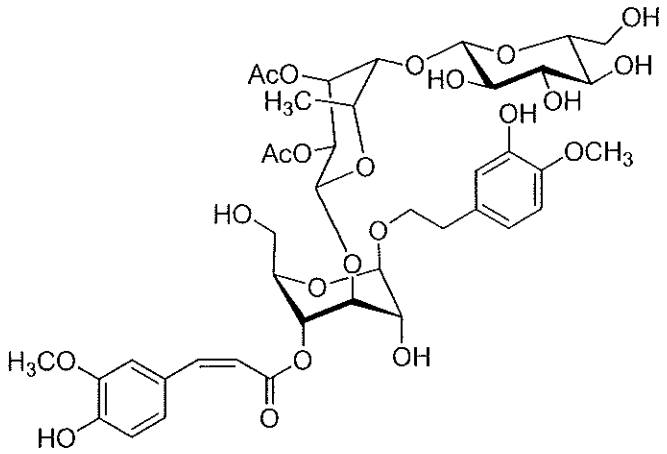
141j: Verbascoside



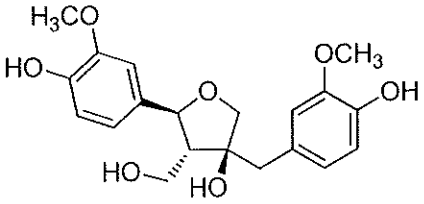
142j: Martynoside



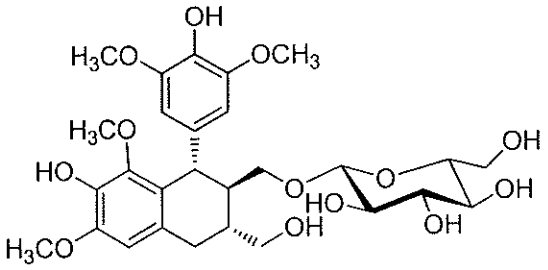
143j: Premnethanoside A



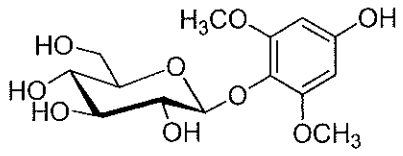
144j: Premnethanoside B



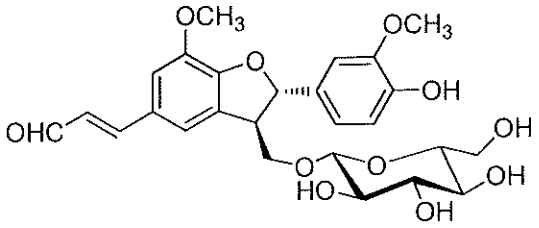
145j: (-)-Olivile



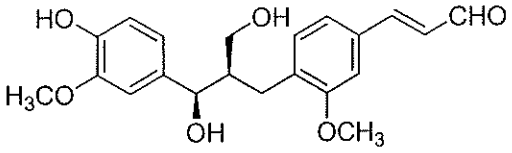
146j: Lyoniresinol 9'-β-D-glucopyranoside



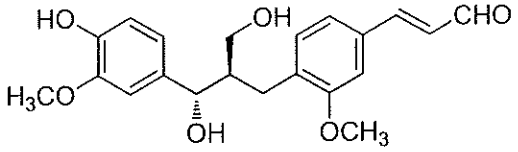
147j: Leonuriside A



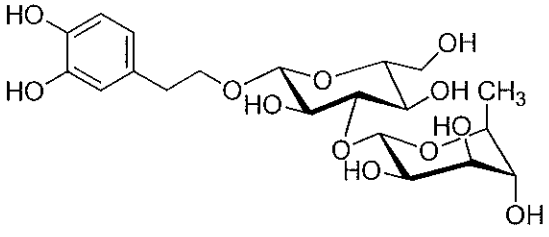
148j: Plucheoside D1



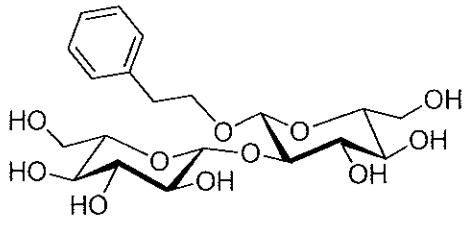
149j: *eryrhro*-(4-Hydroxy-3-methoxy phenyl)-2-{4-[2-formyl-(*E*)-vinyl]-2-methoxyphenoxy}-propan-1,3-diol



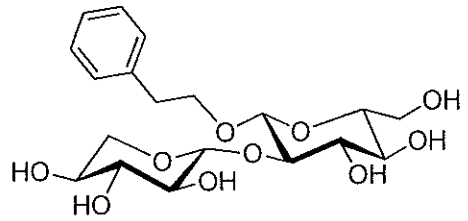
150j: *thero*-(4-Hydroxy-3-methoxy phenyl)-2-{4-[2-formyl-(*E*)-vinyl]-2-methoxyphenoxy}-propan-1,3-diol



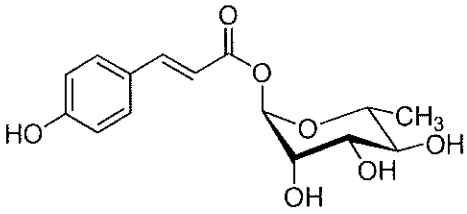
151j: Decaffeoylacteoside



152j: Benzyl alcohol β-D-(2′-*O*-β-D-xylopyranosyl)glucopyranoside

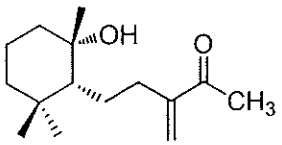


153j: Phenethyl alcohol β-D-(2′-*O*-β-D-glucopyranosyl)glucopyranoside

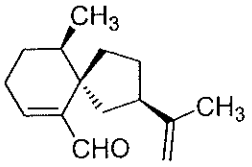


154j: 1-*O*-*trans*-*p*-Coumaroyl-α-L-rhamnopyranoside

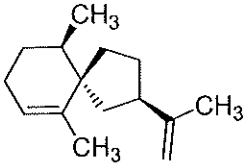
k: sesquiterpenoids



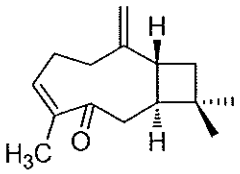
155k: 7α-Hydroxy-6,11-cyclofarnes-3(15)-en-2-one



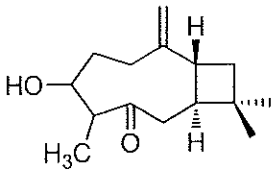
156k: Premnaspiral



157k: Premnaspirodiene

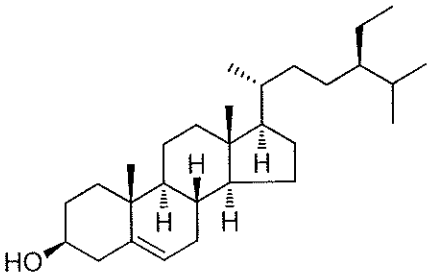


158k: Buddledin C

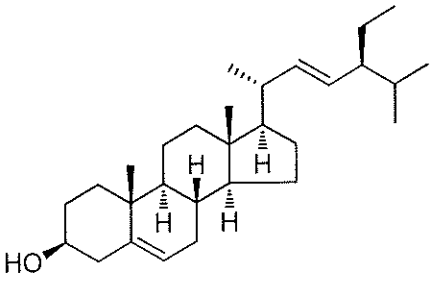


159k: 5-Hydroxy-4,5-dihydrocaryophyllen-3-one

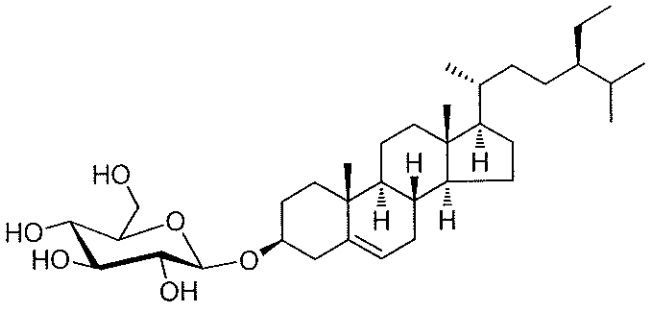
l: steroids



160l: β-Sitosterol

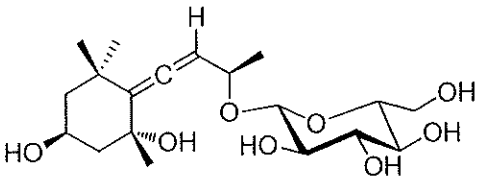


161l: Stigmasterol

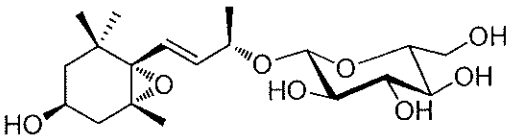


162l: β-Daucosterol

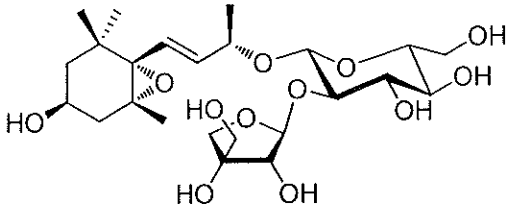
m: terpenoids



163m: 7-(3,5-Dihydroxy-1,1,5-trimethyl cyclohexylidene)-9-methylprop-8-enyl 9-*O*-β-D-glucopyranoside

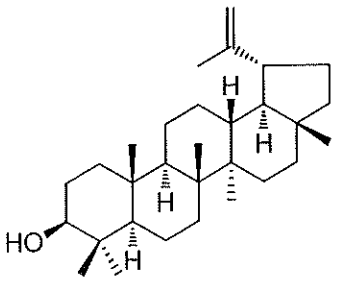


164m: 3-Hydroxy-5,6-epoxy-β-ionol-9-*O*-β-D-glucopyranoside

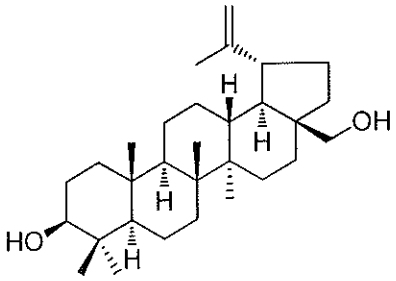


165m: Premnaionoside

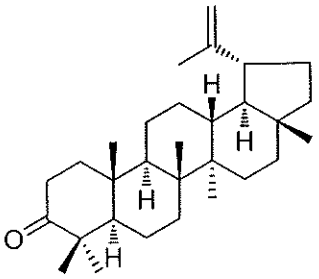
n: triterpenoids



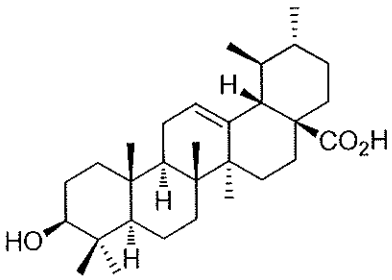
166n: Lupeol



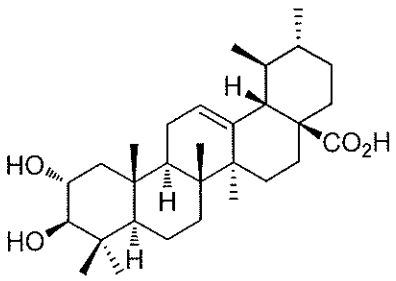
167n: Betulin



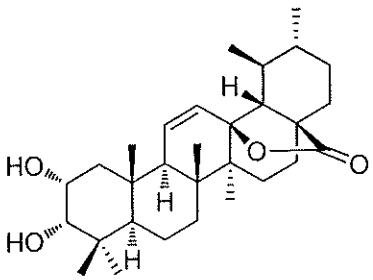
168n: Lupenone



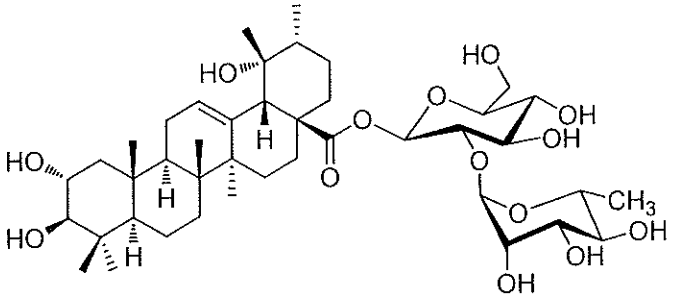
169n: Ursolic acid



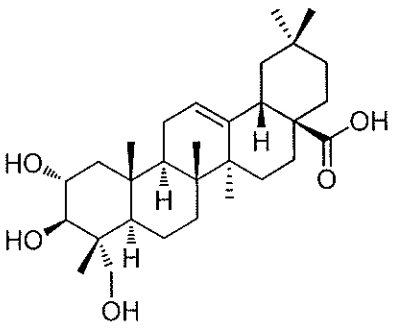
170n: 2α-Hydroxyursolic acid



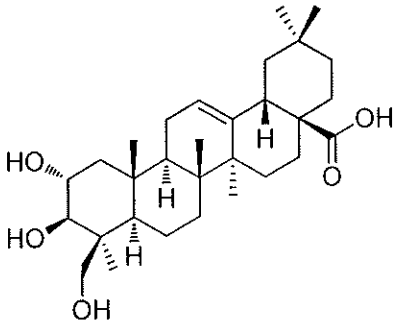
171n: 3-*epi*-Corosolic acid lactone



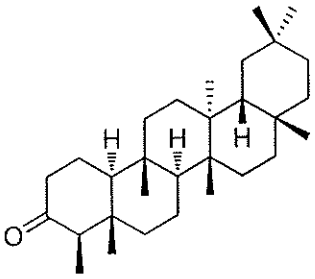
172n: 28-*O*-α-L-Rhamnopyranosyl(1→2)-β-D-glucopyranoside tormentic acid ester



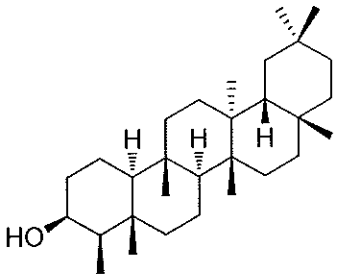
173n: Arjunolic acid



174n: Hyptatic acid A

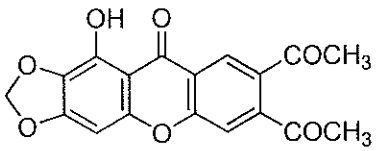


175n: Friedelin

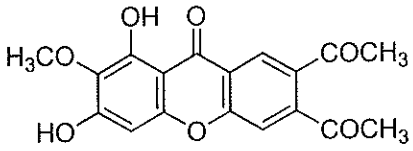


176n: β-Friedelinol

o: xanthones



177o: 1-Hydroxy-2,3-methylenedioxy-6-methoxycarbonyl-7-acetylxanthone



178o: 1,3-dihydroxy-2-methoxy-6-methoxycarbonyl-7-acetylxanthone

1.3 Objectives

Up to the present, the chemical constituents and biological activities of these plants are of interest. This research involved isolation, purification and structure elucidation of chemical constituents isolated from the roots and fruits of *Diosyros wallichii* and the roots and twigs of *Premna obtusifolia* and also evaluation of pure compounds for antibacterial and cytotoxic activities for *D. wallichii* and anti-inflammatory, and antibacterial activities for *P. obtusifolia.*

CHAPTER 2
EXPERIMENTAL

2.1 Instruments and Chemicals

Melting points were determined on the Fisher-John melting point apparatus. UV spectra were recorded on SPECORD S100 (Analytikjena), UV-160A spectrophotometer (Shimadzu) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in MeOH solution. The optical rotations $[\alpha]_D$ were measured in chloroform and methanol solution at the Sodium D line (590 nm) on a JASCO P-1020 digital polarimeter. The IR spectra were measured with a Perkin-Elmer FTS FT-IR and Shimudzu FTIR-8900 IR spectrophotometer. Single Crystal X-ray diffraction measurements were collected using SMART 1-K CDD diffractometer with monochromated Mo-Kα radiation (λ = 0.71073 A) using ω-scan mode and SHELXTL for structure solution and refinement. NMR spectra were recorded using 300, 400 and 500 MHz Bruker FTNMR Ultra Shield^{TM} spectrometers in $CDCl_3$ 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 which was analytical grade reagent. Vacuum liquid chromatography (VLC) and column chromatography (CC) were carried out on silica gel 60H (Merck) and silica gel 100 (Merck), respectively.

2.2 Plant Material

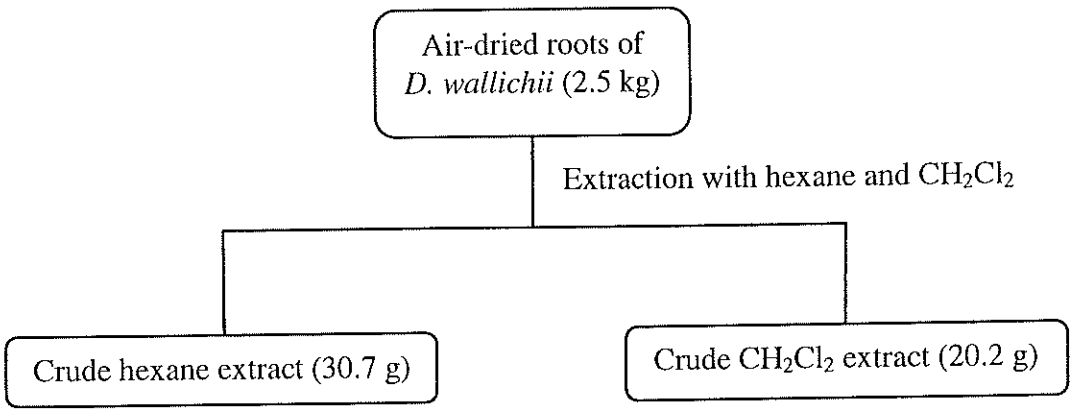
The roots and fruits of *D. wallichii* were collected in December 2008 from Satun Province in the southern part of Thailand. This plant was identified by Prof. Puangpen Sirirugsa and a voucher specimen (No. PSU 0013410) has been deposited at the Herbarium of Department of Biology, Faculty of Science, Prince of Songkla University.

Roots and twigs of *P. obtusifolia* were collected from Satun Province in the southern part of Thailand in 2008 and identified by Assoc. Prof. Dr. Kitichate Sridith, Department of Biology, Faculty of Science, Prince of Songkla University and a voucher specimen (No. PSU 0013409) has been deposited in the Herbarium of the same department.

2.3 Extractions and Isolations

2.3.1 Isolation and chemical investigation of D. wallichii

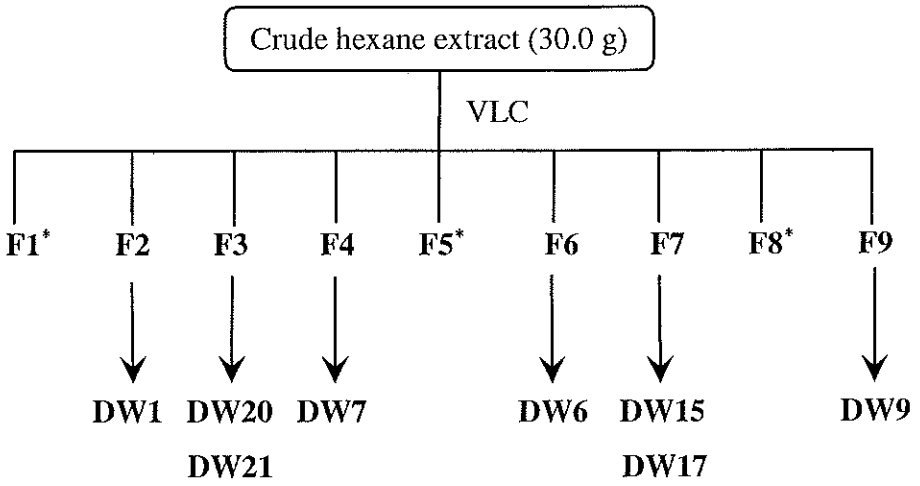
The air-dried and pulverized roots (2.5 kg) were exhaustively extracted with hexane and methylene chloride successively (2 x 10 L for each solvent for one week) at room temperature and evaporated under reduced pressure to provide crude hexane (30.7 g) and CH_2Cl_2 (20.2 g) extracts, respectively. The process of extraction was shown in **Scheme 1**.



Scheme 1 Extraction of the roots of *D. wallichii*.

2.3.1.1 Investigation of the crude hexane extract from the roots of D. wallichii

The crude hexane extract (30.0 g) was subjected to vacuum liquid chromatography (VLC) using hexane as eluent and increasing polarity with EtOAc to give nine fractions (F1-F9).



* No further investigation

Scheme 2 Isolation of compounds **DW1**, **DW6-DW7**, **DW9**, **DW15**, **DW17** and **DW20-DW21** from the hexane extract.

Fraction F2 (8.8 g) was recrystallized from methylene chloride to give **DW1** (4.3 g).

Fraction F3 (3.7 g) was separated by VLC using EtOAc-hexane (2:8, v/v) as eluting solvent to afford five subfractions (F3a-F3e). Subfraction F3b (2.9 g) was purified by VLC with EtOAc-hexane (1:9, v/v) to afford a mixture of **DW20 and DW21** (1.7 g).

Fraction F4 (3.5 g) was separated by VLC using EtOAc-hexane (1:9, v/v) as eluting solvent to afford five subfractions (F4a-F4e). Subfraction F4d (762.3 mg) was purified by VLC with EtOAc-hexane (0.5:9.5, v/v) to afford **DW7** (117.2 mg).

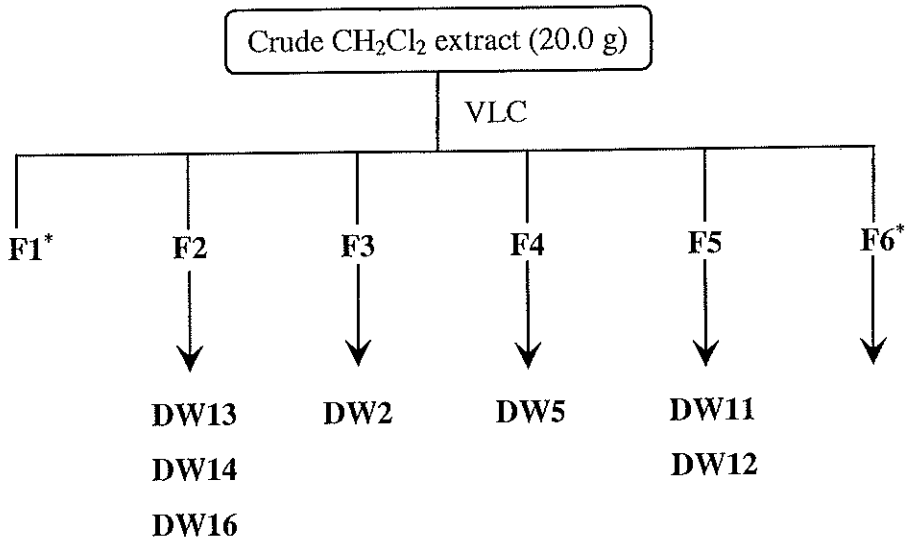
Fraction F6 (1.5 g) was separated by VLC using CH_2Cl_2-hexane (4:6, v/v) as eluting solvent to afford three subfractions (F6a-F6c). Subfraction F6c (428.6 mg) was purified by VLC with EtOAc-hexane (2:8, v/v) to give **DW6** (47.7 mg).

Fraction F7 (6.4 g) was separated by VLC with EtOAc-hexane (3:7, v/v) to afford **DW15** (3.9 g) and **DW17** (509.2 mg).

Fraction F9 (1.3 g) was separated by VLC with EtOAc-CH_2Cl_2 (1:9, v/v) to give **DW9** (156.4 mg).

2.3.1.2 Investigation of the crude CH_2Cl_2 extract from the roots of D. wallichii

The CH_2Cl_2 extract (20.2 g) was separated by VLC using hexane as eluent and increasing polarity with EtOAc to give six fractions (F1-F6).



* No further investigation

Scheme 3 Isolation of compounds **DW2**, **DW5**, **DW11-DW14** and **DW16** from the CH_2Cl_2 extract.

Fraction F2 (3.6 g) was separated by VLC with EtOAc-hexane (2:8, v/v) to afford **DW13** (35.4 mg), **DW14** (3.7 g) and **DW16** (25.6 mg).

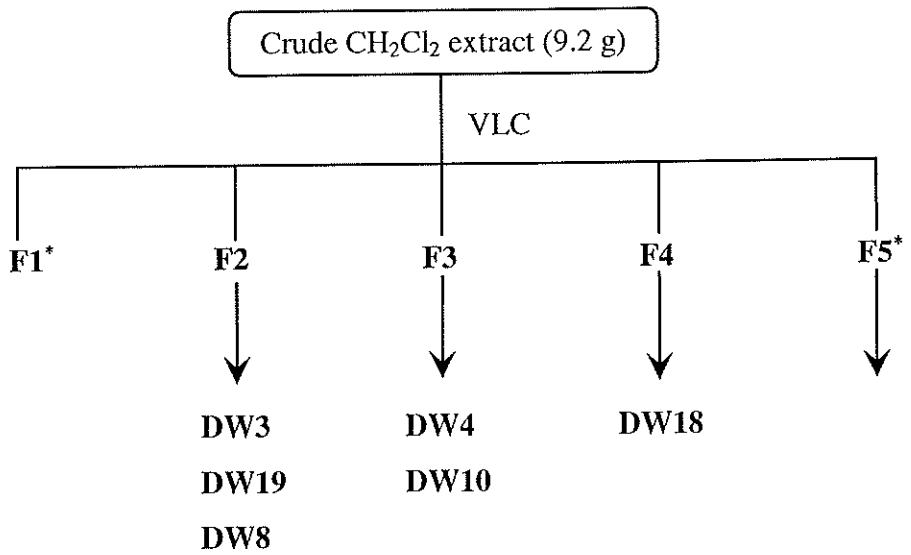
Fraction F3 (1.2 g) was separated by VLC using acetone-hexane (2:9, v/v) as eluting solvent to afford six subfractions (F3a-F3f). Subfraction F3d (352.8 mg) was purified by VLC with EtOAc-hexane (1:9, v/v) to give **DW2** (33.6 mg).

Fraction F4 (612.8 mg) was separated by VLC with Acetone-hexane (1:9, v/v) to afford **DW5** (18.4 mg).

Fraction F5 (2.3 g) was separated by VLC using EtOAc-hexane (3:7, v/v) as eluting solvent to afford four subfractions (F5a-F5d). Subfraction F5c (1.5 g) was separated by VLC using Acetone-hexane (2:8, v/v) as eluting solvent to afford five subfractions (F5c1-F5c5). Subfraction F5c3 (527.2 mg) was purified by VLC with CH_2Cl_2-hexane (4:6, v/v) to give **DW11** (40.4 mg). Subfraction F5c5 (752.3 mg) was purified by VLC with EtOAc-hexane (3:7, v/v) to give **DW12** (32.4 mg).

2.3.1.2 Investigation of the crude CH_2Cl_2 extract from the fresh fruits of D. wallichii

The fresh fruits of *Diospyros wallichii* (0.5 kg) were extracted with MeOH (2 x 2 L for one week) at room temperature. The extract was evaporated to dryness and the residue was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was evaporated and the residue (9.2 g) was subjected to VLC using hexane as eluent and increasing polarity with EtOAc to give five fractions (F1-F5).



* No further investigation

Scheme 4 Isolation of compounds **DW3-DW4, DW8, DW10, DW18** and **DW19** from the CH_2Cl_2 extract

Fraction F2 (1.3 g) was separated by VLC using EtOAc-hexane (1:9, v/v) as eluting solvent to afford five subfractions (F2a-F2e). Subfraction F2d (452.2

mg) was purified by VLC with EtOAc-hexane (0.5:9.5, v/v) to give **DW3** (19.5 mg) and **DW19** (11.7 mg). Subfraction F2e (552.3 mg) was separated by VLC using EtOAc-hexane (0.5:9.5, v/v) as eluting solvent to afford two subfractions (F2e1-F2e2). Subfraction F2e2 (359.3 mg) was purified by VLC with CH_2Cl_2-hexane (3:7, v/v) to afford **DW8** (14.7 mg).

Fraction F3 (3.3 g) was separated by VLC using Acetone-hexane (2:8, v/v) as eluting solvent to afford five subfractions (F3a-F4e). Subfraction F3a (528.1 mg) was purified by VLC with EtOAc-hexane (3:7, v/v) to give **DW4** (10.5 mg) and **DW10** (13.4 mg).

Fraction F4 (1.5 g) was separated by VLC with EtOAc-hexane (5:5, v/v) to afford **DW18** (18.9 mg).

Compound DW1, plumbagin: Orange needles; m.p. 77-79 °C; UV (MeOH) λ_{max} : 209, 264 and 413 nm; IR (thin film) υ_{max}: 3370, 1665, 1645, 1610, 1595, 875 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

Compound DW2, droserone: Orange amorphous powder; m.p. 169-172 °C; UV (MeOH) λ_{max} : 228, 278 and 400 nm; IR (thin film) υ_{max}: 3308, 1643, 1625, 1580, 1435, 1395, 1095, 835 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 5.

Compound DW3, 2,3-epoxyplumbagin: Pale yellow needles; m.p. 97-99 °C; $[\alpha]_D^{28}$ −17.4 ($CHCl_3$, *c* 0.50); UV (MeOH) λ_{max} : 254, 283 and 420 nm; IR (thin film) υ_{max}: 3407, 1655, 1635, 1614, 1577, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 7.

Compound DW4, 2-hydroxymethyl-5-methoxy-1,4-naphthoquinone: Orange powder; m.p. 145-147 °C; UV (MeOH) λ_{max} : 226, 263 and 414 nm; IR (thin film) υ_{max}: 3467, 1651, 1632, 1458, 1377, 1284 1065, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 9.

Compound DW5, diomuscinone: White amorphous solid; m.p. 93-95 °C; UV (MeOH) λ_{max} : 212 and 256 nm; IR (thin film) υ_{max}: 3430, 1640, 1600, 1575, 1490, 1082, 907 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 10.

Compound DW6, isoshinanolone: Colorless oil; $[\alpha]_D^{28}$ +42.5 ($CHCl_3$, *c* 1.00); UV (MeOH) λ_{max}: 217, 259 and 335 nm; IR (thin film) υ_{max}: 3500, 1634, 1580, 1451, 1406, 1341, 1242, 1090, 980, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 12.

Compound DW7, *epi*-isoshinanolone: Colorless oil; $[\alpha]_D^{28}$ –42.5 ($CHCl_3$, *c* 0.70); UV (MeOH) λ_{max} : 213, 256 and 334 nm; IR (thin film) υ_{max}: 3465, 1633, 1578, 1455, 1399, 1362, 1240, 1072, 996 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 14.

Compound DW8, maritinone: Red amorphous powder; m.p. 199-201 °C; UV (MeOH) λ_{max} : 211, 267 and 409 nm; IR (thin film) υ_{max}: 3489, 1661, 1635, 1615, 1583, 883 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz); EIMS *m/z* $[M]^+$ 374.1 (Calcd for $C_{22}H_{14}O_6$, 374.1), see Table 16.

Compound DW9, 5,5'-dihydroxy-2,2'-dimethyl-7,7'-binaphthalen-1,1',4,4'-tetraone: Red solid; m.p. 250 °C (decomposed); UV (MeOH) λ_{max}: 210, 270 and 415 nm; IR (thin film) υ_{max}: 3473, 1664, 1641, 1600, 1418, 1367, 1353, 1261, 774 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 18; HREIMS: *m/z* $[M]^{+\bullet}$ 374.0812 (calcd for $C_{22}H_{14}O_6$, 347.0790).

Compound DW10, methylene-3,3'-biplumbagin: Orang needles; m.p. 202-204 °C; UV (MeOH) λ_{max} : 215, 247, 275 and 410 nm; IR (thin film) υ_{max}: 3570, 2910, 1655, 1632, 1597, 1455, 1389, 1360, 1290, 1195, 960 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz); EIMS *m/z* $[M]^+$ 388.1 (Calcd for $C_{23}H_{16}O_6$, 388.1), see Table 20.

Compound DW11, 2-hydroxymethyl-1,5-dimethoxynaphthalen-4-ol: White powder; m.p. 63-65 °C; UV (MeOH) λ_{max}: 231 and 291 nm; IR (neat) υ_{max}: 3400, 1633, 1613, 1445, 1387, 1262, 1239, 1072, 996, 755, cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 23; HREIMS: *m/z* $[M]^{+\bullet}$ 234.0920 (calcd for $C_{13}H_{14}O_4$, 233.9992).

Compound DW12, 2,2'-bis-hydroxymethyl-1,1',5,5'-tetramethoxy-3,3'-binaphthalen-4,4'-diol: White amorphous powder; m.p. 115-117 °C; UV (MeOH) λ_{max}: 228 and 295 nm; IR (neat) υ_{max}: 3389, 1632, 1605, 1455, 1382, 1357, 1244, 1069, 998, 762 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 24; HREIMS: *m/z* $[M]^{+\bullet}$ 466.1622 (calcd for $C_{26}H_{26}O_8$, 466.1627).

Compound DW13, scopoletin: Yellow oil; UV (MeOH) λ_{max}: 225, 240, 245 and 346 nm; IR (thin film) υ_{max}: 3450, 1725, 1630, 1590, 1450, 1180, 940, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 25.

Compound DW14, lupeol: White solid; m.p. 193-194 °C; $[\alpha]_D^{28}$ +25.0 ($CHCl_3$, *c* 0.20); IR (KBr) υ_{max}: 3343, 2945, 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 26.

Compound DW15, lupenone: White solid; m.p. 163-165 °C; $[\alpha]_D^{28}$ +50.0 ($CHCl_3$, *c* 0.10); IR (KBr) υ_{max}: 2914, 1704, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 28.

Compound DW16, betulin: White solid; m.p. 230-231°C; $[\alpha]_D^{28}$ +16.7° ($CHCl_3$, *c* 0.150); IR (KBr) υ_{max}: 3382, 2942, 1645 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see see Table 30.

Compound DW17, betulinaldehyde: Colorless viscous oil, due to its instability, no IR was obtained. ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 32.

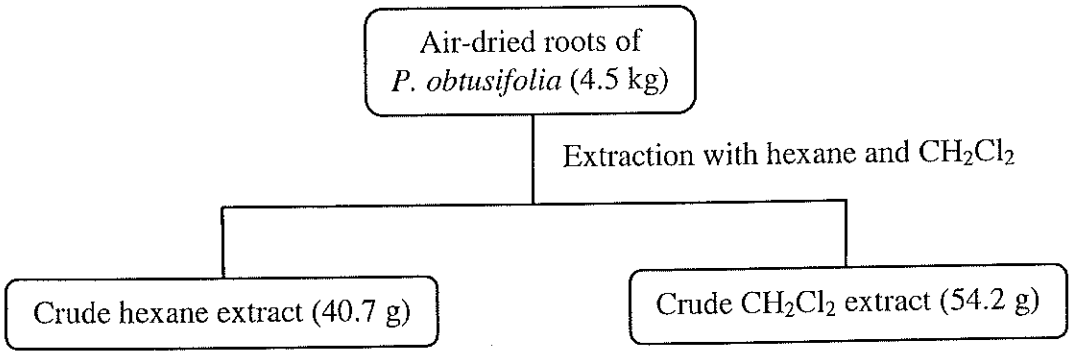
Compound DW18, betulinic acid: White solid; m.p. 279-280 °C; $[\alpha]_D^{28}$ +15.0 ($CHCl_3$, c = 0.10); IR υ_{max}: 3415, 2942, 1686, 1645 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 34.

Compound DW19, 3β,29-dihydroxyolean-12-en-28-oic acid: White amorphous powder; m.p. 334-336 °C; $[\alpha]_D^{26}$ +82.0 ($CHCl_3$; c 0.10); IR (thin film) υ_{max}: 3400, 2950, 1695, 1460, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 37.

Compound DW20, *β*-sitosterol and **DW21**, stigmasterol: White solid; IR (neat) υ_{max}: 3425, 1642 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Figure 77.

2.3.2 Isolation and Chemical Investigation of *P. obtusifolia*

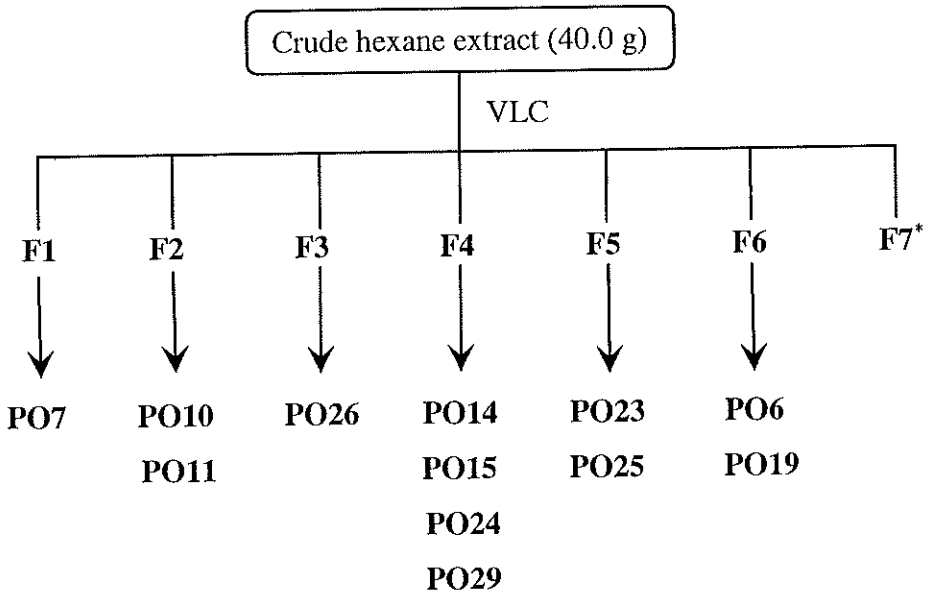
The dried roots of *P. obtusifolia* (4.5 kg) were extracted with hexane and CH_2Cl_2 successively (2 × 10 L for each solvent for one week) at room temperature and evaporated under reduced pressure to provide crude hexane (40.7 g) and CH_2Cl_2 (54.2 g) extracts, respectively. The process of extraction was shown in **Scheme 5**.



Scheme 5 Extraction of the roots of *P. obtusifolia*.

2.3.2.1 Investigation of the crude hexane extract from the roots of P. obtusifolia

The crude hexane extract (40.7 g) was subjected to vacuum liquid chromatography (VLC) using hexane as eluent and increasing polarity with EtOAc to give seven fractions (F1-F7).



* No further investigation

Scheme 6 Isolation of compounds **PO6**, **PO7**, **PO10**, **PO11**, **PO14**, **PO15**, **PO19**, **PO23-PO26** and **PO29** from the hexane extract.

Fraction F1 (3.7 g) was separated by VLC using EtOAc-hexane (0.5:9.5, v/v) as eluting solvent to give three subfractions (F1a-F1c). Subfraction F1a (501.4 mg) was purified by VLC using 100% hexane providing **PO7** (6.4 mg).

Fraction F2 (1.2 g) was separated by VLC using EtOAc-hexane (0.5:9.5, v/v) as eluting solvent to give three subfractions (F2a-F2c). Subfraction F2a (409.2 mg) was purified by VLC using 100% hexane to provide **PO10** (6.1 mg). Subfraction F2c (214.2 mg) was purified by VLC using EtOAc-hexane (1:9, v/v) to afford **PO11** (57.5 mg).

Fraction F3 (4.5 g) was separated by VLC using EtOAc-hexane (1:9, v/v) as eluting solvent to afford seven subfractions (F3a-F3g). Subfraction F3b (728.4 mg) was separated by VLC with CH_2Cl_2-hexane (3:7, v/v) as eluting solvent to afford four subfractions (F3b1-F3b4). Subfraction F3b2 (40.7 mg) was purified by VLC using CH_2Cl_2-hexane (3:7, v/v) to provide **PO26** (3.3 mg).

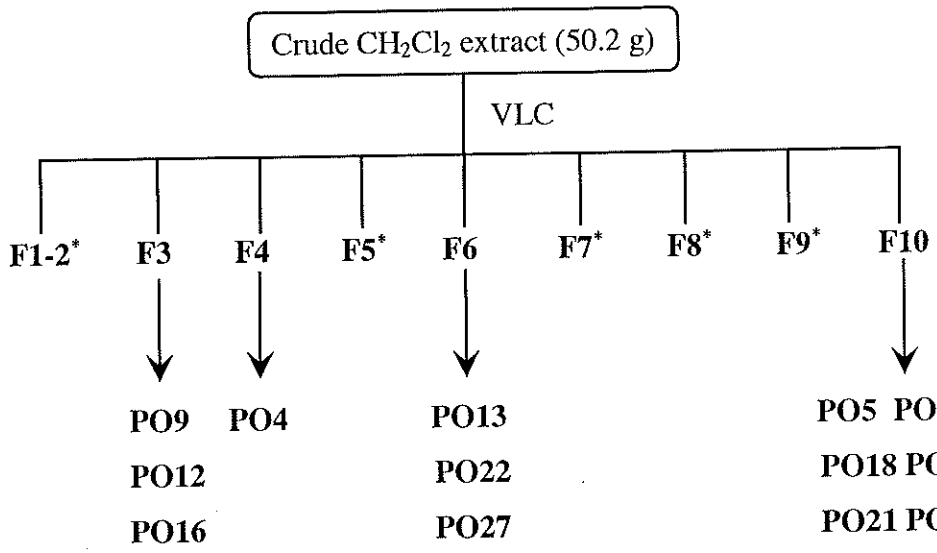
Fraction F4 (9.0 g) was separated by VLC using CH_2Cl_2-hexane (2:8, v/v) as eluting solvent to afford six subfractions (F4a-F4f). Subfraction F4a (5.8 g) was recrystallized from methylene chloride to give **PO15** (95.0 mg). Subfraction F4c (1.4 g) was purified by VLC with EtOAc-hexane (1:9, v/v) to give **PO14** (16.0 mg) and **PO29** (27.2 mg). Subfraction F4e (862.4 mg) was purified by VLC using EtOAc-hexane (1:9, v/v) to provide **PO24** (12.3 mg).

Fraction F5 (3.7 g) was separated by VLC using EtOAc-hexane (0.5:9.5, v/v) as eluting solvent to afford five subfractions (F5a-F5e). Subfraction F5c (726.7 mg) was separated by VLC with EtOAc-hexane (0.5:9.5, v/v) as eluting solvent to give three subfractions (F5c1-F5c3). Subfraction F5c2 (448.1 mg) was purified by VLC using EtOAc-CH_2Cl_2 (0.5:9.5, v/v) to provide **PO25** (35.5 mg) and **PO23** (58.7 mg).

Fraction F6 (15.9 g) was separated by VLC with EtOAc-hexane (1:9, v/v) as eluting solvent to afford six subfractions (F6a-F6f). Subfraction F6d (4.4 g) was separated by VLC using EtOAc-hexane (0.5:9.5, v/v) as eluting solvent to give five subfractions (F6d1-F6d5). Subfraction F6d3 gave **PO19** (18.2 mg) after recrystalliztion from CH_2Cl_2. Subfraction F6f (1.4 g) was purified by VLC using EtOAc-hexane (0.5:9.5, v/v) to provide **PO6** (5.2 mg).

2.3.2.2 Investigation of the crude CH_2Cl_2 extract from the roots of P. obtusifolia

The CH_2Cl_2 extract (50.2 g) was separated by VLC using hexane as eluent and increasing polarity with EtOAc to give ten fractions (F1-F10).



* No further investigation

Scheme 7 Isolation of compounds **PO4**, **PO5**, **PO9**, **PO12**, **PO13**, **PO16-PO18**, **PO20**, **PO21**, **PO22** and **PO27-PO28** from the CH_2Cl_2 extract.

Fraction F3 (7.0 g) was separated by VLC with EtOAc-hexane (0.5:9.5, v/v) as eluting solvent to afford six subfractions (F3a-F3f). Subfraction F3f (1.2 g) was purified by VLC with EtOAc-hexane (2:8, v/v) to provide **PO9** (7.9 mg), **PO16** (16.3 mg) and **PO12** (6.8 mg).

Fraction F4 (1.5 g) was separated by VLC using EtOAc-hexane (1:9, v/v) as eluting solvent to give eight subfractions (F4a-F4h). Subfraction F4c (745.1 mg) was purified by VLC with EtOAc-hexane (1:9, v/v) to provide **PO4** (82.6 mg).

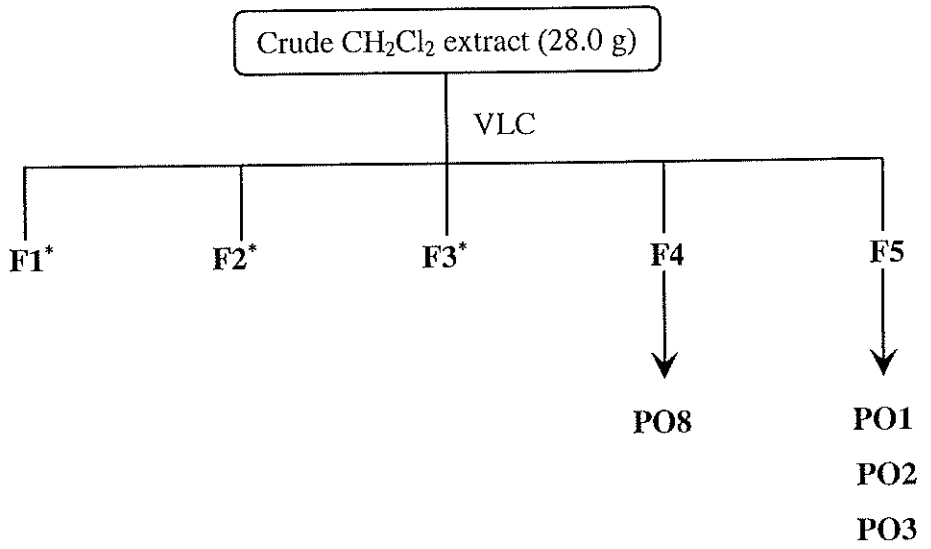
Fraction F6 (6.5 g) was separated by VLC with EtOAc-hexane (0.5:9.5, v/v) as eluting solvent to afford four subfractions (F6a-F6d). Subfraction F6a (1.4 g) was purified by VLC with EtOAc-hexane (1:9, v/v) to give **PO27** (251.7 mg). Subfraction F6c (1.7 g) was purified by VLC with EtOAc-hexane (2:8, v/v) to provide **PO22** (8.3 mg). Subfraction F6d (2.8 g) was recrystallized from methylene chloride to give **PO13** (746.3 mg).

Fraction F10 (20.5 g) was separated by VLC using EtOAc-hexane (3:7, v/v) as eluting solvent to afford ten subfractions (F10a-F10j). Subfraction F10d (4.1 g) was purified by VLC using EtOAc-hexane (3:7, v/v) to provide **PO18** (118.5 mg), **PO17** (15.1 mg) and **PO5** (15.6 mg). Subfraction F10f (4.7 g) was separated by VLC

using EtOAc-hexane (3:7, v/v) as eluting solvent to afford four subfractions (F10f1-F10f4). Subfraction F10f4 (2.2 g) was purified by VLC using EtOAc-hexane (4:6, v/v) to give **PO28** (6.2 mg), **PO20** (147.2 mg) and **PO21** (62.6 mg).

2.3.2.3 Investigation of the crude CH_2Cl_2 extract from the twigs of P. obtusifolia

The twigs of *P. obtusifolia* (2.0 kg) were extracted with CH_2Cl_2 (2 × 2 L for one week) at room temperature and evaporated under reduced pressure to provide crude CH_2Cl_2 extract (28.2 g). The CH_2Cl_2 extract (28.0 g) was subjected to VLC using hexane as eluent and increasing polarity with EtOAc to give five fractions (F1-F5).



* No further investigation

Scheme 8 Isolation of compounds **PO1-PO3** and **PO8** from the CH_2Cl_2 extract.

Fraction F4 (2.8 g) was separated by VLC using EtOAc-hexane (1:9, v/v) as eluting solvent to afford nine subfractions (F4a-F4i). Subfraction F4i (728.2 mg) was purified by VLC with EtOAc-hexane (0.5:9.5, v/v) to give **PO8** (16.4 mg).

Fraction F5 (7.2 g) was separated by VLC using acetone-hexane (2:8, v/v) as eluting solvent to afford five subfractions (F5a-F5e). Subfraction F5a (2.6 g) was purified by VLC with EtOAc-hexane (2:8, v/v) to give **PO1** (12.0 mg) and **PO2**

(13.6 mg). Subfraction F5d (1.4 g) was purified by VLC with EtOAc-hexane (1:9, v/v) to provide **PO3** (35.6 mg).

Compound PO1, isopimara-7,15-dien-1β,3β-diol: White amorphous solid; m.p. 168–170 °C; $[\alpha]_D^{26}$ –18.4 ($CHCl_3$; *c* 0.59); IR (thin film) υ_{max}: 3339, 2968, 2876, 1637, 1457, 1366, 1001, 908, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 38; HREIMS: *m/z* $[M]^{+\bullet}$ 304.2407 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Compound PO2, isopimara-7,15-dien-1β,19-diol: White amorphous solid; m.p. 92–95 °C; $[\alpha]_D^{26}$ –17.5 ($CHCl_3$; *c* 0.71); IR (thin film) υ_{max}: 3344, 2927, 2867, 1690, 1637, 1451, 1215, 1084, 1021, 909, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 39; HREIMS: *m/z* $[M]^{+\bullet}$ 304.2403 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Compound PO3, 13-*epi*-5,15-rosadien-3α,11β-diol: White amorphous solid; m.p. 78–81 °C; $[\alpha]_D^{26}$ –13.6 ($CHCl_3$; *c* 0.09); IR (thin film) υ_{max}: 3409, 2930, 2863, 1615, 1276, 1157, 1114, 1026, 760, 711, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 41; HREIMS: *m/z* $[M]^{+\bullet}$ 304.2397 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Compound PO4, abietatrien-1*β*-ol: Colorless oil; $[\alpha]_D^{26}$ –51.4 ($CHCl_3$; *c* 0.15); UV (EtOH) λ_{max} (log ε): 214 (3.99) and 258 (3.05) nm; IR (thin film) υ_{max}: 3421, 2956, 2969, 1670, 1609, 1495, 1459, 1387, 1217, 1030, 992, 832, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 42; HREIMS: *m/z* $[M]^{+\bullet}$ 286.2295 (calcd for $C_{20}H_{30}O$, 286.2297).

Compound PO5, abietatrien-1*β*,12-diol: Colorless oil; $[\alpha]_D^{26}$ –15.7 ($CHCl_3$; *c* 0.27); UV (EtOH) λ_{max} (log ε): 210 (3.97) and 282 (3.90) nm; IR (thin film) υ_{max}: 3351, 2958, 1507, 1420, 1216, 1005, 891, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C

NMR ($CDCl_3$, 100 MHz), see Table 43; HREIMS: *m/z* $[M]^{+\bullet}$ 302.2254 (calcd for $C_{20}H_{30}O_2$, 302.2246).

Compound PO6, ferruginol: White powder; m.p. 56-58 °C; $[\alpha]_D^{26}$ +42.7 ($CHCl_3$; *c* 0.70); UV (EtOH) λ_{max} (log ε): 213 (4.31) and 258 (3.43) nm; IR (thin film) υ_{max}: 3605, 2914, 2832, 1587 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 45.

Compound PO7, *O*-methyl ferruginol: Colorless oil; $[\alpha]_D^{26}$ +15.0 ($CHCl_3$; *c* 0.10); UV (EtOH) λ_{max} (log ε): 210 (4.24) and 253 (3.35) nm; IR (thin film) υ_{max}: 2978, 2889, 1524 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 47.

Compound PO8, lambertic acid: Amorphous solid; m.p. 251-253 °C; $[\alpha]_D^{26}$ +128 ($CHCl_3$; *c* 0.07); UV (EtOH) λ_{max} (log ε): 215 (4.37) and 283 (3.89) nm; IR (thin film) υ_{max}: 3360, 2800, 1694, 1619, 1580, 976, 857 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 49.

Compound PO9, sugiol: White amorphous solid; m.p. 292-294 °C; $[\alpha]_D^{26}$ +17.7 ($CHCl_3$; *c* 0.11); UV (EtOH) λ_{max} (log ε): 220 (4.29), 233 (4.28) and 286 (3.38) nm; IR (thin film) υ_{max}: 3224, 2928, 2865, 1648, 1590, 1342, 906, 867 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 50.

Compound PO10, royleanone: White amorphous solid; m.p. 179-181 °C; $[\alpha]_D^{26}$ +115.8 ($CHCl_3$; *c* 2.87); UV (EtOH) λ_{max} (log ε): 277 (4.28) and 407 (3.38) nm; IR (thin film) υ_{max}: 3360, 2958, 2927, 1641, 1631, 1603, 1461, 1377, 1252, 959 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 52.

Compound PO11, horminone: Orange crystal; m.p. 170-172 °C; $[\alpha]_D^{26}$ −114.0 ($CHCl_3$; *c* 0.10); UV (EtOH) λ_{max} (log ε): 218 (3.40), 272 (4.2) and 400 (2.00) nm; IR

(thin film) υ_{max}: 3358, 2957, 2868, 1649, 1624, 1600, 1455, 1250, 1060, 947 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 54.

Compound PO12, montbretol: Yellow powder; m.p. 201-203 °C; $[\alpha]_D^{26}$ –27 ($CHCl_3$; *c* 0.17); UV (EtOH) λ_{max} (log ε): 248 (4.25) and 342 (3.42) nm; IR (thin film) υ_{max}: 3328, 2932, 1676, 1635, 1504, 1378, 890 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 57.

Compound PO13, 14-deoxycoleon: Colorless crystals; m.p. 279-280 °C; $[\alpha]_D^{26}$ +58.0 ($CHCl_3$; *c* 0.10); UV (EtOH) λ_{max} (log ε): 210 (4.70), 287 (3.80), and 345 (3.0) nm; IR (thin film) υ_{max}: 3400, 3050, 2960, 2880, 1690, 1660, 1560, 1560, 1320, 1140, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 59.

Compound PO14, taxodion: Yellow oil; $[\alpha]_D^{26}$ +43.9 ($CHCl_3$; *c* 2.03); UV (EtOH) λ_{max} (log ε): 231 (4.17), 254 (4.11), 320 (3.58), 333 (3.26) and 400 (2.05) nm; IR (thin film) υ_{max}: 3530, 2873, 1740, 1643, 1531, 1376, 1288, 912 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 61.

Compound PO15, arucadiol: Orang crystals; m.p. 183-185 °C; $[\alpha]_D^{26}$ +9.9 ($CHCl_3$; *c* 1.00);UV (EtOH) λ_{max} (log ε): 228 (4.58), 270 (4.25), 362 (3.54) and 410 (3.65) nm; IR (thin film) υ_{max}: 3490, 3060, 2970, 2880, 1640, 1590, 1510, 1425, 1040, 870 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 63.

Compound PO16, 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial: Colorless crystals; m.p. 193-195 °C; $[\alpha]_D^{26}$ +12.8 ($CHCl_3$; *c* 0.81); UV (EtOH) λ_{max} (log ε): 218 (4.35), 260 (4.20) and 295 (3.64) nm IR (thin film) υ_{max}: 3434, 2942, 1710, 1666, 1545, 1472, 2310, 865 cm^{-1}); ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 64.

Compound PO17, salvicanaraldehyde: Colorless oil; $[\alpha]_D^{26}$ +38.1 ($CHCl_3$; *c* 0.91); UV (EtOH) λ_{max} (log ε): 231 (4.55) and 281 (3.70) nm; IR (thin film) υ_{max}: 3552, 2951, 1685, 1619, 1440, 1098, 879 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 66.

Compound PO18, 6*α*,11,12-trihydroxy-7*β*,20-epoxy-8,11,13-abietatriene: White solid; m.p. 199–201 °C; $[\alpha]_D^{24}$ –21.1 ($CHCl_3$; *c* 0.02); UV (EtOH) λ_{max} (log ε): 210 (4.29), 222 (4.28) and 276 (3.38) nm; IR (thin film) υ_{max}: 3351, 2958, 2871, 1578, 1446, 1297, 1046, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 68; HREIMS: *m/z* $[M]^{+\bullet}$ 332.1999 (calcd for $C_{20}H_{28}O_4$, 332.1988).

Compound PO19, 5*α*,11,12-trihydroxy-6-oxa-abieta-8,11,13-trien-7-one: Colorless oil; $[\alpha]_D^{24}$ +59.3 ($CHCl_3$; *c* 0.14); UV (EtOH) λ_{max} (log ε): 218 (4.46), 260 (3.95) and 295 (3.60) nm; IR (thin film) υ_{max}: 3452, 2961, 2871, 1682, 1621, 1584, 1417, 1236, 1001, 928, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 69; HREIMS: *m/z* $[M]^{+\bullet}$ 334.1792 (calcd for $C_{19}H_{26}O_5$, 334.1780).

Compound PO20, 5,6-dihydro-6*α*-hydroxysalviasperanol: Amorphous solid; m.p. 188-190 °C; $[\alpha]_D^{26}$ –8.6 ($CHCl_3$; *c* 0.88); UV (EtOH) λ_{max} (log ε): 205 (4.50) and 270 (3.80) nm; IR (thin film) υ_{max}: 3693, 3604, 3551, 1601, 1539, 1451, 1259, 929 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 70.

Compound PO21, salviasperanol: Amorphous solid; m.p. 207-209 °C; $[\alpha]_D^{26}$ –32.5 ($CHCl_3$; *c* 2.10); UV (EtOH) λ_{max} (log ε): 205 (3.13), 275 (4.50) and 280 (4.60) nm; IR (thin film) υ_{max}: 3500, 3420, 1620, 1260, 855 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 72.

Compound PO22, 11,12-dihydroxy-8,11,13-icetexatrien-1-one: Colorless crystals; m.p. 179-182 °C; $[\alpha]_D^{26}$ +107.5 ($CHCl_3$; *c* 0.41); UV (EtOH) λ_{max} (log ε): 219 (4.17), 269 (3.86) and 292 (3.49) nm; IR (thin film) υ_{max}: 3293, 2958, 1686, 1451, 1286,

1158, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 74.

Compound PO23, 11,12-dihydroxy-6,8,11,13-icetexatatraen-1-one: Colorless crystals; m.p. 176–178 °C; $[\alpha]_D^{26}$ –182.2 ($CHCl_3$; *c* 0.21); UV (EtOH) λ_{max} (log ε): 219 (4.17), 269 (3.86) and 292 (3.49) nm; IR (thin film) υ_{max}: 3478, 2959, 2869, 1681, 1629, 1584, 1444, 1306, 759 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 76; HREIMS: *m/z* $[M]^{+\bullet}$ 314.1894 (calcd for $C_{20}H_{26}O_3$, 314.1882).

Compound PO24, obtusinone A: Red amorphous solid; m.p. 135–137 °C; $[\alpha]_D^{24}$ +112.4 ($CHCl_3$; *c* 0.21); UV (EtOH) λ_{max} (log ε): 223 (5.06), 263 (4.97) and 451 (4.20) nm; IR (thin film) υ_{max}: 3327, 2933, 2871, 1691, 1667, 1654, 1598, 1461, 1263, 927, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 77; HREIMS: *m/z* $[M]^{+\bullet}$ 298.1566 (calcd for $C_{19}H_{22}O_3$, 298.1569).

Compound PO25, obtusinone B: Orange amorphous solid; m.p. 89–91 °C; $[\alpha]_D^{24}$ +14.9 ($CHCl_3$; *c* 1.17); UV (EtOH) λ_{max} (log ε): 215 (4.04), 272 (4.12) and 352 (3.23) nm; IR (thin film) υ_{max}: 2961, 2871, 1665, 1634, 1602, 1566, 1459, 1345, 1263, 1167, 946, 772 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 78; HREIMS: *m/z* $[M]^{+\bullet}$ 282.1614 (calcd for $C_{19}H_{22}O_2$, 282.1620).

Compound PO26, obtusinone C: Red amorphous solid; m.p. 104–106 °C; $[\alpha]_D^{24}$ +57.2 ($CHCl_3$; *c* 0.12); UV (EtOH) λ_{max} (log ε): 215 (4.23), 285 (4.15), 353 (3.67) and 411 (2.87) nm; IR (thin film) υ_{max}: 3386, 2962, 2851, 1728, 1650, 1628, 1461, 1414, 1090, 765 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 80; HREIMS: *m/z* $[M]^{+\bullet}$ 298.1563 (calcd for $C_{19}H_{22}O_3$, 298.1569).

Compound PO27, 4*β*,5*β*-dihydroxy-10-*epi*-eudesmane: Colorless oil; $[\alpha]_D^{26}$ –54.0 ($CHCl_3$; *c* 0.84); IR (thin film) υ_{max}: 3400, 2942, 1686, 1645 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 82.

Compound PO28, 4*β*,10*β*-dihydroxyaromadendrane: Colorless prisms; m.p. 138-140 °C; $[\alpha]_D^{26}$ –37.1 ($CHCl_3$; *c* 0.76); IR (thin film) υ_{max}: 3396, 2924, 2862, 1456, 1375, 1109 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 84.

Compound PO29, spathulenol: Colorless oil; $[\alpha]_D^{25}$ +12 ($CHCl_3$; *c* 0.15).; IR (thin film) υ_{max}: 3413, 2945, 1614, 920, 880 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 86.

2.4 Bioassays

2.4.1 Cytotoxic assay

The cancer cell lines MCF-7 (human breast adenocarcinoma) were grown in Dulbecco's Modified Eagle Medium: Nutrient Mixture F12 (D-MEM/F12) supplemented with 10% Fetal bovine serum (FBS). Cells were seeded in 96-well (3000 cell/well) and allowed to adhere for 24 h at 37 °C with 5% CO_2 in fully humidified incubator. Then 100 µl of 25 µg/ml crude extract or five fold diluted pure compound in medium (final concentration 0.008, 0.04, 0.2, 1, 5 µg/ml) were dispensed into wells of the cell plates and incubated further for 72 h. After removal of the sample medium, the cells were topped up with 200 µl D-MEM/F12 medium and incubated. After 72 h. Cells were fixed with cold 40% Trichloroacetic acid and kept at 4 °C for 1 h and washed with tap water. The cells were determined by Sulphorodamine assay. The absorbance was measured at 492 nm using a microplate reader. The results were based on the ability of extracts to inhibit cells growth compared with control (cells in media without extract) and calculated for IC_{50} using

probit analysis. Camptothecin, which was used as a standard, showed cytotoxic activity at <0.024 μg/ml.

2.4.2 Antibacterial assay

The isolated compounds from the roots and fresh fruits of *D. wallichii* and the roots and twigs of *P. obtusifolia* were tested against both Gram-positive and Gram-negative bacteria: *B. subtilis*, *S. aureus*, TISTR517, *E. faecalis* TISTR459, Methicillin-Resistant *S. aureus* (MRSA) ATCC43300, Vancomycin-Resistant *E. faecalis* (VRE) ATCC 51299, *Streptococcus faecalis*, *S. typhi*, *S. sonei* and *P. aeruginosa*. The microorganisms were obtained from the culture collections, Department of Industrial Biotechnology and Department of Pharmacognosy and Botany, PSU, except for the TISTR and ATCC strains, which were obtained from Pharmaceutical Microbial Research Center (MIRCEN), Bangkok, Thailand. The antibacterial assay employed was the same as described in Boonnak et al.. Vancomycin, which was used as a standard, showed antibacterial activity against Vancomycin-Resistant *E. faecalis* (VRE) ATCC 51299 at <2.34 μg/ml.

2.4.3 Anti-inflammatory assay

Inhibitory effects on NO production by murine macrophage-like RAW264.7 cells were evaluated using a modified method from that previously reported (Banskota et al., 2003). Briefly, the RAW264.7 cell line [purchased from Cell Lines Service (CLS)] was cultured in Rosewell Park Memorial Institute (RPMI) medium supplemented with 0.1% sodium bicarbonate and 2 mM glutamine, penicillin G (100 units/ml), streptomycin (100 μg/ml) and 10% fetal calf serum (FCS). The cells were harvested with trypsin–ethylenediaminetetraacetic acid (EDTA) and diluted to a suspension in a fresh medium. The cells were seeded in 96-well plates with 1×10^5 cells/well and allowed to adhere for 1 h at 37 °C in a humidified atmosphere containing 5% CO_2. After that the medium was replaced with a fresh medium containing 50 μg/ml of LPS together with the test samples at various concentrations

and was then incubated for 48 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. Cytotoxicity was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric method. Briefly, after 48 h incubation with the test samples, MTT solution (10 μl, 5 mg/ml in phosphate buffer saline (PBS) was added to the wells. After 4 h incubation, the medium was removed, and isopropanol containing 0.04 M HCl was then added to dissolve the formazan production in the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm. The test compounds were considered to be cytotoxic when the optical density of the sample-treated group was less than 80% of that in the control (vehicle-treated) group. L-NA and caffeic acid phenethylester (CAPE) were used as positive controls. The stock solution of each test sample was dissolved in DMSO, and the solution was added to the medium RPMI (final DMSO is 1%). Inhibition (%) was calculated using the following equation and IC_{50} values were determined graphically (n = 4):

$$\text{Inhibition (\%)} = \frac{A - B}{A - C} \times 100$$

A-C : NO_2^- concentration (μM) [*A* : LPS (+), sample (-); *B* : LPS (+), sample(+); *C* : LPS (-), sample (-)].

The results were expressed as mean ± standard error means (S.E.M) of four determinations at each concentration for each sample. The IC_{50} values were calculated using the Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

2.5 X-ray crystallographic studies of 11,12-dihydroxy-6,8,11,13-icetexatatraen-1-one (PO23) and obtusinone A (PO24)

Crystallographic data were collected at 100.0(1) K with the Oxford Cyrosystem Cobra low-temperature attachment. The data were collected using a Bruker Apex2 CCD diffractometer with a graphite monochromated Mo Kα radiation

at a detector distance of 5 cm and with APEX2 software. The collected data were reduced using *SAINT* program, and the empirical absorption corrections were performed using *SADABS* program. The structures were solved by direct methods and refined by least-squares using the *SHELXTL* software package (Spek, 2009). For 11,12-dihydroxy-6,8,11,13-icetexatatraen-1-one (**PO23**), hydroxy H atom was located from the difference map and refined isotropically. The remaining H atoms were placed in calculated positions with (C-H) = 0.93 for aromatic, 0.98 for CH, 0.97 for CH_2 and 0.96 A° for CH_3 atoms, after checking their positions in the difference map. The U_{iso} values were constrained to be $1.5U_{eq}$ of the carrier atoms for methyl H atoms and$1.2U_{eq}$ for hydroxyl and the other H atoms. Whereas for obtusinone A, all H atoms were located from the difference maps and refined isotropically. The final refinement converged well. Materials for publication were prepared using *SHELXTL* (Spek, 2009) and *PLATON* (Sheldrick, 2008).

Crystal data for 11,12-dihydroxy-6,8,11,13-icetexatatraen-1-one (**PO23**): $C_{20}H_{26}O_3$, M=314.41, 0.53×0.43×0.11 mm, monoclinic, $P2_1/n$, a=9.7176(2) Å, b=14.1949(2) Å, c=12.6935(2) Å, β=107.395(1)°, D_x=1.250 Mg m^{-3}, μ(Mo Kα)=0.082 mm^{-1}, 28,434 reflection measured, 6246 unique reflections, R=0.0364, R_w=0.0929.

Crystal data for obtusinone A (**PO24**): $C_{19}H_{22}O_3$, M=298.37, 0.57×0.26×0.02 mm, triclinic, P-1, a=7.3596(7) Å, b=8.8950(8) Å, c=12.6014(11) Å, α=97.941(2)°, β=106.284(2)°, γ=97.958(2)°, D_x=1.286 Mg m^{-3}, μ(Mo Kα)=0.086 mm^{-1}, 16,608 reflection measured, 4479 unique reflections, R=0.0452, R_w=0.1263.

The crystallographic-information files for 11,12-dihydroxy-6,8,11,13-icetexatatraen-1-one and obtusinone A have been deposited in the Cambridge Crystallographic Data Center as CCDC826321 and CCDC826437, respectively. These data can be obtained free of charge via http://www.ccdc.cam.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

CHAPTER 3
RESULTS AND DISCUSSION

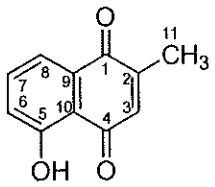
3.1 Structure elucidation of compounds from the roots and fruits of *Diospyros wallichii*

The air-dried and pulverized roots (2.5 kg) of *D. wallichii* were extracted with hexane at room temperature. The solvent was removed under reduced pressure yielding a dark-yellow viscous oil (30.7 g). The residue obtained after hexane extraction was further extracted with CH_2Cl_2 to yield a dark-yellow, resinous extract (20.2 g) after evaporation of the CH_2Cl_2 under vacuum. The hexane and CH_2Cl_2 extracts were subjected to silica gel vacuum liquid chromatography and /or crystallized affording fifteen compounds as two new naphthalene derivatives (**DW11** and **DW12**), one new binaphthoquinone (**DW9**), five known naphthoquinones (**DW1**, **DW2** and **DW5-DW7**), one known coumarin (**DW13**), four known triterpenoids (**DW14-DW17**) and a known mixture of steroids (**DW20** and **DW21**).

The fresh fruits of *Diospyros wallichii* (0.5 kg) were extracted with MeOH at room temperature. The extract was evaporated to dryness and the residue was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was evaporated and the residue (9.2 g) was subjected to silica gel vacuum liquid chromatography and /or crystallization affording six compounds as four known naphthoquinones (**DW3**, **DW4**, **DW8** and **DW10**) and two known triterpenoids (**DW18** and **DW19**).

Their structures were elucidated mainly by 1D and 2D NMR spectroscopic data: ^1H, ^{13}C NMR, DEPT 135°, DEPT 90°, HMQC, HMBC and ^1H-^1H COSY. Mass spectra were determined for three new compounds **DW9**, **DW11** and **DW12** and two known compounds **DW8** and **DW10**. The physical data of the known compounds were also compared with the reported values.

3.1.1 Compound DW1



Compound **DW1** was obtained as orange needles, m.p. 77-79 °C. The UV absorption maxima at λ_{max} 209, 264 and 413 nm indicated a highly conjugated system. Its IR spectrum suggested the presence of carbonyl group (1665 and 1645 cm^{-1}), a hydroxyl group (3370 cm^{-1}) and an aromatic group (1595 cm^{-1}). Furthermore, the presence of two carbonyl groups were shown at δ 183.9 and 189.7 in the ^{13}C NMR spectrum. These data indicated that **DW1** should have a *para*-naphthoquinone chromophore (Lin *et al.*, 1989).

The 11 carbon signals were observed in the ^{13}C NMR spectrum of **DW1** (**Table 3**, **Figure 39**) which were further classified by DEPT experiments as one methyl (δ_C 16.1), four olefinic methine (δ 118.7, 123.7, 134.9 and 135.7), six quaternary olefinic including two carbonyl carbons (δ 114.5, 131.5, 149.2, 160.7, 183.9 and 189.7).

The ^{1}H NMR spectrum of **DW1** (**Table 3, Figure 38**) revealed the presence of three adjacent aromatic proton signals at δ 7.06 (dd, *J*= 8.4, 1.2 Hz), 7.46 (t, *J*= 8.4 Hz), and 7.35 (dd, *J*= 8.4, 1.2 Hz) assignable to H-6, H-7 and H-8, respectively. One broad doublet quinoid proton signal at δ 6.63 (*J*= 1.5 Hz) was due to H-3. An upfiled doublet quinonoid methyl signal at δ 2.01 (*J*= 1.5 Hz) was attached to the C-2 on quinone ring, while a signal at δ 11.79 was due to the presence of a hydrogen bonded hydroxyl at C-5, whose assignments were based on the HMBC experiments (**Table 3**, **Figure 3**). Thus on the basis of its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data with the previous report (Khan et al., 1998; Gu *et al.*, 2004; Tangmouo *et al.*, 2005) (**Table 4**), compound **DW1** was identified as plumbagin.

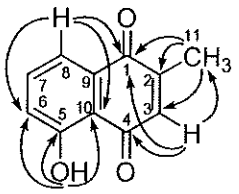


Figure 3 Major HMBC correlations of **DW1**

Table 3 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW1**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CO	183.9		
2	C	149.2		
3	CH	134.9	6.63 br d (1.5)	1, 10, 11
4	CO	189.7		
5	C	160.7		
6	CH	123.7	7.06 dd (8.4, 1.2)	5, 8, 10
7	CH	135.7	7.46 t (8.4)	5, 6, 8, 9
8	CH	118.7	7.35 dd (8.4, 1.2)	1, 6, 10
9	C	131.5		
10	C	114.5		
11	CH_3	16.1	2.01 d (1.5)	1, 2, 3
5-OH			11.79 s	5, 6, 7, 10

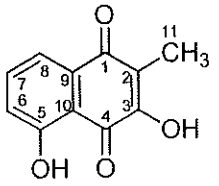
Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 4 Comparison of ^{1}H and ^{13}C NMR spectral data of compounds **DW1** and plumbagin

Position	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	
	DW1	plumbagin	**DW1**	plumbagin
1	183.9	183.9		
2	149.2	149.2		
3	134.9	134.9	6.63 br d (1.5)	6.75 q (2.0)
4	189.7	189.6		
5	160.7	160.6		
6	123.7	123.6	7.06 dd (8.4, 1.2)	7.19 dd, (8.0, 2)
7	135.7	135.7	7.46 t (8.4)	7.55 m
8	118.7	118.7	7.35 dd (8.4, 1.2)	7.55 m
9	131.5	131.5		
10	114.5	114.6		
11	16.1	16.1	2.01 d (1.5)	2.15 d, (2)
5-OH			11.79 s	11.90 s

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.1.2 Compound DW2



Compound **DW2** was isolated as an orange amorphous powder, m.p. 169-172 °C. The UV and IR spectra were similar to those of **DW1**.

The ^{1}H and ^{13}C NMR spectral data of **DW2** (**Table 5**, **Figures 40** and **41**) were similar to those of **DW1** (**Table 3**, **Figures 38** and **39**), except for differences in the substitution pattern of the quinonoid ring in **DW2**, in which one hydroxyl proton were shown at δ 7.24 (br s) instead of an olefinic methine proton at δ 6.63 (br d, *J*= 1.5 Hz) as in **DW1**. The ^{13}C NMR and DEPT experiments also proved the presence of an additional oxy-quaternary carbon at δ_C 152.7 in **DW2**, which was associated with the absence of the ^{1}H NMR signal of H-3 at δ 6.63 (br d, *J*= 1.5 Hz) as in **DW1**. This finding was further supported by HMBC correlations (**Table 5**, **Figure 4**) from H-11 (δ 2.10) to C-3 (δ 152.7). By comparison of the ^{1}H and ^{13}C NMR spectral data with the previously reported data (Gunaherath *et al.*, 1983; Bringmann *et al.*, 2000) (**Table 5** and **6**), therefore compound **DW2** was identified as droserone.

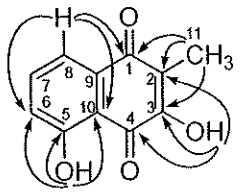


Figure 4 Major HMBC correlations of **DW2**

Table 5 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW2** and ^{13}C NMR of droserone (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[a]		DW2[b]	
1	CO	184.5	184.1		
2	C	121.8	121.8		
3	C	152.8	152.7		
4	CO	184.2	184.5		
5	C	161.2	161.1		
6	CH	123.2	123.1	7.19 dd (7.5, 1.8)	5, 8, 10
7	CH	137.5	137.5	7.62 t (7.5)	5, 6, 9
8	CH	119.7	119.6	7.67 dd (7.5, 1.8)	1, 6, 7, 10
9	C	132.7	132.7		
10	C	112.9	112.9		
11	CH_3	8.8	8.7	2.10 s	1, 2, 3
3-OH				7.24 br s	2, 3, 4
5-OH				11.1 s	5, 6, 7, 10

[a] Spectrum recorded at 150 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

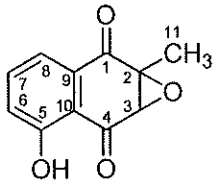
Table 6 Comparison of ^{1}H NMR spectral data of compounds **DW1, DW2** and droserone

Position	**δ_H/ppm (mult., *J* in Hz)**		
	DW1[a]	**DW2**[a]	droserone[b]
3	6.63 br d (1.5)		
6	7.06 dd (8.4, 1.2)	7.19 dd (7.5, 1.8)	7.10–7.70 m
7	7.46 t (8.4)	7.62 t (7.5)	
8	7.35 dd (8.4, 1.2)	7.67 dd (7.5, 1.8)	
11	2.01 d (1.5)	2.10 s	2.10 s
3-OH		7.24 br s	
5-OH	11.79 s	11.1 s	11.03 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 60 (^{1}H NMR) MHz, $CDCl_3$.

3.1.3 Compound DW3



Compound **DW3** was isolated as pale yellow needles, m.p. 97-99 °C; $[\alpha]_D^{28}$ –17.4 ($CHCl_3$, *c* 0.50). The UV and IR spectra were similar to those of **DW1**.

The ^1H and ^{13}C NMR spectral data of **DW3** (**Table 7**, **Figures 42** and **43**) were similar to those of **DW1** (**Table 3**, **Figures 38** and **39**). However, six olefinic carbons can be found in the ^{13}C NMR spectrum of **DW3**, which indicated the disappearance of one double bond of quinonoid ring. The appearances of oxymethine proton as a singlet at δ 3.80 and an oxymethine carbon at δ 61.3 and an oxyquaternary carbon at δ 61.2 in the ^1H and ^{13}C NMR spectra of **DW3**, suggesting an epoxy moiety. This finding was further supported by HMBC correlations (**Table 7**, **Figure 5**) from H-11 (δ 1.75) to C-2 (δ 61.2) and C-3 (δ 61.3). By comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data (Ogihara *et al.*, 1997; Higa *et al*, 2002) (**Table 7** and **8**), therefore compound **DW3** was identified as 2,3-epoxyplumbagin.

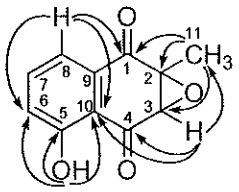


Figure 5 Major HMBC correlations of **DW3**

Table 7 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW3** and ^{13}C NMR of 2,3-epoxyplumbagin (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[a]		DW3[b]	
1	CO	190.8	190.8		
2	C	61.2	61.2		
3	CH	61.3	61.3	3.80 s	2, 4, 10, 11
4	CO	196.6	196.6		
5	C	161.4	161.4		
6	CH	124.1	124.1	7.26 dd (8.1, 1.2)	5, 8, 10
7	CH	137.1	137.1	7.64 t (8.1)	5, 9
8	CH	119.8	119.8	7.58 dd (8.1, 1.2)	1, 6, 10
9	C	132.1	132.2		
10	C	114.4	114.4		
11	CH_3	14.6	14.6	1.75 s	1, 2, 3
5-OH				11.2 s	5, 6, 10

[a] Spectrum recorded at 67.8 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

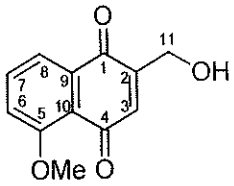
Table 8 Comparison of ^{1}H NMR spectral data of compounds **DW1, DW3** and 2,3-epoxyplumbagin

Position	**δ_H/ppm (mult., *J* in Hz)**		
	DW1[a]	**DW3**[a]	2,3-epoxyplumbagin[b]
3	6.63 br d (1.5)	3.80 s	3.78 s
6	7.06 dd (8.4, 1.2)	7.26 dd (8.1, 1.2)	7.15–7.66 m
7	7.46 t (8.4)	7.64 t (8.1)	
8	7.35 dd (8.4, 1.2)	7.58 dd (8.1, 1.2)	
11	2.01 d (1.5)	1.75 s	1.70 s
5-OH	11.79 s	11.2 s	11.1 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 90 (^{1}H NMR) MHz, $CDCl_3$.

3.1.4 Compound DW4



Compound **DW4** was isolated as an orange powder, m.p. 145-147 °C. The UV and IR spectra were similar to those of **DW1**.

The ^{1}H and ^{13}C NMR spectral data of **DW4** (**Table 9**, **Figures 44** and **45**) were related to those of **DW1** (**Table 3**, **Figures 38** and **39**). The difference in the spectrum of **DW4** was shown as an additional singlet methyl proton signal at δ 4.01 and a methyl carbon signal at δ 56.4 which replaced a hydroxyl group at δ 11.79 in **DW1**, suggesting a methoxyl proton at C-5. Furthermore, the spectrum of **DW4** was shown as the disappearance of a methyl proton at δ 2.01 (H-11) in the ^{1}H NMR of **DW1** but the appearance of a broad singlet of methylene protons at δ 4.64 in **DW4**, whereas the ^{13}C NMR spectrum of **DW4** displayed a signal of oxymethylene carbon at δ 59.7 instead of a methyl carbon at δ 16.1 as in **DW1**. The location of the methoxyl and oxymethylene groups were confirmed by HMBC experiment (**Table 9**, **Figure 6**) in which the methoxyl protons at δ 4.01 showed correlations to C-5 (δ 159.6). Thus, compound **DW4** was identified as 2-hydroxymethyl-5-methoxy-1,4-naphthoquinone, which was previously synthesized by Wurm, 1982. This is the first report of **DW4** from a natural source.

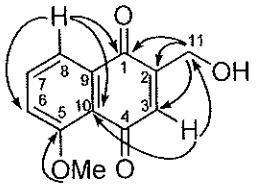


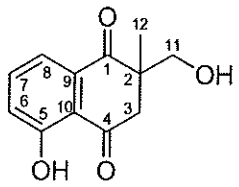
Figure 6 Major HMBC correlations of **DW4**

Table 9 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW4**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CO	185.6		
2	C	146.7		
3	CH	135.7	6.90 br d (1.6)	1, 2, 10, 11
4	CO	184.4		
5	C	159.6		
6	CH	118.1	7.31 d (8.4)	5, 8, 10
7	CH	134.9	7.67 t (8.4)	5, 6, 9
8	CH	119.1	7.73 d (8.4)	1, 6, 10
9	C	134.2		
10	C	119.7		
11	CH_2	59.7	4.64 br s	1, 2, 3, 4
5-OCH_3		56.4	4.01 s	5

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.1.5 Compound DW5



Compound **DW5** was isolated as a white amorphous solid, m.p. 93-95 °C. The IR and UV spectra were closely related to that of **DW4**.

The ^{1}H and ^{13}C NMR spectral data of **DW5** (**Table 10**, **Figures 46** and **47**) resembled those of **DW4** (**Table 9**, **Figures 44** and **45**). The differences were shown in the chemical shifts of the methyl protons which was shown at δ 4.01 in **DW4** but at δ 1.35 in **DW5** and the signal of an aliphatic methine proton at δ 6.90 (br d) in **DW4** was changed to signals of two doublets at δ 2.57 and 3.38 in **DW5**. In addition, two doublets of oxymethylene protons were shown at δ 3.47 and 4.09 (d, *J*= 10.8). The location of the methyl group at C-2 was confirmed by HMBC experiment (**Table 10**, **Figures 7**) in which the methyl proton at δ 1.35 (H-12) showed correlations to C-2 (δ 50.7), C-1 (δ 200.7, C-3 (δ 46.5) and C-11 (δ 67.7). By comparison of the ^{1}H and ^{13}C NMR spectral data with the previously reported data (Miyoshi *et al.*, 1984) (**Table 10, 11** and **22**), therefore compound **DW5** was identified as diomuscinone.

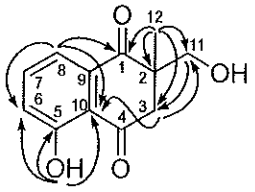


Figure 7 Major HMBC correlations of **DW5**

Table 10 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW5** and ^{13}C NMR of diomuscinone (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[a]		DW5[b]	
1	CO	200.8	200.7		
2	C	50.5	50.7		
3	CH_2	46.6	46.5	2.57 d (17.1)	1, 2, 10, 11, 12
				3.38 d (17.1)	
4	CO	202.8	202.9		
5	C	161.1	161.1		
6	CH	124.0	123.9	7.25 dd (8.4, 1.2)	5, 8, 10
7	CH	136.9	136.9	7.63 t (8.4)	5, 6, 9
8	CH	118.5	117.5	7.53 dd (8.4, 1.2)	1, 6, 10
9	C	134.0	133.9		
10	C	117.5	117.5		
11	CH_2	67.8	67.7	3.47 d (10.8)	1, 3
				4.09 d (10.8)	
12	CH_3	21.4	21.3	1.35 s	1, 2, 3, 11
5-OH				12.00 s	5, 6, 10

[a] Spectrum recorded at 90 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 11 Comparison of ^{1}H NMR spectral data of compounds **DW1, DW5** and diomuscinone

Position	**δ_H/ppm (mult., *J* in Hz)**		
	DW4[a]	**DW5**[a]	diomuscinone[b]
3	6.90 br d (1.6)	2.57 d (17.1)	2.80 d (17.0)
		3.38 d (17.1)	3.43 d (17.0)
6	7.31 d (8.4)	7.25 dd (8.4, 1.2)	7.25–7.4[c]
7	7.67 t (8.4)	7.63 t (8.4)	7.5–7.8[d]
8	7.73 d (8.4)	7.53 dd (8.4, 1.2)	7.5–7.8[d]
11	4.64 br s	3.47 d (10.8)	3.53 d (11.5)
		4.09 d (10.8)	4.06 d (11.5)
12	4.01 s	1.35 s	1.31 s
5-OH		12.00 s	

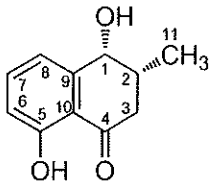
[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 90 (^{1}H NMR) MHz, $CDCl_3$.

[c] Overlapped with the solvent signal.

[d] Complex.

3.1.6 Compound DW6



Compound **DW6** was isolated as a colorless oil; $[\alpha]_D^{28}$ + 42.5 ($CHCl_3$, *c* 1.00). The IR and UV spectra were closely related to those of **DW1**.

The ^1H and ^{13}C NMR spectral data of **DW6** (**Table 12**, **Figures 48** and **49**) were similar to those of **DW1** (**Table 3**, **Figures 38** and **39**). However, a carbonyl and olefinic carbons in quinonoid ring were absence in the ^{13}C NMR spectrum of **DW6**, which indicated the reduction of one carbonyl and one double bond of a quinonoid ring in **DW1**. The appearances of a complex coupling system attributing to a methine at δ 2.33 (m, H-2), which was coupled with the methyl at δ 1.11 (d, *J*= 6.9 Hz, H-11), an oxymethine proton at δ 4.63 (br d, *J*= 2.7 Hz, H-1), and two methylene protons at δ 2.47 (dd, *J*= 17.7, 4.2 Hz, H-3a) and 2.77 (dd, *J*= 17.7, 10.8 Hz, H-3b) were observed. This finding was further supported by HMBC correlations (**Table 12**, **Figure 8**).

The relative stereochemistry at C-1 and C-2 should be α orientations for both hydroxyl and methyl groups. The *cis*-isomer was established with the aid of the coupling constant (*J*= 2.7 Hz) found for H-1, and by supporting from NOESY cross peak of H-1 and H-2. By comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data (Bringmann *et al.*, 1999, $[\alpha]_D^{25}$ + 22.2 ($CHCl_3$, *c* 1.0) (**Table 12**, **13** and **22**), therefore compound **DW6** was identified as isoshinanolone.

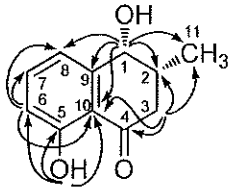


Figure 8 Major HMBC correlations of **DW6**

Table 12 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compounds **DW6** and ^{13}C NMR of isoshinanolone (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[a]		DW6[b]	
1	CH	71.2	70.7	4.63 br d (2.7)	2, 8, 9, 10, 11
2	CH	34.3	34.3	2.33 m	11
3	CH_2	40.7	40.6	2.47 dd (17.7, 4.2)	1, 2, 4, 10, 11,
				2.77 dd (17.7, 10.8)	
4	CO	204.7	205.1		
5	C	162.7	162.3		
6	CH	118.2	117.7	6.86 d (8.1)	6, 7, 8, 10
7	CH	136.9	137.0	7.41 t (8.1)	9
8	CH	118.6	119.0	6.83 d (8.1)	1, 6, 9, 10
9	C	145.0	145.1		
10	C	114.9	114.8		
11	CH_3	16.1	16.1	1.11 d (6.9)	1, 2, 3
5-OH				12.35 s	5, 6, 10

[a] Spectrum recorded at 150.9 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

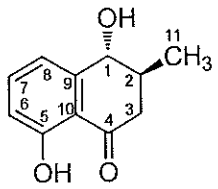
Table 13 Comparison of ^{1}H NMR spectral data of compounds **DW1, DW6** and isoshinanolone

Position	**δ_H /ppm (mult., *J* in Hz)**		
	DW1[a]	**DW6**[a]	isoshinanolone
1		4.63 br d (2.7)	4.75 d d (2.4)
2		2.33 m	2.44 m
3	6.63 br d (1.5)	2.47 dd (17.7, 4.2)	2.56 ddd (17.7, 4.3, 0.9)
		2.77 dd (17.7, 10.8)	2.87 dd (17.7, 11.0)
6	7.06 dd (8.4, 1.2)	6.83 d (8.1)	6.94 dd (8.5, 1.2)
7	7.46 t (8.4)	7.41 t (8.1)	7.48 dd (8.3, 7.3)
8	7.35 dd (8.4, 1.2)	6.86 d (8.1)	6.92 d (7.3)
11	2.01 d (1.5)	1.11 d (6.9)	1.19 d (6.7)
5-OH	11.79 s	12.35 s	12.42 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] spectrum recorded at 250 (^{1}H NMR) MHz, $CDCl_3$.

3.1.7 Compound DW7



Compound **DW7** was isolated as a colorless oil; $[\alpha]_D^{28}$ – 42.5 ($CHCl_3$, *c* 0.70). The IR and UV spectra were closely related to those of **DW6**.

The ^1H and ^{13}C NMR spectral data of **DW7** (**Table 14**, **Figures 50** and **51**) were closely resemble to those of **DW6** (**Table 12**, **Figures 48** and **49**), except that the coupling constant of H-1 in **DW7** was 8.1 Hz, whereas the coupling constant of H-1 in **DW6** was 2.7 Hz. The coupling constant of H-1 in compound **DW7** was in agreement with the respective coupling pattern (axial–equatorial) of H-1 and H-2, indicating the *trans*-isomer. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report (Bringmann *et al.*, 1999 (**Table 14**, **15** and **22**), compound **DW7** was identified as *epi*-isoshinanolone, an epimer of isoshinanolone.

Table 14 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compounds **DW7** and ^{13}C NMR of *epi*-isoshinanolone (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[a]		DW7[b]	
1	CH	73.7	73.6	4.51 d (8.1)	2, 8, 9, 10, 11
2	CH	37.4	38.0	2.27 m	
3	CH_2	43.4	43.4	2.47 dd (17.1, 10.2)	1, 2, 4, 10, 11,
				2.93 dd (17.1, 3.9)	12
4	CO	203.6	203.8		
5	C	162.5	162.7		
6	CH	117.3	117.1	6.92 d (8.4)	6, 7, 8, 10
7	CH	137.0	137.0	7.50 t (8.4)	6, 9
8	CH	117.2	117.4	7.12 d (8.4)	1, 6, 9, 10
9	C	145.9	145.8		
10	C	115.3	115.3		
11	CH_3	17.8	17.4	1.12 d (6.9)	1, 2, 3
5-OH				12.38 s	5, 6, 10

[a] Spectrum recorded at 50 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

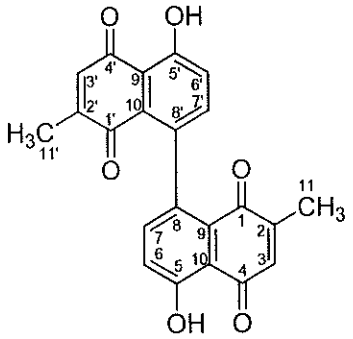
Table 15 Comparison of ^{1}H NMR spectral data of compounds **DW6**, **DW7** and *epi*-isoshinanolone

Position	**δ_H/ppm (mult., *J* in Hz)**		
	DW6[a]	**DW7**[a]	*epi*-isoshinanolone[b]
1	4.63 d (2.7)	4.51 d (8.1)	4.50 d (8.0)
2	2.33 m	2.27 m	2.25 m
3	2.47 dd (17.7, 4.2)	2.47 dd (17.1, 10.2)	2.43 dd (17.0, 10.1)
	2.77 dd (17.7, 10.8)	2.93 dd (17.1, 3.9)	2.91 dd (17.0, 3.8)
6	6.83 d (8.1)	6.92 d (8.4)	7.10 dd (7.6, 1.0)
7	7.41 t (8.1)	7.50 t (8.4)	7.49 t (8.0)
8	6.86 d (8.1)	7.12 d (8.4)	690 ddd (8.4, 1.1, 0.6)
11	1.11 d (6.9)	1.12 d (6.9)	1.19 d (6.5)
5-OH	12.35 s	12.38 s	11.74 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 200 (^{1}H NMR) MHz, $CDCl_3$.

3.1.8 Compound DW8



Compound **DW8** was isolated as a red amorphous powder, m.p. 199-201 °C. Its molecular formula, $C_{22}H_{14}O_6$ ($[M]^+$ 374.1, calcd 374.1), was deduced by EI-MS spectrum. The UV and IR spectra were similar to those of **DW1**.

The ^1H and ^{13}C NMR spectral data of **DW8** (**Table 16**, **Figures 52** and **53**) were closely resemble to those of **DW1** (**Table 3**, **Figures 38** and **39**), except that the ABX system of phenolic ring A was absence, while one pair of *ortho*-coupled aromatic protons at δ 7.21 and 7.29 (d, *J*= 8.7 Hz, H-6 and H-7, respectively) was observed. Thus, the splitting pattern for H-6 and H-7 was simplified due to the absence of H-8, together with the molecular formula, establishing the dimer with linkage at C-8 to C8′ and was supported by HMBC correlation (**Table 16**, **Figure 9**) of H-6 and H-7 to C-8 (δ 128.2). Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report (Gu *et al.*, 2004) (**Tables 16**, **17** and **22**), compound **DW8** was assigned as maritinone.

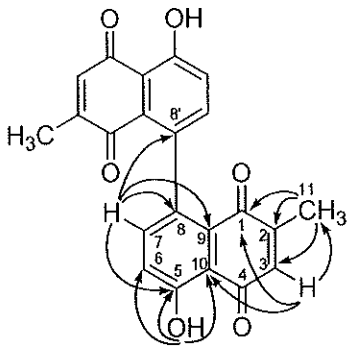


Figure 9 Major HMBC correlations of **DW8**

Table 16 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW8** and ^{13}C NMR of Maritinone (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[a]		DW8[b]	
1	CO	185.1	185.2		
2	C	150.0	150.0		
3	CH	134.9	134.9	6.81 q (1.5)	1, 10, 11
4	CO	190.5	190.5		
5	C	161.3	161.3		
6	CH	124.3	124.3	7.21 d (8.7)	5, 8, 10
7	CH	137.9	138.0	7.29 d (8.7)	5, 6, 8, 9
8	C	128.2	128.2		
9	C	135.5	135.6		
10	C	115.4	115.5		
11	CH_3	16.6	16.6	2.01 d (1.5)	1, 2, 3,
5-OH				12.55 s	5, 6, 7, 10

[a] Spectrum recorded 125 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

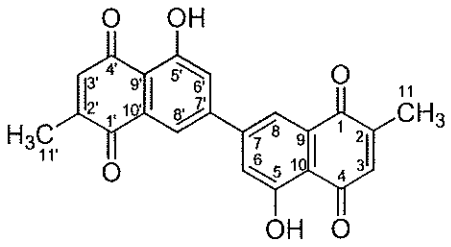
Table 17 Comparison of ^{1}H NMR spectral data of compounds **DW1, DW8** and maritinone

Position	**δ_H/ppm (mult., *J* in Hz)**		
	DW1[a]	**DW8**[a]	maritinone[b]
3	6.63 br d (1.5)	6.81 q (1.5)	6.81 q (1.5)
6	7.06 dd (8.4, 1.2)	7.21 d (8.7)	7.21 d (8.7)
7	7.46 t (8.4)	7.29 d (8.7)	7.29 d (8.7)
8	7.35 dd (8.4, 1.2)		
11	2.01 d (1.5)	2.01 d (1.5)	2.01 d (1.5)
5-OH	11.79 s	12.55 s	12.67 s

[a] Spectra recorded at 500 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

3.1.9 Compound DW9



Compound **DW9** was obtained as a red solid, m.p. 250 °C (decomposed). The HREIMS spectrum showed a molecular ion peak $[M]^+$ at *m/z* 374.0812, corresponding to the molecular formula of $C_{22}H_{14}O_6$. The UV spectrum exhibited bands (λ_{max} 210, 270 and 415 nm) and the IR spectrum showed absorption bands for hydroxyl (3473 cm^{-1}), carbonyl (1664 cm^{-1}) and aromatic ring (1600 cm^{-1}) typical of the general pattern observed for **DW1**. The ^{13}C and ^{1}H NMR spectral data (**Table 18**, **Figures 54** and **55**) revealed only 11 carbon signals and proton signals similar to **DW1** (**Table 3**, **Figures 38** and **39**), but the mass spectrum and the molecular formula revealed 22 carbons, thus suggesting that **DW9** is a symmetrical dimer of **DW1**. The ^{1}H NMR spectrum of **DW9** was comparable to that of **DW1** (**Table 19**) except that there were no clear signals for the *ortho* and *meta*-coupled protons, but the presence of a broad singlet aromatic proton at δ 7.73 (2H, H-6 and H-8). The triplet proton signal of H-7 at δ 7.46 as present in **DW1** was absent in **DW9**, thus suggesting the linkage to be at the 7 to 7′ positions and supporting by HMBC correlation (**Table 18**, **Figure 10**) of H-6 and H-8 to C-7 (δ 131.2). The structure of **DW9** was thus established to be 5,5'-dihydroxy-2,2'-dimethyl-7,7'-binaphthalen-1,1',4,4'-tetraone, a 7-7′-plumbagin dimer, a new compound (Salae *et al.*, 2010).

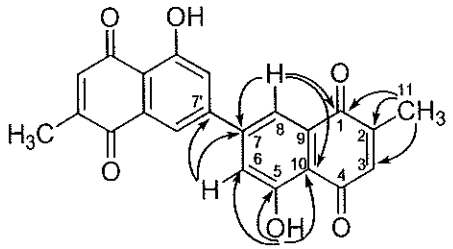


Figure 10 Major HMBC correlations of **DW9**

Table 18 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW9**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CO	183.1		
2	C	149.8		
3	CH	135.5	6.85 q (1.5)	10
4	CO	190.4		
5	C	158.9		
6	CH	118.7	7.73 br s	5, 7, 10
7	C	131.2		
8	CH	137.6	7.73 br s	1, 7, 9, 10
9	C	131.9		
10	C	115.3		
11	CH_3	16.4	2.22 d (1.5)	1, 2, 3
5-OH			12.50 s	5, 7, 10

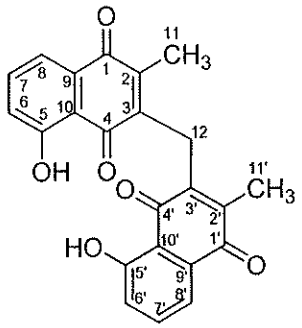
Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 19 Comparison of ^{1}H and ^{13}C NMR spectral data of compounds **DW1** and **DW9**

Position	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	
	DW1	**DW9**	**DW1**	**DW9**
1	183.9	183.1		
2	149.2	149.8		
3	134.9	135.5	6.63 br d (1.5)	6.85 q (1.5)
4	189.7	190.4		
5	160.7	158.9		
6	123.7	118.7	7.06 dd (8.4, 1.2)	7.73 br s
7	135.7	131.2	7.46 t (8.4)	
8	118.7	137.6	7.35 dd (8.4, 1.2)	7.73 br s
9	131.5	131.9		
10	114.5	115.3		
11	16.1	16.4	2.01 d (1.5)	2.22 d (1.5)
12			11.79 s	12.50 s

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.1.10 Compound DW10



Compound **DW10** was isolated as orange needles, m.p. 202-204 °C. The UV and IR spectra showed the characteristic signals of **DW1**. The molecular ion (*m/z* $[M]^+$ 388.1, calcd 388.1) was shown in the EIMS spectrum.

The ^1H and ^{13}C NMR spectral data of **DW10** (**Table 20**, **Figures 56** and **57**) were similar to those of **DW1** (**Table 3**, **Figures 38** and **39**). The difference in the spectrum of **DW10** was shown as the absence of quinonoid proton at δ 6.63 (br d, H-3) in the ^1H NMR of **DW1** and the NMR spectrum of **DW10** showed a singlet signal of methylene protons at δ_H 3.97 (δ_C 25.6), thus suggesting a methylene group attached at C-3. The NMR and EIMS spectral data show that **DW10** is a symmetric dimer of **DW1** linked by a methylene bridge. Since no quinonoid proton was observed, the position of the dimeric linkage must be 3-3′. The HMBC experiment (**Table 20**, **Figure 11**) showed long-range correlations between the methylene proton to C-2 (δ 149.8), C-3 (δ 135.5) and C-4 (δ 189.3). By comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data (Gunaherath *et al.*, 1988; Higa *et al.*, 2002) (**Table 20-22**), therefore compound **DW10** was identified as methylene-3,3'-biplumbagin.

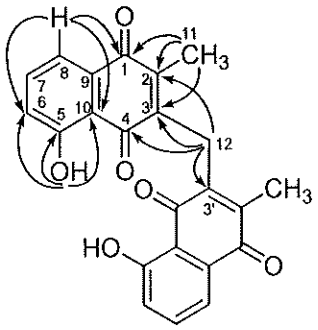


Figure 11 Major HMBC correlations of **DW10**

Table 20 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW10**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CO	184.2		
2	C	149.8		
3	C	135.5		
4	CO	189.3		
5	C	161.3		
6	CH	124.0	7.23 dd, (7.8, 1.5)	5, 8, 10
7	CH	136.1	7.59 t, (7.8)	5, 6, 9
8	CH	119.1	7.64 dd, (7.8, 1.5)	1, 6, 10
9	C	132.0		
10	C	114.7		
11	CH_3	13.4	2.32 s	1, 2, 3, 4
12	CH_2	25.6	3.97 s	2, 3, 4
5-OH			11.9 s	5, 6, 10

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 21 Comparison of ^{1}H NMR spectral data of compounds **DW1, DW10** and methylene-3,3'-biplumbagin (**R**)

Position	δ_H/ppm (mult., *J* in Hz)		
	DW1[a]	**DW10**[a]	**R**[b]
3	6.63 br d (1.5)		
6	7.06 dd (8.4, 1.2)	7.23 dd, (7.8, 1.5)	7.63–7.13 m
7	7.46 t (8.4)	7.59 t, (7.8)	
8	7.35 dd (8.4, 1.2)	7.64 dd, (7.8, 1.5)	
11	2.01 d (1.5)	2.32 s	2.30 s
12		3.97 s	3.96 s
5-OH	11.79 s	11.90 s	11.93 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

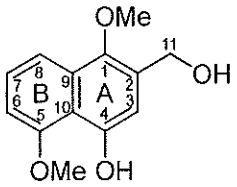
[b] Spectrum recorded at 60 (^{1}H NMR) MHz, $CDCl_3$.

Table 22 Comparison of ^{13}C NMR spectral data of compounds **DW1-DW10**

No.	DW1	DW2	DW3	DW4	DW5	DW6	DW7	DW8	DW9	DW10
1	183.9	184.1	190.8	185.6	200.7	70.7	73.6	185.1	183.1	184.2
2	149.2	121.8	61.2	146.7	50.7	34.3	38.0	150.0	149.8	149.8
3	134.9	152.7	61.3	135.7	46.5	40.6	43.4	134.9	135.5	135.5
4	189.7	184.5	196.6	184.4	202.9	205.1	203.8	190.5	190.4	189.3
5	160.7	161.1	161.4	159.6	161.1	162.3	162.7	161.3	158.9	161.3
6	123.7	123.1	124.1	118.1	123.9	117.7	117.1	124.3	118.7	124.0
7	135.7	137.5	137.1	134.9	136.9	137.0	137.0	138.0	131.2	136.1
8	118.7	119.6	119.8	119.1	117.5	119.0	117.4	128.2	137.6	119.1
9	131.5	132.7	132.2	134.2	133.9	145.1	145.8	135.4	131.9	132.0
10	114.5	112.9	114.4	119.7	117.5	114.8	115.3	115.4	115.3	114.7
11	16.1	8.7	14.6	59.7	67.7	16.1	17.4	16.6	16.4	13.4
12					21.3					25.6

Spectra recorded at 75 (^{13}C NMR) MHz, $CDCl_3$.

3.1.11 Compound **DW11**



Compound **DW11** was obtained as white amorphous powder, m.p. 63-65 °C. The molecular formula $C_{13}H_{14}O_4$, on the basis of $[M]^+$ at *m/z* 234.0920 in the HREIMS suggested seven unsaturations. The UV spectrum exhibited absorption bands at λ_{max} 231 and 291 nm while IR spectrum showed absorption bands for hydroxyl (3400 cm^{-1}) and aromatic ring (1613 cm^{-1}).

The analysis of the ^{13}C NMR spectral data (**Table 23**, **Figure 59**) of **DW11** through DEPT revealed 13 carbons among which are two methoxyls (δ_C 56.1 and 62.3), an oxymethylene (δ_C 60.3), four sp^2 methine and six sp^2 quaternary carbons. Of the six sp^2 quaternary carbons, three were linked to oxygen atoms in view of their deshielded chemical shifts at δ_C 145.5, 150.7 and 156.3. Since two methoxyl groups were present, the third oxygenated quaternary carbon should link to a hydroxyl group. These data together with seven unsaturations suggested a naphthalene skeleton. The ^1H NMR data (**Table 23**, **Figure 58**) showed four aromatic proton signals. The first three signals appeared at δ_H 6.72 (1H, d, *J*= 8.4 Hz, H-6), 7.30 (1H, t, *J*= 8.4 Hz, H-7) and 7.60 (1H, d, *J*= 8.4 Hz, H-8). The fourth aromatic proton signal appeared as a broad singlet at δ_H 6.86 (1H, H-3), hence suggesting a substituent group in ring B and three substituent groups in ring A of a naphthalene skeleton. The two methoxyl protons, a hydroxyl and oxymethylene protons appeared at δ_H 3.81, 3.98, 9.13 and 4.78 ppm, respectively. The HMBC experiment (**Table 23**, **Figure 12**) allowed the assignment of the structure as follow: the methyl singlet of O-CH_3 at δ 3.81 showed correlation with C-1 (δ 145.5) whereas that at δ 3.98 showed correlation with C-5 (δ 156.3), indicating the location of the two O-CH_3 groups at C-1 and C-5, respectively. A methine proton H-3 (δ 6.86) showed clear ^3J correlation with oxymethylene carbon C-11 (δ 60.3), the oxyquaternary carbon C-1 (δ 145.5) and C-10 (δ 115.1) and a ^2J correlation with an oxyquaternary carbon C-4 (δ 150.7), hence the hydroxymethyl

group was located at C-2 and a hydroxyl group at C-4 of ring A. The NOESY experiment (**Figure 12**) revealed interactions between 1-OCH_3/H_2-11, H_2-11/H-3, 1-OCH_3/H-8, 5-OCH_3/H-6, thus supporting the assigned structure. Compound **DW11** was therefore a new compound and characterized as 2-hydroxymethyl-1,5-dimethoxynaphthalen-4-ol, a reduced and 1-*O*-methylated derivative of compound **DW4** (Salae *et al.*, 2010).

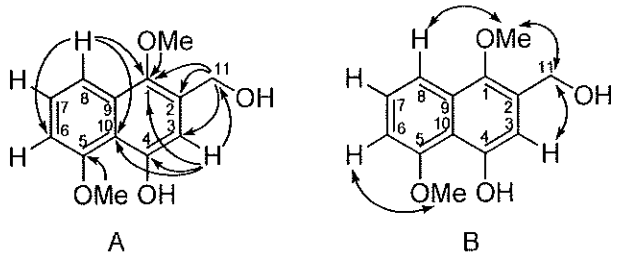


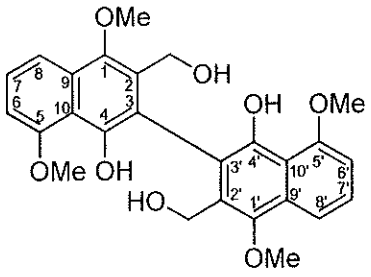
Figure 12 Selected HMBC correlations (A) and NOESY correlations (B) for **DW11**

Table 23 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compounds **DW11**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	C	145.5		
2	C	131.1		
3	CH	109.8	6.86 br s	1, 4, 10, 11
4	C	150.7		
5	C	156.3		
6	CH	104.3	6.72 d (8.4)	5, 8
7	CH	126.1	7.30 t (8.4)	5, 6, 8, 9
8	CH	115.7	7.60 d (8.4)	1, 6, 9, 10
9	C	130.3		
10	C	115.1		
11	CH_2	60.3	4.78 br s	1, 2, 3
1-OCH_3		62.3	3.81 s	1
5-OCH_3		56.1	3.98 s	5
4-OH			9.13 s	

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.1.12 Compound DW12



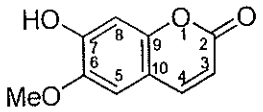
Compound **DW12** was obtained as white amorphous powder, m.p. 115-117 °C. The UV and IR spectra showed the characteristics of **DW11**. The HREIMS spectrum showed a molecular ion peak $[M]^+$ at *m/z* 466.1622, suggesting a molecular formula of $C_{26}H_{26}O_8$. A comparison of the NMR and mass spectral data between compounds **DW11** and **DW12** (**Table 23** and **24**, **Figures 58**, **59**, **60** and **61**) suggested that **DW12** was a dimer of **DW11** with C_2 symmetry (**Table 24**). A broad singlet signal of H-3 at δ 6.86 as shown in **DW11** was absent in **DW12,** suggesting the dimeric linkage between C-3 and C-3′. On the basis of these evidences, compound **DW12** was a new compound and identified as 2,2'-bis-hydroxymethyl-1,1',5,5'-tetramethoxy-3,3'-binaphthalen-4,4'-diol (Salae *et al.*, 2010).

Table 24 Comparison of ^{1}H and ^{13}C NMR spectral data of compounds **DW11** and **DW12**

Position	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	
	DW11	**DW12**	**DW11**	**DW12**
1	145.5	147.8		
2	131.1	131.1		
3	109.8	118.1	6.86 br s	
4	150.7	147.2		
5	156.3	156.3		
6	104.3	105.0	6.72 d (8.4)	6.84 d (8.4)
7	126.1	126.7	7.30 t (8.4)	7.40 t (8.4)
8	115.7	116.5	7.60 d (8.4)	7.80 d (8.4)
9	130.3	130.5		
10	115.1	115.6		
11	60.3	58.2	4.78 br s	4.50 d (11.1)
				4.56 d (11.1)
1-OCH_3	62.3	63.4	3.81 s	
5-OCH_3	56.1	56.2	3.98 s	4.01 s

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.1.13 Compound DW13



Compound **DW13** was obtained as yellow oil. The UV spectrum showed absorption maxima at 225, 240 and 346 nm and IR spectrum absorption bands at ν_{max} 3450, 1725, 1630 and 1590 cm^{-1}, suggesting the presence of a hydroxyl, an unsaturated lactone and aromatic ring groups, respectively.

The ^{13}C NMR and DEPT spectral data (**Table 25**, **Figure 63**) of **DW13** indicated 10 resonances including a methoxy signal at δ 56.3 (OCH_3), a lactone signal at δ 162.1 (C=O), and eight low-field signals at δ 103.2 (CH), 107.9 (CH), 111.2 (C), 112.6 (CH), 143.8 (CH), 144.8 (C), 150.1 (C), and 150.4 (C). These data indicated that **DW13** contained an unsaturated lactone and a methoxyl group attached to aromatic ring.

In the ^1H NMR spectral data (**Table 25**, **Figure 62**) of **DW13**, the characteristic signals of a 6,7-disubstitued coumarin were apparent, with two doublets at δ 6.28 (1H, d, *J*= 9.6 Hz, H-3) and 7.60 (1H, d, *J*= 9.6 Hz, H-4), two aromatic signals at δ 6.86 (1H, s, H-5) and δ 6.88 (1H, s, H-8), and one methoxyl signal at δ 3.93 (3H, s, OCH_3-6). The location of the methoxy group was assigned to C-6 based on the HMBC correlation between a methoxyl signal at δ 3.93 and C-6 at δ_C 144.8 and NOESY cross-peak between OCH_3-6 (δ_H 3.93) and H-5 (δ_H 6.86) (**Table 25**, **Figure 13**). Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report (Razdan *et al.*, 1987) (**Table 25**), compound **DW13** was identified as scopoletin.

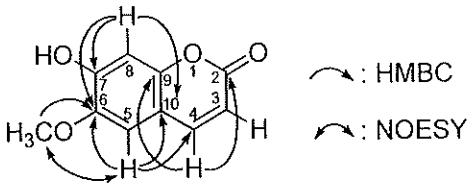


Figure 13 Major HMBC and NOESY correlations of **DW13**

Table 25 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW13** and comparison of ^{1}H and ^{13}C NMR of **DW13** and scopoletin (**R**)

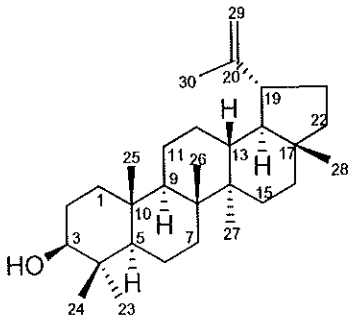
Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)		HMBC*
		R[a]	DW13[b]	R[a]	DW13[b]	
1						
2	CO	160.5	162.1			
3	CH	112.5	112.6	6.26 d (9.4)	6.28 d (9.6)	2, 4,10
4	CH	142.0	143.8	7.58 d (9.4)	7.60 d (9.6)	2, 5, 9, 10
5	CH	107.5	107.9	6.89 s	6.86 s	4, 6, 7, 10
6	C	143.0	144.8			
7	C	149.5	150.4			
8	CH	102.0	103.2	6.82 s	6.88 s	6, 7, 10
9	C	150.0	150.1			
10	C	110.5	111.2			
6-OCH_3		55.2	56.3	3.93 s	3.93 s	6

[a] Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

* For **DW13**

3.1.14 Compound DW14



Compound **DW14** was obtained as a white solid, mp. 193-194 °C, $[\alpha]_D^{28}$ +25.0 ($CHCl_3$, *c* 0.20). It exhibited hydroxyl (3343 cm^{-1}) and double bond (1638 cm^{-1}) absorptions in the IR spectrum and gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^1H NMR spectral data of **DW14** (**Table 26**, **Figure 64**) showed characteristic of lupane triterpenoid as seven methyl singlet signals at δ 0.76, 0.79, 0.83, 0.94, 0.97 and 1.03 including one vinylic methyl at δ 1.68, two protons of an isopropenyl moiety at δ 4.68 (1H, d, *J*= 2.1 Hz) and 4.56 (1H, m) and a typical lupane H_β-19 proton at δ 2.38 (dt, *J*= 11.1, 5.7 Hz). An oxymethine proton was shown at δ 3.19 (1H, dd, *J*= 10.8, 5.1 Hz, H-3). The doublet splitting pattern together with a large coupling constant of H-3 with $J_{ax\text{-}ax}$ = 10.8 Hz and $J_{ax\text{-}aq}$ = 5.1 Hz indicated an axial (α) orientation of H-3. The ^{13}C and DEPT spectral data (**Table 26**, **Figure 65**) of **DW14** showed all 30 carbon signals as seven methyl (δ 14.6, 15.4, 16.0, 16.1, 18.0, 19.3 and 28.0), eleven methylene (δ 18.3, 20.9, 25.2, 27.4, 27.5, 29.9, 34.3, 35.6, 38.7, 40.0 and 109.3), six methine (δ 38.1, 48.0, 48.3, 50.5, 55.3 and 79.0) and six quaternary carbons (δ 37.2, 38.9, 40.8, 42.8, 43.0 and 151.0).

The position of the hydroxyl group at C-3 was determined through an HMBC experiment (**Table 26**, **Figure 14**) in which the oxymethine proton at δ 3.19 (H-3) showed correlations with C-1 (δ 38.7), C-4 (δ 38.9), C-23 (δ 28.0) and C-24 (δ 15.4). The position of a methine proton at C-19 was determined from HMBC correlation of H-19 (δ 2.38) with C-18 (δ 48.3), C-20 (δ 151.0), C-21 (δ 29.9) and C-

30 (δ 19.3). Thus on the basis of its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data with the previous report (Reyolds *et al.*, 1986, $[\alpha]_D^{25}$ +23.0° (EtOH, *c* 0.50); Burns *et al.*, 2000; Thongdeeying 2005) (**Table 26**, **27** and **36**), compound **DW14** was identified as 3β-lupeol.

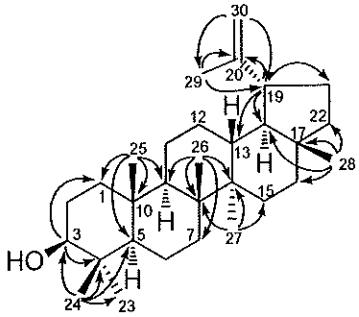


Figure 14 Major HMBC correlations of **DW14**

Table 26 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW14** and ^{13}C NMR of lupeol (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW14[c]	
1	CH_2	38.7	38.7	0.91 m[a]	
2	CH_2	27.4	27.4	1.56 m[a]	
3	CH	79.0	79.0	3.19 dd (10.8, 5.1)	1, 4, 23, 24
4	C	38.8	38.9		
5	CH	55.3	55.3	0.69 m[a]	
6	CH_2	18.3	18.3	1.40, 1.55 m[a]	
7	CH_2	34.2	34.3	1.40 m[a]	
8	C	40.8	40.8		
9	CH	50.3	50.5	1.28 m[a]	
10	C	37.1	37.2		
11	CH_2	20.9	20.9	1.22, 1.45 m[a]	
12	CH_2	25.1	25.2	1.08 m[a]	
13	CH	38.0	38.1	1.67 m[a]	

Table 26 (continued)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW14[c]	
14	C	42.8	42.8		
15	CH_2	27.4	27.5	1.56 m[a]	
16	CH_2	35.6	35.6	1.51 m[a]	
17	C	43.0	43.0		
18	CH	48.3	48.3	1.38 m[a]	
19	CH	48.0	48.0	2.38 dt (11.1, 5.7)	13, 18, 20, 29, 30
20	C	150.9	151.0		
21	CH_2	29.8	29.9	1.94 m[a]	
22	CH_2	40.0	40.0	1.20, 1.40 m[a]	
23	CH_3	28.0	28.0	0.97 s	3, 4, 5, 24
24	CH_3	15.3	15.4	0.76 s	3, 4, 5, 23
25	CH_3	16.1	16.1	0.83 s	1, 5, 9, 10
26	CH_3	15.9	16.0	1.03 s	7, 8, 9, 14
27	CH_3	14.5	14.6	0.94 s	8, 14, 15
28	CH_3	18.0	18.0	0.79 s	16, 17, 18, 22
29	CH_2	109.3	109.3	4.56 m	19, 30
				4.68 d (2.1)	
30	CH_3	19.3	19.3	1.68 s	19, 30

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 125 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

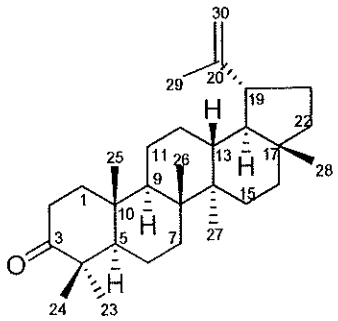
Table 27 Comparison of ^{1}H NMR spectral data of compounds **DW14** and lupeol

Position	δ_H/ppm (mult., *J* in Hz)	
	DW14[a]	lupeol[b]
1	0.91 m	0.90, 1.67 m
2	1.56 m	1.56, 1.60 m
3	3.19 dd (10.8, 5.1)	3.18 dd
5	0.69 m	0.68 m
6	1.40, 1.55 m	1.39, 1.51 m
7	1.40 m	1.39 m
9	1.28 m	1.27 m
11	1.22, 1.45 m	1.23, 1.41 m
12	1.08 m	1.07, 1.67 m
13	1.67 m	1.66 m
15	1.56 m	1.00, 1.68 m
16	1.51 m	1.37, 1.47 m
18	1.38 m	1.36 m
19	2.38 dt (11.1, 5.7)	2.38 dt
21	1.94 m	1.94 m
22	1.20, 1.40 m	1.19, 1.38 m
23	0.97 s	0.96 s
24	0.76 s	0.76 s
25	0.83 s	0.83 s
26	1.03 s	1.03 s
27	0.94 s	0.94 s
28	0.79 s	0.78 s
29	4.56 m	4.56 m
	4.68 d (2.1)	4.69 m
30	1.68 s	1.68 s

[a] Spectrum recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 500 (^{1}H NMR) MHz, $CDCl_3$.

3.1.15 Compound DW15



Compound **DW15** was obtained as a white solid, mp. 163-165 °C, $[\alpha]_D^{28}$ +50.0 ($CHCl_3$, *c* 0.10). It exhibited carbonyl (1704 cm^{-1}) and double bond (1642 cm^{-1}) absorptions in the IR spectrum and gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^1H and ^{13}C NMR spectral data (**Table 28**, **Figures 66** and **67**) of **DW15** showed signals similar to **DW14** (**Table 26**, **Figures 64** and **65**) except that in **DW15** a doublet of doublets signal of a methine proton H-3 disappeared and the carbon signal at C-3 (δ 217.0) was displayed as a carbonyl carbon instead of the oxy-methine carbon at δ 79.0 as in **DW14**. The location of the carbonyl group was confirmed by HMBC experiment (**Table 28**) in which both H_3-24 (δ 1.02) and H_3-23 (δ 1.07) showed long-range correlations with C-3 (δ 217.0), C-4 (δ 46.3) and C-5 (δ 54.3). Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report (Razdan *et al.*, 1988, $[\alpha]_D^{25}$ +50.0 ($CHCl_3$, *c* 0.10); Thongdeeying 2005) (**Table 28**, **29** and **36**), compound **DW15** was identified as lupenone.

Table 28 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW15** and ^{13}C NMR of lupenone (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW15[c]	
1	CH_2	39.6	38.6	0.90 m[a]	
2	CH_2	34.1	33.1	2.49 m[a]	
3	C	217.9	217.0		
4	C	47.2	46.3		
5	CH	55.8	54.3	1.32 m[a]	
6	CH_2	19.6	18.7	1.45 m[a]	
7	CH_2	33.5	32.6	0.87, 1.45 m[a]	
8	C	40.7	39.8		
9	CH	49.7	48.8	1.38 m[a]	
10	C	36.8	35.9		
11	CH_2	21.4	20.5	1.30 m[a]	
12	CH_2	25.1	24.2	1.68 m[a]	
13	CH	38.1	37.2	1.68 m[a]	
14	C	42.7	41.9		
15	CH_2	27.4	26.4	0.82 m[a]	
16	CH_2	35.6	34.5	1.37, 1.50 m[a]	
17	C	42.7	42.0		
18	CH	48.2	47.3	1.38 m[a]	
19	CH	47.8	47.0	2.40 m[a]	18, 20, 21, 29, 30
20	C	150.5	149.8		
21	CH_2	29.8	28.8	1.26, 1.92 m[a]	
22	CH_2	39.9	39.0	1.19, 1.41 m[a]	
23	CH_3	26.6	25.7	1.07 s	3, 4, 5, 24
24	CH_3	21.0	20.0	1.02 s	3, 4, 5, 23
25	CH_3	15.8	15.0	0.93 s	5, 9, 10
26	CH_3	15.4	14.8	1.07 s	7, 8, 9, 14

Table 28 (continued)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW15[c]	
27	CH_3	14.4	13.5	0.96 s	14, 15
28	CH_3	18.0	17.0	0.80 s	17, 18, 22
29	CH_2	109.2	108.1	4.57 m	19, 30
				4.69 d (2.1)	
30	CH_3	19.2	18.3	1.68 s	19, 20, 29

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 75 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 29 Comparison of ^{1}H NMR spectral data of compounds **DW14**, **DW15** and lupenone

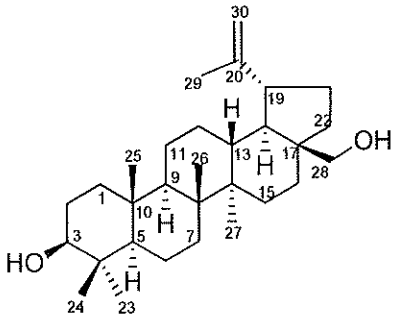
Position	δ_H/ppm (mult., *J* in Hz)		
	DW14	DW15	lupenone
1	0.91 m	0.90 m	
2	1.56 m	2.49 m	
3	3.19 dd (10.8, 5.1)		
5	0.69 m	1.32 m	
6	1.40, 1.55 m	1.45 m	
7	1.40 m	0.87, 1.45 m	
9	1.28 m	1.38 m	
11	1.22, 1.45 m	1.30 m	
12	1.08 m	1.68 m	
13	1.67 m	1.68 m	
15	1.56 m	0.82 m	
16	1.51 m	1.37, 1.50 m	
18	1.38 m	1.38 m	

Table 29 (continued)

Position	δ_H/ppm (mult., *J* in Hz)		
	DW14	**DW15**	lupenone
19	2.38 dt (11.1, 5.7)	2.40 m	2.24–252 m
21	1.94 m	1.26, 1.92 m	1.84–1.97 m
22	1.20, 1.40 m	1.19, 1.41 m	
23	0.97 s	1.07 s	1.04 s
24	0.76 s	1.02 s	1.00 s
25	0.83 s	0.93 s	0.90 s
26	1.03 s	1.07 s	1.02 s
27	0.94 s	0.96 s	0.93 s
28	0.79 s	0.80 s	0.77 s
29	4.56 m	4.57 m	4.55 br s
	4.68 d (2.1)	4.69 d (2.1)	4.66 br s
30	1.68 s	1.68 s	1.66 s

Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

3.1.16 Compound DW16



Compound **DW16** was obtained as a white solid, m.p. 230-231°C, $[\alpha]_D^{28}$ +16.7° ($CHCl_3$, *c* 0.15). The IR spectrum showed the characteristics of **DW14** and gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^1H and ^{13}C NMR spectral data of **DW16** (**Table 30**, **Figures 68** and **69**) and **DW14** (**Table 26**, **Figures 64** and **65**) revealed close structural similarity. The difference in the spectrum of **DW16** was shown as only six singlet signals of methyl groups at δ 0.76, 0.82, 0.97, 0.98, 1.02 and 1.68. In addition, the AB system of oxymethylene protons was shown at δ 3.80 (1H, dd, *J*= 10.8, 1.5 Hz) and 3.33 (1H, dd, *J*= 10.8) which was not observed in **DW14**. On the basis of HMBC experiment (**Table 30**), the oxymethylene protons (2H-28) showed long-range correlation with C-16 (δ 29.2), C-17 (δ 47.5) and C-22 (δ 34.0), thus the oxymethylene protons were located at C-28 (δ 60.6). This compound was assigned as betulin by comparison of its spectral data (**Table 30**, **31** and **36**) with those reported in the literature (Monago *et al.*, 1984, $[\alpha]_D$ +19° ($CHCl_3$, *c* 0.15); Tinto *et al.*, 1992; Thongdeeying 2005).

Table 30 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **DW16** and ^{13}C NMR of betulin (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW16[c]	
1	CH_2	38.8	38.7	0.90, 1.70 m[a]	
2	CH_2	27.2	27.4	1.59 m[a]	
3	CH	78.9	79.0	3.19 dd (10.8, 5.1)	1, 4, 23, 24
4	C	38.9	38.9		
5	CH	55.3	55.3	0.68 m[a]	
6	CH_2	18.3	18.3	1.41 m[a]	
7	CH_2	34.3	34.2	1.04, 1.40 m[a]	
8	C	40.9	40.9		
9	CH	50.4	50.4	1.27 m[a]	
10	C	37.2	37.2		
11	CH_2	20.9	20.8	1.28, 1.46 m[a]	
12	CH_2	25.3	25.2	1.68 m[a]	
13	CH	27.3	37.3	1.67 m[a]	
14	C	42.7	42.7		
15	CH_2	27.0	27.0	1.11, 1.66 m[a]	
16	CH_2	29.2	29.2	1.20, 1.98 m[a]	
17	C	47.8	47.5		
18	CH	48.8	48.8	1.60 m[a]	
19	CH	47.8	47.5	2.38 m[a]	18, 20, 21, 29, 30
20	C	150.6	150.5		
21	CH_2	29.8	29.8	1.91 m[a]	
22	CH_2	34.0	34.0	1.80, 1.88 m[a]	
23	CH_3	28.0	28.0	0.97 s	3, 4, 5, 24
24	CH_3	15.4	15.4	0.76 s	3, 4, 5, 23
25	CH_3	16.1	16.1	0.82 s	1, 5, 9
26	CH_3	16.0	16.0	1.02 s	7, 8, 9, 14

Table 30 (continued)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW16[c]	
27	CH_3	14.8	14.8	0.98 s	8, 13,14, 15
28	CH_2	60.2	60.6	3.33 d (10.8)	16, 17, 22
				3.80 dd (10.8, 1.5)	
29	CH_2	109.6	109.7	4.58 m	19, 20, 30
				4.68 d (2.1)	
30	CH_3	19.1	19.1	1.68 s	19, 20, 29

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 31 Comparison of ^{1}H NMR spectral data of compounds **DW14, DW16** and betulin

Position	δ_H /ppm (mult., *J* in Hz)		
	DW14[a]	DW16[a]	betulin[b]
1	0.91 m	0.90, 1.70 m	
2	1.56 m	1.59 m	
3	3.19 dd (10.8, 5.1)	3.19 dd (10.8, 5.1)	3.20 dd (10.1, 4.7)
5	0.69 m	0.68 m	
6	1.40, 1.55 m	1.41 m	
7	1.40 m	1.04, 1.40 m	
9	1.28 m	1.27 m	
11	1.22, 1.45 m	1.28, 1.46 m	
12	1.08 m	1.68 m	
13	1.67 m	1.67 m	
15	1.56 m	1.11, 1.66 m	
16	1.51 m	1.20, 1.98 m	

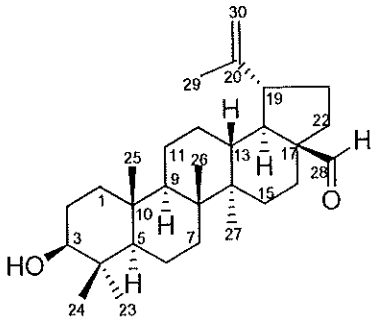
Table 31 (continued)

Position	δ_H/ppm (mult., *J* in Hz)		
	DW14[a]	**DW16**[a]	betulin[b]
18	1.38 m	1.60 m	
19	2.38 dt (11.1, 5.7)	2.38 m	
21	1.94 m	1.91 m	
22	1.20, 1.40 m	1.80, 1.88 m	
23	0.97 s	0.97 s	0.96 s
24	0.76 s	0.76 s	0.76 s
25	0.83 s	0.82 s	0.82 s
26	1.03 s	1.02 s	0.97 s
27	0.94 s	0.98 s	0.98 s
28	0.79 s	3.33 d (10.8)	3.33 d (11.0)
		3.80 dd (10.8, 1.5)	3.79 d (11.0)
29	4.56 m	4.58 m	4.58 m
	4.68 d (2.1)	4.68 d (2.1)	4.68 m
30	1.68 s	1.68 s	1.65 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

3.1.17 Compound DW17



Compound **DW17** was obtained as a colorless viscous oil. It gave a purple vanillin-sulfuric acid test. Due to its instability, no IR spectrum was obtained.

The ^{1}H and ^{13}C NMR spectral data (**Table 32, Figures 70** and **71**) of **DW17** were similar to those of **DW14** (**Table 26**, **Figures 64** and **65**), except that **DW17** had only six methyl singlets at δ 0.75, 0.82, 0.92, 0.96, 0.98 and 1.70 and showed additional signal of aldehydic proton at δ 9.68 (1H, d, *J*= 1.5 Hz). The signal of a methine proton at δ 2.86 (H-19) in **DW17** was shifted more downfield than **DW14** (δ 2.38). On the basis of the HMBC (**Table 32**), the aldehyde group was located at C-28 (δ 206.7) from correlation of H-28 (δ 9.68) with C-17 (δ 59.3) and C-18 (δ 48.1). Compound **DW17** was identified as betulinaldehyde by comparison of its spectral data with those reported in the literature (Macias *et al*, 1994) (**Table 32, 33** and **36**).

Table 32 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compounds **DW17** and ^{13}C NMR of betulinaldehyde (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW17[c]	
1	CH_2	38.7	38.7	0.91, 1.67 m[a]	
2	CH_2	27.3	27.4	1.58, 1.66 m[a]	
3	CH	78.9	79.0	3.18 dd (10.8, 5.1)	23, 24
4	C	38.8	38.8		
5	CH	55.5	55.3	0.67 m[a]	
6	CH_2	18.2	18.3	1.40, 1.55 m[a]	
7	CH_2	34.3	34.3	1.38, 1.44 m[a]	
8	C	40.8	40.8		
9	CH	50.4	50.5	1.26 m[a]	
10	C	37.1	37.2		
11	CH_2	20.7	20.7	1.27, 1.46 m[a]	
12	CH_2	25.5	25.2	1.75 m[a]	
13	CH	38.7	38.7	2.03 m[a]	
14	C	42.5	42.6		
15	CH_2	29.2	29.3	1.46 m[a]	
16	CH_2	28.8	28.8	1.17, 2.12 m[a]	14, 17, 20, 28
17	C	59.3	59.3		
18	CH	48.0	48.1	1.73 m[a]	
19	CH	47.5	47.5	2.86 dt (10.8, 5.7)	18, 21, 30
20	C	149.7	149.7		
21	CH_2	29.8	29.9	1.24, 1.89 m[a]	
22	CH_2	33.2	33.2	1.34, 1.80 m[a]	
23	CH_3	27.9	28.0	0.96 s	3, 4, 5, 24
24	CH_3	15.4	15.3	0.75 s	3, 4, 5, 23
25	CH_3	15.9	16.1	0.82 s	1, 5, 9, 10
26	CH_3	16.1	16.0	0.92 s	7, 8, 9, 14

Table 32 (continued)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW17[c]	
27	CH_3	14.2	14.3	0.98 s	8, 14, 15
28	CHO	205.6	206.7	9.68 d (1.5)	17, 18
29	CH_2	110.1	110.2	4.63 m	19, 30
				4.76 m	
30	CH_3	19.0	19.0	1.70 s	19, 20, 29

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 33 Comparison of ^{1}H NMR spectral data of compounds **DW14, DW17** and betulinaldehyde

Position	δ_H/ppm (mult., *J* in Hz)		
	DW14[a]	DW17[a]	betulin[b]
1	0.91 m	0.91, 1.67 m	0.90, 1.65 m
2	1.56 m	1.58, 1.66 m	1.54, 1.59 m
3	3.19 dd (10.8, 5.1)	3.18 dd (10.8, 5.1)	3.17 dd (11.1, 5.0)
5	0.69 m	0.67 m	0.67 m
6	1.40, 1.55 m	1.40, 1.55 m	1.36, 1.55 m
7	1.40 m	1.38, 1.44 m	1.40 m
9	1.28 m	1.26 m	1.28 m
11	1.22, 1.45 m	1.27, 1.46 m	1.24, 1.42 m
12	1.08 m	1.75 m	1.02, 1.74 m
13	1.67 m	2.03 m	2.01 m
15	1.56 m	1.46 m	1.17 m
16	1.51 m	1.17, 2.12 m	1.42, 2.06 m

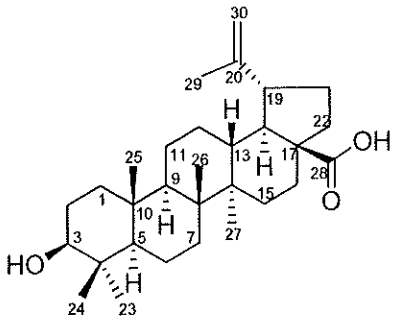
Table 33 (continued)

Position	δ_H/ppm (mult., *J* in Hz)		
	DW14[a]	**DW17**[a]	betulinaldehyde[b]
18	1.38 m	1.73 m	1.71 m
19	2.38 dt (11.1, 5.7)	2.86 dt (10.8, 5.7)	2.85 m
21	1.94 m	1.24, 1.89 m	1.45, 1.87 m
22	1.20, 1.40 m	1.34, 1.80 m	1.33, 1.74 m
23	0.97 s	0.96 s	0.95 s
24	0.76 s	0.75 s	0.74 s
25	0.83 s	0.82 s	0.80 s
26	1.03 s	0.92 s	0.90 s
27	0.94 s	0.98 s	0.96 s
28	0.79 s	9.68 d (1.5)	9.66 d
29	4.56 m	4.63 m	4.62
	4.68 d (2.1)	4.76 m	4.74
30	1.68 s	1.70 s	1.68 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

3.1.18 Compound DW18



Compound **DW18** was obtained as a white solid, mp. 279-280 °C, $[\alpha]_D^{28}$ +15.0 ($CHCl_3$, *c* 0.10). It exhibited hydroxyl (3415 cm^{-1}) and a carboxyl (1686 cm^{-1}) absorption in the IR spectrum. It also gave a purple vanillin-sulfuric acid test indicating a triterpene.

The ^1H and ^{13}C NMR spectral data (**Table 34**, **Figures 72** and **73**) of **DW18** were similar to those of **DW14** (**Table 26**, **Figures 64** and **65**). The difference in the spectrum of **DW18** was shown as disappearance of a methyl signal at δ_H 0.79 (H_3-28, s, δ_C 18.0) in **DW14** and the appearance of a carboxyl signal at δ_C 179.6 (C-28) in **DW18**. The location of the carboxyl group was confirmed by HMBC experiment (**Table 34**) in which the methylene proton signals at δ 1.93 (1H, m, H-22a) and 1.40 (1H, m, H-22b) showed correlations with C-17 (δ 55.3), C-18 (δ 48.3) and C-28 (δ 179.6). Thus on the basis of its spectroscopic data and comparison with the previous report (Tinto *et al.*, 1992, $[\alpha]_D^{28}$ +6.8° (pyridine, *c* 2.00); Peng *et al.*, 1998; Thongdeeying 2005) (**Table 34-36**), compound **DW18** was identified as betulinic acid.

Table 34 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compounds **DW18** and ^{13}C NMR of betulinic acid (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW18[c]	
1	CH_2	38.5	37.7	0.87, 1.64 m[a]	
2	CH_2	28.2	26.4	1.55 m[a]	
3	CH	78.1	78.0	3.19 dd (10.8, 5.4)	
4	C	39.4	37.9		
5	CH	55.9	54.4	0.69 m[a]	
6	CH_2	18.7	17.3	1.35, 1.48 m[a]	
7	CH_2	34.7	33.3	1.35 m[a]	
8	C	41.0	39.7		
9	CH	50.9	49.5	1.20 m[a]	
10	C	37.5	36.2		
11	CH_2	21.1	19.8	1.42 m[a]	
12	CH_2	26.0	24.5	1.67 m[a]	
13	CH	39.2	37.4	2.20 m[a]	
14	C	42.8	41.4		
15	CH_2	30.2	28.7	1.14, 1.23 m[a]	
16	CH_2	32.8	31.2	2.22 m[a]	
17	C	56.6	55.3		
18	CH	49.7	48.3	1.55 m[a]	
19	CH	47.7	45.9	3.00 m[a]	18, 20, 21, 29, 30
20	C	151.4	149.4		
21	CH_2	31.1	29.6	1.89 m[a]	
22	CH_2	37.4	36.0	1.40, 1.93 m[a]	17, 18, 28
23	CH_3	28.5	27.0	0.97 s	3, 4, 5, 24
24	CH_3	16.2	14.3	0.75 s	3, 4, 5, 23
25	CH_3	16.3	15.1	0.82 s	1, 5, 9,10
26	CH_3	16.2	15.0	0.94 s	7, 8, 9, 14

Table 34 (continued)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW18[c]	
27	CH_3	14.8	13.7	0.98 s	8, 13,14, 15
28	CO	180.0	179.6		
29	CH_2	109.2	108.7	4.74 br s	19, 20, 30
				4.61 br s	
30	CH_3	19.3	18.4	1.69 s	19, 20, 29

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 35 Comparison of ^{1}H NMR spectral data of compounds **DW14, DW18** and betulinic acid

Position	δ_H/ppm (mult., *J* in Hz)		
	DW14	DW18	betulinic acid
1	0.91 m	0.87, 1.64 m	
2	1.56 m	1.55 m	
3	3.19 dd (10.8, 5.1)	3.19 dd (10.8, 5.4)	3.19 dd (10.0, 4.7)
5	0.69 m	0.69 m	
6	1.40, 1.55 m	1.35, 1.48 m	
7	1.40 m	1.35 m	
9	1.28 m	1.20 m	
11	1.22, 1.45 m	1.42 m	
12	1.08 m	1.67 m	
13	1.67 m	2.20 m	
15	1.56 m	1.14, 1.23 m	
16	1.51 m	2.22 m	
18	1.38 m	1.55 m	

Table 35 (continued)

Position	δ_H/ppm (mult., *J* in Hz)		
	DW14	**DW18**	betulinic acid
19	2.38 dt (11.1, 5.7)	3.00 m	2.99 ddd (11.0, 11.0, 5.5)
21	1.94 m	1.89 m	
22	1.20, 1.40 m	1.40, 1.93 m	
23	0.97 s	0.97 s	0.93 s
24	0.76 s	0.75 s	0.75 s
25	0.83 s	0.82 s	0.82 s
26	1.03 s	0.94 s	0.96 s
27	0.94 s	0.98 s	0.97 s
28	0.79 s		
29	4.56 m	4.61 br s	4.60 d (1.5)
	4.68 d (2.1)	4.74 br s	4.73 d (1.5)
30	1.68 s	1.69 s	1.68 s

Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

Table 36 Comparison of ^{13}C NMR spectral data of compounds **DW14-DW18**

Position	**DW14**	**DW15**	**DW16**	**DW17**	**DW18**
1	38.7	38.6	37.7	38.7	38.7
2	27.4	33.1	26.4	27.4	27.4
3	79.0	217.0	78.0	79.0	79.0
4	38.9	46.3	37.9	38.9	38.8
5	55.3	54.3	54.4	55.3	55.3
6	18.3	18.7	17.3	18.3	18.3
7	34.3	32.6	33.3	34.2	34.3
8	40.8	39.8	39.7	40.9	40.8
9	50.5	49.7	49.5	50.4	50.5
10	37.2	36.8	36.2	37.2	37.2

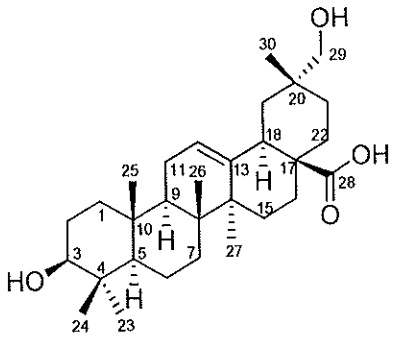
Table 36 (continued)

Position	DW14	DW15	DW16	DW17	DW18
11	20.9	21.4	19.8	20.8	20.7
12	25.2	25.1	24.5	25.2	25.2
13	38.1	38.1	37.4	37.3	38.7
14	42.8	42.7	41.4	42.7	42.6
15	27.5	27.4	28.7	27.0	29.3
16	35.6	35.6	31.2	29.2	28.8
17	43.0	42.7	55.3	47.5	59.3
18	48.3	48.2	48.3	48.8	48.1
19	48.0	47.8	45.9	47.5	47.5
20	151.0	150.5	149.4	150.5	149.7
21	29.9	29.8	29.6	29.8	29.9
22	40.0	39.9	36.0	34.0	33.2
23	28.0	26.6	27.0	28.0	28.0
24	15.4	21.0	14.3	15.4	15.3
25	16.1	15.8	15.1	16.1	16.1
26	16.0	15.4	15.0	16.0	16.0
27	14.6	13.5	13.7	14.8	14.3
28	18.0	17.0	179.6	60.6	206.7
29	109.3	108.1	108.7	109.7	110.2
30	19.3	18.3	18.4	19.1	19.0

Assignments were based on DEPT 90° and DEPT 135°experiments.

Spectra recorded at 75 (^{13}C NMR) MHz, $CDCl_3$.

3.1.19 Compound DW19



Compound **DW19** was isolated as an amorphous powder, m.p. 334-336 °C; $[\alpha]_D^{26}$ +82.0 ($CHCl_3$; *c* 0.10). It exhibited hydroxyl (3400 cm^{-1}) and carbonyl (1695 cm^{-1}) absorptions in the IR spectrum.

The ^{13}C and DEPT spectral data (**Table 37**, **Figure 75**) of **DW19** showed all 30 carbon signals as six methyl (δ 15.3, 15.4, 17.0, 19.0, 26.0 and 28.0), eleven methylene (δ 18.3, 23.0, 23.4, 27.2, 27.6, 28.8, 31.6, 32.6, 38.4, 40.1, and 74.3), five methine (δ 40.2, 47.6, 55.2 79.0 and 123.0), seven quaternary carbons (δ 35.7, 37.1, 38.7, 39.3, 41.6, 46.8 and 143.3) and a carboxyl carbon (δ 182.0).

The ^1H NMR spectral data (**Table 37**, **Figure 74**) of **DW19** showed signals for six tertiary methyl groups at δ_H 0.76, 0.77, 091, 096, 0.98 and 1.14, whish correlated in the HMQC experiments with the carbon signals at δ_C 15.3, 15.5, 17.0, 19.0, 26.0 and 28.0, respectively. A further feature was the signal at 5.30 (1H, br s) typical of H-12 of oleanene skeleton, which was confirmed by the presence of the signals at δ_C 123.0 and 143.3 attributable to C-12 and C-13 in the ^{13}C NMR spectrum (**Table 37**, **Figures 73**). A signal at δ_C 182.0 and the carbon resonances of ring D in the ^{13}C NMR spectrum similar to those of oleanolic acid suggested the occurrence of a free carboxylic group at C-28 position. The presence of one secondary alcoholic function was also deduced from the signal at δ_H 3.22 (1H, dd, *J*= 10.2, 4.8 Hz), which correlated in the HMQC spectrum with the carbon resonance at δ_C 79.0. This signal is typical of ring A of a 3β-hydroxy substituted oleanolic acid (Maillard *et al.*, 1992). The NMR spectra of **DW19** contained one methyl group less than oleanolic acid and one signal at δ 3.28 (2H, s) in the ^1H NMR spectrum that correlated to δ 74.3 in the

^{13}C NMR spectrum, suggesting that one of the methyl groups of the oleanolic acid was replaced by a hydroxymethyl group. This group was located at C-29 on the basis of HMBC correlations (**Table 37**, **Figure 15**) from the methyl at δ 0.96 (CH_3-30) to C-29 (δ 74.3). Compound **DW19** was identified as 3β,29-dihydroxyolean-12-en-28-oic acid by comparison of its spectral data with those reported in the literature (Ahmad *et al*, 1984, $[\alpha]_D$ +70.4 (CH_3OH; *c* 0.3); (**Table 37**).

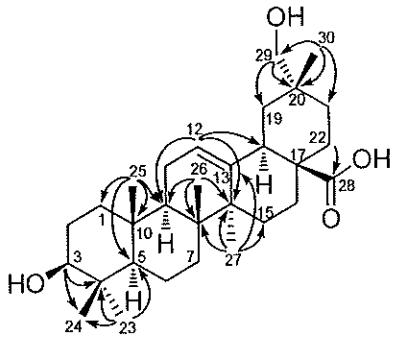


Figure 15 Major HMBC correlations of **DW19**

Table 37 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compounds **DW19** and ^{13}C NMR of 3β,29-dihydroxyolean-12-en-28-oic acid (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW19[c]	
1	CH_2	38.9	38.4	1.13, 1.60 m[a]	
2	CH_2	28.1	27.2		
3	CH	78.1	79.0	3.22 dd (10.2, 4.8)	23, 24
4	C	39.4	37.1		
5	CH	55.9	55.2	0.71 m[a]	
6	CH_2	18.8	18.3	1.39, 1.59 m[a]	
7	CH_2	33.3	32.6	1.30, 1.44 m[a]	
8	C	39.8	39.3		
9	CH	48.2	47.6	1.57 m[a]	

Table 37 (continued)

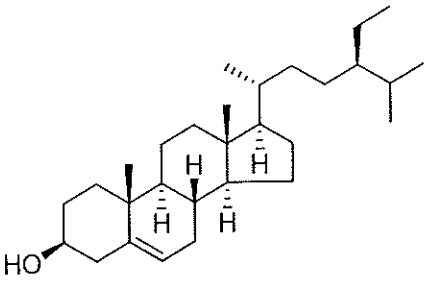
Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		DW19[c]	
10	C	37.4	38.7		
11	CH_2	23.8	23.4	1.65, 1.98 m[a]	
12	CH	122.5	123.0	5.30 br s	9, 14, 18
13	C	144.9	143.3		
14	C	42.2	41.6		
15	CH_2	28.2	27.6		
16	CH_2	23.8	23.0	1.63, 1.70 m[a]	
17	C	47.2	46.8		
18	CH	41.4	40.2	1.13 m[a]	
19	CH_2	41.4	40.1	2.87 dd (13.8, 3.9), 1.70 m	18, 21, 30
20	C	36.6	35.7		
21	CH_2	29.1	28.8		
22	CH_2	32.7	31.6	1.62, 1.80 m[a]	28
23	CH_3	28.8	28.0	0.98 s	3, 4, 5, 24
24	CH_3	16.5	15.3	0.77 s	3, 4, 5, 23
25	CH_3	15.5	15.4	0.91 s	1, 5, 9, 10
26	CH_3	17.4	17.0	0.76 s	8, 9, 14
27	CH_3	26.2	26.0	1.14 s	8, 13, 14, 15
28	CO	180.2	182.0		
29	CH_2	73.9	74.3	3.28 s	19, 20, 21, 30
30	CH_3	19.8	19.0	0.96 s	19, 20, 21, 29

[a] Deduced from HMQC experiment.

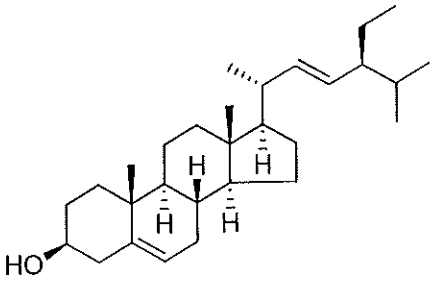
[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.1.20 Compounds DW20 and DW21



DW20: β-Sitosterol



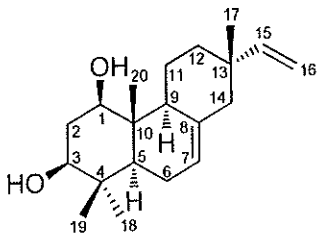
DW21: Stigmasterol

The mixture of **DW20** and **DW21** was isolated as a white solid. Its IR spectrum showed absorption bands at 3425 (hydroxyl) and 1642 cm^{-1} (double bond). The ^1H NMR (**Figure 76**) spectral data contained an oxymethine proton at δ 3.57-3.47 (m), three olefinic protons at δ 5.36-5.34 (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 ^1H NMR data was corresponded to previous reported data of β-sitosterol and stigmasterol. Thus, this mixture was identified as β-sitosterol (**DW20**) and stigmasterol (**DW21**) (Cheenpracha, 2004).

3.2 Structure elucidation of compounds from the roots and twigs of *Premna obtusifolia*

The air-dried roots and twigs of *P. obtusifolia* (4.5 kg) were extracted with hexane and methylene chloride successively at room temperature. The hexane and CH_2Cl_2 extracts were subjected to silica gel vacuum liquid chromatography repeatedly and /or crystallization to give eleven new compounds as two isopimarane diterpenoids (**PO1** and **PO2**), one rosane diterpenoid (**PO3**), four abietane diterpenoids (**PO4**, **PO5**, **PO18** and **PO19**), four icetexane diterpenoids (**PO23-PO26**), together with nineteen known compounds (**PO6**, **PO7**, **PO8-PO15**, **PO16**, **PO17**, **PO20-PO22** and **PO27-PO29**). Their structures were elucidated by spectroscopic analysis, mainly UV, IR, 1D and 2D NMR (^1H, ^{13}C, DEPT, COSY, NOESY, HMQC and HMBC) and HREIMS spectroscopic techniques. The known compounds were identified by comparison of their spectroscopic and physical data with those reported in the literature. In addition, the structures of compounds **PO10**, **PO11**, **PO13**, **PO20**, **PO23** and **PO25** were additionally confirmed by X-ray diffraction analysis.

3.2.1 Compound PO1



Compound **PO1** was obtained as a white amorphous solid, m.p. 168–170 °C, $[\alpha]_D^{26}$ –18.4 ($CHCl_3$; *c* 0.59) which exhibited a molecular ion $[M]^{+\bullet}$ peak at 304.2407 (calcd 304.2402) in the HREIMS corresponding to a molecular formula $C_{20}H_{32}O_2$ with five degrees of unsaturation. The IR spectrum showed strong absorption bands for hydroxyl at 3339 cm^{-1} and double bond at 1637 cm^{-1}.

The combined analysis of the ^{13}C NMR and DEPT spectra of **PO1** (**Table 38**, **Figure 78**) revealed the presence of 20 carbon signals assigned to four methyls, six methylenes, six methines including those of two oxymethines, and two olefinic methine carbons and four quaternary carbons.

The occurrence in the ^1H NMR spectrum of **PO1** (**Table 38**, **Figure 77**) of a vinyl group with three dd resonances at δ_H 5.80 (*J*= 17.4 and 10.8 Hz, H-15), δ_H 4.92 (*J* = 17.4 and 1.2 Hz, H_b-16) and δ_H 4.86 (*J* = 10.8 and 1.2 Hz, H_a-16) and the presence of a broad doublet for an olefinic methine proton at δ_H 5.39 (*J*= 3.0 Hz, H-7) and four methyl group singlets suggested a pimarane type skeleton (Wenkert *et al.*, 1972). Two oxymethine carbons resonated at δ_C 79.0 (C-1) and 76.1 (C-3). Their positions were deduced from the HMBC correlations (**Table 38**, **Figure 16**) observed between H-1 (δ_H 3.55) and C-2 (δ 37.9) and C-10 (δ 41.5) and between H-3 (δ_H 3.33) and C-2 (δ 37.9) and C-4 (δ 38.7), respectively. The oxymethine protons H-1 and H-3 both resonated as a doublet of doublets with large and small coupling constants of 12.0 and 4.5 Hz and 12.0 and 4.2 Hz, respectively. The magnitude of these coupling constants suggested the α axial position for both of these protons. These assignments were further supported from NOESY experiments (**Figure 16**). An olefinic methine proton at δ 5.39 (H-7) showed HMBC correlations (**Table 38**) with the carbons at δ

46.7 (C-14), 48.7 (C-5) and 52.4 (C-9) and COSY cross-peak with the methylene protons H_2-6 at δ 1.97-2.04 and 2.03-2.11.

The stereochemistry at C-13 was established by comparison of the ^{13}C NMR chemical shifts of C-12, C-13, C-14, C-15, C-16 and C-17 with those of isopimarane diterpenoids (Wenkert *et al.*, 1972; Lago *et al.*, 2000; Passannanti *et al.*, 1984; Rijo *et al.*, 2009): isopimaradiene (Wenkert *et al.*, 1972) establishing an axial position for the Me-17 and an equatorial position for the vinyl group. Compound **PO1** was therefore a new compound which identified as isopimara-7,15-dien-1β,3β-diol (Salae *et al.*, 2012).

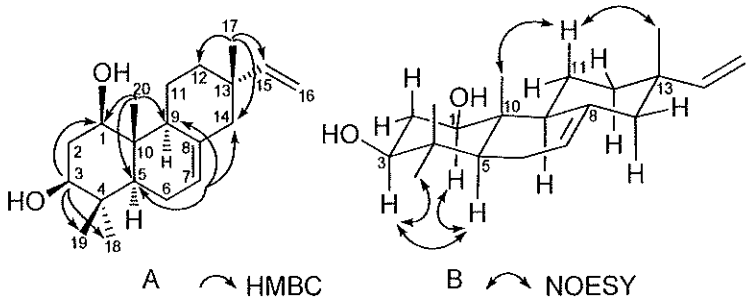


Figure 16 Selected HMBC correlations (A) and NOESY correlations (B) for **PO1**

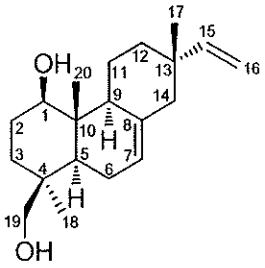
Table 38 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO1**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CH	79.0	3.55 dd (12.0, 4.5)	2, 3, 9, 10, 20
2	CH_2	37.9	1.71 q (12.0)	1, 3, 4,10
			1.80–1.90 m[a]	
3	CH	76.1	3.33 dd (12.0, 4.2)	1, 2, 4, 18, 19
4	C	38.7		
5	CH	48.7	1.02 dd (11.7, 4.8)	4, 7, 9, 10, 18, 19, 20
6	CH_2	22.6	1.97–2.04 m[a]	5, 7, 8, 10
			2.03–2.11 m[a]	
7	CH	121.2	5.39 br d (3.0)	5, 6, 9, 14
8	C	136.1		
9	CH	52.4	1.81–1.91 m[a]	
10	C	41.5		
11	CH_2	23.9	1. 37–1.53 m[a]	8, 9, 12, 14, 17
			2.10–2.21 m[a]	
12	CH_2	36.5	1.33–1.53 m[a]	9, 11, 13, 14, 17
13	C	36.8		
14	CH_2	46.7	1.91 d (15.3)	9, 12, 13, 17
			1.98 d (15.3)	
15	CH	150.3	5.80 dd (17.4, 10.8)	12, 13, 14, 17
16	CH_2	109.2	4.86 dd (10.8, 1.2)	13, 15
			4.92 dd (17.4, 1.2)	
17	CH_3	21.5	0.87 s	12, 13, 14, 15
18	CH_3	28.3	0.98 s	3, 4, 5, 19
19	CH_3	15.7	0.88 s	3, 4, 5, 18
20	CH_3	8.3	0.89 s	1, 5, 9, 10

[a] Deduced from HMQC experiment.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.2.2 Compound PO2



Compound **PO2** was also obtained as a white amorphous solid, m.p. 92–95 °C, $[\alpha]_D^{26}$ –17.5 ($CHCl_3$; *c* 0.71). The HREIMS showed a molecular ion $[M]^{+\bullet}$ peak at *m/z* 304.2403 (calcd 304.2402) consistent with the molecular formula $C_{20}H_{32}O_2$ and like **PO1** implied five degrees of unsaturation. The IR spectrum proved the existence of hydroxyl (3344 cm^{-1}) and double bond (1637 cm^{-1}).

The ^1H and ^{13}C NMR spectroscopic data of **PO2** (**Table 39**, **Figures 79** and **80**) indicated that **PO2** had the same isopimarane diterpene skeleton as **PO1** (**Table 38**, **Figures 77** and **78**). Compound **PO2**, however, showed the presence of an AB system (δ_H 3.40 and 3.84 (d, *J*= 10.8 Hz, H_2-19)) for a hydroxymethyl group attached to the quaternary carbon C-4, instead of the singlet for the C-19 protons at δ_H 0.88 in the ^1H NMR spectrum of compound **PO1** and the signal of an oxymethine proton at δ_H 3.33 (H-3) in **PO1** was replaced by multiplet signals of methylene protons at δ 1.00-1.16 and 1.84-1.97 in **PO2**. The ^{13}C NMR and DEPT spectra confirmed the presence of oxymethylene at δ_C 64.2 and the methylene at δ_C 33.1. This finding was further confirmed by the HMBC correlations from H_3-18 (δ 0.95) to C-19 (δ 64.2) and C-3 (δ 33.1).

The stereochemistry at the stereogenic centers was consistent with that of **PO1** based on the analysis of the NOESY experiment. The location of the primary alcohol at C-19 was found to be β-oriented due to NOESY cross-peak between H_3-19/H_3-20. Compound **PO2** was therefore a new compound which elucidated as isopimara-7,15-dien-1β,19-diol (Salae *et al.*, 2012).

Table 39 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO2**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CH	81.4	3.45 dd (11.1, 5.1)	2, 3, 9, 10, 20
2	CH_2	28.7	1.53–1.67 m[a]	
3	CH_2	33.1	1.00–1.16 m[a]	
			1.84–1.97 m[a]	
4	C	37.8		
5	CH	51.3	1.20–1.32 m[a]	4, 6, 7, 10, 18, 20
6	CH_2	22.6	1.90–2.04 m[a]	
7	CH	121.4	5.37 br s	5, 9
8	C	136.3		
9	CH	52.6	1.87–1.96 m[a]	1, 5, 7, 8, 12
10	C	41.2		
11	CH_2	23.9	1.37–1.45 m[a]	
			2.13–2.27 m[a]	
12	CH_2	36.7	1.35–1.51 m[a]	
13	C	36.5		
14	CH_2	46.8	1.83–2.04 m[a]	
15	CH	150.4	5.80 dd (17.7, 10.8)	12, 13, 14, 17
16	CH_2	109.1	4.85 dd (10.8, 1.2)	13, 15
			4.91 dd (17.7, 1.2)	
17	CH_3	21.4	0.87 s	12, 13, 14, 15
18	CH_3	26.6	0.95 s	3, 4, 5, 19
19	CH_2	64.2	3.40 d (10.8)	3, 4, 5, 18
			3.84 d (10.8)	
20	CH_3	9.5	0.87 s	3, 4, 18

[a] Deduced from HMQC experiment.

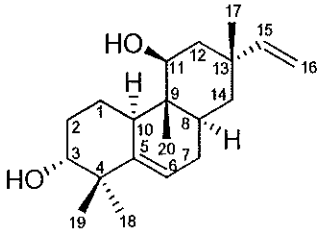
Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 40 Comparison of ^{1}H and ^{13}C NMR spectral data of compounds **PO1** and **PO2**

Position	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	
	PO1	**PO2**	**PO1**	**PO2**
1	79.0	81.4	3.55 dd (12.0, 4.5)	3.45 dd (11.1, 5.1)
2	37.9	28.7	1.71 q (12.0)	1.53–1.67 m
			1.80–1.90 m	
3	76.1	33.1	3.33 dd (12.0, 4.2)	1.00–1.16 m
				1.84–1.97 m
4	38.7	37.8		
5	48.7	51.3	1.02 dd (11.7, 4.8)	1.20–1.32 m
6	22.6	22.6	1.97–2.04 m	1.90–2.04 m
			2.03–2.11 m	
7	121.2	121.4	5.39 br d (3.0)	5.37 br s
8	136.1	136.3		
9	52.4	52.6	1.81–1.91 m	1.87–1.96 m
10	41.5	41.2		
11	23.9	23.9	1. 37–1.53 m	1.37–1.45 m
			2.10–2.21 m	2.13–2.27 m
12	36.5	36.7	1.33–1.53 m	1.35–1.51 m
13	36.8	36.5		
14	46.7	46.8	1.91 d (15.3)	1.83–2.04 m
			1.98 d (15.3)	
15	150.3	150.4	5.80 dd (17.4, 10.8)	5.80 dd (17.7, 10.8)
16	109.2	109.1	4.86 dd (10.8, 1.2)	4.85 dd (10.8, 1.2)
			4.92 dd (17.4, 1.2)	4.91 dd (17.7, 1.2)
17	21.5	21.4	0.87 s	0.87 s
18	28.3	26.6	0.98 s	0.95 s
19	15.7	64.2	0.88 s	3.40 d (10.8), 3.84 d (10.8)
20	8.3	9.5	0.89 s	0.87 s

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.2.3 Compound PO3



Compound **PO3** was isolated as white amorphous solid, m.p. 78–81 °C, $[\alpha]_D^{26}$ –13.6 ($CHCl_3$; *c* 0.09). Its molecular formula $C_{20}H_{32}O_2$ was established by the HREIMS which exhibited a molecular ion $[M]^{+\bullet}$ peak at *m/z* 304.2397 (calcd 304.2402) suggesting five degrees of unsaturation. The IR spectrum showed absorption bands for hydroxyl (3409 cm^{-1}) and double bond (1615 cm^{-1}).

The ^1H NMR spectrum of **PO3** (**Table 41**, **Figure 81**) displayed four tertiary methyl groups (δ_H 1.12, 0.97, 0.95 and 0.69) and four olefinic protons of a vinyl group (δ_H 5.75, 5.02 and 5.00), a trisubstituted double bond (δ_H 5.56) and two oxymethine protons (δ_H 3.60 and 3.21). The ^{13}C NMR spectrum (**Table 41**, **Figure 82**) revealed the presence of 20 carbons including two oxymethine (δ_C 77.9 and 77.0), a vinyl group (δ_C 146.4 and 112.1), a trisubstituted double bond (δ_C 145.8 and 117.9) and four methyl groups (δ_C 31.3, 24.6, 21.0 and 6.3). Pimarane, isopimarane and rosane skeletons have four angular methyl groups and can be differentiated by the relative stereochemistry at C-3 and the position of the methyl at C-20. Rosanes arise from migration of the C-10 methyl group of pimaranes to the C-9 position and occurs in both enantiomeric series (Santos *et al.*, 2006).

The placement of a methyl group at C-9 in a rosane skeleton for **PO3** was established from the HMBC correlations (**Table 41**, **Figure 17**) of CH_3-20 at δ 0.69 to the carbons at δ 36.6 (C-8), 40.1 (C-9), 45.9 (C-10) and 77.9 (C-11), and of an oxymethine at δ 3.60 (H-11) to the carbons at δ 6.3 (C-20), 36.6 (C-8), 37.7 (C-13) and 45.9 (C-10). This also established the hydroxyl group at C-11. Furthermore the correlations of H-3 at δ 3.21 to the carbons at δ 21.0 (C-19), 24.6 (C-18), 27.4 (C-1), 42.1 (C-4) and 145.8 (C-5), of an olefinic methine at δ 5.56 (H-6) to the carbons at δ

29.3 (C-7), 36.6 (C-8), 42.1 (C-4) and 45.9 (C-10), of the methyl group at δ 0.97 (CH_3-17) to the carbons at δ 37.7 (C-13), 39.4 (C-14), 43.3 (C-12) and 146.4 (C-15) allowed the placement of another hydroxyl group at C-3 and double bonds at C-5/C-6 and C-15/C-16, respectively.

The stereochemistry of **PO3** was determined by a NOESY experiment (**Figure 17**) in which the key correlations are as follows: CH_3-20/H-1β and H-12β; H-12β/CH_3-17; CH_3-18/H-10; H-10/H-11; H-3/H-1β; H-11/H-10 and H-12α. These data were in accordance with the β-orientations of CH_3-20, CH_3-17, and H-3 and the α-orientations of H-10, H-11 and CH_3-18. It was notable that in a previously isolated 11β-hydroxy-rosa-5,15-diene, that Me-17 and Me-20 had a *trans* stereochemical relationship (Geis *et al.*, 2000) whereas this relationship was *cis* in compound **PO3**. Further, the configuration at C-13 in **PO3** is the same as that in compounds **PO1** and **PO2**. Therefore, the structure of **PO3** is represented as 13-*epi*-5,15-rosadien-3α,11β-diol, a new compound (Salae *et al.*, 2012).

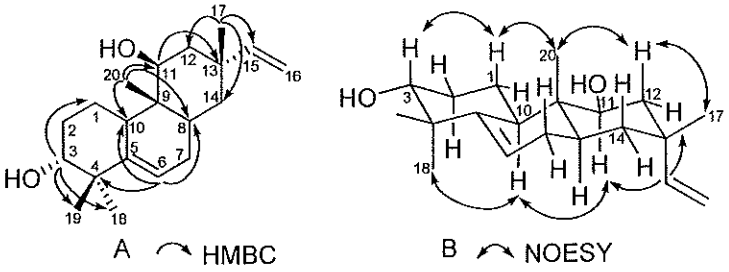


Figure 17 Selected HMBC correlations (A) and NOESY correlations (B) for **PO3**

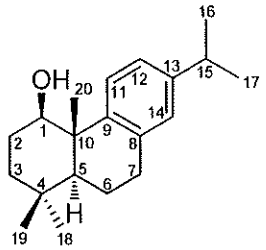
Table 41 ^{1}H, ^{13}C NMR and HMBC spectral data of compound **PO3**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CH_2	27.4	1.10 qd (13.2, 3.6)	2, 3, 5, 9, 10
			2.43 dq (13.2, 3.6)	
2	CH_2	30.7	1.49–1.66 m[a]	1, 3, 4, 10
			1.74–1.82 m[a]	
3	CH	77.0	3.21 dd (11.7, 4.5)	1, 2, 4, 18, 19
4	C	42.1		
5	C	145.8		
6	CH	117.9	5.56 td (4.5, 2.1)	4, 7, 8, 10
7	CH_2	29.3	1.69–1.78 m[a]	6, 8, 9, 14
8	CH	36.6	1.26–1.37 m[a]	7, 9, 10, 13
9	C	40.1		
10	CH	45.9	2.11 br d (13.2)	
11	CH	77.9	3.60 dd (12.0, 4.2)	8, 9, 10, 12, 13, 20
12	CH_2	43.3	1.43 t (12.0)	9, 11, 13, 14, 15, 17
			1.66 ddd (12.0, 4.2, 2.1)	
13	C	37.7		
14	CH_2	39.4	1.18–1.29 m[a]	7, 8, 9, 12, 13, 15, 17
			1.30–1.41 m[a]	
15	CH	146.4	5.75 dd (17.4, 11.1)	12, 13, 14, 17
16	CH_2	112.1	5.00 dd (17.4, 1.2)	13, 15, 17
			5.02 dd (11.1, 1.2)	
17	CH_3	31.3	0.97 s	12, 13, 14, 15
18	CH_3	24.6	1.12 s	3, 4, 5, 19
19	CH_3	21.0	0.95 s	3, 4, 5, 18
20	CH_3	6.3	0.69 s	8, 9, 10, 11

[a] Deduced from HMQC experiment.

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.2.4 Compound PO4



Compound **PO4** was isolated as a colorless oil with $[\alpha]_D^{26}$ −51.4 ($CHCl_3$; *c* 0.15). The molecular formula was determined to be $C_{20}H_{30}O$ from a molecular ion $[M]^{+\bullet}$ peak at *m/z* 286.2295 (calcd 286.2297) in HREIMS, implying six degrees of unsaturation. The IR spectrum showed absorption bands for hydroxyl (3421 cm^{-1}) and aromatic (1670 cm^{-1}). The presence of an aromatic ring was supported by the UV spectrum at λ_{max} 214 and 258 nm.

The ^{13}C NMR and DEPT spectra of **PO4** (**Table 42**, **Figure 84**) indicated 20 carbon signals, of five methyls, four methylenes, six methines and five quaternary, suggesting that **PO4** should contain three rings. The ^{13}C NMR spectra indicated the presence of six aromatic carbons (δ 146.9, 146.0, 135.5, 126.9, 126.7 and 123.9) and one oxymethine (δ 77.6).

Examination of the ^1H NMR spectrum of **PO4** (**Table 42**, **Figure 83**) and ^1H-^1H COSY spectrum indicated the presence of an isopropyl group as evidenced from two methyl doublets at δ_H 1.21 (d, *J*= 7.2 Hz, H_3-16 and H_3-17) which were coupled to a methine proton at δ 2.80 (sept, *J*= 7.2 Hz, H-15), two *ortho*-coupled aromatic protons at δ 8.07 (d, *J*= 8.4 Hz, H-11) and δ 6.97 (br d, *J*= 8.4 Hz, H-12), and an aromatic singlet signal at δ 6.87 (s, H-14). The spectrum also showed two geminal methyl protons at δ_H 0.89 (s, H_3-18) and δ_H 0.92 (s, H_3-19) and tertiary methyl protons at δ_H 1.21 (s, H_3-20). In addition, an oxymethine proton was displayed at δ 3.85 (dd, *J*= 9.9 and 6.0 Hz, H-1). This combination of signals suggested an abietane skeleton comparable to that of abietatrien-3β-ol (Urones *et al.*, 1988) except for the position of an oxymethine in ring A of **PO4**. The hydroxyl group was suggested to be at C-1 from the downfield shift of C-10 (δ 43.6 in **PO4** vs. δ 37.4 in

abietatrien-3β-ol), and this was confirmed by the HMBC correlations (**Table 42**, **Figure 18**) of the methyl protons CH_3-20 at δ1.21 to C-1 (δ77.6).

The relative stereochemistry of an oxymethine H-1 was assigned as α-axial from a doublet of doublets signal with *J*= 9.9 and 6.0 Hz and from the NOESY experiment (**Figure 18**) which showed cross-peaks between H-5, H-1, Me-18 and H-7α. Compound **4** was a new compound and assigned as abietatrien-1β-ol (Salae *et al.*, 2012).

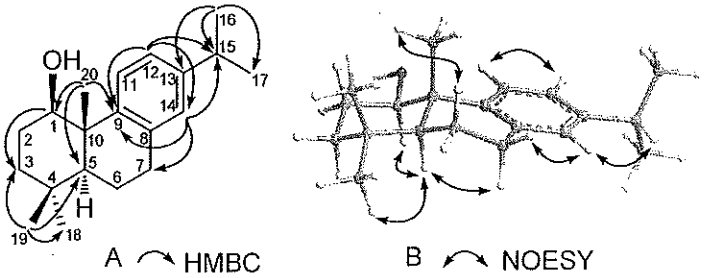


Figure 18 Selected HMBC correlations (A) and NOESY correlations (B) for **PO4**

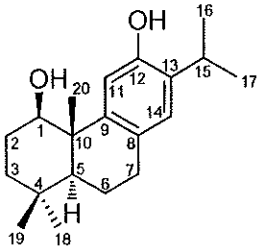
Table 42 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO4**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CH	77.6	3.85 dd (9.9, 6.0)	2, 3, 9, 10, 20
2	CH_2	30.2	1.65–1.73 m[a]	1, 3, 10
			1.74–1.84 m[a]	
3	CH_2	39.8	1.30 ddd (13.2, 12.3, 5.7)	1, 2, 4, 5, 18, 19
			1.45 td (13.2, 3.6)	
4	C	33.4		
5	CH	49.6	1.26 dd (11.4, 4.5)	1, 3, 7, 10, 18, 19, 20
6	CH_2	19.2	1.78–1.85 m[a]	4, 5, 7, 8, 10
			1.87–1.95 m[a]	
7	CH_2	29.8	2.70–2.85 m[a]	5, 6, 8, 9, 14
			2.92 ddd (17.1, 7.5, 3.6)	
8	C	135.5		
9	C	146.9		
10	C	43.6		
11	CH	126.9	8.07 d (8.4)	8, 10, 12, 13, 20
12	CH	123.9	6.97 br d (8.4)	8, 9, 14, 15
13	C	146.0		
14	CH	126.7	6.87 s	7, 9, 11, 12, 15
15	CH	33.5	2.80 sept (7.2)	12, 13, 14, 16, 17
16	CH_3	24.1	1.21 d (7.2)	13, 15, 17
17	CH_3	24.1	1.21 d (7.2)	13, 15, 16
18	CH_3	32.8	0.89 s	3, 4, 5, 19
19	CH_3	21.3	0.92 s	3, 4, 5, 18
20	CH_3	17.4	1.21 s	1, 5, 9, 10

[a] Deduced from HMQC experiment.

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.2.5 Compound PO5



Compound **PO5** was obtained as a colorless oil with $[\alpha]_D^{26}$ −15.7 ($CHCl_3$; *c* 0.27). Its molecular formula $C_{20}H_{30}O_2$ was determined from a molecular ion $[M]^{+\bullet}$ peak at *m/z* 302.2254 (calcd 302.2246) in the HREIMS, indicating an increment of one oxygen atom from **PO4**. The IR spectrum indicated the existence of hydroxyl (3351 cm^{-1}) and aromatic (1507 cm^{-1}) groups.

The ^1H and ^{13}C NMR spectral data of **PO5** (**Tables 43**, **Figure 85** and **86**) were similar to those of **PO4** (**Tables 42** and **44**, **Figure 83** and **84**), except for differences in the substitution pattern of the phenyl ring in **PO5**, in which two *para* protons were shown at δ 7.72 (s, H-11) and δ 6.81 (s, H-14) instead of *ortho*-coupled protons at δ 8.07 and 6.97 and a singlet at δ 6.87 as in **PO4**. The ^{13}C NMR and DEPT experiments also proved the presence of an additional oxyquaternary carbon at δ_C 151.0 in **PO5**, which was associated with the absence of the ^1H NMR signal of H-12 at δ 6.97 (br d) as in **PO4**. This finding was further supported by HMBC correlations (**Tables 43**) from H-14 (δ 6.81), and H-15 (δ 3.20) to C-12 (δ 151.0). The foregoing spectral data and literature survey provided evidence of **PO5** as a ferruginol derivative (Harrison *et al.*, 1987).

The relative configuration of H-1 in **PO5** was suggested to be α-oriented as in **PO4**. Thus, compound **PO5** was a new compound and identified as 1β-hydroxyferruginol (abietatrien-1β,12-diol) (Salae *et al.*, 2012).

Table 43 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO5**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CH	78.2	3.80 dd (10.4, 4.8)	9, 10, 20
2	CH_2	30.1	1.65–1.85 m[a]	1, 3, 10
3	CH_2	39.7	1.35 dd (13.2, 3.6)	1, 2, 4, 5, 18, 19
			1.48 td (13.2, 3.6)	
4	C	33.3		
5	CH	49.8	1.26 dd (12.4, 3.2)	1, 4, 7, 10, 18, 19, 20
6	CH_2	19.2	1.70–1.90 m[a]	4, 5, 7, 8, 10
7	CH_2	29.6	2.65–2.76 m[a]	5, 6, 8, 9
			2.84 ddd (16.4, 7.2, 2.4)	
8	C	127.3		
9	C	147.0		
10	C	43.4		
11	CH	113.7	7.72 s	8, 9, 10, 12, 13
12	C	151.0		
13	C	132.4		
14	CH	126.3	6.81 s	7, 9, 11, 12, 15
15	CH	26.6	3.20 sept (6.8)	12, 13, 14, 16, 17
16	CH_3	22.6	1.22 d (6.8)	13, 15, 17
17	CH_3	22.7	1.21 d (6.8)	13, 15, 16
18	CH_3	32.7	0.91 s	3, 4, 5, 19
19	CH_3	21.3	0.91 s	3, 4, 5, 18
20	CH_3	17.8	1.21 s	1, 5, 9, 10

[a] Deduced from HMQC experiment.

Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

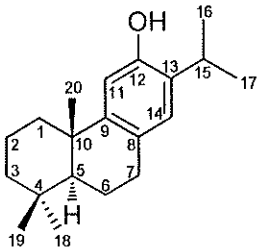
Table 44 Comparison of ^{1}H and ^{13}C NMR spectral data of compounds **PO4** and **PO5**

Position	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	
	PO4[a]	**PO5**[b]	**PO4**[a]	**PO5**[b]
1	77.6	78.2	3.85 dd (9.9, 6.0)	3.80 dd (10.4, 4.8)
2	30.2	30.1	1.65–1.73 m	1.65–1.85 m
			1.74–1.84 m	
3	39.8	39.7	1.30 ddd (13.2, 12.3,	1.35 dd (13.2, 3.6)
			5.7) 1.45 td (13.2, 3.6)	1.48 td (13.2, 3.6)
4	33.4	33.3		
5	49.6	49.8	1.26 dd (11.4, 4.5)	1.26 dd (12.4, 3.2)
6	19.2	19.2	1.78–1.85 m	1.70–1.90 m
			1.87–1.95 m	
7	29.8	29.6	2.70–2.85 m	2.65–2.76 m
			2.92 ddd (17.1, 7.5, 3.6)	2.84 ddd (16.4, 7.2, 2.4)
8	135.5	127.3		
9	146.9	147.0		
10	43.6	43.4		
11	126.9	113.7	8.07 d (8.4)	7.72 s
12	123.9	151.0	6.97 br d (8.4)	
13	146.0	132.4		
14	126.7	126.3	6.87 s	6.81 s
15	33.5	26.6	2.80 sept (7.2)	3.20 sept (6.8)
16	24.1	22.6	1.21 d (7.2)	1.22 d (6.8)
17	24.1	22.7	1.21 d (7.2)	1.21 d (6.8)
18	32.8	32.7	0.89 s	0.91 s
19	21.3	21.3	0.92 s	0.91 s
20	17.4	17.8	1.21 s	1.21 s

[a] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

3.2.6 Compound PO6



Compound **PO6** was isolated as a white solid, m.p. 56-58 °C, $[\alpha]_D^{26}$ +42.7 ($CHCl_3$; *c* 0.70). The UV and IR spectrum showed absorption bands similar to those of **PO5**.

The ^1H and ^{13}C NMR spectral data of **PO6** (**Table 45**, **Figures 87** and **88**) were similar to those of **PO5** (**Table 43**, **Figure 85** and **86**). The difference in the spectrum of **PO6** was shown as the disappearance of an oxymethine proton at δ 3.80 (H-1) in the ^1H NMR of **PO5** and the ^{13}C NMR spectrum of **PO6** displayed a signal of methylene carbon at δ 38.8 instead of an oxymethine carbon at δ 78.2, thus suggesting a methylene group at C-1. This finding was further confirmed by the HMBC correlations (**Table 45**, **Figure 19**) from H_3-20 (δ 1.15) to C-1 (δ 38.8). By comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data [Tezuka et al., 1998; Lee *et al.*, 2005; Ryu *et al.*, 2010, $[\alpha]_D^{24}$ +51.7 (MeOH; *c* 0.50)] (**Table 46**), therefore compound **PO6** was identified as ferruginol.

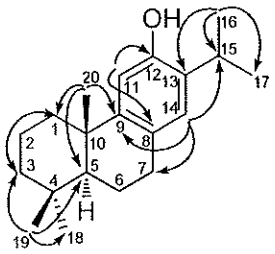


Figure 19 Selected HMBC correlations for **PO6**

Table 45 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO6** and ^{13}C NMR of ferruginol (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO6[c]	
1	CH_2	38.8	38.8	1.37 dd (12.6, 3.6)	2, 3, 5, 10, 20
				2.23 br d (12.6)	
2	CH_2	19.2	19.2	1.30–1.48 m[a]	1, 3, 4, 10
				1.50–1.90 m[a]	
3	CH_2	41.7	41.7	1.20 m, 1.46 m[a]	1, 2, 4, 5, 18, 19
4	C	33.4	33.4		
5	CH	50.3	50.3	1.29 dd (12.3, 2.4)	1, 3, 7, 9, 19, 20
6	CH_2	19.3	19.3	1.30–1.48 m[a]	4, 5, 7, 8, 10
				1.50–1.90 m[a]	
7	CH_2	29.7	29.7	2.70–2.90 m[a]	5, 6, 8, 9, 14
8	C	127.3	127.3		
9	C	146.8	146.8		
10	C	37.5	37.5		
11	CH	110.9	111.0	6.62 s	8, 10, 12
12	C	150.6	150.6		
13	C	131.3	131.4		
14	CH	126.6	126.6	6.83 s	7, 8, 9, 12, 13, 15
15	CH	26.8	26.8	3.11 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	22.7	22.7	1.23 d (6.9)	13, 15, 17
17	CH_3	22.5	22.6	1.21 d (6.9)	13, 15, 16
18	CH_3	33.3	33.3	0.93 s	3, 4, 5, 19
19	CH_3	21.6	21.6	0.90 s	3, 4, 5, 18
20	CH_3	24.7	24.8	1.15 s	1, 5, 9, 10
12-OH				4.73 s	

[a] Deduced from HMQC experiment, [b] Spectrum recorded at 125 (^{13}C NMR) MHz, $CDCl_3$. [c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

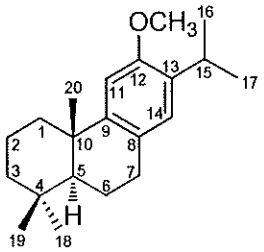
Table 46 Comparison of ^{1}H NMR spectral data of compounds **PO5**, **PO6** and ferruginol

Position	δ_H/ppm (mult., *J* in Hz)		
	PO5[a]	**PO6**[a]	ferruginol[b]
1	3.80 dd (10.4, 4.8)	1.37 dd (12.6, 3.6)	1.37 td (13.5,3.5)
		2.23 br d (12.6)	2.15 dtd (13.5, 3.5, 1.5)
2	1.65–1.85 m	1.30–1.48 m	1.58 dq (13.5, 3.5)
		1.50–1.90 m	1.72 qt (13.5, 3.5)
3	1.35 dd (13.2, 3.6)	1.20 m	1.20 td (13.5, 3.5)
	1.48 td (13.2, 3.6)	1.46 m	1.46 dtd (13.5, 3.5, 1.5)
5	1.26 dd (12.4, 3.2)	1.29 dd (12.3, 2.4)	1.31 dd (12.5, 2.0)
6	1.70–1.90 m	1.30–1.48 m	1.66 dddd (13.5, 12.7,
		1.50–1.90 m	11.0, 7.0)
			1.85 ddt (13.5, 7.5, 2.0)
7	2.65–2.76 m	2.70–2.70 m	2.76 ddd (16.5, 11.0, 7.5)
	2.84 ddd (16.4, 7.2, 2.4)		2.85 ddd (16.7, 7.0, 2.0)
11	7.72 s	6.62 s	6.61 s
14	6.81 s	6.83 s	6.81 s
15	3.20 sept (6.8)	3.11 sept (6.9)	3.11 sept (7.0)
16	1.22 d (6.8)	1.23 d (6.9)	1.22 d (7.0)
17	1.21 d (6.8)	1.21 d (6.9)	1.24 d (7.0)
18	0.91 s	0.93 s	0.93 s
19	0.91 s	0.90 s	0.91 s
20	1.21 s	1.15 s	1.16 s
12-OH		4.73 br s	4.60 br s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 500 (^{1}H NMR) MHz, $CDCl_3$.

3.2.7 Compound PO7



Compound **PO7** was isolated as a colorless oil with $[\alpha]_D^{26}$ +15.0 ($CHCl_3$; *c* 0.10). The UV and IR spectrum showed absorption bands similar to those of **PO6**.

The ^1H and ^{13}C NMR spectral data of **PO7** (**Table 47**, **Figures 89** and **90**) were related to those of **PO6** (**Table 45**, **Figures 87** and **88**). The difference in the spectrum of **PO7** was shown as an additional singlet methyl proton signal at δ 3.80 and a methyl carbon signal at δ 55.6 replaced a hydroxyl group at δ 4.73 in **PO6**, thus suggesting a methoxyl proton at C-12. The location of the methoxyl group was confirmed by HMBC experiment (**Table 47**) in which the methoxyl protons at δ 3.80 showed correlations to C-12 (δ 155.0). By comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data (katoh *et al.*, 2007) (**Table 48**), therefore compound **PO7** was identified as *O*-methyl ferruginol.

Table 47 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO7** and ^{13}C NMR of *O*-methyl ferruginol (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO7[c]	
1	CH_2	38.9	38.9	1.43 m[a]	2, 3, 5, 10, 20
				2.24 br d (12.3)	
2	CH_2	19.2	19.2	1.60–1.90 m[a]	
3	CH_2	41.7	41.7	1.22 m[a]	1, 2, 4, 5, 18, 19
				1.48 m[a]	
4	C	33.5	33.5		
5	CH	50.5	50.5	1.33 dd (12.3, 2.4)	1, 3, 7, 9, 19, 20
6	CH_2	19.4	19.4	1.60–1.90 m[a]	
7	CH_2	29.8	29.8	2.71–2.90 m[a]	5, 6, 8, 9, 14
8	C	126.9	126.9		
9	C	148.1	148.1		
10	C	37.9	37.9		
11	CH	106.6	106.6	6.72 s	8, 12, 13
12	C	155.0	155.0		
13	C	134.3	134.3		
14	CH	126.4	126.4	6.83 s	7, 9, 12, 15
15	CH	26.5	26.5	3.21 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	22.7	22.7	1.19 d (6.9)	13, 15, 17
17	CH_3	22.9	22.9	1.17 d (6.9)	13, 15, 16
18	CH_3	33.3	33.3	0.94 s	3, 4, 5, 19
19	CH_3	21.6	21.6	0.92 s	3, 4, 5, 18
20	CH_3	24.8	24.8	1.20 s	1, 5, 9, 10
12-OCH_3		55.6	55.6	3.80 s	12

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H) and 75 (^{13}C NMR) MHz, $CDCl_3$.

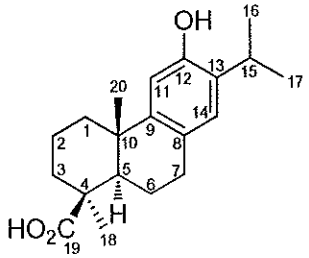
Table 48 Comparison of ^{1}H NMR spectral data of compounds **PO6**, **PO7** and *O*-methyl ferruginol

Position	δ_H/ppm (mult., *J* in Hz)		
	PO6[a]	**PO7**[a]	*O*-methyl ferruginol[b]
1	1.37 dd (12.6, 3.6)	1.43 m	1.32–1.51 m
	2.23 br d (12.6)	2.24 br d (12.3)	2.23–2.27 m
2	1.30–1.48 m	1.60–1.90 m	1.58–1.78 m
	1.50–1.90 m		
3	1.20 m, 1.46 m	1.22 m	1.15–1.25 m
		1.48 m	1.32–1.51 m
5	1.29 dd (12.3, 2.4)	1.33 dd (12.3, 2.4)	1.32–1.51 m
6	1.30–1.48 m	1.60–1.90 m	1.83–1.89
	1.50–1.90 m		
7	2.70–2.79 m	2.71–2.90 m	2.73–2.89 m
	2.80–2.90 m		
11	6.62 s	6.72 s	6.48 s
14	6.83 s	6.83 s	6.72 s
15	3.11 sept (6.9)	3.21 sept (6.9)	3.21 sept (7.0)
16	1.23 d (6.9)	1.19 d (6.9)	1.19 d (7.0)
17	1.21 d (6.9)	1.17 d (6.9)	1.19 d (7.0)
18	0.93 s	0.94 s	0.94 s
19	0.90 s	0.92 s	0.92 s
20	1.15 s	1.20 s	1.17 s
12-OH	4.73 s		
12-OCH_3		3.80 s	3.79 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

3.2.8 Compound PO8



Compound **PO8** was isolated as a white amorphous solid, mp. 251-253 °C, $[\alpha]_D^{26}$ +128 ($CHCl_3$; *c* 0.07). It exhibited hydroxyl (3360 cm^{-1}), carbonyl (1694 cm^{-1}) and double bond (1619 cm^{-1}) absorptions in the IR spectrum. The UV spectrum showed absorption bands similar to those of **PO6**.

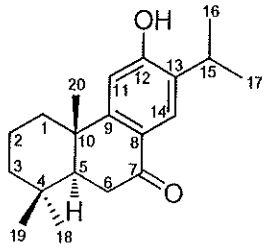
The ^1H and ^{13}C NMR spectral data (**Table 49**, **Figures 91** and **92**) of **PO8** were comparable to those of **PO6** (**Table 45**, **Figures 87** and **88**). The difference in the spectrum of **PO8** was shown as disappearance of a methyl signal at δ_H 0.90 (H_3-19, s, δ_C 21.6) in **PO6** and the appearance of a carboxyl signal at δ_C 182.0 (C-19) in **PO8**. The location of the carboxyl group was confirmed by HMBC experiment (**Table 49**) in which the methyl proton signal at δ 1.31 (1H, s, H-18) showed correlations with C-19 (δ 182.0), C-3 (δ 37.4), C-4 (δ 43.7) and C-5 (δ 52.7). Thus on the basis of its spectroscopic data and comparison with the previous report (Campello *et al.*, 1975; Cambie *et al.*, 1983), compound **PO8** was identified as lambertic acid.

Table 49 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO8**

Position	DEPT	δ_C/ppm	δ_H/ppm (multiplicity, *J*/Hz)	HMBC
1	CH_2	39.3	1.40 dd (13.2, 3.9)	3, 5, 10, 20
			2.17 m	
2	CH_2	19.9	1.61 m	1, 3
			2.01 m	
3	CH_2	37.4	1.10 m	1, 4, 5
			2.26 m	
4	C	43.7		
5	CH	52.7	1.53 dd (12.3 1.5)	4, 6, 7, 10, 19, 20
6	CH_2	21.1		
7	CH_2	31.2	2.70 m	5, 6, 8, 9, 14
			2.82 m	
8	C	127.2		
9	C	146.4		
10	C	38.2		
11	CH	111.8	6.63 s	8, 10, 12
12	C	150.9		
13	C	131.9		
14	CH	126.5	6.82 s	7, 9, 12, 15
15	CH	26.7	3.12 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	22.5	1.23 d (6.9)	13, 15, 17
17	CH_3	22.6	1.22 d (6.9)	13, 15, 16
18	CH_3	28.7	1.31 s	3, 4, 5, 19
19	CO_2H	182.0		
20	CH_3	23.1	1.10 s	1, 5, 9, 10
12-OH			2.20–2.80 br	

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.2.9 Compound PO9



Compound **PO9** was obtained as a white amorphous solid, m.p. 292-294 °C, $[\alpha]_D^{26}$ +17.7 ($CHCl_3$; *c* 0.11). It UV and IR spectra showed absorptions for hydroxyl, a conjugated carbonyl, and a conjugated aromatic functional groups.

The ^1H and ^{13}C NMR spectral data of **PO9** (**Table 50**, **Figures 93** and **94**) were similar to those that of **PO6** (**Table 45**, **Figures 87** and **88**). The presence of an isopropyl group followed from two doublets of methyl proton signals at δ 1.27 and 1.24 (each 3H, H_3-16 and H_3-17, respectively) and a septet methine proton signal at δ 3.15 (H-15). Three singlet methyl proton signals were at δ 0.92 (H-18), 0.99 (H-19), and 1.22 (H-20). Other signals were a separate methylene doublet of doublets proton signals at δ 2.58 and 2.69 (H-6), two singlet aromatic methine proton signals at δ 6.70 (H-11) and 7.91 (H-14). When compared to compound **PO6**, an additional carbonyl group was observed at δ 198.5 in the ^{13}C NMR spectrum of **PO9** and the only downfield aromatic methine proton signal was shown at δ 7.91 (H-14), which suggested the presence of a keto group instead of a methylene group at C-7, which was confirmed by the HMBC experiment (**Table 50**) from H-14 (δ 7.91) and H-5 (δ 1.85) to C-7 (δ 198.5). By comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data [Ara *et al.*, 1988; Benjamin, 2003; Kolak *et al.*, 2005; Marcos *et al.*, 2010, $[\alpha]_D^{24}$ +51.7 ($CDCl_3$; *c* 0.1)] (**Table 51**), compound **PO9** was identified as sugiol.

Table 50 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO9** and ^{13}C NMR of sugiol (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO9[c]	
1	CH_2	37.3	37.9	1.50 m[a]	2, 3, 5, 10
				2.20 br d (12.6)	
2	CH_2	18.4	18.9	1.68 m[a]	1, 3, 4, 10
				1.77 tt (13.5, 3.3)	
3	CH_2	40.9	41.4	1.26 m[a]	1, 4
				1.53 dd (12.6, 3.3)	
4	C	32.7	33.3		
5	CH	49.2	49.5	1.85 dd (12.9, 4.5)	1, 4, 7, 9, 10, 19, 20
6	CH_2	35.4	36.1	2.58 dd (18.0, 12.9)	4, 5, 7, 8, 10
				2.69 dd (18.0, 4.5)	
7	CO	199.2	198.5		
8	C	122.4	124.7		
9	C	156.3	156.4		
10	C	37.4	37.9		
11	CH	109.0	110.1	6.70 s	8, 9, 10, 12, 13
12	C	160.3	158.1		
13	C	133.0	132.6		
14	CH	125.6	126.6	7.91 s	7, 9, 12, 15
15	CH	26.1	26.8	3.15 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	21.8	22.3	1.27 d (6.9)	13, 15, 17
17	CH_3	21.6	22.4	1.24 d (6.9)	13, 15, 16
18	CH_3	31.9	32.6	0.92 s	3, 4, 5, 19
19	CH_3	20.7	21.4	0.99 s	3, 4, 5, 18
20	CH_3	22.5	23.2	1.22 s	1, 5, 9, 10
12-OH				5.60 br s	

[a] Deduced from HMQC experiment, [b] Spectrum recorded at 125 (^{13}C NMR) MHz, $CDCl_3$.[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

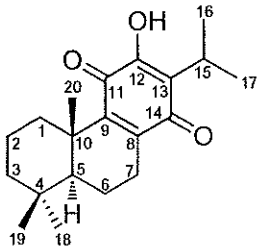
Table 51 Comparison of ^{1}H NMR spectral data of compounds **PO6**, **PO9** and sugiol

Position	δ_H/ppm (mult., *J* in Hz)		
	PO6[a]	**PO9**[a]	sugiol[b]
1	1.37 dd (12.6, 3.6)	1.50 m	1.56 m
	2.23 br d (12.6)	2.20 br d (12.6)	2.25 br dt (13.0, 2.0)
2	1.30–1.48 m	1.68 m	
	1.50–1.90 m	1.77 tt (13.5, 3.3)	
3	1.20 m, 1.46 m	1.26 m	
		1.53 dd (12.6, 3.3)	
5	1.29 dd (12.3, 2.4)	1.85 dd (12.9, 4.5)	
6	1.30–1.48 m	2.58 dd (18.0, 12.9)	2.52 dd (17.5, 13.5)
	1.50–1.90 m	2.69 dd (18.0, 4.5)	2.64 dd (17.5, 3.5)
7	2.70–2.79 m		
	2.80–2.90 m		
11	6.62 s	6.70 s	6.70 s
14	6.83 s	7.91 s	7.87 s
15	3.11 sept (6.9)	3.15 sept (6.9)	3.20 sept (7.0)
16	1.23 d (6.9)	1.27 d (6.9)	1.22 d (7.0)
17	1.21 d (6.9)	1.24 d (6.9)	1.22 d (7.0)
18	0.93 s	0.92 s	0.95 s
19	0.90 s	0.99 s	0.95 s
20	1.15 s	1.22 s	1.22 s
12-OH	4.73 s	5.60 br s	

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

3.2.10 Compound PO10



Compound **PO10** was obtained as a white amorphous solid, m.p. 179-181 °C, $[\alpha]_D^{26}$ +115.8 ($CHCl_3$; *c* 2.87). Its IR spectrum showed hydroxyl (3360 cm^{-1}) and carbonyl (1641 and 1631 cm^{-1}) and the UV absorptions at λ_{max} 277 and 407 nm suggested the presence of a *para*-benzoquinone element.

The ^{13}C NMR spectrum and DEPT experiment of **PO10** (**Table 52**, **Figures 95** and **96**) indicated that **PO10** has a total of 20 carbons, which is consistent with a diterpene skeleton. Two new signals of carbonyl carbons appeared at δ 183.4 (C-11) and 187.5 (C-14). In addition, in the ^1H NMR spectrum, two singlet signals for the olefinic methine protons at δ 6.62 and 6.83 of **PO6** were disappeared and the downfield protons H_2-7 (δ 2.33, ddd, *J*= 20.7, 11.4, 7.2 Hz and 2.71 ddd, *J*= 20.7, 5.2, 1.2) were shown in **PO10** indicating the existence of a *para*- benzoquinone group. This finding was further confirmed by the HMBC correlations (**Table 52**, **Figure 20**) from H_2-7 (δ 2.33 and 2.71) to C-14 (δ 187.5) and 12-OH (δ 7.23 to C-11 (δ 183.4). Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report [Tezuka *et al.*, 1998; Benjamin Rodrigues 2003; kolak *et al.*, 2005, $[\alpha]_D^{26}$ +129.6 ($CHCl_3$; *c* 0.1)] (**Table 53**), compound **PO10** was assigned as royleanone. X-ray crystallographic analysis of **PO10** (Hoong-Kun *et al.*, 2011) was also carried out and gave ORTEP drawing as shown in **Figure 21**.

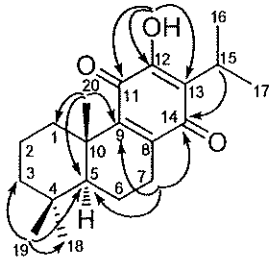


Figure 20 Selected HMBC correlations of **PO10**

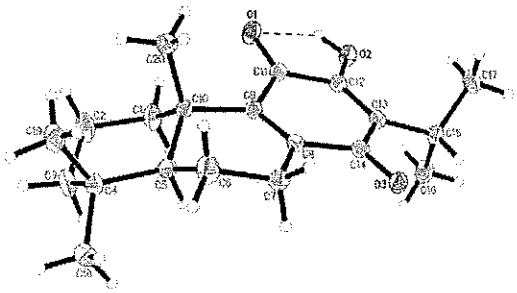


Figure 21 *ORTEP* drawing of **PO10**

Table 52 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO10** and ^{13}C NMR of royleanone (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO10[c]	
1	CH_2	36.2	36.3	1.15 m[a], 2.75 m[a]	2, 3, 10
2	CH_2	18.9	18.9	1.51 m[a]	1, 3, 4, 10
				1.72 tt (13.8, 3.3)	
3	CH_2	41.3	41.3	1.21 m[a], 1.46 m[a]	1, 2, 4, 18, 19
4	C	33.4	33.5		
5	CH	51.7	51.7	1.10 m[a]	4, 7, 9, 10, 18, 20
6	CH_2	17.4	17.4	1.38 m[a]	4, 5, 7, 8, 10
				1.86 dd (13.2, 7.2)	
7	CH_2	26.7	26.7	2.33 ddd (20.7, 11.4, 7.2)	5, 6, 8, 9, 14
				2.71 ddd (20.7, 5.2, 1.2)	
8	C	146.0	146.0		
9	C	146.5	146.5		
10	C	38.4	38.4		
11	CO	183.4	183.4		
12	C	150.5	150.5		
13	C	123.7	123.7		
14	CO	187.5	187.5		
15	CH	24.1	24.1	3.15 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	19.9	19.8	1.21 d (6.9)	13, 15, 17
17	CH_3	20.0	19.9	1.20 d (6.9)	13, 15, 16
18	CH_3	33.5	33.4	0.93 s	3, 4, 5, 19
19	CH_3	21.8	21.7	0.90 s	3, 4, 5, 18
20	CH_3	20.0	20.1	1.25 s	1, 5, 9, 10
12-OH				7.23 s	11, 12, 13

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

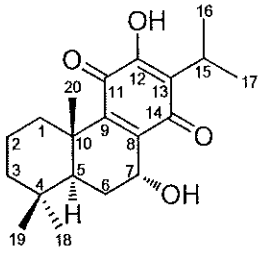
Table 53 Comparison of ^{1}H NMR spectral data of compounds **PO6**, **PO10** and royleanone

Position	δ_H/ppm (mult., *J* in Hz)		
	PO6[a]	**PO10**[a]	royleanone[b]
1	1.37 dd (12.6, 3.6)	1.15 m	1.12 td (13.5, 3.5)
	2.23 br d (12.6)	2.75 m	2.75 ddt (13.5, 3.5, 1.5)
2	1.30–1.48 m	1.51 m	1.53 dq (13.5, 3.5)
	1.50–1.90 m	1.72 tt (13.8, 3.3)	1.72 qt (13.5, 3.5)
3	1.20 m, 1.46 m	1.21 m	1.20 m
		1.46 m	1.46 dtd (13.5, 3.5, 1.5)
5	1.29 dd (12.3, 2.4)	1.10 m	1.10 dd (12.5, 1.5)
6	1.30–1.48 m	1.38 m	1.38 dddd (13.5, 12.5,
	1.50–1.90 m	1.86 dd (13.2, 7.2)	11.5, 6.0)
			1.87 ddt (13.5, 7.5, 1.5)
7	2.70–2.79 m	2.33 ddd (20.7, 11.4,	2.34 ddd (21.0, 11.5,
	2.80–2.90 m	7.2)	7.5)
		2.71 ddd (20.7, 5.2, 1.2)	2.71 ddd (21.0, 6.0, 1.5)
11	6.62 s		
14	6.83 s		
15	3.11 sept (6.9)	3.15 sept (6.9)	3.15 sept (7.0)
16	1.23 d (6.9)	1.21 d (6.9)	1.21 d (7.0)
17	1.21 d (6.9)	1.20 d (6.9)	1.20 d (7.0)
18	0.93 s	0.93 s	0.93 s
19	0.90 s	0.90 s	0.90 s
20	1.15 s	1.25 s	1.25 s
12-OH	4.73 s	7.23 s	7.23 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

3.2.11 Compound PO11



Compound **PO11** was obtained as orange crystals, m.p. 170-172 °C, $[\alpha]_D^{26}$ −114.0 ($CHCl_3$; *c* 0.10). The UV and IR spectrum were closely related to that of **PO10**.

The ^1H and ^{13}C NMR spectral data of **PO11** (**Table 54**, **Figures 97** and **98**) resembled those of **PO10** (**Table 52**, **Figures 95** and **96**), except that an additional oxymethine proton signal at δ 4.72 (*J*= 4.8, 1.5 Hz) was observed in **PO11**. The linkage position for the oxymethine proton at C-7 was established by HMBC correlation (**Table 54**) to C-5, C-6, C-8, C-9 and C-14, and the configuration of hydroxyl group was determined to be α due to absence of cross peak between H-7 (δ_H 4.72) and H-5 (δ_H 1.53) in the NOESY experiment. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report [Tezuka *et al.*, 1998; Benjamin Rodrigues 2003, $[\alpha]_D^{26}$ −128.2 ($CHCl_3$; *c* 0.1)] (**Table 55**), compound **PO11** was assigned as horminone. X-ray crystallographic analysis of **PO11** (Abdul Razak *et al.*, 2010) was also carried out and gave ORTEP drawing as shown in **Figure 22**.

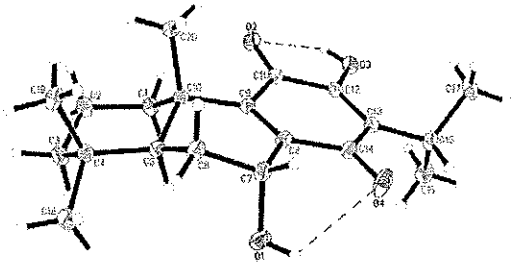


Figure 22 *ORTEP* drawing of **PO11**

Table 54 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO11** and ^{13}C NMR of horminone (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO11[c]	
1	CH_2	35.8	35.7	1.19 m[a]	3, 5, 10, 20
				2.70 m[a]	
2	CH_2	18.9	18.8	1.50 m[a]	1, 3, 4, 10
				1.74 m[a]	
3	CH_2	41.1	41.1	1.23 m[a]	1, 2, 4, 5, 18, 19
				1.48 m[a]	
4	C	33.0	33.0		
5	CH	45.7	45.7	1.53 m[a]	1, 3, 4, 6, 7, 10
6	CH_2	25.8	25.7	1.59 m[a]	4, 5, 7, 8, 10
				1.96 d (12.6)	
7	CH	63.2	63.2	4.72 dd (4.8, 1.5)	5, 6, 8, 9, 14
8	C	143.2	143.1		
9	C	147.8	147.8		
10	C	39.1	39.1		
11	CO	183.9	183.9		
12	C	151.1	151.2		
13	C	124.2	124.0		
14	CO	189.1	189.1		
15	CH	24.0	24.0	3.16 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	19.8	19.8	1.20 d (6.9)	13, 15, 17
17	CH_3	19.9	19.9	1.19 d (6.9)	13, 15, 16
18	CH_3	33.1	33.1	0.99 s	3, 4, 5, 19
19	CH_3	21.7	21.2	0.90 s	3, 4, 5, 18
20	CH_3	18.4	18.4	1.25 s	1, 5, 9, 10
12-OH				7.39 s	11, 12, 13

[a] Deduced from HMQC experiment, [b] Spectrum recorded at 125 (^{13}C NMR) MHz, $CDCl_3$.[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 55 Comparison of ^{1}H NMR spectral data of compounds **PO10**, **PO11** and royleanone

Position	δ_H/ppm (mult., *J* in Hz)		
	PO10[a]	**PO11**[a]	**royleanone**[b]
1	1.15 m	1.19 m	1.18 td (13.5, 3.5)
	2.75 m	2.70 m	2.70 ddt (13.5, 3.5, 1.5)
2	1.51 m	1.50 m	1.47 dtd (13.5, 3.5, 1.5)
	1.72 tt (13.8, 3.3)	1.74 m	1.73 qt (13.5, 3.5)
3	1.21 m	1.23 m	1.25 dt (13.5, 3.5)
	1.46 m	1.48 m	1.56 dt (13.5, 3.5)
5	1.10 m	1.53 m	1.54 br d (13.5)
6	1.38 m	1.59 m	1.61 td (13.5, 4.5)
	1.86 dd (13.2, 7.2)	1.96 d (12.6)	1.97 br d (13.5)
7	2.33 ddd (20.7, 11.4, 7.2)	4.72 dd (4.8, 1.5)	4.73 br d (4.5)
	2.71 ddd (20.7, 5.2, 1.2)		
15	3.15 sept (6.9)	3.16 sept (6.9)	3.16 sept (7.0)
16	1.21 d (6.9)	1.20 d (6.9)	1.21 d (7.0)
17	1.20 d (6.9)	1.19 d (6.9)	1.22 d (7.0)
18	0.93 s	0.99 s	0.98 s
19	0.90 s	0.90 s	0.90 s
20	1.25 s	1.25 s	1.21 s
12-OH	7.23 s	7.39 s	7.33 br s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

Table 56 Comparison of ^{13}C NMR spectral data of compounds **PO4-PO9**

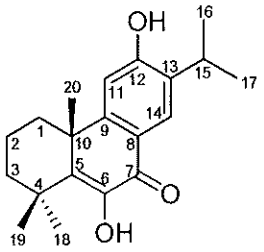
Position	**PO4**[a]	**PO5**[b]	**PO6**[a]	**PO7**[a]	**PO8**[a]	**PO9**[a]	**PO10**[a]	**PO11**[a]
1	77.6	78.2	38.8	38.9	39.3	37.9	36.3	35.7
2	30.2	30.1	19.2	19.2	19.9	18.9	18.9	18.8
3	39.8	39.7	41.7	41.7	37.4	41.4	41.3	41.1
4	33.4	33.3	33.4	33.5	43.7	33.3	33.5	33.0
5	49.6	49.8	50.3	50.5	52.7	49.5	51.7	45.7
6	19.2	19.2	19.3	19.4	21.1	36.1	17.4	25.7
7	29.8	29.6	29.7	29.8	31.2	198.5	26.7	63.2
8	135.5	127.3	127.3	126.9	127.2	124.7	146.0	143.1
9	146.9	147.0	146.8	148.1	146.4	156.4	146.5	147.8
10	43.6	43.4	37.5	37.9	38.2	37.9	38.4	39.1
11	126.9	113.7	111.0	106.6	111.8	110.1	183.4	183.9
12	123.9	151.0	150.6	155.0	150.9	158.1	150.5	151.2
13	146.0	132.4	131.4	134.3	131.9	132.6	123.7	124.0
14	126.7	126.3	126.6	126.4	126.5	126.6	187.5	189.1
15	33.5	26.6	26.8	26.5	26.7	26.8	24.1	24.0
16	24.1	26.6	22.6	22.7	22.5	22.3	19.8	19.8
17	24.1	22.7	22.7	22.9	22.6	22.4	19.9	19.9
18	32.8	32.7	33.3	33.3	28.7	32.6	33.4	33.1
19	21.3	21.3	21.6	21.6	182.0	21.4	21.7	21.2
20	17.4	17.8	24.8	24.8	23.1	23.2	20.1	18.4

Assignments were based on DEPT 90° and DEPT 135°experiments.

[a] Spectrum recorded at 75 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

3.2.12 Compound PO12



Compound **PO12** was isolated as a yellow powder, m.p. 201-203 °C, $[\alpha]_D^{26}$ –27 ($CHCl_3$; *c* 0.17). The UV absorption maxima at λ_{max} 248 and 342 nm indicated an aromatic system. The IR spectrum confirmed the presence of the aromatic system (1635 and 1504 cm^{-1}), conjugated carbonyl (1676 cm^{-1}), and hydroxyl (3328 cm^{-1}) groups.

The ^{13}C NMR and DEPT spectral data of **PO12** (**Table 57**, **Figure 100**) indicated 20 resonances including a conjugated carbonyl signal at δ 179.7 (C=O) and eight low-field signals at δ 111.4 (CH), 120.9 (C), 125.6 (CH), 133.8 (C), 141.1 (C), 143.8 (C), 154.9 (C), and 157.7 (C). These data suggested that **PO12** contained a carbonyl, a double bond, and a phenyl groups.

The ^1H NMR spectral data of **PO12** (**Table 57**, **Figure 99**) were similar to those of **PO9** (**Table 50**, **Figures 93** and **94**), except for the absence of the aliphatic methine proton (H-5) and methylene protons AX signals in **PO9**. Thus, compound **PO12** was deduced to be a 5,6-dehydrogented derivative of **PO9**. In addition, the singlet signal at δ 7.16 was deduced to be a hydroxyl group at C-6. The double bond and hydroxyl group were assigned to C-5 and C-6 by HMBC correlations (**Table 57**) of CH_3-20/C-5 (δ_C 141.1), CH_3-18 and 19/C-5 (δ_C 141.1), and OH-6/C-6 (δ_C 143.8). Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report (Su *et al.*, 1994, $[\alpha]_D^{26}$ –8.5 ($CHCl_3$; *c* 0.9) (**Table 57** and **58**), compound **PO12** was assigned as montbretrol.

Table 57 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO12** and ^{13}C NMR of montbretrol (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO12[c]	
1	CH_2	33.6	33.6	1.78 m[a]	2, 3, 10, 20
				2.28 m[a]	
2	CH_2	17.6	17.6	1.77 m[a]	1, 3, 4, 10
				1.93 m[a]	
3	CH_2	37.9	37.9	1.47 m[a]	1, 2, 4, 18, 19
				1.94 m[a]	
4	C	35.9	35.9		
5	C	141.1	141.1		
6	C	143.7	143.8		
7	CO	179.7	179.7		
8	C	120.9	120.9		
9	C	154.9	154.9		
10	C	40.3	40.3		
11	CH	111.4	111.4	6.90 s	8, 9, 10, 13
12	C	157.7	157.7		
13	C	133.8	133.8		
14	CH	125.6	125.6	8.02 s	7, 9, 12, 15
15	CH	26.9	26.9	3.19 sept (6.8)	12, 13, 14, 16, 17
16	CH_3	22.3	22.3	1.31 d (6.8)	13, 15, 17
17	CH_3	22.5	22.5	1.28 d (6.8)	13, 15, 16
18	CH_3	28.2	28.2	1.45 s	3, 4, 5, 19
19	CH_3	27.5	27.6	1.45 s	3, 4, 5, 18
20	CH_3	35.1	35.1	1.50 s	1, 5, 9, 10
12-OH				5.85 s	11, 12, 13

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 75 (^{13}C NMR) MHz, $CDCl_3$.

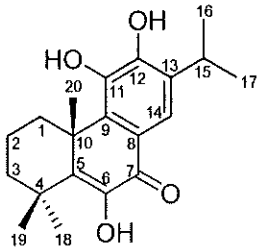
[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 58 Comparison of ^{1}H NMR spectral data of compounds **PO9**, **PO12** and montbretrol

Position	δ_H/ppm (mult., *J* in Hz)		
	PO9	**PO12**	montbretrol
1	1.50 m[a]	1.78 m	
	2.20 br d (12.6)	2.28 m	
2	1.68 m[a]	1.77 m	
	1.77 tt (13.5, 3.3)	1.93 m	
3	1.26 m[a]	1.47 m	
	1.53 dd (12.6, 3.3)	1.94 m	
5	1.85 dd (12.9, 4.5)		
6	2.58 dd (18.0, 12.9)		
	2.69 dd (18.0, 4.5)		
11	6.70 s	6.90 s	6.82 s
14	7.91 s	8.02 s	7.99 s
15	3.15 sept (6.9)	3.19 sept (6.8)	3.16 sept (7.0)
16	1.27 d (6.9)	1.31 d (6.8)	1.25 d (7.0)
17	1.24 d (6.9)	1.28 d (6.8)	1.28 d (7.0)
18	0.92 s	1.45 s	1.41 s
19	0.99 s	1.45 s	1.41 s
20	1.22 s	1.50 s	1.46
6-OH		7.16 s	
12-OH	5.60 br s	5.85 s	

Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

3.2.13 Compound PO13



Compound **PO13** was obtained as colorless crystals, m.p. 279-280 °C; $[\alpha]_D^{26}$ +58.0 ($CHCl_3$; *c* 0.10). The UV and IR spectrum were closely related to that of **PO12**.

The ^1H and ^{13}C NMR spectral data of **PO13** (**Table 59**, **Figures 101** and **102**) were resemble to those of **PO12** (**Table 57**, **Figures 99** and **100**). The difference in the spectrum of **PO7** was shown as the absence of a singlet olefinic methine proton signal at δ 6.72 and the presence of a downfield proton signal at δ 3.05 (H-1β, m), which suggested the presence of a hydroxyl group at C-11. By comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data (Topcu *et al.*, 1996; Benjamin Rodrigues 2003; Fraga *et al.*, 2005) (**Table 59** and **60**), therefore compound **PO13** was identified as 14-deoxycoleon. X-ray crystallographic analysis of **PO13** (Salae *et al.*, 2009) was also carried out and gave ORTEP drawing as shown in **Figure 23**.

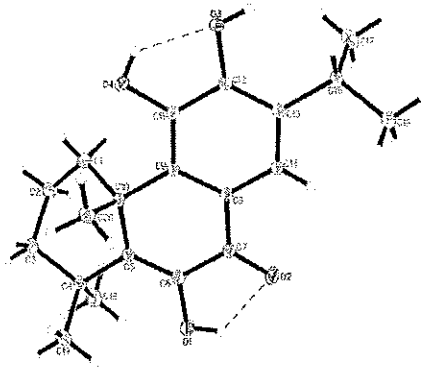


Figure 23 *ORTEP* drawing of **PO13**

Table 59 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO13** and ^{13}C NMR of 14-deoxycoleon (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO13[c]	
1	CH_2	30.3	29.7	1.68 m[a]	2, 3, 5, 10, 20
				3.05 m[a]	
2	CH_2	17.8	17.7	1.64 m[a]	1, 3, 4, 10
				1.88 m[a]	
3	CH_2	36.3	36.4	1.40 dd (5.7, 3.3)	1, 2, 4, 18, 19
				2.04 dt (18.3, 5.7)	
4	C	36.4	36.5		
5	C	143.2	144.7		
6	C	140.7	142.6		
7	CO	179.8	180.4		
8	C	120.1	120.5		
9	C	138.1	138.5		
10	C	40.6	41.0		
11	C	142.9	141.8		
12	C	145.3	146.6		
13	C	132.6	134.0		
14	CH	116.4	116.2	7.70 s	7, 9, 12, 15
15	CH	27.0	26.9	3.20 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	22.3	22.7	1.23 d (6.9)	13, 15, 17
17	CH_3	22.5	22.4	1.25 d (6.9)	13, 15, 16
18	CH_3	27.3	27.9	1.45 s	3, 4, 5, 19
19	CH_3	27.9	27.2	1.45 s	3, 4, 5, 18
20	CH_3	27.9	27.5	1.67 s	1, 5, 9, 10
6-OH				7.15 s	5, 6, 7

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 125 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

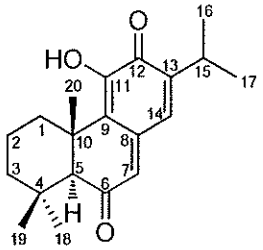
Table 60 Comparison of ^{1}H NMR spectral data of compounds **PO12**, **PO13** and 14-deoxycoleon (**R**)

Position	δ_H/ppm (mult., *J* in Hz)		
	PO12[a]	**PO13**[a]	14-deoxycoleon[b]
1	1.78 m	1.68 m	1.71 m
	2.28 m	3.05 m	2.93 m
2	1.77 m	1.64 m	1.61 m
	1.93 m	1.88 m	1.86 m
3	1.47 m	1.40 dd (5.7, 3.3)	1.41 m
	1.94 m	2.04 dt (18.3, 5.7)	2.01 td (13.0, 5.0)
11	6.90 s		
14	8.02 s	7.70 s	7.70 s
15	3.19 sept (6.8)	3.20 sept (6.9)	3.04 sept (6.8)
16	1.31 d (6.8)	1.23 d (6.9)	1.27 d (6.8)
17	1.28 d (6.8)	1.25 d (6.9)	1.30 d (6.8)
18	1.45 s	1.45 s	1.43 s
19	1.45 s	1.45 s	1.44 s
20	1.50 s	1.67 s	1.67 s
6-OH	7.16 s	7.15 s	7.09 s
12-OH	5.85 s		

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 500 (^{1}H NMR) MHz, $CDCl_3$.

3.2.14 Compound PO14



Compound **PO14** was isolated as a yellow oil, $[\alpha]_D^{26}$ +43.9 ($CHCl_3$; *c* 2.03). The IR spectrum had bands for hydroxyl (3530 cm^{-1}), a conjugate carbonyl (1740 and 1643 cm^{-1}) and a double bond (1531 cm^{-1}) groups which was confirmed by the UV absorption maxima at λ_{max} 206, 320, 333 and 400 nm.

The ^{13}C NMR and DEPT spectral data of **PO14** (**Table 61**, **Figure 104**) displayed five methyl, three methylene, four methine, and eight quaternary carbons including two carbonyl signals at δ 181.7 and 201.1 (C=O), five low-field signals at δ 125.3 (C), 134.0 (CH), 136.1 (CH), 139.9 (C), 145.0 (C), and 145.3 (C).

The ^1H NMR spectral data of **PO14** (**Table 61**, **Figure 103**) revealed three singlets at δ 1.27×2 and 1.12, indicating the presence of three methyl groups at C-18, C-19 and C-20 and two methyl doublets at δ 1.18 and 1.16 (each 3H, d, *J*= 6.8 Hz, H-16 and H-17), together with a methine signal at δ 3.07 (1H, sept, *J*= 6.8 Hz, H-15), showing the presence of an isopropyl group, thus suggested that **PO14** was an abietane-type diterpene. Downfield signals were observed at δ 6.21 (1H, s, H-7) and 6.89 (1H, s, H-14) and an exchangeable enol proton at δ 7.58 (H-11). The signal at δ 2.60 was assigned to be H-5, which exhibited HMBC correlations (**Table 61**) with C-1 (δ_C 37.0), C-3 (δ_C 42.5), C-4 (δ_C 32.8), C-9 (δ_C 125.3), C-10 (δ_C 42.9), C-18 (δ_C 33.2) and C-19 (δ_C 22.1). The HMBC correlations (**Table 61**) of OH/C-9 (δ_C 125.3), C-11 (δ_C 145.3) and C-12 (δ_C 181.7), and H-20/C-1 (δ_C 37.0), C-5 (δ_C 63.0), C-9 (δ_C 125.3) and C-10 (δ_C 42.9) confirmed the hydroxyl group at C-11. The proton H-15 has HMBC correlations with δ_C 181.7 and 136.1 [resonating with δ 6.89 (s)], and therefore they were assigned to be C-12 and C-14, respectively. Two singlet olefinic protons at δ 6.21 and 6.89 exhibited the NOESY correlations, and thus δ 6.21 is at C-

7. Thus on the basis of its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data with the previous report (Kupchan *et al.*, 1968; Tezuka et al., 1998, $[\alpha]_{D}^{26}$ +38.9 ($CHCl_3$; *c* 0.1); Benjamin Rodriguez, 2003) (**Table 61** and **62**), compound **PO14** was identified as taxodione.

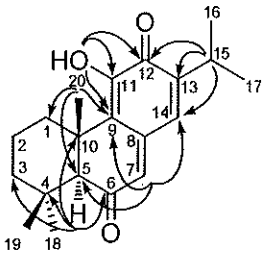


Figure 24 Major selected HMBC correlations of **PO14**

Table 61 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO14** and ^{13}C NMR of taxodione (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO14[c]	
1	CH_2	37.1	37.0	1.72 m[a]	2, 3, 5, 10, 20
				2.94 m[a]	
2	CH_2	18.6	18.5	1.58 m[a]	1, 3, 4, 10
				1.75 m[a]	
3	CH_2	42.6	42.5	1.16 m[a]	1, 2, 4, 18, 19
				1.42 m[a]	
4	C	32.9	32.8		
5	CH	63.1	63.0	2.60 s	1, 4, 6, 9, 18, 19
6	CO	201.0	201.1		
7	CH	134.0	134.0	6.21 s	5, 9, 14
8	C	140.0	139.9		
9	C	125.7	125.3		
10	C	42.9	42.9		
11	C	145.0	145.3		
12	CO	181.7	181.7		
13	C	145.4	145.0		
14	CH	136.2	136.1	6.89 s	7, 8, 9, 12, 15
15	CH	27.2	27.1	3.07 sept (6.8)	12, 13, 14, 16, 17
16	CH_3	21.3	21.8	1.18 d (6.8)	13, 15, 17
17	CH_3	21.6	21.6	1.16 d (6.8)	13, 15, 16
18	CH_3	33.3	33.2	1.27 s	3, 4, 5, 19
19	CH_3	22.1	22.1	1.27 s	3, 4, 5, 18
20	CH_3	21.9	21.2	1.12 s	1, 5, 9, 10
11-OH				7.58 s	9, 11, 12

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

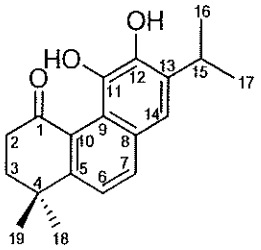
[c] Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

Table 62 Comparison of ^{1}H NMR spectral data of compounds **PO14** and taxodione

Position	δ_H /ppm (mult., *J* in Hz)	
	PO14	taxodione
1	1.72 m	1.75 m
	2.94 m	2.94 m
2	1.58 m	1.59 dq (13.5, 3.5)
	1.75 m	1.71 qt (13.5, 3.5)
3	1.16 m	1.20 m
	1.42 m	1.41 dtd (13.5, 3.5, 1.5)
5	2.60 s	2.60 s
7	6.21 s	6.21 s
14	6.89 s	6.88 s
15	3.07 sept (6.8)	3.07 sept (7.0)
16	1.18 d (6.8)	1.18 d (7.0)
17	1.16 d (6.8)	1.16 d (7.0)
18	1.27 s	1.12 s
19	1.27 s	1.27 s
20	1.12 s	1.27 s
11-OH	7.58 s	7.57 s

Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

3.2.15 Compound PO15



Compound **PO15** was obtained as orange crystals, m.p. 183-185 °C, $[\alpha]_D^{26}$ +9.9 ($CHCl_3$; *c* 1.00). The IR spectrum showed bands for hydroxyl (3490 cm^{-1}), a carbonyl (1640 cm^{-1}) and for a double bond (1590 cm^{-1}) groups and the UV absorption maxima at λ_{max} 270, 362 and 410 nm indicating the presence of an aromatic system.

The ^1H NMR spectral data of **PO15** (**Table 63**, **Figure 105**) showed the existence of signals for an isopropyl group (δ 1.21, 6H, d, *J*= 6.8 Hz and δ 3.26, septet, *J*= 6.8 Hz) as well as two methyl groups as a singlet at δ 1.13 (6H). Signals for two protons with an AX pattern were found at δ 6.62 (d, *J*= 12.0 Hz, H-6) and δ 7.34 (d, *J*= 12.0 Hz, H-7), a geminal dimethyl group at δ 1.13 (s, 6H, H-18 and H-19, respectively) and two methylene groups at δ 2.42 (t, *J*= 6.6 Hz, H-2) and δ 1.87 (t, *J*= 6.6 Hz, H-3) were observed. Another aromatic proton at δ 6.77 (s, H-14) and one hydrogen bonded phenolic functions at δ 6.38 (s, OH-11).

The 19 carbon signals observed in the ^{13}C NMR and DEPT spectral data of **PO15** (**Table 63**, **Figure 106**) showed the presence of a carbonyl group at δ 200.5, ten olefinic carbons at δ 117.6 (CH), 121.9 (C), 124.1 (CH), 127.7 (C), 128.2 (C), 132.4 (C), 138.2 (CH), 140.0 (CH), 145.6 (C), 156.8 (C), suggesting that **PO15** contained two rings and a norditerpenoid structure. Careful analysis of the 1D and 2D NMR spectra indicated that **PO15** could be ascribed to a 20-norabietane diterpenoid by the absence of the characteristic methyl (CH_3-20) for a simple abietane diterpenoid. This finding was further confirmed by the HMBC correlations (**Table**

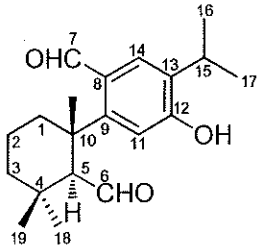
63). Compound **PO15** was identified as arucadiol by comparison of its spectral data with those reported in the literature (Michavila *et al.*, 1986).

Table 63 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO15**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CO	200.5		
2	CH_2	36.6	2.42 t (6.6)	1, 3, 4, 10
3	CH_2	33.4	1.87 t (6.6)	1, 4, 5, 18, 19
4	C	35.0		
5	C	156.8		
6	CH	124.1	6.62 d (12.0)	4, 5, 7, 8, 10
7	CH	140.0	7.34 d (12.0)	5, 8, 9, 14
8	C	127.7		
9	C	121.9		
10	C	128.2		
11	C	138.2		
12	C	145.6		
13	C	132.4		
14	CH	117.6	6.77 s	7, 9, 12, 15
15	CH	27.3	3.26 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	22.5	1.21 d (6.9)	13, 15, 17
17	CH_3	22.5	1.21 d (6.9)	13, 15, 16
18	CH_3	27.2	1.13 s	3, 4, 5, 19
19	CH_3	27.2	1.13 s	3, 4, 5, 18
11-OH			6.38 s	11, 12, 13

Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

3.2.16 Compound PO16



Compound **PO16** was isolated as colorless crystals, m.p. 193-195 °C, $[\alpha]_D^{26}$ +12.8 ($CHCl_3$; *c* 0.81). The IR spectrum showed absorption bands at 3434 cm^{-1} for hydroxyl group, 1710 cm^{-1} and 1666 cm^{-1} for carbonyl groups, 1545 cm^{-1} for aromatic groups and the UV absorption maxima at λ_{max} 218, 260 and 295 nm which indicated the presence of an aromatic system.

The ^1H NMR spectral data of **PO16** (**Table 64**, **Figure 107**) showed two aromatic proton singlets at δ 7.00 and 7.84, an isopropyl moiety with a doublet for two methyls at δ 1.26 and 1.27 (*J*= 6.9 Hz) and a septet for a proton at δ 3.22, three singlet quaternary methyl groups at δ 0.71, 1.03 and 1.50, a doublet aliphatic methine proton at δ 3.16, and two aldehyde protons at δ 9.86 (d, *J*= 4.2 Hz) and 10.44 (s). Its ^{13}C NMR and DEPT spectral data (**Table 64**, **Figure 108**) exhibited five methyls, three methylenes, four methines, six quaternary carbons and two aldehyde carbons. The HMBC correlations (**Table 64**) for the C-6 aldehyde at δ 206.9 with the H-5 proton in the aliphatic methine proton at δ 3.16, and the C-7 aldehyde at δ 192.1 with the H-14 aromatic proton at δ 7.84, suggested that **PO16** must possess a 6,7-*seco*-abietane skeleton. The relative stereochemistry at C-5 position was determined from the NOESY spectrum. Since the methyl group at C-10 could be assigned with a β-configuration for biogenetic reasons and no correlation between this methyl group and the proton at C-5 was observed, H-5 was determined to have an α-configuration. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report (Kuo *et al.*, 2001, $[\alpha]_D^{26}$ +20.0 ($CHCl_3$; *c* 1.2); Katoh *et al.*, 2007) (**Table 65**), compound **PO16** was assigned as 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial.

Table 64 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO16** and ^{13}C NMR of 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO16	
1	CH_2	37.4	37.4	1.55–1.80 m[a]	3, 5, 9, 10, 20
				2.31 m[a]	
2	CH_2	19.3	19.3	1.60–1.80 m[a]	1, 3, 4, 10
3	CH_2	37.6	37.7	1.55–1.80 m[a]	1, 2, 4, 18, 19
4	C	33.5	33.4		
5	CH	64.6	64.5	3.16 d (4.2)	1, 3, 4, 6, 10, 18, 19
6	CHO	206.2	206.9	9.86 d (4.2)	
7	CHO	192.6	192.1	10.44 s	8, 9, 14
8	C	127.9	127.3		
9	C	151.1	151.0		
10	C	40.4	40.5		
11	CH	114.8	115.0	7.00 s	8, 9, 10, 13
12	C	157.9	158.4		
13	C	132.4	132.4		
14	CH	134.6	135.0	7.84 s	7, 8, 9, 12, 15
15	CH	26.7	26.7	3.22 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	22.2	22.2	1.26 d (6.9)	13, 15, 17
17	CH_3	22.2	22.2	1.27 d (6.9)	13, 15, 16
18	CH_3	30.6	30.7	0.71 s	3, 4, 5, 19
19	CH_3	27.4	27.3	1.03 s	3, 4, 5, 18
20	CH_3	28.1	28.0	1.50 s	1, 5, 9, 10
12-OH				7.13 br s	

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

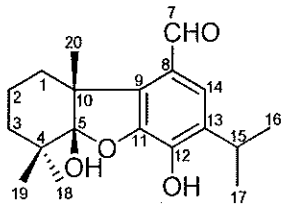
Table 65 Comparison of ^{1}H NMR spectral data of compounds **PO16** and 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial

Position	δ_H/ppm (mult., *J* in Hz)	
	PO16[a]	12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial[b]
1	1.55–1.80 m	1.50–1.83 m
	2.31 m	2.28–2.34 m
2	1.60–1.80 m	1.50–1.83 m
3	1.55–1.80 m	1.50–1.83 m
5	3.16 d (4.2)	3.13 d (3.8)
6	9.86 d (4.2)	9.86 d (3.8)
7	10.44 s	10.47 s
11	7.00 s	6.96 s
14	7.84 s	7.84 s
15	3.22 sept (6.9)	3.18 sept (7.0)
16	1.26 d (6.9)	1.26 d (6.9)
17	1.27 d (6.9)	1.27 d (6.9)
18	0.71 s	0.70 s
19	1.03 s	1.01 s
20	1.50 s	1.50 s
12-OH	7.13 br s	7.57 s

[a] Spectrum recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

3.2.17 Compound PO17



Compound **PO17** was isolated as a colorless oil with $[\alpha]_D^{26}$ +38.1 ($CHCl_3$; *c* 0.91). The UV and IR spectra were closely related to those of **PO16**. The ^{13}C NMR and DEPT spectral data of **PO17** (**Table 66**, **Figure 110**) showed 19 carbons made up of five methyls, three methylenes, three methines, and eight quaternary carbons and one aldehyde carbon (δ_C 190.2). The ^{1}H and ^{13}C NMR spectroscopic data (**Table 66**, **Figures 109** and **110**) of **PO17** were resembled to those of **PO16** (**Table 64**, **Figure 107** and **108**). The difference in the spectrum of **PO17** was the absence of a doublet of an aldehydic proton signal at δ9.86 in the ^{1}H NMR of **PO16** and the ^{13}C NMR spectrum of **PO17** displayed of a carbon at δ 114.8, suggesting a hemiacetal group and thus the ether linkage to tertiary hydroxyl group at C-5. The linkage position for the hemiacetal was established as C-5 by HMBC correlations (**Table 66**, **Figures 25**) for the CH_3-18, CH_3-19 and CH_3-20 to C-5 (**Table 66**). By comparison of the ^{1}H and ^{13}C NMR spectral data with the previously reported data (Luis *et al.*, 1993) (**Table 67**), therefore compound **PO17** was identified as salvicanaraldehyde.

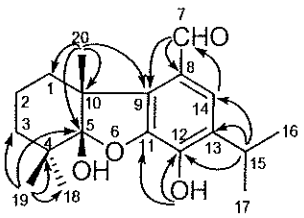


Figure 25 Major selected HMBC correlations of **PO17**

Table 66 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO17** and ^{13}C NMR of salvicanaraldehyde (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO17[c]	
1	CH_2	39.4	39.7	1.42 m[a]	2, 3
				2.25 m[a]	
2	CH_2	17.6	17.6	1.30–1.70 m[a]	
3	CH_2	37.5	37.4	1.30–1.70 m[a]	
4	C	37.8	37.9		
5	C	115.0	114.8		
6					
7	CHO	189.7	190.2	10.02 s	8, 9
8	C	134.6	125.8		
9	C	126.7	137.7		
10	C	51.3	51.1		
11	C	137.6	143.0		
12	C	144.2	144.1		
13	C	142.0	134.6		
14	CH	125.0	124.2	7.31 s	7, 9, 13, 15
15	CH	30.1	27.1	3.27 sept (6.8)	12, 13, 14, 16, 17
16	CH_3	19.3	22.4	1.26 d (6.8)	13, 15, 17
17	CH_3	22.4	22.4	1.25 d (6.8)	13, 15, 16
18	CH_3	26.4	26.2	1.24 s	3, 4, 5, 19
19	CH_3	24.5	24.5	1.21 s	3, 4, 5, 18
20	CH_3	17.7	19.7	1.72 s	1, 5, 9, 10
12-OH				5.61 br s	12, 13

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 50 (^{13}C NMR) MHz, C_6D_6.

[c] Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

Table 67 Comparison of ^{1}H NMR spectral data of compounds **PO16**, **PO17** and salvicanaraldehyde

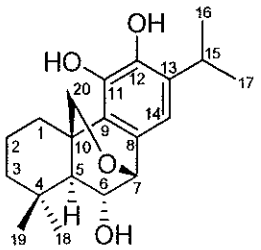
Position	δ_H/ppm (mult., *J* in Hz)		
	PO16[a]	**PO17**[b]	salvicanaraldehyde[c]
1	1.50–1.83 m	1.42 m	2.22 m
	2.28–2.34 m	2.25 m	
2	1.50–1.83 m	1.30–1.70 m	
3	1.50–1.83 m	1.30–1.70 m	
5	3.13 d (3.8)		
6	9.86 d (3.8)		
7	10.47 s	10.02 s	10.04 s
11	6.96 s		
14	7.84 s	7.31 s	7.32 s
15	3.18 sept (7.0)	3.27 sept (6.8)	3.28 hept
16	1.26 d (6.9)	1.26 d (6.8)	1.27 d
17	1.27 d (6.9)	1.25 d (6.8)	1.27 d
18	0.70 s	1.24 s	1.27 s
19	1.01 s	1.21 s	1.27 s
20	1.50 s	1.72 s	1.67 s
12-OH	7.57 s	5.61 br s	5.48 s

[a] Spectrum recorded at 300 (^{1}H NMR) MHz, $CDCl_3$

[b] Spectrum recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

[c] Spectrum recorded at 200 (^{1}H NMR) MHz, $CDCl_3$.

3.2.18 Compound PO18



Compound **PO18** was obtained as a white solid, m.p. 199–201 °C; $[\alpha]_D^{24}$ –21.1 ($CHCl_3$; *c* 0.02). The molecular formula $C_{20}H_{28}O_4$ was determined by HREIMS (calcd 332.1988, found 332.1999 $[M]^{+\bullet}$), requiring seven degrees of unsaturation. The UV absorption (λ_{max} 210 and 276 nm) indicated an aromatic ring while the IR (3351 and 1578 cm^{-1}) spectrum indicated hydroxyl and aromatic groups, respectively. The spectral data indicated that **PO18** possessed an abietane type skeleton.

The ^{13}C NMR spectral data (**Table 68**, **Figure 112**) revealed 20 carbon signals identified as four methyls (δ_C 22.2, 22.7(×2) and 33.5), four methylenes (δ_C 18.8, 29.8, 41.3 and 66.9), five methines (δ_C 26.9, 56.3, 68.9, 73.8 and 115.9) and seven quaternary carbons (δ_C 33.5, 41.2, 127.8, 128.3, 133.3, 139.9 and 142.1). A singlet at δ 6.65 in the ^1H NMR spectrum was ascribed to the aromatic H-14. The characteristic signals of an isopropyl group bound to an aromatic ring, as well as two broad singlets exchangeable with D_2O corresponding to the hydroxy phenolic groups at C-11 and C-12 positions were also observed in the ^1H NMR spectrum (**Table 68**, **Figures 111**), whose data suggested a penta-substituted aromatic ring. Two methyl singlets at δ_H 0.97and 1.06 were ascribed to the C-18 and C-19 methyl groups. The angular methyl group (C-20) frequently found in abietane-type diterpenes was not observed. Instead doublets for two nonequivalent geminal protons were evident at δ_H 4.20 and 2.89 (*J*= 8.4 Hz, H_2-20). A doublet at δ_H 4.46 (*J*= 4.0 Hz, H-7) was observed, the chemical shift of the latter suggested its connection to a hydroxyl group. The ethereal function must be cyclic as indicated by its molecular formula and two ^{13}C NMR signals due to two oxygenated sp^3 carbons at δ 66.9 (C-20) and δ 73.8 (C-

7). The proton H-7 was coupled with a proton resonanced as a triplet at δ 3.98 (*J*= 4.0 Hz, H-6; δc 68.9) whose chemical shift of the latter suggested a connection to a hydroxyl group. The proton H-6 was also coupled with H-5 (δ 1.04, d, *J*= 4.0 Hz). The coupling constants 4.0 Hz of H-6, H-5, H-7 were in agreement with that of 16-hydroxyisorosmanol (Luis *et al.*, 1993) (*J*= 4.4 Hz) indicated for H-7. NOESY experiments also supported these assignments (**Figure 25**). The NMR data suggested that **PO18** was similar in structure to 6-*epi*-demethylesquirolin D, (Esquivel *et al.*, 1995) with **PO18** however, having a C-20 methylene group rather than a C-20 oxymethine carbon as found in the latter known compound. Therefore, compound **PO18** was a new compound and characterized as 6α,11,12-trihydroxy-7β,20-epoxy-8,11,13-abietatriene (Salae *et al.*, 2012).

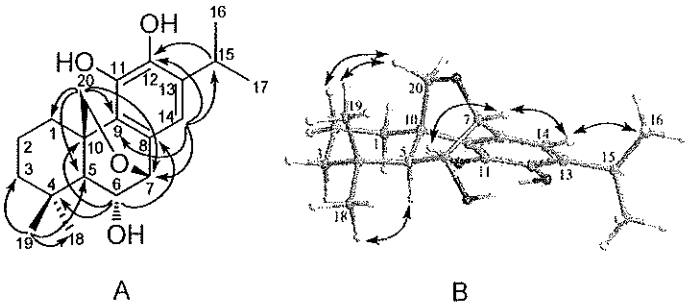


Figure 26 Selected HMBC correlations (A) and NOESY correlations (B) for **PO18**

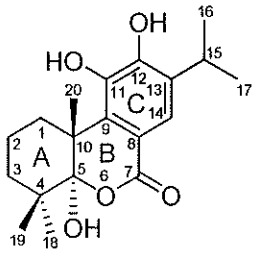
Table 68 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO18**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CH_2	29.8	2.09 br d (13.6)	3, 5, 10, 20
			2.60 td (13.6, 4.8)	
2	CH_2	18.8	1.50–1.60 m[a]	2, 3, 9, 10, 20
3	CH_2	41.3	1.20–1.25 m[a]	1, 4, 5, 18
			1.45–1.55 m[a]	
4	C	33.5		
5	CH	56.3	1.04 d (4.0)	3, 4, 6, 10, 18, 19, 20
6	CH	68.9	3.98 t (4.0)	4, 7, 8
7	CH	73.8	4.46 d (4.0)	5, 6, 9, 14, 20
8	C	127.8		
9	C	128.3		
10	C	41.2		
11	C	139.9		
12	C	142.1		
13	C	133.3		
14	CH	115.9	6.65 s	7, 9, 10, 11, 12, 15
15	CH	26.9	3.09 sept (6.8)	12, 13, 14, 16, 17
16	CH_3	27.7	1.16 d (6.8)	13, 15, 17
17	CH_3	22.7	1.17 d (6.8)	13, 15, 16
18	CH_3	33.5	0.97 s	3, 4, 5, 19
19	CH_3	22.2	1.06 s	3, 4, 5, 18
20a	CH_2	66.9	2.89 d (8.4)	1, 5, 7, 9, 10
			4.02 d (8.4)	
11-OH			6.33 s	9, 11, 12
12-OH			6.86 s	

[a] Deduced from HMQC experiment.

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.2.19 Compound PO19



Compound **PO19** was isolated as a colorless oil with $[\alpha]_D^{24}$ +59.3 ($CHCl_3$; *c* 0.14). Its molecular formula, $C_{19}H_{26}O_5$ was established by HREIMS (calcd 334.1780, found 334.1792 $[M]^{+\bullet}$), with seven degrees of unsaturation. The IR spectrum showed a conjugated carbonyl (1682 cm^{-1}) and hydroxyl (3452 cm^{-1}) groups. The maximum absorption bands at λ_{max} 218, 260 and 295 nm in the UV spectrum indicated the presence of aromatic and conjugated carbonyl groups.

The ^{13}C NMR spectral data of **PO19** (**Table 69**, **Figure 114**) revealed 19 carbon signals which were sorted by a DEPT experiment into five methyls (δ 17.5, 22.3, 22.4, 24.4 and 26.3), three methylenes (δ 17.6, 37.9 and 38.3), two methines (δ 27.1 and 123.3), three tetrasubstituted sp^3 carbons (δ 37.6, 51.3 and 114.9), five tetrasubstituted sp^2 carbons (δ 118.0, 134.0, 137.2, 143.0 and 143.2) and a lactone carbonyl (δ 172.1).

The ^1H and ^{13}C NMR spectroscopic data of **PO19** (**Table 69**, **Figures 113** and **114**) were similar to those of **PO18** (**Table 68**, **Figures 111** and **112**) for the signals corresponding to the A and C rings. The major differences were the NMR signals corresponding to the ring B of **PO19**. The molecular formula of **PO19** and the presence of a lactone carbonyl carbon at δ 172.1 suggested a 6,7-*seco*-abietane skeleton. This was supported by the HMBC correlations (**Table 69**, **Figures 27**) of H-14 at *δ* 7.49 to the carbons at δ 27.1 (C-15), 51.3 (C-10), 137.2 (C-9), 143.2 (C-12) and 172.1 (C-7). Furthermore the HMBC correlations of CH_3-20 at δ 1.26 with the carbons at δ 38.3 (C-1), 51.3 (C-10), 114.9 (C-5) and 137.2 (C-9) placed an OH at C-5. The structure of **PO19** was closely related to cupresol (Jolad *et al.*, 1984) except for

the presence of a hydroxyl group at C-11 in ring C of **PO19** instead of an aromatic proton and 5β-OH in cupresol (Jolad *et al.*, 1984).

The relative stereochemistry of a tertiary hydroxyl at C-5 in **PO19** should be α-oriented indicating a *trans*-juction for the A and B rings because the methyl protons of **PO19** at C-18 were shifted more downfield than cupresol (δ 1.21 vs. δ 0.69). Thus, compound **PO19** was a new compound and characterized as 5α,11,12-trihydroxy-6-oxa-abieta-8,11,13-trien-7-one (Salae *et al.*, 2012).

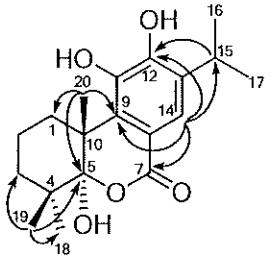


Figure 27 Major selected HMBC correlations of **PO19**

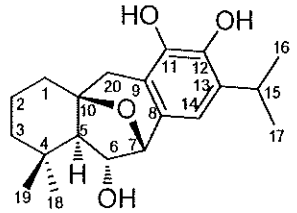
Table 69 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO19**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1a	CH_2	38.3	1.40–1.50 m[a]	
			2.46 br d (12.6)	
2	CH_2	17.6	1.50–1.55 m[a]	
3	CH_2	37.9	1.37–1.49 m[a]	
4	C	37.6		
5	C	114.9		
6				
7	CO	172.1		
8	C	118.0		
9	C	137.2		
10	C	51.3		
11	C	143.0		
12	C	143.2		
13	C	134.0		
14	CH	123.3	7.49 s	7, 9, 10, 12, 15
15	CH	27.1	3.25 sept (7.2)	12, 13, 14, 16, 17
16	CH_3	22.4	1.25 d (7.2)	13, 15, 17
17	CH_3	22.3	1.25 d (7.2)	13, 15, 16
18	CH_3	24.4	1.21 s	3, 4, 5, 19
19	CH_3	26.3	1.24 s	3, 4, 5, 18
20	CH_3	17.5	1.26 s	1, 5, 9, 10

[a] Deduced from HMQC experiment

Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

3.2.20 Compound PO20



Compound **PO20** was isolated as an amorphous solid, m.p. 188-190 °C, $[\alpha]_D^{26}$ –8.6 ($CHCl_3$; *c* 0.88). The IR spectrum displayed the absorption bands due to aromatic ring (1601 and 1539 cm^{-1}) and hydroxyl groups (3693 and 3604 cm^{-1}). The UV spectrum showed the absorption maxima at λ_{max} 205 and 270 nm, indicative of a simple phenolic ring.

The ^1H NMR spectrum of **PO20** (**Table 70**, **Figure 115**) showed signals for an isopropyl group (δ 1.16, 6H, d, *J*= 6.8 Hz and δ 3.14, septet, *J*= 6.8 Hz) as well as two methyl groups as a singlet at δ 0.91 and 0.92. The H-7, H-6 and H-5 protons appeared at δ 4.57 (d, *J*= 6.4 Hz, H-7), 4.17 (t, *J*= 6.4 Hz, H-6) and 1.26 (d, *J*= 6.4 Hz, H-5), as detected by a ^1H-^1H COSY experiment. One sharp singlet proton at δ 6.47 was assigned as an aromatic proton at C-14. Finally, an AB system at δ 2.41 and 2.68 (d, *J*=16.4 Hz) suggested the methylene protons at C-20 in **PO20**.

The ^{13}C NMR and DEPT spectral data of **PO20** (**Table 70**, **Figure 116**) showed four methyls, four methylenes, five methines (including an olefinic one and two oxygenated ones), and seven quaternary carbons (including six olefinic ones and an oxyquternary one). Considering the structures of the compounds previously isolated from this genus, along with showing a clearly AB system at δ 2.41 and 2.68 (*J*= 16.4 Hz) characteristic of the C-20 methylene protons of an icetexane skeleton (Esquivel *et al.*, 1995), **PO20** could be ascribed to be an icetexane or abietane diterpenoid (Rasool *et al.*, 1991; Fraga *et al.*, 1986; Hasegawa *et al.*, 1985). Careful analysis of the 1D and 2D NMR spectra indicated that **PO20** could be ascribed to be an icetexane diterpenoid by the absence of the characteristic quaternary carbon (C-10) and a methyl group (Me-20) for a simple abietane diterpenoid. A comparison of the ^1H and ^{13}C NMR spectral data of **PO20** with those of brussonol (Fraga *et al.*, 2005)

indicated that they were strikingly similar except that the presence of a triplet at δ 4.17 (*J*= 6.4 Hz) was assigned to H-6, geminal to the secondary hydroxyl group in **PO20**. The hydroxyl group was located at C-6 by HMBC correlation (**Table 70, Figures 28**)

The relative stereochemistry at C-6 should be an α orientation for hydroxyl group because the *trans* fusion depicted in **PO20** was established with the aid of the coupling constant (*J*= 6.4 Hz) found for H-6, and by comparison with related compound **PO18**. Thus on the basis of its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data with the previous report [Esquivel *et al.*, 1995, $[\alpha]_D^{26}$ –20.0 ($CHCl_3$; *c* 0.1)] (**Table 70** and **71**), compound **PO20** was assigned as 5,6-dihydro-6α-hydroxysalviasperanol. X-ray crystallographic analysis of **PO20** (Asik *et al.*, 2010) was also carried out and gave ORTEP drawing as shown in **Figure 29**.

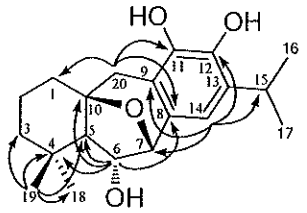


Figure 28 Major selected HMBC correlations of **PO20**

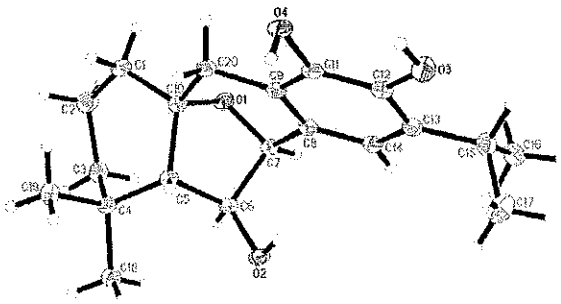


Figure 29 *ORTEP* drawing of **PO20**

Table 70 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO20** and ^{13}C NMR of 5,6-dihydro-6α-hydroxysalviasperanol (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO20[c]	
1	CH_2	29.6	29.5	1.45–1.60 m[a]	
				1.70–1.80 m[a]	
2	CH_2	15.2	15.1	1.70–1.80 m[a]	
3	CH_2	30.0	30.3	1.45–1.60 m[a]	
4	C	31.3	31.2		
5	CH	58.1	57.4	1.26 d (6.4)	3, 6, 7, 10, 18, 20
6	CH	77.9	77.9	4.17 t (6.4)	4, 5, 7, 8
7	CH	78.4	78.2	4.57 d (6.4)	5, 8, 9, 10 , 14
8	C	126.6	126.2		
9	C	117.3	117.6		
10	C	80.5	80.9		
11	C	142.3	142.7		
12	C	140.6	141.4		
13	C	132.3	133.0		
14	CH	115.7	115.6	6.47 s	7, 9, 12, 15
15	CH	27.2	26.9	3.14 sept (6.8)	12, 13, 14, 16, 17
16	CH_3	22.6	22.6	1.16 d (6.8)	13, 15, 17
17	CH_3	22.6	22.7	1.16 d (6.8)	13, 15, 16
18	CH_3	30.1	29.9	0.91 s	3, 4, 5, 19
19	CH_3	22.8	22.8	0.92 s	3, 4, 5, 18
20	CH_2	30.4	39.9	2.41 d (16.4)	1, 5, 8, 9, 10, 11
				2.68 d (16.4)	

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 50 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

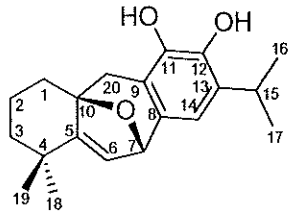
Table 71 Comparison of ^{1}H NMR spectral data of compounds **PO20** and 5,6-dihydro-6α-hydroxysalviasperanol

Position	δ_H /ppm (mult., *J* in Hz)	
	PO20[a]	5,6-dihydro-6α-hydroxysalviasperanol[b]
1	1.45–1.60 m	
	1.70–1.80 m	
2	1.70–1.80 m	
3	1.45–1.60 m	
5	1.26 d (6.4)	
6	4.17 t (6.4)	4.30 t (6.2)
7	4.57 d (6.4)	4.72 d (6.2)
14	6.47 s	6.54 s
15	3.14 sept (6.8)	3.12 sept (6.8)
16	1.16 d (6.8)	1.24 d (6.8)
17	1.16 d (6.8)	1.23 d (6.8)
18	0.91 s	1.03 s
19	0.92 s	1.01 s
20	2.41 d (16.4)	2.43 d (16.0)
	2.68 d (16.4)	2.76 d (16.0)

[a] Spectrum recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 200 (^{1}H NMR) MHz, $CDCl_3$.

3.2.21 Compound PO21



Compound **PO21** was isolated as an amorphous solid, m.p. 207-209 °C, $[\alpha]_D^{26}$ –32.5 ($CHCl_3$; *c* 2.10). The IR and UV spectral data were closely related to that of **PO20**.

The close similarity of the ^1H and ^{13}C NMR spectral data of **PO21** (**Table 72**, **Figures 117** and **118**) to that of an icetexane diterpene, compound **PO20** (**Table 70**, **Figures 115** and **116**), indicated that **PO21** has the same 7,10-hemiketalic icetexane skeleton as **PO20**. Analysis of the HMQC and HMBC spectra supported this structure. However, instead of the hydroxy group as in **PO20**, a doublet, observed in the ^1H NMR spectrum of **PO21** at δ 6.03 (*J*= 2.0 Hz) was assigned to the olefinic proton at C-6. The ^{13}C NMR and DEPT experiments also proved the presence of two additional sp^2 carbon atoms at δ 149.1 (C-5) and δ 128.2 (C-6) in **PO21**, which was associated with the absence of the ^1H NMR signal of H-5 at δ 1.26 and 4.17 as in **PO20**. This finding was further supported by HMBC correlations (**Table 72**) from CH_3-18, CH_3-19, CH_2-20 and H-7 to C-5 (δ 149.1). By comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data [Esquivel *et al.*, 1995, $[\alpha]_D^{26}$ –31.5 ($CHCl_3$; *c* 0.2)] (**Table 73**), therefore compound **PO21** was identified as salviasperanol.

Table 72 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO21** and ^{13}C NMR of salviasperanol (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO21[c]	
1	CH_2	38.6	38.5	1.77 m[a]	3, 5, 10, 20
				2.13 m[a]	
2	CH_2	19.0	18.9	1.78 m[a]	
3	CH_2	40.0	40.0	1.35 m[a]	
				1.58 m[a]	
4	C	33.6	33.6		
5	C	149.5	149.1		
6	CH	128.3	128.2	6.03 d (2.0)	4, 5, 7, 10
7	CH	79.9	80.5	5.08 d (2.0)	5, 6, 8, 9, 10 , 14
8	C	131.4	130.5		
9	C	117.0	117.4		
10	C	83.8	84.0		
11	C	142.6	143.0		
12	C	139.9	141.1		
13	C	131.0	131.9		
14	CH	112.1	111.8	6.41 s	7, 9, 12, 13, 15
15	CH	27.0	26.8	3.20 sept (6.8)	12, 13, 14, 16, 17
16	CH_3	22.4	22.3	1.25 d (6.8)	13, 15, 17
17	CH_3	22.8	22.6	1.17 d (6.8)	13, 15, 16
18	CH_3	29.9	29.6	1.01 s	3, 4, 5, 19
19	CH_3	27.7	27.3	1.12 s	3, 4, 5, 18
20	CH_2	30.2	30.5	2.64 d (16.8)	1, 5, 8, 9, 10, 11
				2.98 d (16.8)	

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 50 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

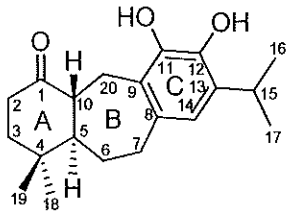
Table 73 Comparison of ^{1}H NMR spectral data of compounds **PO20**, **PO21** and salviasperanol

Position	δ_H/ppm (mult., *J* in Hz)		
	PO20[a]	**PO21**[a]	salviasperanol[b]
1	1.45–1.60 m	1.77 m	
	1.70–1.80 m	2.13 m	
2	1.70–1.80 m	1.78 m	
3	1.45–1.60 m	1.35 m	
		1.58 m	
5	1.26 d (6.4)		
6	4.17 t (6.4)	6.03 d (2.0)	6.04 dd (2.1, 0.8)
7	4.57 d (6.4)	5.08 d (2.0)	5.07 d (2.1)
14	6.47 s	6.41 s	6.43 s
15	3.14 sept (6.8)	3.20 sept (6.8)	3.08 sept (6.8)
16	1.16 d (6.8)	1.25 d (6.8)	1.24 d (6.8)
17	1.16 d (6.8)	1.17 d (6.8)	1.30 d (6.8)
18	0.91 s	1.01 s	1.02 s
19	0.92 s	1.12 s	1.12 s
20	2.41 d (16.4)	2.64 d (16.8)	2.65 d (16.5)
	2.68 d (16.4)	2.98 d (16.8)	2.88 d (16.5)

[a] Spectra recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 200 (^{1}H NMR) MHz, $CDCl_3$.

3.2.22 Compound PO22



Compound **PO22** was isolated as colorless crystals, m.p. 179-182 °C, $[\alpha]_D^{26}$ +107.5 ($CHCl_3$; *c* 0.41). The IR spectrum displayed the absorption bands due to carbonyl (1686 cm^{-1}) and hydroxyl groups (3293 cm^{-1}). The UV spectrum was closely related to that of **PO20**.

The ^1H NMR spectral data of **PO22** (**Table 74**, **Figure 119**) displayed signals for an isopropyl group as two methyl doublets at δ 1.23 and 1.20 (*J* = 6.9 Hz, CH_3-16 and CH_3-17) and a septet at δ 3.23 (*J* = 6.9 Hz, H-15). Signals for two tertiary methyl groups were evident as two singlets at δ 1.08 (CH_3-18) and 0.91 (CH_3-19). In addition, two methine protons were shown as resonances at δ 1.14 (1H, m, H-5) and 2.70 (1H, dd, *J* = 12.0, 6.6 Hz, H-10) while the methylene protons were displayed at δ 3.15 (1H, d, *J* = 14.4 Hz, H-20a), 2.60 (1H, dd, *J* = 14.4, 6.6 Hz, H-20b), δ 2.27 (1H, ddd, *J* = 13.5, 4.5, 2.7 Hz, H-2a), δ 2.53 (1H, dt, *J* = 13.5, 6.3 Hz, H-2b), δ 1.54 (1H, dt, *J* = 13.5, 4.5 Hz, H-3a), δ 1.70 (1H, ddd, *J* = 13.5, 6.3, 2.7 Hz, H-3b), δ 1.57 (1H, m, H-6a), δ 1.91 (1H, dddd, *J* = 16.5, 6.0, 5.7, 2.7 Hz, H-6b), δ 2.60 (1H, m, H-7a), and δ 2.99 (1H, ddd, *J* = 15.6, 10.5, 5.7 Hz, H-7b).

The ^{13}C NMR and DEPT spectral data of **PO22** (**Table 74**, **Figure 120**) displayed 20 carbon signals in which contained four methyls, five methylenes, four methines (including one olefinic carbon), and seven quaternary carbons (including five olefinic ones and a carbonyl one). A ketone carbon signal at δ 217.2 was also observed. These data indicated an icetexane diterpene skeleton similar to that of rosmaridiphrenol (Houlihan et al., 1984) except for the location of carbonyl in **PO22** was attached to cyclohexane in ring A. This finding was further supported by HMBC correlations (**Table 75**).

The relative stereochemistry of **PO22** was deduced by analysis of its NOESY spectrum. Correlations from Me-19 to H-10 and Me-18 to H-5 revealed that H-10 and H-5 were in β and α configuration, respectively. By comparison of the ^{1}H and ^{13}C NMR spectral data with the previously reported data [Pertino *et al.*, 2010, $[\alpha]_D^{26}$ +23 ($CHCl_3$; *c* 0.2)] (**Table 75**), therefore compound **PO22** was identified as 11,12-dihydroxy-8,11,13-icetexatrien-1-one.

Table 74 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO22** (300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$)[b] and ^{13}C NMR of 11,12-dihydroxy-8,11,13-icetexatrien-1-one (100 MHz, $CDCl_3$)[a]

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[a]		PO22[b]	
1	CO	217.2	217.2		
2	CH_2	38.4	38.5	2.27 ddd (13.5, 4.5, 2.7)	1, 3, 4, 10
				2.53 td (13.5, 6.3)	
3	CH_2	42.2	42.2	1.54 td (13.5, 4.5)	1, 2, 4, 4, 18,
				1.70 ddd (13.5, 6.3, 2.7)	19
4	C	33.6	33.6		
5	CH	48.9	48.9	1.14 m	4, 6, 10, 18, 19
6	CH_2	24.5	24.5	1.57 m	4, 7, 8, 10
				1.91 dddd (16.5, 6.0, 5.7,2.7)	
7	CH_2	30.3	30.4	2.60 m	6, 8, 9, 14
				2.99 ddd (15.6, 10.5, 5.7)	
8	C	130.3	130.4		
9	C	122.8	120.7		
10	CH	49.5	49.5	2.70 dd (12.0, 6.6)	1, 4, 5, 6, 9, 20
11	C	141.8	140.1		
12	C	142.6	141.8		
13	C	131.7	131.8		
14	CH	117.7	117.9	6.47 s	7, 9, 12, 13, 15
15	CH	27.1	27.2	3.23 sept (6.9)	12, 14, 16, 17
16	CH_3	22.1	22.1	1.23 d (6.9)	13, 15, 17
17	CH_3	22.9	22.9	1.20 d (6.9)	13, 15, 16
18	CH_3	19.9	19.9	1.08 s	3, 4, 5, 19
19	CH_3	28.9	29.0	0.91 s	3, 4, 5, 18
20	CH_2	22.7	22.7	2.60 dd (14.4, 6.6)	1, 8, 9, 10, 11
				3.15 d (14.4)	
11-OH				7.96 br s	
12-OH				5.76 s	11, 12, 13

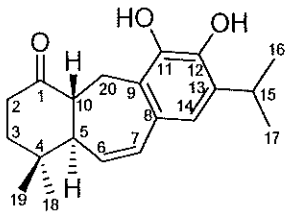
Table 75 Comparison of ^{1}H spectrum data of compounds **PO22** and 11,12-dihydroxy-8,11,13-icetexatrien-1-one (**R**)

Position	δ_H/ppm (mult., *J* in Hz)	
	PO22[b]	**R**[a]
2	2.27 ddd (13.5, 4.5, 2.7)	2.22 ddd (14.1, 4.3, 2.8)
	2.53 td (13.5, 6.3)	2.52 ddd (14.1, 14.1, 6.3)
3	1.54 td (13.5, 4.5)	1.54 ddd (14.1, 13.1, 4.3)
	1.70 ddd (13.5, 6.3, 2.7)	1.69 ddd (14.1, 13.1, 4.3)
5	1.14 m	1.13 ddd 13.9, 11.9, 2)
6	1.57 m, 1.91 dddd (16.5, 6.0, 5.7,2.7)	1.52 m
		1.91 m
7	2.60 m, 2.99 ddd (15.6, 10.5, 5.7)	2.68 m
		2.98 ddd
10	2.70 dd (12.0, 6.6)	2.70 m
14	6.47 s	6.47 s
15	3.23 sept (6.9)	3.23 dq (6.8, 6.8)
16	1.23 d (6.9)	1.23 d (6.8)
17	1.20 d (6.9)	1.20 d (6.8)
18	1.08 s	1.07 s
19	0.91 s	0.90 s
20	2.60 dd (14.4, 6.6)	2.58 d (14.0)
	3.15 d (14.4)	3.14 d (14.0)
11-OH	7.96 br s	
12-OH	5.76 s	

[a] Spectra recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectrum recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

3.2.23 Compound PO23



Compound **PO23** was isolated as colorless crystals after recrystallization from CH_2Cl_2, m.p. 176–178 °C, $[\alpha]_D^{24}$ −182.2 ($CHCl_3$; *c* 2.93). A molecular formula $C_{20}H_{26}O_3$ was determined by HREIMS (calcd 314.1882, found 314.1894 $[M]^{+\bullet}$), implying eight degrees of unsaturation. The UV spectrum of **PO23** showed absorption maxima at λ_{max} 219, 269, and 292 nm, indicating the presence of a conjugated system. Its IR spectrum showed absorption bands at 3478, 1681, and 1629 cm^{-1} suggested the presence of hydroxyl, carbonyl, and aromatic functional groups in the molecule. The subsequent X-ray structure (**Figure 31**) showed an icetexane-type diterpenoid skeleton whose structure could be derived from abietane (Simmons *et al.*, 2009). The structure was also established from analysis of its spectroscopic data.

The ^1H NMR spectral data of **PO23** (**Table 76**, **Figure 121**) displayed signals for an isopropyl group as two methyl doublets at δ 1.21 (*J* = 6.8 Hz, CH_3-16 and CH_3-17) and a septet at δ 3.27 (*J*= 6.8 Hz, H-15). Signals for two tertiary methyl groups were evident as two singlets at δ 0.90 (CH_3-18) and 1.24 (CH_3-19). Furthermore two methine protons were shown as resonances at δ 1.90 (1H, ddd, *J*= 13.6, 6.0, 1.6 Hz, H-5) and 3.31 (1H, dd, *J*= 13.6, 6.0 Hz, H-10) while three olefinic methine protons were displayed at δ 5.90 (1H, dd, *J*= 10.4, 6.0 Hz, H-6), 6.57 (s, H-14) and 6.62 (1H, dd, *J*= 10.4, 6.0 Hz, H-7), whose assignments were based on the HMBC experiments (**Table 76**, **Figures 30**). A ketone carbon signal at δ 216.6 was also observed. These data indicated an icetexane diterpene skeleton similar to that of 11,12-dihydroxy-8,11,13-icetexatrien-1-one (**PO22**) except for the replacement of -CH_2-CH_2- in the heptacyclic of **PO22** by -CH=CH- moiety in **PO23**. The placement of a double bond at C-6 and C-7 was confirmed by the HMBC experiment (**Table 76**, **Figure 30**). The NOESY spectrum showed the correlations between CH_3-18/H-5, H-

10/CH_3-19 whose data together with that from X-ray data were in accordance with the β-orientation of H-10, CH_3-19 and α-orientation of H-5 and CH_3-18. Based on these data, compound **PO23** was a new compound and identified as 11,12-dihydroxy-6,8,11,13-icetexatatraen-1-one (Salae *et al.*, 2012).

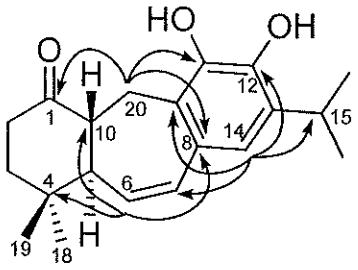


Figure 30 Major selected HMBC correlations of **PO23**

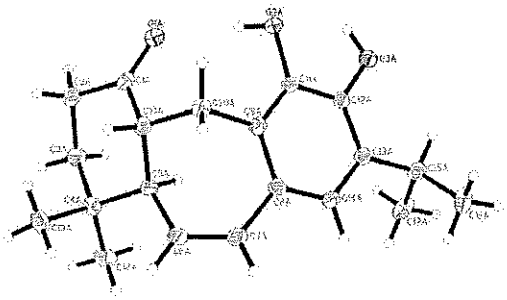


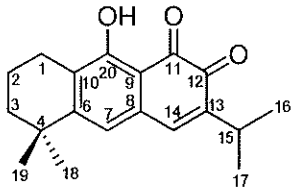
Figure 31 *ORTEP* drawing of **PO23**

Table 76 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO23**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CO	216.6		
2	CH_2	38.2	2.33 ddd (13.6, 4.8, 2.0)	1, 3, 4, 10
			2.53 td (13.6, 6.4)	
3	CH_2	41.5	1.52 td (13.6, 4.8)	1, 2, 4, 5, 18, 19
			1.75 ddd (13.6, 6.4, 2.0)	
4	C	32.6		
5	CH	51.6	1.90 ddd (13.6, 6.0, 1.6)	1, 3, 7, 10, 18, 19, 20
6	CH	129.0	5.90 dd (10.4, 6.0)	4, 5, 8, 9, 10
7	CH	131.3	6.62 dd (10.4, 6.0)	5, 8, 9, 14
8	C	130.1		
9	C	122.7		
10	CH	60.1	3.31 dd (13.6, 6.0)	1, 4, 5, 6, 9, 20
11	C	140.5		
12	C	142.6		
13	C	132.1		
14	CH	117.7	6.57 s	7, 9, 12, 15
15	CH	27.1	3.27 sept (6.8)	12, 13, 14, 16, 17
16	CH_3	22.7	1.21 d (6.8)	13, 15, 17
17	CH_3	22.3	1.21 d (6.8)	13, 15, 16
18	CH_3	29.2	0.90 s	3, 4, 5,19
19	CH_3	19.9	1.24 s	3, 4, 5, 18
20	CH_2	24.3	2.30 dd (14.0, 6.8)	1, 5, 8, 9, 10, 11
			3.03 d (14.0)	
11-OH			8.01 s	9, 11, 12
12-OH			6.00 s	11, 12, 13

Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

3.2.24 Compound PO24



Compound **PO24** was isolated as a red amorphous solid, m.p. 135–137 °C; $[\alpha]_D^{24}$ +112.4 ($CHCl_3$; *c* 0.21), which was further recrystallized from hexane/CH_2Cl_2 to yield orange single crystals. A molecular formula $C_{19}H_{22}O_3$ was implied by HREIMS (calcd 298.1569, found 298.1566 $[M]^{+\bullet}$). Strong absorption bands for a hydroxyl (3327 cm^{-1}), a quinoid carbonyl (1691 and 1667 cm^{-1}) and an aromatic (1654 and 1598 cm^{-1}) groups were shown in the IR spectrum, while the absorption bands (223, 263 and 451 nm) in the UV spectrum indicated quinone group. Signals for two carbonyl groups (δ_C 180.9 and 181.8) were evident from the ^{13}C NMR spectrum. The X-ray data of **PO24** (**Figure 33**) showed a linear tricyclic of cyclohexane-*ortho*-naphthoquinone skeleton.

The 19 carbon signals were observed in the ^{13}C NMR spectrum of **PO24** (**Table 77**, **Figure 124**) which were further classified by DEPT experiments as four methyl, three methylene, two olefinic methine, one methine, six quaternary olefinic, two carbonyl, and one quaternary carbons.

The ^1H NMR spectrum of **PO24** (**Table 77**, **Figure 123**) showed signals for two methyls at δ 1.29, (s, CH_3-18 and CH_3-19), an isopropyl moiety (δ 1.16, d, J = 6.9 Hz, CH_3-16 and CH_3-17) and δ 3.06, 1H, sept, J = 6.9 Hz, H-15), two singlets of the olefinic methine protons at δ 7.06 and 6.80 (s, H-14 and H-7, respectively), and a chelated hydroxyl proton (δ 12.50, s). The COSY spectrum showed cross-peaks between methylene protons: H_2-2 (δ 1.65-1.75, 1.77-1.89) and H_2-1 (δ 2.67)/H_2-3 (δ 1.60-1.75). On the basis of the above spectroscopic and X-ray crystallographic data, compound **PO24** was a new compound and designated as obtusinone A (Salae *et al.*, 2012).

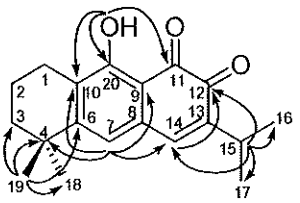


Figure 32 Major selected HMBC correlations of **PO24**

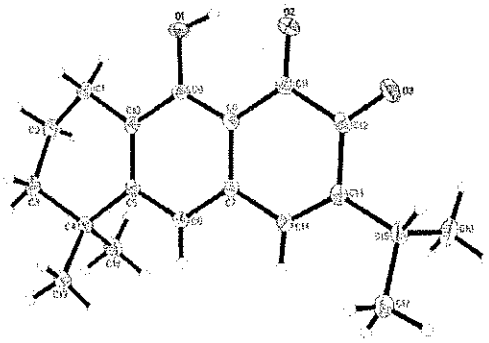


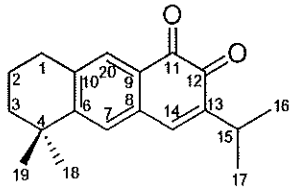
Figure 33 *ORTEP* drawing of **PO24**

Table 77 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO24**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CH_2	23.1	2.67 t (6.3)	2, 3, 5, 8, 9, 10
2	CH_2	18.2	1.65–1.75 m	1, 3, 4, 10
			1.77–1.89 m	
3	CH_2	38.0	1.60–1.75 m	1, 2, 4, 5, 18, 19
4	C	34.8		
5				
6	C	158.0		
7	CH	121.0	6.80 s	4, 5, 8, 9, 10, 11, 14
8	C	131.8		
9	C	111.1		
10	C	128.8		
11	CO	181.8		
12	CO	180.9		
13	C	145.0		
14	CH	138.4	7.06 s	6, 7, 8, 9, 12, 13, 15
15	CH	26.9	3.06 sept (6.9)	12, 13, 14, 16, 17
16	CH_3	21.6	1.16 d (6.9)	13, 15, 17
17	CH_3	21.6	1.16 d (6.9)	13, 15, 16
18	CH_3	30.5	1.29 s	3, 4, 5, 19
19	CH_3	30.5	1.29 s	3, 4, 5, 18
20	C	165.2		
20-OH			12.50 s	5, 8, 9, 10, 11

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.2.25 Compound PO25



Compound **PO25** was also isolated as an orange amorphous solid, m.p. 89–91 °C, $[\alpha]_D^{24}$ +14.9 ($CHCl_3$; *c* 1.17). The HREIMS of **PO25** showed a molecular ion $[M]^{+\bullet}$ peak at *m/z* 282.1614 (calcd 282.1620), consistent with the molecular formula $C_{19}H_{22}O_2$ with nine degrees of unsaturation similar to that of **PO24**. The UV and IR spectra were similar to those of **PO24**. The ^1H and ^{13}C NMR spectral data (**Table 78**, **Figures 125** and **126**) of **PO25** were very similar to those of **PO24** (**Table 77** and **79**, **Figures 123** and **124**) except for the presence of a singlet signal of an aromatic methine proton at δ 7.72 in **PO25** instead of a chelated hydroxyl proton at δ 12.50 as in **PO24**. This aromatic methine proton showed HMBC correlations (**Table 78**, **Figures 34**) with δ 34.6 (C-4), δ 155.0 (C-6), δ 133.0 (C-8) and δ 179.6 (C-11) which supported its location at C-20. Thus, **PO25** was a new compound and designated as obtusinone B (Salae *et al.*, 2012).

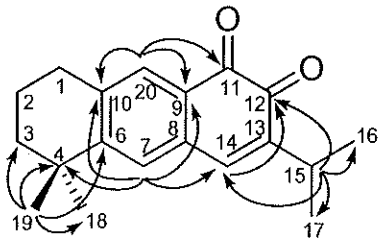


Figure 34 Major selected HMBC correlations of **PO25**

Table 78 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO25**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CH_2	30.4	2.78 t (6.4)	2, 3, 5, 9, 10
2	CH_2	19.1	1.75–1.85 m	1, 3, 4, 10
3	CH_2	38.5	1.65–1.71 m	1, 2, 4, 5, 18, 19
4	C	34.6		
5				
6	C	155.0		
7	CH	128.0	7.22 s	4, 8, 10, 11
8	C	133.0		
9	C	127.7		
10	C	138.6		
11	CO	179.6		
12	CO	181.2		
13	C	145.2		
14	CH	139.1	7.13 s	6, 8, 12, 15
15	CH	26.9	3.05 sept (6.8)	12, 13, 14, 16, 17
16	CH_3	21.6	1.16 d (6.8)	13, 15, 17
17	CH_3	21.6	1.16 d (6.8)	13, 15, 16
18	CH_3	31.3	1.32 s	3, 4, 5, 19
19	CH_3	31.3	1.32 s	3, 4, 5, 18
20	CH	131.2	7.72 s	4, 6, 7, 11

Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

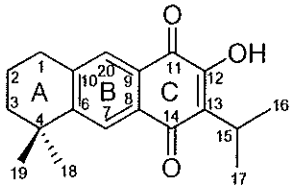
Table 79 Comparison of ^{1}H and ^{13}C NMR spectral data of compounds **PO24** and **PO25**

Position	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	
	PO24[a]	**PO25**[b]	**PO24**[a]	**PO25**[b]
1	23.1	30.4	2.67 t (6.3)	2.78 t (6.4)
2	18.2	19.1	1.65–1.75 m	1.75–1.85 m
			1.77–1.89 m	
3	38.0	38.5	1.60–1.75 m	1.65–1.71 m
4	34.8	34.6		
5				
6	158.0	155.0		
7	121.0	128.0	6.80 s	7.22 s
8	131.8	133.0		
9	111.1	127.7		
10	128.8	138.6		
11	181.8	179.6		
12	180.9	181.2		
13	145.0	145.2		
14	138.4	139.1	7.06 s	7.13 s
15	26.9	26.9	3.06 sept (6.9)	3.05 sept (6.8)
16	21.6	21.6	1.16 d (6.9)	1.16 d (6.8)
17	21.6	21.6	1.16 d (6.9)	1.16 d (6.8)
18	30.5	31.3	1.29 s	1.32 s
19	30.5	31.3	1.29 s	1.32 s
20	165.2	131.2		7.72 s
20-OH			12.50 s	

[a] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

3.2.26 Compound PO26



Compound **PO26** was obtained as a red amorphous solid, m.p. 104–106 °C, $[\alpha]_D^{24}$ +57.2 ($CHCl_3$; *c* 0.12). The molecular formula $C_{19}H_{22}O_3$ as determined by the HREIMS (calcd 298.1569, found 298.1563 $[M]^{+\bullet}$) indicating nine degrees of unsaturation. The IR spectrum of **PO26** exhibited bands for carbonyl (1728 and 1650 cm^{-1}), hydroxyl (3386 cm^{-1}) and aromatic (1628 cm^{-1}) groups. Furthermore, the presence of two carbonyl groups at δ 184.8 and 181.8 in the ^{13}C NMR spectrum and the UV absorption maxima at λ_{max} 215, 285, 353 and 411 nm indicated that **PO26** should have a *para*-naphthoquinone chromophore (Lin *et al.*, 1989).

The ^{1}H and ^{13}C NMR spectral data of **PO26** (**Table 80**, **Figures 127** and **128**) were comparable to those of **PO25** (**Table 78** and **81**, **Figures 125** and **126**) except for the differences in ring C in which that of **PO25** was an *ortho*-quinonoid but that of **PO26** was a *para*-quinonoid. A singlet signal of an aromatic methine proton H-14 at δ_H 7.13: δc 139.1 shown in **PO25** was replaced by a hydroxyl signal at δ 7.41 in **PO26** whose location at C-12 was suggested from the HMBC correlations (**Table 80**, **Figures 35**) of H-15 at δ 3.40 with the carbons at δ 128.4 (C-13), 152.7 (C-12) and 184.8 (C-14). Furthermore an aromatic proton H-7 which appeared at δ 7.22 in **PO25** was shifted more downfield in **PO26** to δ 8.06 supporting a carbonyl group at

C-14. Based on all of the spectroscopic evidence, the structure of **PO26** was a new compound and characterized as obtusinone C (Salae *et al.*, 2012).

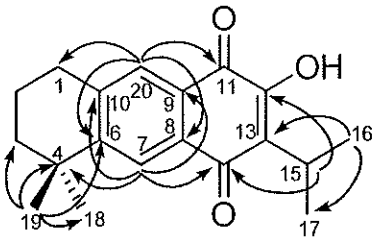


Figure 35 Major selected HMBC correlations of **PO26**

Table 80 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO26**

Position	DEPT	δ_C/ppm	δ_H/ppm (mult., *J* in Hz)	HMBC
1	CH_2	30.8	2.86 t (6.3)	2, 3, 5, 9, 10
2	CH_2	19.1	1.80–1.85 m	1, 3, 4, 10
3	CH_2	38.6	1.65–1.75 m	1, 2, 4, 5, 18, 19
4	C	34.9		
5				
6	C	154.1		
7	CH	125.7	8.06 s	1, 4, 7, 8, 10, 11, 14
8	C	131.1		
9	C	126.3		
10	C	141.9		
11	CO	181.8		
12	C	152.7		
13	C	128.4		
14	CO	184.8		
15	CH	24.6	3.40 sept (7.2)	12, 13, 14, 16, 17
16	CH_3	19.9	1.30 d (7.2)	13, 15, 17
17	CH_3	19.9	1.30 d (7.2)	13, 15, 16
18	CH_3	31.3	1.33 s	3, 4, 5, 19
19	CH_3	31.3	1.33 s	3, 4, 5, 18
20	CH	127.0	7.72 s	1, 5, 7, 11, 14
12-OH			7.41 s	11, 12, 13

Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

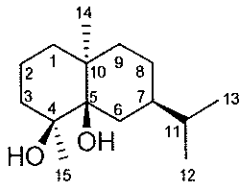
Table 81 Comparison of ^{1}H and ^{13}C NMR spectral data of compounds **PO25** and **PO26**

Position	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	
	PO25[a]	**PO26**[b]	**PO25**[a]	**PO26**[b]
1	30.4	30.8	2.78 t (6.4)	2.86 t (6.3)
2	19.1	19.1	1.75–1.85 m	1.80–1.85 m
3	38.5	38.6	1.65–1.71 m	1.65–1.75 m
4	34.6	34.9		
5				
6	155.0	154.1		
7	128.0	125.7	7.22 s	8.06 s
8	133.0	131.1		
9	127.7	126.3		
10	138.6	141.9		
11	179.6	181.8		
12	181.2	152.7		
13	145.2	128.4		
14	139.1	184.8	7.13 s	
15	26.9	24.6	3.05 sept (6.8)	3.40 sept (7.2)
16	21.6	19.9	1.16 d (6.8)	1.30 d (7.2)
17	21.6	19.9	1.16 d (6.8)	1.30 d (7.2)
18	31.3	31.3	1.32 s	1.33 s
19	31.3	31.3	1.32 s	1.33 s
20	131.2	127.0	7.72 s	7.72 s
12-OH				7.41 s

[a] Spectra recorded at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

3.2.27 Compound PO27



Compound **PO27** was isolated as a colorless oil, $[\alpha]_D^{26}$ –54.0. Its IR spectrum showed an absorption band at 3400 cm^{-1}, indicating the presence of hydroxyl groups.

The ^{13}C NMR and DEPT spectra indicated that **PO27** (**Table 82**, **Figure 130**) contained 15 resonances including two methine, six methylene, four methyl carbons as well as three quaternary carbons. Two oxygenated quaternary carbon signals at δ 74.6 and 78.6 in the ^{13}C NMR spectrum indicated the presence of two tertiary hydroxyl groups. The ^{1}H and ^{13}C NMR spectra of **PO27** suggested an eudesmane framework with oxygenated function at C-4 (δ_C 74.6) and C-5 (δ_C 78.6). Analysis of the ^{1}H NMR spectrum of **PO7** (**Table 82**, **Figure 129**) suggested an isopropyl [δ 0.92 (3H, d, *J*= 6.9 Hz, H-12), 0.89 (3H, d, *J*= 6.9 Hz, H-13), 2.02 (1H, dqq, *J*= 10.5, 6.9, 6.9 Hz, H-11)], two methyl [δ 1.01 (3H, s, H-14), 1.27 (3H, s, H-15). The above-mentioned ^{1}H and ^{13}C NMR spectra of **PO27** showed a close similarity to those of dihydrokongol (Eduardo *et al.*, 1979). However, the methine carbon signal at δ 51.7 of C-5 in dihydrokngol was absent in the ^{13}C NMR spectrum of **PO27**. Instead, an oxyquaternary carbon signal appearing at δ 78.6 was observed. This suggested that **PO27** was a 5-hydroxyl derivative of dihydrokongol. This conclusion was confirmed by HMBC experiment (**Table 82**, **Figure 36**). The relative configuration of **PO27** was derived from NOESY correlation of H-14/H-6α, H-14/H-15 and 6α/7α (**Figure 36**). Thus on the basis of its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data with the previous report (Jakupovic *et al.*, 1992) (**Table 82** and **83**), compound **PO27** was identified as 4β,5β-dihydroxy-10-*epi*-eudesmane.

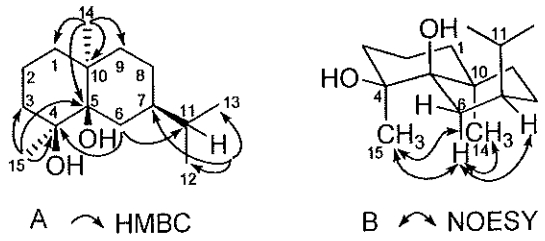


Figure 36 Selected HMBC correlations (A) and NOESY correlations (B) for **PO27**

Table 82 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO27** and ^{13}C NMR of 4β,5β-dihydroxy-10-*epi*-eudesmane (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO12[c]	
1	CH_2	33.7	33.7	1.53–1.68 m[a]	
2	CH_2	19.2	19.2	1.44–1.66 m[a]	
3	CH_2	37.0	37.0	1.88, 1.45 m[a]	
4	C	74.6	74.6		
5	C	78.8	78.6		
6	CH_2	26.0	26.1	1.87, 1.65 m[a]	
7	CH	40.5	40.7	1.34 m[a]	
8	CH_2	22.4	22.4	1.81, 1.65 m[a]	
9	CH_2	34.7	34.7	1.87, 0.90 m[a]	
10	C	37.6	37.5		
11	CH	29.3	29.7	2.02 dqq (10.5, 6.9, 6.9)	7, 8, 12, 13
12	CH_3	21.9	21.9	0.92 d (6.9)	7, 11, 13
13	CH_3	22.9	22.9	0.89 d (6.9)	7, 11, 12
14	CH_3	22.7	22.7	1.01 s	3, 4, 5
15	CH_3	24.0	24.0	1.27 s	1, 5, 9, 10

[a] Deduced from HMQC experiment.

[b] Spectrum recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

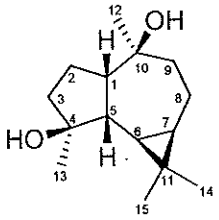
Table 83 Comparison of ^{1}H NMR spectral data of compounds **PO27** and 4β,5β-dihydroxy-10-*epi*-eudesmane

Position	δ_H/ppm (mult., *J* in Hz)	
	PO27[a]	4β,5β-dihydroxy-10-*epi*-eudesmane[b]
1	1.53–1.68 m	1.48–1.67m
	0.97 m	097 m
2	1.44–1.66 m	1.48–1.67m
3	1.88 m	1.86 br ddd
	1.45 m	1.45 m
6	1.87 m	1.89 ddd (15, 2, 0.5)
	1.65 m	1.66 dd (15, 6.5)
7	1.34 m	1.34 m
8	1.81 m	1.71 dddd
	1.65 m	1.62 m
9	1.87 m	1.86 br ddd (14, 13, 3.5)
	0.90 m	0.88 ddd
11	2.02 dqq (10.5, 6.9, 6.9)	2.01 dqq (10.5, 7, 7)
12	0.92 d (6.9)	0.93 d (7)
13	0.89 d (6.9)	0.89 d (7)
14	1.01 s	1.01 s
15	1.27 s	1.27 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

3.2.28 Compound PO28



Compound **PO28** was isolated as an optically active colorless prism with m.p. 138–140 °C, $[\alpha]_D^{26}$ –37.1 ($CHCl_3$; *c* 0.76). The IR spectrum indicated the presence of hydroxyl group (3396 cm^{-1}). Analysis the ^{13}C NMR and DEPT spectra indicated that **PO28** (**Table 84**, **Figure 132**) contained 15 resonances including four methyl, four methylene, four methine and three quaternary carbons including two oxygenated quaternary carbon signals (δ_C 75.0 and 80.3).

The ^1H NMR spectrum of **PO28** (**Table 84**, **Figure 131**) showed two methine protons [δ 0.42 (1H, dd, *J*= 10.8, 9.6 Hz, H-6), 0.64 (1H, ddd, *J*= 11.2, 9.6, 6.4 Hz, H-7)] and two methyl groups [δ 1.04 (6H, s, H-14 and H-15)], characteristic for the presence of a cyclic propane in the aromadendrane skeleton (Vlakhov *et al.*, 1967). The coupling constant (*J*= 9.6 Hz) between H-6 and H-7 suggested that H-6 and H-7 were *cis* form (Bohlmann et al., 1983). Furthermore, two methine protons at δ 1.49-1.88 (1H, m, H-1), 1.20 (1H, m, H-5), four methylenes [δ 1.49-1.88 (6H, m, H-2, H-3 and H-9), 0.89 (2H, m, H-8)] and two methyls [δ 1.17 (3H, s, H-12), 1.25 (3H, s, H-13)] were also observed. The HMBC experiment (**Table 84**, **Figure 37**) showed correlations between H-12 (δ 1.17) and C-10 (δ 75.0) and H-13 (δ 1.25) and C-4 (δ 80.3), suggesting the presence of a tertiary hydroxyl group. According to the above data, the structure of **PO28** is similar to aromadendrane-4α, 10β-diol, which was previously isolated from *Brasilia sickie* by Bohlmann *et al.* However, the NOESY experiment (**Figure 37**) indicated that the relative stereochemistry of hydroxyl groups at C-4 and C-10 both had β-orientations. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report [Wu *et*

al., 2000, $[\alpha]_D^{25}$ –20.2 ($CHCl_3$; *c* 0.24)] (**Table 84** and **85**), compound **PO28** was identified as 4β,10β-dihydroxy aromadendrane.

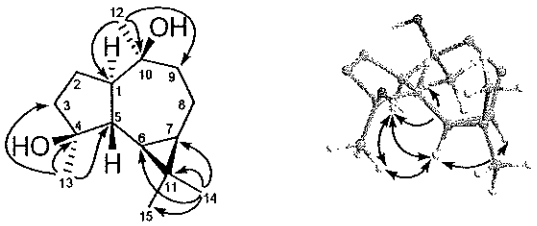


Figure 37 Selected HMBC correlations (A) and NOESY correlations (B) for **PO28**

Table 84 ^1H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO28** and ^{13}C NMR of 4β,10β-dihydroxy aromadendrane (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[a]		PO28[b]	
1	CH	56.4	56.4	1.49–1.88 m[a]	
2	CH_2	23.8	23.7	1.49–1.88 m[a]	
3	CH_2	41.1	41.1	1.49–1.88 m[a]	
4	C	80.3	80.3		
5	CH	48.4	48.4	1.20 m[a]	
6	CH	28.3	28.3	0.42 dd (10.8, 9.6)	4, 15
7	CH	26.6	26.6	0.64 ddd (11.2, 9.6, 6.4)	5, 15
8	CH_2	20.1	20.1	0.89 m[a]	
9	CH_2	44.4	44.4	1.49–1.88 m[a]	
10	C	75.0	75.0		
11	C	19.5	19.5		
12	CH_3	20.3	20.3	1.17 s	1, 9, 10
13	CH_3	23.4	24.5	1.25 s	3, 4, 5
14	CH_3	16.4	16.4	1.04 s	6, 7, 11, 15
15	CH_3	28.6	28.6	1.04 s	6, 7, 11, 14

[a] Deduced from HMQC experiment.

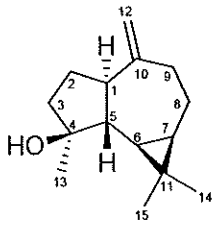
Spectra recorded at 400 (^1H NMR) and 100 (^{13}C NMR) MHz, $CDCl_3$.

Table 85 Comparison of ^{1}H NMR spectral data of compounds **PO28** and 4β,10β-dihydroxy aromadendrane

Position	δ_H/ppm (mult., *J* in Hz)	
	PO28	4β,10β-dihydroxy aromadendrane
1	1.49–1.88 m	1.50–1.90 m
2	1.49–1.88 m	1.50–1.90 m
3	1.49–1.88 m	1.50–1.90 m
5	1.20 m	1.18 dd (10.7, 10.7)
6	0.42 dd (10.8, 9.6)	0.40 dd (10.7, 9.6)
7	0.64 ddd (11.2, 9.6, 6.4)	0.62 ddd (11.0, 9.6, 6.0)
8	0.89 m	0.88 m
9	1.49–1.88 m	1.50–1.90 m
12		1.15 s
13	1.17 s	1.23 s
14	1.25 s	1.01 s
15	1.04 s	1.01 s

Spectra recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

3.2.29 Compound PO29



Compound **PO29** was isolated as an optically active colorless oil with $[\alpha]_D^{25}$ +12 ($CHCl_3$; *c* 0.15). The IR spectrum indicated the presence of hydroxyl group (3413 cm^{-1}) and double bond (1614 cm^{-1}). The ^1H and ^{13}C NMR spectral data of **PO29** (**Table 86**, **Figures 133** and **134**) were very similar to those of **PO28** (**Table 84**, **Figures 131** and **132**) except for the presence of two singlet signals of two olefinic protons due to terminal methylene at δ 4.66 (1H, s, H-12a) and 4.68 (1H, s, H-12b) in **PO29** instead of methyl protons at δ 1.17 (H_3-12) as in **PO28**. This finding was further confirmed by the HMBC correlations (**Table 86**) from H-9 (δ 2.42) to C-10 (δ 153.4) and C-12 (δ 106.3). By comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data [Iwabuchi *et al.*, 1989, $[\alpha]_D^{22}$ +5.3 ($CHCl_3$; *c* 3.70)] (**Table 87**), therefore compound **PO29** was identified as spathulenol.

Table 86 ^{1}H, ^{13}C NMR, DEPT and HMBC spectral data of compound **PO29** and ^{13}C NMR of spathulenol (**R**)

Position	DEPT	δ_C/ppm		δ_H/ppm (mult., *J* in Hz)	HMBC
		R[b]		PO29[c]	
1	CH	53.4	53.4	2.22 m[a]	5, 9
2	CH_2	26.8	26.7	1.58, 1.85 m[a]	
3	CH_2	41.8	41.7	1.55, 1.78 m[a]	
4	C	153.5	153.4		
5	CH	54.5	54.2	1.31 m[a]	
6	CH	30.0	29.9	0.46 dd (11.1, 9.6)	3, 7, 8, 11, 15
7	CH	27.7	27.5	0.70 m[a]	6, 11, 15
8	CH_2	24.9	24.7	1.01, 2.00 m[a]	
9	CH_2	39.0	38.8	2.42 dd (13.3, 6.1)	1, 7, 8, 10, 12
				2.02 m[a]	
10	C	80.9	80.8		
11	C	20.3	20.2		
12	CH_2	106.3	106.3	4.66 br s	1, 9, 10
				4.68 br s	
13	CH_3	26.1	26.0	1.28 s	3, 4, 5
14	CH_3	16.4	16.3	1.03 s	6, 7, 11, 15
15	CH_3	28.7	28.6	1.05 s	6, 7, 11, 14

[a] Deduced from HMQC experiment.

[b] Spectra recorded at 100 (^{13}C NMR) MHz, $CDCl_3$.

[c] Spectra recorded at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz, $CDCl_3$.

Table 87 Comparison of ^{1}H NMR spectral data of compounds **PO29** and spathulenol

Position	δ_H/ppm (mult., *J* in Hz)	
	PO29[a]	spathulenol[b]
1	2.22 m	
2	1.58, 1.85 m	
3	1.55, 1.78 m	
5	1.31 m	
6	0.46 dd (11.1, 9.6)	0.47 dd (11.4, 9.5)
7	0.70 m	0.71 ddd (11.4, 9.4, 5.9)
8	1.01, 2.00 m	
9	2.42 dd (13.3, 6.1)	2.42 m
	2.02 m	
12	4.66 br s	4.67 br s
	4.68 br s	4.69 br s
13	1.28 s	1.28 s
14	1.03 s	1.04 s
15	1.05 s	1.06 s

[a] Spectra recorded at 300 (^{1}H NMR) MHz, $CDCl_3$.

[b] Spectra recorded at 400 (^{1}H NMR) MHz, $CDCl_3$.

3.3 Biological activities of the isolated compounds from *D. wallichii* and *P. obtusifolia*

In this research, the isolated compounds from the roots and fresh fruits of *D. wallichii* and the roots and twigs of *P. obtusifolia* were tested for antibacterial activities against both Gram-positive bacteria: *B. subtilis*, *S. aureus*, TISTR517, *E. faecalis* TISTR459, Methicillin-Resistant *S. aureus* (MRSA) ATCC43300, Vancomycin-Resistant *E. faecalis* (VRE) ATCC 51299, *Streptococcus faecalis*, and Gram-negative bacteria: *S. typhi*, *S. sonei* and *P. aeruginosa* (**Table 88**, **89**, **90** and **91**). The roots and twigs of *P. obtusifolia* were also tested for anti-inflammatory activity against LPS-induced NO production in RAW264.7 cell line (**Table 92**). In addition, the roots and fresh fruits of *D. wallichii* were also tested for cytotoxic activity against MCF-7 human cancer cell line. (**Table 89**)

3.3.1 Activity of isolated compounds from *D. wallichii* on antibacterial and cytotoxic activities

The antibacterial activities of naphthalene derivatives **DW1-DW6**, **DW8**, **DW9**, **DW11** and **DW12** were reported in **Table 88**. Compound **DW1** showed significant antibacterial activity against *B. subtilis* and *E. faecalis*, but moderate activity against MRSA, VRE and *S. sonei*. Compounds **DW4** and **DW5** exhibited moderate antibacterial activity against *B. subtilis* and *S. sonei*, while compound **DW1** also exhibited moderate antibacterial activity against *E. faecalis* and MRSA. Antifungal activity against *C. albicans* of all compounds was inactive. In addition, the cytotoxity against MCF-7 cell line of compounds **DW1-DW6**, **DW8**, **DW9**, **DW11** and **DW12** was also evaluated. All compounds except compound **DW6** were active. Compounds **DW8** and **DW9** exhibited better activity than the rest of the tested compounds with respective IC_{50} values of 0.06 and 0.09 μg/ml. Structure-activity relationships for cytotoxic activity against MCF-7 human cancer cell line should be indicated as a binapthoquinone skeleton was necessary for increasing the activity: mononapthoquinone skeleton of compound **DW1** was less active with the IC_{50} value

of 0.46 μM, while binapthoquinone skeleton of compound **DW8** and **DW9** were most active with respective IC_{50} values of 0.06 and 0.09 μM.

Table 88 Antibacterial activities against gram-positive bacteria of compounds **DW1-DW6, DW8, DW9, DW11** and **DW12** from *D. wallichii*

Compounds	Antibacterial activities (MIC, μg/mL)				
	B. subtilis	*S. aureus*	*E. faecalis*	*MRSA*	*VRE*
DW1	4.68	37.5	4.68	9.37	9.37
DW2	150	>300	300	>300	150
DW3	75	150	75	18.75	75
DW4	9.37	37.5	9.37	9.37	37.5
DW5	9.37	75	18.75	18.75	37.5
DW6	300	>300	300	>300	>300
DW8	150	>300	150	>300	>300
DW9	150	>300	300	150	300
DW11	75	300	150	150	150
DW12	>300	>300	>300	>300	>300
Vancomycin*	<2.34	<2.34	<2.34	<2.34	<2.34

* Standard drug.

Table 89 Antibacterial activities against gram-negative bacteria and cytotoxic against MCF-7 cell line of compounds **DW1-DW6, DW8, DW9, DW11** and **DW12** from *D. wallichii*

Compounds	Antibacterial activities (MIC, μg/mL)			Cytotoxic activity
	S. typhi	*S. sonei*	*P. aeruginosa*	MCF-7[a] (IC_{50}, μg/mL)
DW1	37.5	9.37	150	0.46
DW2	>300	150	>300	0.29
DW3	300	75	300	0.35
DW4	37.5	9.37	>300	0.80
DW5	75	9.37	300	0.86
DW6	>300	300	>300	9.57
DW8	>300	>300	>300	0.06
DW9	>300	150	>300	0.09
DW11	300	150	>300	0.78
DW12				0.51
Camptothecin*				<0.024
Vancomycin*	<2.34	<2.34	<2.34	<2.34

* Standard drug.

[a] Human breast adenocarcinoma cell line.

3.3.2 Activity of isolated compounds from *P. obtusifolia* on antibacterial and anti-inflammatory activities

The antibacterial activities of the compounds **PO4**, **PO11**, **PO13**, **PO15-PO23-PO25** and **PO27** are reported in **Table 90** and **91**. Compound **PO13** showed significant antibacterial activity against *B. subtilis*, *S. aureus*, *E. faecalis*, MRSA, VRE, *S. typhi* and *S. sonei* but weak activity against *P. aeruginosa*. Compound **PO21** showed significant antibacterial activity against *S. sonei* but moderate activity against *B. subtilis*, *E. faecalis*, MRSA and VRE while compound **PO20** also exhibited moderate antibacterial activity against MRSA. All compounds were found to be inactive against *C. albicans*. In addition the crude hexane and CH_2Cl_2 extracts exhibited potent inhibitory activity against LPS-induced NO production in RAW264.7 cell lines with IC_{50} values of 4.3 and 6.1 μg/ml, respectively. Therefore some isolated compounds were evaluated for their anti-NO activity, the results of which are shown in **Table 92**. Compound **PO24** (IC_{50} = 1.7 μM) possessed the highest activity followed by compounds **PO13**, **PO14** and **PO25** (IC_{50} = 6.1, 7.8 and 6.2 μM, respectively) whereas other compounds exhibited moderate and mild activites. The inhibitory activities of most isolates were much stronger than that of NO synthase inhibitor (Tewtrakul et al., 2009) L-Nitroarginine (L-NA), IC_{50} = 61.8 μM except for compounds **PO19** and **PO27** which showed weaker activity (IC_{50} = >100 μM). Compound **PO24** also showed higher inhibitory activity than caffeic acid phenethylester (CAPE) (IC_{50} = 5.2 μM). Structure-activity relationships for anti-inflammatory activity should be suggested as a hydroxyl group on an *ortho*-napthoquinone skeleton was necessary for increasing the activity: compound **PO24** with a hydroxyl group was strongly active (IC_{50} = 1.7 μM), whereas compound **PO25** was less active (IC_{50} = 6.2 μM).

Table 90 Antibacterial activities against gram-positive bacteria of compounds **PO4, PO11, PO13, PO15-PO21, PO23-PO25** and **PO27** from *P.obtusifolia*

Compounds	Antibacterial activities (MIC, μg/mL)				
	B. subtilis	*S. aureus*	*E. faecalis*	*MRSA*	*VRE*
PO4	300	300	300	300	300
PO11	300	>300	300	>300	>300
PO13	4.68	2.34	4.68	2.34	2.34
PO15	>300	>300	>300	>300	>300
PO16	300	300	300	300	300
PO17	300	150	300	150	150
PO18	37.5	37.5	37.5	37.5	37.5
PO19	>300	>300	>300	>300	>300
PO20	37.5	75	75	9.37	75
PO21	9.37	18.75	9.37	9.37	9.37
PO23	75	75	75	75	75
PO24	150	75	75	37.5	37.5
PO25	75	75	75	37.5	75
PO27	150	>300	150	300	300
Vancomycin*	<2.34	<2.34	<2.34	<2.34	<2.34

* Standard drug.

Table 91 Antibacterial activities against gram-negative bacteria of compounds **PO4**, **PO11**, **PO13**, **PO15-PO21**, **PO23-PO25** and **PO27** from *P.obtusifolia*

Compounds	Antibacterial activities (MIC, μg/mL)		
	S. typhi	*S. sonei*	*P. aeruginosa*
PO4	300	300	300
PO11	>300	300	>300
PO13	2.46	4.68	150
PO15	>300	>300	>300
PO16	300	300	300
PO17	150	150	>300
PO18	150	37.5	150
PO19	>300	>300	>300
PO20	75	18.75	>300
PO21	37.5	2.34	>300
PO23	75	37.5	150
PO24	75	75	>300
PO25	75	75	>300
PO27	>300	75	>300
Vancomycin*	<2.34	<2.34	<2.34

* Standard drug.

Table 92 Inhibitory effects on NO production[c] of compounds **PO3-PO7, PO11-PO15, PO17-PO25, PO27** and **PO28** from *P.obtusifolia*

NO.	% Inhibition at various concentrations (μM)					IC_{50} (μM)
	0	3	10	30	100	
PO3	0.0±8.8		13.8±0.5	16.5±1.2	51.6±1.1[b]	98.1
PO4	0.0±5.4		0.9±1.2	34.5±0.6[b]	95.3±2.5[b,d]	36.8
PO5	0.0±5.4		11.7±2.2	66.5±2.6[b]	102.3±0.5[b,d]	23.9
PO7	0.0±5.4		7.9±1.5	13.5±1.5[a]	80.0±1.2[b,d]	65.9
PO11	0.0±5.4		−27.0±1.2	10.1±1.9	98.4±0.3[b,d]	47.1
PO12	0.0±5.4		−7.0±1.7	7.0±1.2	77.9±1.4[b,d]	59.2
PO13	0.0±5.4	9.1±2.8	96.4±0.6[b,d]	98.9±1.6[b,d]	99.6±1.0[b,d]	6.1
PO14	0.0±6.1	5.7±1.1	70.3±1.4[b]	99.8±1.5[b,d]	99.8±1.5[b,d]	7.8
PO15	0.0±5.4		−5.4±0.9	59.7±1.1[b]	94.1±3.6[b,d]	31.3
PO17	0.0±6.1		2.7±1.5	25.9±1.4[b]	96.9±0.5[b,d]	37.8
PO18	0.0±5.4		−7.6±0.7	−0.7±1.4[b]	79.9±1.7[b]	73.6
PO19	0.0±6.1		3.8±1.0	4.4±1.0	35.5±0.6[b]	>100
PO20	0.0±6.1		6.9±0.9	33.2±1.0[b]	96.0±1.1[b,d]	35.0
PO21	0.0±6.1		6.9±0.9	52.8±0.9[b]	96.9±1.1[b,d]	29.3
PO22	0.0±6.1		6.9±1.3	59.4±1.1[b]	97.6±1.0[b,d]	27.6
PO23	0.0±8.8		13.4±1.0	48.8±2.0[b]	94.7±1.3[b,d]	29.1
PO24[e]	0.0±6.1	79.2±1.9[b,d]	99.3±1.5[b,d]	99.8±1.4[b,d]	100.5±1.4[b,d]	1.7
PO25	0.0±6.1	7.6±1.5	94.6±1.1[b,d]	97.1±0.7[b,d]	99.1±0.7[b,d]	6.2
PO27	0.0±8.8		−20.9±1.9	9.1±0.8	51.0±1.2[b]	>100
PO28	0.0±8.8		11.4±0.5	17.9±0.9	54.5±0.9[b]	73.6
L-NA	0.0±9.9	11.7±4.6	20.2±0.2	34.7±0.4[a]	71.6±1.2[b]	61.8
CAPE	0.0±9.9	30.7±3.2[a]	68.6±3.4[b]	98.7±1.2[b,d]	98.9±2.1[b,d]	5.6

Statistical significance, [a]$p < 0.05$, [b]$p < 0.001$.

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

[d] Cytotoxic effect was observed.

[e] Only **PO24** exhibited on NO production with 25.8±1.3 μM (% Inhibition at various concentrations of 1 μM. L-NA= L-Nitroarginine, CAPE= Caffeic acid phenethylester.

CHAPTER 4
CONCLUSION

A new binaphthoquinone, named 5,5'-dihydroxy-2,2'-dimethyl-7,7'-binaphthalen-1,1',4,4'-tetraone (**DW9**), and two new naphthalene derivatives, named 2-hydroxymethyl-1,5-dimethoxynaphthalen-4-ol (**DW11**) and 2,2'-bis-hydroxymethyl-1,1',5,5'-tetramethoxy-3,3'-binaphthalen-4,4'-diol (**DW12**), were isolated from the roots and fruits of *D. wallichii*, together with eighteen known compounds including nine naphthoquinones: plumbagin (**DW1**), droserone (**DW2**), 2,3-epoxyplumbagin (**DW3**), 2-hydroxymethyl-5-methoxy-1,4-naphthoquinone (**DW4**), diomuscinone (**DW5**), isoshinanolone (**DW6**) *epi*-isoshinanolone Maritinone (**DW8**) and methylene-3,3'-biplumbagin (**DW10**), one coumarin: scopoletin (**DW13**), six triterpenoids: lupeol (**DW14**), lupenone (**DW15**), betulin (**DW16**), betulinaldehyde (**DW17**), betulinic acid (**DW18**) and 3β,29-dihydroxyolean-12-en-28-oic acid (**DW19**), and a mixture of steroids: β-sitosterol (**DW20**) and stigmasterol (**DW21**). The structures of the new compounds were elucidated by spectroscopic analysis, mainly 1D and 2D NMR techniques (^{1}H, ^{13}C, COSY, NOESY, HMQC and HMBC) and of the known compounds by comparison of their physical, UV, IR, ^{1}H and ^{13}C NMR data with those of published compounds. Antibacterial and cytotoxic activities of the isolates were also evaluated. It was found that **DW1** possessed the most potent antibacterial activities against *B. subtilis* and *E. faecalis*, but moderate activity against MRSA, VRE and *S. sonei*. Compounds **DW4** and **DW5** exhibited moderate antibacterial activity against *B. subtilis* and *S. sonei*, while compound **DW1** also exhibited moderate antibacterial activity against *E. faecalis* and MRSA. Compounds **DW8** and **DW9** exhibited better activity than the rest of the tested compounds with respective IC_{50} values of 0.06 and 0.09 μg/ml. Structure-activity relationships for cytotoxic activity against MCF-7 human cancer cell line should be indicated as a binapthoquinone skeleton was necessary for increasing the activity: mononapthoquinone skeleton of compound **DW1** was less active with the IC_{50} value

of 0.46 μM, while binapthoquinone skeleton of compound **DW8** and **DW9** were most active with respective IC_{50} values of 0.06 and 0.09 μM.

From the roots and twigs of *P. obtsifolia* were isolated eleven new compounds as two isopimarane diterpenoids, named isopimara-7,15-dien-1β,3β-diol (**PO1**) and isopimara-7,15-dien-1β,19-diol (**PO2**), one rosane diterpenoid named 13-*epi*-5,15-rosadien-3α,11β-diol (**PO3**), four abietane diterpenoids, named abietatrien-1β-ol (**PO4**), 1β-hydroxyferruginol (**PO5**), 6α,11,12-trihydroxy-7β,20-epoxy-8,11,13-abietatriene (**PO18**) and 5α,11,12-trihydroxy-6-oxa-abieta-8,11,13-trien-7-one (**PO19**), and four icetexane diterpenoids, named 11,12-dihydroxy-6,8,11,13-icetexatatraen-1-one (**PO23**) and obtusinone A-C (**PO24-PO26**) along with eighteen known compounds including sixteen abietane-type diterpenoids: ferruginol (**PO6**), *O*-methyl ferruginol (**PO7**), lambertic acid (**PO8**), sugiol (**PO9**), royleanone (**PO10**), horminone (**PO11**), montbretrol (**PO12**), 14-deoxycoleon (**PO13**), taxodione (**PO14**), arucadiol (**PO15**), 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (**PO16**), salvicanaraldehyde (**PO17**), three icetexane-type diterpenoids: 5,6-dihydro-6α-hydroxysalviasperanol (**PO20**), salviasperanol (**PO21**) and 11,12-dihydroxy-8,11,13-icetexatrien-1-one (**PO22**), and three sesquiterpenoids: 4β,5β-dihydroxy-10-*epi*-eudesmane (**PO27**), 4β,10β-dihydroxy aromadendrane (**PO28**) and spathulenol (**PO29**). In addition, the structures of **PO10**, **PO11**, **PO13**, **PO20**, **PO22** and **PO25** were additionally confirmed by X-ray diffraction analysis. Antibacterial and anti-inflammatory activities of the isolates were also evaluated. Compound **PO13** showed significant antibacterial activities against *B. subtilis*, *S. aureus*, *E. faecalis*, MRSA, VRE, *S. typhi* and *S. sonei* but weak activity against *P. aeruginosa*. Compound **PO21** showed significant antibacterial activities against *S. sonei* but moderate activity against *B. subtilis*, *E. faecalis*, MRSA and VRE while compound **PO20** also exhibited moderate antibacterial activity against MRSA. All compounds were found to be inactive against *C. albicans*. Compound **PO24** (IC_{50} = 1.7 μM) possessed the highest anti-inflammatory activity followed by compounds **PO13**, **PO14** and **PO25** (IC_{50} = 6.1, 7.8 and 6.2 μM, respectively) whereas other compounds exhibited moderate and mild activites. The inhibitory activities of most isolates were much stronger than that of NO synthase inhibitor (Tewtrakul et al., 2009) L-Nitroarginine (L-NA), IC_{50} = 61.8

μM except for compounds **PO19** and **PO27** which showed weaker activity (IC_{50} = >100 μM). Compound **PO24** also showed higher inhibitory activity than caffeic acid phenethylester (CAPE) (IC_{50} = 5.2 μM). Structure-activity relationships for anti-inflammatory activity should be suggested as a hydroxyl group on an *ortho*-napthoquinone skeleton was necessary for increasing the activity: compound **PO24** with a hydroxyl group was strongly active (IC_{50} = 1.7 μM), whereas compound **PO25** was less active (IC_{50} = 6.2 μM).

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APPENDIX

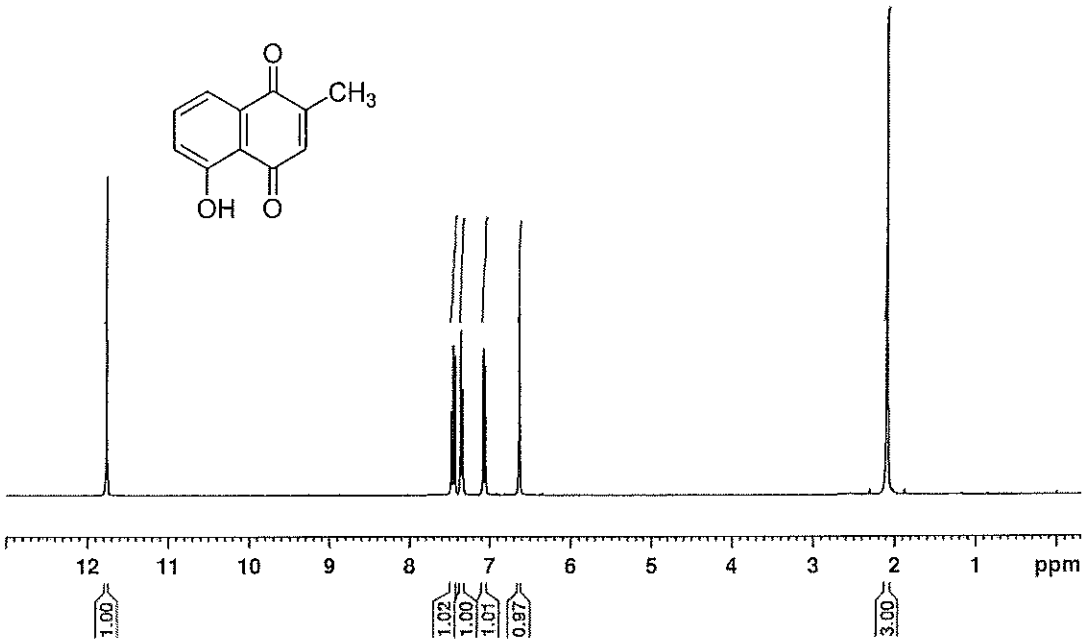

Figure 38 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW1**

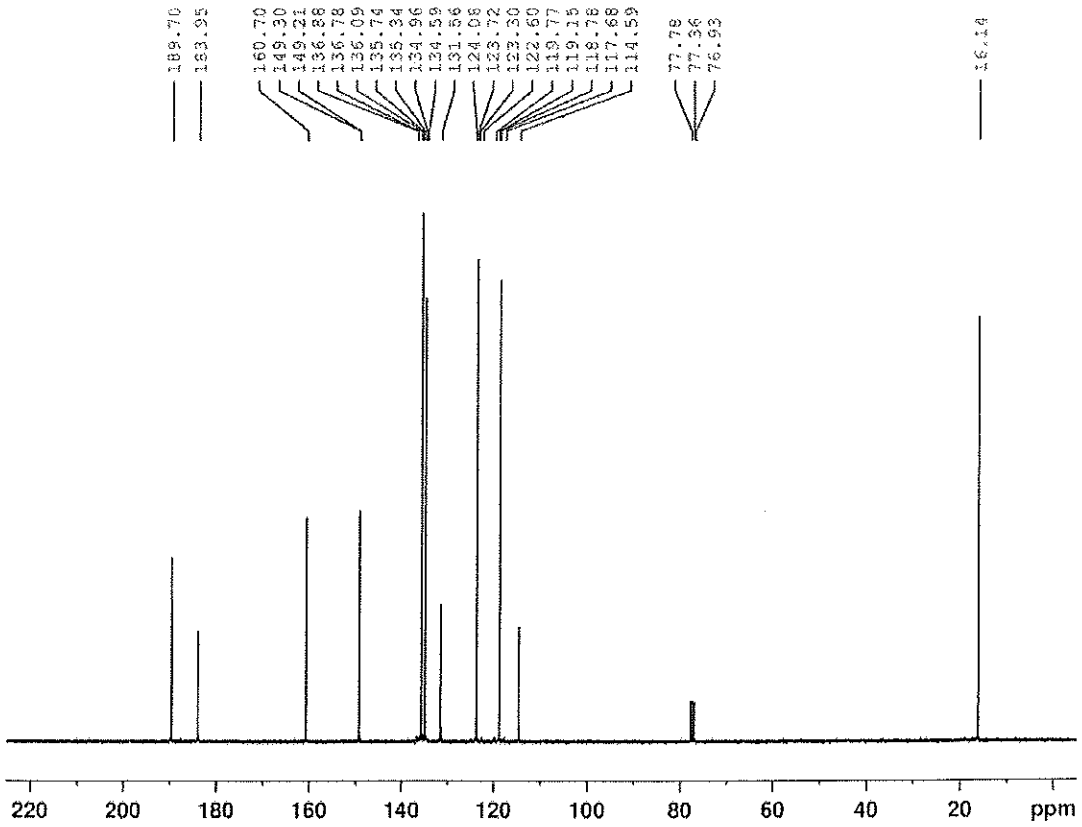

Figure 39 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW1**

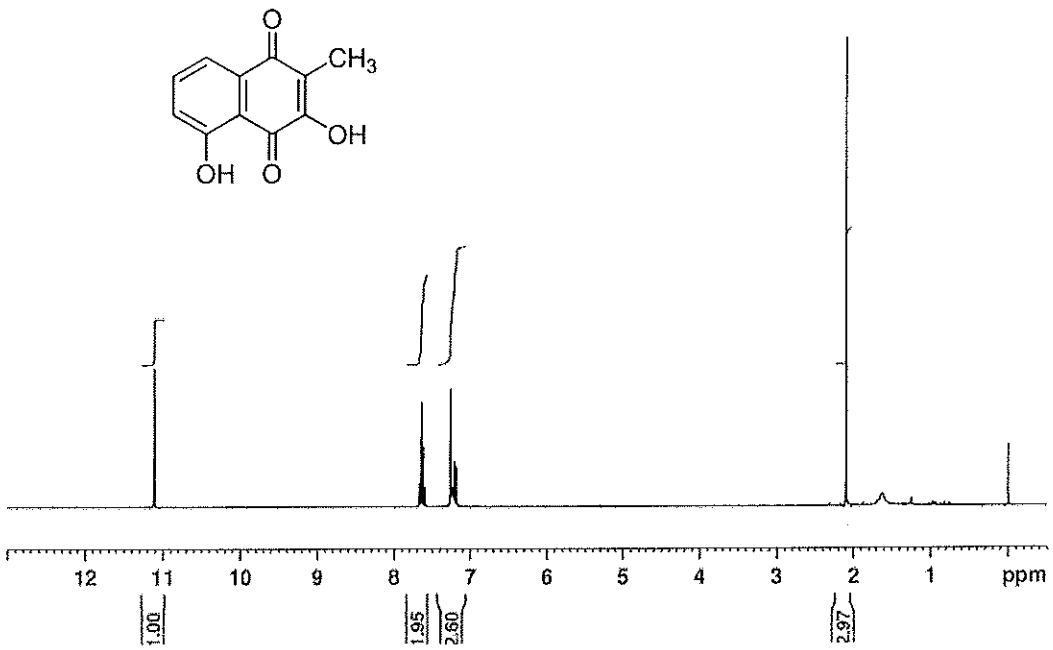

Figure 40 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW2**

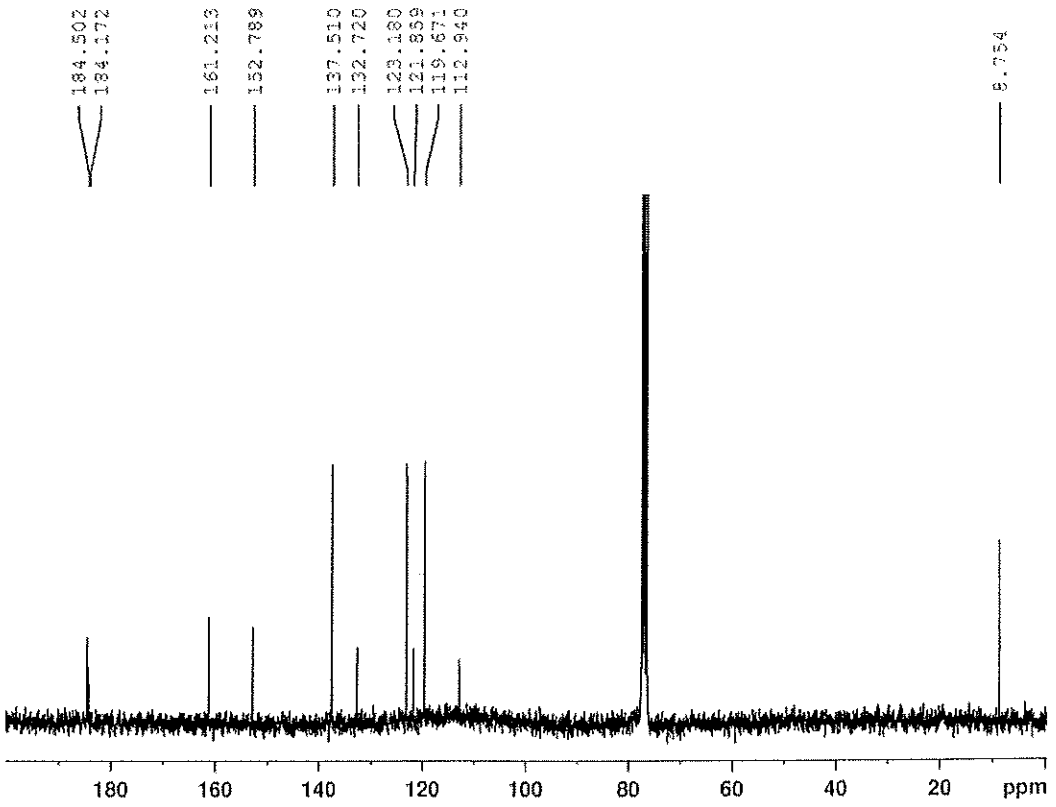

Figure 41 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW2**

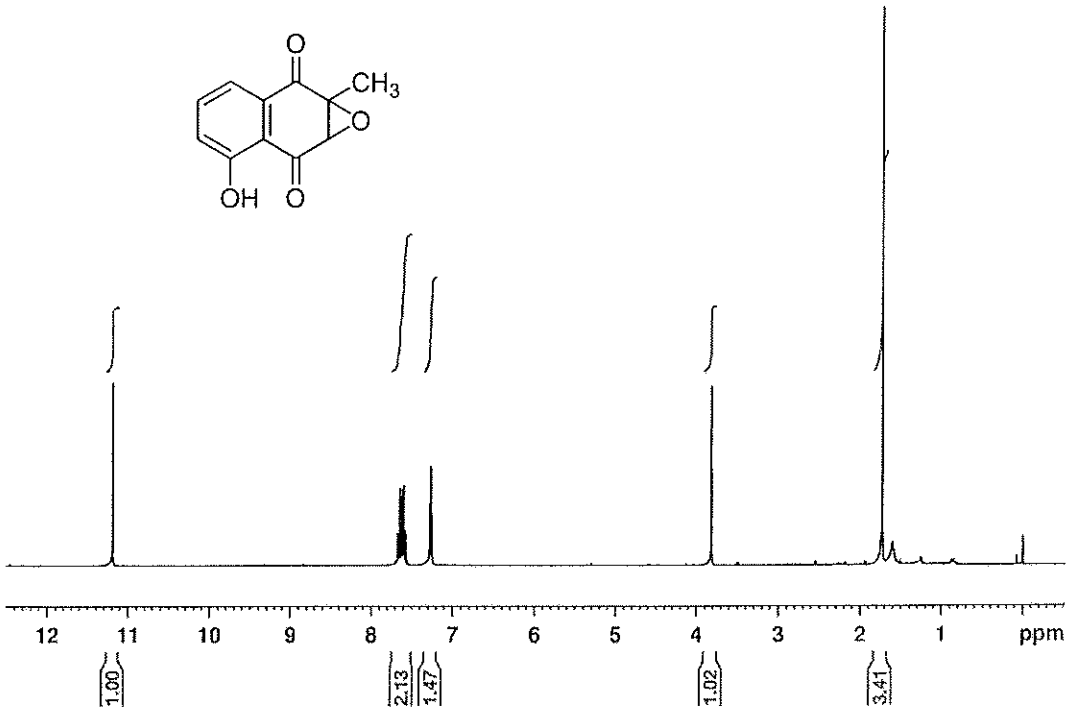

Figure 42 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW3**

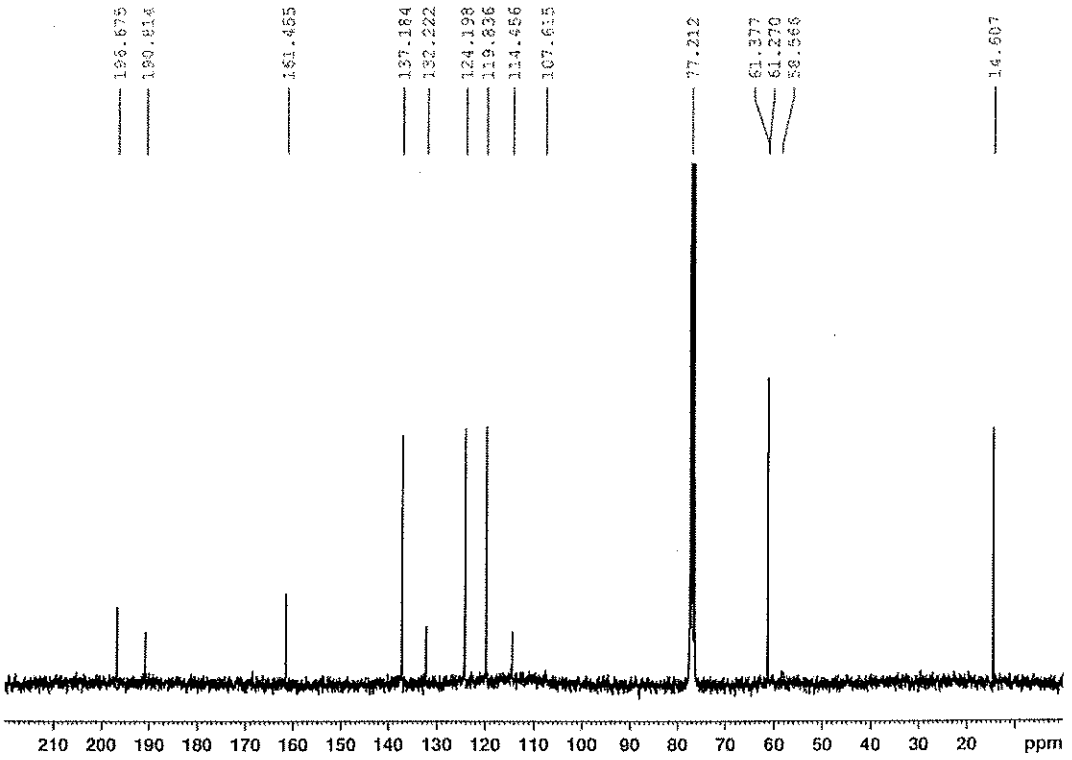

Figure 43 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW3**

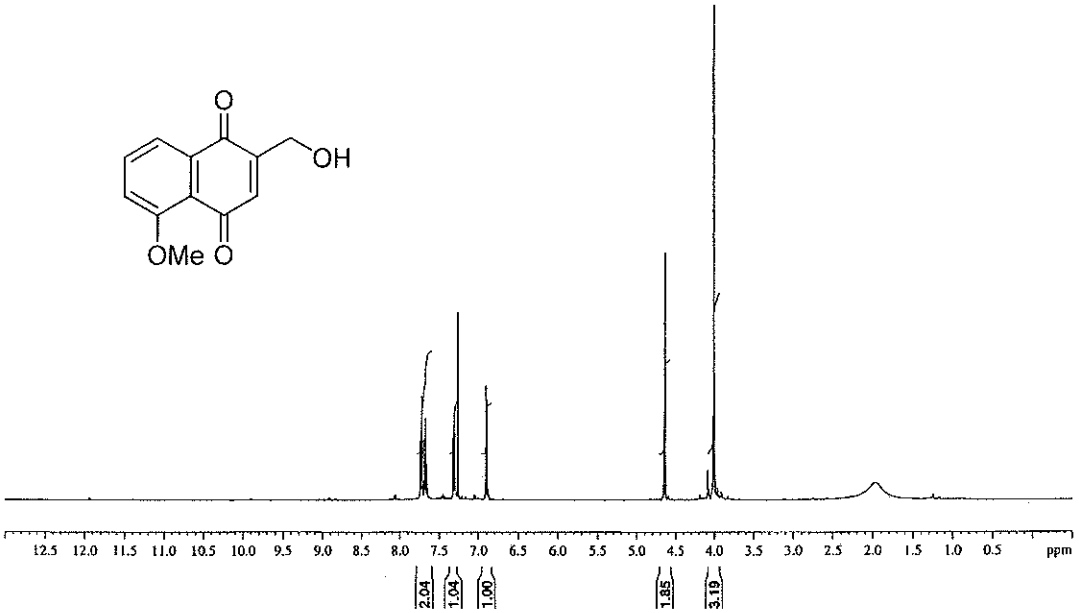

Figure 44 ^{1}H NMR (400 MHz) ($CDCl_3$) spectrum of compound **DW4**

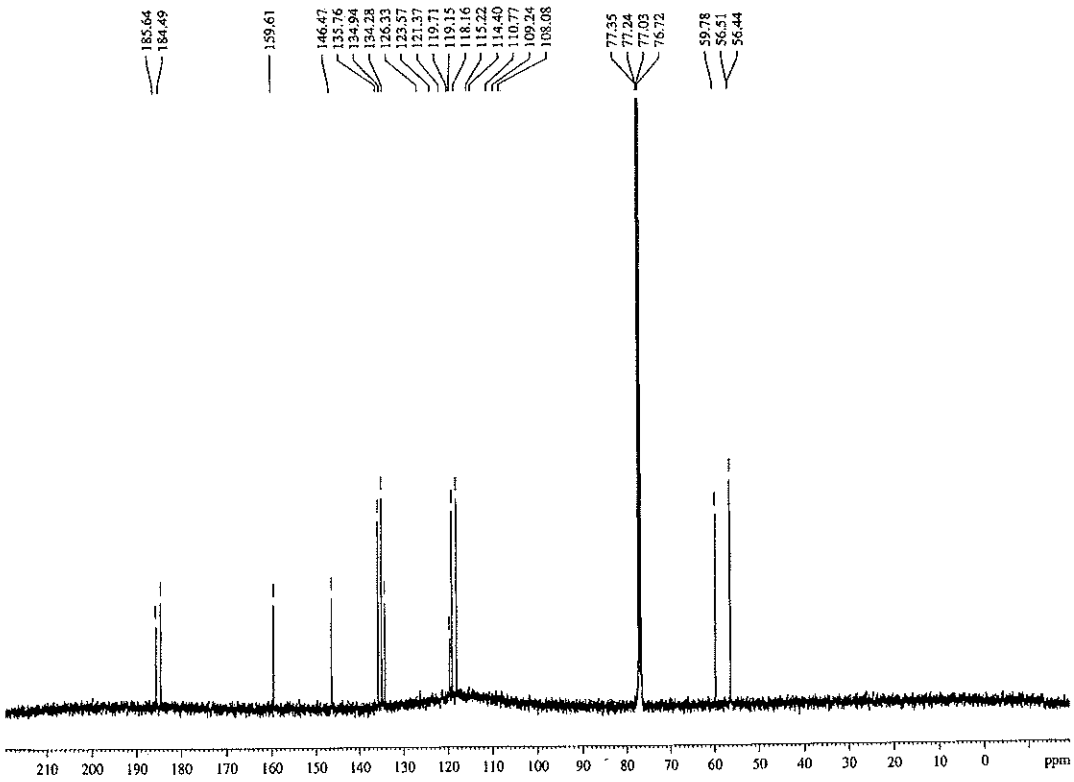

Figure 45 ^{13}C NMR (100 MHz) ($CDCl_3$) spectrum of compound **DW4**

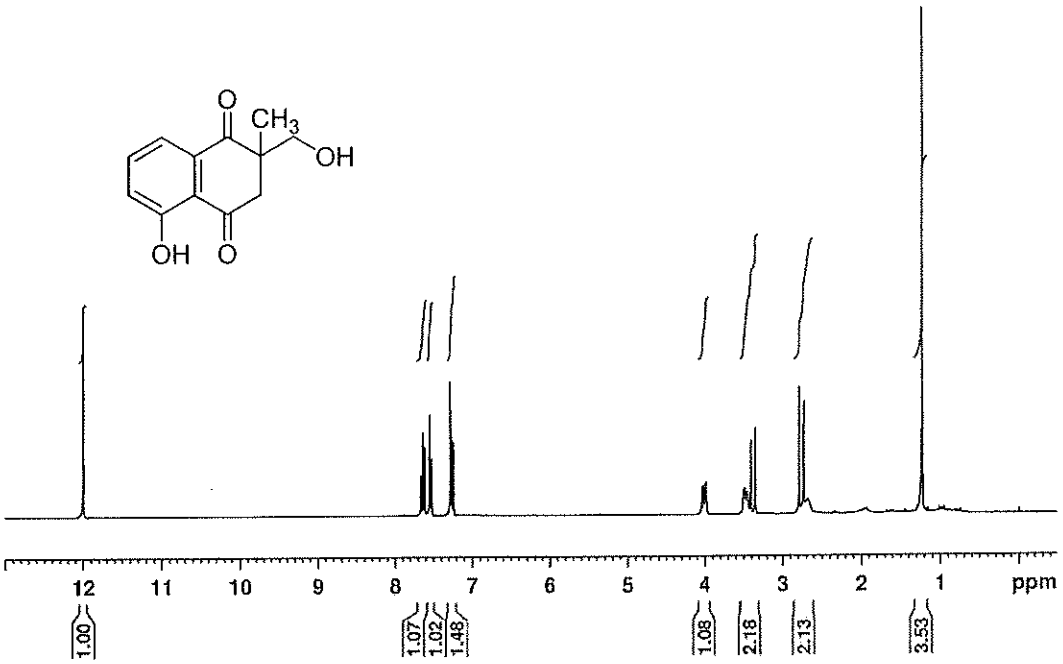

Figure 46 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW5**

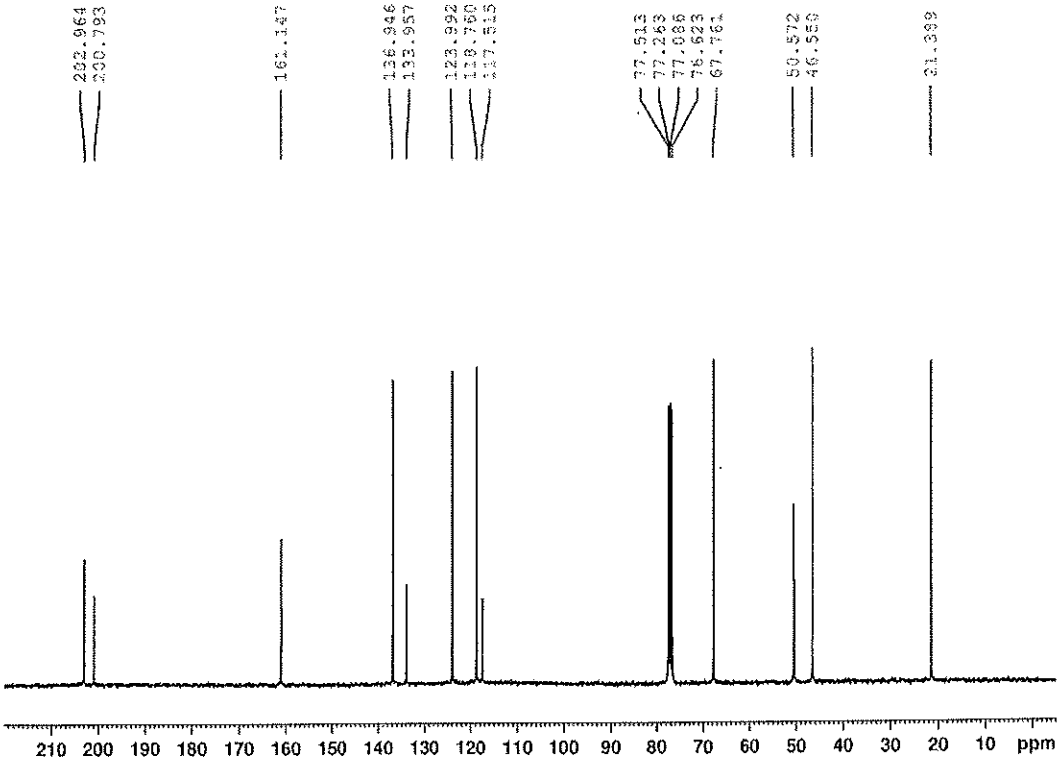

Figure 47 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW5**

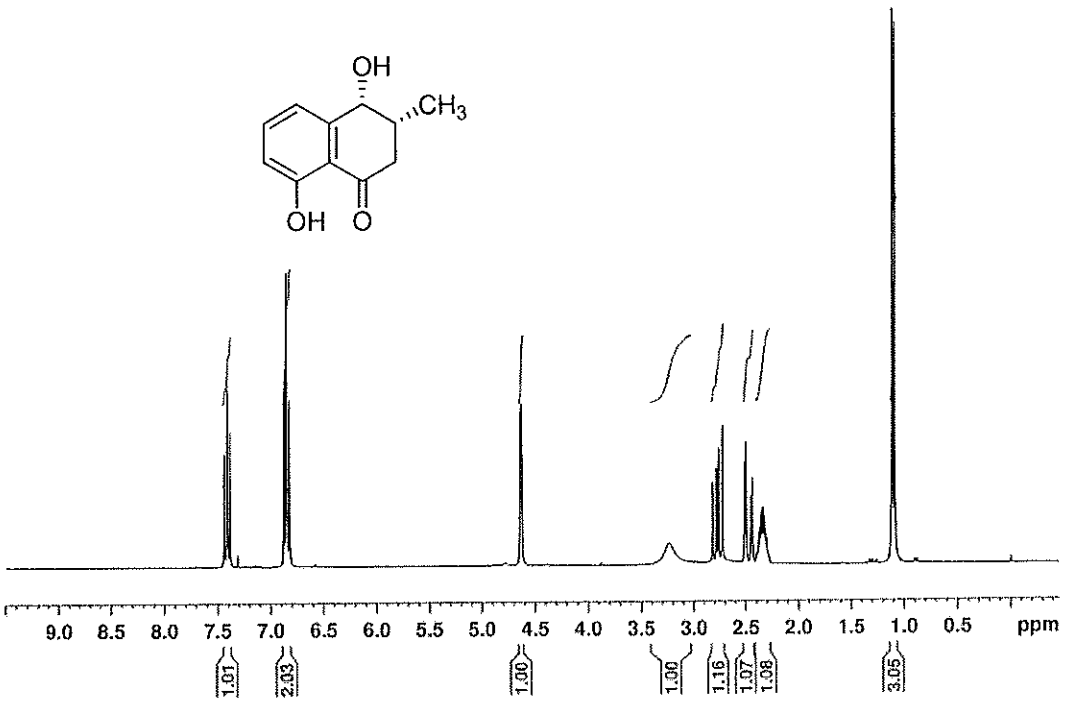

Figure 48 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW5**

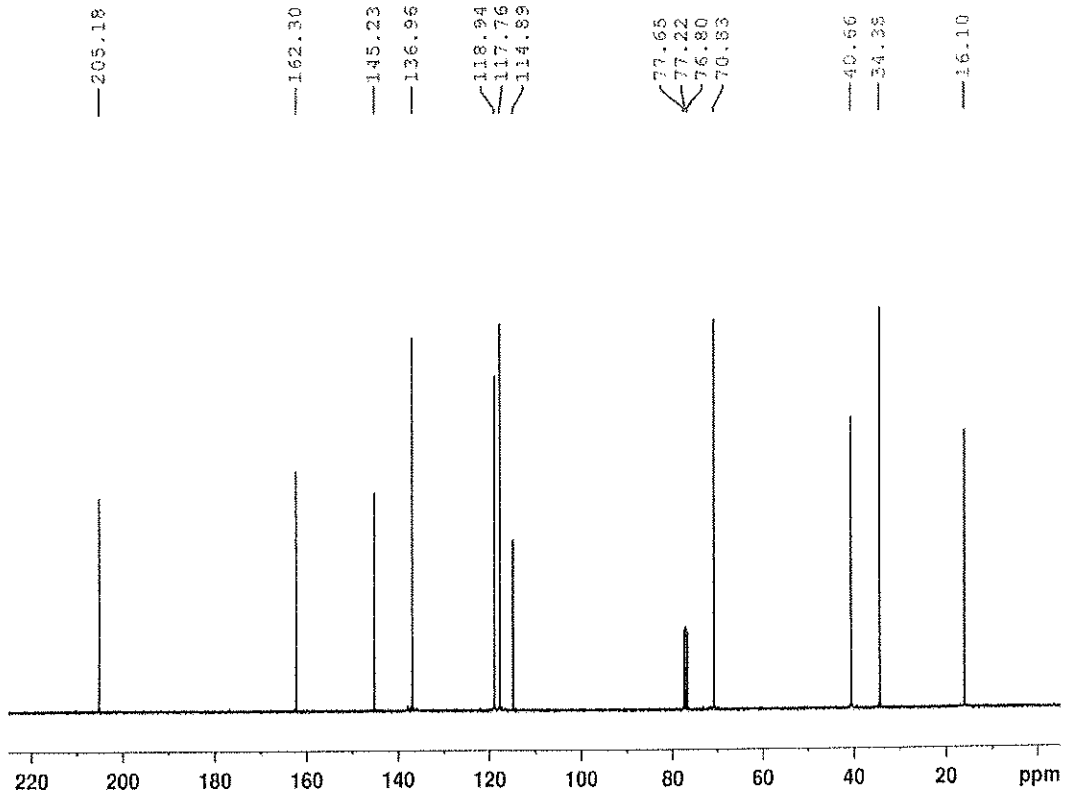

Figure 49 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW5**

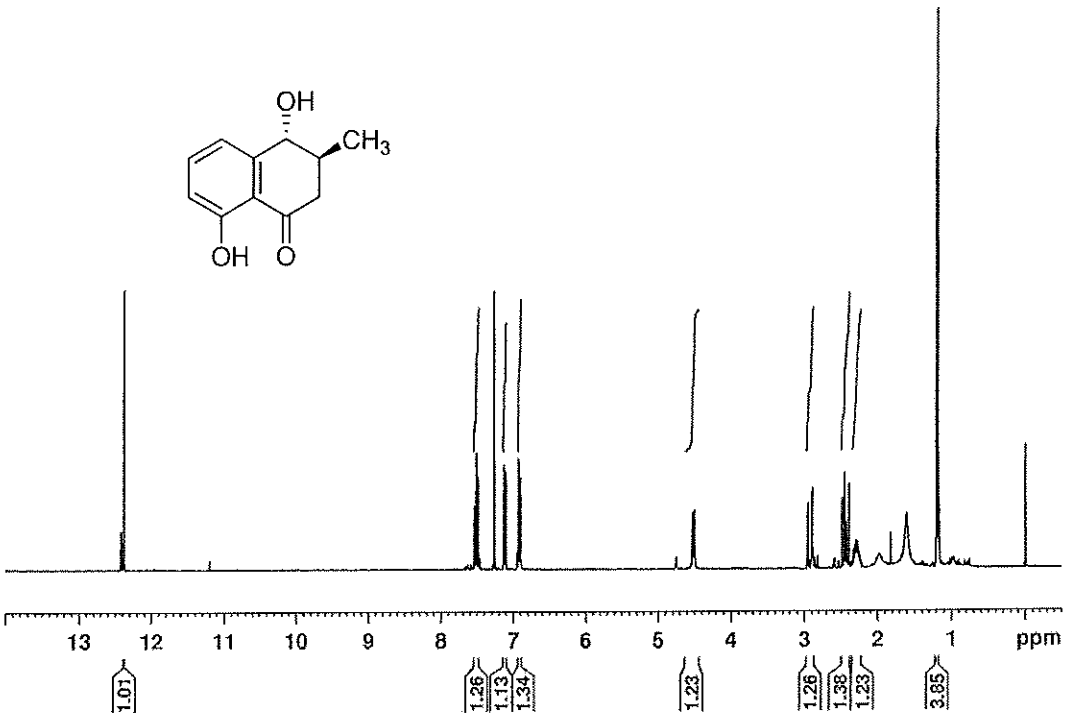

Figure 50 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW7**

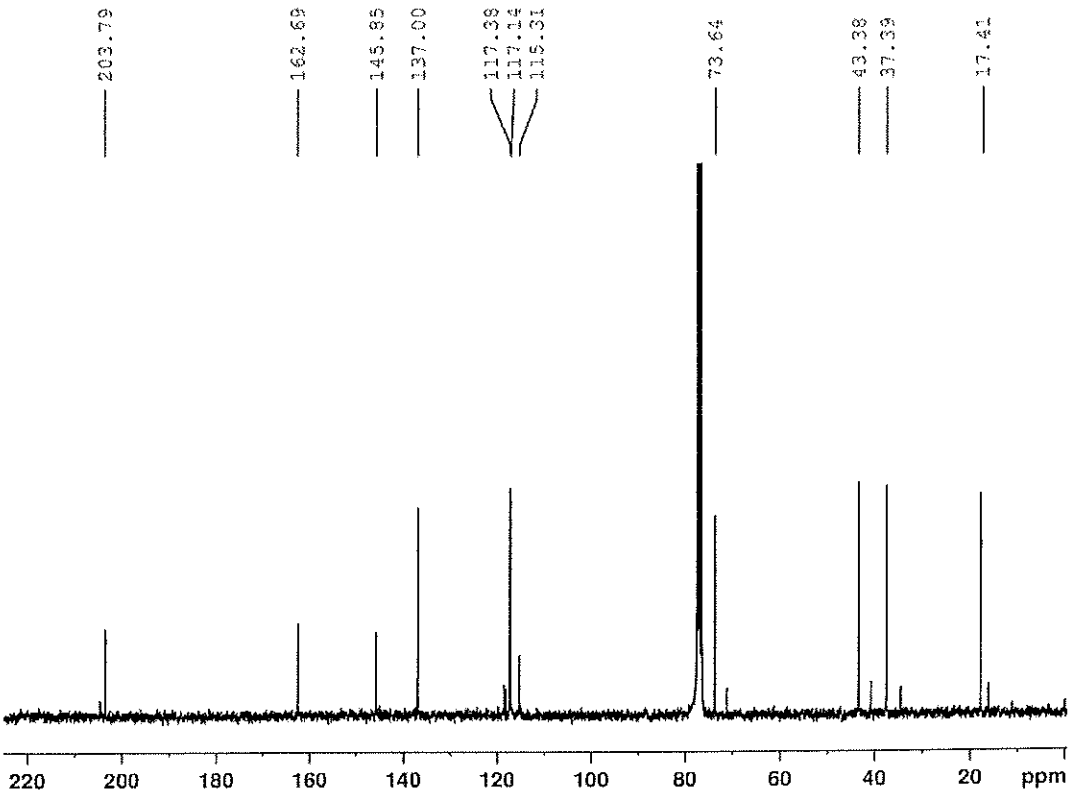

Figure 51 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW7**

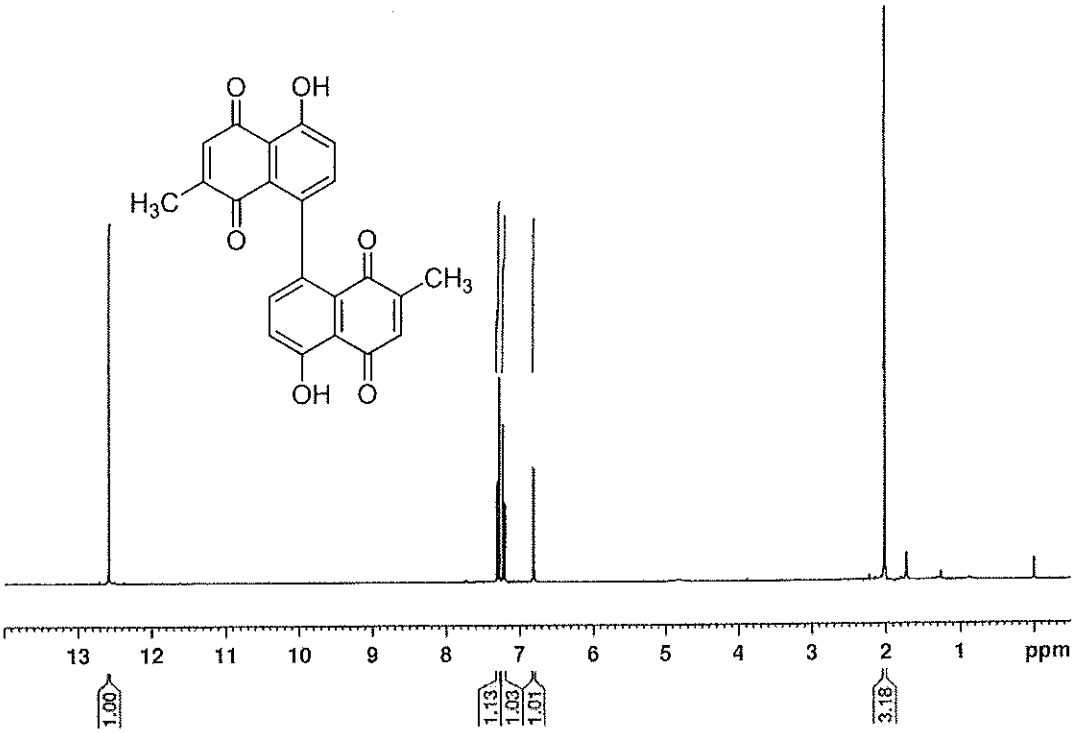

Figure 52 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW8**

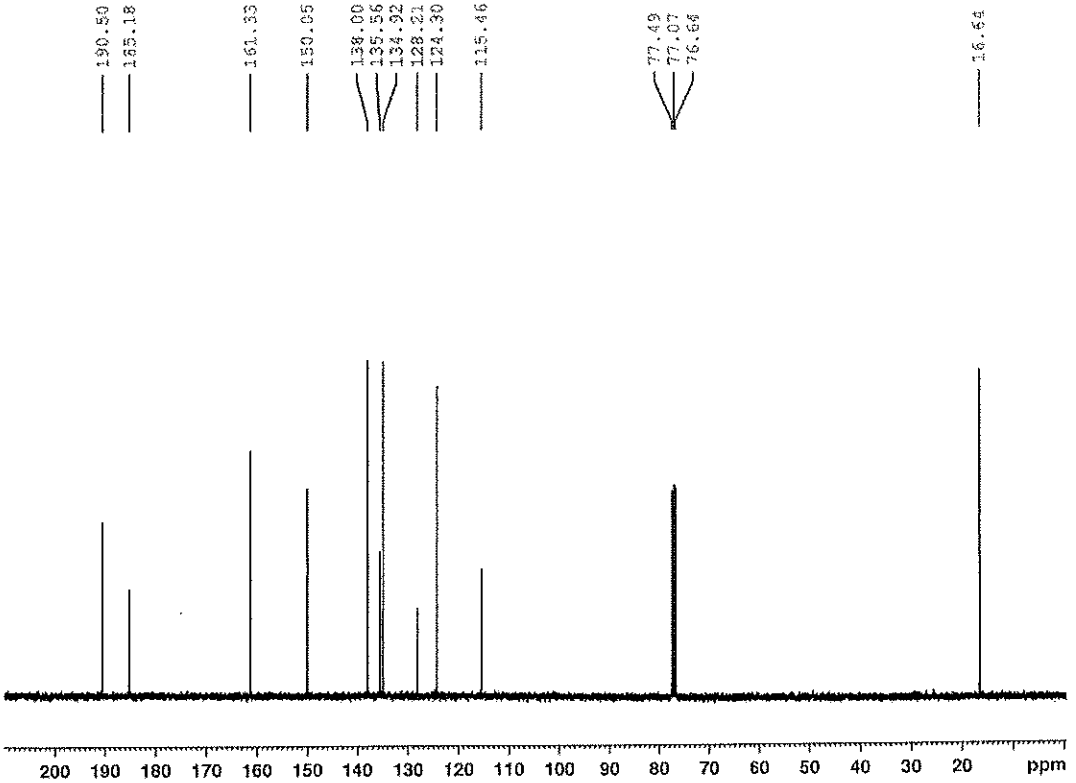

Figure 53 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW8**

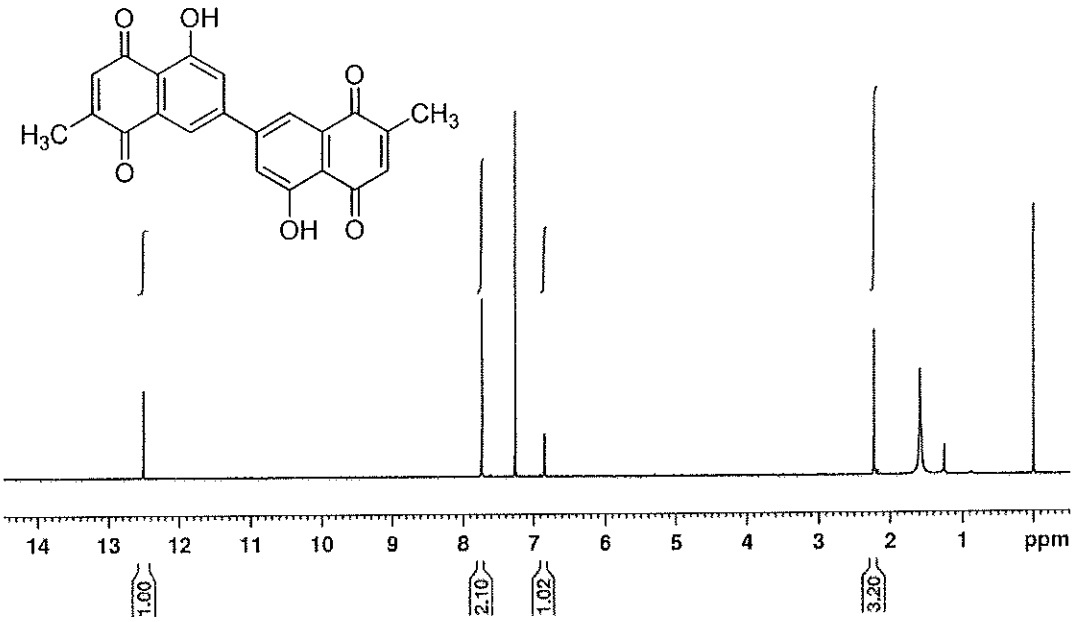

Figure 54 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW9**

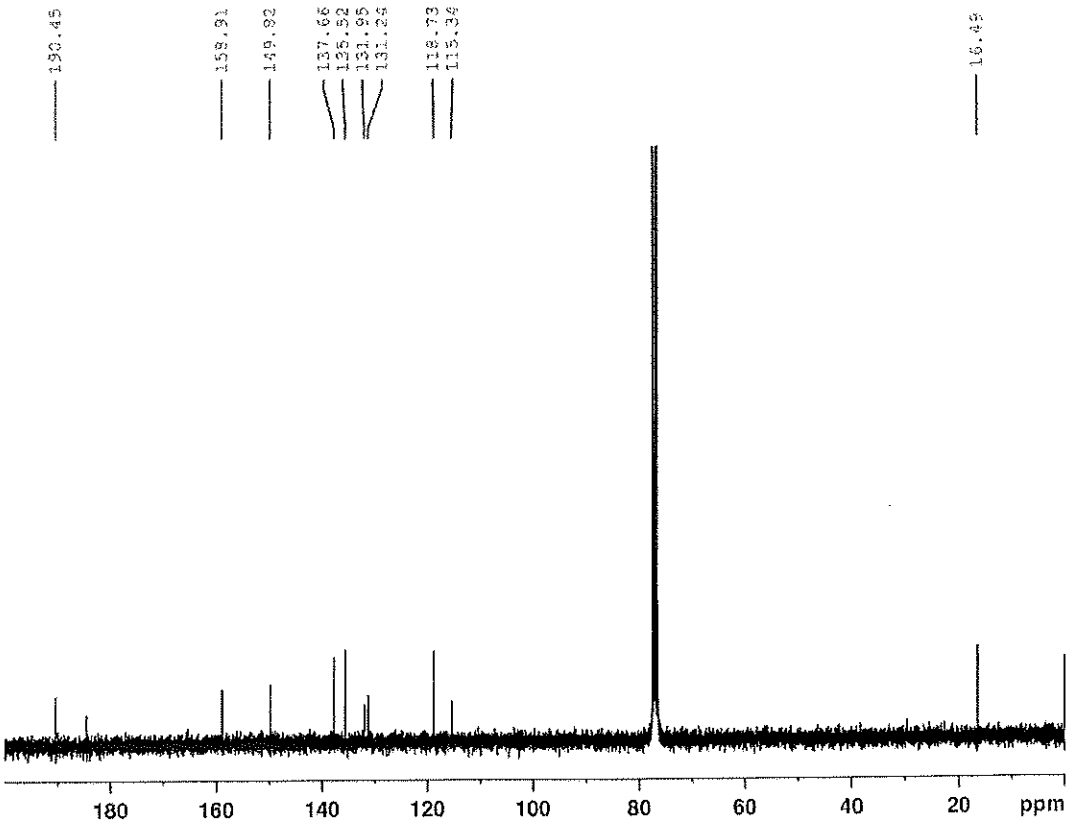

Figure 55 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW9**

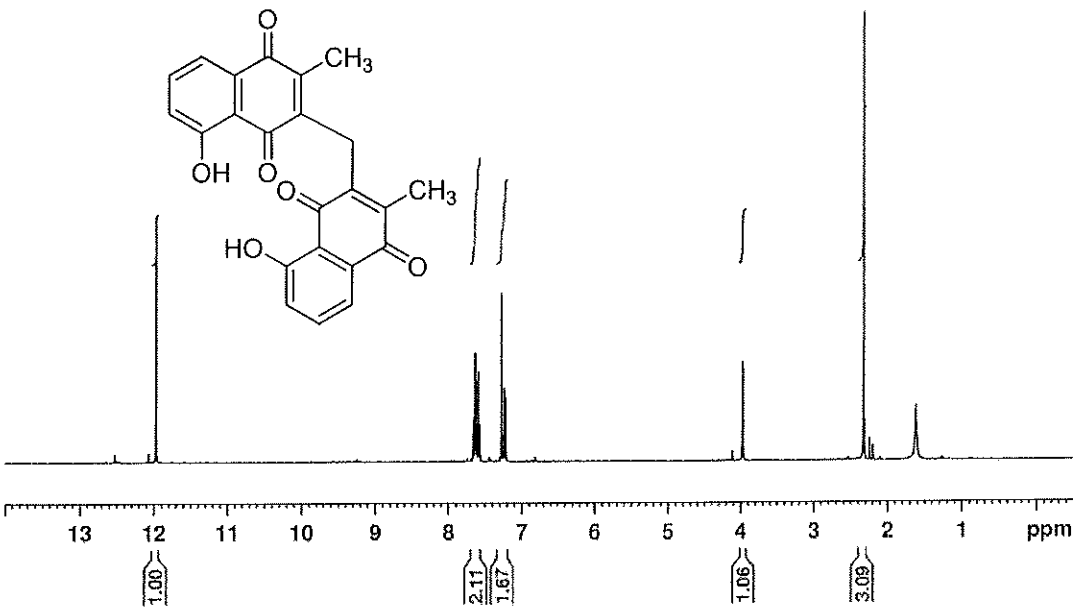

Figure 56 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW10**

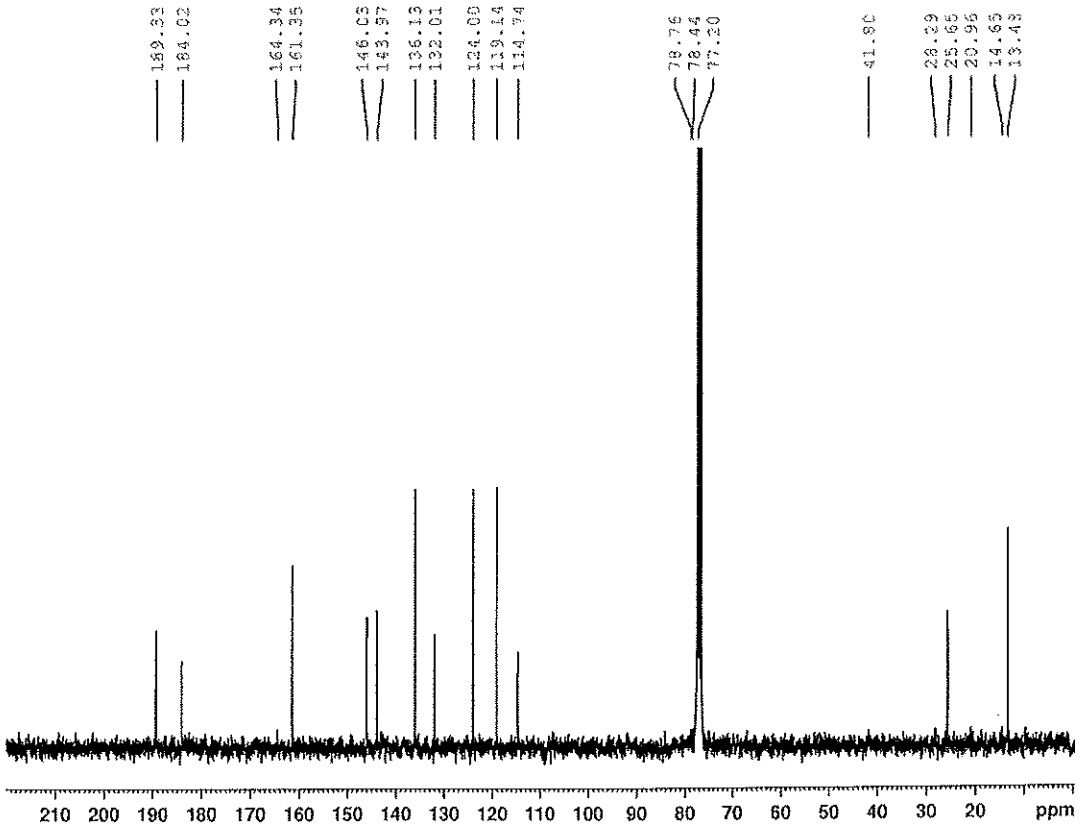

Figure 57 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW10**

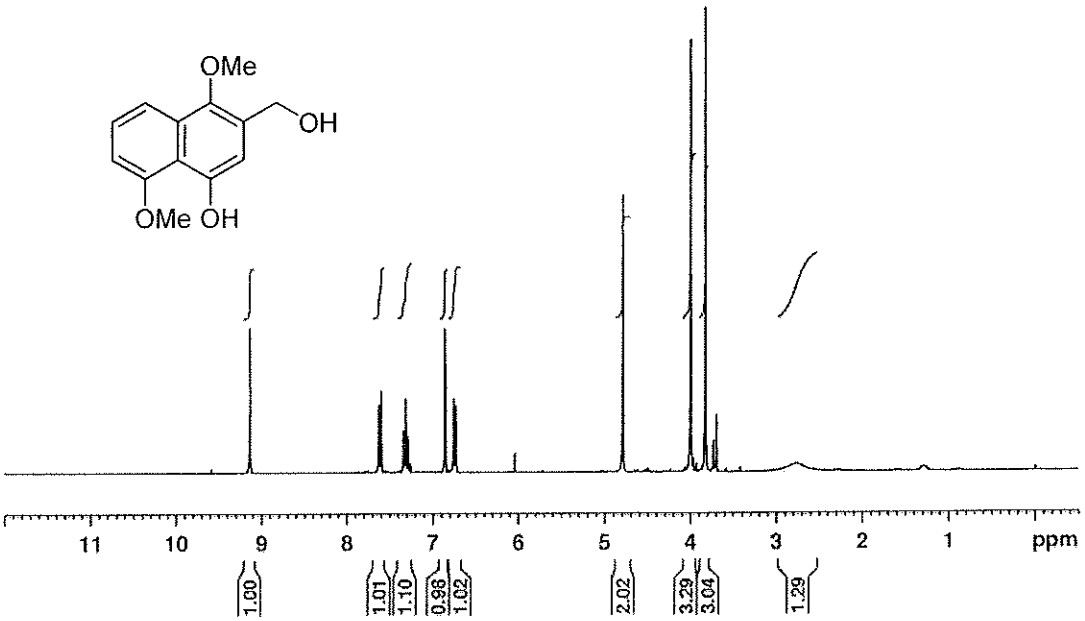

Figure 58 ^{1}H NMR (400 MHz) ($CDCl_3$) spectrum of compound **DW11**

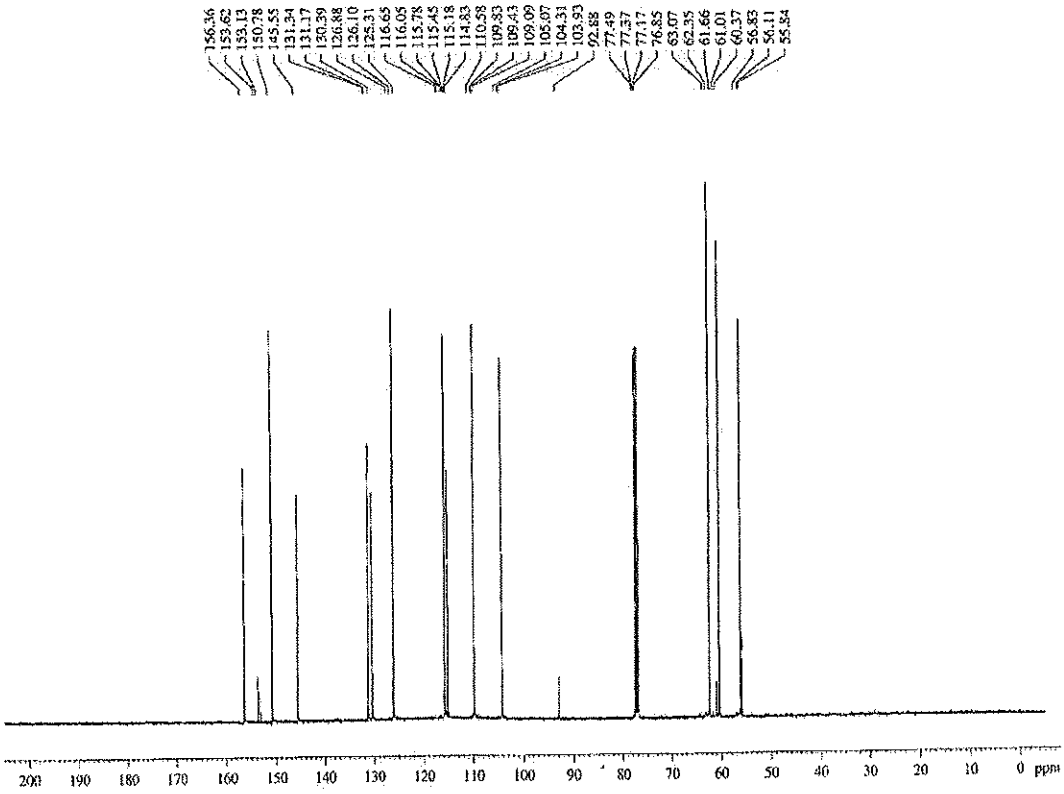

Figure 59 ^{13}C NMR (100 MHz) ($CDCl_3$) spectrum of compound **DW11**

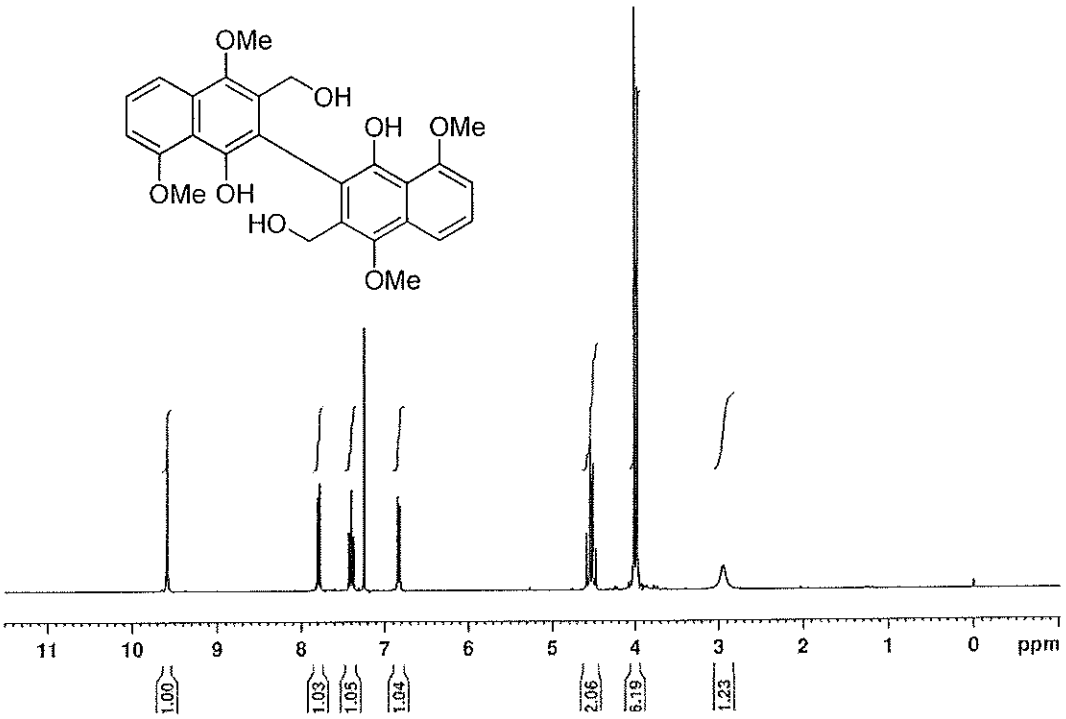

Figure 60 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW12**

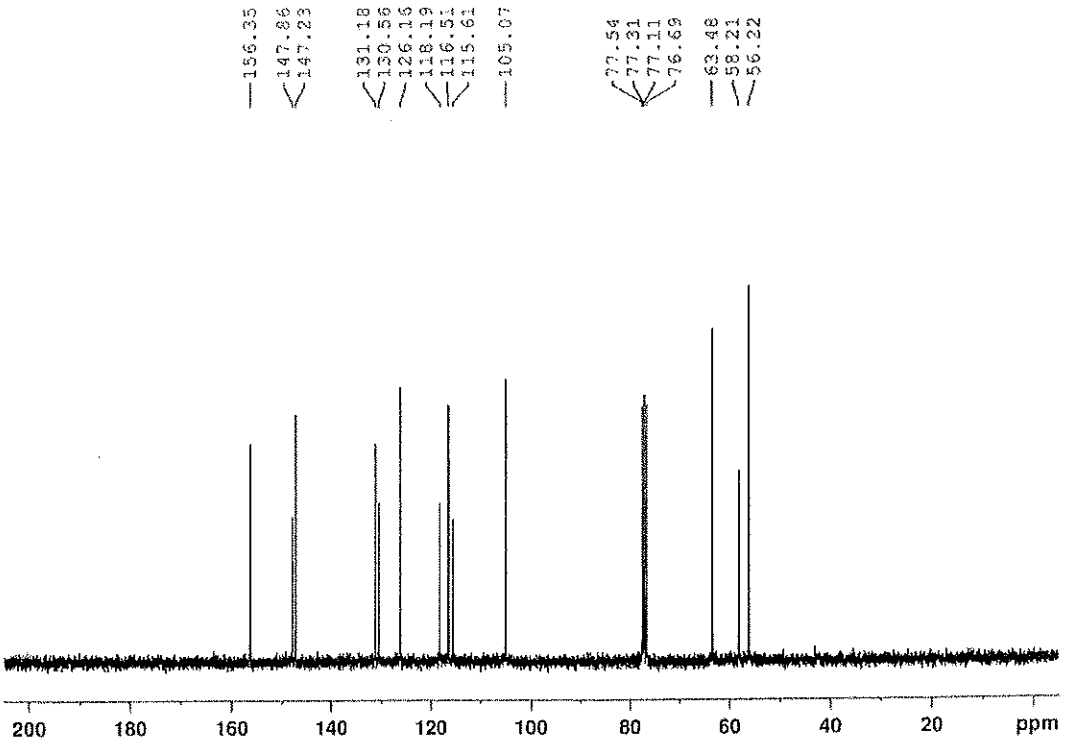

Figure 61 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW12**

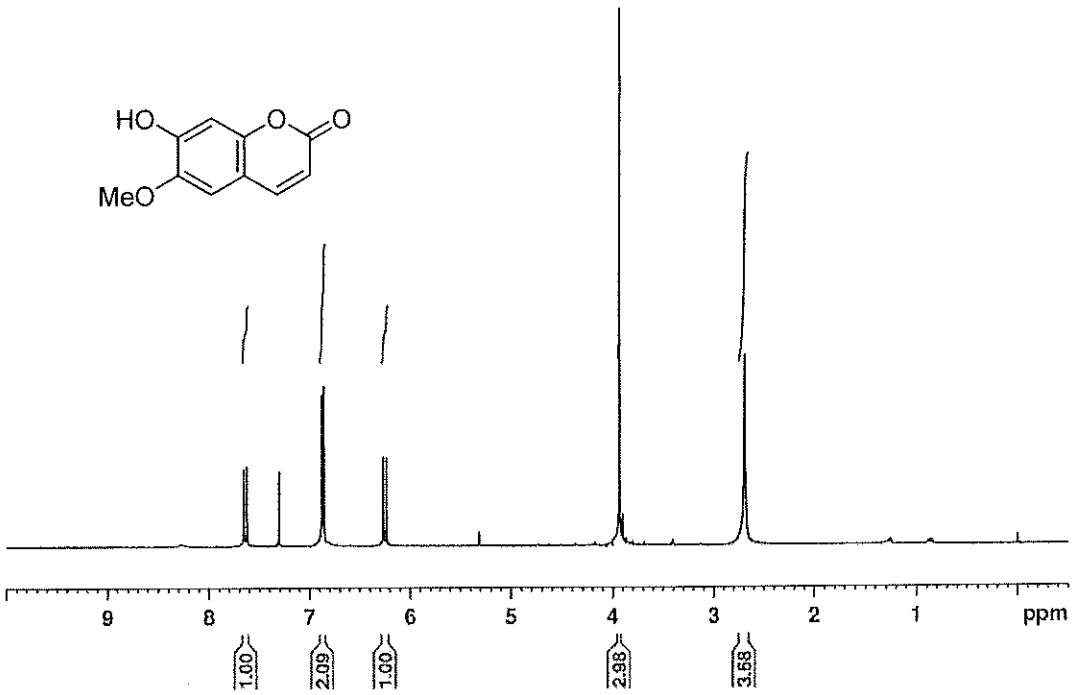

Figure 62 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW13**

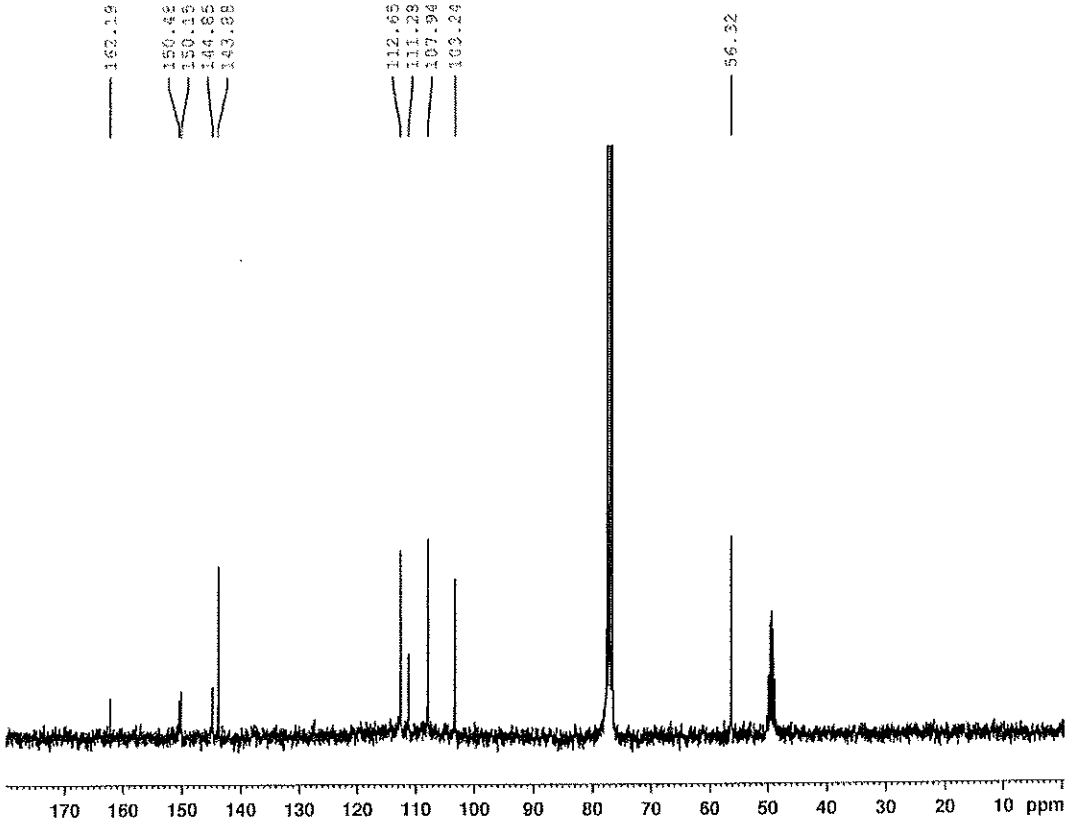

Figure 63 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW1**

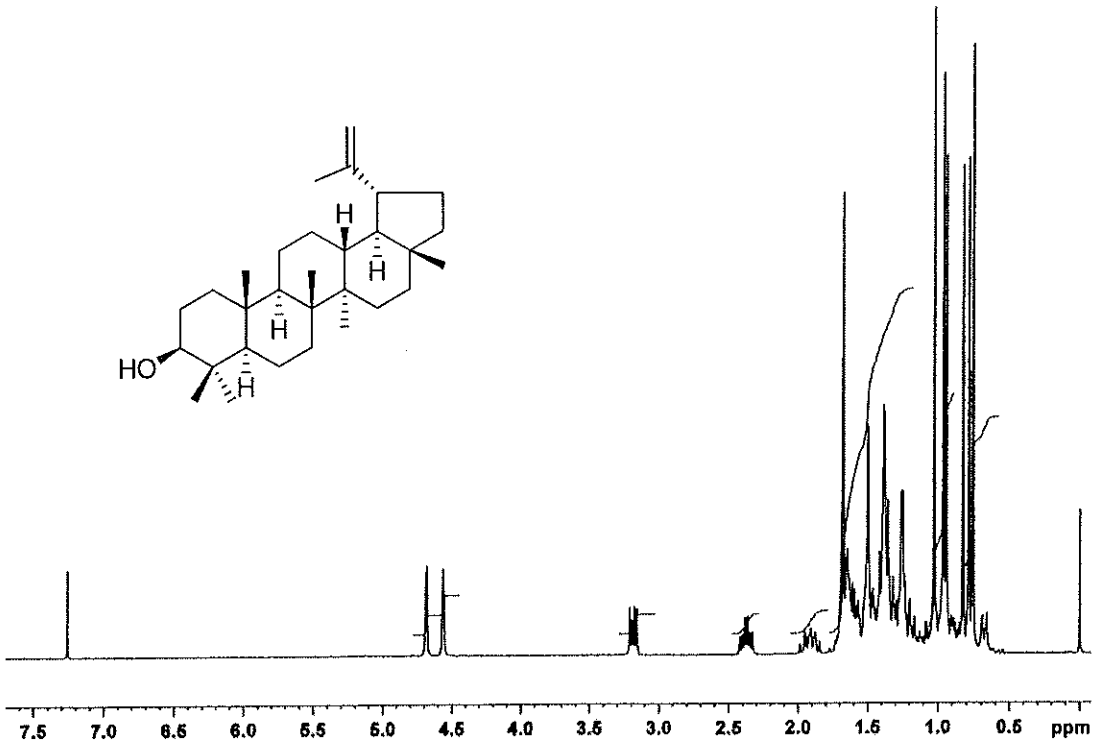

Figure 64 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW14**

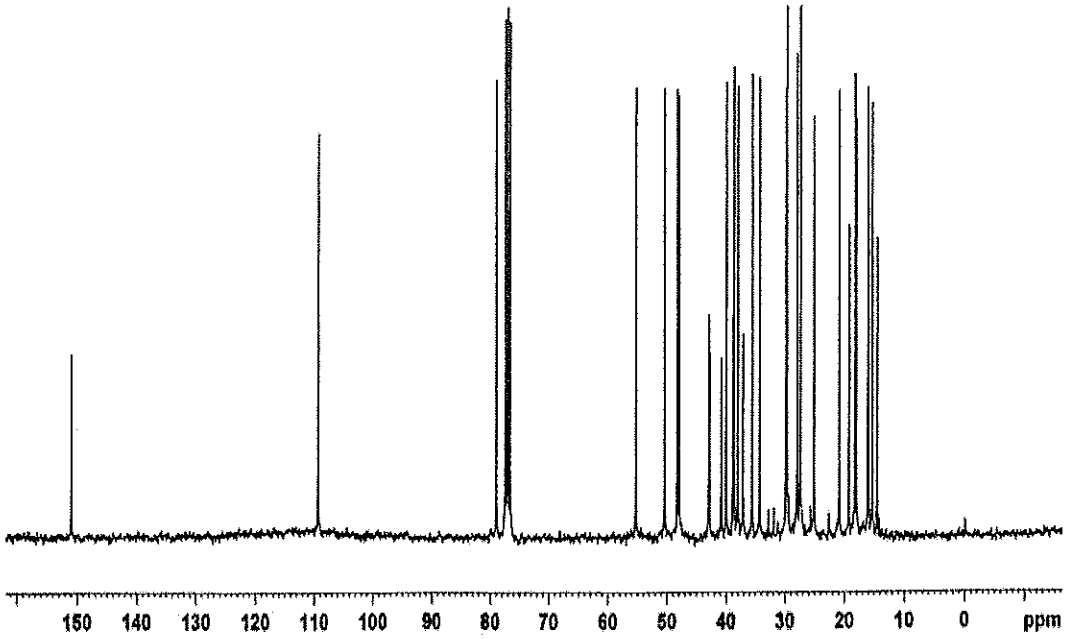

Figure 65 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW14**

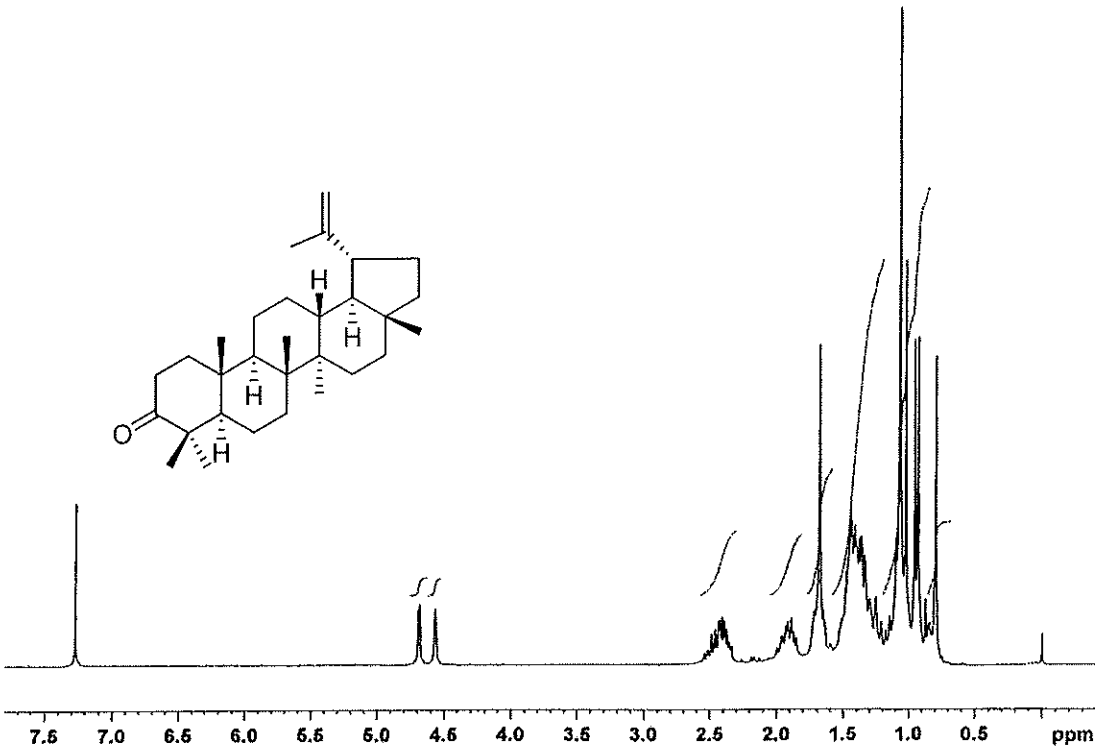

Figure 66 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW15**

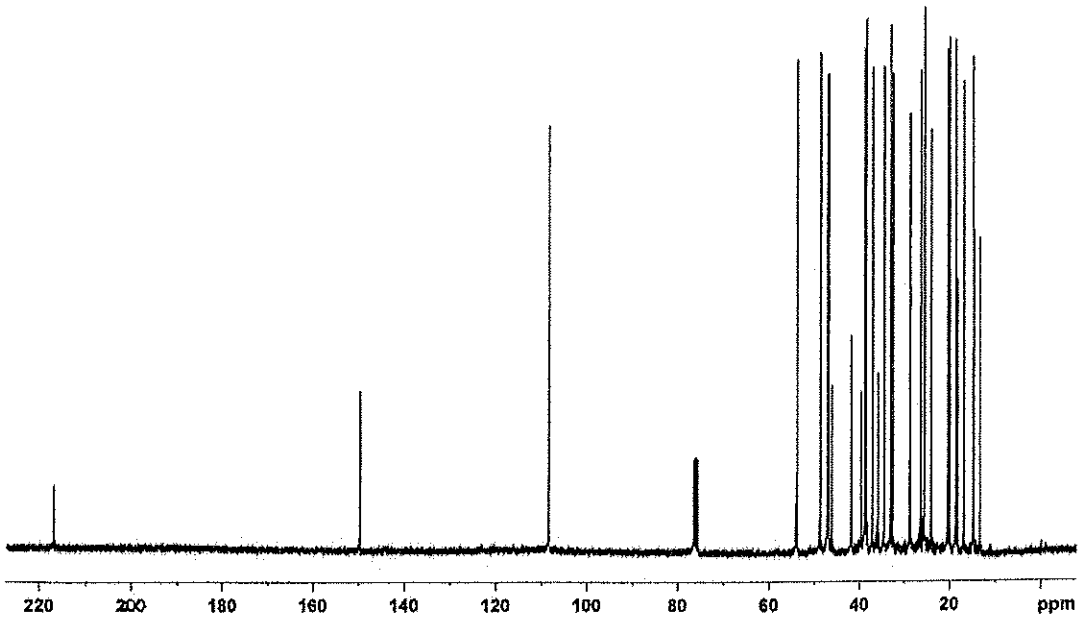

Figure 67 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW15**

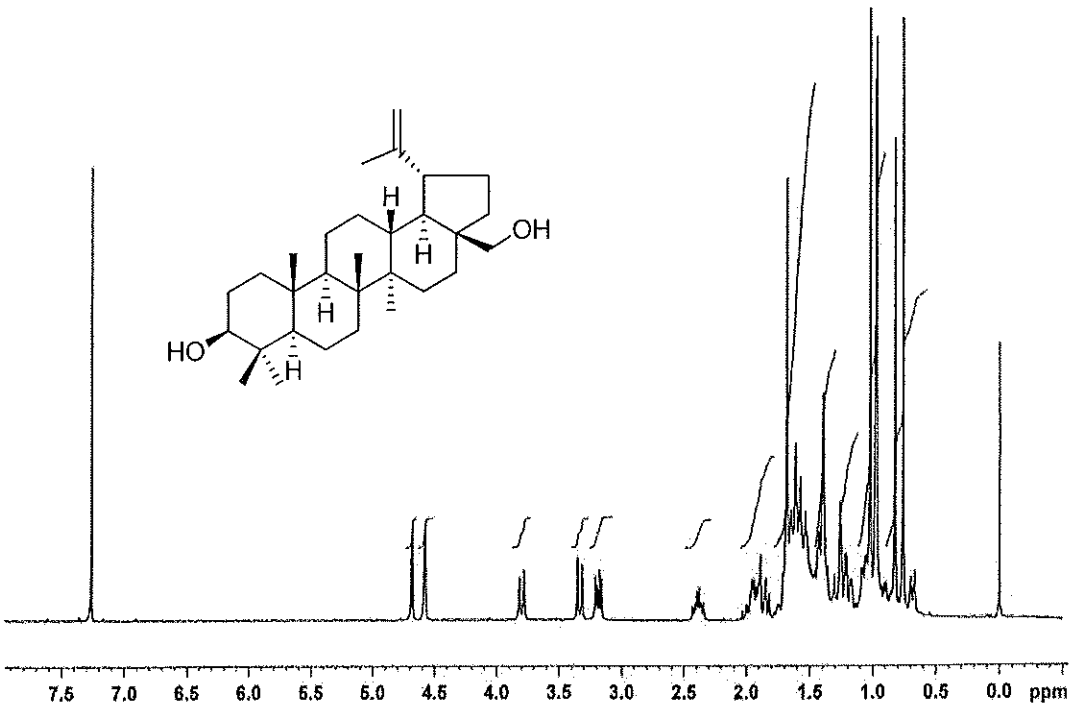

Figure 68 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW16**

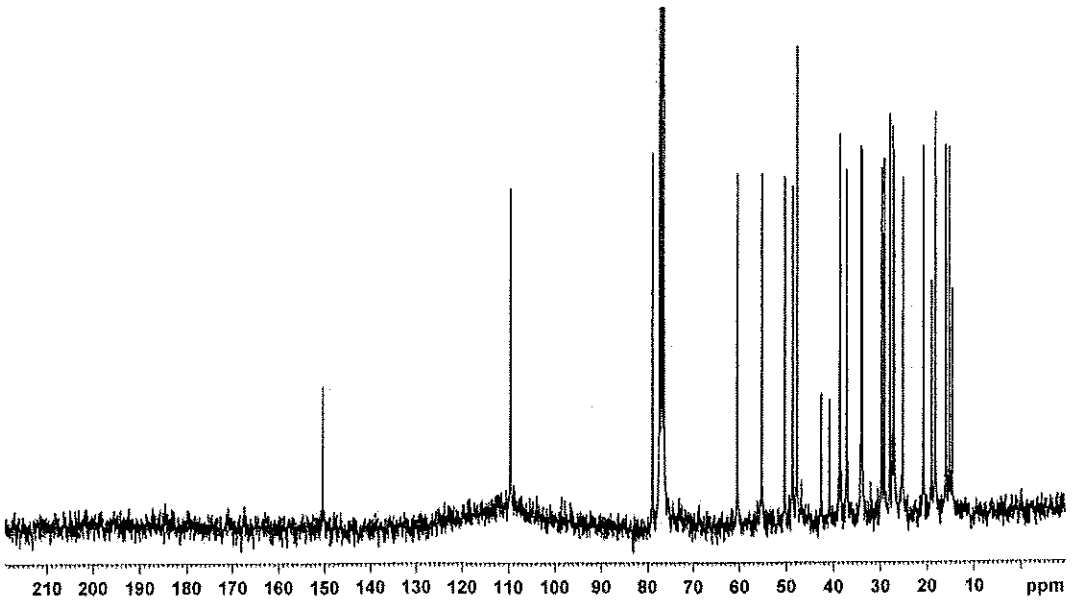

Figure 69 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW16**

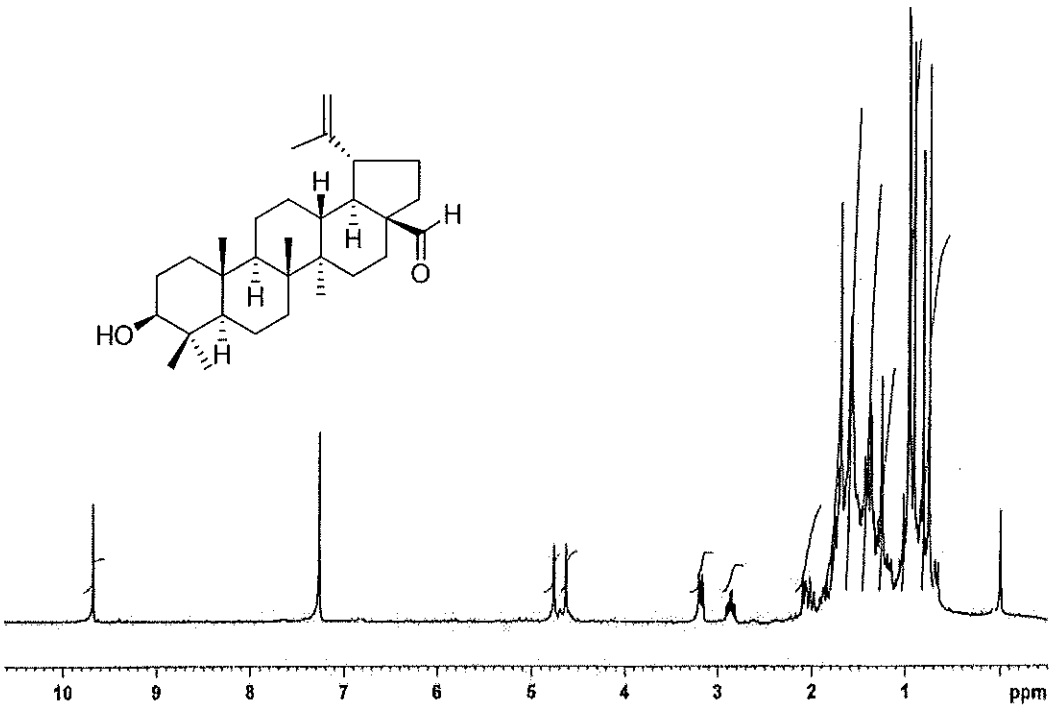

Figure 70 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW17**

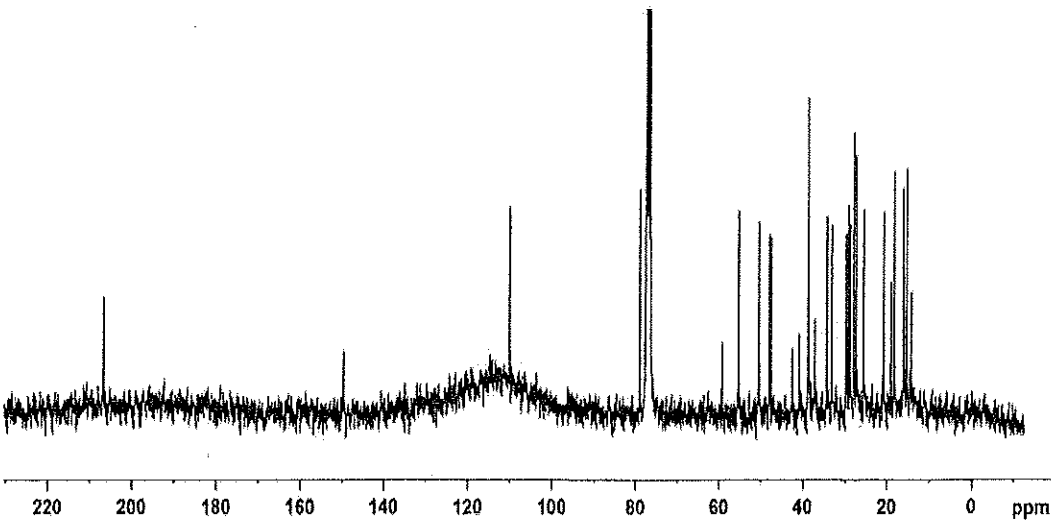

Figure 71 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW17**

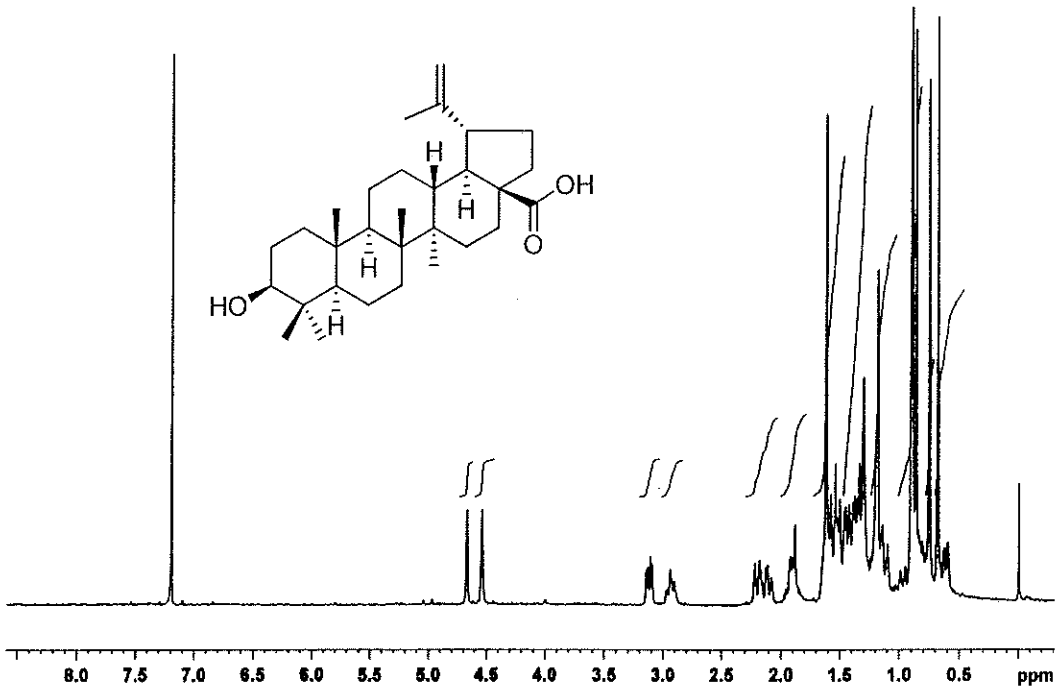

Figure 72 ^{1}H NMR (300 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **DW18**

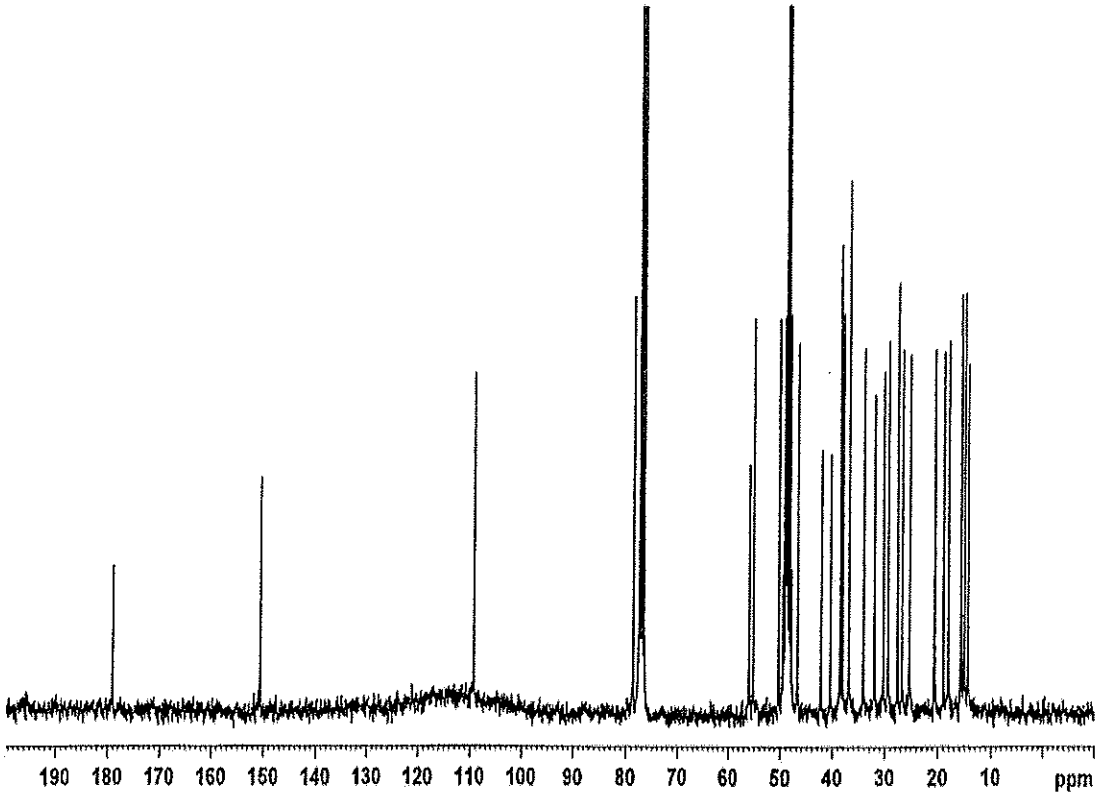

Figure 73 ^{13}C NMR (75 MHz) ($CDCl_3$+CD_3OH) spectrum of compound **DW18**

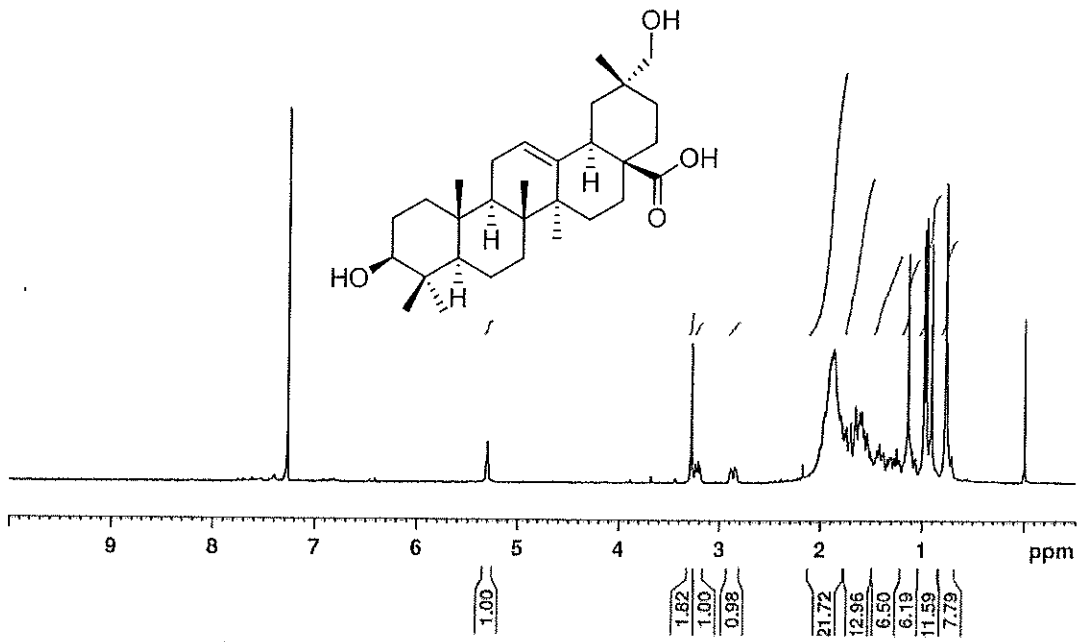

Figure 74 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **DW19**

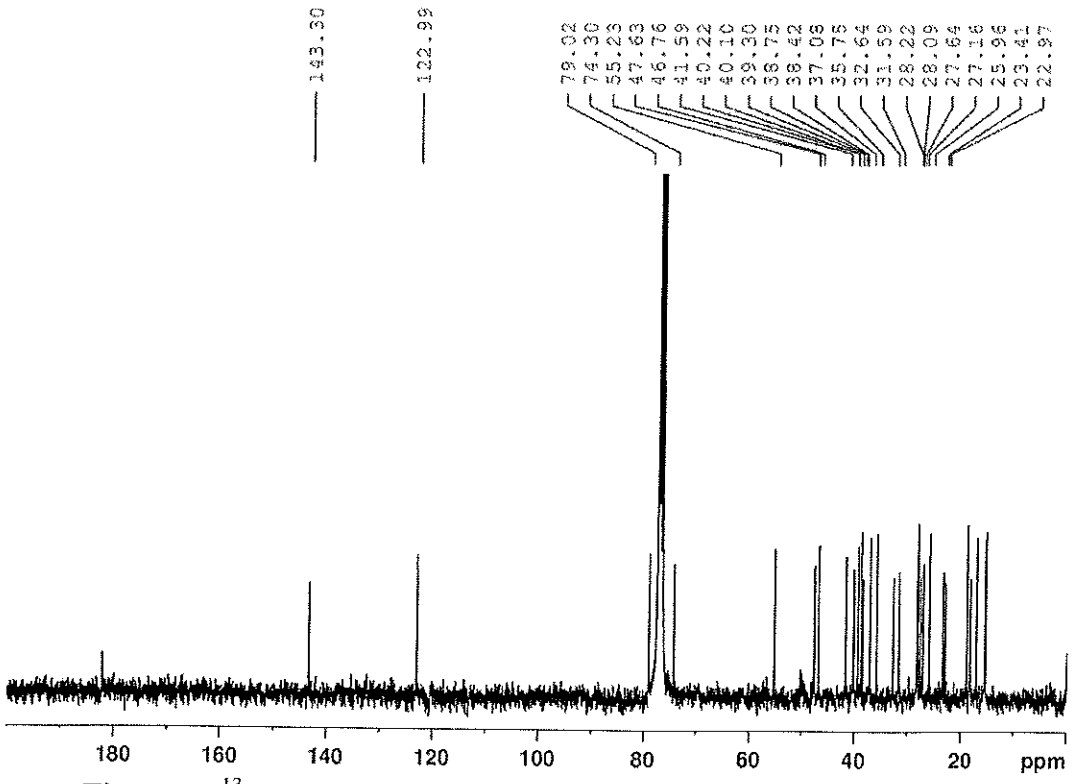

Figure 75 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **DW19**

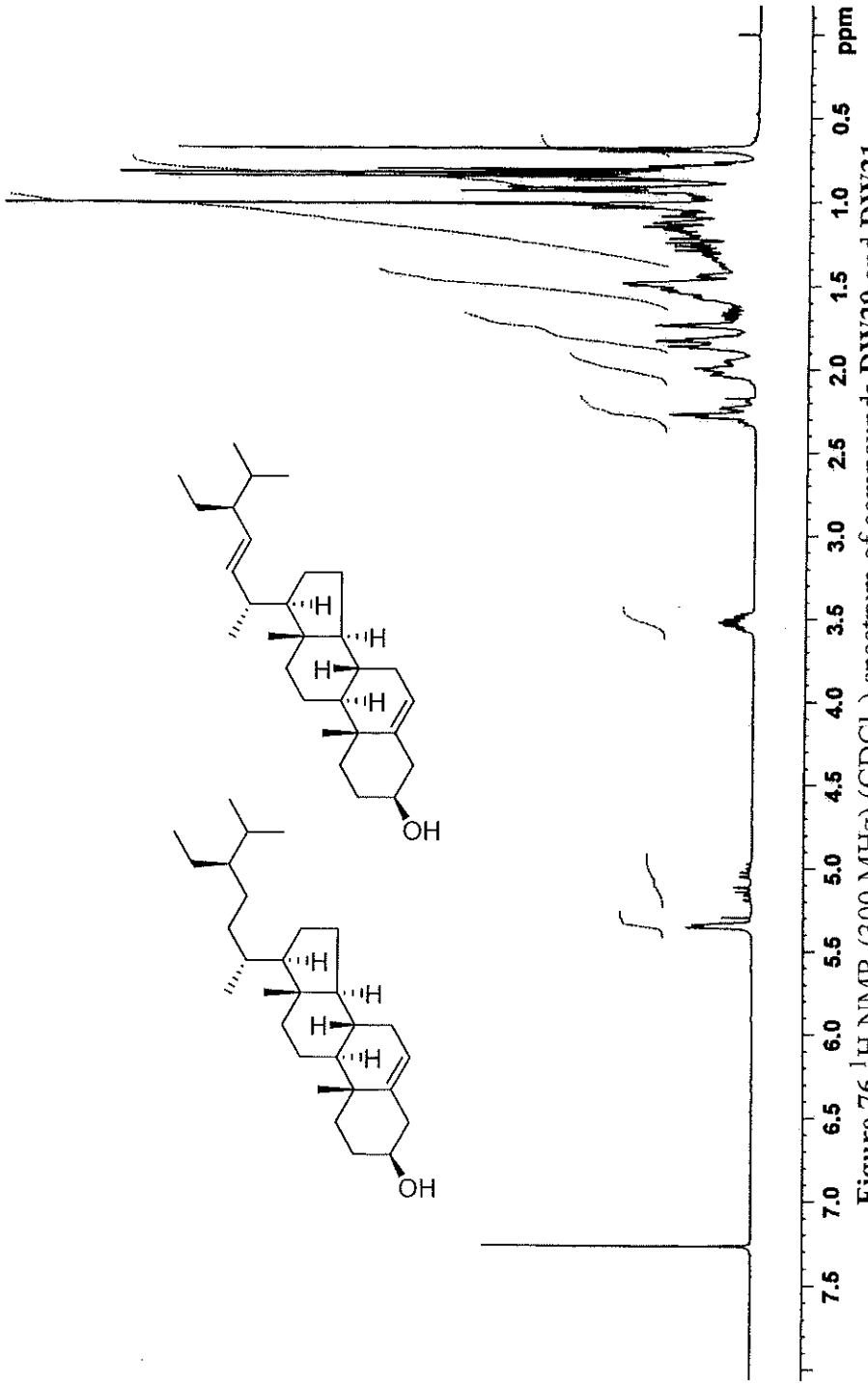

Figure 76 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compounds **DW20** and **DW21**

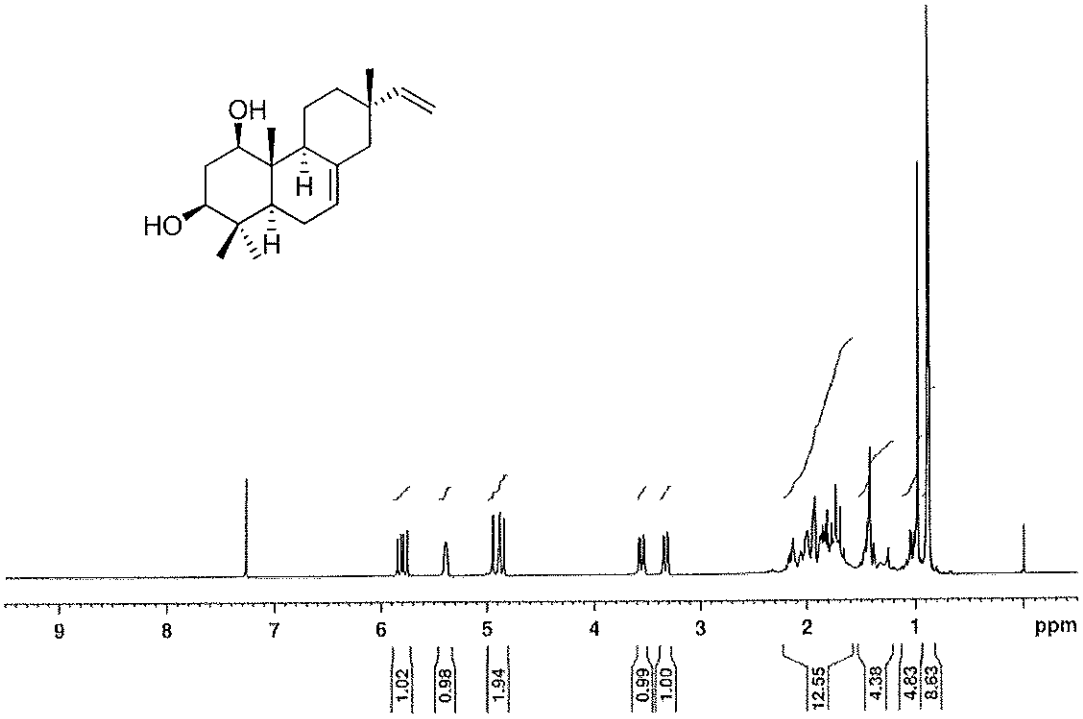

Figure 77 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO1**

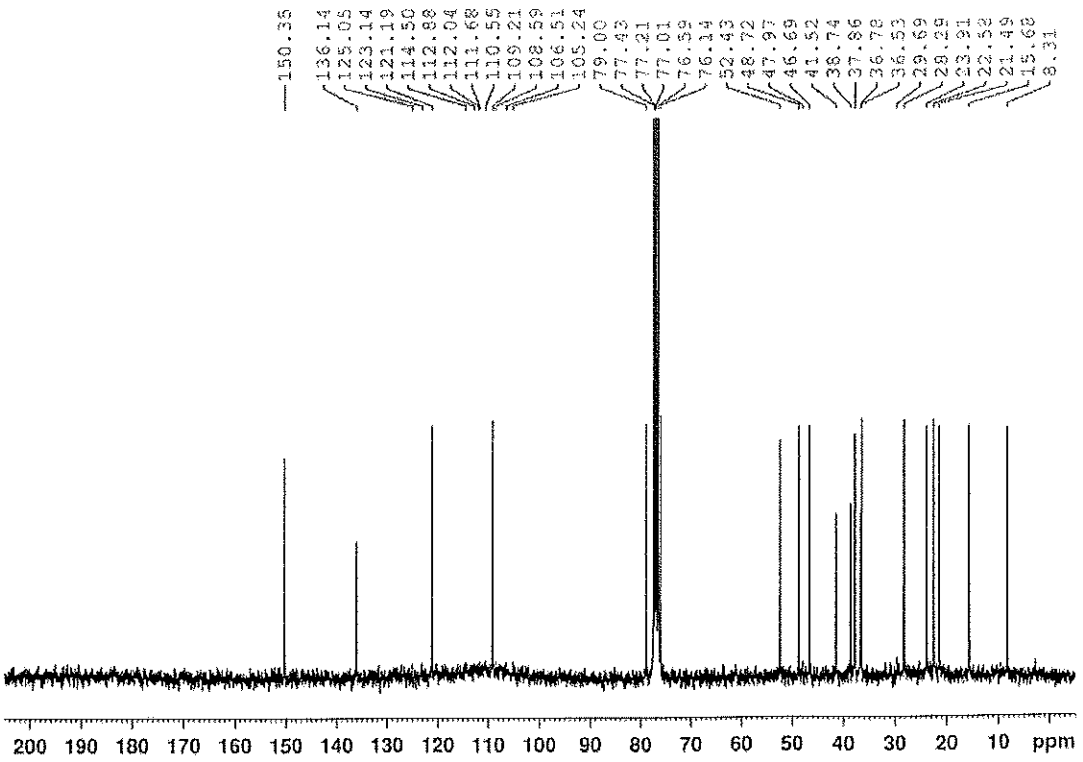

Figure 78 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO1**

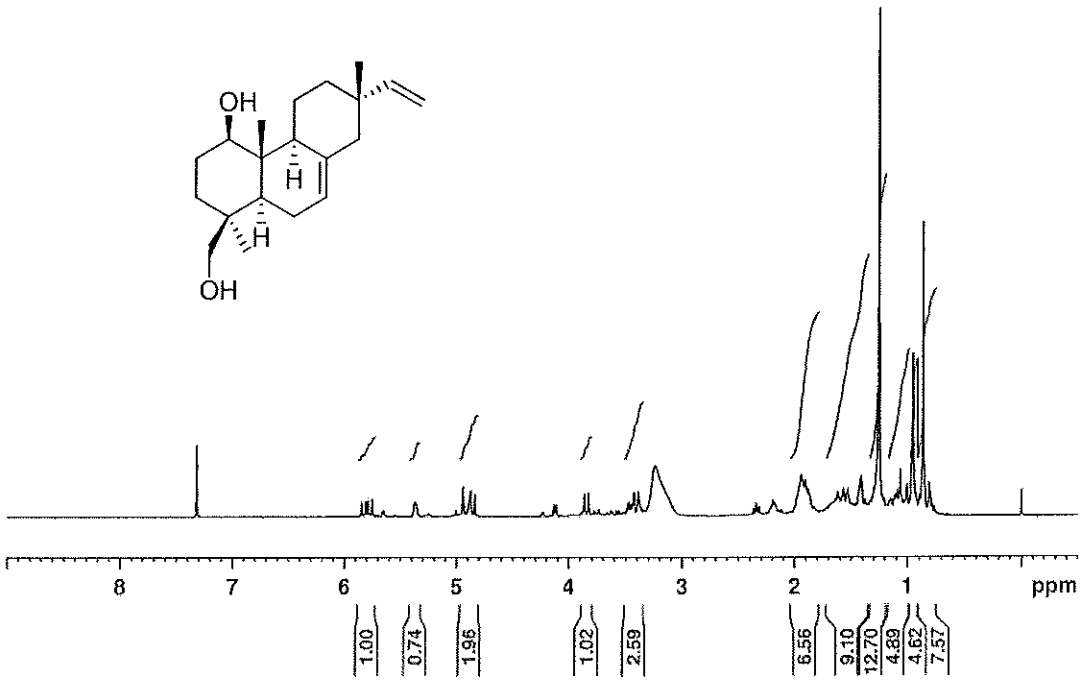

Figure 79 ^{1}H NMR (300 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **PO2**

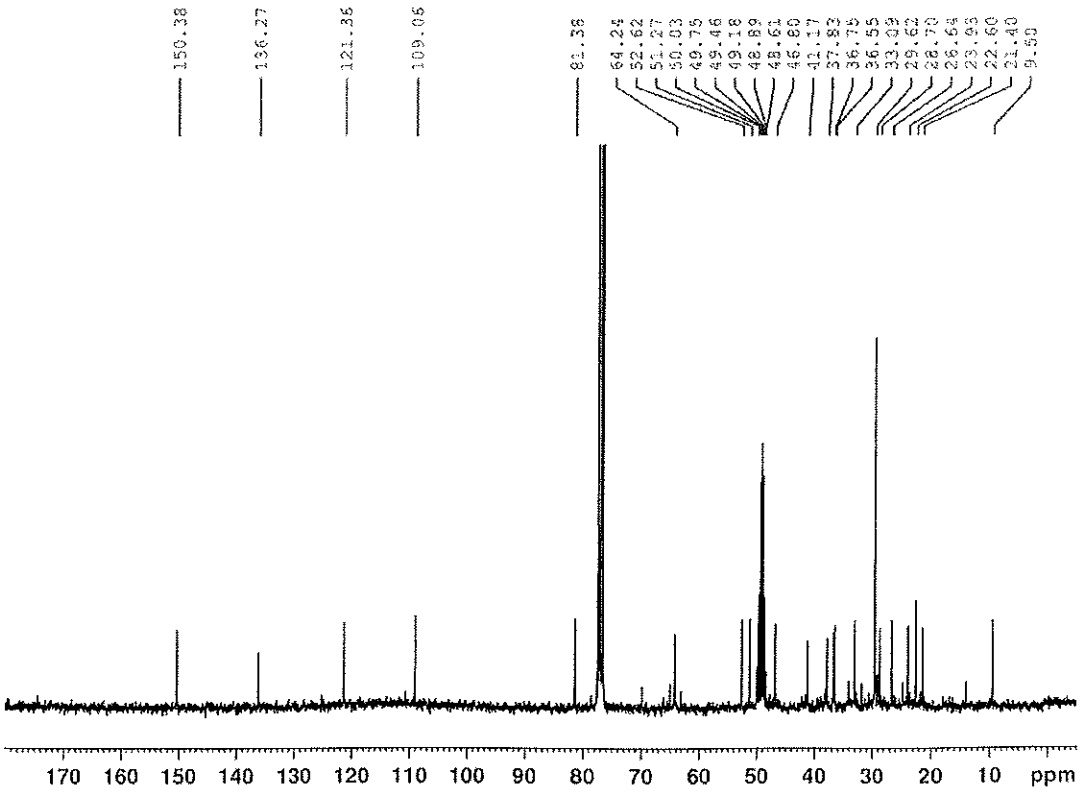

Figure 80 ^{13}C NMR (75 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **PO2**

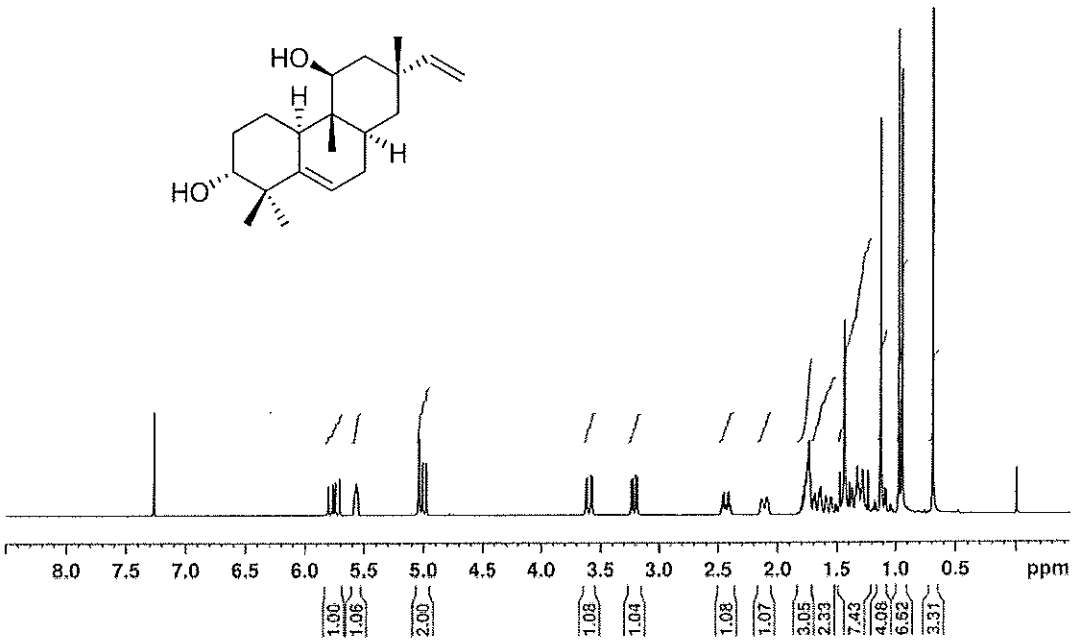

Figure 81 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO3**

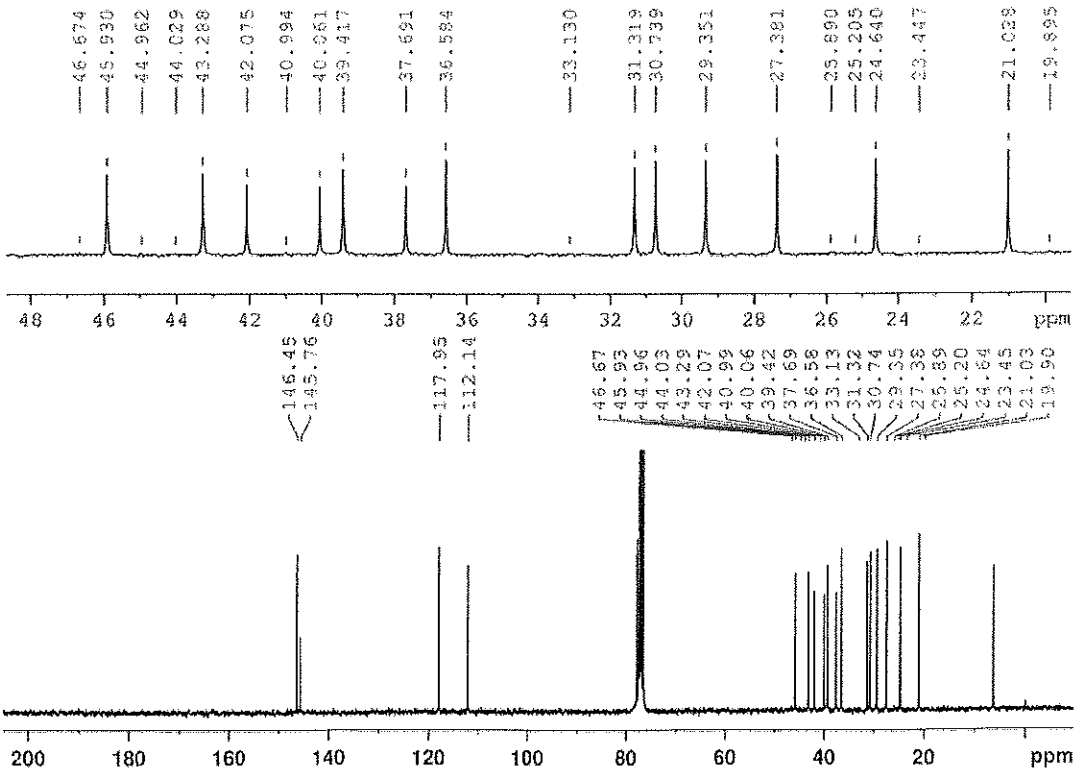

Figure 82 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO3**

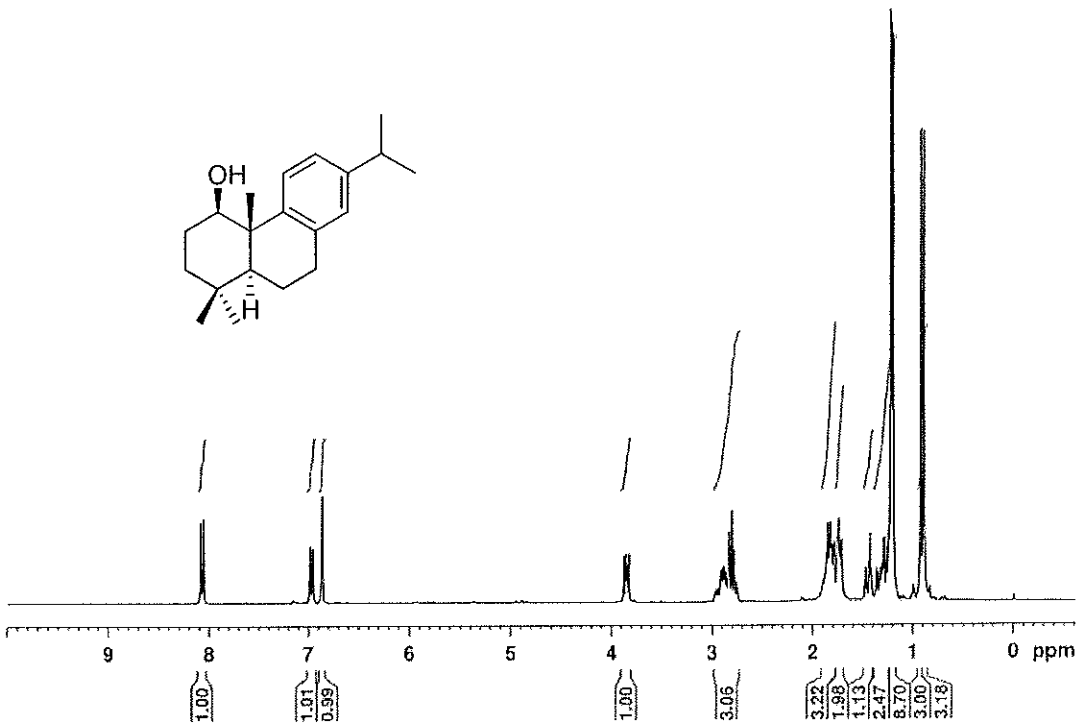

Figure 83 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO4**

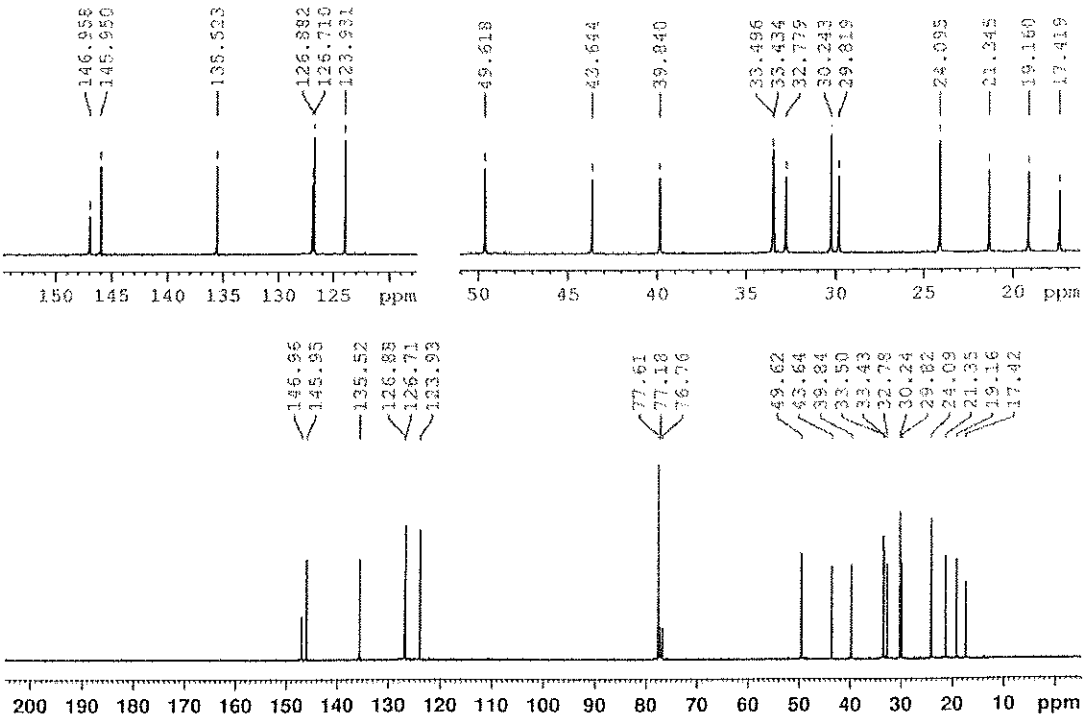

Figure 84 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO4**

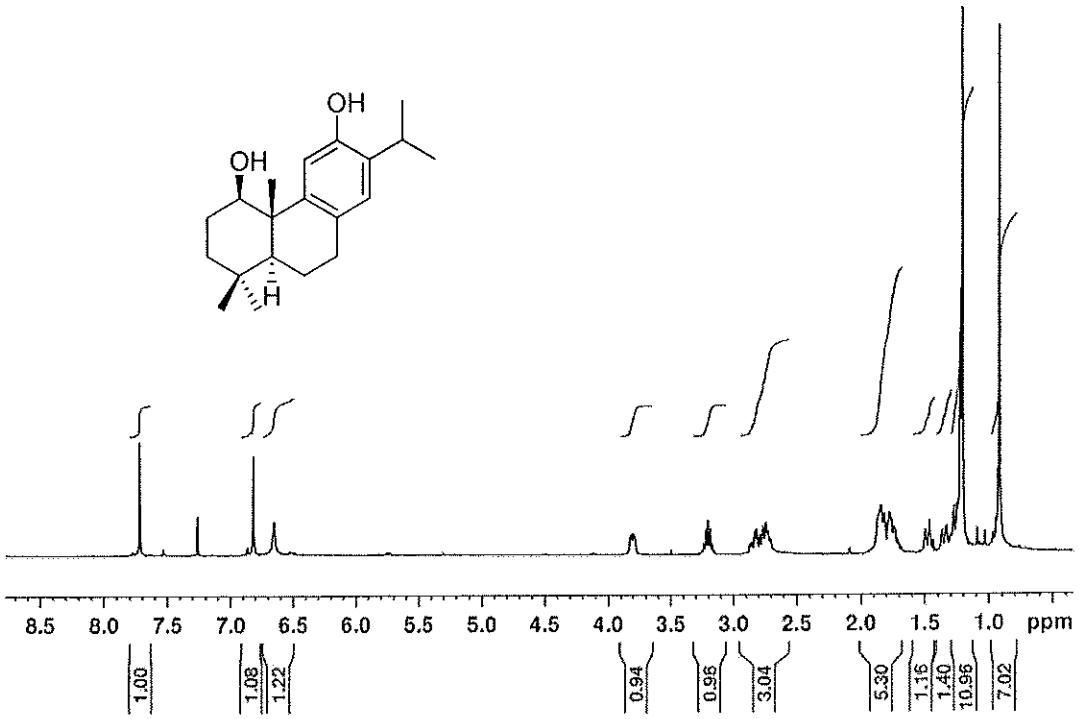

Figure 85 ^{1}H NMR (400 MHz) ($CDCl_3$) spectrum of compound **PO5**

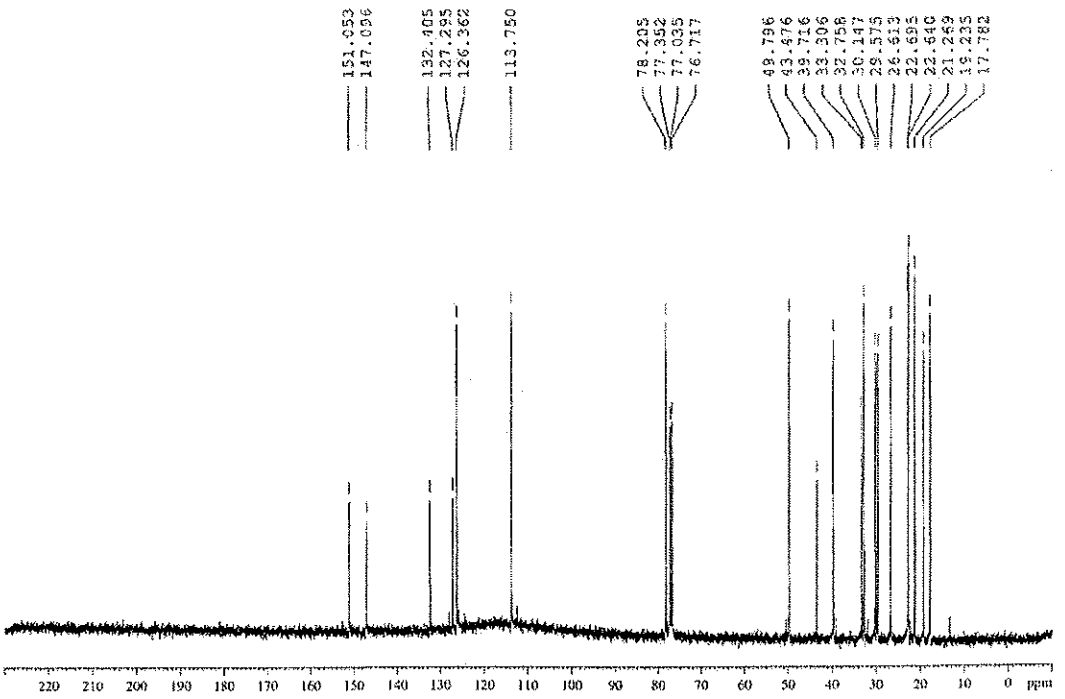

Figure 86 ^{13}C NMR (100 MHz) ($CDCl_3$) spectrum of compound **PO5**

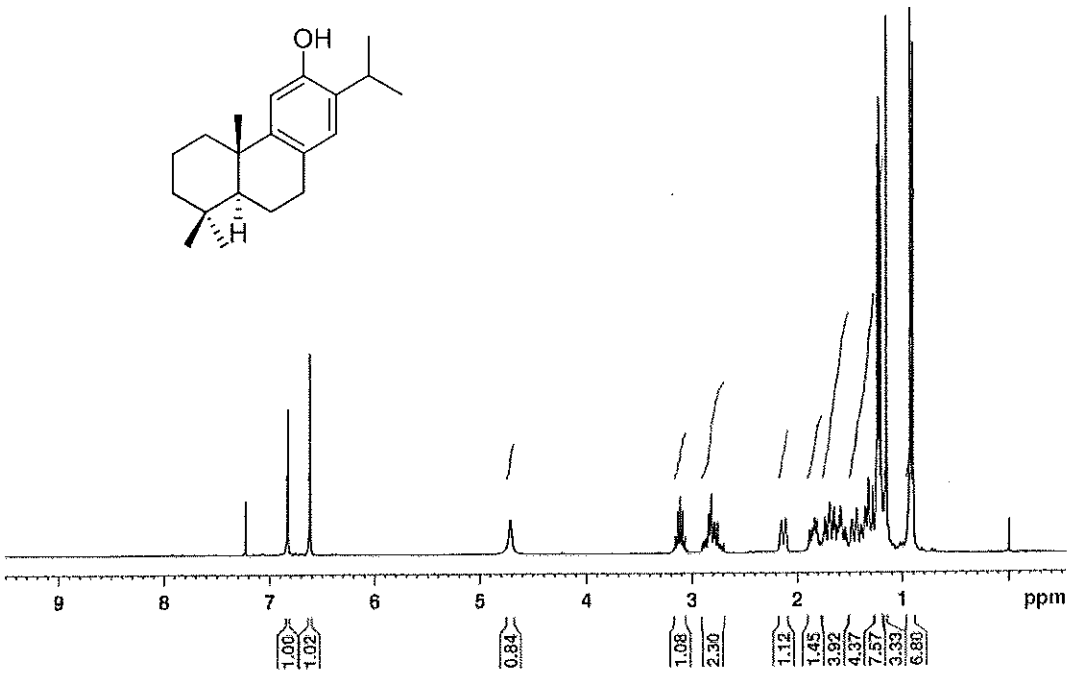

Figure 87 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO6**

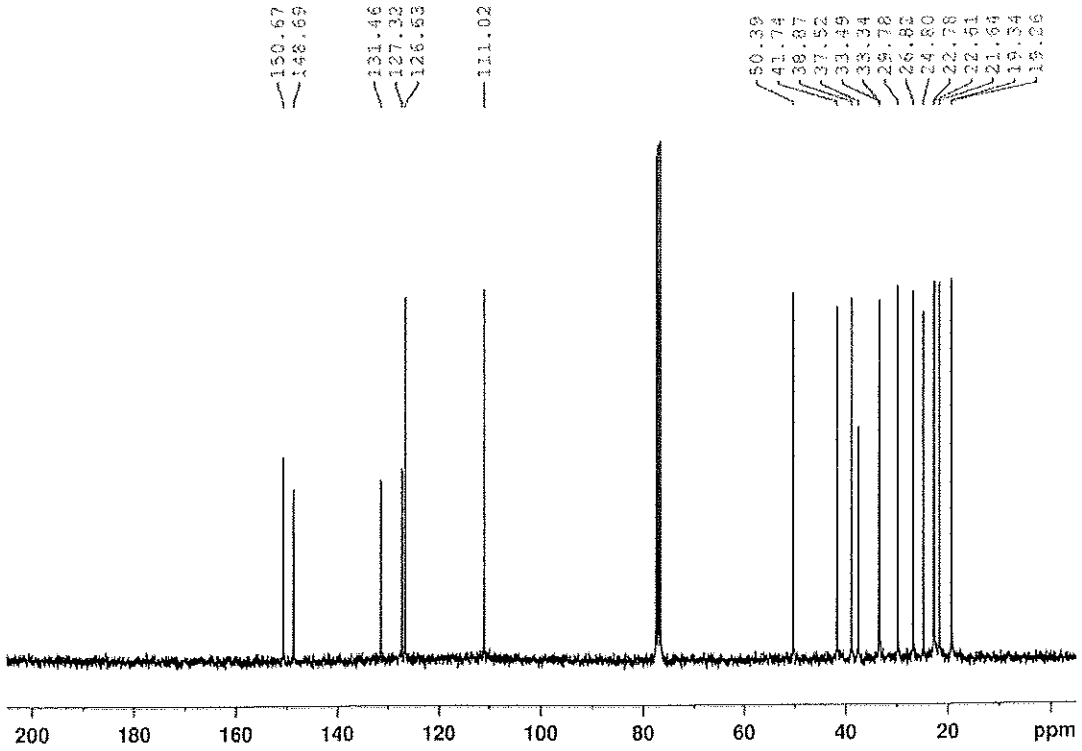

Figure 88 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO6**

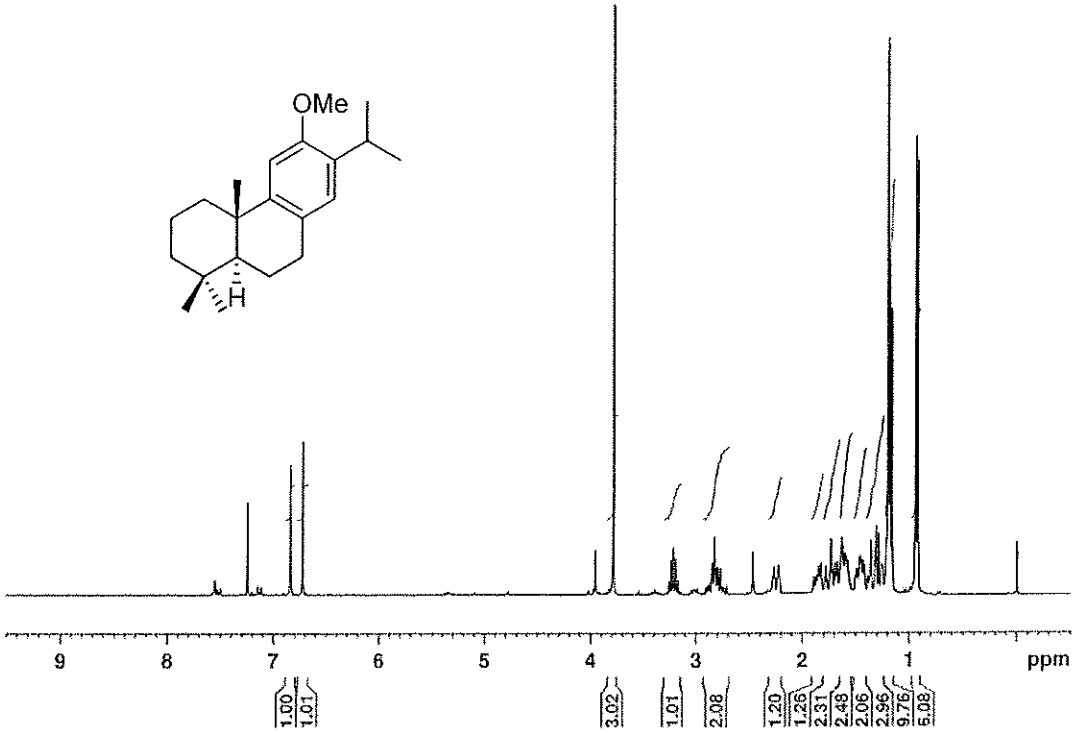

Figure 89 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO7**

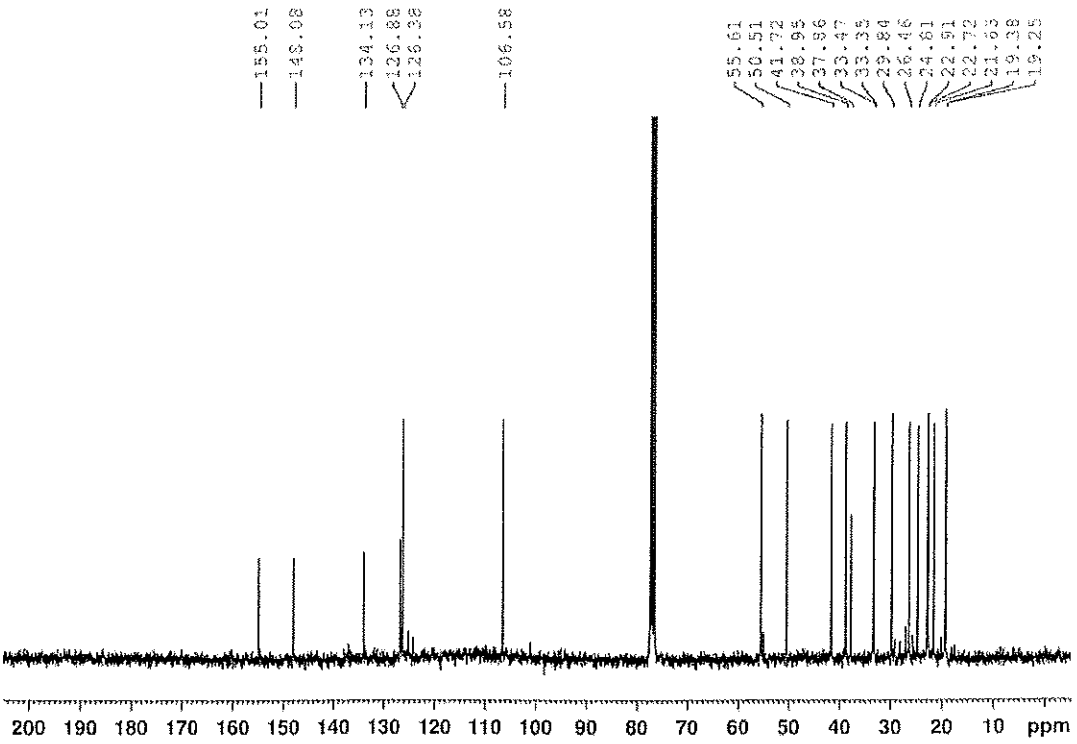

Figure 90 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO7**

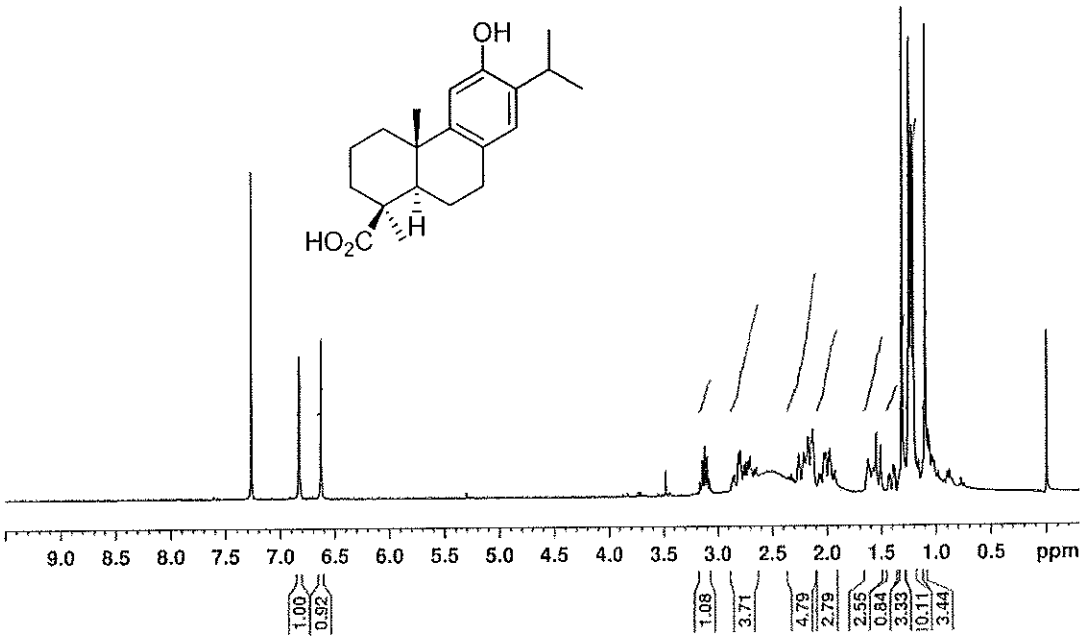

Figure 91 ^{1}H NMR (300 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **PO8**

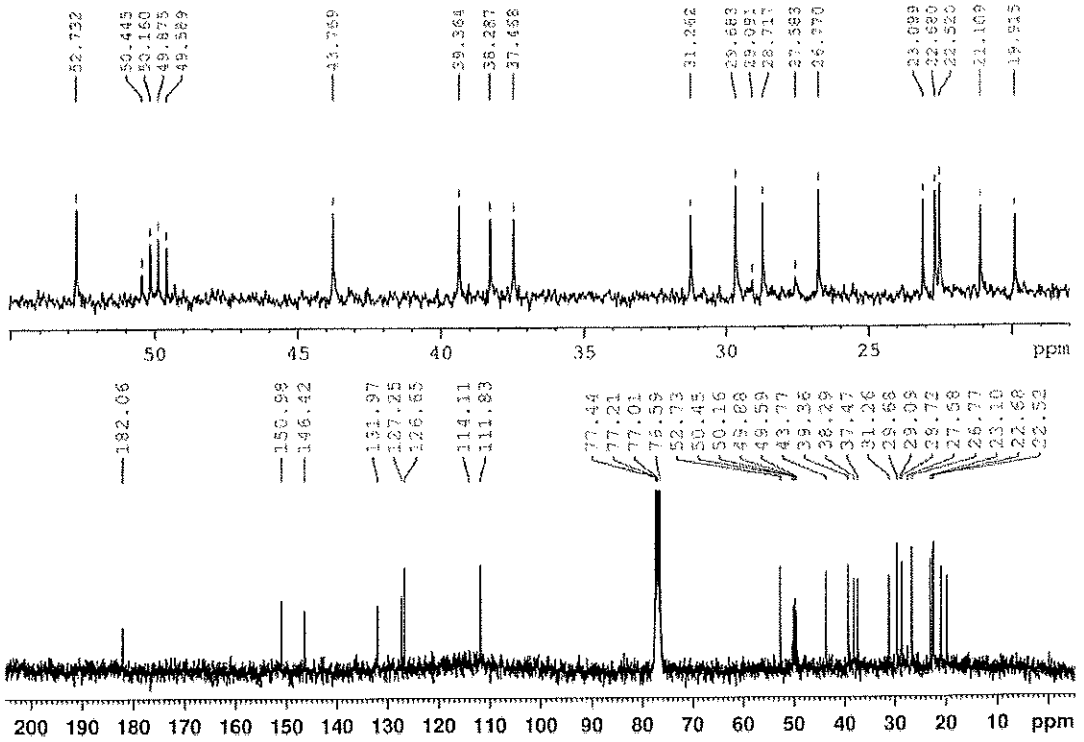

Figure 92 ^{13}C NMR (75 MHz) ($CDCl_3$+CD_3CD) spectrum of compound **PO8**

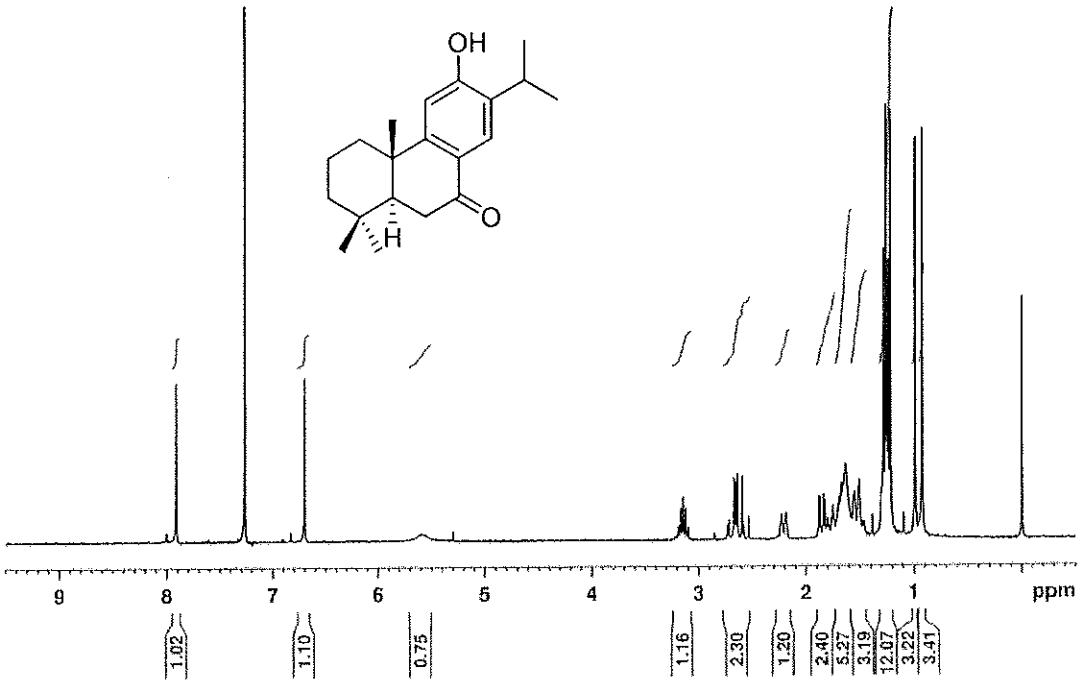

Figure 93 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO9**

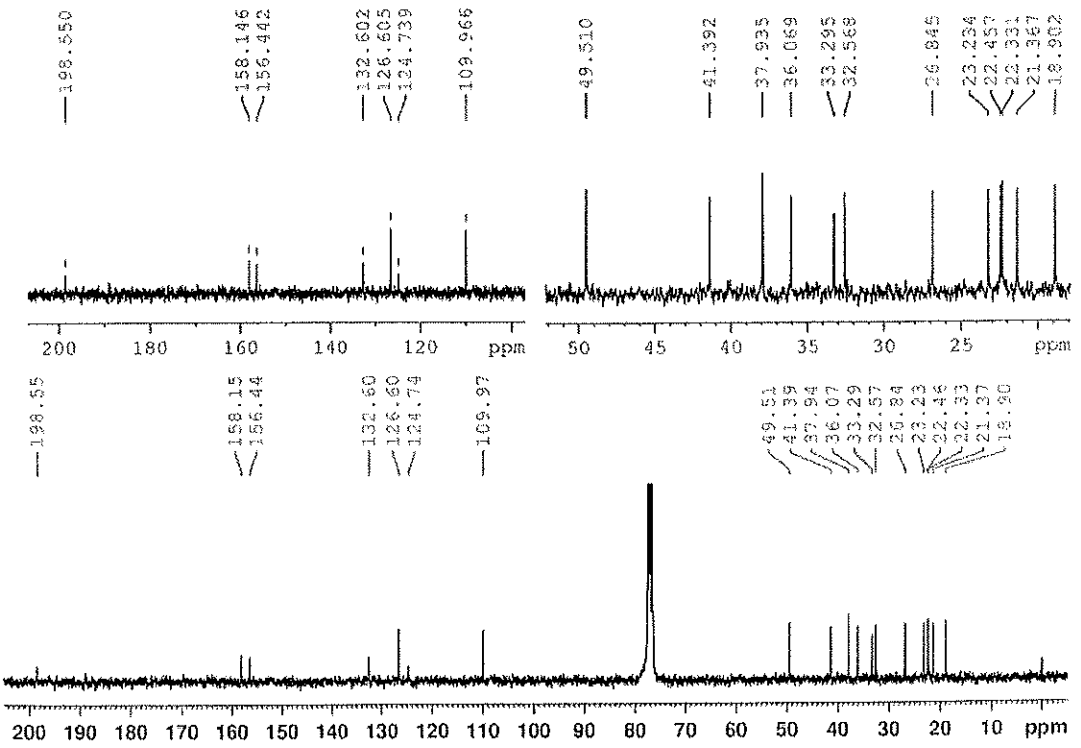

Figure 94 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO9**

Figure 95 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO10**

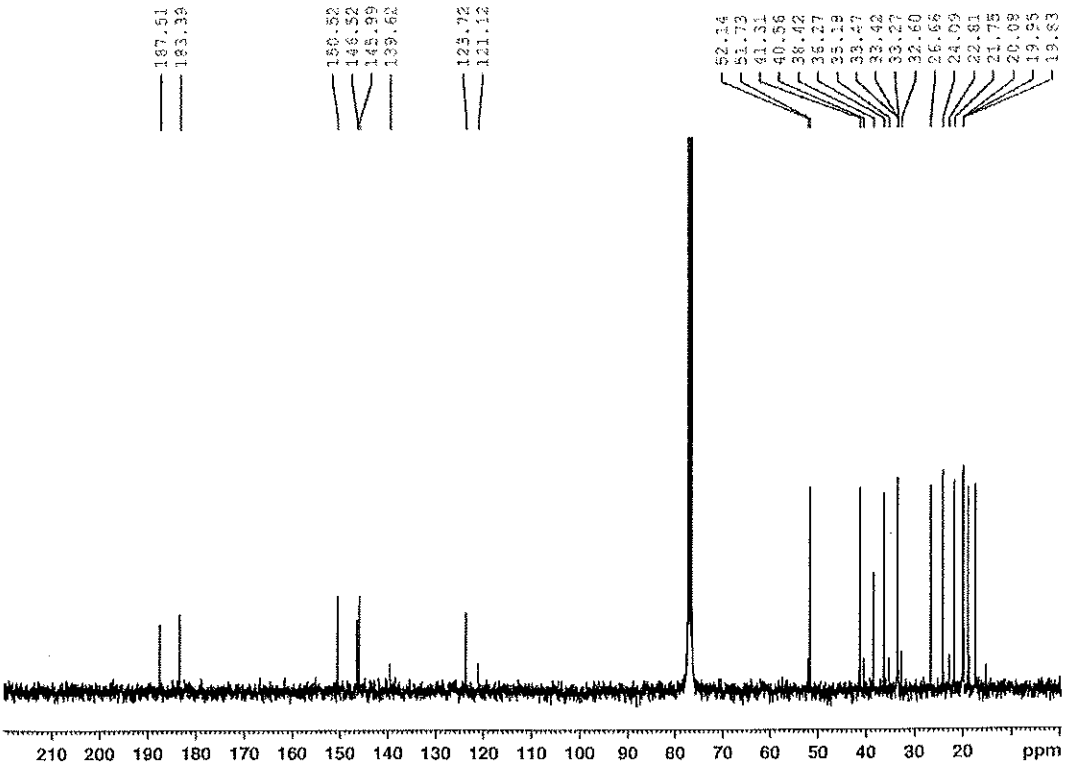

Figure 96 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO10**

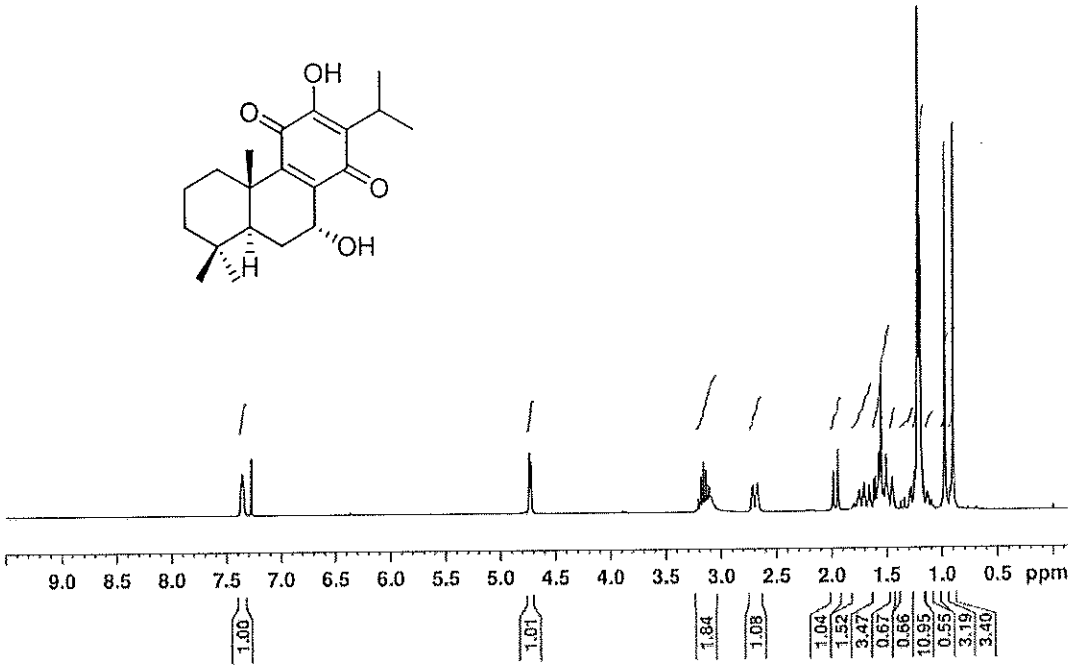

Figure 97 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO11**

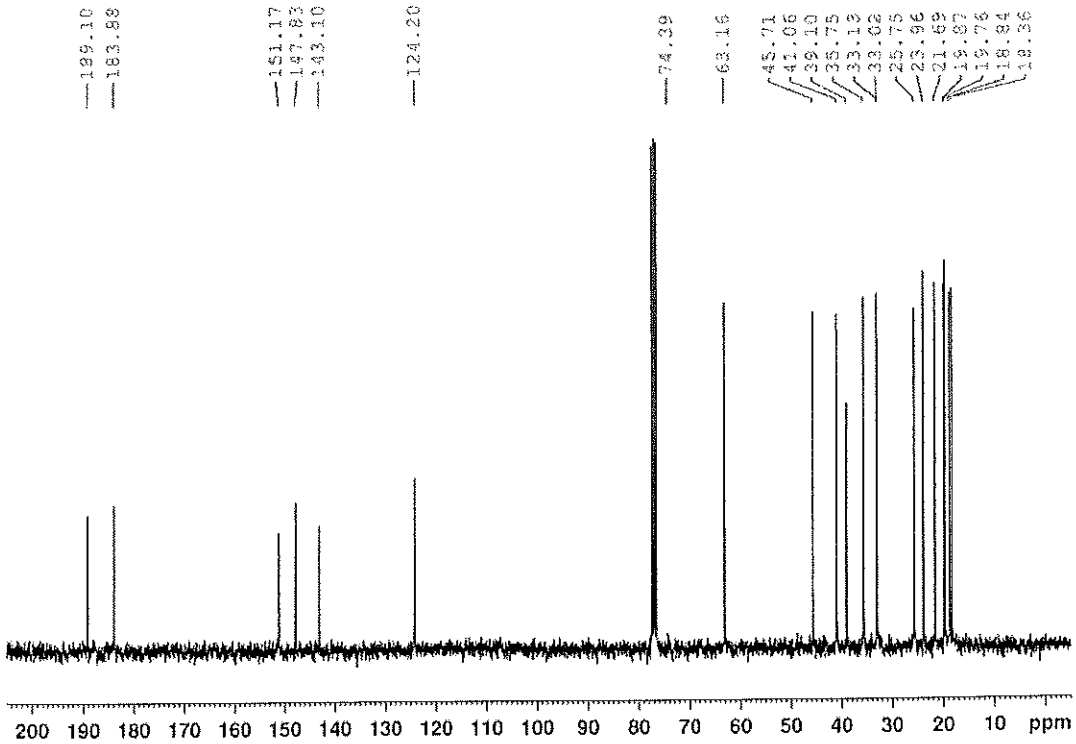

Figure 98 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO11**

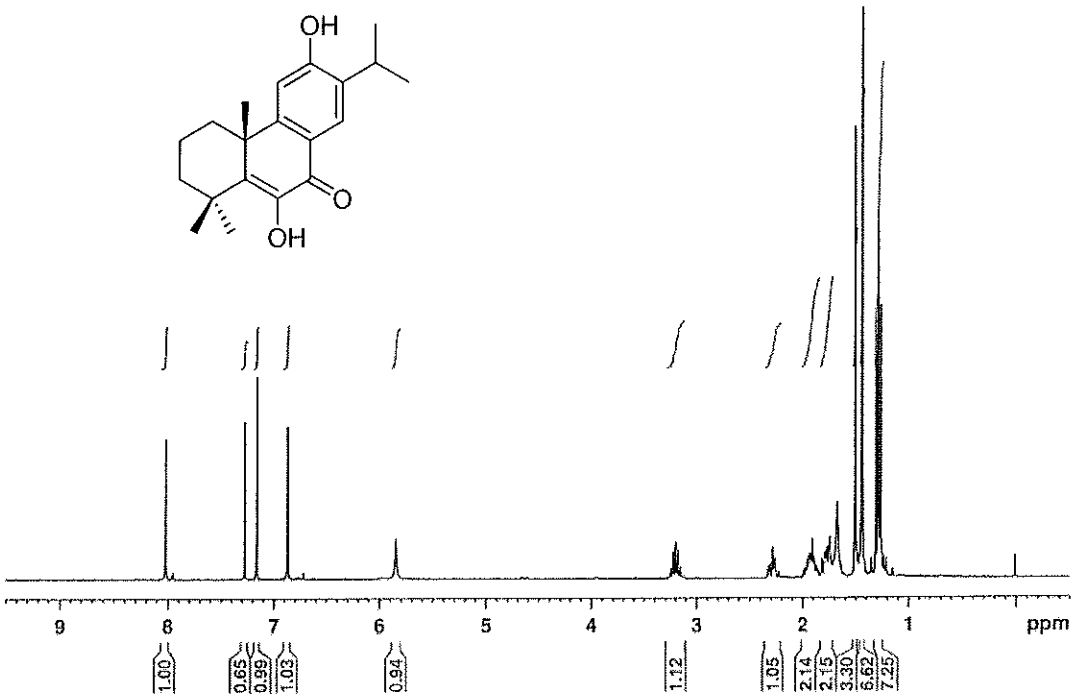

Figure 99 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO12**

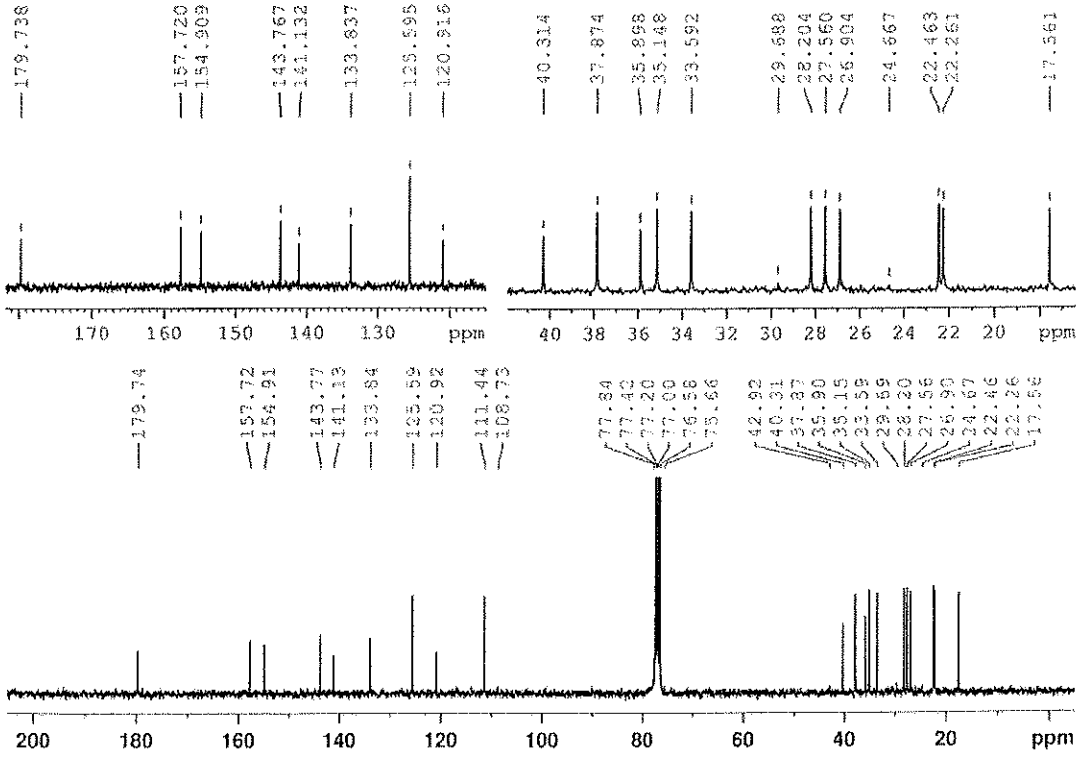

Figure 100 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO12**

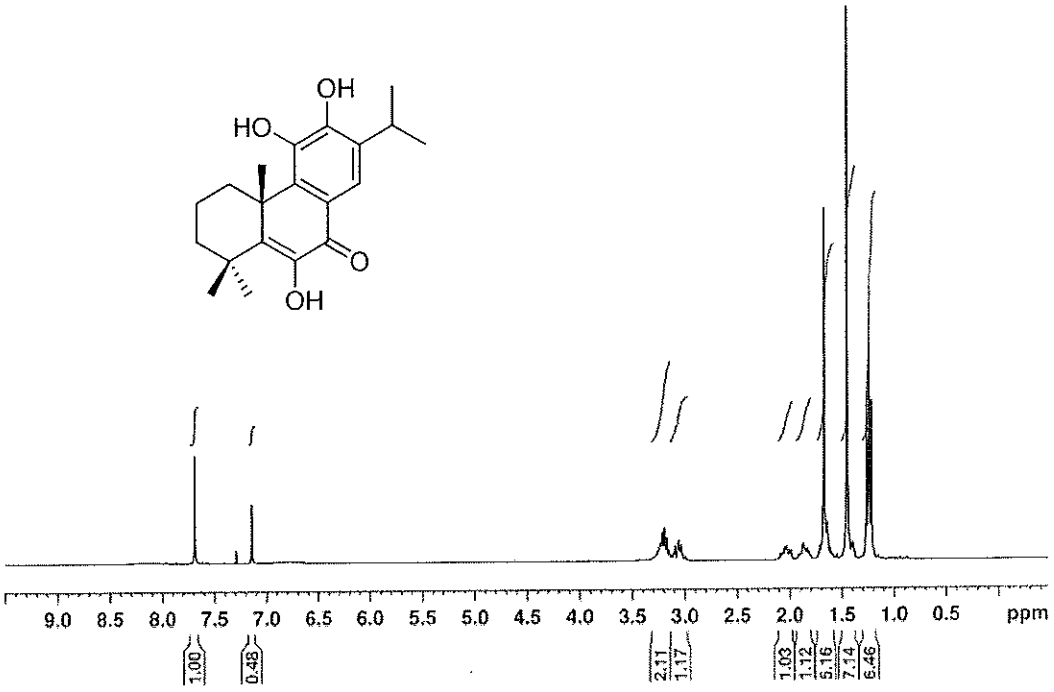

Figure 101 ^{1}H NMR (300 MHz) ($CDCl_3$+CD_3CD) spectrum of compound **PO13**

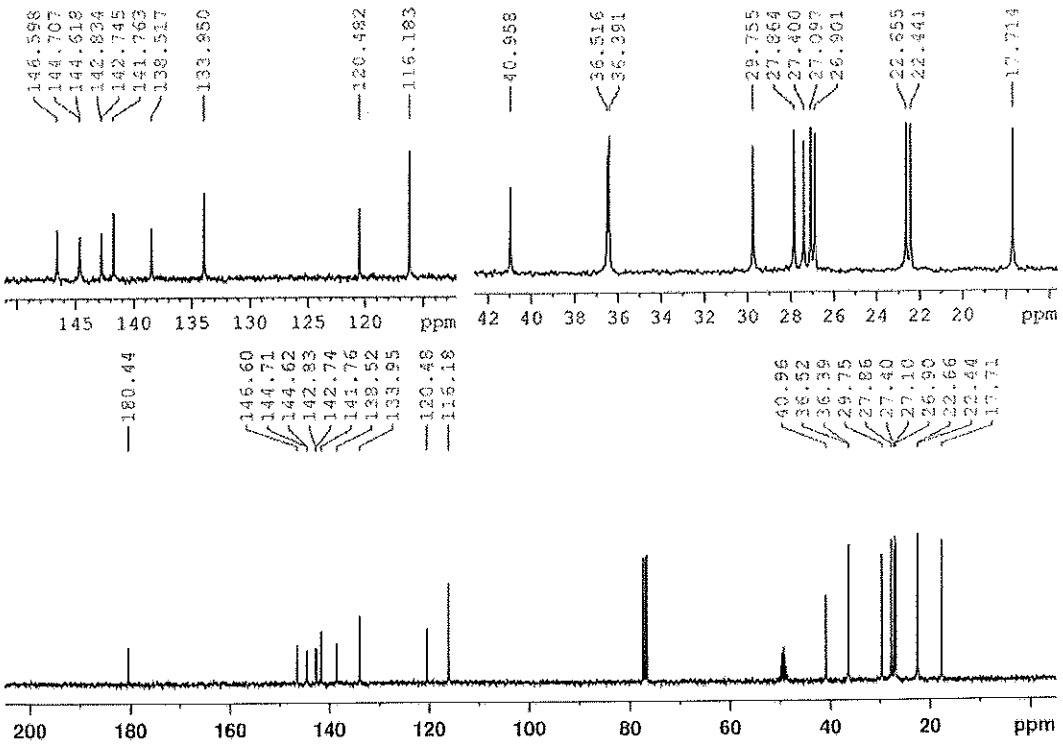

Figure 102 ^{13}C NMR (75 MHz) ($CDCl_3$+CD_3CD) spectrum of compound **PO13**

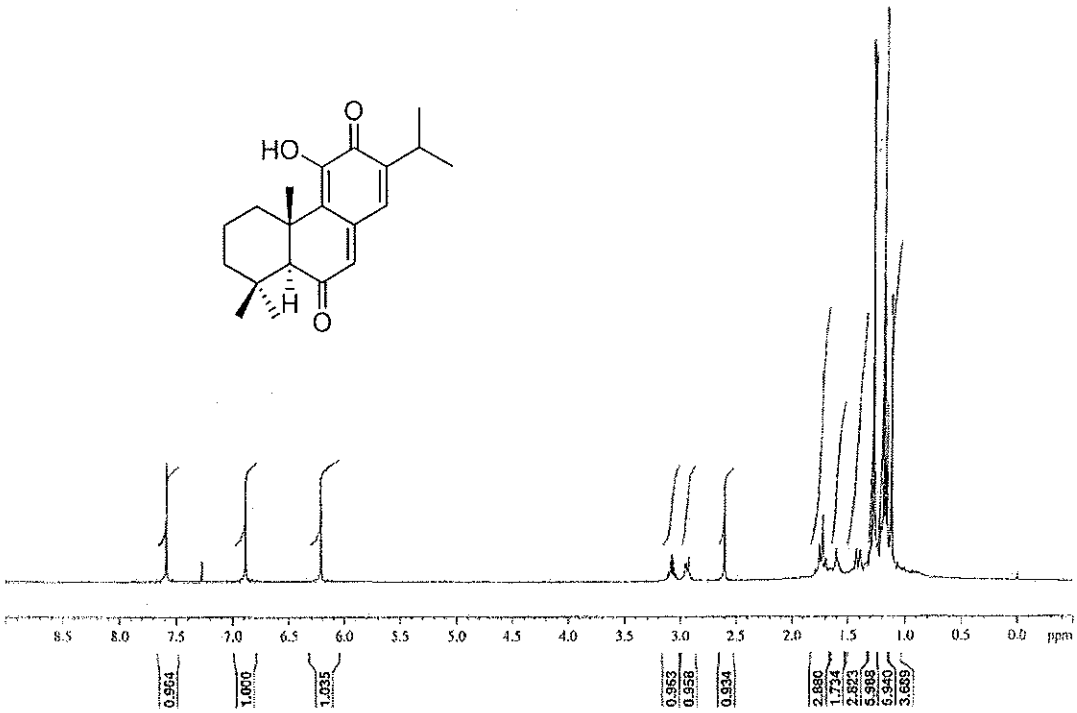

Figure 103 ^{1}H NMR (400 MHz) ($CDCl_3$) spectrum of compound **PO14**

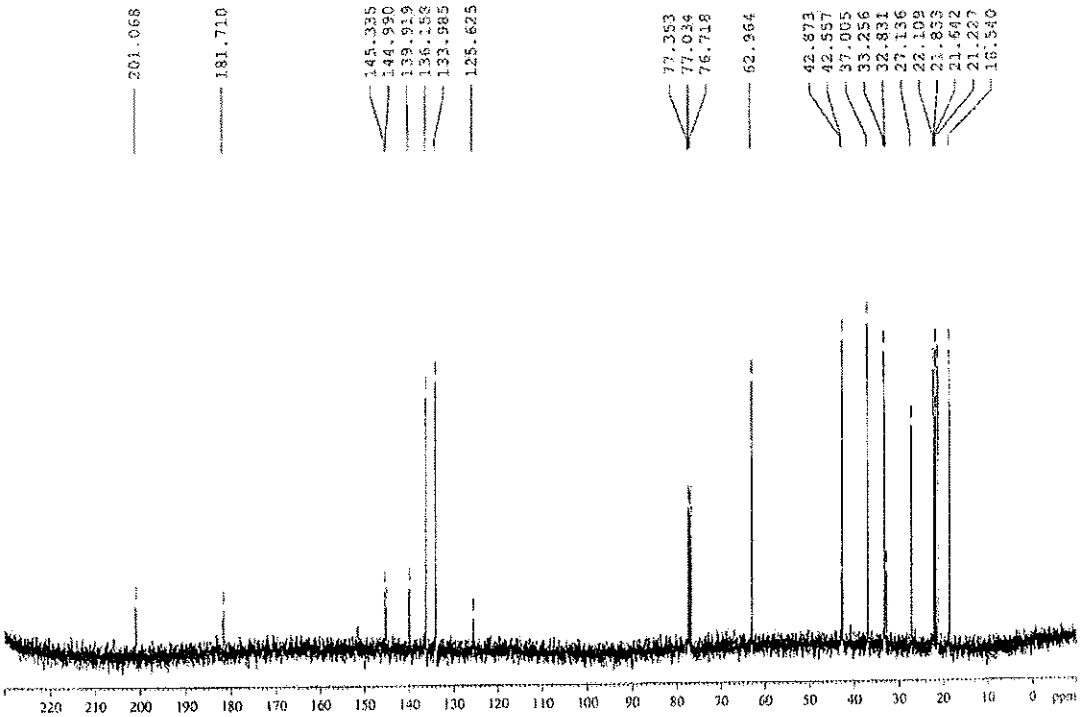

Figure 104 ^{13}C NMR (100 MHz) ($CDCl_3$) spectrum of compound **PO14**

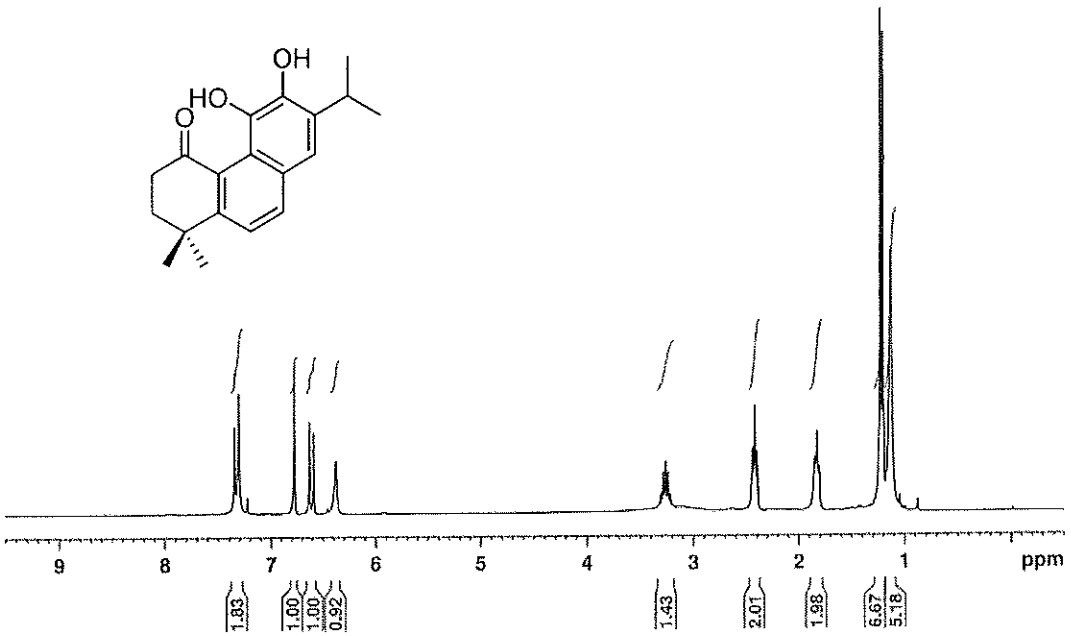

Figure 105 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO15**

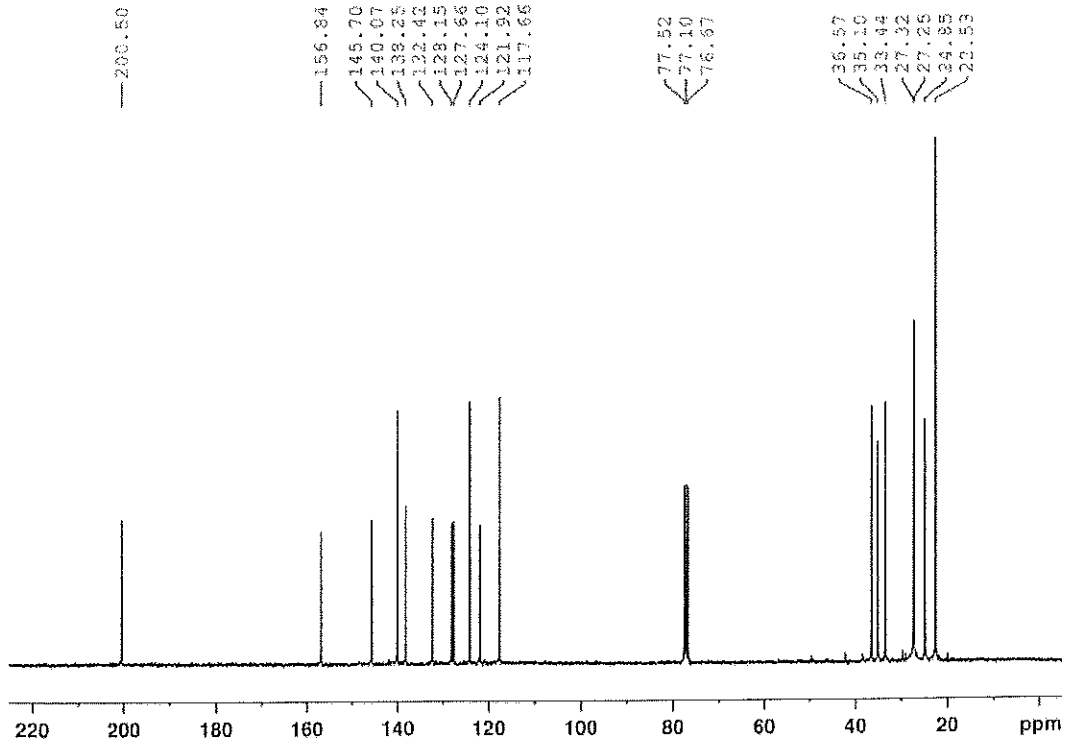

Figure 106 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO15**

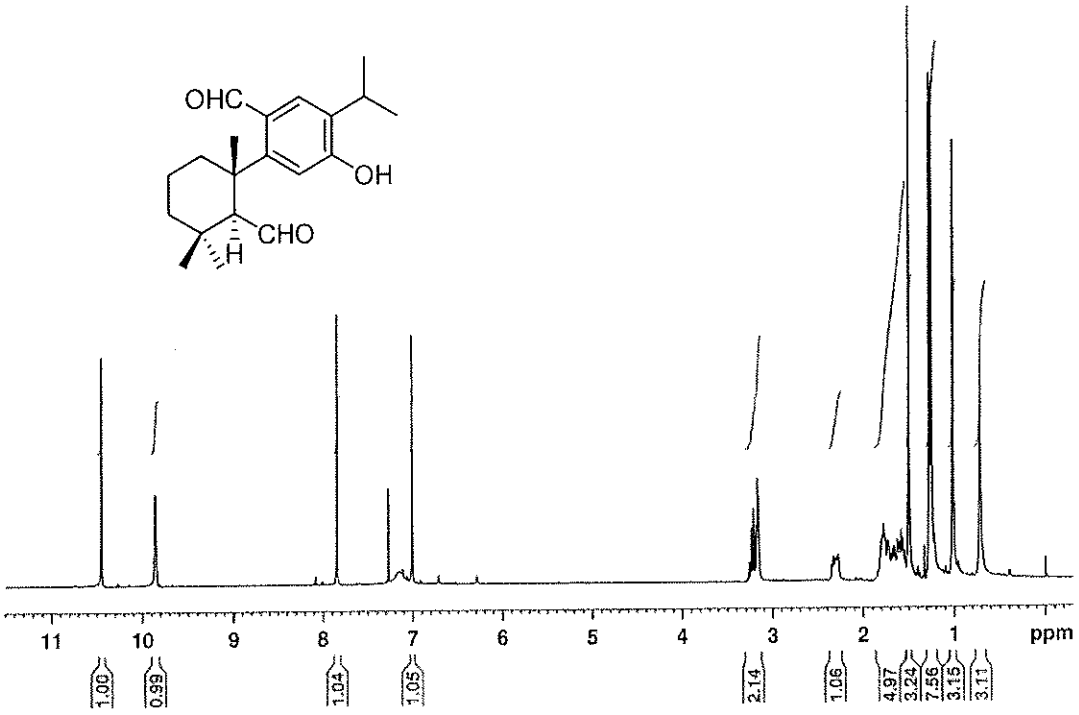

Figure 107 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO16**

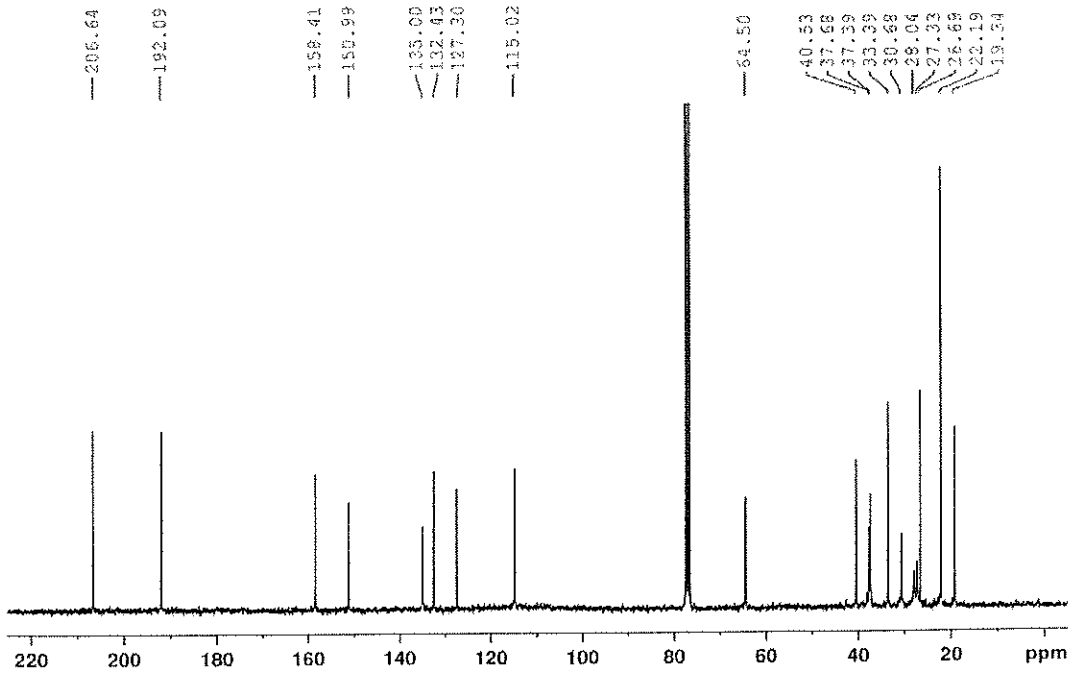

Figure 108 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO16**

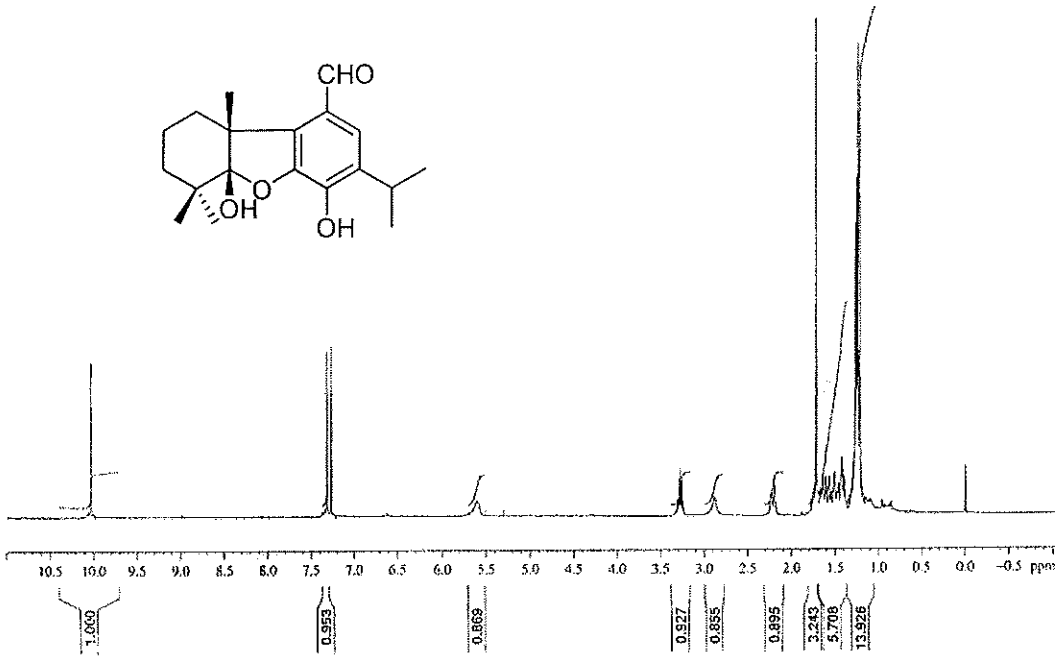

Figure 109 ^{1}H NMR (400 MHz) ($CDCl_3$) spectrum of compound **PO17**

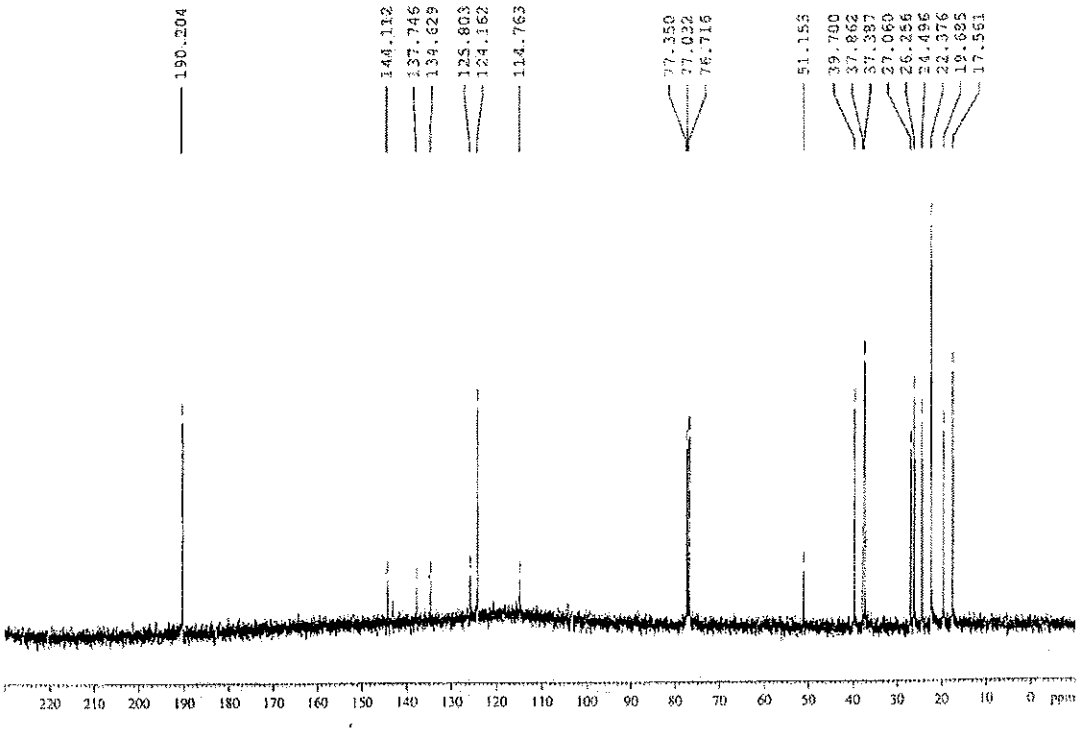

Figure 110 ^{13}C NMR (100 MHz) ($CDCl_3$) spectrum of compound **PO17**

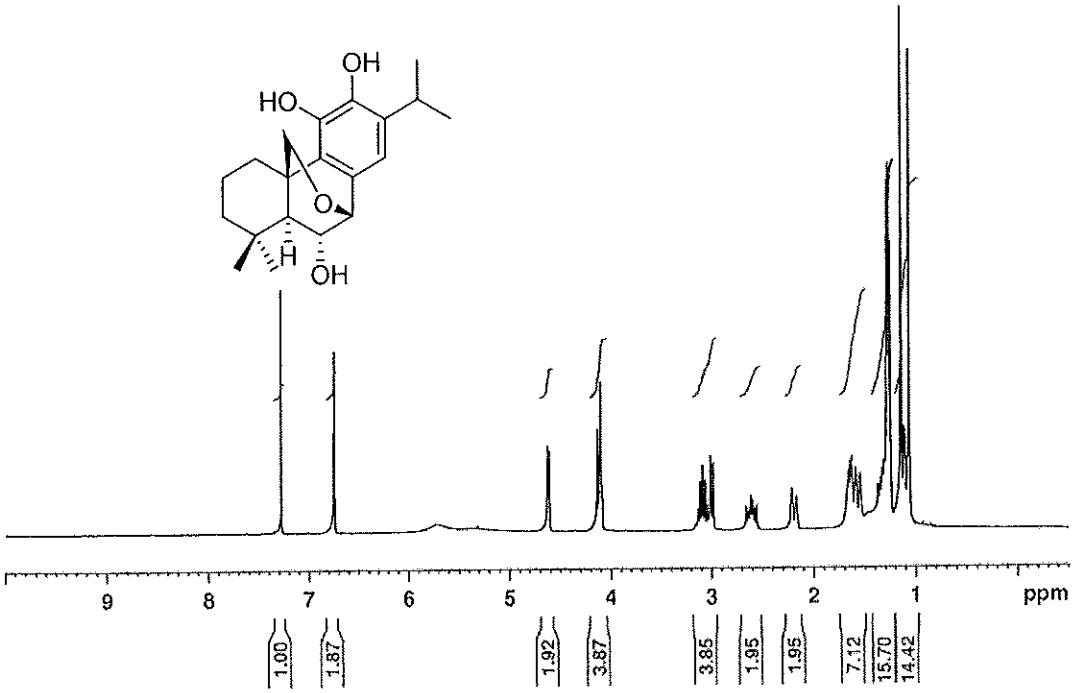

Figure 111 ^{1}H NMR (400 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **PO18**

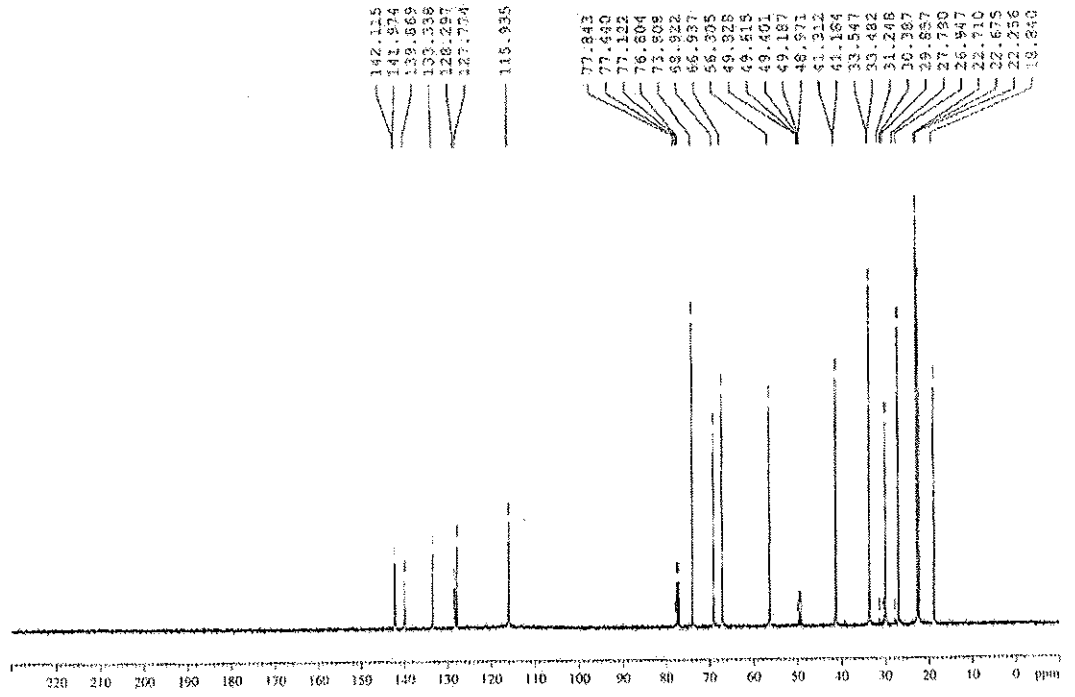

Figure 112 ^{13}C NMR (100 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **PO18**

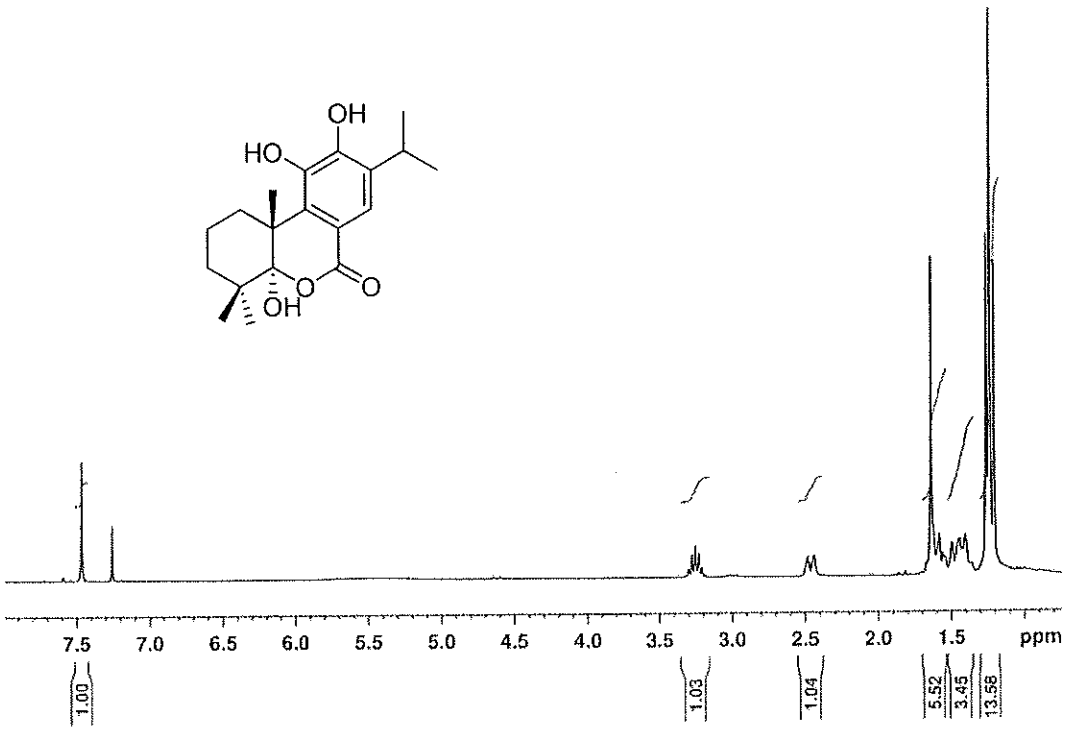

Figure 113 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO19**

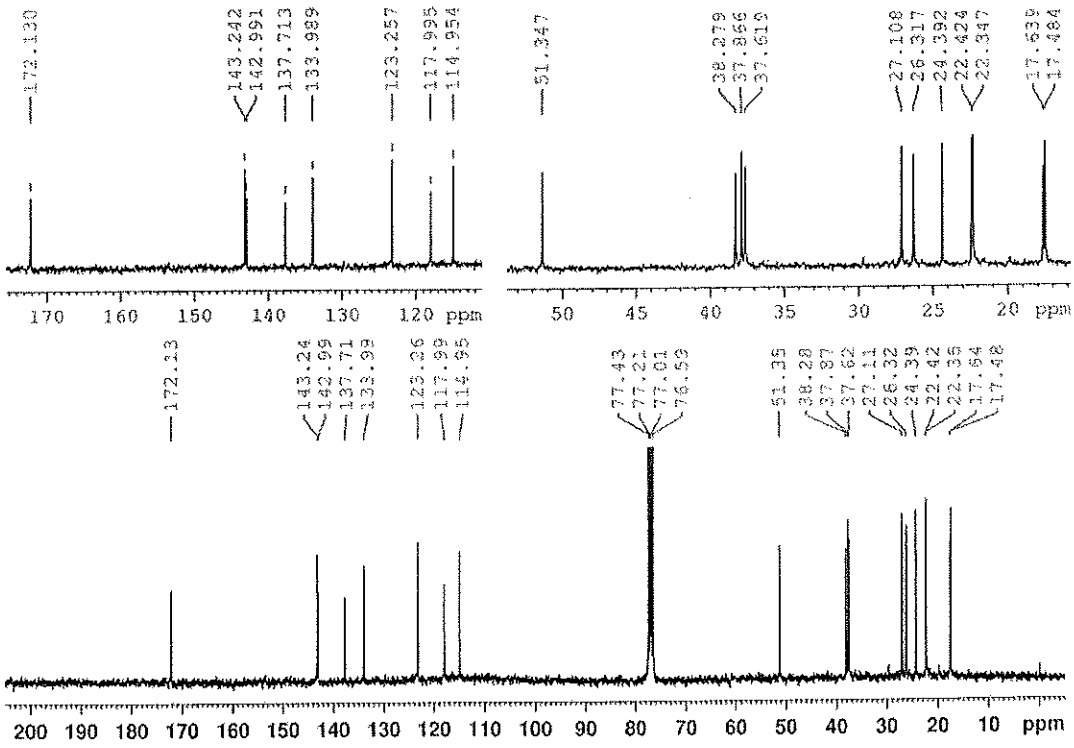

Figure 114 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO19**

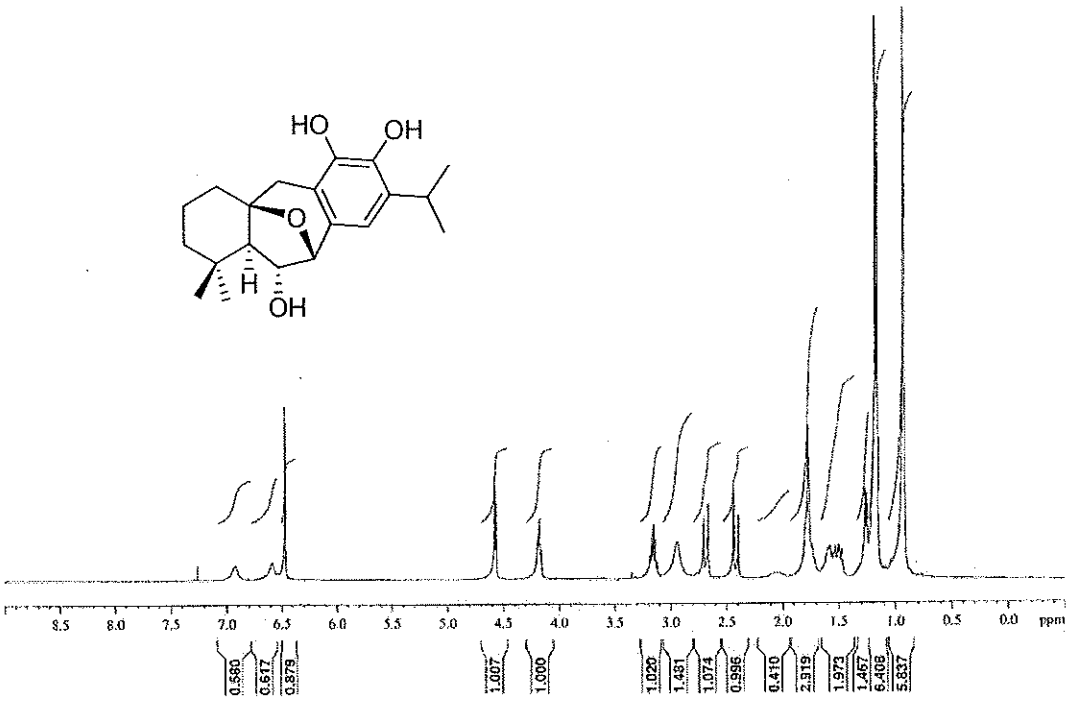

Figure 115 ^{1}H NMR (400 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **PO20**

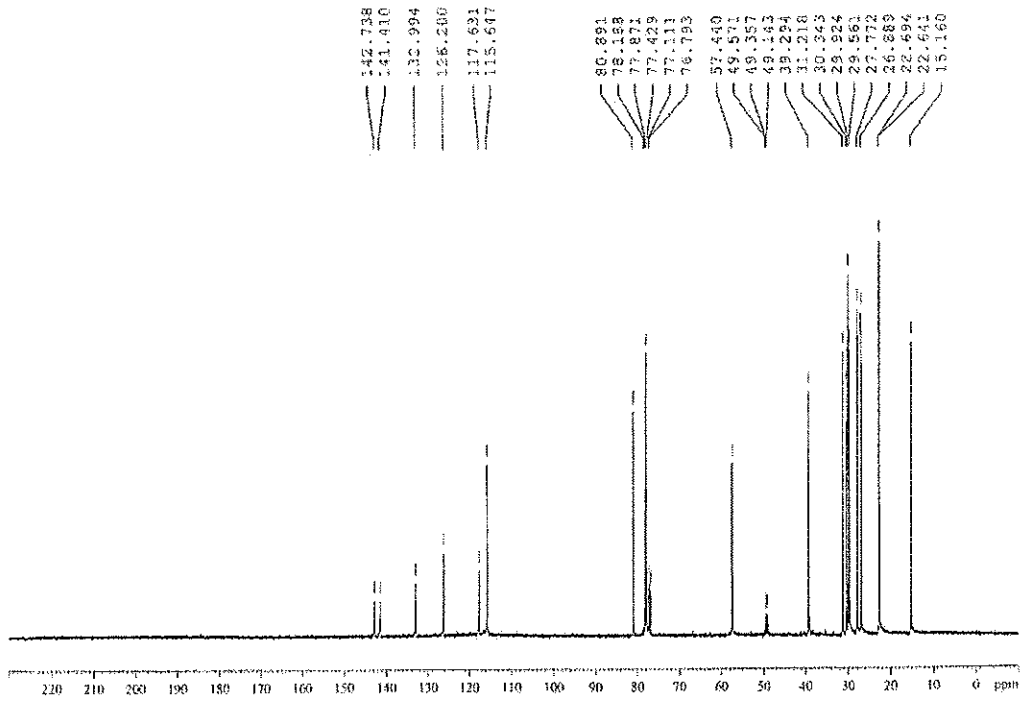

Figure 116 ^{13}C NMR (100 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **PO20**

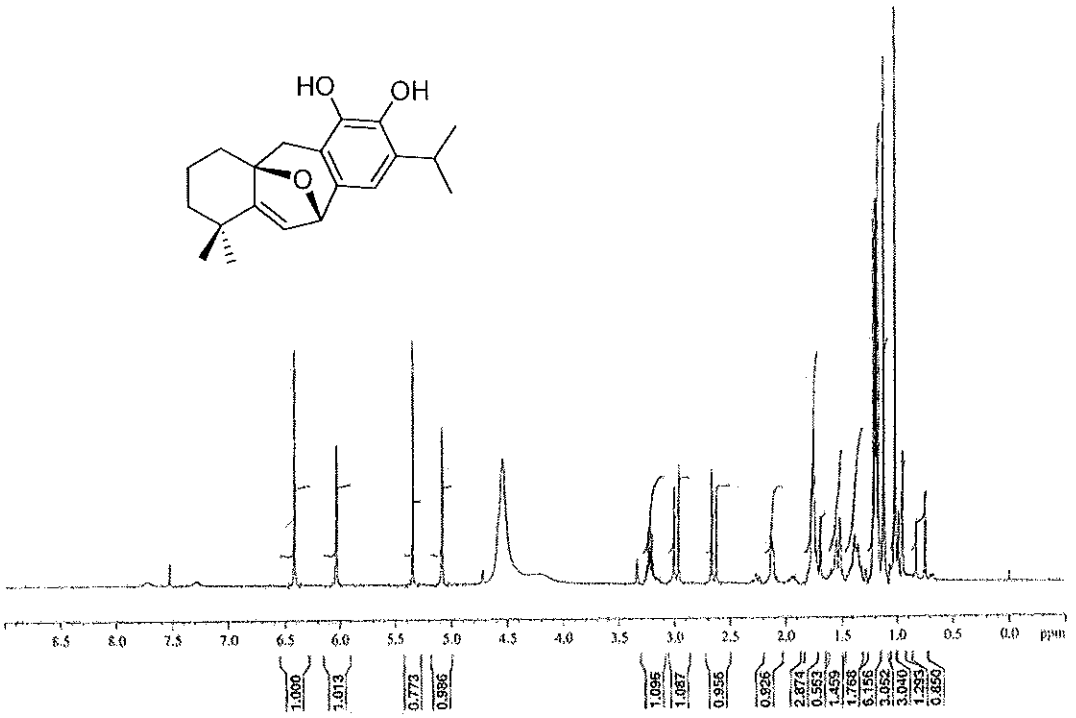

Figure 117 ^{1}H NMR (400 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **PO21**

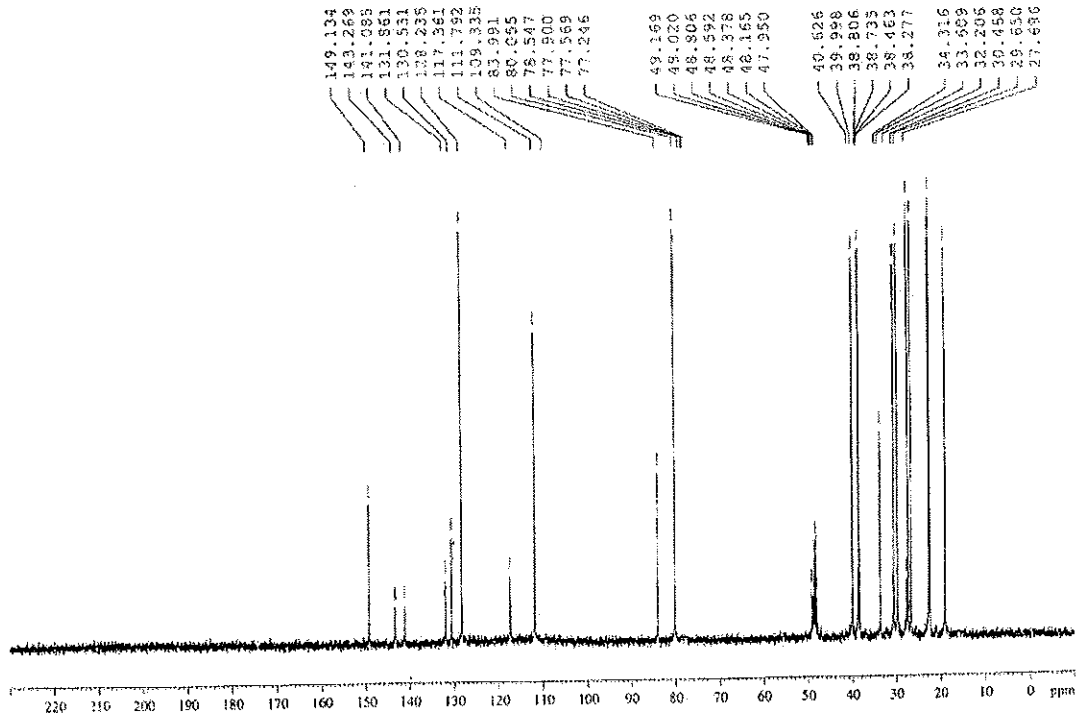

Figure 118 ^{13}C NMR (100 MHz) ($CDCl_3$+CD_3OD) spectrum of compound **PO21**

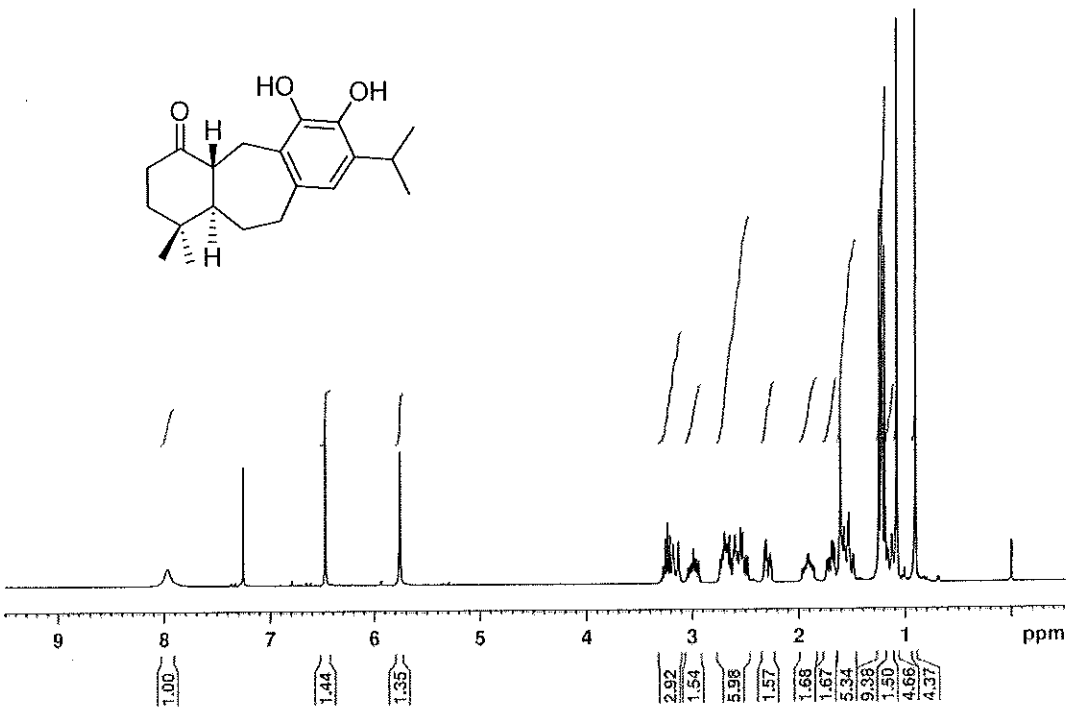

Figure 119 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO22**

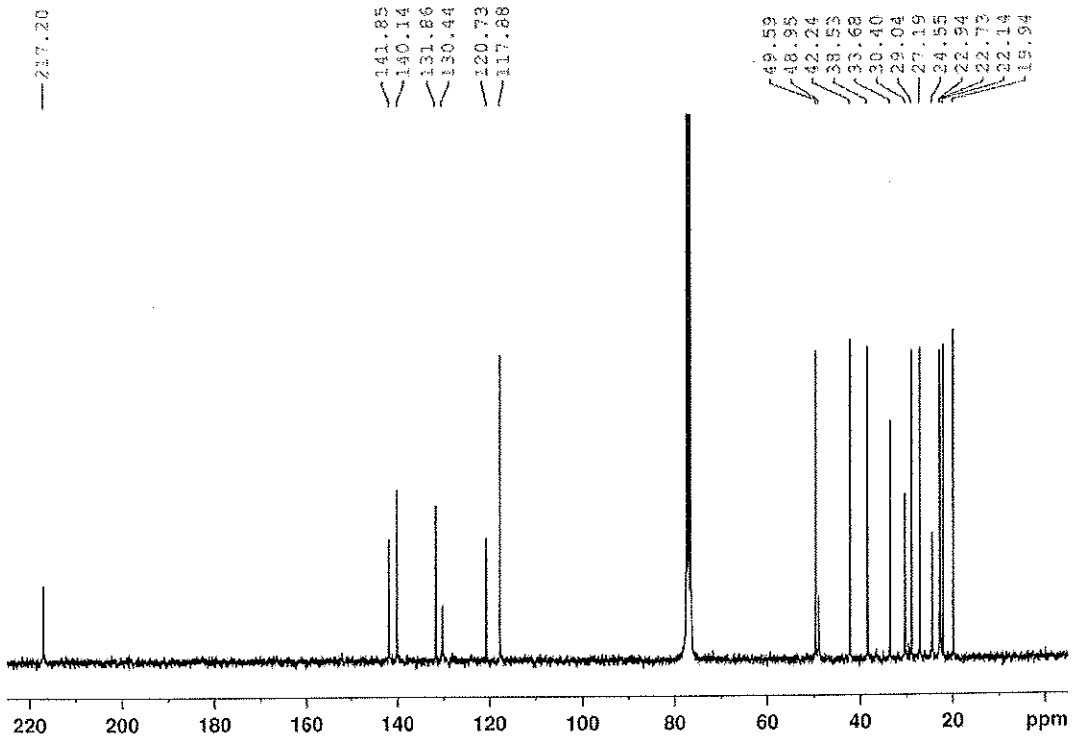

Figure 120 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO22**

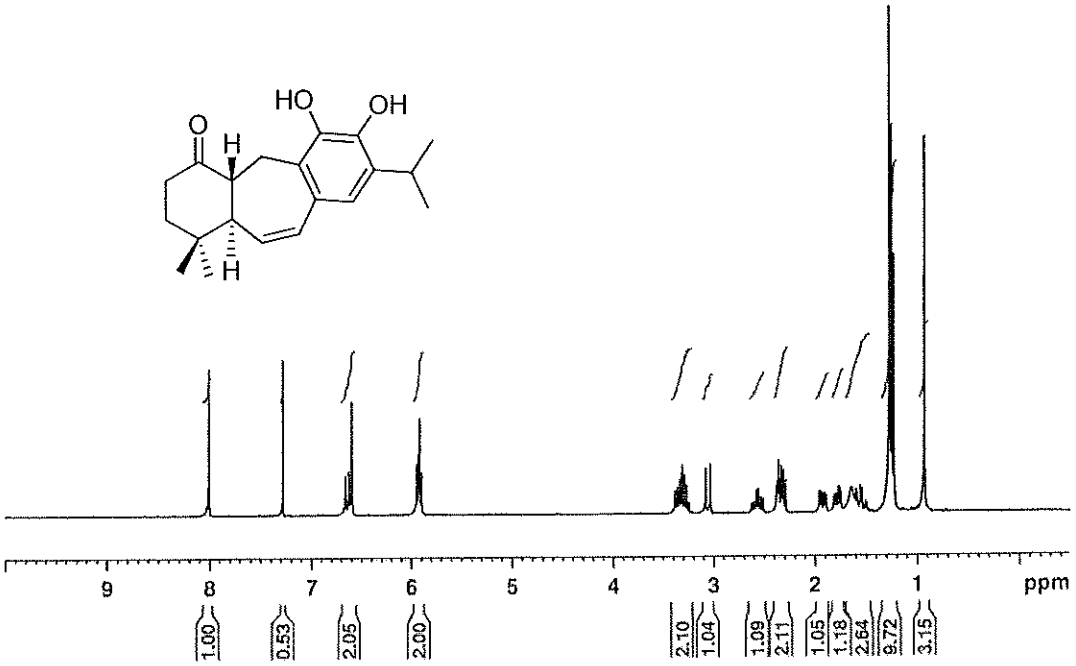

Figure 121 ^{1}H NMR (400 MHz) ($CDCl_3$) spectrum of compound **PO23**

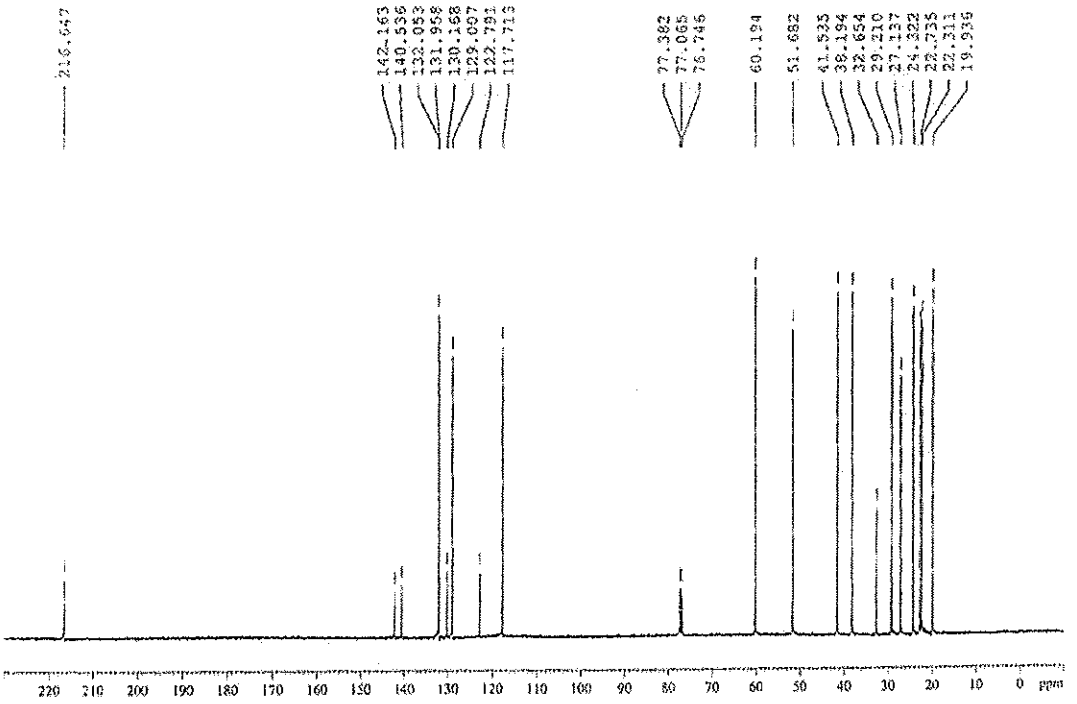

Figure 122 ^{13}C NMR (100 MHz) ($CDCl_3$) spectrum of compound **PO23**

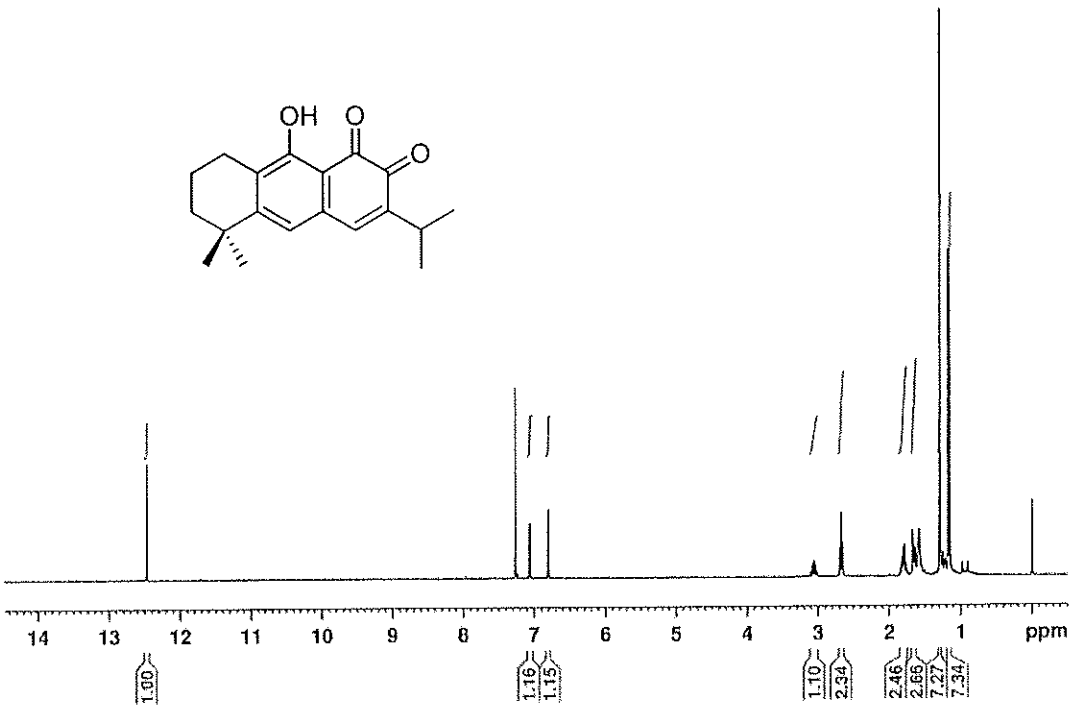

Figure 123 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO24**

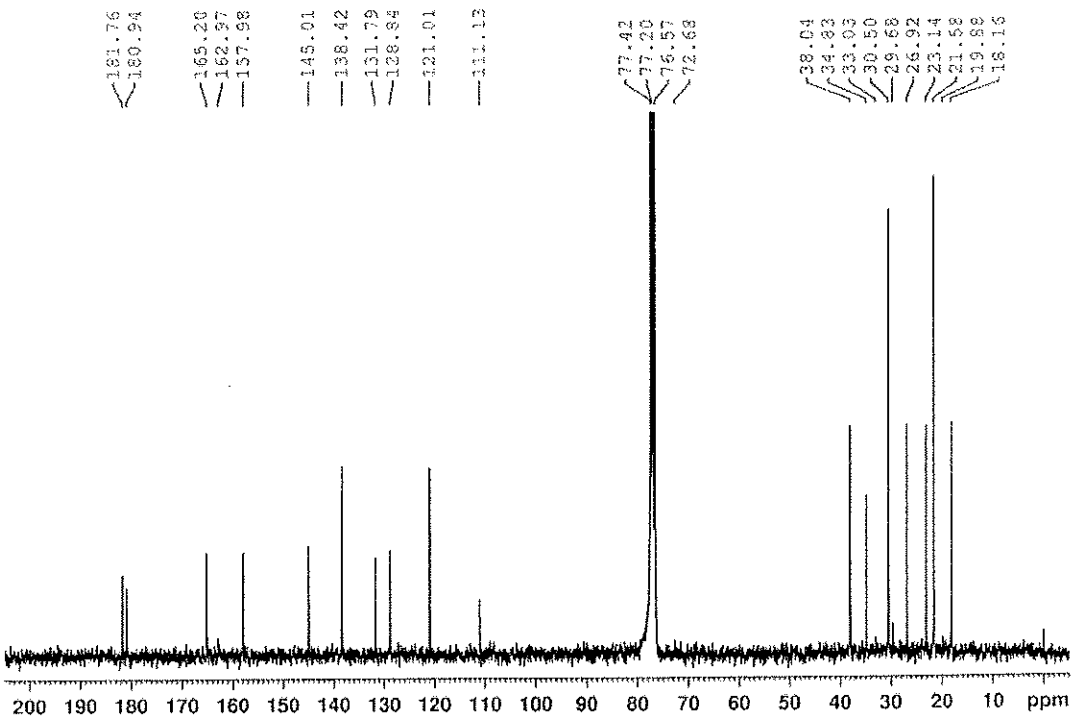

Figure 124 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO24**

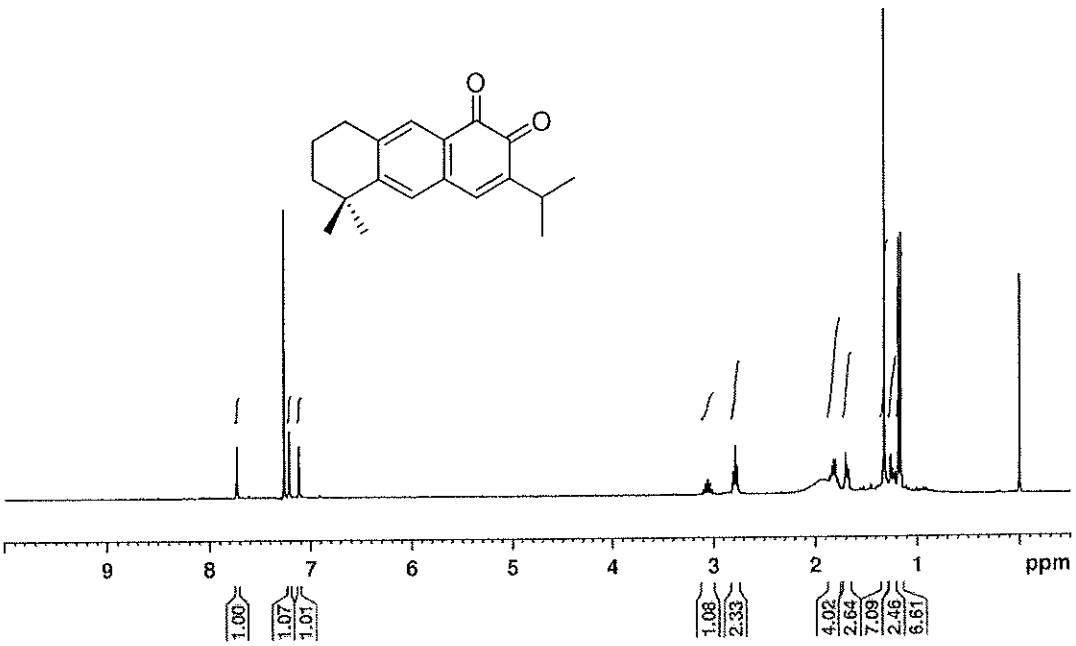

Figure 125 ^{1}H NMR (400 MHz) ($CDCl_3$) spectrum of compound **PO25**

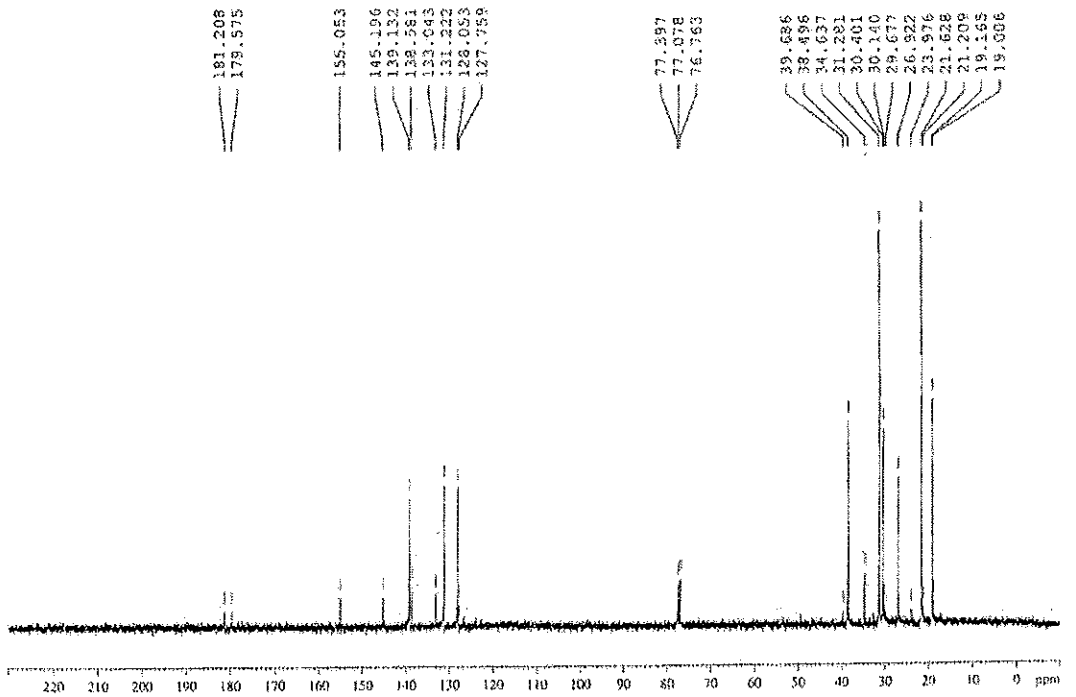

Figure 126 ^{13}C NMR (100 MHz) ($CDCl_3$) spectrum of compound **PO25**

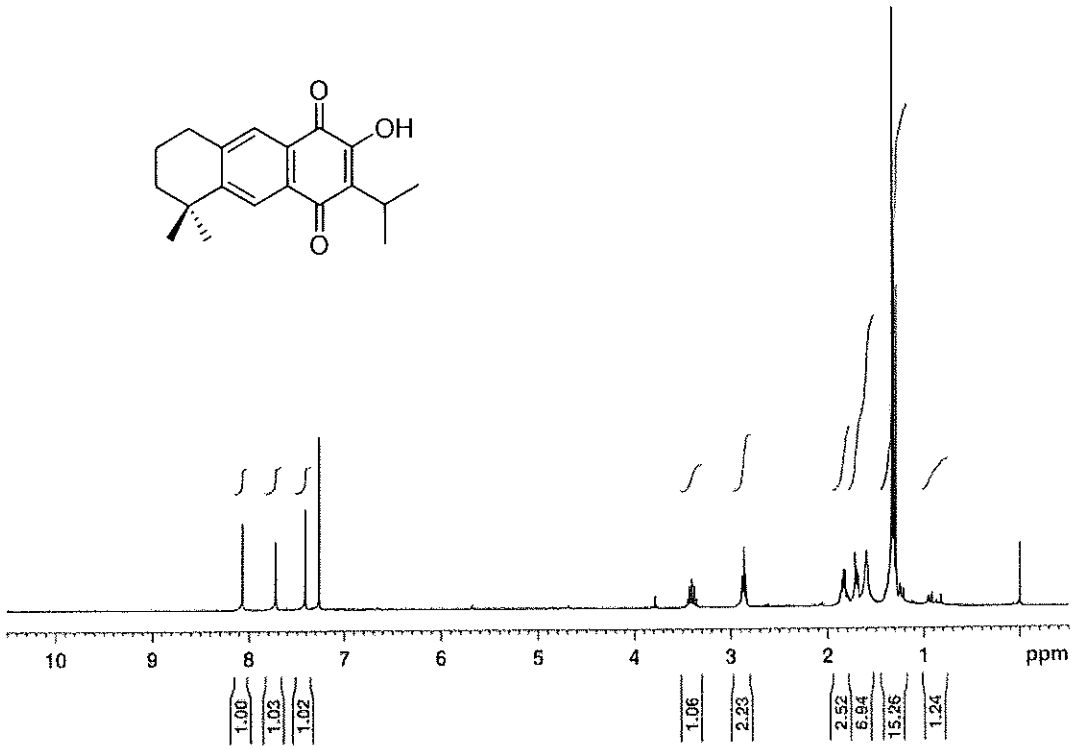

Figure 127 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO26**

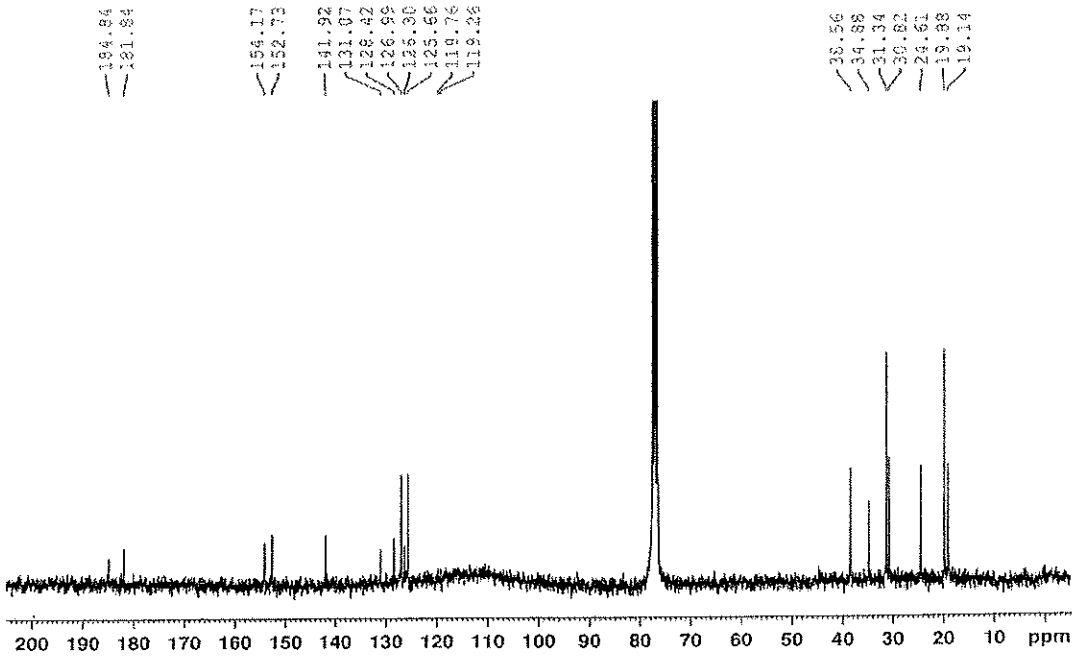

Figure 128 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO26**

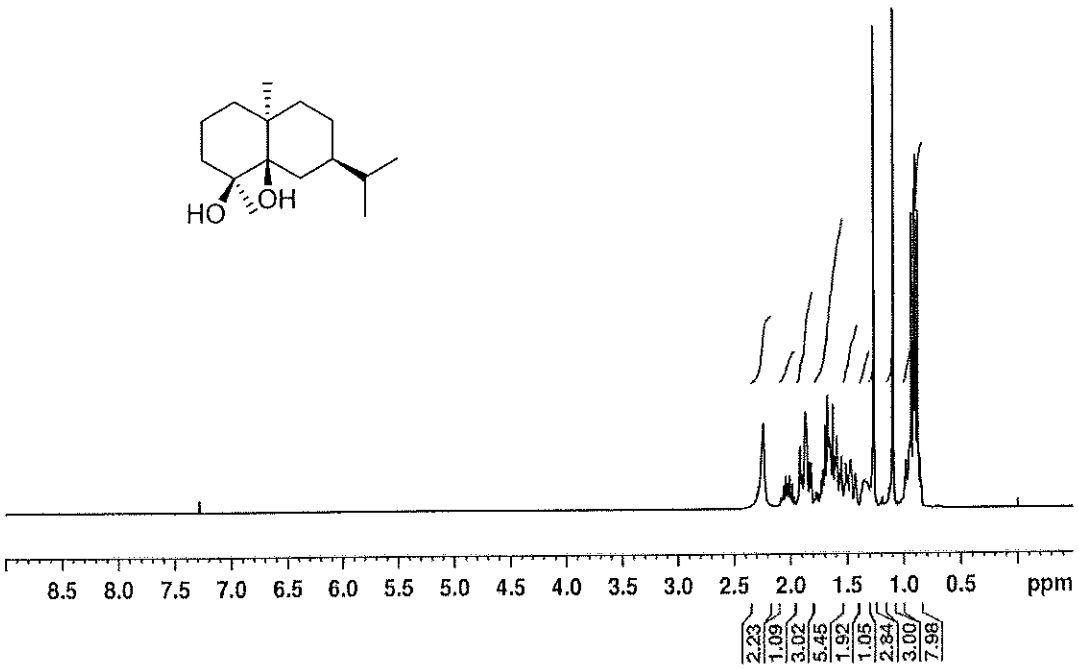

Figure 129 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO27**

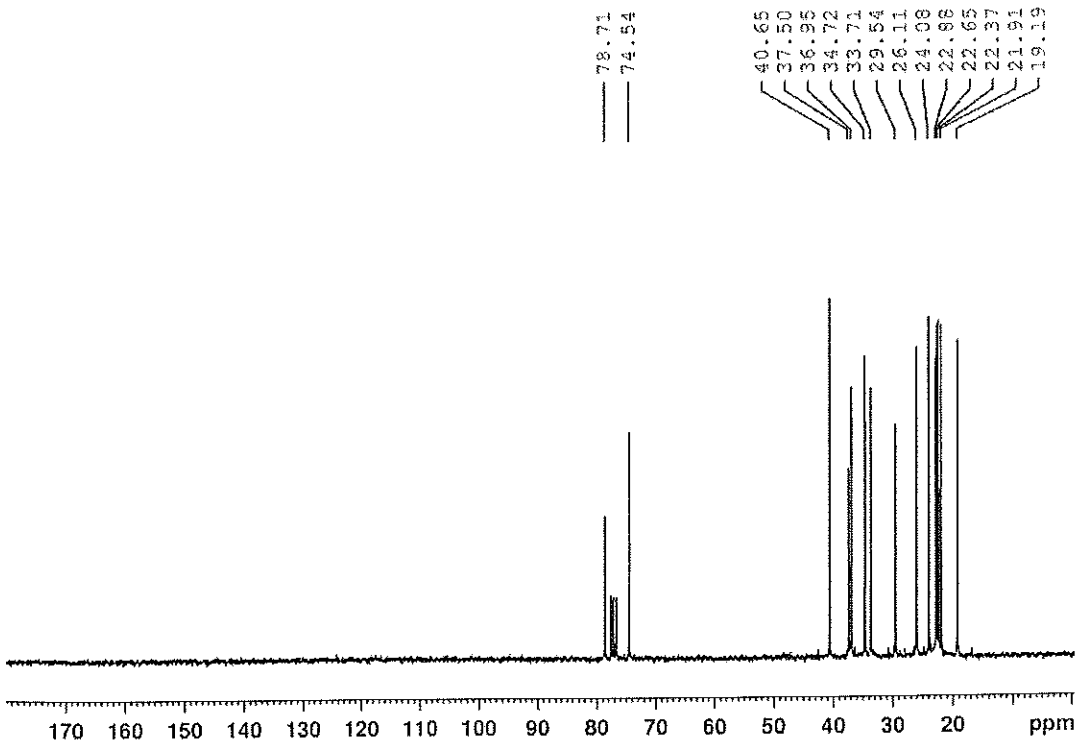

Figure 130 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO27**

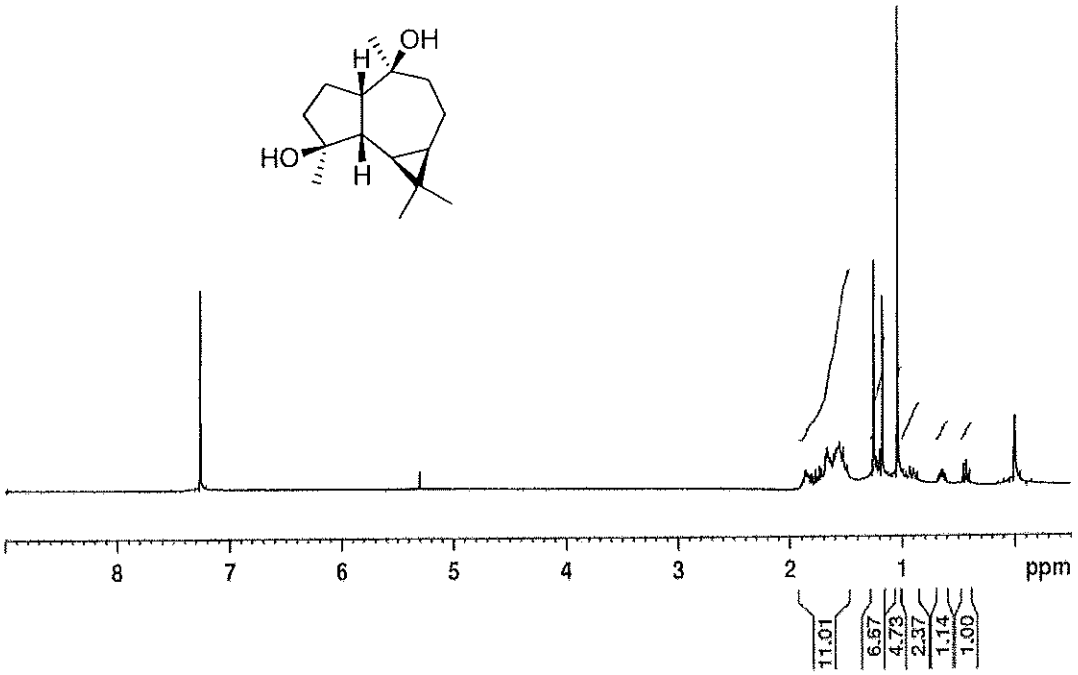

Figure 131 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO28**

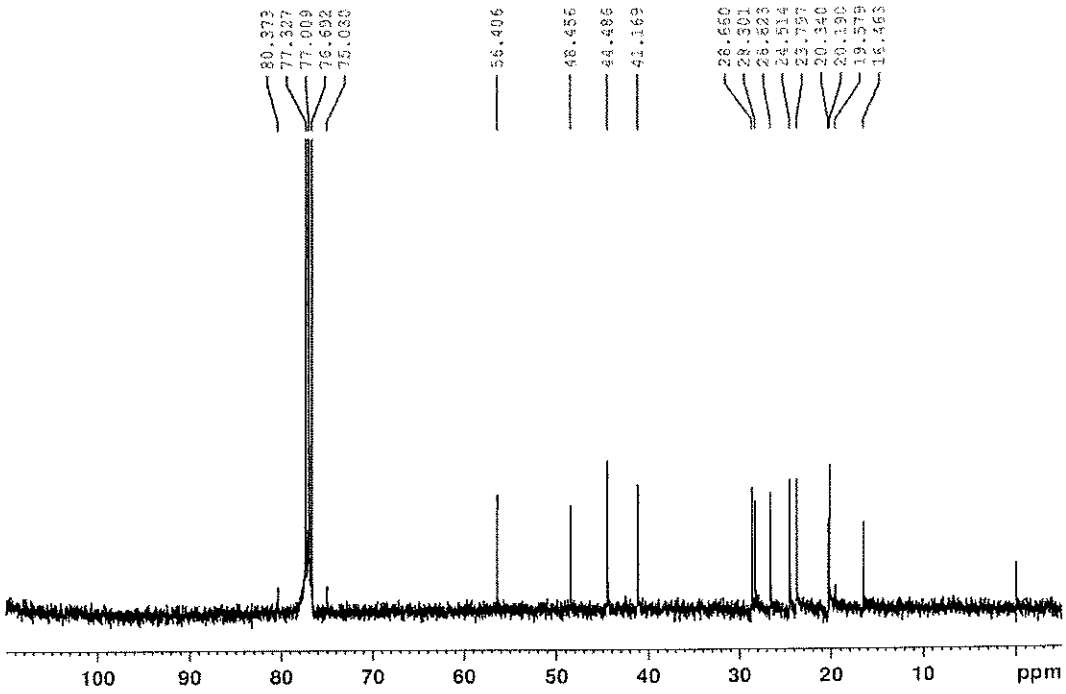

Figure 132 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO28**

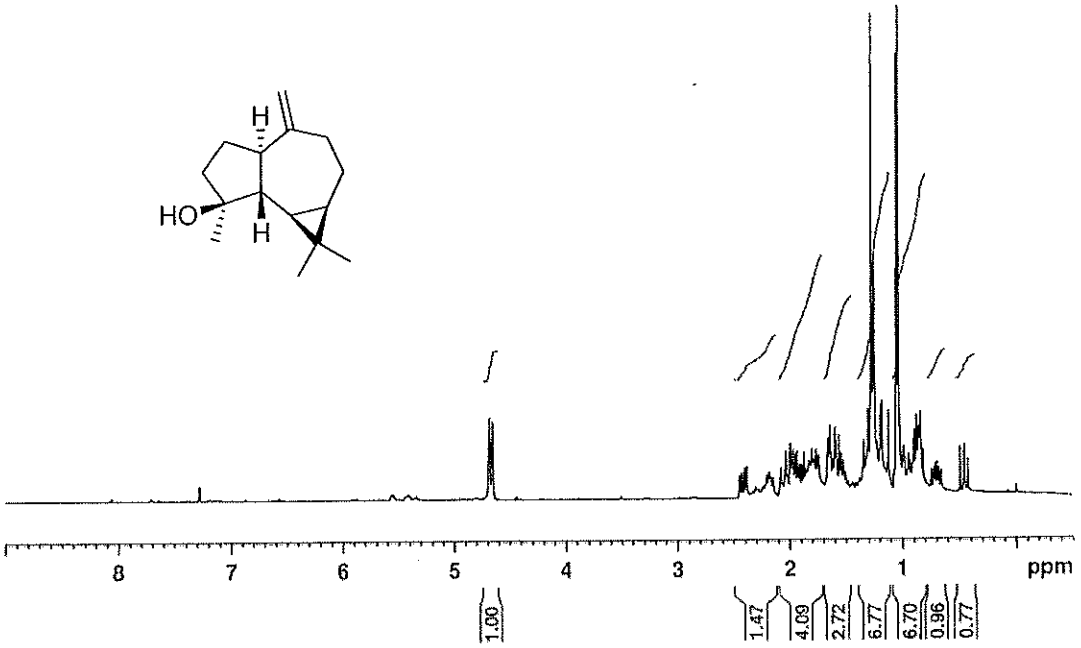

Figure 133 ^{1}H NMR (300 MHz) ($CDCl_3$) spectrum of compound **PO29**

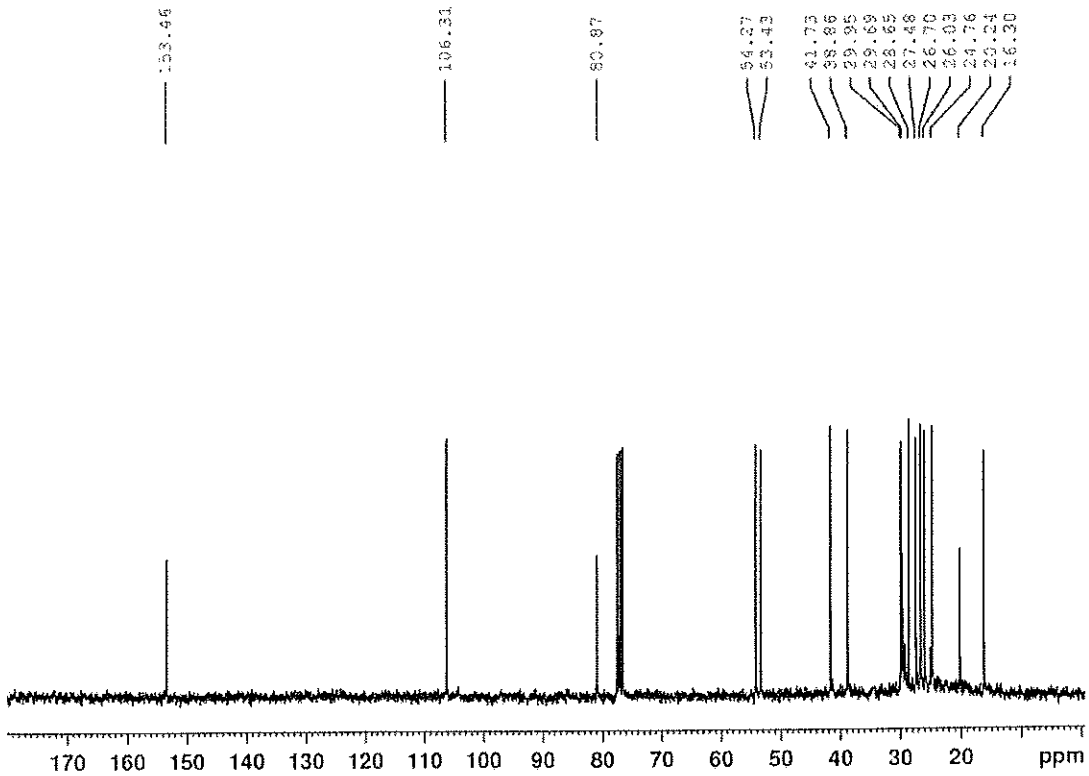

Figure 134 ^{13}C NMR (75 MHz) ($CDCl_3$) spectrum of compound **PO29**

VITAE

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Student ID 5010230030

Educational Attainment

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M. Sc. (Organic Chemistry)	Prince of Songkla University	2007

Scholarship Awards during Enrolment

1. The Center for Innovation in Chemistry, (PERCH-CIC), Commission on Higher Education, Ministry of Education
2. The Thesis Research Fund through the Graduate School, Prince of Songkla University

List of Publication and Proceeding

Publications

Salae, A. W.; Chantrapromma, S.; Hoong Kun, F.; Karalai, C. 2009. 6-Hydroxysalvinolone, Acta Cryst., E65, o2379–o2380.

Salae, A. W.; Karalai, C.; Ponglimanont, C.; Kanjana-Opas, A.; Yuenyongsawad, S. 2010. Naphthalene derivatives from *Diospyros wallichii*, Can. J. Chem., 88, 922–927.

Salae, A. W.; Rodjun, A.; Karalai, C.; Ponglimanont, C.; Chantrapromma, S.; Kanjana-Opas, A.; Tewtrakul, S.; Hoong-Kun, F. 2012. Potential anti-inflammatory diterpenes from *Premna obtusifolia*, Tetrahedron, 68, 819–829.

Proceedings

Salae, A. W.; Karalai, C.; Ponglimanont, 2008. Chemical constituents from the roots of *Diospyros wallichii*. The 6th Regional IMT-GT UNINET Conference. The Gurney Resort Hotel & Residences, Penang, Malaysia. 28-30 August 2008. (oral presentation)

Salae, A. W.; Karalai, C.; Ponglimanont, 2008. "Naphthoquinones and lupine-type triterpenoids from the roots of *Diospyros wallichii*". The 34th Congress on Science and Technology of Thailand, Queen Sirikit National Convention Center, Bangkok, Thailand, October 31- November 2, 2008. (poster presentation)

Salae, A. W.; Karalai, C.; Ponglimanont, 2009. "Chemical constituents from the roots of *Diospyros wallichii*", PERCH Conference VI. Jomtein Palm Beach Resort, Pattaya, Chonburi, Thailand, 3-6 May, 2009. (poster presentation)

Salae, A. W.; Karalai, C.; Ponglimanont, 2011. "Diterpenoids from the roots of *Premna obtusifolia*", PERCH Conference VII. Jomtein Palm Beach Resort, Pattaya, Chonburi, Thailand, 4-7 May, 2011. (poster presentation)