Chemical Constituents from the Stem Bark of *Oroxylum indicum* (L.) Benth. ex Kurz.

Saowanee Maungjunburee

A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title Chemical Constituents from the Stem Bark of *Oroxylum indicum* (L.) Benth. ex Kurz.
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Major Program Chemical Studies

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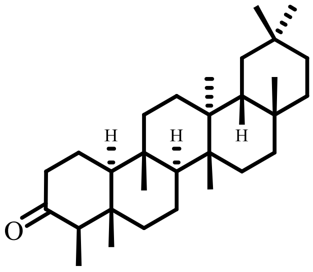
ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมีจากเปลือกต้นเพกา (*Oroxylum indicum* (L.) Benth. ex Kurz.)
ผู้เขียน	นางสาวเสาวณีย์ เมืองจันทร์บุรี
สาขาวิชา	เคมีศึกษา
ปีการศึกษา	2553

บทคัดย่อ

การศึกษาองค์ประกอบทางเคมีของเปลือกต้นเพกา (*Oroxylum indicum* (L.) Benth. ex Kurz) แยกได้สารประกอบที่มีรายงานการวิจัยแล้ว 15 สาร เป็นสารกลุ่ม flavonoids 9 สารคือ tectochrysin **(SM4)**, luteolin **(SM5),** 5,7-dihydroxy-3-methoxyflavone **(SM6)**, chrysin **(SM8)**, galangin **(SM9)**, apigenin **(SM10)**, 5,7,4'-trihydroxy-3-methoxyflavone **(SM12)**, kaempferol **(SM13)** และ scutellarein **(SM14)**, triterpenoids 2 สาร คือ friedelin **(SM1)** และ betulinic acid **(SM2),** isocoumarin 1 สาร คือ (*R*)-(-)-mellein **(SM3)**, benzofuranone 1 สาร คือ rengyolone **(SM7)** และอนุพันธ์ของกรดเบนโซอิก 2 สาร คือ 4-hydroxybenzoic acid **(SM11)** และ 3,4-dihydroxybenzoic acid **(SM15)** โครงสร้างของสารประกอบเหล่านี้วิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี UV IR NMR MS และ เปรียบเทียบกับสารที่มีรายงานการวิจัยแล้ว



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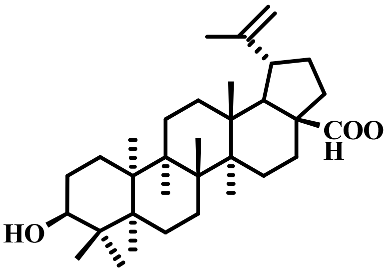

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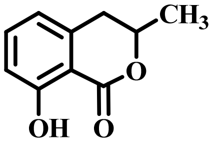

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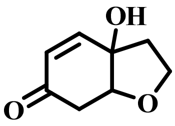

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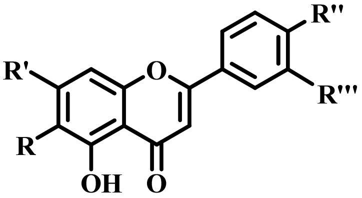
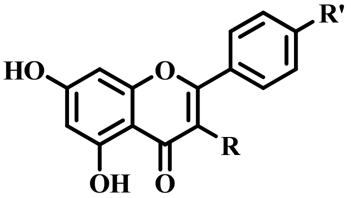
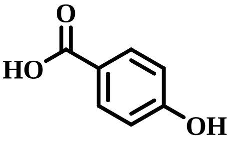


SM4 : R = R'' = R''' = H, R' = OMe
SM5 : R = H, R' = R'' = R''' = OMe
SM8 : R = R'' = R''' = H , R' = OH
SM10 : R = R''' = H, R' = R'' = OH
SM14 : R = R' = R'' = OH, R''' = H



SM6 : R = OMe, R' = H
SM9 : R = OH, R' = H
SM12 : R = OMe, R' = OH
SM13 : R = R' = OH



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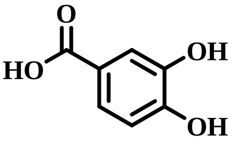

SM15

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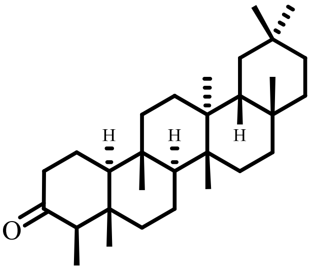
ABSTRACT

Investigation of the chemical constituents from the stem bark of *Oroxylum indicum* (L.) Benth. ex Kurz. yielded fifteen known compound; nine flavonoids: tectochrysin **(SM4)**, luteolin **(SM5)**, 5,7-dihydroxy-3-methoxyflavone **(SM6)**, chrysin **(SM8)**, galangin **(SM9)**, apigenin **(SM10)**, 5,7,4'-trihydroxy-3-methoxyflavone **(SM12)**, kaempferol **(SM13)** and scutellarein **(SM14)**, two triterpenoids: friedelin **(SM1)** and betulinic acid **(SM2)**, one isocoumarin: (*R*)-(-)-mellein **(SM3)**, one benzofuranone: rengyolone. **(SM7)** and two benzoic acid derivatives: 4-hydroxybenzoic acid **(SM11)** and 3,4-dihydroxybenzoic acid **(SM15)**, Their structures were determined on the basis of UV, IR, NMR MS and by comparison their spectroscopic data with those reported.



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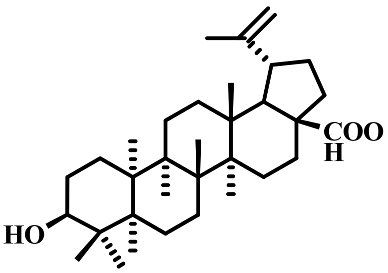

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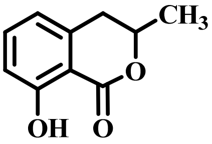

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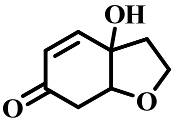

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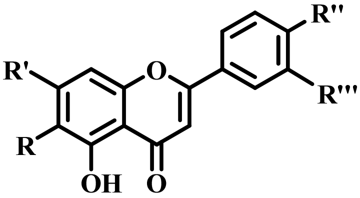

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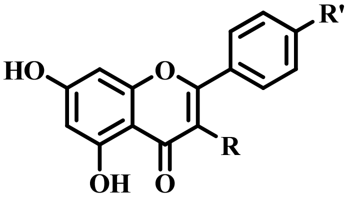

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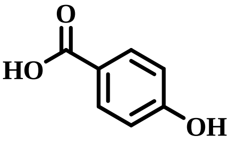

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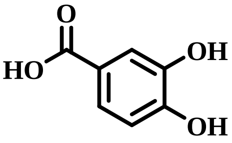

SM15

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Saowanee Maungjunburee

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this research is to investigate the chemical constituents of *Oroxylum indicum* (L.) Benth. ex Kurz.. It is a part of the basic research on the utilization of Thai medicinal plants. This research will contribute significantly to scientific basis of traditional medicine. Sixteen pure compounds have been isolated from this plant. Some of the compounds showed strong antibacterial activity. Moreover, many compounds of flavonoids type have been reported to have cytotoxic, anti-imflammatory, antimicrobial and antioxidation activities. So, further study on the biological activity of the isolated compounds should be performed which can lead to active compounds. Therefore Thai plant can be utilized as a natural resource of potential drugs.

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LIST OF ABBREVIATIONS AND SYMBOLS

s = singlet
d = doublet
t = triplet
m = multiplet
dd = doublet of doublet
dt = doublet of triplet
td = triplet of doublet
ddd = doublet of doublet of doublet
br s = broad singlet
g = gram
kg = kilogram
mg = milligram
% = percent
nm = nanometer
mp. = melting point
cm^{-1} = reciprocal centimeter (wave number)
δ = chemical shift relative to TMS
J = coupling constant
λ_{max} = maximum wavelength
ν = absorption frequencies
ε = molar extinction coefficient
$^{\circ}C$ = degree celcius
MHz = megahertz
ppm = part per million
IR = Infrared
UV = Ultraviolet-Visible
NMR = Nuclear Magnetic Resonance
2D NMR = Two Dimentional Nuclear Magnetic Resonance
COSY = Correlated Spectroscopy

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

DEPT = Distortionless Enhancement by Polarization Transfer
HMBC = Heteronuclear Multiple Bond Correlation
HMQC = Heteronuclear Multiple Quantum Coherence
CC = Column chromatography
TMS = Tetramethylsilane
Acetone-d_6 = Deuteroacetone
DMSO-d_6 = Deuterodimethylsulphoxide
$CDCl_3$ = Deuterochloroform
MeOH = Methanol
CH_2Cl_2 = Dichloromethane
TLC = Thin-Layer Chromatography
MIC = Minimum Inhibition Concentration

CHAPTER 1
INTRODUCTION

1.1 Introduction

The family Bignoniaceae, comprises about 120 genus and 800 species, growing mainly in Africa and Central and south America. (Spichiger, 2004). Twenty-four genus have been found in Thailand, they are shown as follows: *Campsis, Crescentia, Dolichandrone, Fernandoa, Heterophragma, Jacaranda, Kigelia, Mansoa, Markhamia, Millingtonia, Nyctocalos, Oroxylum, Pajanelia, Pandorea, Pauldopia, Podranea, Pyrostegia, Radermachera, Santisukia, Saritaea, Spathodea, Stereospermum, Tabebuia, Tecoma* (Smitinand, 1980).

Oroxylum indicum (L.) Benth. ex Kurz is in *Oroxylum* Genus, (Bignoniaceae), also locally known as “Pa-ka” or “Sonapatha” is a widely used medicinal plant in south Asia, southeast Asia and China. It is a commonly used herbal medicine in Ayurvedic system. Roots, leaves and stems of *O. indicum* have been used as a single drug or as a component of certain compound drug preparations in the Indian. Ayurvedic system of medicine have used the plant for treatment of various disorders as well as used as a tonic and Rasayana drug (Anonymous *et al*., 1998), in Chinese medicine for curing stomach disorders, diarrhea, dysentery and rheumatic swelling. In Thailand, the fruits and flowers are consumed as a common part of the diet in the north and northeastern areas (Mitsuru *et al.,* 2001). The plant has been reported to possess anti-inflammatory, diuretic, anti-arthritic, antifungal and antibacterial activity (Warrier *et al*., 1995). Roots are used for the treatment of tuberculosis (Bhattacharja *et al.,* 2000) dropsy (Naomita *et al.,* 2004) and nasopharyngeal cancer (Mao *et al.,* 2002). The fruits are used in treating bronchitis, leucoderma, and helminthosis (Parrotta *et al.,* 2001 and Dalal *et al.,* 2004). It is used as an astringent, carminative, diuretic, stomachic, and aphrodiasic and is valued for stimulating digestion, curing fevers, coughs and other respiratory disorders (John *et al.,* 2001).

The stem bark and leaves of this plant is reported to contain flavonoids namely, chrysin, oroxylin-A, scutellarin, baicalein (Sankara *et al.,* 1972). Seeds of this plant are reported to contain ellagic acid (Vasanth *et al.,* 1991). Various other effects like antibacterial, analgesic and gastro-protective properties of "Pa-ka" have also been reported. It is a tree that is found generally in damp region. In the present review an attempt has been made to compile and critically analyse various published reports on *O. indicum*.

O. indicum is a medium sized tree growing 8-12 meters in height. The bark is grayish brown in color with corky lenticels. The leaves, very large, 0.5-1.5 meter in length, 2-3 pinnate, leaflets 12 cm long and 8 cm broad. The flowers are reddish-purple outside and pale, spinkish-yellow within, numerous, in large erect racemes. The fruits are flat capsules, 0.33-1 meter long and 5-10 cm broad, sword-shaped. The seeds numerous, flat and winged all around, except at the base. The plant flowers in June-July and bears fruits in November.

1.2 Review of Literatures

1.2.1 The Chemical Constituents and Biological Activity of the family Bignoniaceae

Known phytochemistry of Bignoniaceae consist of flavonoids, iridoids, phenylpropanoid, triterpenoids, anthraquinones, naphroquinones etc. *Crescentia* genera have been reported in the chemical composition of *Crescentia cujete* and *Crescentia alata*. Both plants contained the iridoid compounds. The constituents of the fruits of *C. cujete* were reported that found 16 iridoids including 8 new compounds, name crescentins I-V and crescentosides A-C. The fruits of this plant have been used as a Vietnamese folk medicine, as an expectorant, antitussive, laxative and for stomach disorders (Kaneko *et al*., 1997). Four new iridoids were isolated from the pulp of the fruits of *C. alata* (Valladares *et al*., 2007). The pulp of the fruits from this plant has been used to relieve different respiratory infections, cough, asthma, bronchitis, tuberculosis, and breast pain (Argueta *et al.,* 1994).

There was only one report for the chemical composition of the genus *Dolichandrone*. Phenolic glycosides were isolated from the branches of *D. serrulata*. The flower of this plant has a bitter taste and has been used as a vegetable. The bark is used in Thai traditional medicine as an antifever and anti-inflammatory agent (Sinaphet *et al*., 2006).

Fernandoa adenophylla, known as “Khae-hang-khang”, have been used in Thai traditional medicine. The leaves are used for external treatment of skin diseases. A new lignan glycoside (fernandoside) and a new phenylpropanoid glycoside (2''-*O*-β-apiosylverbascoside) were isolated from the leaves and branches of *F. adenophylla* together with 12 known compounds (Kanchanapoom *et al*., 2001).

There were many reports in *Kigelia africana* which was a tropical tree used in African folk medicine. The fruits were used for emollient, anti-eczema, anti-psoriasis, and skin-firming properties and as dressing for ulcers and wounds. The root bark was also used for the treatment of venereal diseases, haemorrhoids, and rheumatism (Irvine, 1961; Oliver-Bever, 1986). Steroids, iridoids, and coumarins have been isolated from the root bark (Houghton *et al*., 1993) and flavonoids and iridoids from the fruit and leaves (Gouda *et al*., 2003). Dichloromethane extracts from the root and stem bark containing naphthoquinones showed anti-trypanosomal and anti-microbial activities and cytotoxicity against melanoma and renal carcinoma cells (Jackson *et al*., 2000; Moideen *et al*., 1999; Akunyili *et al*., 1991; Houghton *et al*., 1994). A polar extract of fruit from *K. africana* indicated the presence of verminoside as a major constituent, and of a series of polyphenols such as verbascoside. In vitro assays showed that verminoside had significant anti-inflammatory effects. Verminoside and verbascoside had also cytotoxic effect and cutaneous irritation (Picerno *et al*., 2005).

There are three plants in *Markhamia* genera found in Thailand. The roots of *M. obtusifolia* were used for the treatment of hookworm in parts of Tanzania (Chhabra and Mahunnah, 1994). Ursolic acid, pomolic acid and 2-*epi*-tormentic acid were isolated from the leaves of *M. obtusifolia* and found that pomolic acid was the most active compound with MIC value of 12.5 μg/ml for *Candida albicans* isolated from dog and 25.0 μg/m for *C. albicans* from cat (Nchu *et al*., 2010). Three new phenylpropanoid glycosides, named luteoside A-C were isolated together with the known compounds verbascoside and isoverbascoside from the roots of *M. lutea*. All five phenylpropanoid glycosides exhibited potent *in vitro* activity against respiratory syncytial virus (Kernan *et al*., 1998). The leaves of *M. lutea* exhibited significant *in vitro* anti-parasitic activity and low cytotoxicity against MRC5 and KB cells. The isolation of ethyl acetate extract of this plant led to three new cycloartane triterpenoids, musambins A-C, as well as three glycoside derivatives, musambiosides A-C, along with the known phaeophorbide A, *β*-sitosterol and pentacyclic triterpenes arjunic acid (Lacroix *et al*., 2009). The isolation of the leaves and branches of *M. stipulata* furnished five verbascoside derivatives (markhamiosides A-E), and one hydroquinone derivative (markhamioside F) along with 13 known compounds (Kanchanapoom *et al*., 2002).

Two anthraquinones, zenkequinone A and B were isolated from the stem bark of *Stereospermum zenkeri* together with six known compounds. Zenkequinone B showed the best antibacterial activity against gram-negative *Pseudomonas aeruginosa* (Lenta *et al*., 2007). *S. chelonoides* have previously been reported in antipyretic property and was also useful in excessive thirst, cough and asthma (Ghani *et al*., 1998). The isolation of this plant led to a new anthraquinone, stereochenol A, and a new naphthoquinone, stereochenol B along with sterekunthal B and sterequinone C (Haque *et al*., 2006). *S. cylindricum* is a Thai medicinal plant for antifever purposes, as well as an anti-inflammatory agent. A new lignan glycoside, a new phenolic diglycoside and a new iridoid glycoside, as well as 12 known compounds were isolated from the leaves and branches of this plant (Kanchanapoom *et al*., 2006). *S. personatum* is widely advocated as a diuretic, anti-inflammatory and in preparations for hemorrhoids, vescle calculi and for cardiotonic, diabetic, cancer, renal, and hyperacidity disease condition (Varier, 1994). Two new anthraquinones, sterequinones A and D and a new naphthoquinone, sterequinone E, along with a known naphthoquinone, sterekunthal B, have been isolated from the stem bark of S. personatum (Kumar *et al.,* 2003). The extraction of both stem and stem bark of this plant led to the isolation of free-radical-scavenging and xanthine oxidase inhibitory molecules along with three new anthraquinones, sterequinone F-H, a new naphthoquinones, sterequinone I, two new phenyl ethyl esters and a new 3,4,5-trimethoxycinnamyl ether, together with known compounds (Kumar *et al*., 2005). The use of *S. kunthianum* still plays an important role in malarial treatment (Gessler *et al.,* 1994; Benoit-Vical *et al*., 1998). Five novel antiplasmodial compounds were isolated from the root bark of this plant (Onegi *et al.,* 2002).

Tabebuia species can be found throughout central and south America. The bark of *T. impetiginosa* has been used as a folk medicine for treating diabetes, ulcers and syphilis (Hashimoto, 1996). The isolation of two cyclopentene dialdehydes from the bark of *T. impetiginosa* was reported. These compounds showed anti-inflammatory activity (Koyama *et al.,* 2000). The isolation of MeOH extract the bark of *T. impetiginosa* isolate nineteen glycosides, consisting of four iridoid glycosides, two lignan glycosides, two isocoumarin glycosides, three phenylethanoid glycosides and eight phenolic glycosides. Sixteen in nineteen compounds were determined as the new compounds (Warashina *et al.,* 2004). Further study on the constituents from the bark of this plant afforded eleven compounds, consisting of four iridoid glycosides, one phenylethanoid glycoside, five phenolic glycosides, and one lignan glycoside, along with seven known compounds (Warashina *et al.,* 2005). Thirteen new phenolic glycosides were obtained by the study of constituents from the bark of this plant (Warashina *et al*., 2006). From the extraction of *T. avellanedae,* two new iridoids and a new phenylethanoid glycoside have been isolated together with twelve known compounds. The isolated compounds inhibited nitric oxide production in lipopolysaccharide-activated macrophage-like J774.1 cells (Awale *et al*., 2005). Before that, the separation by HPLC method of the inner bark of this plant isolate nine naphthoquinones (Steinert *et al.,* 1995). *T. ochracea* is native to tropical America plant. Its stem bark has been used for many years as an antimalarial and for its healing action on ulcers (Gentry, 1982; Bernal, 1989). Fractionation of the inner stem bark of this plant led to the isolation of two new naphtho[2,3-*b*]furan-4,9-diones together with four known naphthofurandiones (Diaz and Medina, 1996).

Flavones were the major components isolated from the stem and root bark of *O. indicum* genus contains oroxylin A, baicalein, chrysin, scutellarin-7-rutinoside, traces of alkaloid, tanic acid and sitosterol, and is used for treating rheumatism (Subramanian *et al*., 1972). Baicalein is reported to possess an anti-inflammatory, antiulcer, antioxidant, hepatoprotective and immunomodulatory activity, while chrysin and baicalein both are reported to have antibacterial, antifungal and antiviral activity. Furthermore, biochanin-A possesses anti-fungal action and tumor necrosis factor-α. Ellagic acid is an important polyphenolic compound (Lien *et al.,* 2003). Most plant extracts are composed of complex phytoconstituents. Moreover, the seeds are purgative and show the presence of terpenes, alkaloids, and saponins (Bhattacharje *et al*., 2000). The chemical constituents isolated from the family Bignoniaceae which have been found in Thailand were shown below (The literature survey from SciFinder Scholar databases).

Table 1 Compounds from the Bignoniaceae family

Genus	Compound	No.	Reference
Crescentia			
C. alata			
pulp	6*β*,7*β*,8*α*,10-tetrahydroxy-*cis*-2-oxabicyclo[4.3.0]nonan-3-one	**1**	Valladares *et al.*, 2007
	6*β*,7*β*,8*α*,10-tetra-*p*-hydroxybenzoyl-*cis*-2-oxabicyclo [4.3.0]nonan-3-one	**2**	
	1*β*,6*β*,7*α*,8*α*,10-pentahydroxy-*cis*-2-oxabicyclo[4.3.0]nonane	**3**	
	6*β*-hydroxy-2-oxabicyclo[4.3.0]Δ^{8-9}-nonen-1-one	**4**	
C. cujete			
fruits	agnuside	**5**	Kaneko *et al.*, 1997
	ajugol	**6**	
	aucubin	**7**	
	5,7-bisdeoxycynanchoside	**8**	
	crescentin I	**9**	
	crescentin II	**10**	
	crescentin III	**11**	
	crescentin IV	**12**	
	crescentin V	**13**	
	crescentoside A	**14**	
	crescentoside B	**15**	
	crescentoside C	**16**	
	6-*O*-*p*-hydroxybenzoyl-6-epiaucubin	**17**	
	6-*O*-*p*-hydroxybenzoylajugol	**18**	
	ningpogenin	**19**	

Table 1 (continued)

Genus	Compound	No.	Reference
Dolichandrone			
D. serrulata			
branches	2"-*O*-apiosylverbascoside	**20**	Sinaphet *et al.*, 2006
	decaffeoylverbascoside	**21**	
	dolichandroside	**22**	
	isoverbascoside	**23**	
	ixoside	**24**	
	luteoside B	**25**	
	markhamioside A	**26**	
	verbascoside	**27**	
Fernandoa			
F. denophylla			
leaves and branches	2"-*O*-apiosylverbascoside	**20**	Kanchanapoom *et al.*, 2001
	decaffeoylverbacoside	**21**	
	fernandoside	**28**	
	isoverbascoside	**23**	
	(+)-lyoniresinol 3*a*-*O*-*β*-glucoside	**29**	
	salidroside	**30**	
	verbascoside	**27**	
Kigelia			
K. africana			
fruits	verbascoside	**27**	Picerno *et al.*, 2005
	verminoside	**31**	

Table 1 (continued)

Genus	Compound	No.	Reference
Markhamia			
M. lutea			
roots	isoverbascoside	**23**	Kernan *et al.*, 1998
	luteoside A	**32**	
	luteoside B	**33**	
	luteoside C	**34**	
	verbascoside	**27**	
M. obtusifolia			
leaves	pomolic acid	**35**	Nchu *et al.*, 2010
	epi-tormentic acid	**36**	
	ursolic acid	**37**	
M. stipulata			
leaves and branches	ajugol	**6**	Kanchanapoom *et al.*, 2002
	2''-*O*-apiosylverbascoside	**20**	
	decaffeoylverbascoside	**21**	
	isoverbascoside	**23**	
	luteoside A	**32**	
	luteoside B	**33**	
	markhamioside A	**38**	
	markhamioside B	**39**	
	markhamioside C	**40**	
	markhamioside D	**41**	
	markhamioside E	**42**	
	markhamioside F	**43**	
	(6*S*,9*R*)-roseoside	**44**	
	sequinoside K	**45**	
	verbascoside	**27**	

Table 1 (continued)

Genus	Compound	No.	Reference
Stereospermum			
S. kunthianum			
root bark	anthrakunthone	**46**	Onegi *et al.*, 2002
	pinnatal	**47**	
	pyranokunthone A	**48**	
	pyranokunthone B	**49**	
	sterekunthal A	**50**	
	sterekunthal B	**51**	
stem and stem bark	coniferaldehyde	**52**	Kumar *et al.*, 2005
	(+)-cycloolivil	**53**	
	dehydro-α-lapachone	**54**	
	7'-hydroxydivanillyltetrahydrofuran	**55**	
	2-(4'-hydroxyphenyl)ethyl nonacosanoate	**56**	
	2-(4'-hydroxyphenyl)ethyl undecanoate	**57**	
	lapachol	**58**	
	2-methoxy-4-[3'-(3",4",5"-trimethoxyphenyl) allyloxymethyl]phenol	**59**	
	norviburtinal	**60**	
	(-)-olivil	**61**	
	pinoresinol	**62**	
	(-)-secoisolariciresinol	**63**	
	sinapaldehyde	**64**	
	(-)-specioside	**65**	
	sterequinone F	**66**	
	sterequinone G	**67**	
	sterequinone H	**68**	
	sterequinone I	**69**	
	3,4,5-trimethoxycinnamaldehyde	**70**	

Table 1 (continued)

Genus	Compound	No.	Reference
S. zenkeri stem bark	(-)-verminoside	**71**	Lenta *et al.*, 2007
	p-coumaric acid	**72**	
	sterequinone F	**66**	
	zenkequinone A	**73**	
	zenkequinone B	**74**	
Tabebuia *T. avellanedae* inner bark	3,4-dimethoxybenzoic acid	**75**	Awale *et al.*, 2005
	6-*O*-(3,4-dimethoxybenzoyl)-ajugol	**76**	
	3,4-dimethoxyphenyl 1-*O*-β-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside	**77**	
	3,4-dimethoxyphenyl 1-*O*-β-D-[5-*O*-(4-hydroxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside	**78**	
	3,4-dimethoxyphenyl 1-*O*-β-D-[5-*O*-(4-methoxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside	**79**	
	4-hydroxybenzoic acid	**80**	
	6-*O*-(4-hydroxybenzoyl)-ajugol	**81**	
	2-(4-hydroxyphenyl)ethyl-1-*O*-β-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside	**82**	
	2-(4-hydroxyphenyl)ethyl-1-*O*-β-D-[5-*O*-(4-hydroxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside	**83**	

Table 1 (continued)

Genus	Compound	No.	Reference
	2-(4-hydroxyphenyl)ethyl-1-*O*-*β*-D-[5-*O*-(4-methoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside	**84**	
	4-methoxybenzoic acid	**85**	
	6-*O*-(4-methoxybenzoyl)-ajugol	**86**	
	3,4,5-trimethoxyphenyl 1-*O*-*β*-D-[5-*O*-(4-methoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside	**87**	
T. impetiginosa			
bark	2-formyl-5-(3',4'-dimethoxybenzoyloxy)-3-methyl-2-cyclopentene-1-acetaldehyde	**88**	Koyama *et al.,* 2000
	2-formyl-5-(4'-methoxybenzoyloxy)-3-methyl-2-cyclopentene-1-acetaldehyde	**89**	
bark	2,4-dimethoxyphenyl 1-*O*-*β*-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside	**90**	Warashina *et al*., 2004
	(-)-6-hydroxymelleinyl 1-*O*-*β*-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside	**91**	
	(-)-6-hydroxymelleinyl 1-*O*-*β*-D-[5-*O*-(3,4,5-trimethoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside	**92**	
	2-(4-hydroxyphenyl) ethyl-1-*O*-*β*-D-[5-*O*-(3,4,5-trimethoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside	**93**	

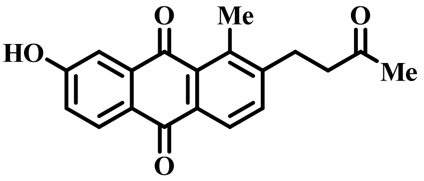
Table 1 (continued)

Genus	Compound	No.	Reference
	4-methoxyphenyl 1-*O*-*β*-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside	**94**	
	6-*O*-(3,4,5-trimethoxybenzoyl)-ajugol	**95**	
	3,4,5-methoxyphenyl 1-*O*-*β*-D-[5-*O*-(3,4-dimethoxybenzoyl)]apiofuranosyl (1→6)-*β*-D-glucopyranoside	**96**	
Oroxylum			
O.indicum			
stem bark	5,7-dihydroxy-6-methoxyflavone.	**97**	Kumar *et al.*, 1938
root and root bark	baicalein	**98**	Subramanian *et al.*, 1972
	baicalein-7-glucuronide	**99**	
	chrysin	**100**	
	chrysin-6-methoxyflavone	**101**	
	scutellarein	**102**	
	scutellarein 7-rutinoside	**103**	
leaves	9,10-anthracenedione	**104**	Dey *et al.*, 1978
root	ellagic acid	**105**	Vasanth *et al.*, 1991
stem bark	dodecanyl oroxylopterocarpan	**106**	Ali *et al.*, 1999
	heptyl oroxylopterocarpan	**107**	
	hexyl oroxylopterocarpan	**108**	
	oroxylopterocarpan	**109**	

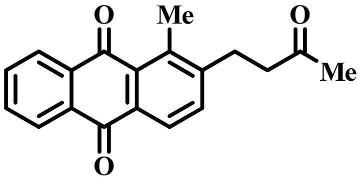
Table 1 (continued)

Genus	Compound	No.	Reference
stem bark	baicalein-7-*O*-caffeate	**110**	Dinda *et al.*, 2007
	baicalein-7-*O*-glucoside	**111**	
	8,8''-bisbaicalein	**112**	
	6-hydroxyluteolin	**113**	
	6-methoxyluteolin	**114**	
leaves	baicalein-7-*O*-diglucoside	**115**	Yuan *et al.*, 2008
	chrysin-7-*O*-glucuronide	**116**	
stem bark	chrysin-7-*O*-glucoside	**117**	Babu *et al.*, 2010
	dihydro oroxylin A	**118**	
	5-hydroxy-4',7-methoxyflavone	**119**	
	7-*O*-methylchrysin	**120**	
	oroxylin A	**121**	

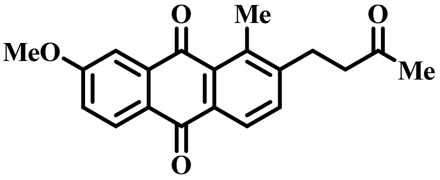
1. anthraquinones



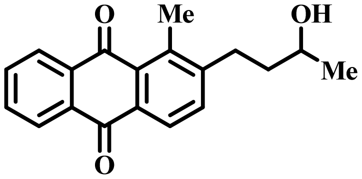
46 : anthrakunthone



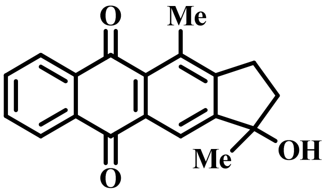
66 : sterequinone F



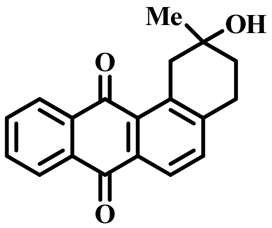
67 : sterequinone G



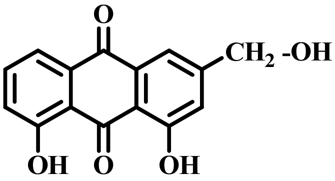
68 : sterequinone H



73 : zenkequinone A

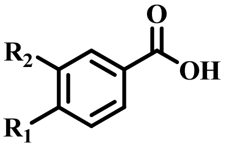


74 : zenkequinone B



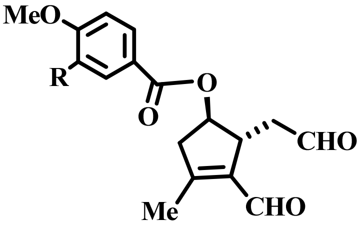
104 : 9,10-anthracenedione

2. benzoic acid derivatives



75 : R_1 = R_2 = OMe : 3,4-dimethoxybenzoic acid
80 : R_1 = OH, R_2 = H : 4-hydroxybenzoic acid
85 : R_1 = OMe, R_2 = H : 4-methoxybenzoic acid

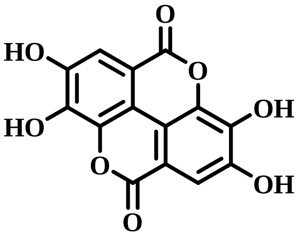
3. cyclopentane dialdehyde



88 : R = OMe : 2-formyl-5-(3',4'-dimethoxybenzoyloxy)-
3-methyl-2-cyclopentene-1-acetaldehyde

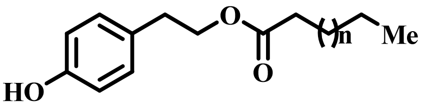
89 : R = H : 2-formyl-5-(4'-methoxybenzoyloxy)-
3 - methyl-2-cyclopentene-1-acetaldehyde

4. ellagic acid



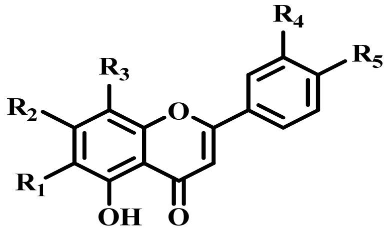
105 : ellagic acid

5. esters

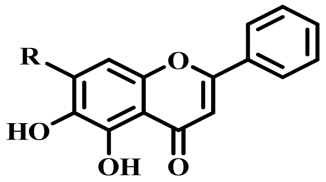


56 : n = 25 : 2-(4'-hydroxyphenyl)ethyl nonacosanoate
57 : n = 7 : 2-(4'-hydroxyphenyl)ethyl undecanoate

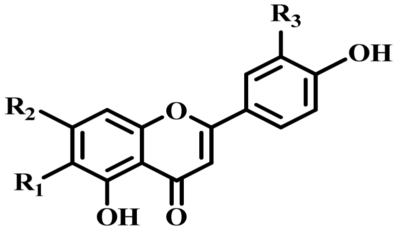
6. Flavones



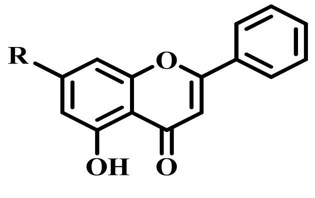
97 : R_1 = OMe, R_2 = OH, R_3 = R_4 = R_5 = H : 5,7-dihydroxy-6-methoxyflavone
112 : R_1 = OMe, R_4 = OH, R_2 = R_5 = H, R_3 = baicalein : 8,8"-bisbaicalein
119 : R_1 = R_3 = R_4 = H , R_2 = R_5 = OMe : 5-hydroxy-4',7-methoxyflavone



98 : R = H : baicalein
99 : R = glucuronide : baicalein-7-glucuronide
110 : R = *O*-caffeate : baicalein-7-*O*-caffeate
111 : R = *O*-glucoside : baicalein-7-*O*-glucoside
115 : R = *O*-diglucoside : baicalein-7-*O*-diglucoside

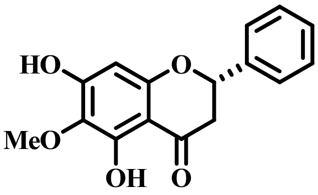


103 : R_1 = OH, R_2 = *O*-rutinoside, R_3 = H : scutellarein 7-rutinoside
102 : R_1 = R_2 = OH, R_3 = H : scutellarein
114 : R_1 = OMe, R_2 = H, R_3 = OH : 6-methoxyluteolin
113 : R_1 = R_3 = OH, R_2 = H : 6-hydroxyluteolin



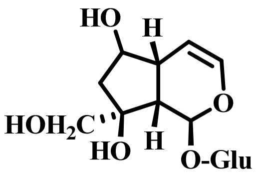
100 : R = OH : chrysin
116 : R = glucuronide : chrysin-7-*O*-glucuronide
117 : R = *O*-glucoside : chrysin-7-*O*-glucoside
120 : R = OMe : 7-*O*-methylchrysin

7. flavanones

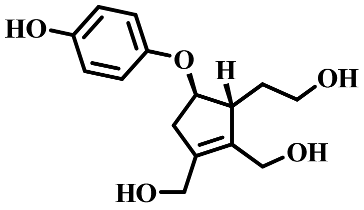


118 : dihydro oroxylin A

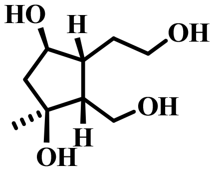
8. iridoids



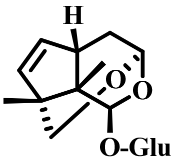
8 : 5,7-bisdeoxycynanchoside



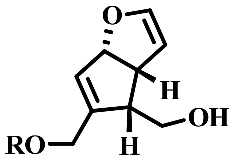
11 : crescentin III



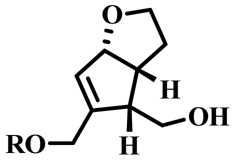
12 : crescentin IV



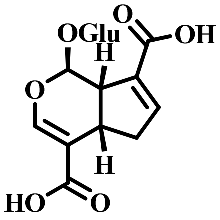
16 : crescentoside C



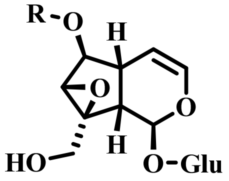
13 : R = H : crescentin V
15 : R = Glu : crescentoside B



14 : R = Glu : crescentoside A
19 : R = H : ningpogenin

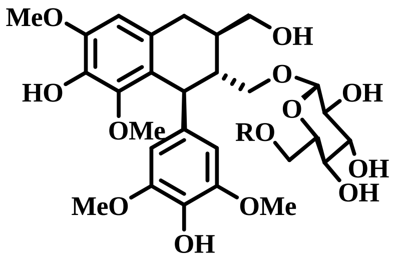


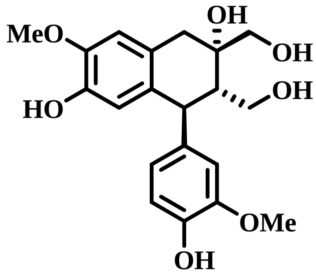
24 : crescentoside B

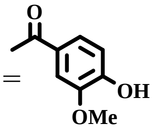


65 : R = 4-hydroxycinnamoyl
: (-)-specioside
71 : R = 3,4-dihydroxycinnamoyl
: (-)-verminoside

9. lignans

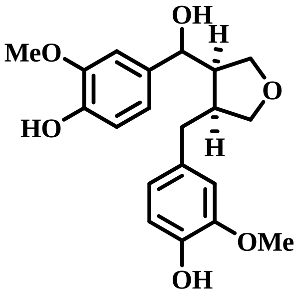


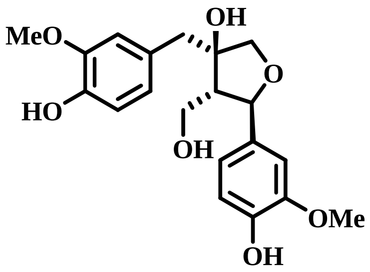


28 : R =  : fernandoside

53 : (+)-cycloolivil

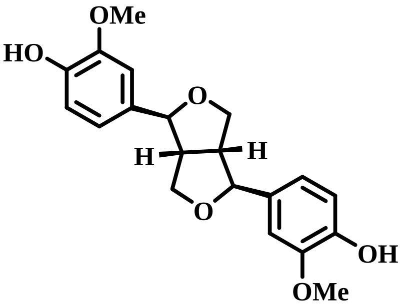
29 : R = H : (+)-lyoniresinol 3a-*O*-β-glucoside

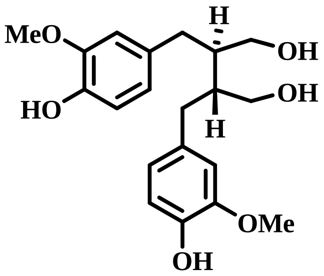




55 : 7'-hydroxydivanillyltetrahydrofuran

61 : (-)-olivil

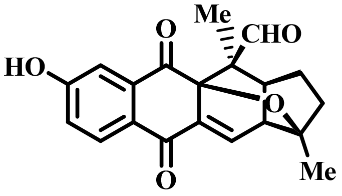




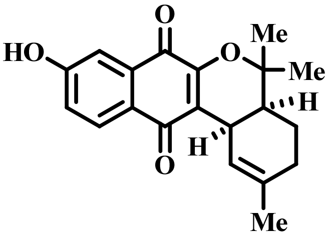
62 : pinoresinol

63 : (-)-secoisolariciresinol

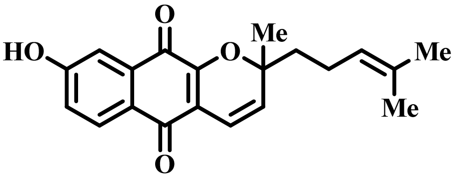
10. naphthoquinones



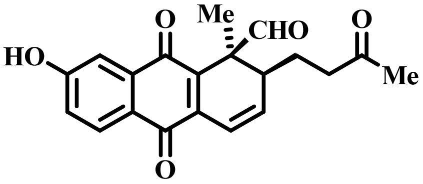
47 : pinnatal



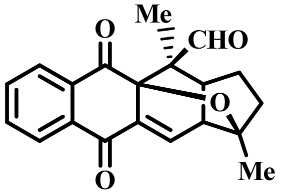
48 : pyranokunthone A



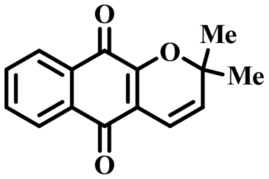
49 : pyranokunthone B



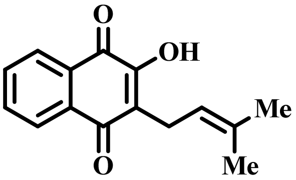
50 : sterekunthal A



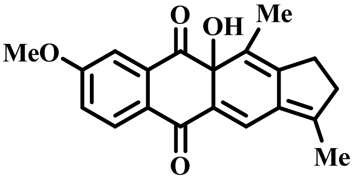
51 : sterekunthal B



54 : dehydro-α-lapachone

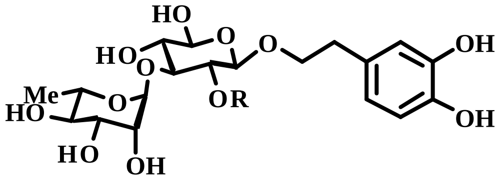


58 : lapachol

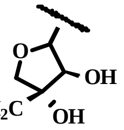


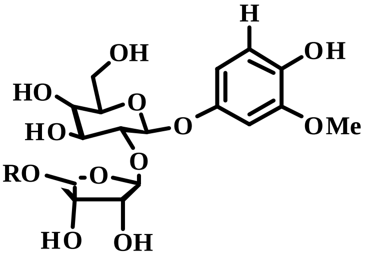
69 : sterequinone I

11. phenolic glycosides

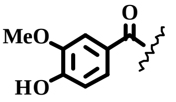


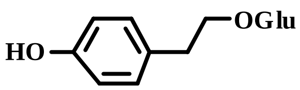
21 : R = H : decaffeoylverbascoside

38 : R =  : markhamioside A

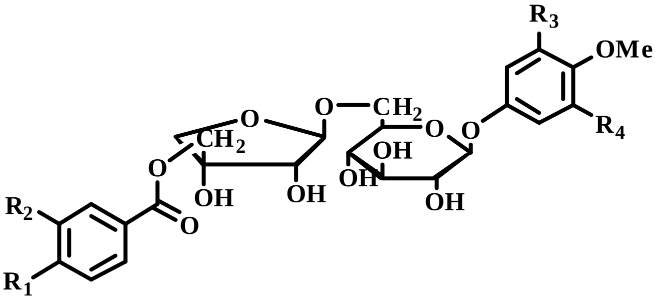


43 : R = H : markhamioside F

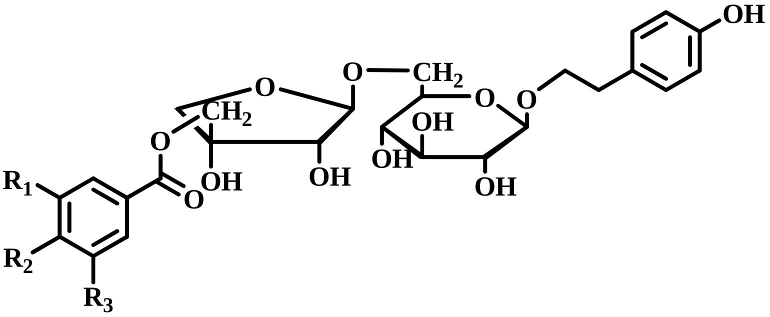
45 : R =  : sequinoside K



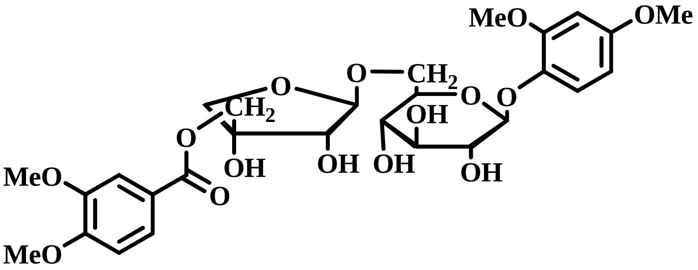
30 : salidroside



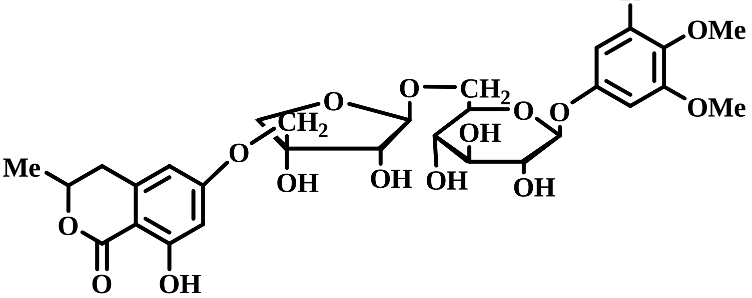
77 : R_1 = R_2 = R_4 = OMe, R_3 = H
: 3,4-dimethoxyphenyl 1-*O*-β-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside
78 : R_1 = OH, R_2 = R_3 = H, R_4 = OMe
: 3,4-dimethoxyphenyl 1-*O*-β-D-[5-*O*-(4-hydroxybenzoyl)]-apiofuranosyl-(1→ 6)-β-D-glucopyranoside
79 : R_1 = R_4 = OMe, R_2 = R_3 = H
: 3,4-dimethoxyphenyl 1-*O*-β-D-[5-*O*-(4-methoxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside
87 : R_1 = R_3 = R_4 = OMe, R_2 = H
: 3,4,5-trimethoxyphenyl 1-*O*-β-D-[5-*O*-(4-methoxybenzoyl)]-apiofuranosyl-(1 → 6)-β-D-glucopyranoside
94 : R_1 = R_2 = OMe, R_3 = R_4 = H
: 4-methoxyphenyl 1-*O*-β-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside
96 : R_1 = R_2 = R_3 = R_4 = OMe
: 3,4,5-methoxyphenyl 1-*O*-β-D-[5-*O*-(3,4-dimethoxybenzoyl)]apiofuranosyl-(1→6)-β-D-glucopyranoside



82 : $R_1 = R_2 = OMe, R_3 = H$
: 2-(4-hydroxyphenyl)ethyl-1-*O*-*β*-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl- (1⟶ 6)-*β*-D-glucopyranoside
83 : $R_1 = OH, R_2 = R_3 = H$
: 2-(4-hydroxyphenyl)ethyl-1-*O*-*β*-D-[5-*O*-(4-hydroxybenzoyl)]-apiofuranosyl- (1⟶ 6)-*β*-D-glucopyranoside
84 : $R_1 = R_3 = H, R_2 = OMe$
: 2-(4-hydroxyphenyl)ethyl-1-*O*-*β*-D-[5-*O*-(4-methoxybenzoyl)]-apiofuranosyl-(1⟶ 6)-*β*-D-glucopyranoside
93 : $R_1 = R_2 = R_3 = OMe$
: 2-(4-hydroxyphenyl)ethyl-1-*O*-*β*-D-[5-*O*-(3,4,5-trimethoxybenzoyl)]-apiofuranosyl-(1⟶ 6)-*β*-D-glucopyranoside

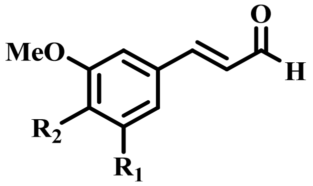


90 : 2,4-dimethoxyphenyl 1-*O*-*β*-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1⟶ 6)-*β*-D-glucopyranoside

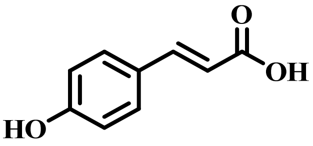


91 : R = H
: (-)-6-hydroxymelleinyl 1-*O*-*β*-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1⟶ 6)-*β*-D-glucopyranoside
92 : R = OMe
: (-)-6-hydroxymelleinyl 1-*O*-*β*-D-[5-*O*-(3,4,5-trimethoxybenzoyl)]-apiofuranosyl-(1⟶ 6)-*β*-D-glucopyranoside

12. phenylpropanoids

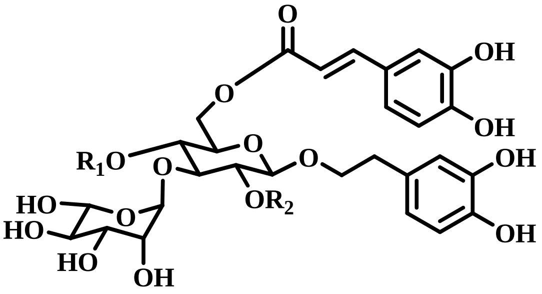


52 : R_1 = H, R_2 = OH : coniferaldehyde
64 : R_1 = OMe, R_2 = OH : sinapaldehyde
70 : R_1 = R_2 = OMe : 3,4,5-trimethoxycinnamaldehyde

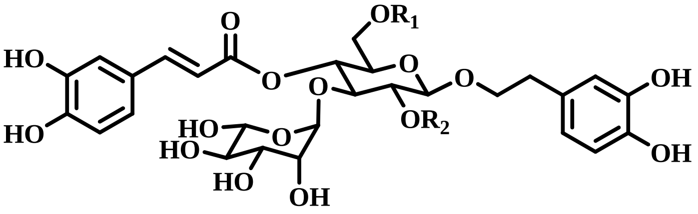


72 : *p*-coumaric acid

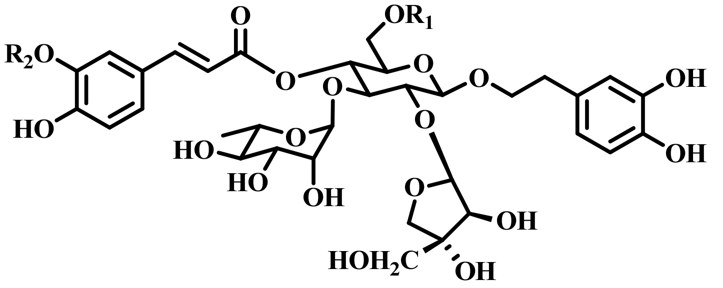
13. phenylpropanoid glycosides



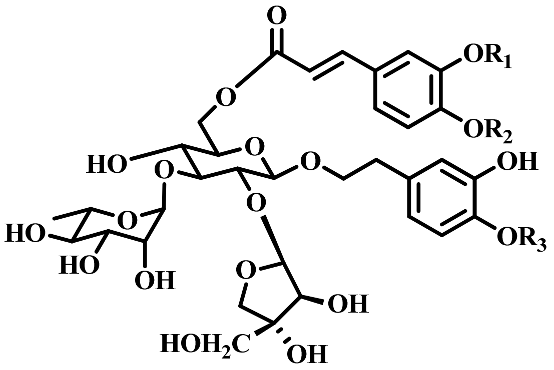
	R_1	R_2	
23 :	H	H	: isoverbascoside
40 :	H	arabinosyl	: markhamioside C



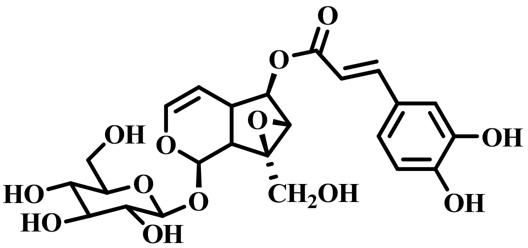
	R_1	R_2	
27 :	H	H	: verbascoside
41 :	Ac	arabinosyl	: markhamioside D
42 :	Ac	galactosyl	: markhamioside E



	R_1	R_2	
20 :	H	H	: 2''-*O*-apiosylverbascoside
22 :	H	Me	: dolichandroside
23 :	Ac	H	: luteoside A

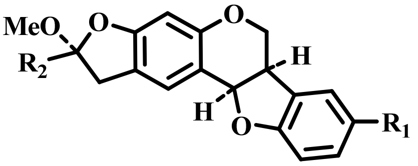


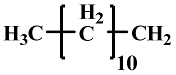
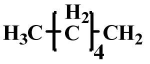
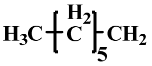
	R_1	R_2	R_3	
33 :	H	H	H	: luteoside B
34 :	Me	Me	H	: luteoside C
39 :	Me	Me	Me	: markhamioside B



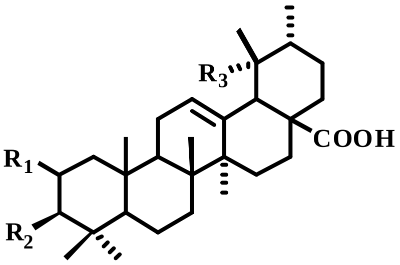
31 : verminoside

14. pterocarpans



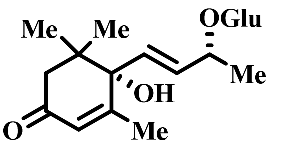
	R_1	R_2	
106 :	H	 $H_3C-[\overset{H_2}{C}]_{10}-CH_2$	: dodecanyl oroxylopterocarpan
107 :	OH	 $H_3C-[\overset{H_2}{C}]_{4}-CH_2$	: heptyl oroxylopterocarpan
108 :	OH	 $H_3C-[\overset{H_2}{C}]_{5}-CH_2$	: hexyl oroxylopterocarpan
109 :	H	OMe	: oroxylopterocarpan

15. triterpenoids

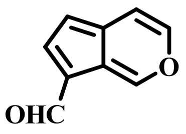


	R_1	R_2	R_3	
35 :	H	OH	OH	: pomolic acid
36 :	OH	OH	OH	: *epi*-tormentic acid
37 :	H	OH	H	: ursolic acid

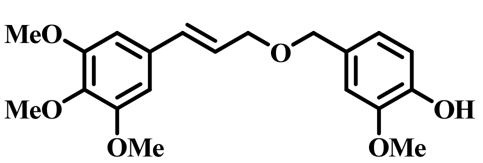
16. others



44 : (6*S*,9*R*)-roseoside



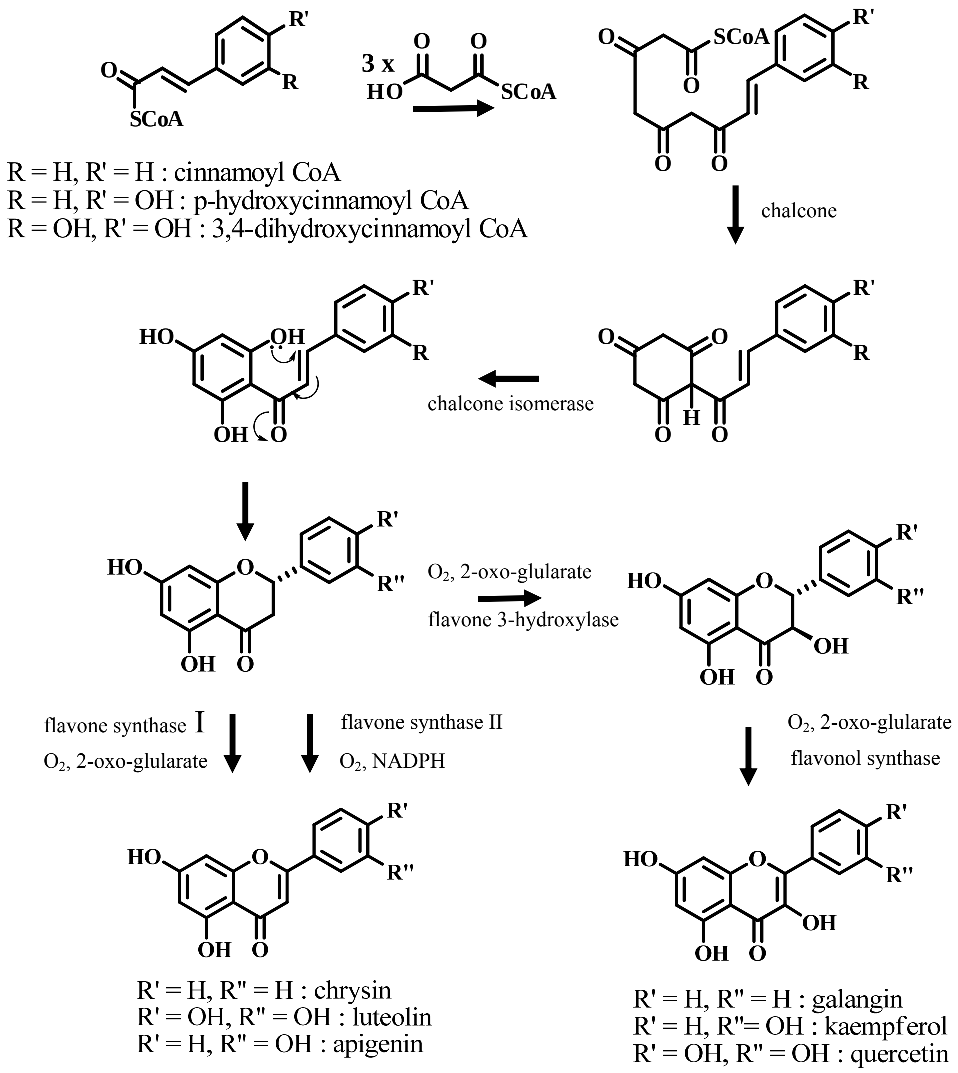
60 : norviburtinal



59 : 2-methoxy-4-[3'-(3",4",5"-trimethoxyphenyl)allyloxymethyl]phenol

1.2.2 Biogenetic pathway of flavonoid compounds

Most flavonoids contain a six-membered heterocyclic ring, formed by Michael-type nucleophilic attack of a phenol group onto the unsaturated ketone to give a flavanone. Flavanones can then give rise to many variants on this basic skeleton, e.g. flavones, flavonols, anthocyanidins, and catechins.



R = H, R' = H : cinnamoyl CoA
R = H, R' = OH : p-hydroxycinnamoyl CoA
R = OH, R' = OH : 3,4-dihydroxycinnamoyl CoA

R' = H, R" = H : chrysin
R' = OH, R" = OH : luteolin
R' = H, R" = OH : apigenin

R' = H, R" = H : galangin
R' = H, R"= OH : kaempferol
R' = OH, R" = OH : quercetin

Scheme 1 Biogenetic pathway of flavones and flavonols

1.3 The Objective

The objective of this work is to investigate the chemical constituents from the stem bark of *O. indicum*.

CHAPTER 2
EXPERIMENTAL

2.1 Instruments and Chemicals

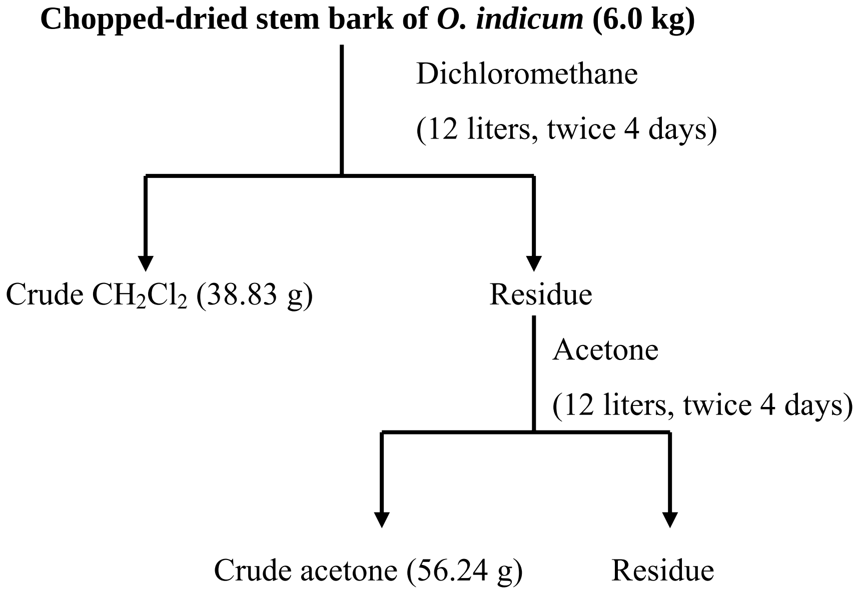
Melting point was recorded in ^{o}C on a digital Electrothermal 9100 Melting Point Apparatus. Ultraviolet spectra were measured with a UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol solution. The optical rotation $[\alpha]_D$ was measured in methanol solution with Sodium D line (590 nm) on a JASCO P-1020 digital polarimeter. The IR spectra were measured with a Perkin-Elmer 783 FTS165 FT-IR spectrophotometer. ^{1}H and ^{13}C – Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra Shield^{TM} 300 MHz spectrometer at Department of Chemistry, Faculty of Science, Prince of Songkla University. Spectra were recorded in deuterochloroform and dimethylsulfoxide-d_6 as δ value in ppm down field from TMS (internal standard δ 0.00) and coupling constant (J) are expressed in hertz. Spectra were recorded in deuterochloroform and dimethylsulfoxide-d_6 as δ value in ppm down field from TMS (internal standard δ 0.00) and coupling constant (J) are expressed in hertz. EI mass spectra were measured on MAT 95 XL Mass spectrometer Quick column chromatography (QCC) and column chromatography were performed by using silica gel 60 H (Merck) and silica gel 100 (70-230 Mesh ASTM, Merck), respectively. For thin-layer chromatography (TLC), aluminum sheets of silica gel 60 F_{254} (20×20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized under ultraviolet light. Solvents for extraction and chromatography were distilled at their boiling ranges prior to use.

2.2 Plant Material

The stem bark of *O. indicum* was collected from Amphur Chian Yai, Nakhonsithammarat province in the southern part of Thailand in September 2008. Identification was made by Mr.Ponlawat Pattarakulpisutti, Department of Biology, Faculty of Science, Prince of Songkla University. The specimen (S.maungjun 1: Chian Yai 26/10/2009) have been deposited in the Herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla, Thailand.

2.3 Extraction and Isolation

Chopped-dried stem bark of *O. indicum* (6.0 kg) was immersed successively at room temperature in dichloromethane and acetone (each extract 4 days). After removal of solvents, the yellow-brown viscous liquid of dichloromethane extract (38.83 g) and the dark-brown viscous liquid of acetone extract (56.24 g) were obtained. The process of extraction was shown in **Scheme 2**.



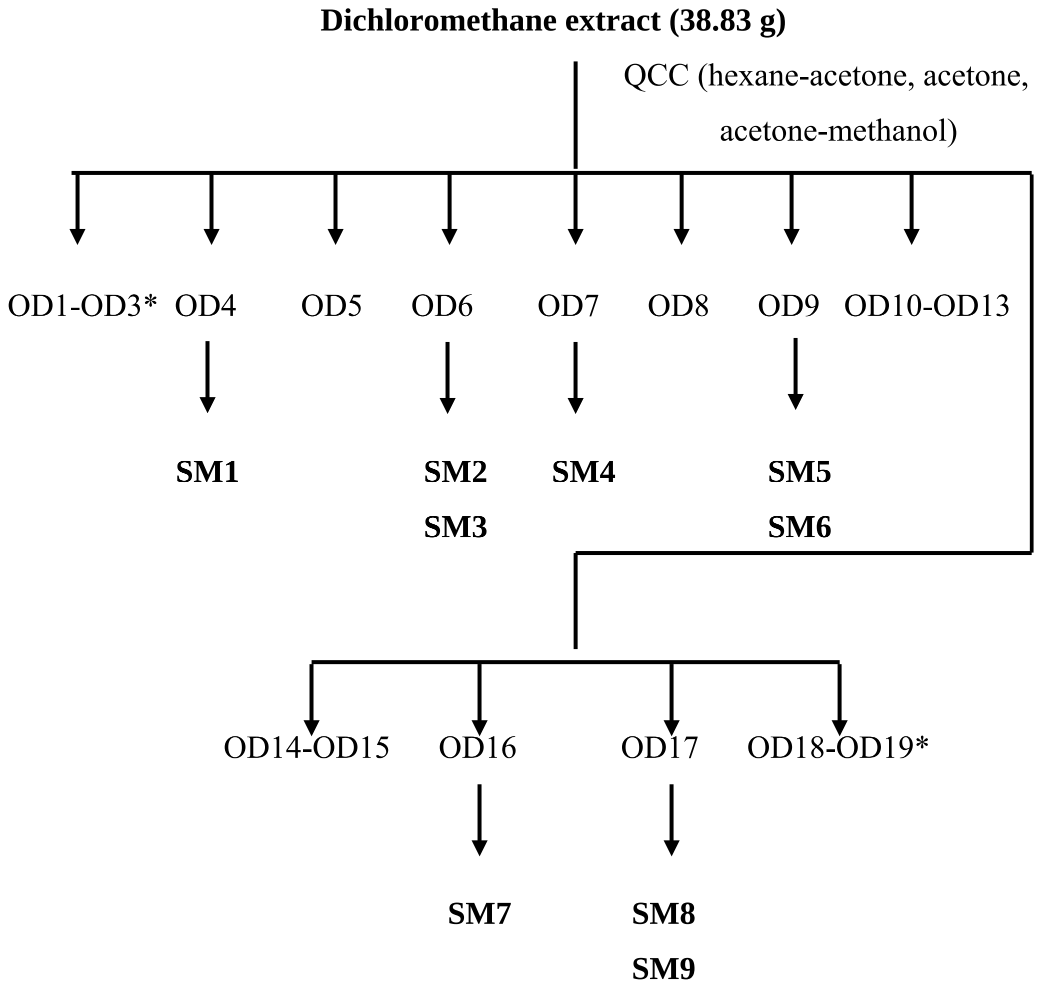
Scheme 2 Extraction of the crude extracts from the stem bark of *O. indicum*

2.3.1 Purification of dichlotomethane extract

The dichloromethane extract (38.83 g) was chromatographed on quick column chromatography over silica gel 60 using mixed hexane-acetone, acetone and mixed acetone-methanol as eluents. On the basis of their TLC characteristics, the fractions containing the same major components were combined to give fractions OD1-OD19 **(Table 2)**. Further purification of subfractions gave nine pure compounds **(Scheme 3)**.

Table 2 Physical characteristic and weights of fractions obtained from QCC of the dichloromethane extract

Fraction	Weight (g)	Physical characteristic
OD1	3.2323	yellow gel
OD2	4.1251	yellow gel
OD3	0.9723	orange gel
OD4	1.0286	yellow viscous liquid
OD5	1.2444	yellow viscous liquid
OD6	0.6503	yellow-brown solid
OD7	1.3411	yellow-brown solid
OD8	0.3770	yellow-brown solid
OD9	0.6819	yellow solid
OD10	0.6756	yellow solid
OD11	0.1922	dark-brown solid
OD12	0.3219	dark-brown solid
OD13	0.5691	dark-brown solid
OD14	1.4389	dark-brown viscous liquid
OD15	0.2792	dark-brown solid
OD16	1.8119	dark-brown solid
OD17	6.0414	dark-brown solid
OD18	0.2399	brown solid
OD19	2.1109	brown solid



* No further investigation

Scheme 3 Isolation of compounds **SM1-SM9** from dichloromethane extract of the stem bark of *O. indicum*

Fraction OD4 (1.0286 g) was purified by column chromatography over silica gel and eluted with hexane-dichloromethane (4:1) to give fractions OD4.1-OD4.6. Subfraction OD4.2 (41.2 mg) was filtered and washed with hexane to give **SM1** (12 mg) as a white solid.

Fraction OD6 (650.3 mg) was further purified by column chromatography over silica gel and eluted with hexane-acetone (95:5) solvent system. The subfractions containing similar components were combined to give fractions OD6.1-OD6.6. A white solid of **SM2** (7.4 mg) was obtained from fraction OD6.2.

Fraction OD6.4 was rechromatograped on column chromatography and eluted with hexane-acetone (9:1) solvent system to give a brown gum of **SM3** (19.5 mg).

Fraction OD7 (1.3411 g) was chromatographed on column chromatography and elution was conducted with hexane-acetone (4:1) to afford 7 fractions (OD7.1-OD7.7). The subfraction OD7.4 (136.8 mg) was rechromatographed using hexane-acetone (5:1) as an eluent to afford a yellow solid of **SM4** (9.3 mg).

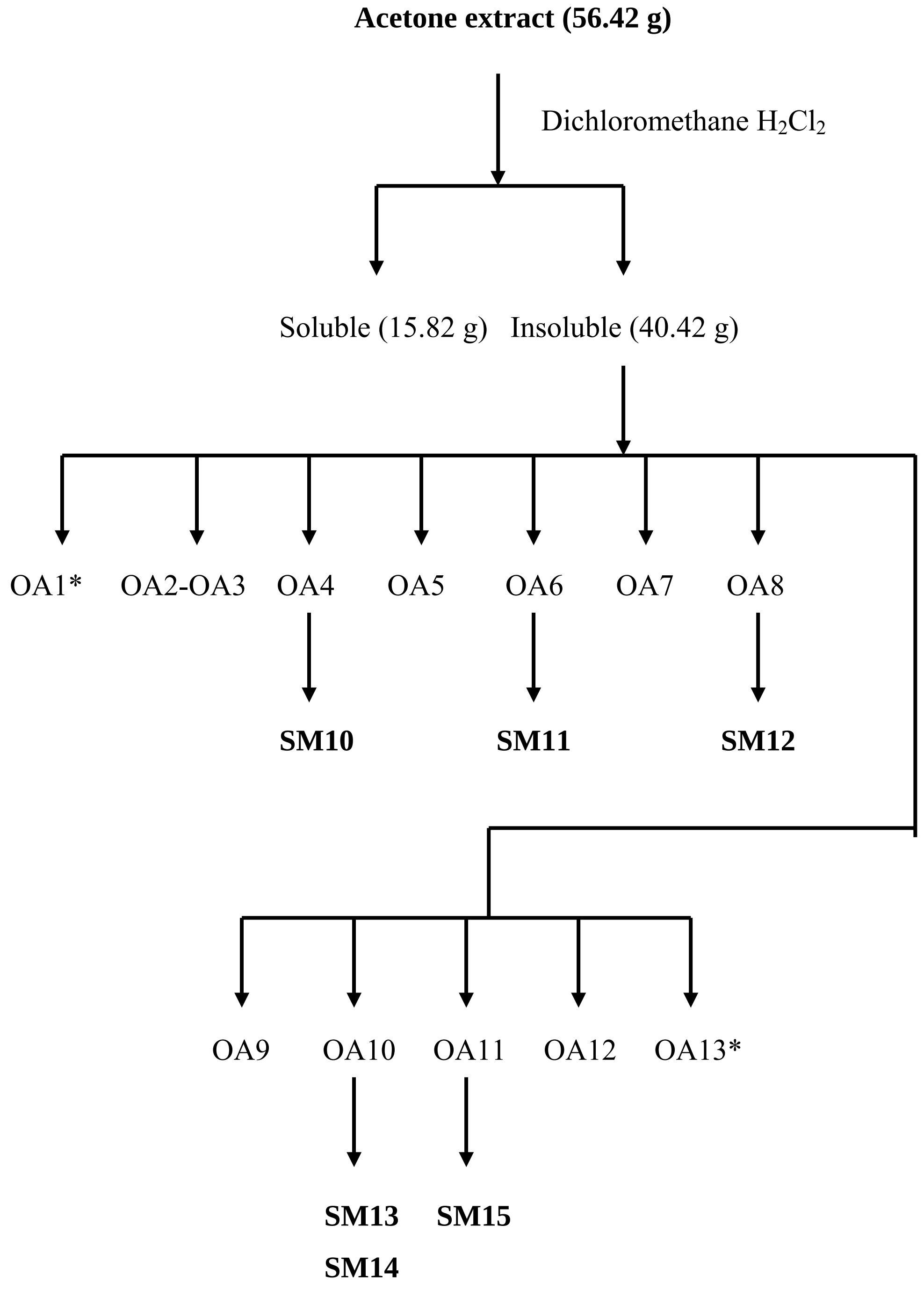
Fraction OD9 (681.9 mg) was further purified by column chromatography over silica gel and eluted with hexane-acetone (9:1 to 8:2). The subfractions containing similar components were combined to give fractions OD9.1-OD9.9. **SM5** (13.7 mg) was obtained as a yellow solid from fraction OD9.3. Subfraction OD9.7 which contained one major component was further purified by crystallization from hexane-acetone (7:1). A yellow solid of **SM6** (60.4 mg) which formed was filtered.

Fraction OD16 (1.8119 g) was further separated by column chromatography over silica gel and eluted with hexane-acetone (7:3) solvent system. The subfractions containing similar components were combined to give 8 fractions (OD16.1-OD16.8). Subfraction OD16.4 (278.4 mg) was further purified by column chromatography over Sephadex^{TM} LH-20 and eluted with dichloromethane-methanol (4:1) to give fractions OD16.4.1-OD16.4.4. Subfraction OD16.4.3 (17.2 mg) was further purified by column chromatography over silica gel and eluted with hexane-dichloromethane-acetone (3:1:1) solvent system to give yellow viscous liquid of **SM7** (7.5 mg).

Fraction OD17 (6.0414 g) was purified by column chromatography over silica gel and eluted with a gradient of dichloromethane-methanol (98:2 to 95:5) to give subfractions OD17.1-OD17.14. Subfraction OD17.2 (368.4 mg) was rechromatographed on column chromatography and eluted with hexane-acetone (8:2 to 7:3) to afford a yellow solid of **SM8** (58.5 mg). Fraction OD17.7 (258.3 mg) was further purified by column chromatography eluted with acetone-hexane (2:8) to give a yellow solid of **SM9** (75.9 mg).

2.3.2 Purification of acetone extract

The dark-brown viscous liquid of acetone extract (56.24 g) was dissolved in dichloromethane to give the soluble fraction (15.82 g) and insoluble fraction (40.42 g). The insoluble fraction was purified by column chromatography over silica gel and eluted with acetone-hexane (3:7 to 5:5) solvent system to give fractions OA1-OA13 **(Table 3)**. Further purification of subfractions gave six pure compounds (**Scheme 4)**.



Scheme 4 Isolation of compounds **SM10-SM15** from acetone extract of the stem bark of *O. indicum*
]

Table 3 Physical characteristic and weights of fractions obtained from QCC of the acetone extract

Fraction	**Weight (g)**	**Physical characteristic**
OA1	4.2131	yellow viscous liquid
OA2	2.8120	yellow viscous liquid
OA3	0.4281	yellow-brown viscous liquid
OA4	0.2576	yellow-brown viscous liquid
OA5	1.6992	yellow-brown solid
OA6	4.3222	yellow solid
OA7	3.9564	yellow solid
OA8	1.2591	red-brown viscous liquid
OA9	1.7344	red-brown solid
OA10	2.5428	dark-brown solid
OA11	3.1215	dark-brown solid
OA12	2.9976	dark-brown solid
OA13	6.8765	brown solid

Fraction OA4 (257.6 mg) was purified by column chromatography over silica gel and eluted with hexane-acetone (8:2) to give 8 fractions (OA4.1-OA4.8). Fraction OA4.5 (56.3 mg) was rechromatographed on column chromatography and eluted with hexane-acetone (9:1 to 8:2). The subfractions containing similar components were combined to give fractions OA4.5.1-OA4.5.4. Subfraction OA4.5.3 (12.4 mg) was further purified by preparative TLC with hexane-acetone (4:1) to give a yellow solid of **SM10** (4.5 mg).

Fraction OA6 (4.3222 g) was purified by column chromatography over Sephadex^{TM} LH-20 and eluted with dichloromethane-methanol (9:1 to 6:4) to give fractions OA6.1-OA6.9. Subfraction OA6.4 (357.7 mg) was purified by column chromatography over Sephadex^{TM} LH-20 and eluted with dichloromethane-methanol (9:1) to give fractions OA6.4.1-OD6.4.9. Subfraction OA6.4.1.5 (57.8 mg) was further purified by column chromatography over Sephadex^{TM} LH-20 and eluted with dichloromethane-methanol (9:1) to give colorless gum of **SM11** (7.4 mg).

Fraction OA8 (1.2591 g) was purified by column chromatography over Sephadex^{TM} LH-20 and eluted with dichloromethane-methanol (4:1) to afford a yellow solid of **SM12** (8.6 mg).

Fraction OA10 (2.5428 g) was separated by column chromatography over Sephadex^{TM} LH-20 and eluted with dichloromethane-methanol (9:1 to 7:3) to give subfractions OA10.1-OA10.8 and to give a yellow solid of **SM13** (20 mg). Fraction OA10.4 (80.6 mg) was further purified by column chromatography over Sephadex^{TM} LH-20 and eluted with dichloromethane-methanol (9:1) to give fractions OA10.4.1-OA10.4.5. Subfraction OA10.4.4 (44 mg) was further purified by column chromatography over Sephadex^{TM} LH-20 and eluted with dichloromethane-methanol (9:1) to give a yellow solid of **SM14** (5 mg).

Fraction OA11 (3.1215 g) was purified by column chromatography over Sephadex^{TM} LH-20 and eluted with dichloromethane-methanol (4:1) to afford fractions OA11.1-OA11.9. Subfraction OA11.6 (59.0 mg) was further purified by column chromatography over silica gel and eluted with a gradient of dichloromethane-methanol (97:3 to 95:5) to give 4 fractions (OA11.6.1-OA11.6.4). Subfraction OA11.6.2 (10.4 mg) was further purified by preparative TLC with dichloromethane-methanol (95:5) to give a brown gum of **SM15** (2.4 mg).

SM1

mp. 245-247 °C

$[\alpha]_D^{28}$: - 28.2° (*c* 0.63, $CHCl_3$)

IR (Neat) ν (cm^{-1}) : 1715 (C=O stretching)

^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data see **Table 4**

SM2

mp. 280-282 °C

$[\alpha]_D^{28}$: +18.7° (*c* 0.03, $CHCl_3$)

IR (Neat) ν (cm^{-1}) : 3415 (O-H stretching), 1686 (C=O stretching) and 1645 (C=C stretching)

^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data see **Table 5**

SM3

IR (Neat) ν (cm^{-1}) : 3438 (O-H stretching), 1680 (C=O stretching)

^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data see **Table 6**

SM4

IR (Neat) ν (cm^{-1}) : 3156 (O-H stretching), 1606 (C=O stretching)

^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data see **Table 8**

SM5

IR (Neat) ν (cm^{-1}) : 3323 (O-H stretching), 1631 (C=O stretching)

^1H NMR and ^{13}C NMR ($CDCl_3$+DMSO-d_6) spectral data see **Table 9**

SM6

UV (CH_3OH) λ_{max} nm (log ε) : 222 (4.57), 272 (4.70) and 317 (4.60)

IR (Neat) ν (cm^{-1}) : 3403 (O-H stretching), 1611 (C=O stretching)

^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data see **Table 10**

SM7

UV (CH_3OH) λ_{max} nm (log ε) : 228 (3.38) and 271 (3.83)

IR (Neat) ν (cm^{-1}) : 3375 (O-H stretching), 1691 (C=O stretching)

^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data see **Table 11**

SM8

UV (CH_3OH) λ_{max} nm (log ε) : 224 (4.56), 245 (4.62), 273 (4.67) and 312 (4.59)

IR (Neat) ν (cm^{-1}) : 3343 (O-H stretching), 1692 (C=O stretching)

^1H NMR and ^{13}C NMR ($CDCl_3$) spectral data see **Table 13**

SM9

UV (CH_3OH) λ_{max} nm (log ε) : 219 (4.23), 273 (4.17) and 321 (3.95) nm

IR (Neat) ν (cm^{-1}) : 3417 (O-H stretching), 1662 (C=O stretching)

^1H NMR and ^{13}C NMR ($CDCl_3$+DMSO-d_6) spectral data see **Table 14**

SM10

UV (CH_3OH) λ_{max} nm (log ε) : 223 (3.92), 274 (4.15) and 333 (4.07)

IR (Neat) ν (cm^{-1}) : 3428 (O-H stretching), 1661 (C=O stretching)

^{1}H NMR and ^{13}C NMR ($CDCl_3$+DMSO-*d*$_6$) spectral data see **Table 15**

SM11

IR (Neat) ν (cm^{-1}) : 3372 (O-H stretching), 1650 (C=O stretching)

^{1}H NMR and ^{13}C NMR (Acetone-*d*$_6$) spectral data see **Table 16**

SM12

UV (CH_3OH) λ_{max} nm (log ε) : 227 (3.76), 269 (4.14) and 336 (3.64)

IR (Neat) ν (cm^{-1}) : 3342 (O-H stretching), 1598 (C=O stretching)

^{1}H NMR and ^{13}C NMR ($CDCl_3$+DMSO-*d*$_6$) spectral data see **Table 17**

SM13

UV (CH_3OH) λ_{max} nm (log ε) : 231 (3.31) and 272 (3.87)

IR (Neat) ν (cm^{-1}) : 3480 (O-H stretching), 1598 (C=O stretching)

^{1}H NMR and ^{13}C NMR ($CDCl_3$+DMSO-*d*$_6$) spectral data see **Table 18**

SM14

UV (CH_3OH) λ_{max} nm (log ε) : 226 (3.88), 275 (4.10) and 332 (3.80)

IR (Neat) ν (cm^{-1}) : 3318 (O-H stretching)

^{1}H NMR and ^{13}C NMR ($CDCl_3$+DMSO-*d*$_6$) spectral data see **Table 19**

SM15

^{1}H NMR and ^{13}C NMR ($CDCl_3$+DMSO-*d*$_6$) spectral data see **Table 20**

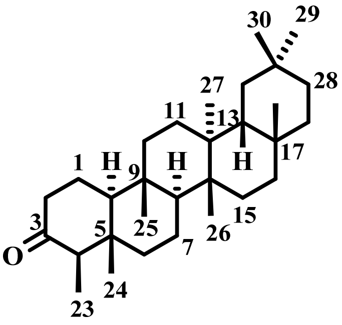
CHAPTER 3

RESULT AND DISCUSSION

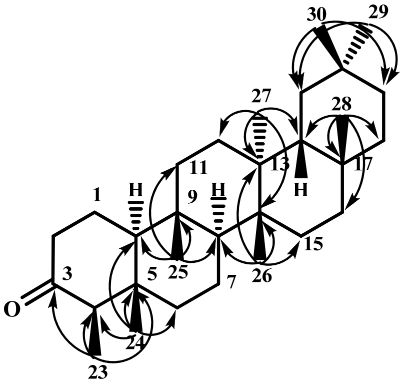
3.1 Structural Determination

The stem bark of *Oroxylum indicum* (L.) Benth. ex Kurz was extracted with methylene chloride and acetone, successively. Separation of methylene chloride extract by column chromatography produced nine compounds whereas purification of acetone extract gave six compounds. They were identified as friedelin **(SM1)**, betulinic acid **(SM2)**, (*R*)-(-)-mellein **(SM3)**, tectochrysin **(SM4)**, luteolin **(SM5**), 5,7-dihydroxy-3-methoxyflavone **(SM6)**, Rengyolone. **(SM7)**, chrysin **(SM8)**, galangin **(SM9)**, apigenin **(SM10)**, 4-hydroxybenzoic acid **(SM11)**, 5,7,4'-trihydroxy-3-methoxyflavone **(SM12),** kaempferol **(SM13)**, scutellarein **(SM14)** and 3,4-dihydroxybenzoic acid **(SM15)**. Their structures were elucidated by 1D and 2D spectroscopic data. The physical data of the known compounds were also compared with the reported values.

SM1: friedelin



SM1 was obtained as a white solid, mp 245-247 °C $[\alpha]_D{}^{28}$: -28.2° (*c* 0.63, $CHCl_3$). The IR spectrum showed absorption bands for carbonyl group at 1715 cm^{-1}. The ^{13}C NMR spectral data (**Table 4**) showed 30 signals for 30 carbons. Analysis of DEPT 90° and DEPT 135° spectra of this compound suggested the presence of eight methyl (δ_C 6.8, 14.7, 17.9, 18.7, 20.3, 31.8, 32.1 and 35.0), eleven methylene (δ_C 18.2, 22.3, 30.5, 32.4, 32.8, 35.3, 35.6, 36.0, 39.3, 41.3 and 41.5), four methine (δ_C 42.8, 53.1, 58.2 and 59.5) and seven quaternary carbons (δ_C 28.2, 30.0, 37.4, 38.3, 39.7, 42.2 and 213.3). The ^1H NMR spectral data showed characteristic of friedelin as one methyl doublet at δ 0.89 (H-23, *d*, J = 6.3 Hz) and seven methyl singlets at δ 0.72, 0.87, 0.95, 1.00, 1.01, 1.05 and 1.18. The HMBC correlation of a methyl group (CH_3-23) to C-3 (δ 213.3), C-4 (δ 58.2) and C-5 (δ 42.2) indicated that the methyl group was placed at C-4. Thus on the basis of its spectroscopic data and comparison with the previously reported data of friedelin (Ahad *et al.*, 1991), **SM1** was therefore assigned as friedelin.



Selected HMBC correlations of **SM1**

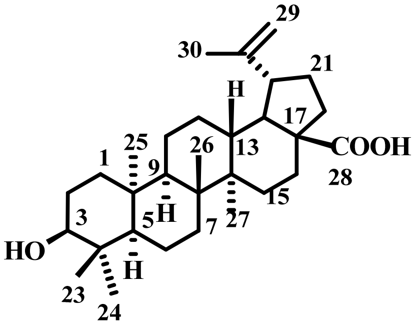
Table 4 ^{1}H, ^{13}C NMR and HMBC spectral data of **SM1**

Position	**δ_H/ppm (multiplicity, *J*/Hz)**	**δ_C (C-Type)**	**HMBC**
1	1.64 (*m*), 1.69 (*m*)	22.3 (CH_2)	-
2	2.36 (*m*), 2.23 (*m*)	41.5 (CH_2)	-
3	-	213.3 (C)	-
4	2.24 (*m*)	58.2 (CH)	-
5	-	42.2 (C)	-
6	2.44 (*m*), 1.78 (*m*)	41.3 (CH_2)	-
7	1.52 (*m*), 1.39 (*m*)	18.2 (CH_2)	-
8	1.42 (*m*)	53.1 (CH)	-
9	-	37.4 (C)	-
10	1.56 (*m*)	59.5 (CH)	-
11	1.61 (*m*), 1.43 (*m*)	35.6 (CH_2)	-
12	1.46 (*m*), 1.34 (*m*)	30.5 (CH_2)	-
13	-	39.7 (C)	-
14	-	38.3 (C)	-
15	1.51 (*m*), 1.29 (*m*)	32.4 (CH_2)	-
16	1.61 (*m*), 1.36 (*m*)	36.0 (CH_2)	-
17	-	30.0 (C)	-
18	1.53 (*m*)	42.8 (CH)	-
19	1.62 (*m*), 1.49 (*m*)	35.3 (CH_2)	-
20	-	28.2 (C)	-

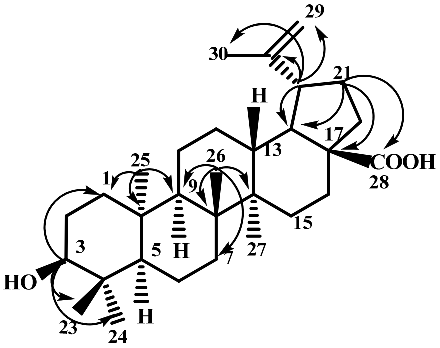
Table 4 (continued)

Position	**δ_H/ppm (multiplicity, *J*/Hz)**	**δ_C (C-Type)**	**HMBC**
21	1.48 (*m*), 0.93 (*m*)	39.3 (CH_2)	-
22	1.50 (*m*), 1.26 (*m*)	32.8 (CH_2)	-
23	0.89 (*d,* 6.3)	6.8 (CH_3)	C-3, C-4, C-5
24	0.72 (*s*)	14.7 (CH_3)	C-4, C-5, C-6, C-10
25	0.87 (*s*)	17.9 (CH_3)	C-8, C-9, C-10, C-11
26	1.01 (*s*)	18.7 (CH_3)	C-8, C-13, C-14, C-15
27	1.05 (*s*)	20.3 (CH_3)	C-12, C-13, C-14, C-18
28	1.18 (*s*)	32.1 (CH_3)	C-16, C-17, C-18, C-22
29	1.00 (*s*)	31.8 (CH_3)	C-19, C-20, C-21
30	0.95 (*s*)	35.0 (CH_3)	C-19, C-20, C-21

SM2: betulinic acid



SM2 was obtained as a white solid, mp. 280-282 °C , $[\alpha]_D^{28}$: +18.7° (*c* 0.03, $CHCl_3$). The FT-IR spectrum showed absorption band of a hydroxyl group at 3415 cm^{-1} and a carbonyl group at 1686 cm^{-1}. The ^1H NMR spectrum (**Table 5**) showed the presence of five methyl singlets (δ 0.75 (H-24), δ 0.82 (H-25), δ 0.94 (H-26), δ 0.97 (H-23) and δ 0.98 (H-27)). In addition, the presence of an isopropenyl group was shown in a lowfield methyl signal at δ 1.69 (H-30), and two vinylic proton at δ 4.61 (*br s*) and 4.74 (*br s*). These data indicated that this compound belonged to the lupine family. The ^1H NMR spectrum further showed a typical lupane H_β-19 proton at δ 3.01 (*m*) and an oxymethine proton at δ 3.19 (*dd*, J = 10.8, 5.4 Hz, H-3). The large coupling constant between H-3 and H-2 with $J_{ax\text{-}ax}$ = 10.8 Hz indicating that 3-hydroxy group was at β-face. The ^{13}C NMR spectral data displayed a signal of a carboxyl carbon at δ 179.1, thus suggesting a carboxylic functionality at C-28, confirming by the HMBC correlations of methylene proton (H-22, δ 1.41 and 1.93) to C-17 (δ 56.1), C-18 (δ 49.1) and C-28 (δ 179.1). Thus on the basis of its spectroscopic data and comparison with the previous report (Macias *et al.*, 1994 and Thongdeeying, 2005), **SM2** was assigned as betulinic acid.



Selected HMBC correlations of **SM2**

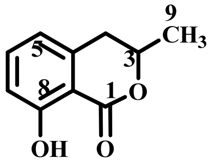
Table 5 ^{1}H, ^{13}C NMR and HMBC spectral data of **SM2**

Position	δ_H/ppm (multiplicity, *J*/Hz)	δ_C (C-Type)	HMBC
1	0.88 (*m*), 1.65 (*m*)	38.7 (CH_2)	-
2	1.57 (*m*), 1.61 (*m*)	26.9 (CH_2)	-
3	3.19 (*dd,* 10.8, 5.4)	78.7 (CH)	C-1, C-23, C-24
4	-	38.7 (C)	-
5	0.69 (*m*)	55.3 (CH)	C-4, C-6, C-7, C-9
6	1.36 (*m*), 1.51 (*m*)	18.2 (CH_2)	-
7	1.38 (*m*)	34.2 (CH_2)	-
8	-	40.6 (C)	-
9	1.26 (*m*)	50.5 (CH)	-
10	-	37.1 (C)	-
11	1.23 (*m*), 1.43 (*m*)	20.8 (CH_2)	-
12	1.69 (*m*)	25.4 (CH_2)	-
13	2.22 (*m*)	38.2 (CH)	-
14	-	42.3 (C)	-
15	1.51 (*m*), 1.51 (*m*)	29.6 (CH_2)	-
16	1.40 (*m*), 2.25 (*m*)	32.2 (CH_2)	-
17	-	56.1 (C)	-
18	1.58 (*m*)	49.1 (CH)	-
19	3.01 (*m*)	46.9 (CH)	C-18, C-20, C-21, C-29, C-30

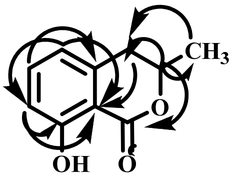
Table 5 (continued)

Position	**δ_H/ppm (multiplicity, *J*/Hz)**	**δ_C (C-Type)**	**HMBC**
20	-	150.7 (C)	-
21	1.42 (*m*), 1.91 (*m*)	30.5 (CH_2)	-
22	1.41 (*m*), 1.93 (*m*)	37.1 (CH_2)	C-17, C-18, C-28
23	0.97 (*s*)	27.6 (CH_3)	C-3, C-4, C-5, C-24
24	0.75 (*s*)	15.2 (CH_3)	C-3, C-4, C-5, C-23
25	0.82 (*s*)	15.9 (CH_3)	C-1, C-5, C-9, C-10
26	0.94 (*s*)	15.6 (CH_3)	C-7, C-8, C-9, C-14
27	0.98 (*s*)	14.5 (CH_3)	C-8, C-13, C-14, C-15
28	-	179.1 (C=O)	-
29	4.61 (*br s*), 4.74 (*br s*)	109.3 (CH_2)	C-19, C-30
30	1.69 (*s*)	19.1(CH_3)	C-19, C-20, C-29

SM3: (*R*)-(-)-mellein



SM3 was obtained as a brown gum, $[\alpha]^{29}_D$ = -72° (*c* 0.07, $CDCl_3$). The IR spectrum showed the absorption bands of O-H stretching at 3438 cm^{-1}, C=O stretching at 1680 cm^{-1}. The ^1H NMR spectrum (**Table 6**) showed a sharp singlet signal of a chelated hydroxy proton at δ 10.95 (8-OH), and three coupled aromatic protons H-7, H-6 and H-5 at δ 6.80 (*d*), δ 7.32 (*t*) and δ 6.61 (*d*). The spectrum further showed a doublet signal of methylene proton at δ 2.85 (H-4), a sextet signal of an oxymethine proton at δ 4.65 (H-3) and a doublet signal of methyl proton at δ 1.47 (H-9). The ^{13}C-NMR spectrum showed signals of one carbonyl carbon (δ 169.9), three quaternary aromatic carbon (δ 162.6, 139.4 and 108.3), four methine carbons (δ 136.1, 117.9, 116.2 and 76.1), one methylene carbon (δ 34.6), one methyl carbon (δ 20.7). The HMBC correlations of H-3 to C-4a (δ 139.4) and C-1 (δ 169.9) and of H-4 to C-8a (δ 108.3), C-5 (δ 117.9) and C-9 (δ 20.7) suggested the point of attachment of $-CH_2-CH(CH_3)-O-CO-$ unit to C-4a and C-8a of an aromatic ring. Thus **SM3** was assigned to be 8-hydroxy-3-methylisochroman-1-one, which was known as mellein (Dimitriadis *et al.*, 1997). Mellein has been reported to have two stereoisomer, (*R*)-(-)-mellein ($[\alpha]^{29}_D$ = -101.3 ° (*c* 0.07, $CDCl_3$)), and (*S*)-(+)-mellein ($[\alpha]^{29}_D$ = +88.6° (*c* 0.27, $CDCl_3$)). **SM3** has optical rotation of $[\alpha]^{29}_D$ = -72° (*c* 0.07, $CDCl_3$), it was therefore concluded that **SM3** is amixture with the excess of (*R*)-(-)-mellein.



Selected HMBC correlations of **SM3**

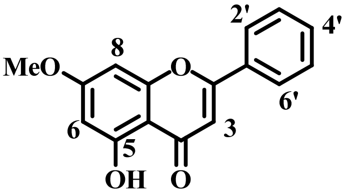
Table 6 ^{1}H, ^{13}C NMR and HMBC spectral data of **SM3**

Position	δ_{H} **(multiplicity, J/Hz)**	δ_{C} **(C-Type)**	**HMBC**
1	-	169.9 (C=O)	-
3	4.65 (*sext,* 6.6)	76.1 (CH)	C-1, C-4, C-4a, C-9
4	2.85 (*d*, 6.6)	34.6 (CH$_2$)	C-3, C-5, C-8a, C-9
4a	-	139.4 (C)	-
5	6.61 (*d*, 7.9)	117.9 (CH)	C-4a, C-6, C-7, C-8a
6	7.32 (*t*, 7.9)	136.1 (CH)	C-4a
7	6.80 (*d*, 7.9)	116.2 (CH)	C-5, C-8, C-8a
8	-	162.6 (C)	-
8a	-	108.3 (C)	-
9	1.47 (*d*, 6.6)	20.7 (CH$_3$)	C-3, C-4
8-OH	10.95 (*s*)	-	C-6, C-7, C-8, C-8a

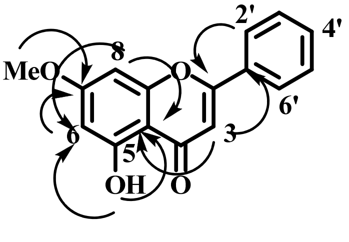
Table 7 ^{1}H-^{1}H COSY spectral data of **SM3**

Proton (δ)		Correlated proton (δ)
H-3 (4.65)	⟷	H-4 (2.85), H-9 (1.47)
H-4 (2.85)	⟷	H-3 (4.65)
H-5 (6.61)	⟷	H-6 (7.32)
H-6 (7.32)	⟷	H-5 (6.61), H-7 (6.80)
H-7 (6.80)	⟷	H-6 (7.32)
H-9 (1.47)	⟷	H-3 (4.65)

SM4: Tectochrysin



SM4 was obtained as a yellow soild, mp. 162-163 °C. The IR spectrum showed the absorption bands of O-H stretching at 3156 cm^{-1}, C=O stretching at 1636 cm^{-1}. The ^{1}H NMR spectral data (**Table 8**) showed the resonances of a flavone proton at *δ* 6.67 (*s*, H-3) and a hydrogen-bonded hydroxyl proton at *δ* 12.73 (*s*, 5-OH). The appearance of meta-coupled signals at *δ* 6.51 (*d*) and 6.39 (*d*) with coupling constant of 2.4 Hz were assigned for aromatic protons H-6 and H-8. The spectrum further showed a doublet of doublet signal of equivalent aromatic protons H-2'/H-6' at *δ* 7.90 (2H, *dd, J*= 7.5, 2.4 Hz) and a multiplet signals of aromatic protons H-3'/H-4', H5' at *δ* 7.57-7.52. The ^{1}H NMR spectrum exhibited the resonances of a methoxyl group at *δ* 3.89. The ^{13}C-NMR spectrum exhibited the resonances of one carbonyl carbon (*δ* 183.4), six quaternary aromatic carbon (*δ* 165.6, 164.0, 162.2, 158.0, 131.4 and 105.8), eight methine carbons (*δ* 131.8, 129.1×2, 128.6×2, 105.5, 98.2 and 92.7). The HMBC correlations of H-6 to C-8, C-7, and C-4a confirmed the position of H-6. The methoxyl group was placed at C-7 because the correlation of *δ* 3.89 (7-OCH$_3$) to *δ* 165.6 (C-7). In addition, the correlations of H-3 and H-2' to the C-2 confirmed the position of H-3 and the phenyl ring. **SM4** then was assigned to be 5-hydroxy-7-methoxy-flavone which was known as tectochrysin (Sutthanut *et al.*, 2007).

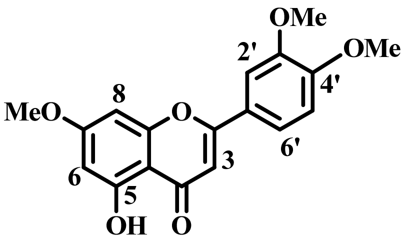


Selected HMBC correlations of **SM4**

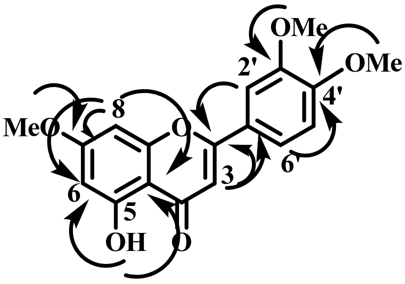
Table 8 ^{1}H, ^{13}C NMR and HMBC spectral data of **SM4**

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**	**HMBC**
2	-	164.0 (C)	-
3	6.67 (*s*)	105.8 (CH)	C-2, C-4a, C-1'
4	-	182.5 (C=O)	-
4a	-	105.5 (C)	-
5	-	162.2 (C)	-
6	6.39 (*d*, 2.4)	98.2 (CH)	C-8, C-7, C-5, C-4a
7	-	165.6 (C)	-
8	6.51 (*d*, 2.4)	92.7 (CH)	C-7, C-6, C-4a, C-8a
8a	-	158.0 (C)	-
1'	-	131.4 (C)	-
2', 6'	7.90 (*dd*, 7.5, 2.4)	129.1 (CH)	C-2, C-4'
3', 5'	7.57-7.52 (*m*)	128.6 (CH)	C-4'
4'	7.57-7.52 (*m*)	131.8 (CH)	-
5-OH	12.73 (*s*)	-	C-4a, C-5, C-6
7-OMe	3.89 (*s*)	55.8 (CH_3)	C-7

SM5: Luteolin



SM5 was obtained as a yellow soild, mp. 329-330 °C. The IR spectrum showed the absorption bands of O-H stretching at 3323 cm^{-1}, C=O stretching at 1631 cm^{-1}. The ^{1}H NMR spectrum (**Table 9**) indicated the presence of a hydrogen-bonded hydroxyl group 5-OH at δ 12.66 (*s*). The flavone proton was resonated at δ 5.98. The resonances of aromatic protons at δ 6.74 and 6.39 with J = 2.1 Hz were assigned for those of H-8 and H-6. The ring B of flavone was assigned to 1,2,4-trisubstitued benzene by the resonances at δ 7.61 (1H, *d*, J = 9.0, H-6'), 7.59 (1H, *s*, H-2') and 7.13 (1H, *d*, J = 9.0, H-5'). The remaining signals exhibited three methoxy protons at δ 3.88×2 and 3.81. The position of methoxyl groups were confirmed by the HMBC correlations of δ 3.88 and H-8 (δ 6.74) to C-7 (δ 170.3), and of δ 3.88 and H-5' (δ 7.13) to C-3' (δ 143.5) and of δ 3.81 and H-6' (δ 7.61), H-2' (δ 7.59) to C-4' (δ 155.4). The ^{13}C-NMR experiment indicated the presence of a carbonyl carbon (δ 182.5), eight quaternary aromatic carbons (δ 170.3, 166.1, 161.4, 160.7, 155.4, 143.5, 125.5 and 110.4), six methine carbons (δ 125.7, 120.3, 117.0, 103.0, 97.6 and 92.8). **SM5** was then assigned to be 5-hydroxy-7,3',4'-trimethoxyflavone which was known as luteolin (Herrera *et al*., 1996).

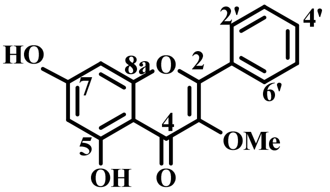


Selected HMBC correlations of **SM5**

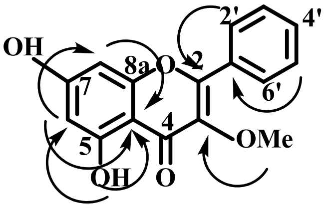
Table 9 ^{1}H, ^{13}C NMR and HMBC spectral data of **SM5**

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**	**HMBC**
2	-	160.7 (C)	-
3	5.98 (*s*)	92.8 (CH)	C-2, C-4a, C-1'
4	-	182.5 (C=O)	-
4a	-	110.4 (C)	-
5	-	166.1 (C)	-
6	6.39 (*d,* 2.1)	103.0 (CH)	C-5, C-8
7	-	170.3 (C)	-
8	6.74 (*d,* 2.1)	97.6 (CH)	C-6, C-7, C-8a
8a	-	161.4 (C)	-
1'	-	125.5 (C)	-
2'	7.59 (*s*)	125.7 (CH)	C-2, C-4'
3'	-	143.5 (C)	-
4'	-	155.4 (C)	-
5'	7.13 (*d,* 9.0)	120.3 (CH)	C-1', C-3', C-4'
6'	7.61 (*d,* 9.0)	117.0 (CH)	C-2, C-4'
5-OH	12.66 (*s*)	-	C-6, C-5, C-4a
7-OMe	3.88 (*s*)	55.8 (CH_3)	C-7
3'-OMe	3.88 (*s*)	55.8 (CH_3)	C-3'
4'-OMe	3.81 (*s*)	55.8 (CH_3)	C-4'

SM6: 5,7-dihydroxy-3-methoxyflavone



SM6 was obtained as a yellow soild. The IR spectrum showed the absorption bands of O-H stretching at 3403 cm^{-1}, C=O stretching at 1611 cm^{-1}. The UV spectrum showed maximum absorption bands at 222, 272 and 317 nm. The ^{1}H NMR spectrum (**Table 10**) showed the signals of a chelated hydroxy proton at δ 13.00 (*s*, 5-OH), a methoxyl group at δ 4.06 (*s*, 3-OMe) and *meta* aromatic protons at δ 6.67 (*s*, H-6) and δ 6.60 (*s*, H-8). The spectrum further showed equivalent aromatic protons H-2'/H-6' at δ 7.90 (*dd*, 7.5, 2.1 Hz) and aromatic protons H-3'/H-5', H-4' at δ 7.57-7.50. The methoxyl group was placed at C-3 according to the HMBC of 3-OCH_3 to C-3 (δ 130.4). Therefore, **SM6** was assigned to be 5,7-dihydroxy-3-methoxyflavone (Kalff *et al.*, 1925).

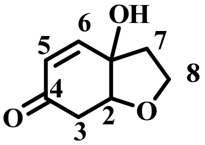


Selected HMBC correlations of **SM6**

Table 10 ^{1}H, ^{13}C NMR and HMBC spectral data of **SM6**

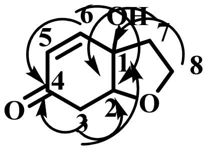
Position	**δ_H (multiplicity, *J*/Hz)**	**δ_C (C-Type)**	**HMBC**
2	-	164.13 (C)	-
3	-	130.4 (C)	-
4	-	183.0 (C=O)	-
4a	-	131.3 (C)	-
5	-	152.1 (C)	-
6	6.67 (*s*)	105.2 (CH)	C-7, C-5, C-4a, C-4
7	-	153.2 (C)	-
8	6.60 (*s*)	93.5 (CH)	C-7, C-6, C-4a
8a	-	155.2 (C)	-
2', 6'	7.90 (*dd*, 7.5, 2.1)	126.3 (CH)	C-2, C-4', C-2', C-6'
3', 5'	7.57-7.50 (*m*)	129.1 (CH)	C-3', C-5'
4'	7.57-7.50 (*m*)	131.9 (CH)	-
5-OH	13.00 (*s*)	-	C-6, C-5, C-4a
3-OMe	4.06 (*s*)	60.9 (CH_3)	-

SM7: Rengyolone



SM7 was obtained as yellow viscous liquid. The IR spectrum showed the absorption bands of O-H stretching at 3375 cm^{-1}, C=O stretching at 1691 cm^{-1}. The UV spectrum showed maximum absorption bands at 228 and 271 nm. The ^{13}C NMR spectrum (**Table 11**) showed the resonance of α,β-unsaturated carbonyl carbon at δ 196.4. The α-olefinic proton (H-5) resonated as doublet at δ 6.24 (J = 10.2 Hz) while β-olefinic proton (H-6) resonated at δ 6.76 (J = 10.2, 1.5 Hz) as a doublet of doublet due to being coupled by H-5 and also by a methine proton H-2 (*via* w-coupling, J = 1.5 Hz). The methine proton H-2 was further coupled by non-equivalent methylene proton H$_a$-3 (δ 2.78, *dd, J* = 17.1, 5.1 Hz) and H$_b$-3 (δ 2.61, *dd, J* = 17.1, 5.7 Hz), it thus resonated as a doublet of doublet of doublet (J = 5.7, 5.1, 1.5 Hz) at δ 4.25. The location of H-6 and H-5 was supported by HMBC correlations of H-6 to C-2 and H-5 to C-1, C-3, C-4. The presence of a hydrofuran ring was suggested from the resonances of non-equivalent oxymethylene protons H$_a$-8 (δ 4.09, *td, J* = 8.7, 6.6 Hz), H$_b$-8 (δ 3.97, *td, J* = 8.7, 6.6 Hz) and non-equivalent methylene protons H$_a$-7 (δ 2.34, *ddd, J* = 13.2, 8.7, 6.6 Hz), H$_b$-7 (δ 2.23, *ddd, J* = 13.2, 8.7, 6.6 Hz). The HMBC correlations of H-8 to C-1, of H-7 to C-1, C-2 and of H-3 to C-1, C-2 revealed that the furan and cyclohexenone moiety were joined by C-1 and C-2. The ^{13}C NMR spectrum showed signals of unsaturated carbonyl carbon at δ 196.4 (C=O), three methylene carbons at δ 66.2, 40.2, 39.6, three methine carbons at δ 147.4, 128.9, 81.7 and one quaternary at δ 75.8. . These assignments indicated that **SM7** was 3*a*-hydroxy-3,3*a*,7,7*a*-tetrahydrobenzofuran-6(2*H*)-one which was known as rengyolone. Rengyolone has been obtained as (*S*)-(+)-rengyolone, $[\alpha]^{29}_{D}$ = +48.6° (*c* 0.3, MeOH) (Tuntiwachwuttikul *et al*., 2003) and racemic mixture (±)-rengyolone, $[\alpha]^{29}_{D}$ = -1.8° (*c* 2.4, MeOH) (Hase *et al*., 1995). The optical rotation value of SM7, $[\alpha]^{29}_{D}$ = -11°

(*c* 0.3, MeOH, suggested that it was obtained as a mixture of (+)-rengyolone and (-)-rengyolone.



Selected HMBC correlations of **SM7**

Table 11 ^{1}H, ^{13}C NMR and HMBC spectral data of **SM7**

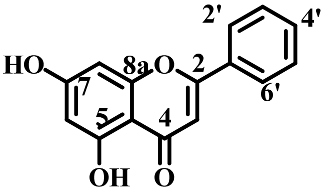
Position	δ_H (multiplicity, *J*/Hz)	δ_C (C-Type)	HMBC
1	-	75.8 (C)	-
2	4.25 (*ddd,* 5.7, 5.1, 1.5)	81.7 (CH)	C-1
3	2.78 (*dd,* 17.1, 5.1)	40.2 (CH_2)	C-1, C-2, C-4
	2.61 (*dd,* 17.1, 5.7)		C-1, C-2, C-4
4	-	196.4 (C=O)	-
5	6.24 (*d,* 10.2)	128.9 (CH)	C-1,C-3, C-4
6	6.76 (*dd,* 10.2, 1.5)	147.4 (CH)	C-2
7	2.34 (*ddd,* 13.2, 8.7, 6.6)	39.6 (CH_2)	C-1, C-2, C-6, C-8
	2.23 (*ddd,* 13.2, 8.7, 6.6)		C-1, C-2, C-6, C-8
8	4.09 (*td,* 8.7, 6.6)	66.2 (CH_2)	C-1
	3.91 (*td,* 8.7, 6.6)		C-1

Table 12 ^{1}H-^{1}H COSY spectral data of **SM7**

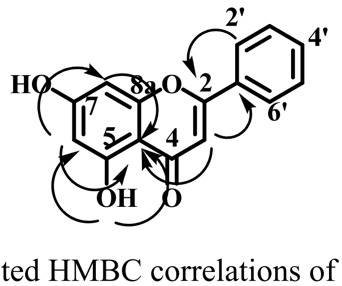
Proton (δ_{ppm})		Correlated proton (δ_{ppm})
H-2 (4.25)	⟷	H-6 (6.76), H-3 (2.78), H-3 (2.61)
H-3 (2.78)	⟷	H-3 (2.61), H-2 (4.25)
H-3 (2.61)	⟷	H-3 (2.78), H-2 (4.25)
H-5 (6.24)	⟷	H-6 (6.76)
H-6 (6.76)	⟷	H-5 (6.24), H-2 (4.25)
H_a-7 (2.34)	⟷	H_b-7 (2.23), H_a-8 (4.09), H_b-8 (3.97)

H_b-7 (2.23)	⟷	H_a-7 (2.34), H_a-8 (4.09), H_b-8 (3.97)
H_a-8 (4.09)	⟷	H_b-8 (3.97), H_a-7 (2.34), H_b-7 (2.23)
H_b-8 (3.97)	⟷	H_a-8 (4.09), H_a-7 (2.34), H_b-7 (2.23)

SM8: Chrysin



SM8 was obtained as a yellow soild, mp. 275-277 °C. The IR spectrum showed the absorption bands of O-H stretching at 3343 cm^{-1}, C=O stretching at 1692 cm^{-1}. The UV spectrum showed maximum absorption bands at 224, 245 and 273 nm. The ^{1}H NMR spectra (**Table 13**) showed signals of a chelated hydroxyl proton (5-OH) at δ 12.66, *meta* aromatic proton (H-6) at δ 6.29 and (H-8) at δ 6.43, equivalent aromatic protons H-2'/H-6' at δ 7.84, aromatic protons H-3'/H-5', H-4' at δ 7.51-7.46. The chemical shift values and coupling patterns of all protons and carbon signals were similar to those of relevant protons of 5-hydroxy-7-methoxy-2-phenyl-4*H*-chromen-4-one (**SM4**). The difference was the appearance of a hydroxyl group at δ 10.01 instead of a methoxyl group at δ 3.89. Therefore, **SM8** was assigned to be 5,7-dihydroxyflavone which was known as chrysin (Chen *et al.*, 2003).

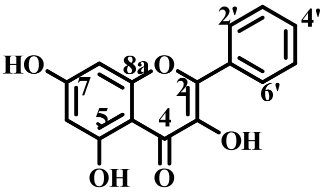


Selected HMBC correlations of **SM8**

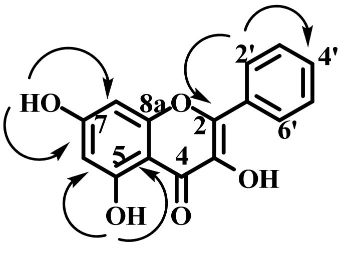
Table 13 ^{1}H, ^{13}C NMR and HMBC spectral data of **SM8**

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**	**HMBC**
2	-	163.6 (C)	-
3	6.58 (*s*)	105.5 (CH)	C-2, C-4a, C-1'
4	-	182.4 (C=O)	-
4a	-	105.5 (C)	-
5	-	164.4 (C)	-
6	6.29 (*d*, 2.1)	99.7 (CH)	C-8, C-7, C-5, C-4a
7	-	162.1 (C)	-
8	6.43 (*d*, 2.1)	94.3 (CH)	C-7, C-6, C-8a
8a	-	158.0 (C)	-
1'	-	126.2 (C)	-
2', 6'	7.84 (*dd*, 8.1, 2.1)	126.2 (CH)	C-2, C-4'
3', 5'	7.51-7.46 (*m*)	128.6 (CH)	C-4'
4'	7.51-7.46 (*m*)	131.6 (CH)	C-1', C-2'
5-OH	12.66 (*s*)	-	C-6, C-5, C-4a
7-OH	10.01 (*s*)	-	-

SM9: Galangin



SM9 was obtained as a yellow soild, mp 215-217 °C. The IR spectrum showed the absorption bands of O-H stretching at 3417 cm^{-1}, C=O stretching at 1662 cm^{-1}. The UV spectrum showed maximum absorption bands at 219, 273 and 321 nm. The ^{1}H NMR spectra (**Table 14**) showed signals of a chelated hydroxyl proton (5-OH) at δ 12.75, two phenolic hydroxyl groups (7-OH) at δ 8.57 and δ 7.71 (3-OH), *meta* aromatic proton (H-6) at δ 6.70 and (H-8) at δ 6.82. The spectrum further showed equivalent aromatic protons H-2'/H-6' at δ 7.94, aromatic protons H-3'/H-5', H-4' at δ 7.59-7.54. The chemical shift values and coupling patterns of all proton and carbon signals were similar to relevant protons of 5,7-dihydroxy-3-methoxy-2-phenyl-4*H*-chromen-4-one **(SM6)**. The replacing of a methoxyl proton signal at δ 4.06 by a hydroxyl proton signal at δ 7.71 suggested that **SM9** was a flavanol namely 3,5,7-trihydroxyflavone which was known as galangin (Facundo *et al*., 2003).

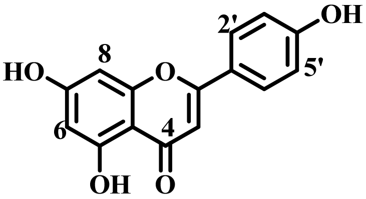


Selected HMBC correlations of **SM9**

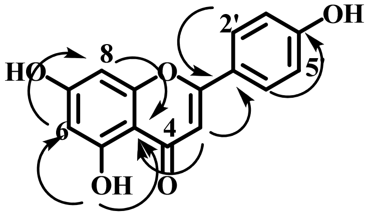
Table 14 ^{1}H, ^{13}C NMR and HMBC spectral data of **SM9**

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**	**HMBC**
2	-	148.1 (C)	-
3	-	137.2 (C)	-
4	-	183.0 (C=O)	-
4a	-	104.3 (C)	-
5	-	161.8 (C)	-
6	6.70 (*s*)	100.1 (CH)	C-5, C-6
7	-	166.4 (C)	-
8	6.82 (*s*)	89.8 (CH)	C-6, C-7, C-8a
8a	-	158.9 (C)	-
1'	-	135.5 (C)	-
2', 6'	7.94 (*dd,* 8.1, 1.5)	126.8 (CH)	C-4', C-6'
3', 5'	7.59-7.54 (*m*)	124.3 (CH)	C-4', C-1'
4'	7.59-7.54 (*m*)	129.6 (CH)	C-1', C-2'
3-OH	7.71 (*s*)	-	C-2, C-3, C-4
5-OH	12.75 (*s*)	-	C-6, C-5, C-4a
7-OH	8.57 (*s*)	-	-

SM10: Apigenin



SM10 was obtained as a yellow soild, mp. 346-348 °C. The IR spectrum showed the absorption bands of O-H stretching at 3428 cm^{-1}, C=O stretching at 1661 cm^{-1}. The UV spectrum showed maximum absorption bands at 223, 274 and 333 nm. The ^{1}H NMR spectrum (**Table 15**) showed the resonances of a hydrogen-bonded hydroxyl group 5-OH at δ 12.84 (*s*) and non chelated proton 7-OH and 4'-OH at δ 10.12 (*br s*) and 9.77 (*br s*), respectively. The most shielded aromatic proton signal (δ 6.50) was assigned for H-3 according to resonance effect of the oxygen atom. The HMBC correlations of H-3 to C-2, C-4a and C-1' supported the assignment of H-3. The resonances of aromatic protons at δ 6.29 and 6.44 with J = 1.8 Hz were assigned for that of H-6 and H-8. The HMBC correlations of H-6 to C-5 (δ 158.6.), C-8 (δ 89.7) and that of H-8 to C-6 (δ 94.2), C-7 (δ 160.2) and C-8a (δ 153.2.) confirmed the assignment of H-6 and H-8. The remaining signal exhibiting as AA'BB' at δ 7.75 (2H, *d, J* = 8.7 Hz) and 6.95 (2H, *d, J* = 8.7 Hz) was proposed for the signal of H-2', H-6' and H-3', H-5', respectively. The assignment of H-2'/H-6' and H-3'/H-5' were confirmed by HMBC cross peaks of H-2'/H-6' to C-2 (δ 162.8), C-4' (δ 159.8) and H-3'/H-5' to C-1' (δ 117.6.), C-4' (δ 159.8). The ^{13}C-NMR spectrum experiment indicated the presence of a carbonyl carbon (δ 181.1), seven quaternary aromatic carbons (δ 162.8, 160.2, 159.8, 158.6, 153.2, 117.6 and 99.7), seven methine carbons (δ 123.2 ×2, 111.2×2, 98.4, 94.2 and 89.7). **SM10** was then assigned to be 5,7,4'-trihydroxyflavone which was known as apigenin (Nakasugi *et al.*, 2000).

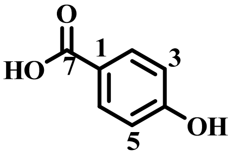


Selected HMBC correlations of **SM10**

Table 15 ^{1}H, ^{13}C NMR spectral data of **SM10**

Position	**δ_H (multiplicity, *J*/Hz)**	**δ_C (C-Type)**	**HMBC**
2	-	162.8 (C)	-
3	6.50 (*s*)	98.4 (CH)	C-2, C-4a, C-1'
4	-	181.1 (C=O)	-
4a	-	99.7 (C)	-
5	-	158.6 (C)	-
6	6.29 (*d,* 1.8)	94.2 (CH)	C-5, C-8
7	-	160.2 (C)	-
8	6.44 (*d*, 1.8)	89.7 (CH)	C-6, C-7, C-8a
8a	-	153.2 (C)	-
1'	-	117.6 (C)	-
2', 6'	7.75 (*d*, 8.7)	123.3 (CH)	C-2, C-4'
3', 5'	6.95 (*d,* 8.7)	111.8 (CH)	C-4', C-1'
4'	-	159.8 (C)	-
5-OH	12.84 (*s*)	-	C-6, C-5, C-4a
7-OH	10.12 (*s*)	-	-
4'-OH	9.77 (*s*)	-	-

SM11: 4-hydroxybenzoic acid

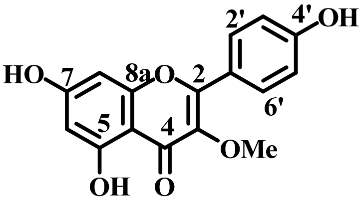


SM11 was obtained as a colorless gum. The IR spectrum exhibited absorption bands at 3372 and 1650 cm^{-1} for a hydroxyl group and a carbonyl group of a carboxylic acid, respectively. The ^{1}H NMR spectrum **(Table 16)** showed the presence of 1,4 di-substituted benzene [δ_H 7.93 (*d*, J = 8.7 Hz, 2H) and 6.84 (*d*, J = 8.7 Hz, 2H)]. The ^{13}C NMR spectrum showed the carbonyl carbon signal of the carboxylic acid at δ_C 166.9. Comparison of its ^{1}H and ^{13}C NMR data with those of 4-hydroxybenzoic acid indicated that **SM11** was 4-hydroxybenzoic acid which was previously isolated from *Oryza sativa* (Cho *et al*., 1998).

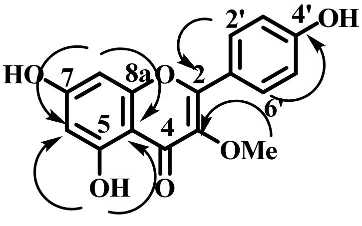
Table 16 ^{1}H, ^{13}C NMR spectral data of **SM11**

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**
1	-	121.7 (C)
2,6	7.93 (*d*, 8.7)	131.8 (CH)
3,5	6.84 (*d*, 8.7)	115.1 (CH)
4	-	161.8 (C)
7	-	166.9 (C=O)

SM12: 5,7,4'-trihydroxy-3-methoxyflavone



SM12 was obtained as a yellow soild, The IR spectrum showed the absorption bands of O-H stretching at 3342 cm^{-1}, C=O stretching at 1598 cm^{-1}. The UV spectrum showed maximum absorption bands at 227, 269 and 336 nm. The ^{1}H NMR spectrum (**Table 17**) showed the resonances of a hydrogen bonded hydroxyl group at δ 13.00 (5-OH), two hydroxyl groups at δ 9.80 (7-OH) and 9.68 (4'-OH) and a methoxyl group at δ 3.92 (3-OCH$_3$). The spectrum further showed signals of *meta*-protons H-6 and H-8 at δ 6.50 and 6.55, respectively. The *ortho* coupling pattern in aromatic region at δ 7.55 (*d*) and 6.96 (*d*) were proposed for the characteristic signals of H-2'/H-6' and H-3'/H-5', respectively. The methoxyl group was placed at C-3 according to the ^{3}J HMBC correlation of this methoxyl group to the high field oxygenated quaternary carbon (δ 130.4, C-3). Therefore, **SM12** was assigned to be 5,7,4'-trihydroxy-3-methoxyflavone (Oesterle *et al.*, 1917).

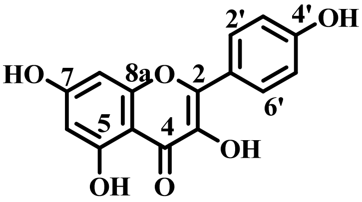


Selected HMBC correlations of **SM12**

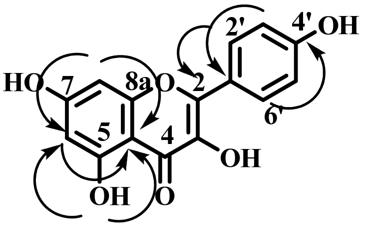
Table 17 ^{1}H, ^{13}C NMR spectral data of **SM12**

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**	**HMBC**
2	-	160.8 (C)	-
3	-	130.4 (C)	-
4	-	197.8 (C=O)	-
4a	-	131.6 (C)	-
5	-	153.2 (C)	-
6	6.50 (*s*)	105.4 (CH)	C-7
7	-	164.7 (C)	-
8	6.55 (*s*)	94.0 (CH)	C-4a, C-5, C-8a
8a	-	156.4 (C)	-
1'	-	121.8 (C)	-
2', 6'	7.55 (*d,* 9.0)	128.3 (CH)	C-2, C-6'
3', 5'	6.96 (*d,* 9.0)	116.2 (CH)	C-1'
4'	-	155.6 (C)	-
5-OH	13.00 (*s*)	-	C-6, C-5, C-4a
7-OH	9.80 (*s*)	-	-
4'-OH	9.68 (*s*)	-	-
3-OMe	3.92 (*s*)	60.1 (CH_3)	C-3

SM13: Kaempferol



SM13 was obtained as a yellow solid, mp. 274-276 °C. The UV spectrum exhibited absorption maxima at 231 and 272 nm. The IR spectrum showed the absorption bands of O-H stretching at 3480 cm^{-1}, C=O stretching at 1598 cm^{-1}. The ^{1}H NMR spectrum **(Table 18)** showed the signal of a chelated hydroxyl group (δ 12.62, 5-OH), two hydroxyl groups (δ 10.12, 7-OH and δ 8.47, 4'-OH), *meta*-aromatic protons (δ 6.50, H-8 and 6.52, H-6) and two sets of *ortho*-aromatic protons (δ 7.68, *d, J* = 9.0 Hz and δ 6.85, *d, J* = 9.0 Hz). These chemical shift values and coupling patterns were similar to those of relevant protons of **SM12** but without the signals of methoxyl group. The ^{13}C-NMR and HMBC correlations confirmed that **SM13** was then assigned to be 3,5,7,4'-tetrahydroxyflavone which was known as kaempferol (Liu *et al*., 2008).

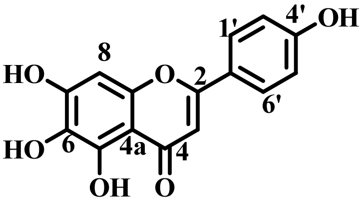


Selected HMBC correlations of **SM13**

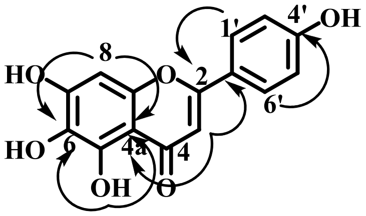
Table 18 ^{1}H, ^{13}C NMR spectral data of **SM13**

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**	**HMBC**
2	-	145.8 (C)	-
3	-	137.8 (C)	-
4	-	181.1 (C=O)	-
4a	-	103.2 (C)	-
5	-	158.9 (C)	-
6	6.52 (*s*)	101.2 (CH)	C-7, C-4a
7	-	162.8 (C)	-
8	6.50 (*s*)	92.8 (CH)	C-4a, C-5, C-8a
8a	-	151.8 (C)	-
1'	-	120.6 (C)	-
2', 6'	7.68 (*d,* 9.0)	126.7 (CH)	C-4', C-6'
3', 5'	6.85 (*d,* 9.0)	114.8 (CH)	C-4', C-1'
4'	-	159.8 (C)	-
5-OH	12.92 (*s*)	-	C-6, C-5, C-4a
7-OH	10.12 (*s*)	-	-
4'-OH	8.47 (*s*)	-	-

SM14: Scutellarein



SM14 was obtained as a yellow soild, mp 235-237 °C. The UV spectrum exhibited absorption maxima at 226, 275 and 332 nm. The IR spectrum showed the absorption bands of O-H stretching at 3318 cm^{-1}. The ^{1}H NMR spectrum (**Table 19**) showed the characteristic resonances of a flavone proton at δ_H 6.43 (*s*, H-3), a chelated hydroxyl proton at δ_H 12.62 (*s*, 5-OH) and an aromatic proton at δ_H 6.47 (*s*, H-8). In addition, a pair of doublet aromatic protons with J = 9.0 (δ_H 7.68, 2H and 6.84, 2H) revealed the presence of 1,4 di-substitued benzene of ring-B. The ^{13}C NMR spectrum indicated the presence of a carbonyl carbon (δ_C 181.1), eight quaternary carbons (δ_C 162.8, 159.8, 151.8, 148.9, 145.8, 127.9, 120.6 and 103.2), six methine carbons (δ_C 126.7×2, 114.8×2, 101.2, and 92.8). The HMBC correlations of H-3 to C-2, C-4, C-4a and C-1' supported the assignment of H-3. The chelated hydroxyl group correlated with carbon signals at δ_C 145.8, 127.9 and 103.2. The signal at δ_C 103.2 was attributed to C-4a because of the correlation of H-3 to that while the signals at δ_C 145.8 and 127.9 were attributed to C-5 and C-6, respectively. The C-6 position was connected to a hydroxyl group although the signal was resonated at highfield, but it was affected by two *ortho*- and one *para*- donating groups. Thus **SM14** was assigned as 5,6,7,4'-tetrahydroxyflavone which was corresponded to scutellarein (Subramanian *et al*., 1972).

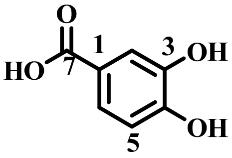


Selected HMBC correlations of **SM14**

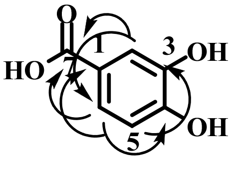
Table 19 ^{1}H, ^{13}C NMR spectral data of **SM14**

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**	**HMBC**
2	-	162.8 (C)	-
3	6.43 (*s*)	101.2 (CH)	C-2, C- 4, C-4a, C- 1'
4	-	181.1 (C=O)	-
4a	-	103.2 (C)	-
6	-	127.9 (C)	-
7	-	151.8 (C)	-
8	6.47 (*s*)	92.8 (CH)	C-4a, C- 6, C-7, C-8a
8a	-	148.9 (C)	-
1'	-	120.6 (C)	-
2', 6'	7.68 (*d,* 9.0)	126.7 (CH)	C-2, C-4'
3', 5'	6.84 (*d,* 9.0)	114.8 (CH)	C-1', C-4'
4'	-	159.8 (C)	-
5-OH	12.62 (*s*)	145.8 (C)	C-6, C-5, C-4a

SM15: 3,4-dihydroxybenzoic acid



SM15 was obtained as a brown gum. The ^{1}H NMR spectrum (**Table 20**) displayed signals for three aromatic protons of a 1,2,4-trisubstitued benzene [δ_H 7.56 (*d*, J = 2.1 Hz, 1H), 7.48 (*dd*, J = 8.4, 2.1 Hz, 1H) and 6.88 (*d*, J = 8.4 Hz, 1H)]. The aromatic proton resonating at δ_H 7.56, 7.48 and 6.88 were attributed to H-2, H-6 and H-5, respectively, according to their multiplicity and coupling constants. The ^{13}C NMR spectrum showed the signal of a carbonyl carbon of the carboxylic acid at δ_C 173.1. The HMBC correlations of H-2 (δ_H 7.56) and H-6 (δ_H 7.48) with C-7 (δ_C 173.1) indicating the both low field aromatic protons were *ortho* position to the carbonyl group. In addition, the aromatic proton H-5 correlated with C-1 (δ_C 127.2) and C-3 (δ_C 149.1) in HMBC experiment confirming that the proton H-5 was *meta* position to the carbonyl group. According to the carbon chemical shifts, the substituents at C-3 (δ_C 149.1) and C-4 (δ_C 154.3) were hydroxyl groups. Therefore, **SM15** was identified as protocatechuic acid.



Selected HMBC correlations of compound **SM15**

Table 20 ^{1}H, ^{13}C NMR spectral data of compounds **SM15**

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**	**HMBC**
1	-	127.2 (C)	-
2	7.56 (*d,* 2.1)	121.7 (CH)	C-3, C-5, C-6, C-7
3	-	149.1 (C)	-
4	-	154.3 (C)	-
5	6.88 (*d,* 8.4)	119.6 (CH)	C-1, C-3, C-4, C-6
6	7.48 (*dd,* 8.4, 2.1)	127.6 (CH)	C-2, C-4, C-7
7	-	173.1 (C=O)	-

3.2 Review of biological activities of the known compounds obtained from this study

Biological activities of some compounds obtained from this study have been previously investigated. Based on the search from SciFinder Scholar, the biological activities of 5-hydroxy-7-methoxyflavone (**SM4**), 5-hydroxy-3,7,4'-trimethoxyflavone **(SM5)**, 5,7-dihydroxy-3-methoxyflavone (**SM6**), rengyolone **(SM7)**, 5,7-dihydroxyflavone **(SM8)**, 3,5,7-trihydroxyflavone **(SM9)**, 5,7,4'-trihydroxyflavone **(SM10)** and 3,5,7,4'-tetrahydroxyflavone **(SM13)** are summarized.

5-hydroxy-7-methoxyflavone (tectochrysin**, SM4**) was examined for anti-inflammatory activity (Tewtrakul *et al*., 2009; Cho *et al*., 2004). It was also strong anti-oxidant (Lee *et al*., 2003).

5-hydroxy-3,7,4'-trimethoxyflavone **(SM5)** showed significant cytotoxicity in the NCI human tumor cell line (growth inhibition, GI_{50} 54 μM) (Beutler *et al*., 1998). It exhibited the moderate inflammatory activity (Tewtrakul *et al*., 2009).

5,7 dihydroxy-3-methoxyflavone **(SM6)** inhibited the growth of *Staphylococcus aureus* (Torrenegra *et al*., 1989). It also exhibited significant anti-platelet aggregation activity in vitro (Chen *et al*., 2007).

Rengyolone **(SM7)** have been reported to showed anti-inflammatory activity (Kim *et al*., 2006).

Chrysin (5,7-dihydroxyflavone, **SM8**) is known to have multiple biological activities, such as anti-inflammation (Ko *et al*., 2003; Kim *et al*., 2002; Hougee *et al*., 2005), anti-cancer (Koganov *et al*., 1999; Habtemariam *et al*., 1997), anti-oxidation (Machala *et al*., 2001; Lapidot *et al*., 2002). In addition, chrysin showed antibacterial activity against *Staphylococcus aureus* with MIC value of 103 μg/mL (Alcaraz *et al*., 2000).

Galangin (3,5,7-trihydroxyflavone, **SM9)** have been reported to show antibacterial activity against *Staphylococcus epidermidis* with MIC value of 6.3 μg/mL (Nishino *et al*., 1987).

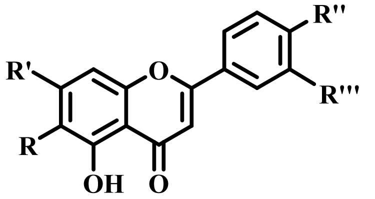
Apigenin (5,7,4'-trihydroxyflavone, **SM10)** is used to evaluate inflammatory activity (Hougee *et al*., 2005).

Kaempferol (3,5,7,4'-tetrahydroxyflavone, **SM13**) was observed for antibacterial activity against *Staphylococcus epidermidis* with MIC value of 50 μg/mL (Nishino *et al*., 1987). There was the report of kaempferol on anti-inflammatory effect (Comalada *et al*., 2006).

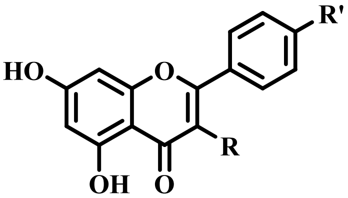
Conclusion

Investigation of the chemical constituents from the stem bark of *Oroxylum indicum* (L.) Benth. ex Kurz. resulted in the isolation of fifteen compounds including nine flavonoids: **SM4**, **SM5**, **SM6**, **SM8**, **SM9**, **SM10**, **SM12**, **SM13** and **SM14**, two triterpenoids: **SM1** and **SM2**, one isocoumarin: **SM3**, one benzofuranone: **SM7** and two benzoic acid derivatives: **SM11** and **SM15**. Compounds **SM1**, **SM2**, **SM3**, **SM5**, **SM6**, **SM8**, **SM9**, **SM11**, **SM12**, **SM14** and **SM15** were obtained for the first time from this plant. Further study on biological activity of the isolated

Flavonoids

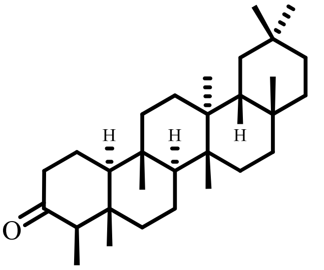


SM4 : R = R'' = R''' = H, R' = OMe : tectochrysin
SM5 : R = H, R' = R'' = R''' = OMe : luteolin
SM8 : R = R'' = R''' = H, R' = OH : chrysin
SM10 : R = R''' = H, R' = R'' = OH : apigenin
SM14 : R = R' = R'' = OH, R''' = H : scutellarein

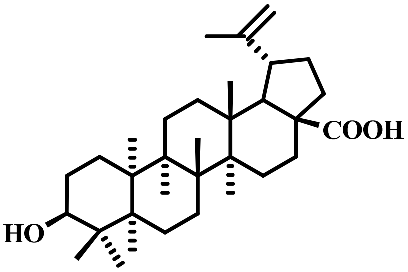


SM6 : R = OMe, R' = H : 5,7-dihydroxy-3-methoxyflavone
SM9 : R = OH, R' = H : galangin
SM12 : R = OMe, R' = OH : 5,7,4'-trihydroxy-3-methoxyflavone
SM13 : R = R' = OH : kaempferol

Triterpenoids

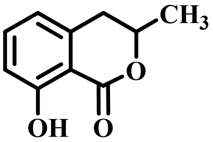


SM1 : friedelin



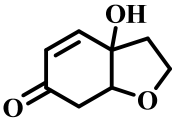
SM2 : betulinic acid

Isocoumarin



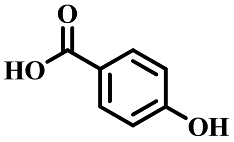
SM3 : (*R*)-(-)-mellein

Benzofuranone

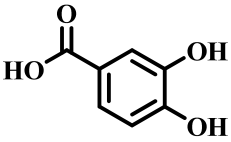


SM7 : rengyolone

Benzoic acid derivatives



SM11 : 4-hydroxybenzoic acid



SM15 : protocatechuic acid

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APPENDIX

1. Spectrum of compounds SM1-SM15

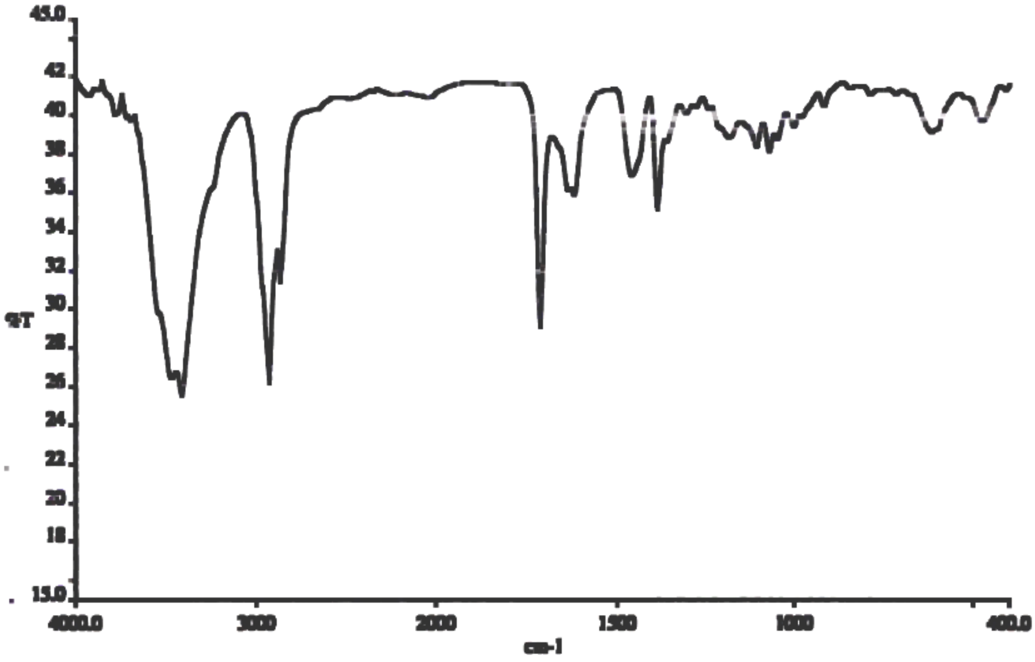


Figure A-1 FT-IR (Neat) spectrum of **SM1**

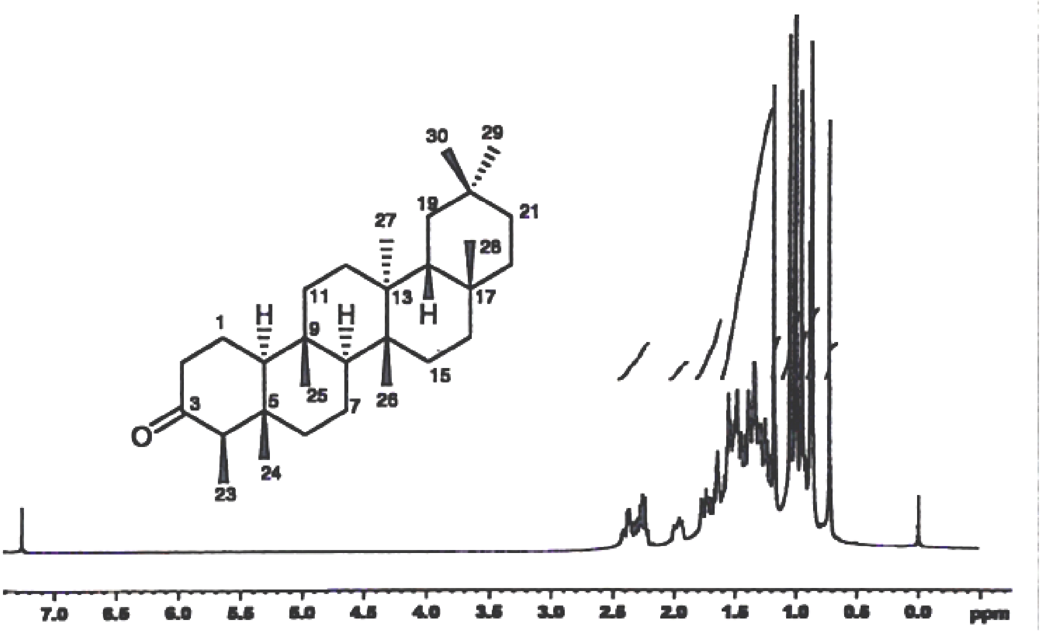


Figure A-2 ^{1}H-NMR (300 MHz) ($CDCl_3$) spectrum of **SM1**

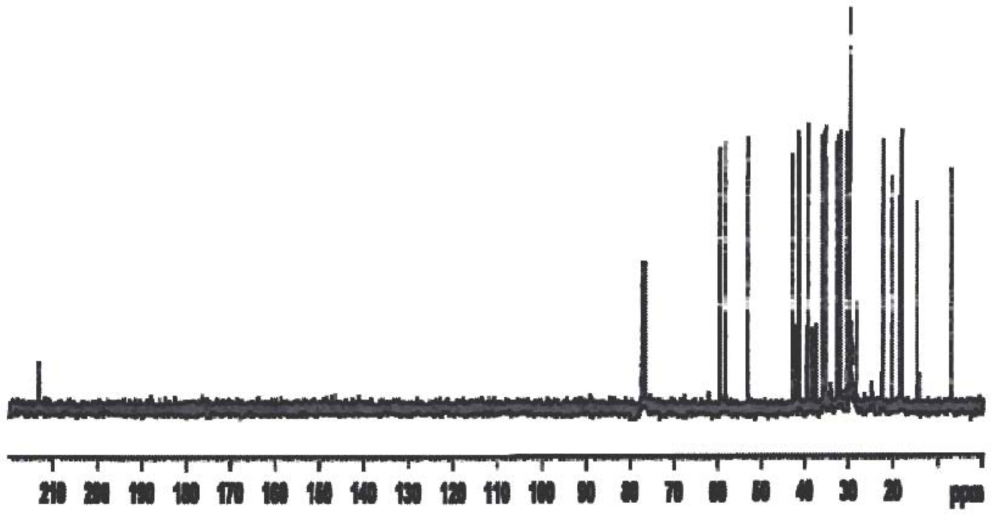


Figure A-3 ^{13}C-NMR (75 MHz) ($CDCl_3$) spectrum of **SM1**

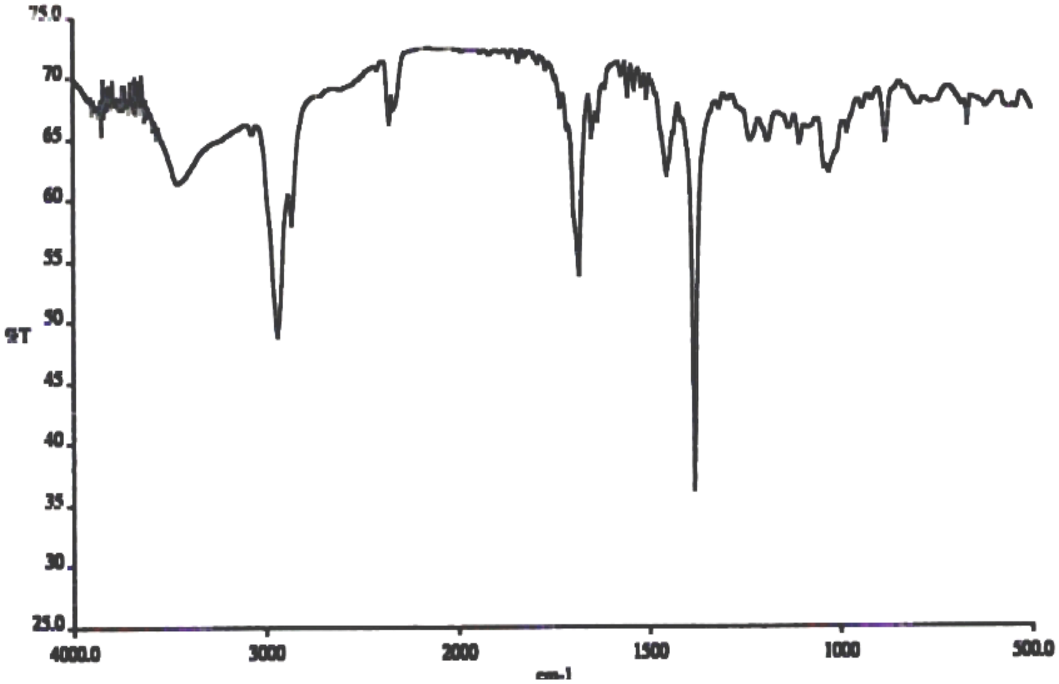


Figure A-4 FT-IR (Neat) spectrum of **SM2**

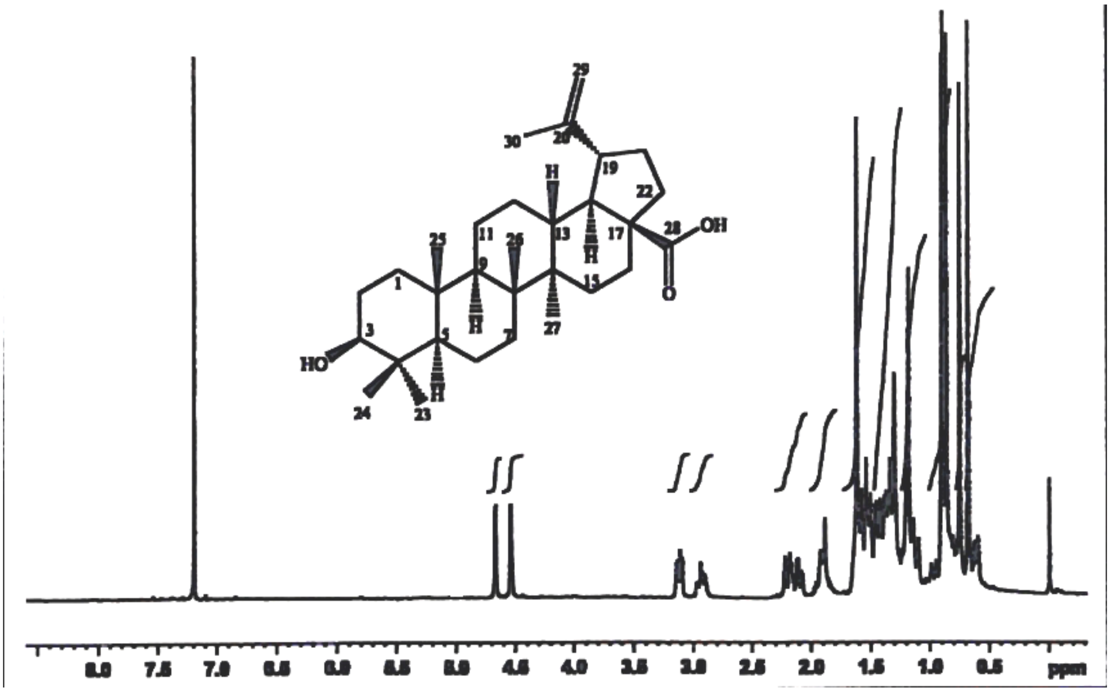


Figure A-5 ^{1}H-NMR (300 MHz) (CDCl$_3$) spectrum of **SM2**

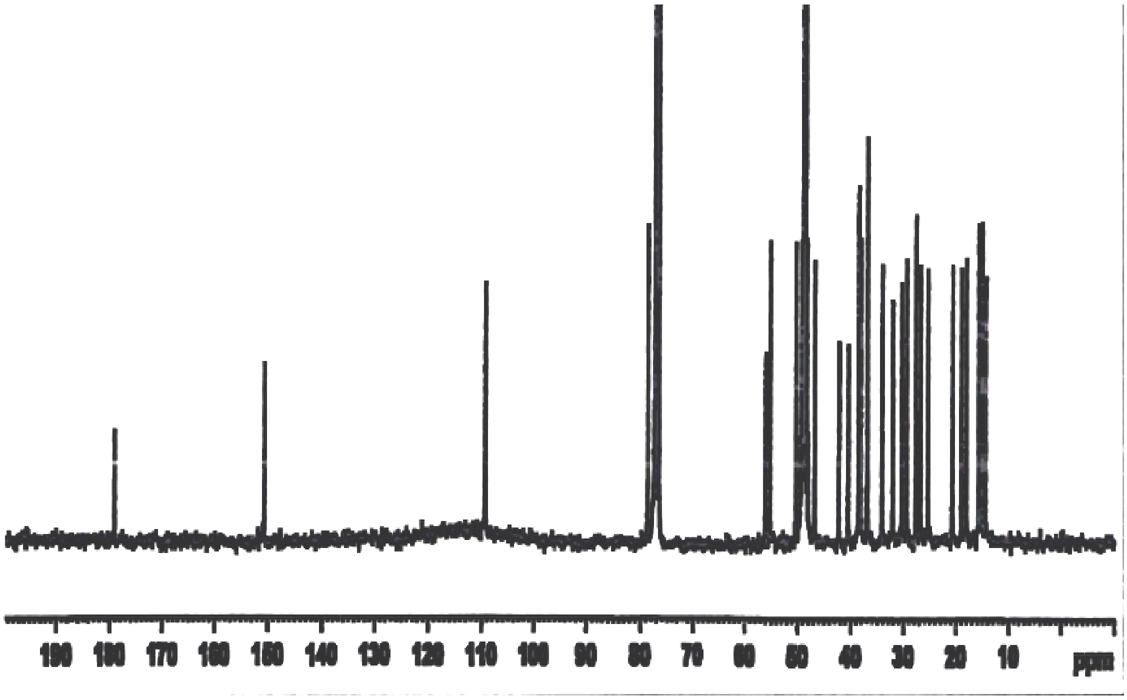


Figure A-6 ^{13}C-NMR (75 MHz) (CDCl$_3$) spectrum of **SM2**

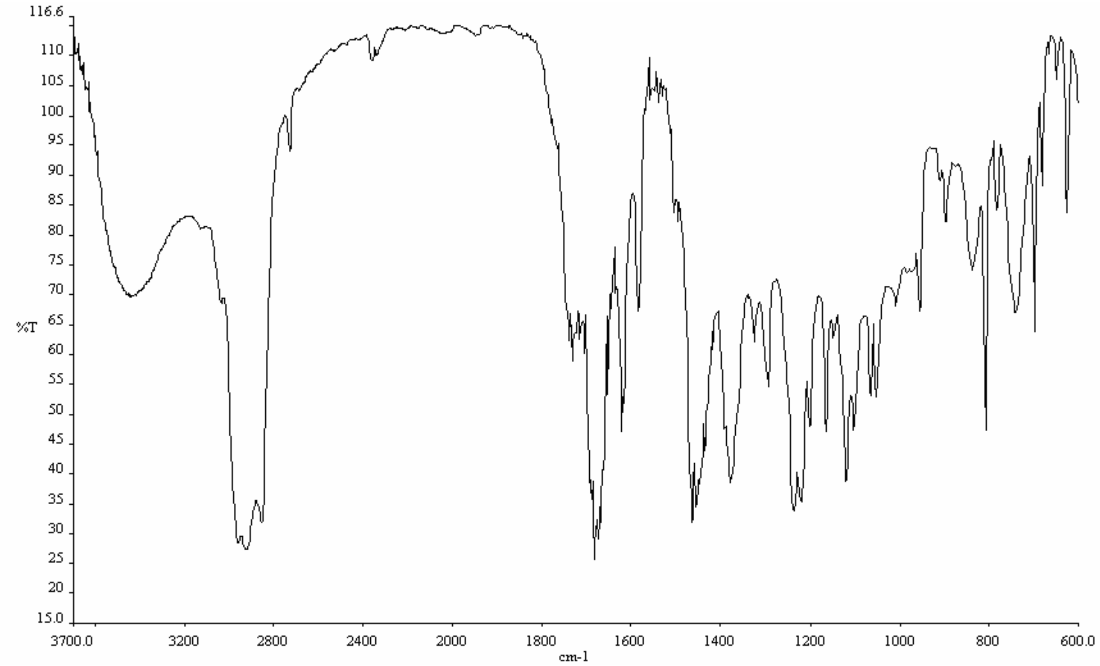


Figure A-7 FT-IR (Neat) spectrum of **SM3**

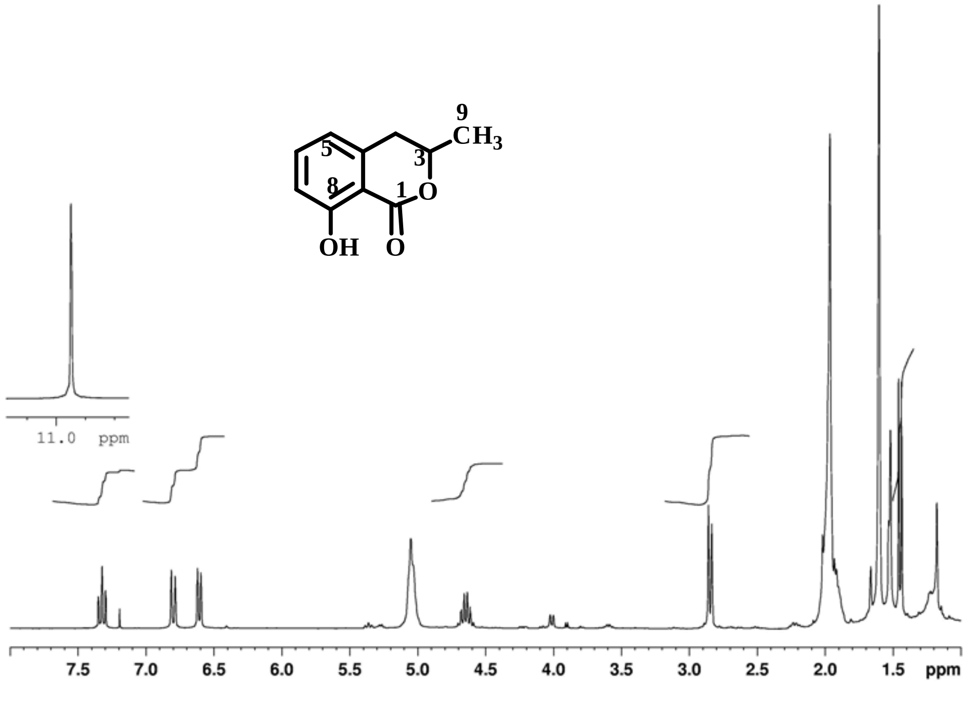


Figure A-8 ^{1}H-NMR (300 MHz) ($CDCl_3$) spectrum of **SM3**

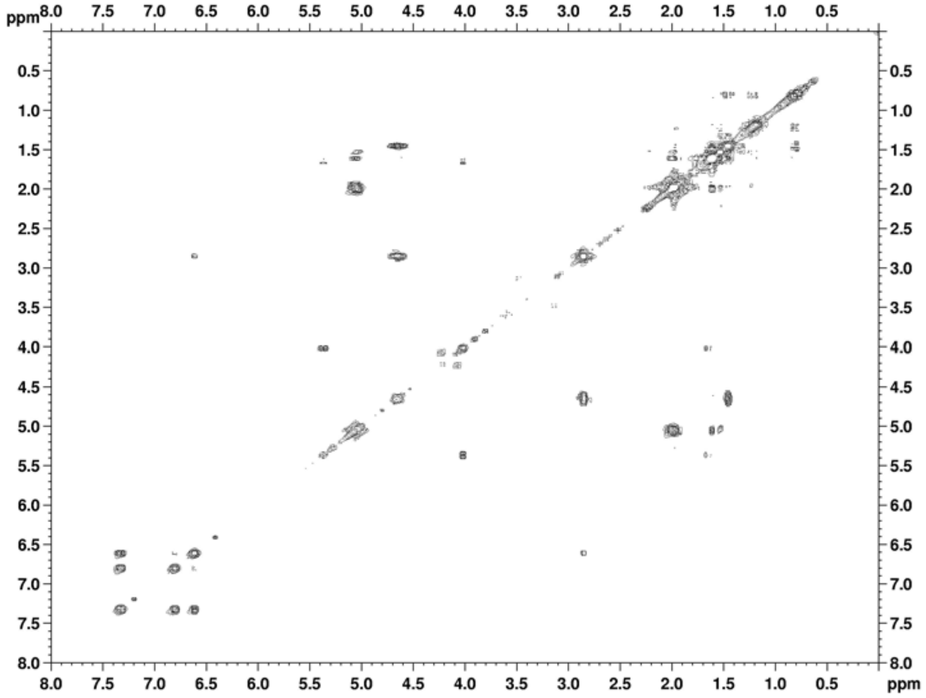


Figure A-9 ^{1}H-^{1}H COSY spectrum of **SM3**

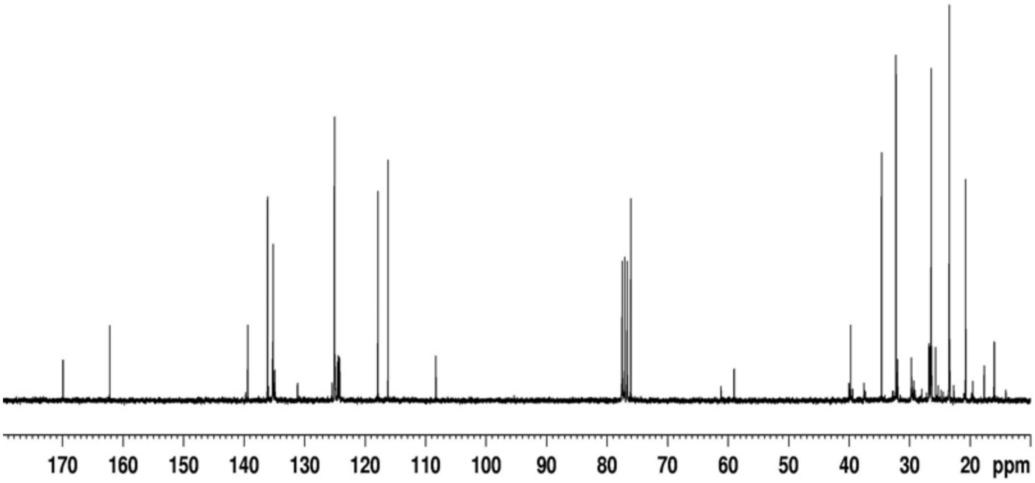


Figure A-10 ^{13}C-NMR (75 MHz) (CDCl$_3$) spectrum of **SM3**

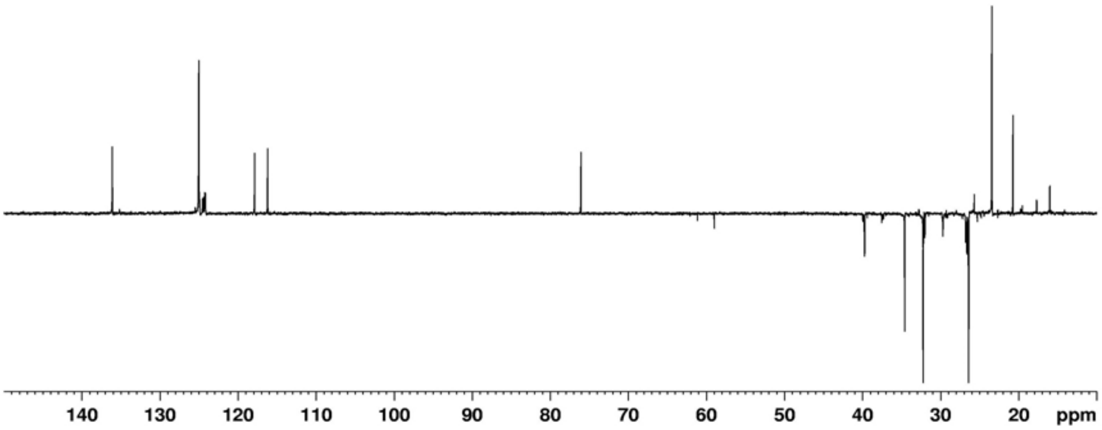


Figure A-11 DEPT 135^{o} ($CDCl_3$) spectrum of **SM3**

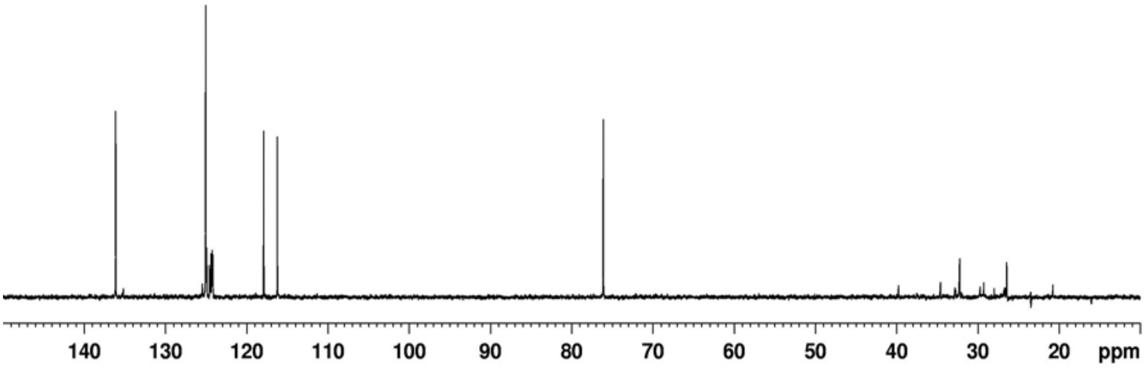


Figure A-12 DEPT 90^{o} ($CDCl_3$) spectrum of **SM3**

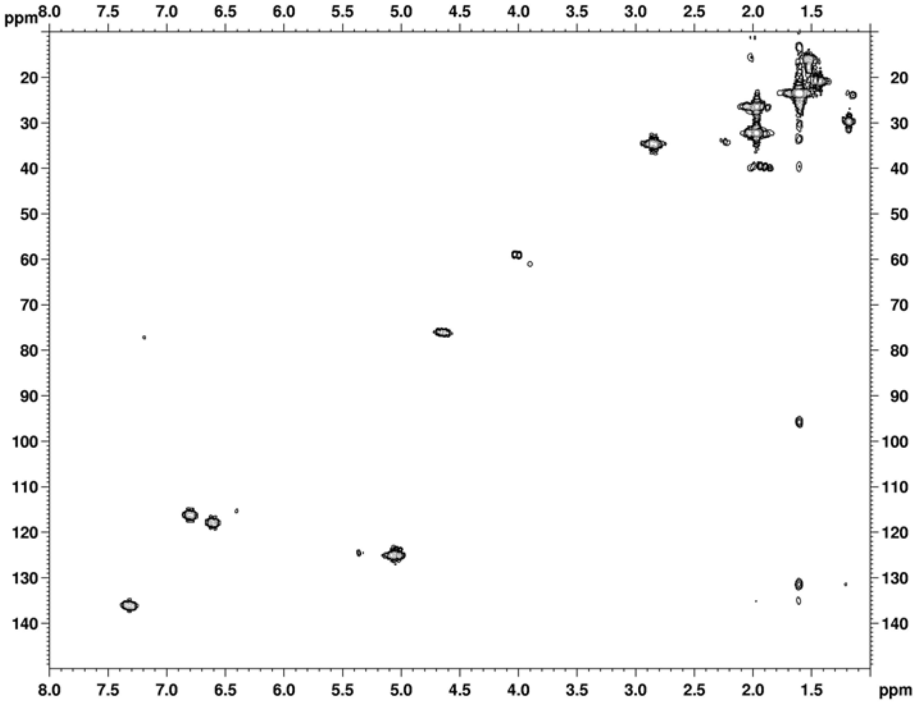


Figure A-13 2D HMQC spectrum of **SM3**

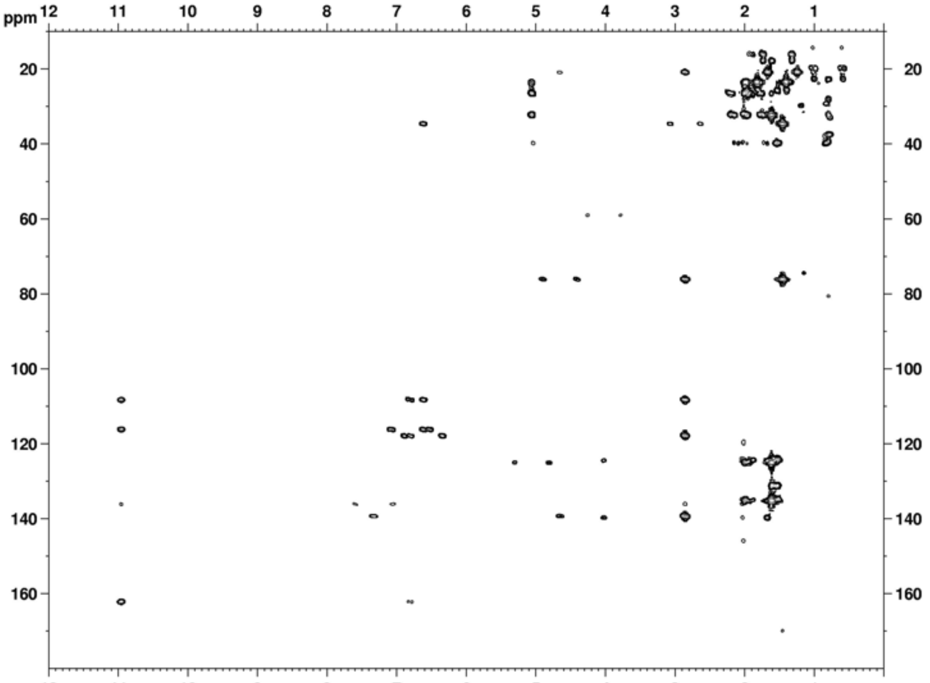


Figure A-14 2D HMBC spectrum of **SM3**

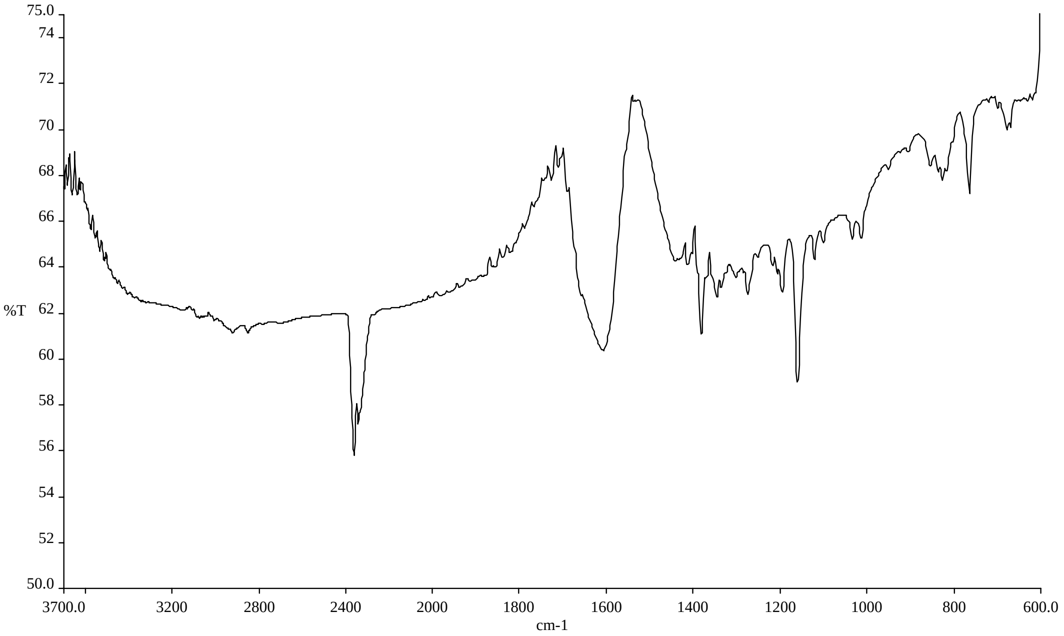


Figure A-15 FT-IR (Neat) spectrum of **SM4**

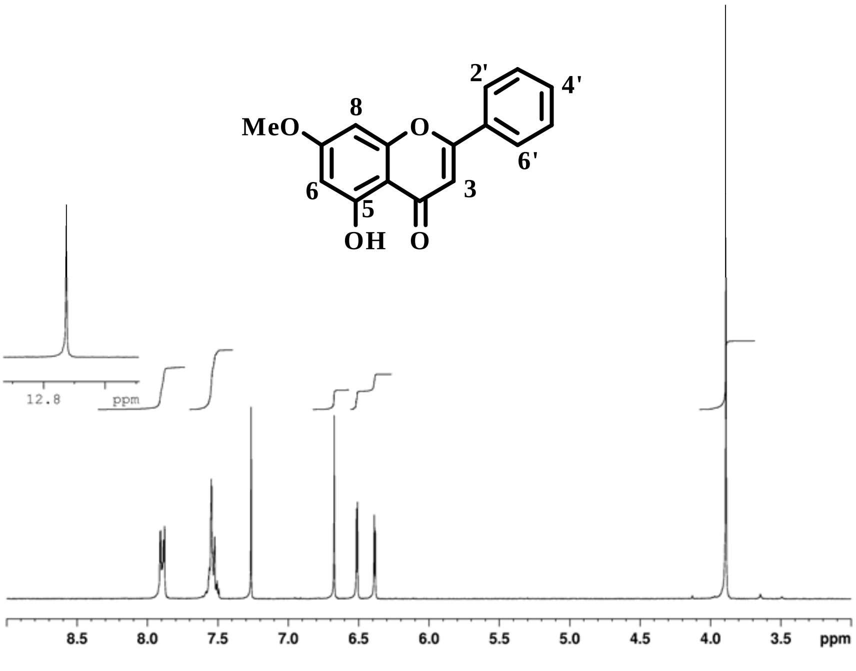


Figure A-16 ^{1}H-NMR (300 MHz) (CDCl$_3$) spectrum of **SM4**

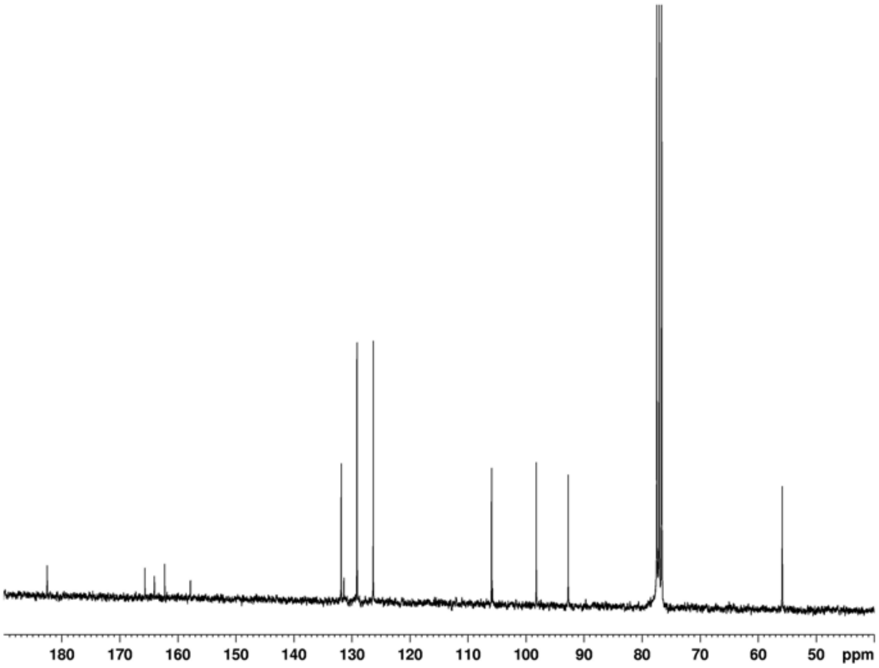


Figure A-17 ^{13}C-NMR (75 MHz) ($CDCl_3$) spectrum of **SM4**

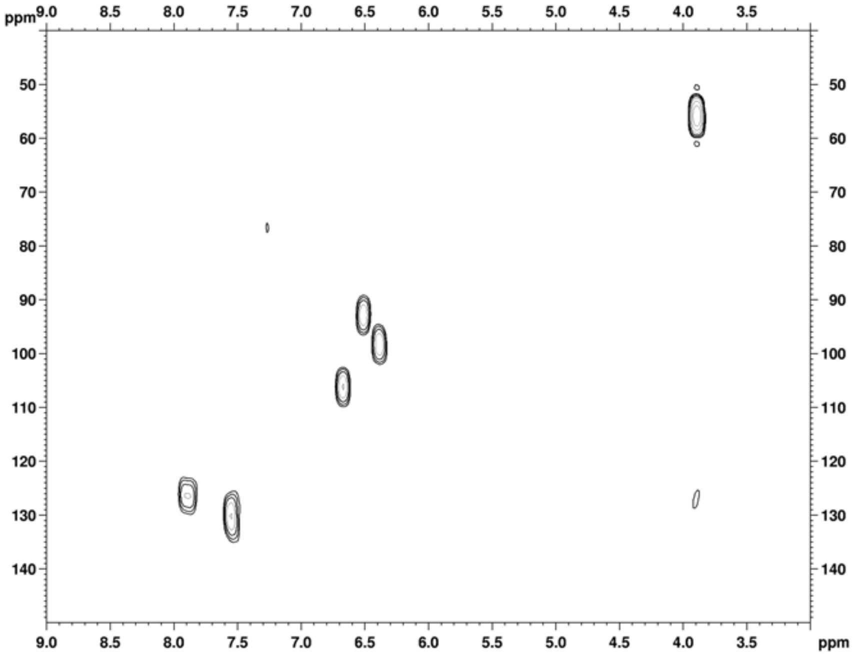


Figure A-18 2D HMQC spectrum of **SM4**

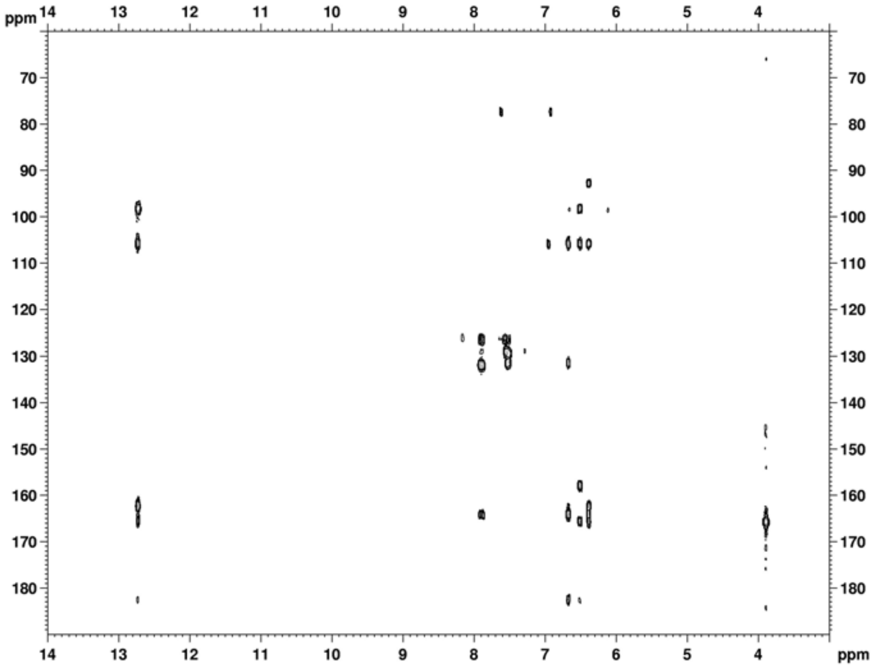


Figure A-19 2D HMBC spectrum of **SM4**

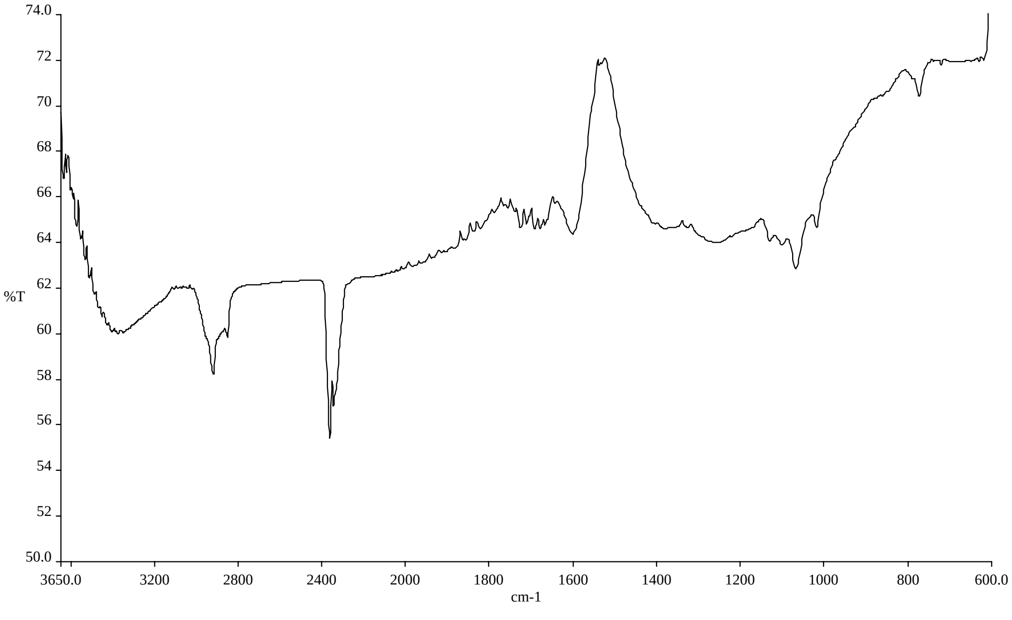


Figure A-20 FT-IR (Neat) spectrum of **SM5**

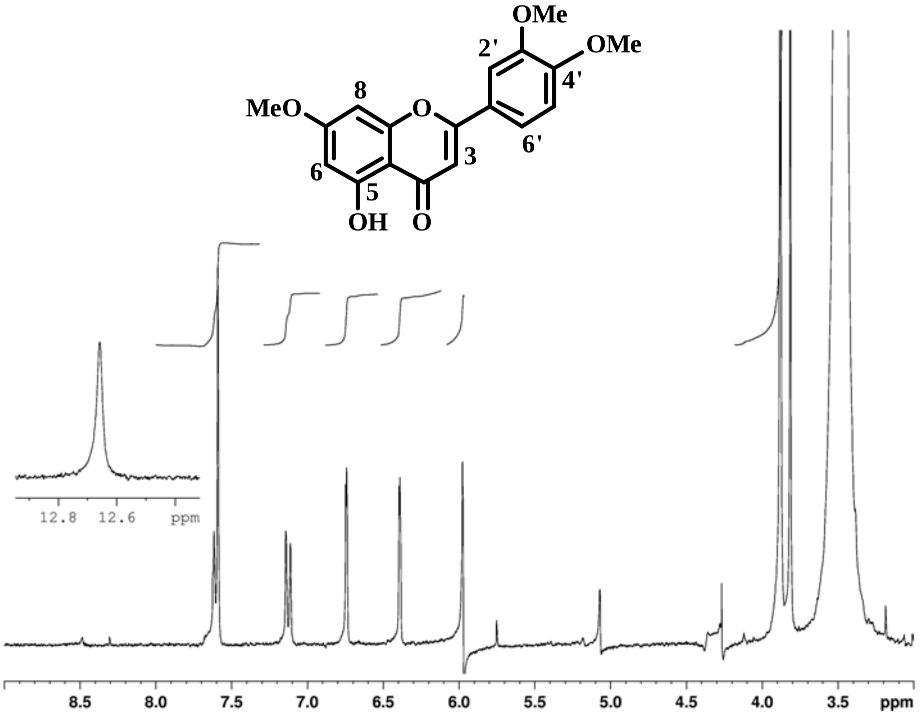


Figure A-21 ^{1}H-NMR (300 MHz) ($CDCl_3$+ DMSO-d_6) spectrum of **SM5**

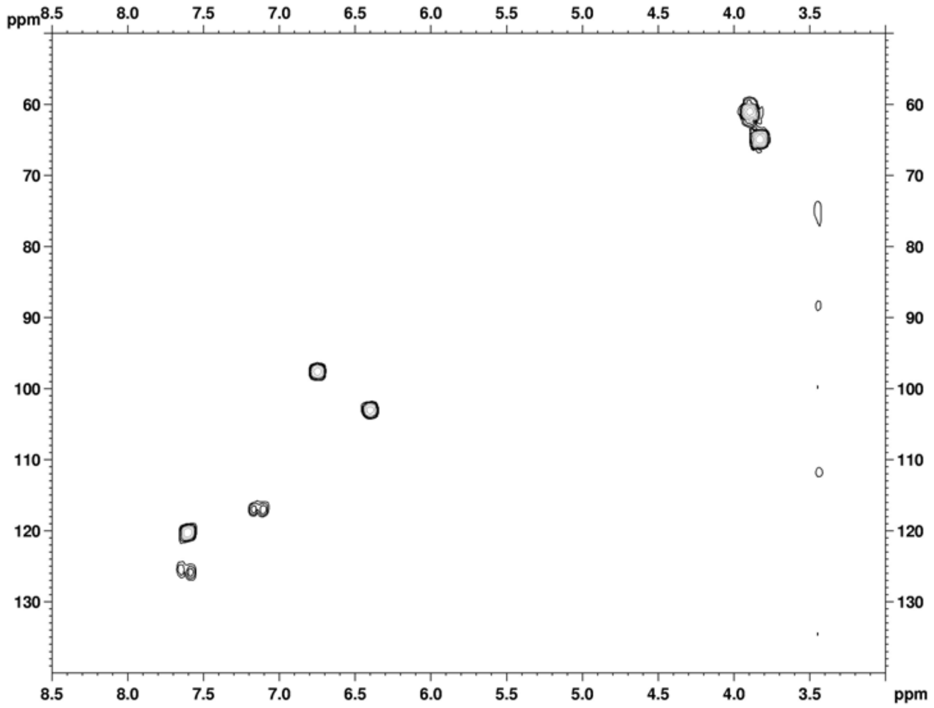


Figure A-22 2D HMQC spectrum of **SM5**

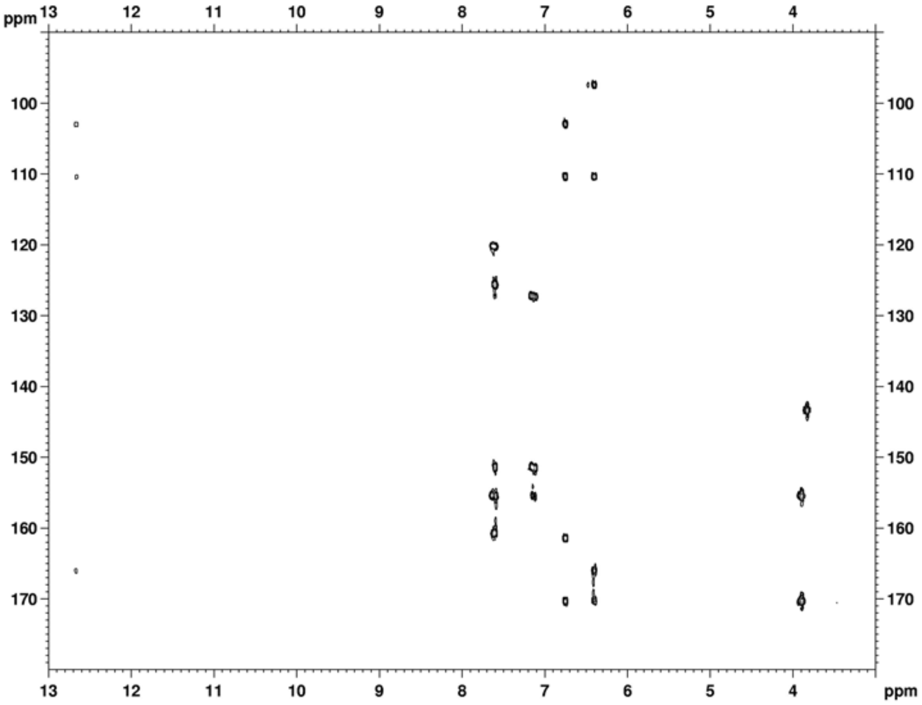


Figure A-23 2D HMBC spectrum of **SM5**

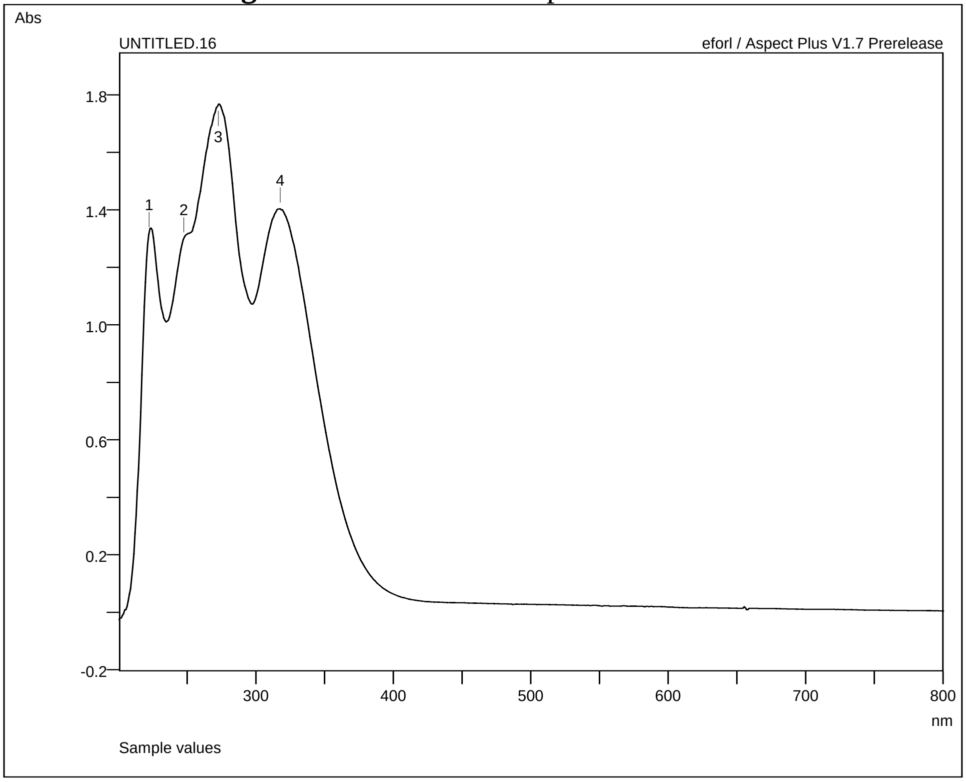


Figure A-24 UV (CH_3OH) spectrum of **SM6**

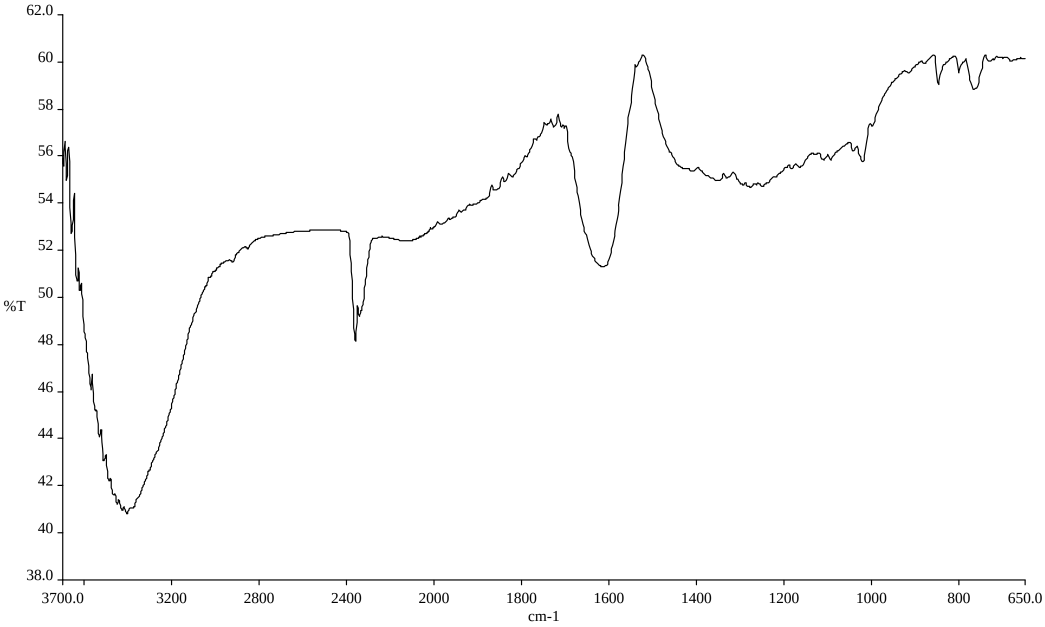


Figure A-25 FT-IR (Neat) spectrum of **SM6**

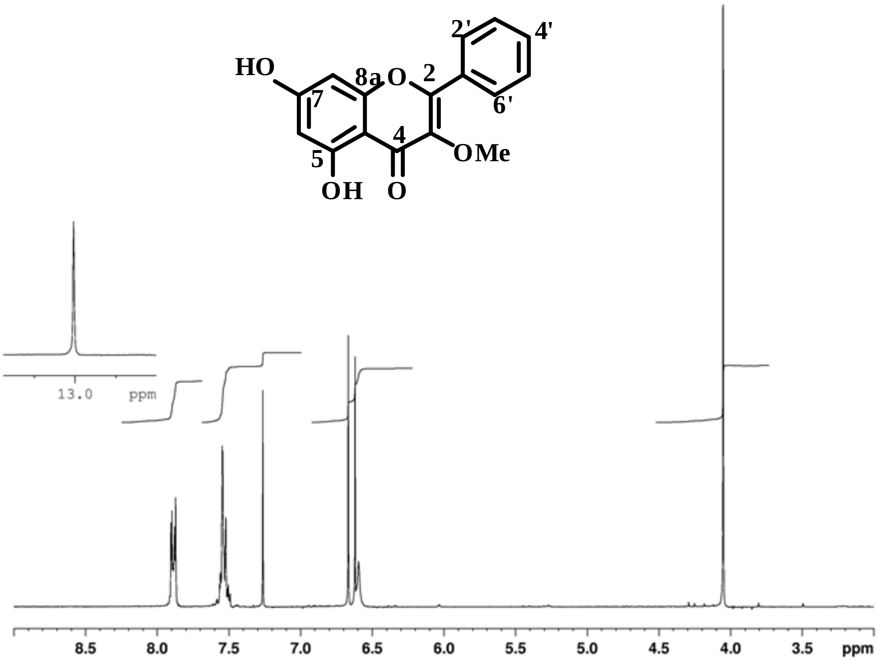


Figure A-26 ^{1}H-NMR (300 MHz) ($CDCl_3$) spectrum of **SM6**

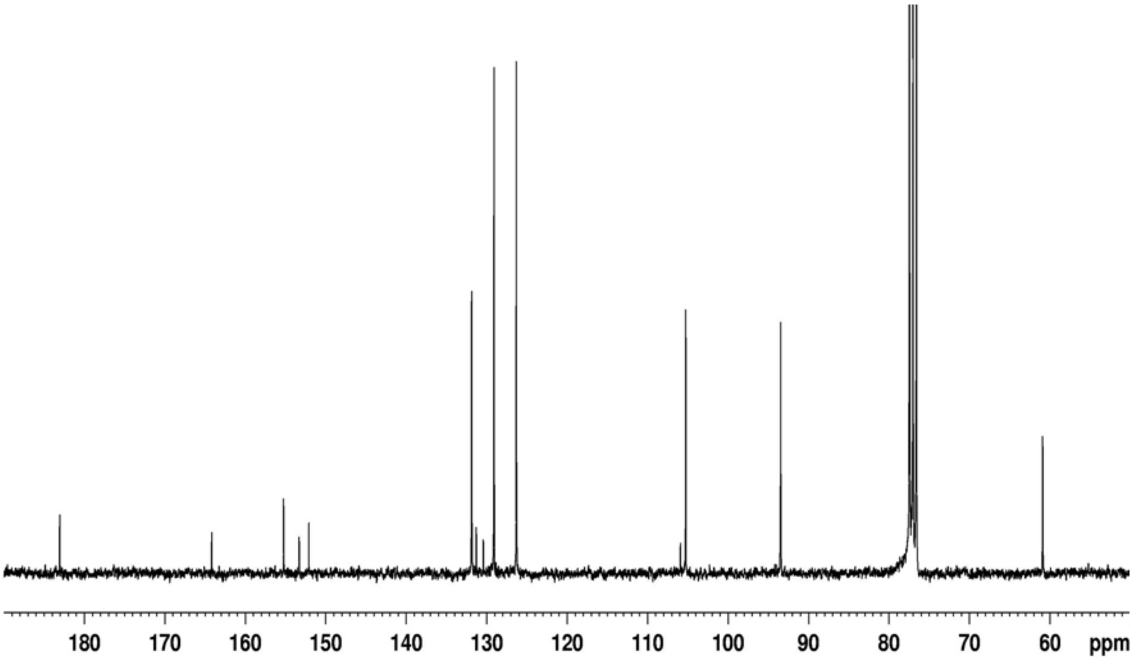


Figure A-27 ^{13}C-NMR (75 MHz) (CDCl$_3$) spectrum of **SM6**

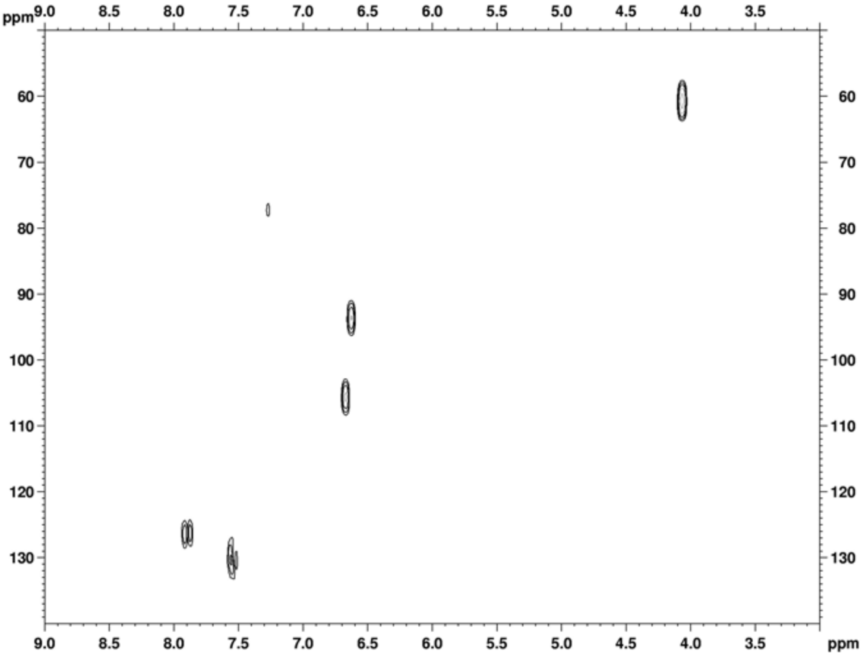


Figure A-28 2D HMQC spectrum of **SM6**

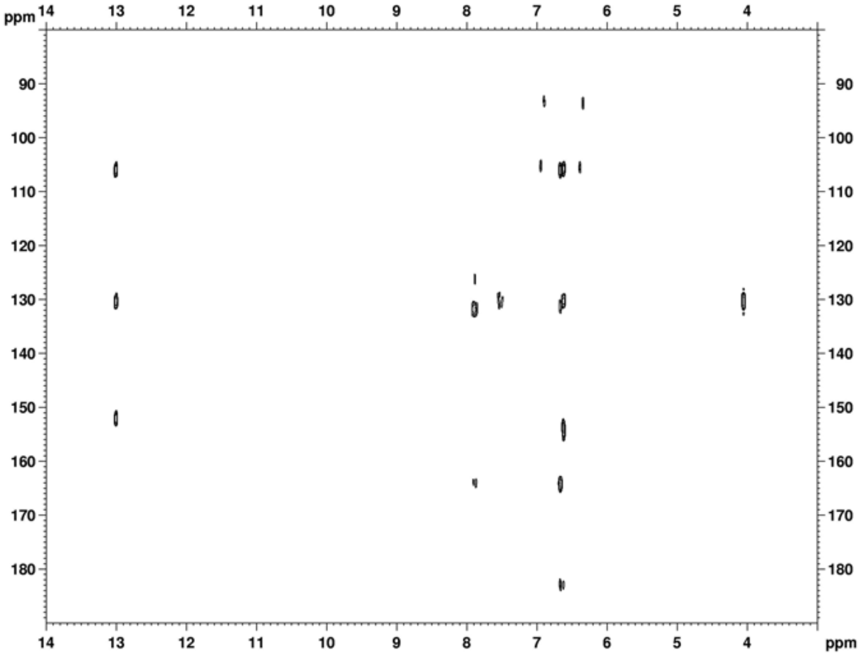


Figure A-29 2D HMBC spectrum of **SM6**

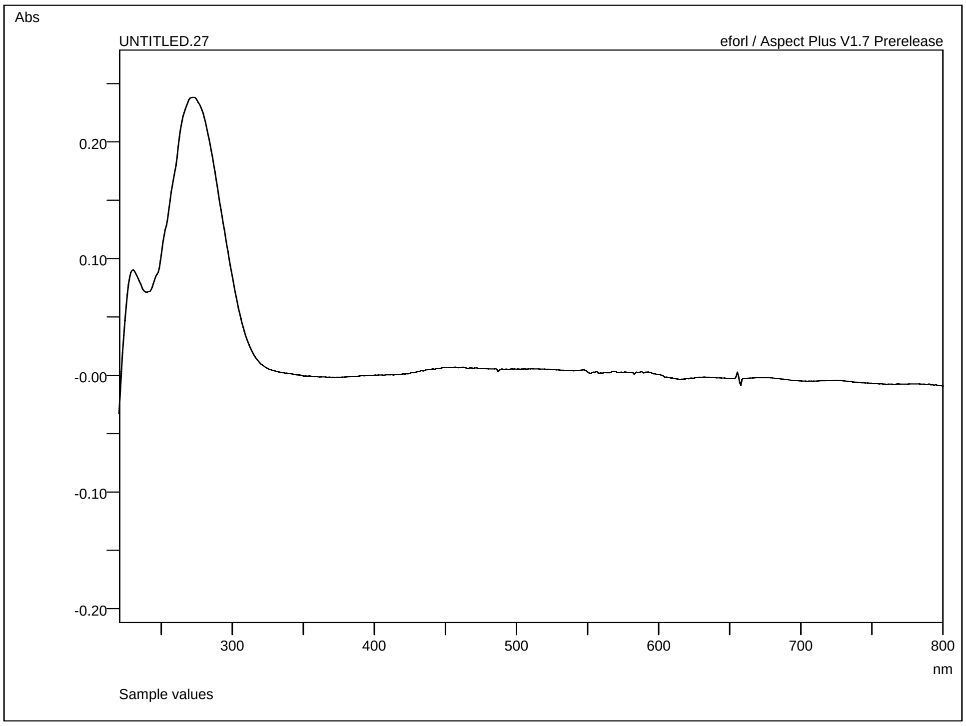


Figure A-30 UV (CH_3OH) spectrum of **SM7**

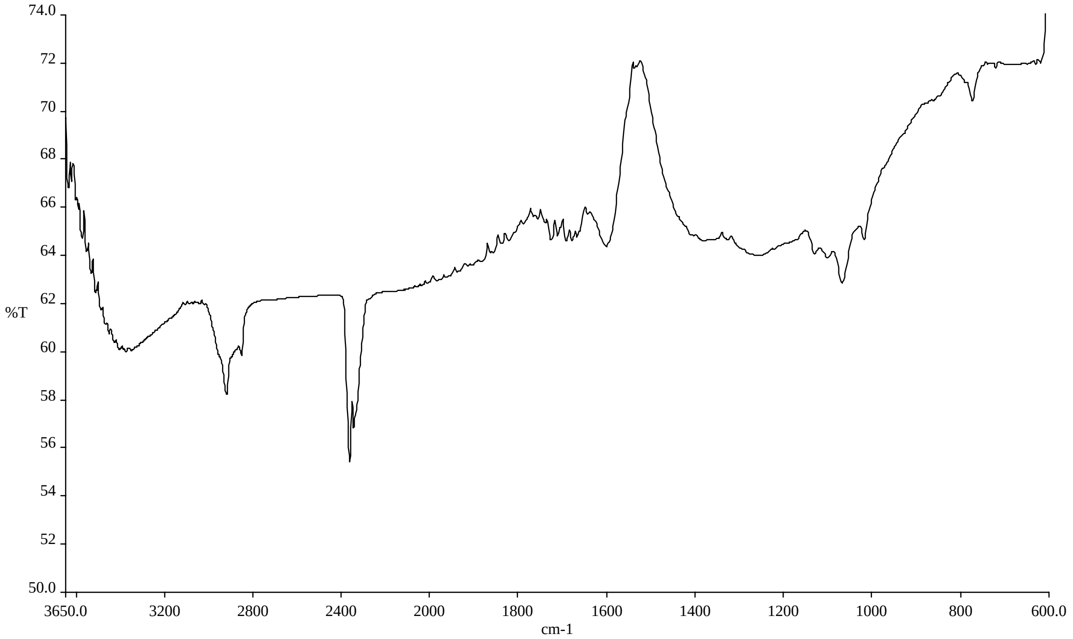


Figure A-31 FT-IR (Neat) spectrum of **SM7**

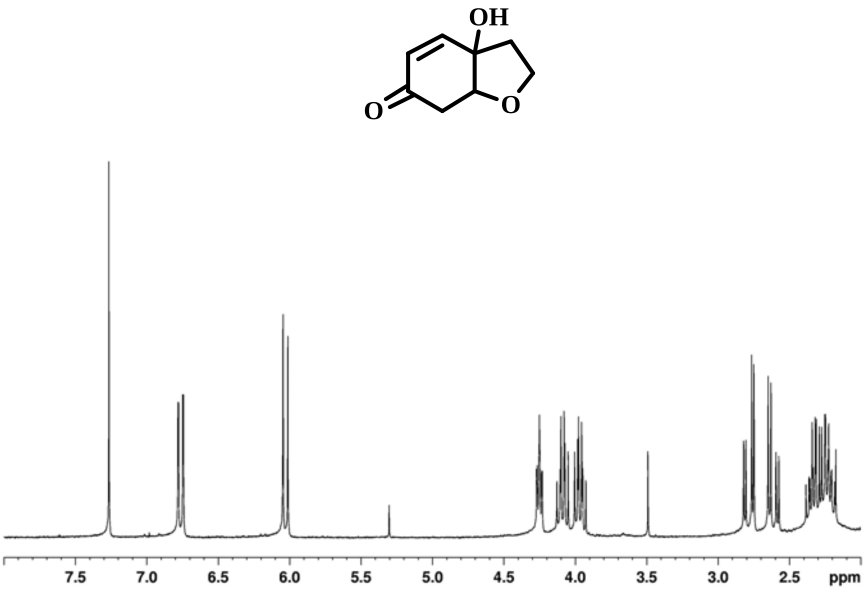


Figure A-32 ^{1}H-NMR (300 MHz) ($CDCl_3$) spectrum of **SM7**

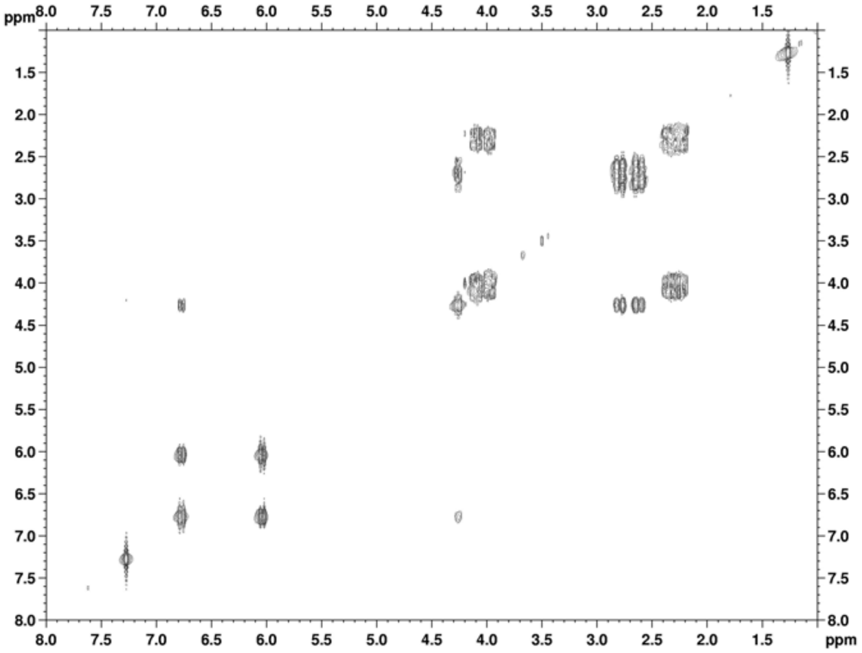


Figure A-33 ^{1}H-^{1}H COSY spectrum of **SM7**

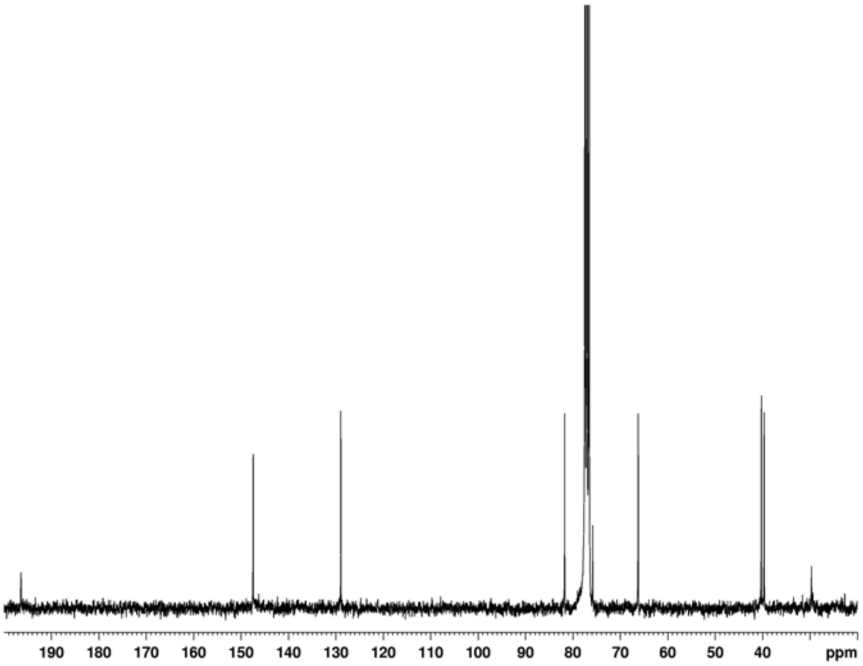


Figure A-34 ^{13}C-NMR (75 MHz) (CDCl$_3$) spectrum of **SM7**

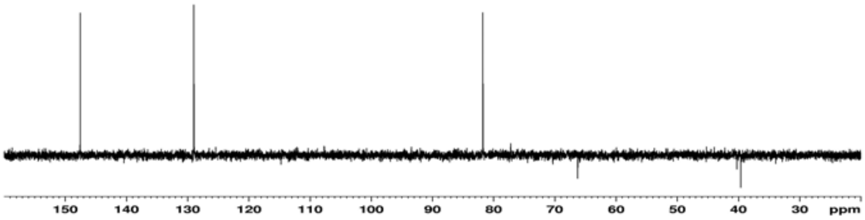


Figure A-35 DEPT 135° ($CDCl_3$) spectrum of **SM7**

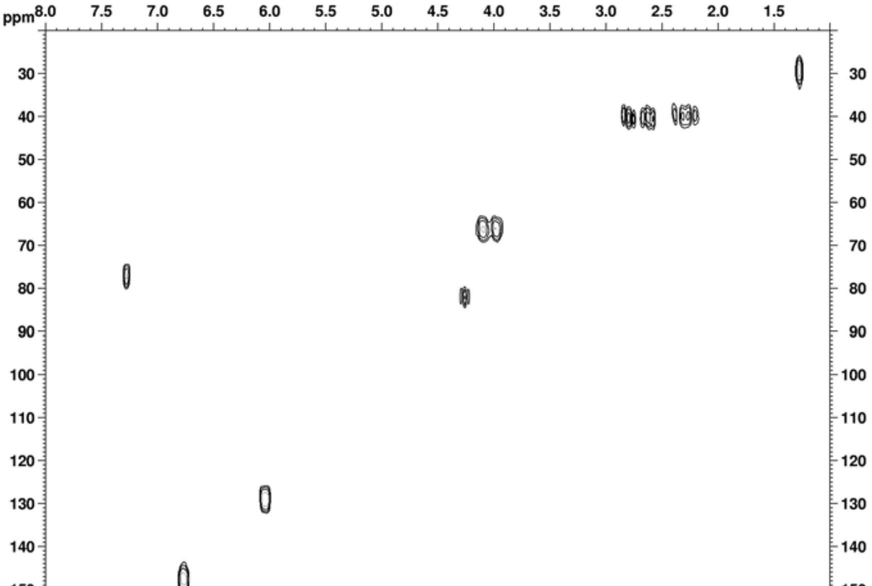


Figure A-36 2D HMQC spectrum of **SM7**

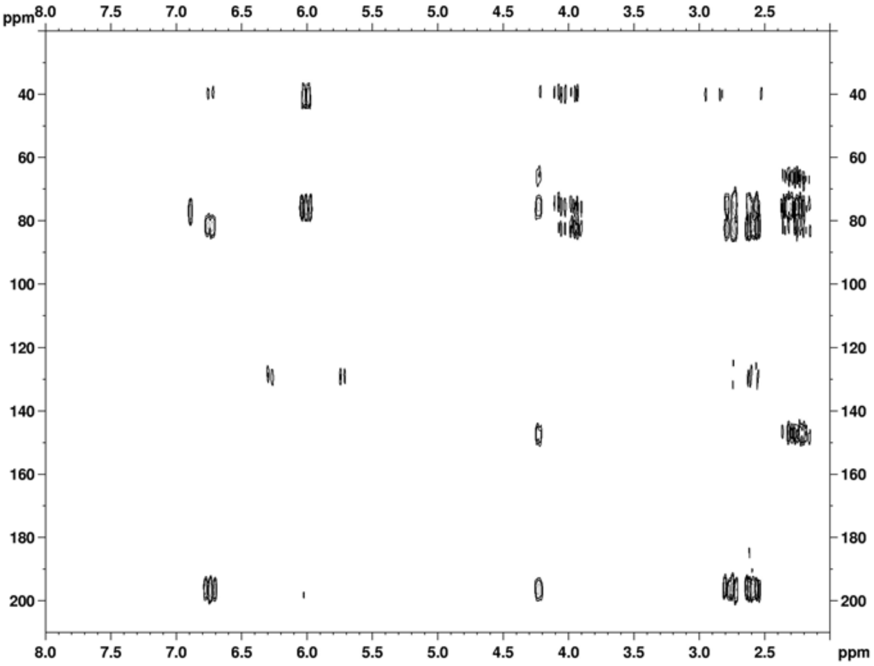


Figure A-37 2D HMBC spectrum of **SM7**

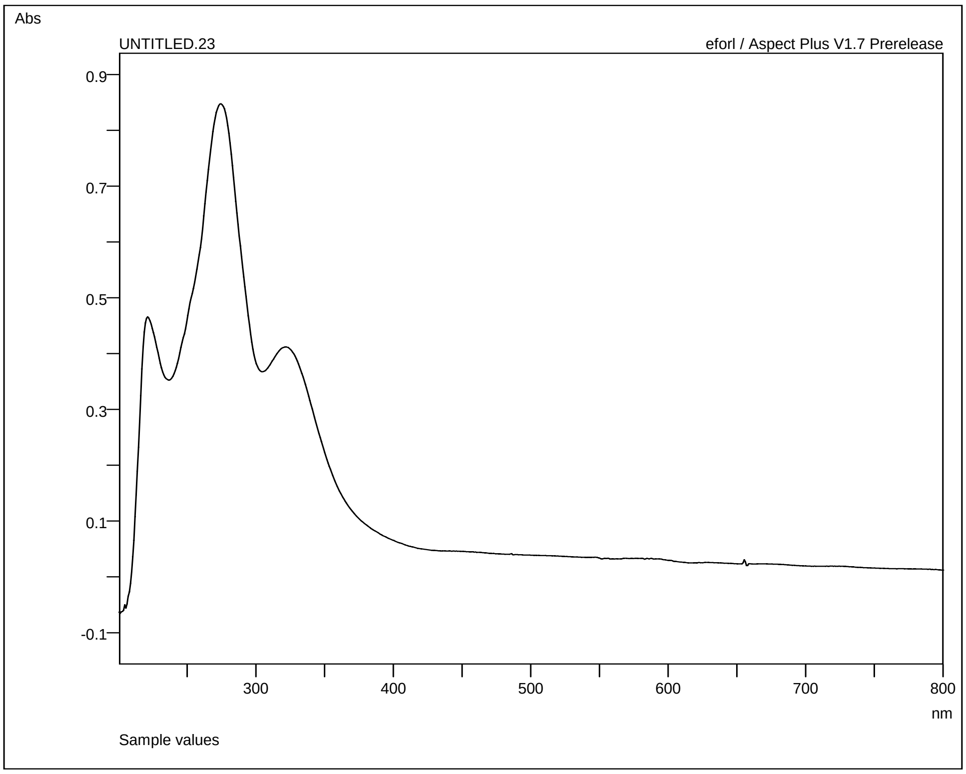


Figure A-38 UV (CH_3OH) spectrum of **SM8**

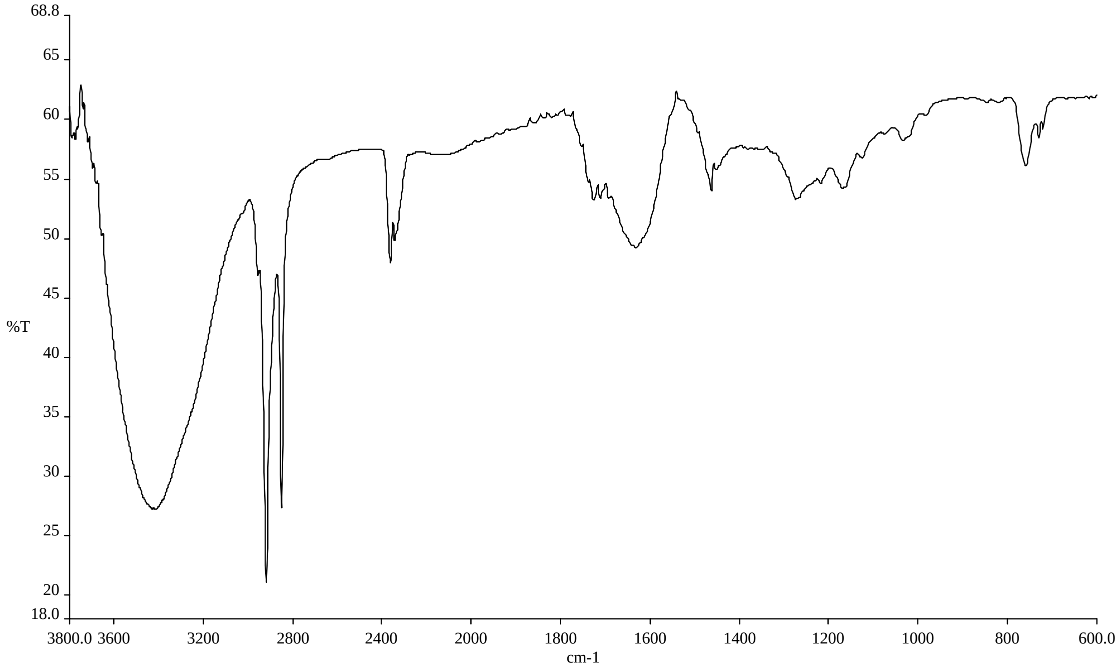


Figure A-39 FT-IR (Neat) spectrum of **SM8**

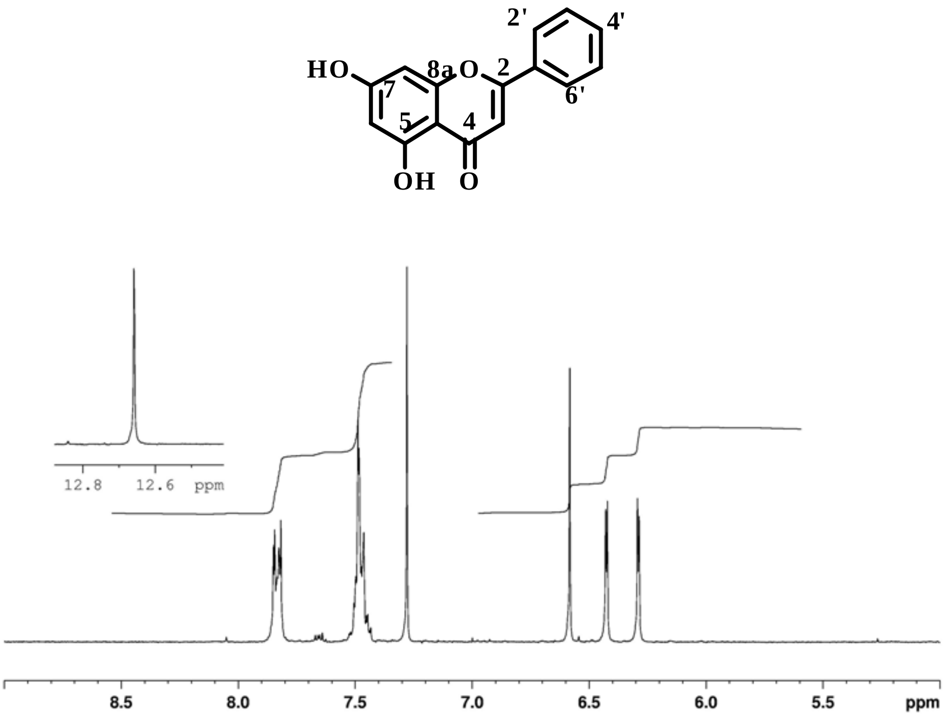


Figure A-40 ^{1}H-NMR (300 MHz) (CDCl$_3$) spectrum of **SM8**

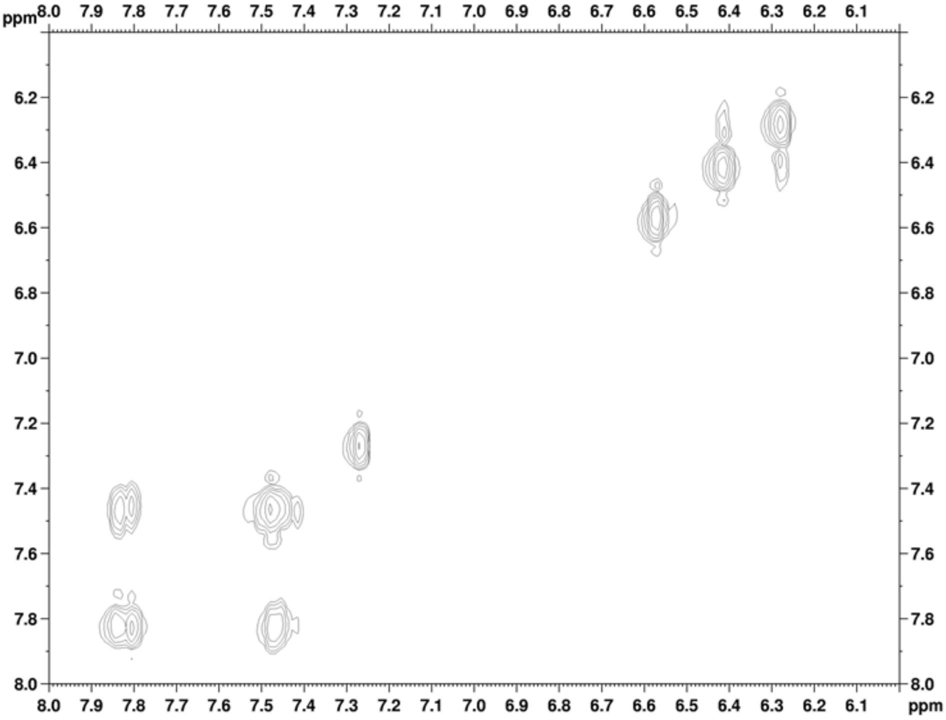


Figure A-41 ^{1}H-^{1}H COSY spectrum of **SM8**

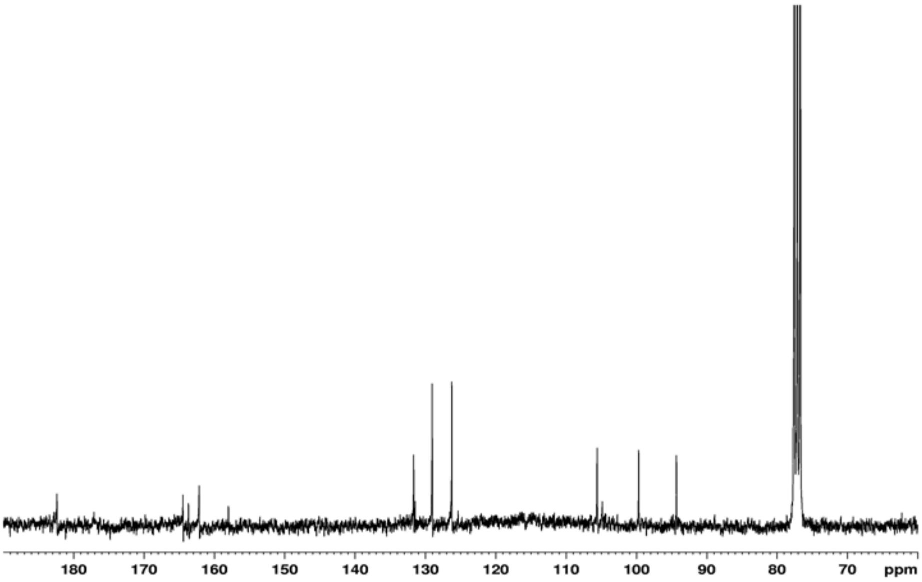


Figure A-42 ^{13}C-NMR (75 MHz) (CDCl$_3$) spectrum of **SM8**

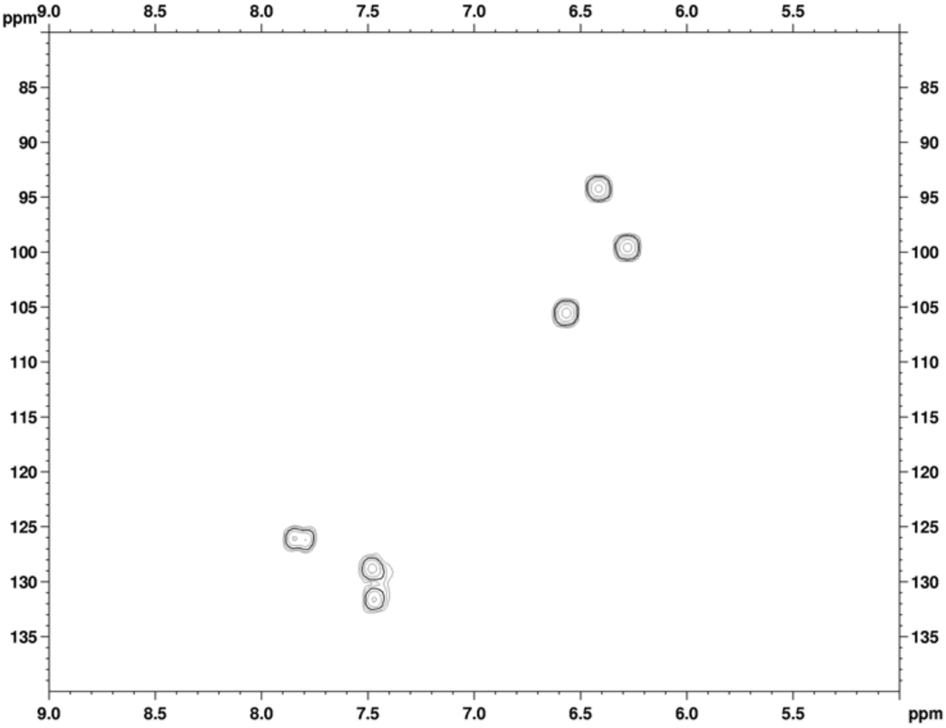


Figure A-43 2D HMQC spectrum of **SM8**

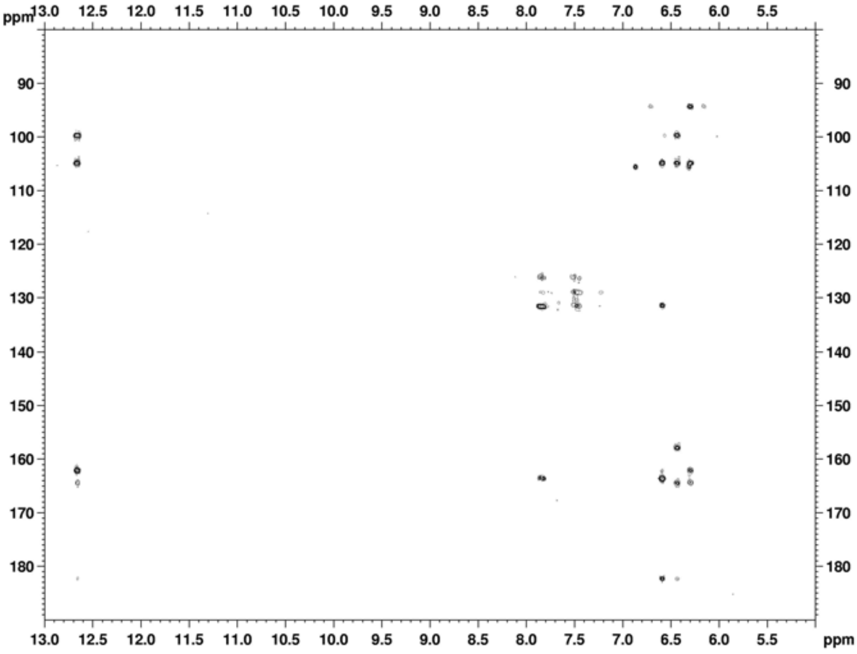


Figure A-44 2D HMBC spectrum of **SM8**

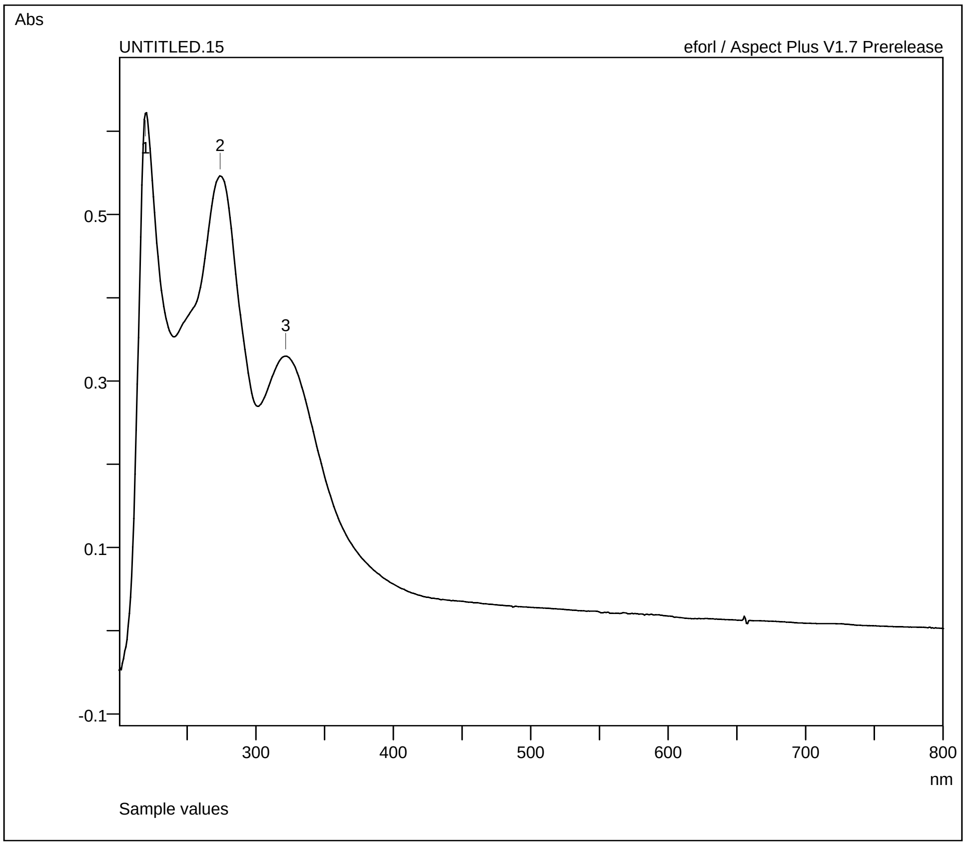


Figure A-45 UV (CH_3OH) spectrum of **SM9**

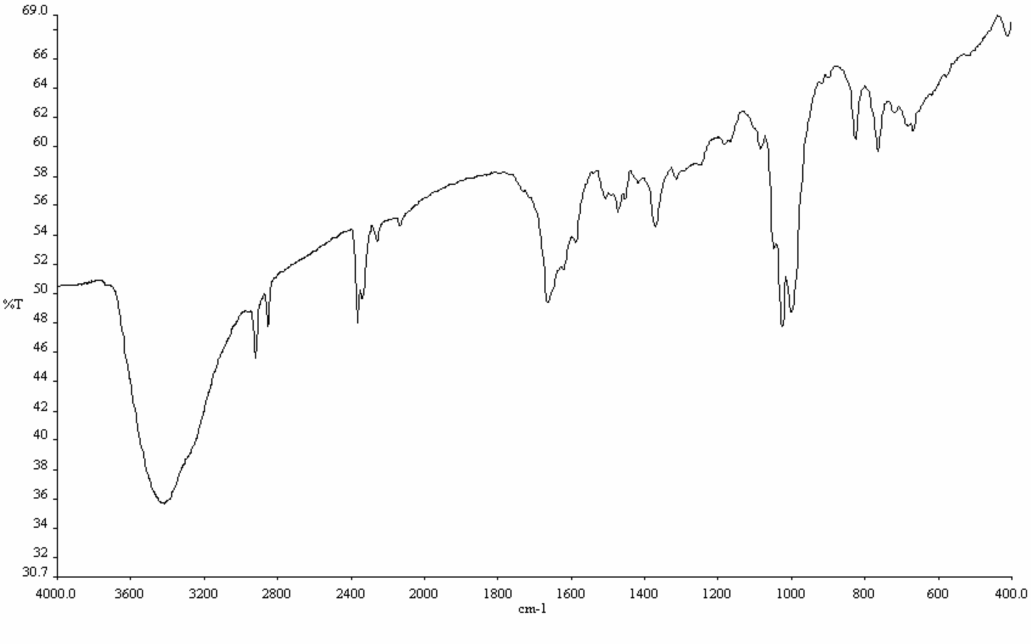
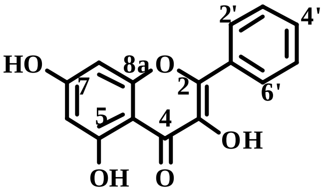


Figure A-46 FT-IR (Neat) spectrum of **SM9**



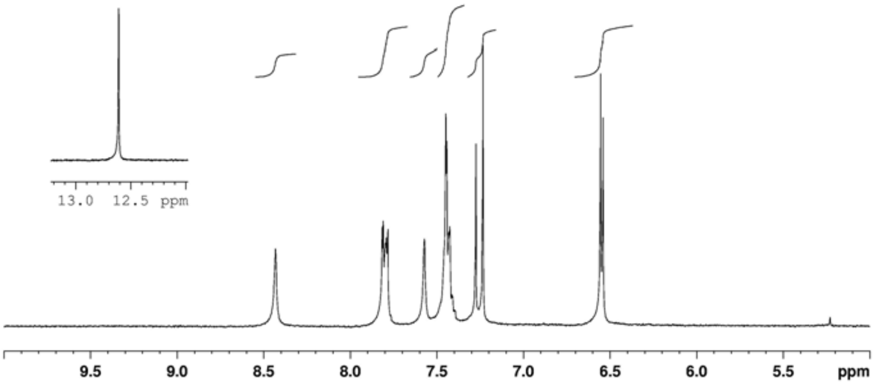


Figure A-47 ^{1}H-NMR (300 MHz) ($CDCl_3$+DMSO-d_6) spectrum of **SM9**

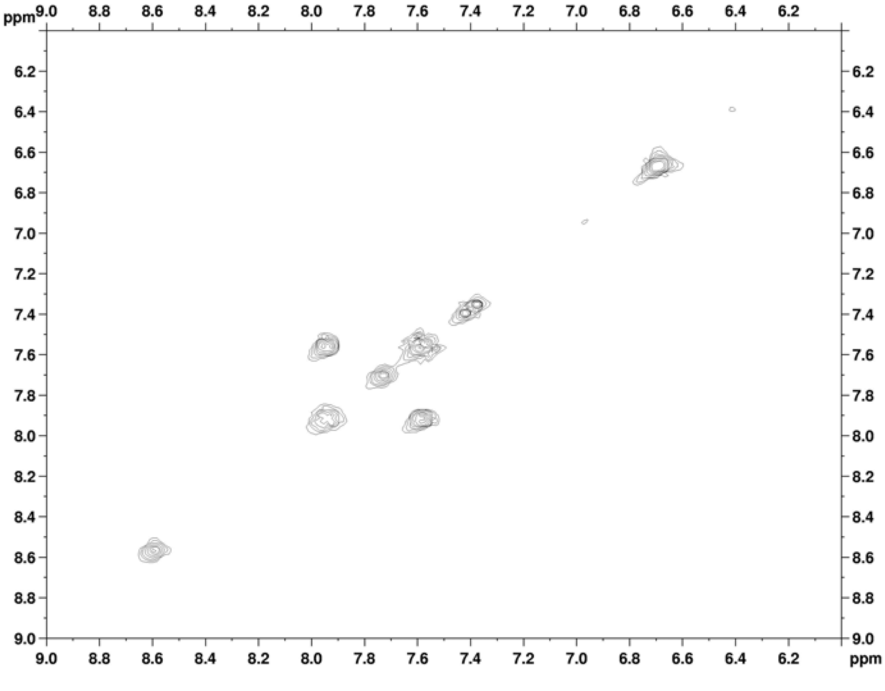


Figure A-48 ^{1}H-^{1}H COSY spectrum of **SM9**

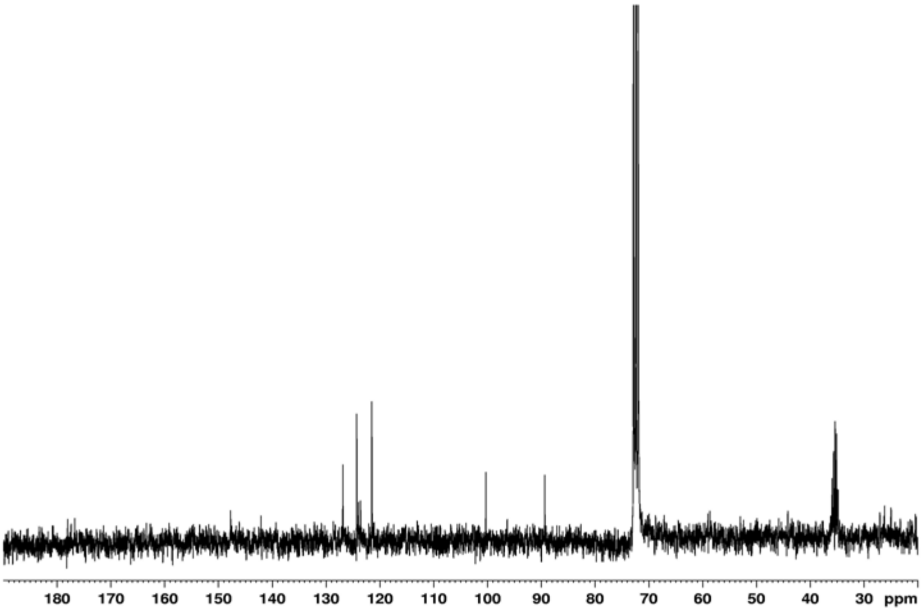


Figure A-49 ^{13}C-NMR (75 MHz) (CDCl$_3$+DMSO-d_6) spectrum of **SM9**

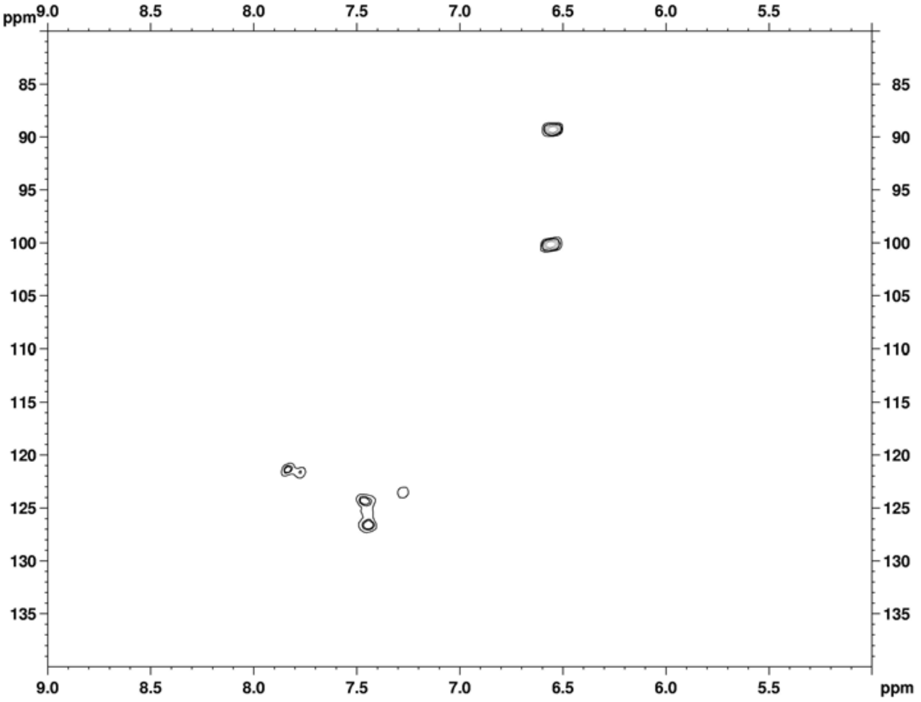


Figure A-50 2D HMQC spectrum of **SM9**

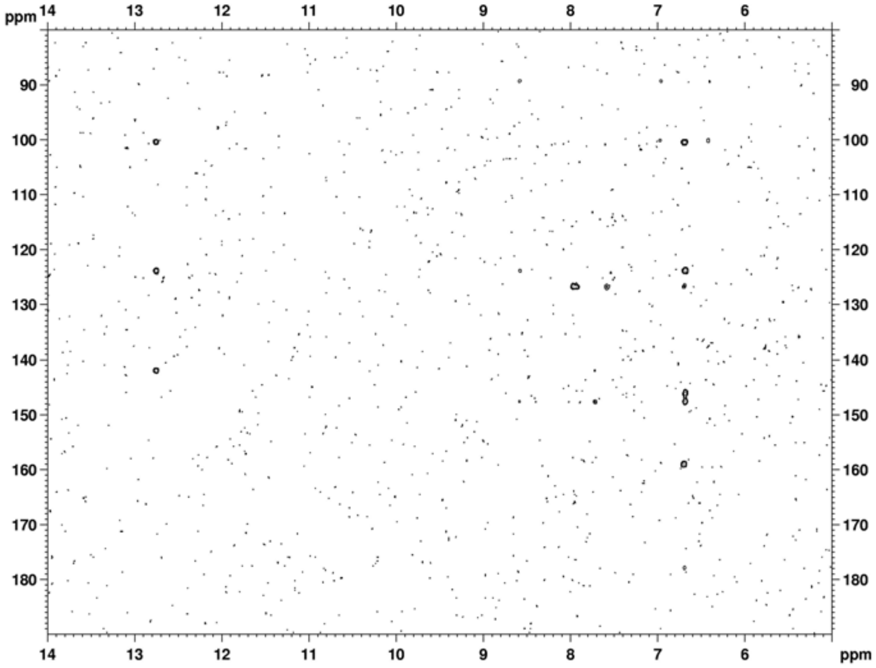


Figure A-51 2D HMBC spectrum of **SM9**

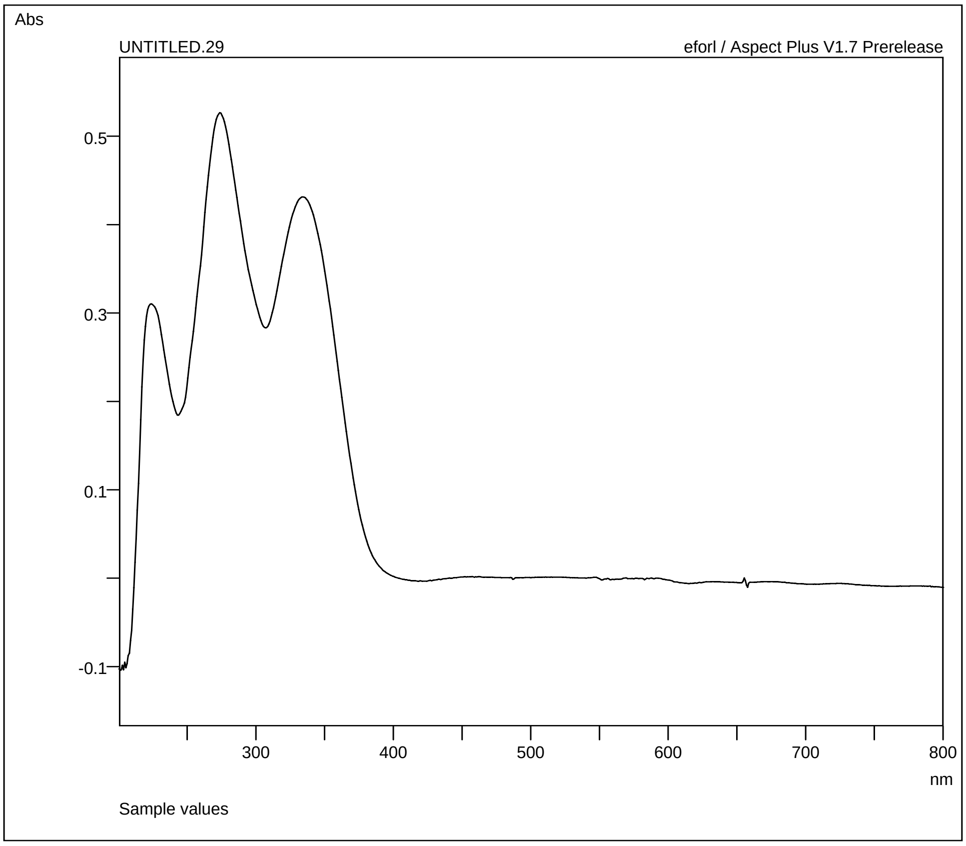


Figure A-52 UV (CH_3OH) spectrum of **SM10**

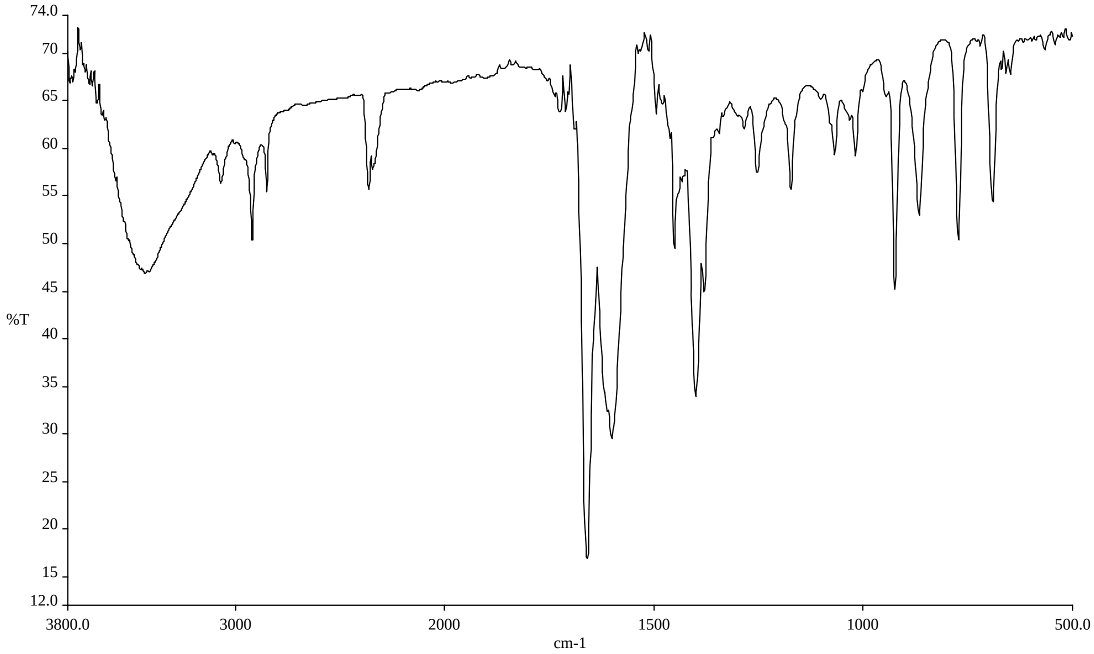


Figure A-53 FT-IR (Neat) spectrum of **SM10**

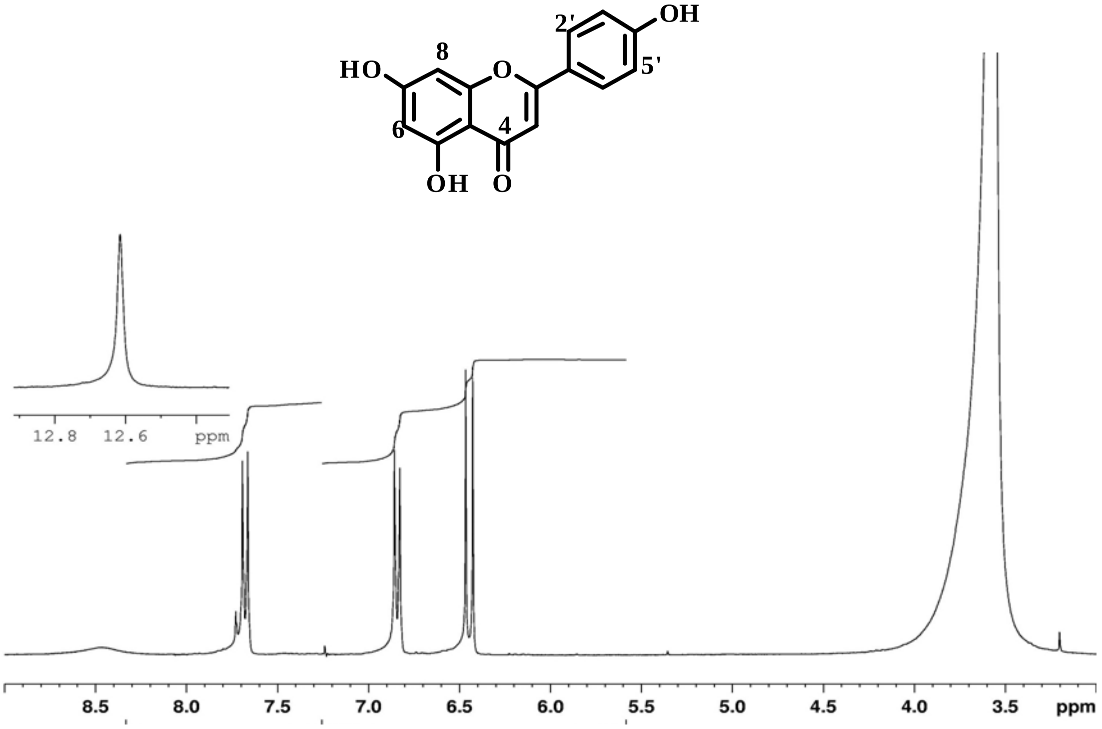


Figure A-54 ^{1}H-NMR (300 MHz) (CDCl$_3$+ DMSO- d_6) spectrum of **SM10**

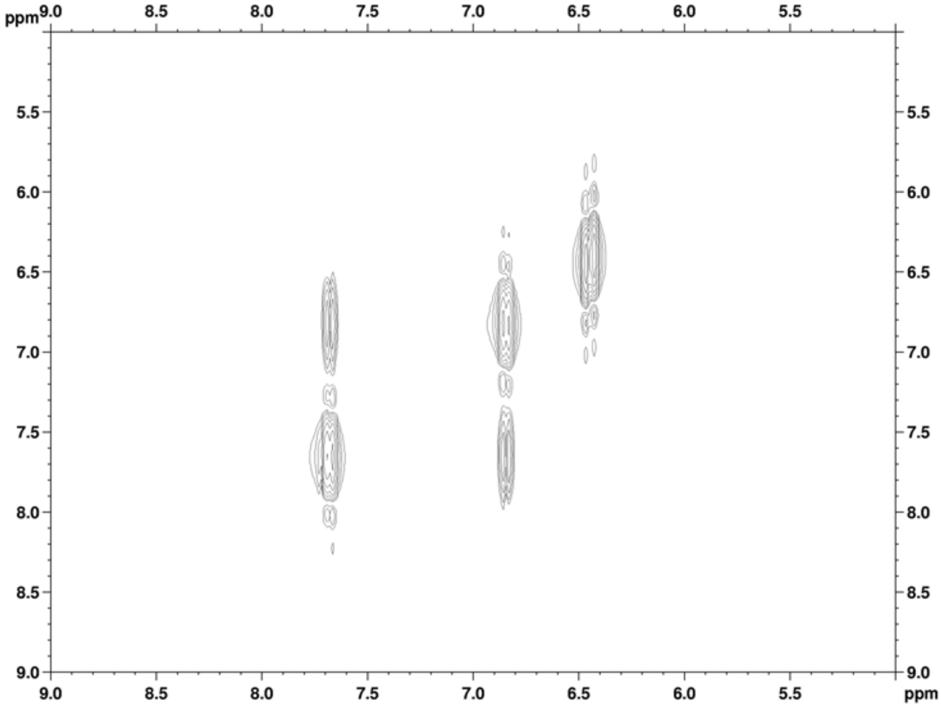


Figure A-55 ^{1}H-^{1}H COSY spectrum of **SM10**

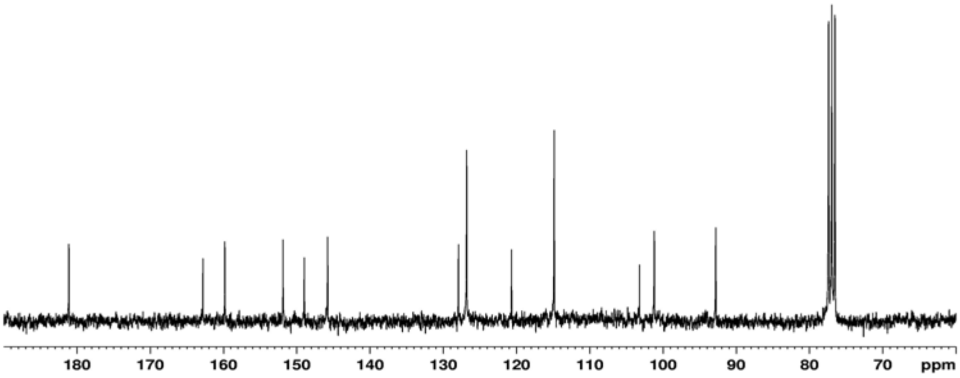


Figure A-56 ^{13}C-NMR (75 MHz) (CDCl$_3$+ DMSO-d_6) spectrum of **SM10**

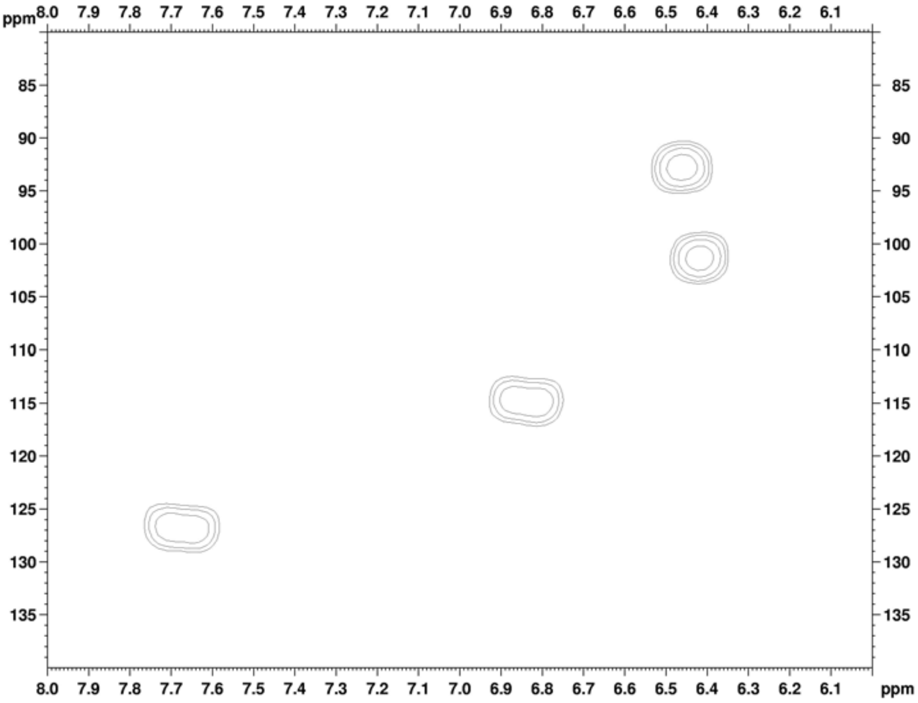


Figure A-57 2D HMQC spectrum of **SM10**

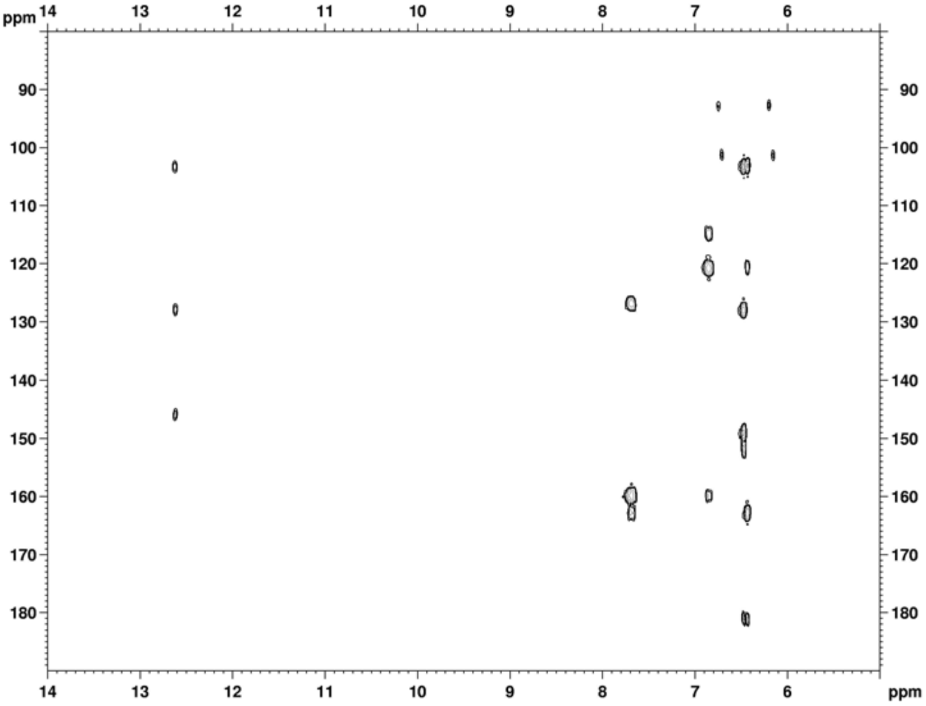


Figure A-58 2D HMBC spectrum of **SM10**

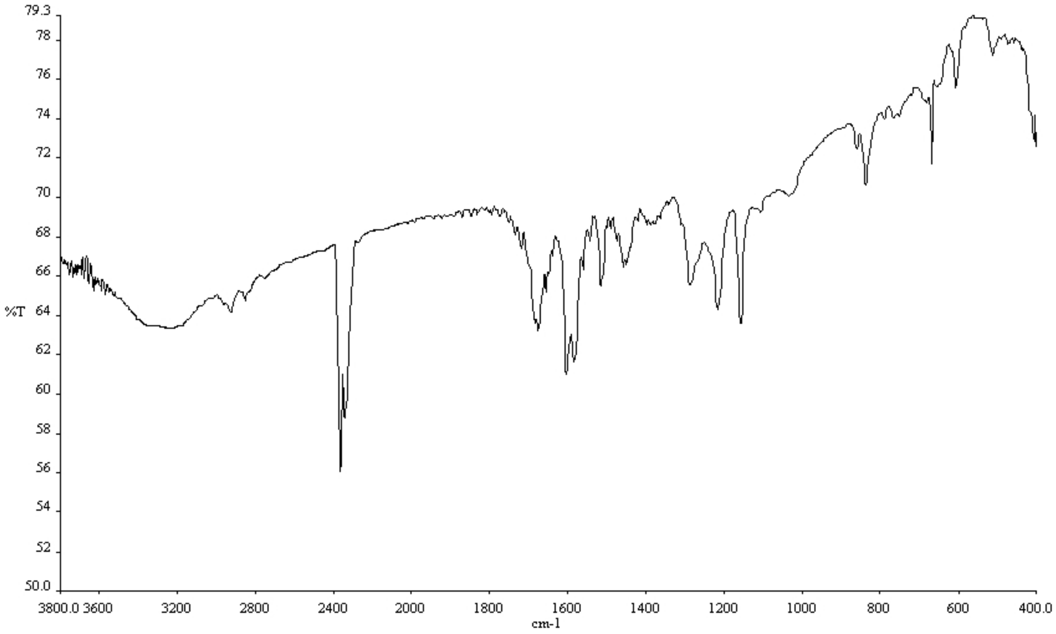


Figure A-59 FT-IR (Neat) spectrum of **SM11**

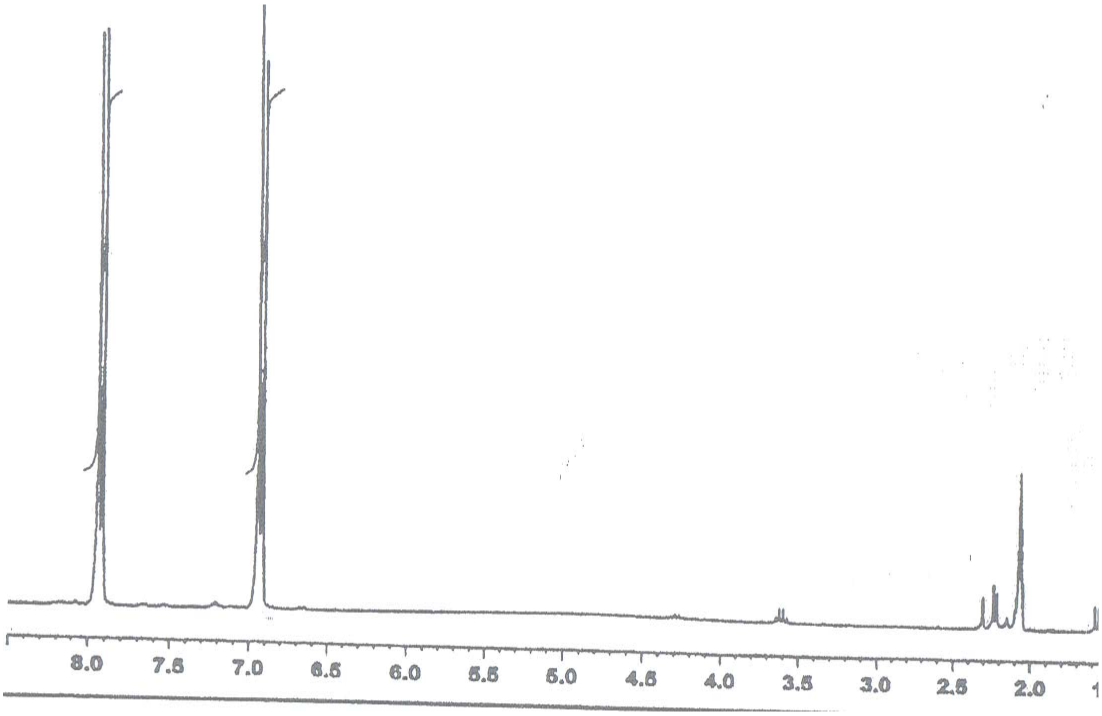


Figure A-60 ^{1}H-NMR (300 MHz) (Acetone-d_6) spectrum of **SM11**

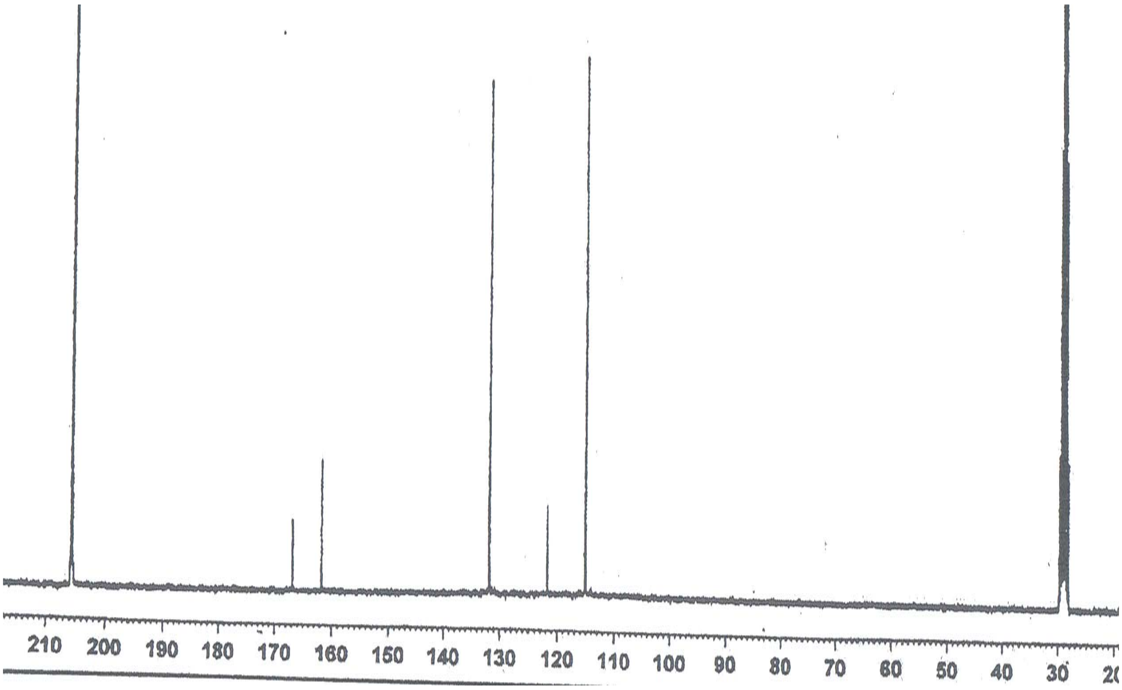


Figure A-61 ^{13}C-NMR (75 MHz) (Acetone-d_6) spectrum of **SM11**

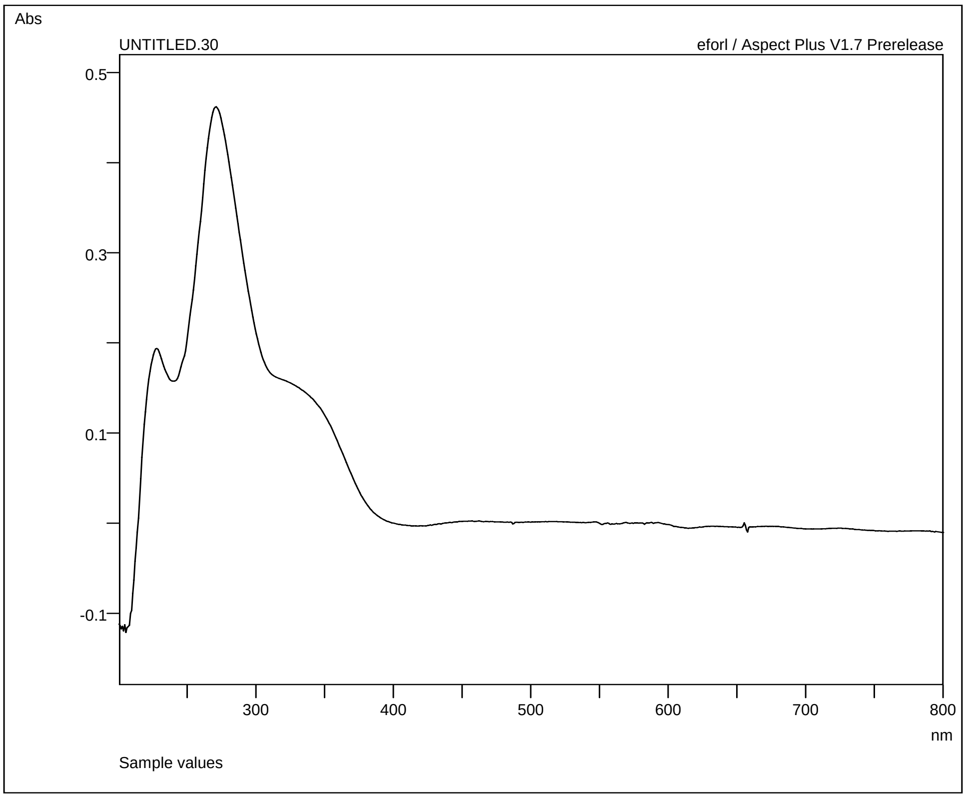


Figure A-62 UV (CH_3OH) spectrum of **SM12**

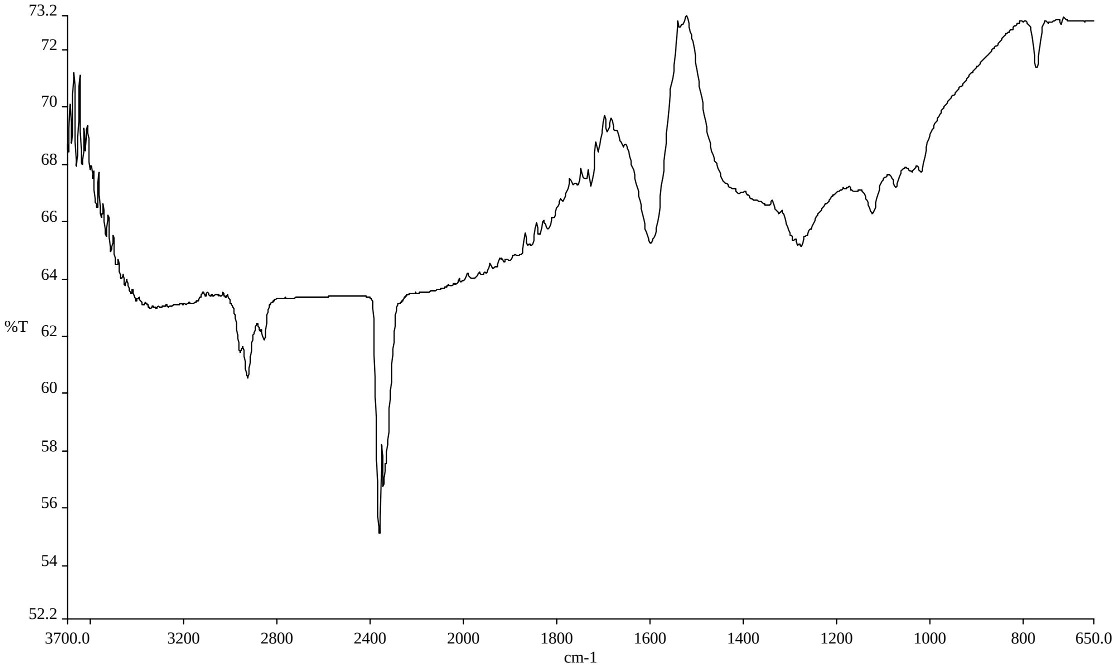


Figure A-63 FT-IR (Neat) spectrum of **SM12**

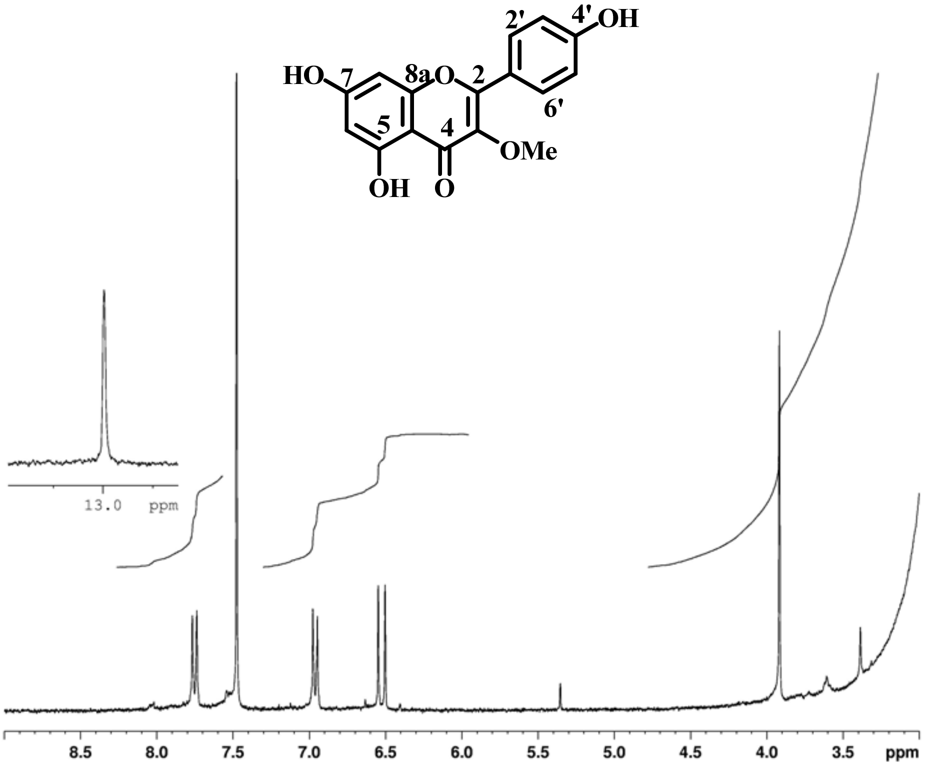


Figure A-64 ^{1}H-NMR (300 MHz) ($CDCl_3$+DMSO-d_6) spectrum of **SM12**

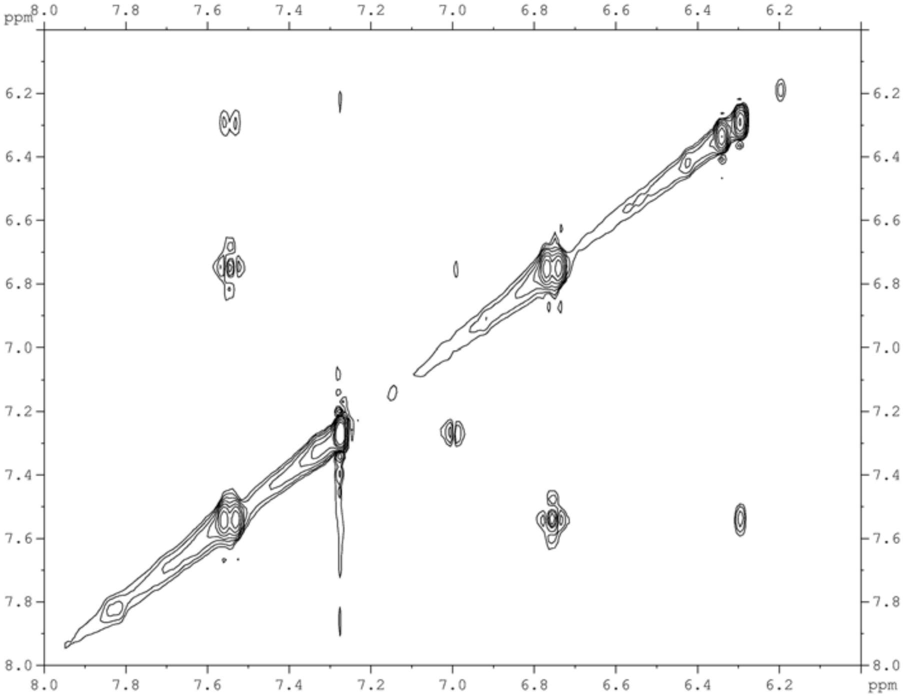


Figure A-65 ^{1}H-^{1}H COSY spectrum of **SM12**

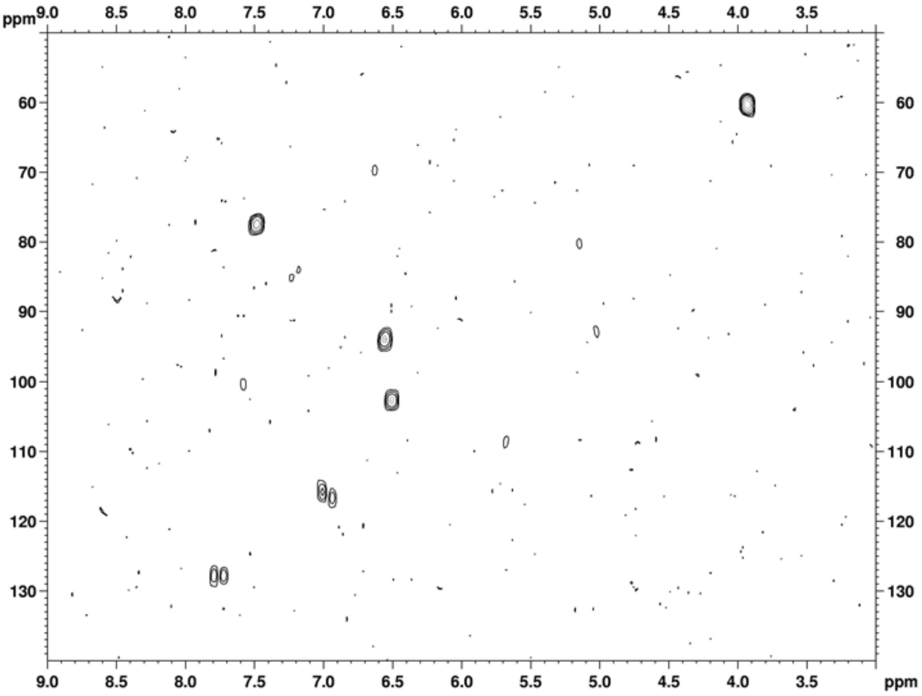


Figure A-66 2D HMQC spectrum of **SM12**

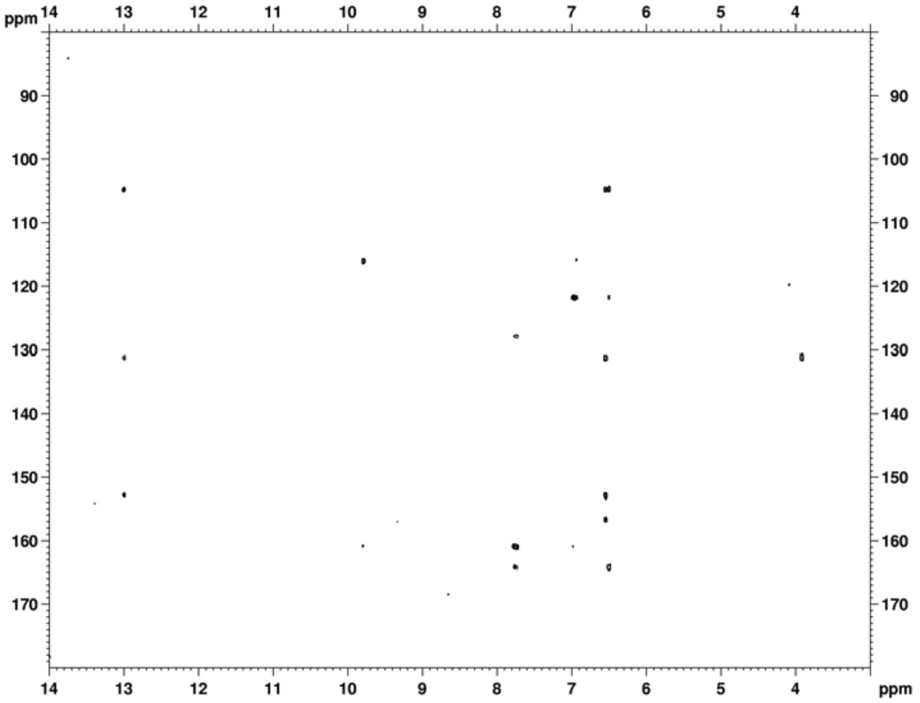


Figure A-67 2D HMBC spectrum of **SM12**

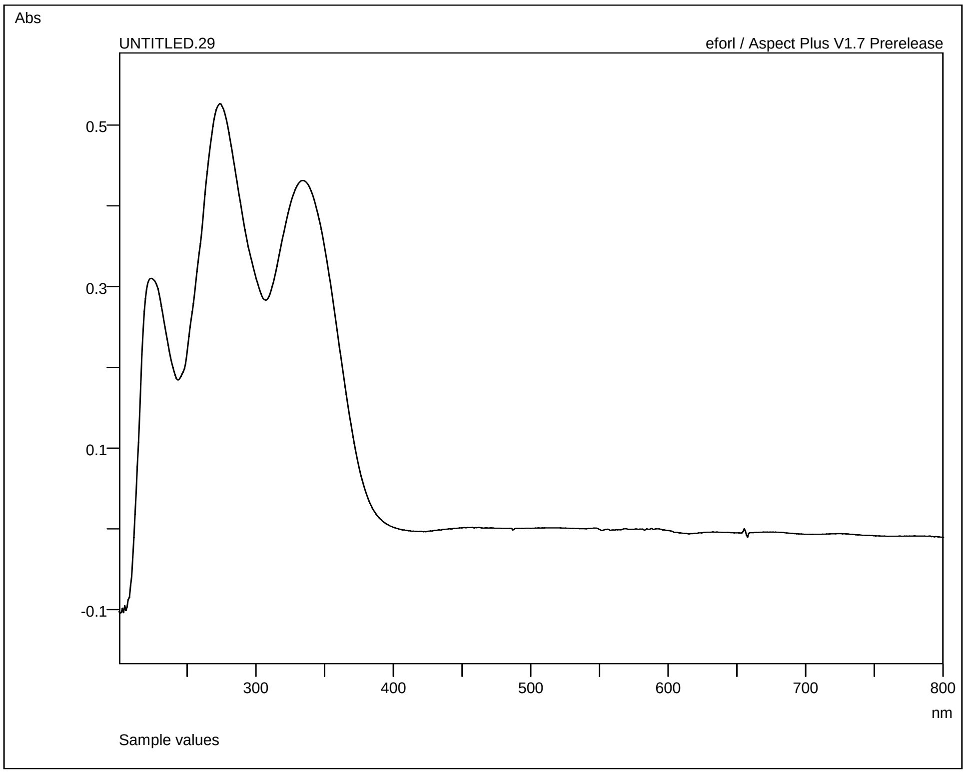


Figure A-68 UV (CH_3OH) spectrum of **SM13**

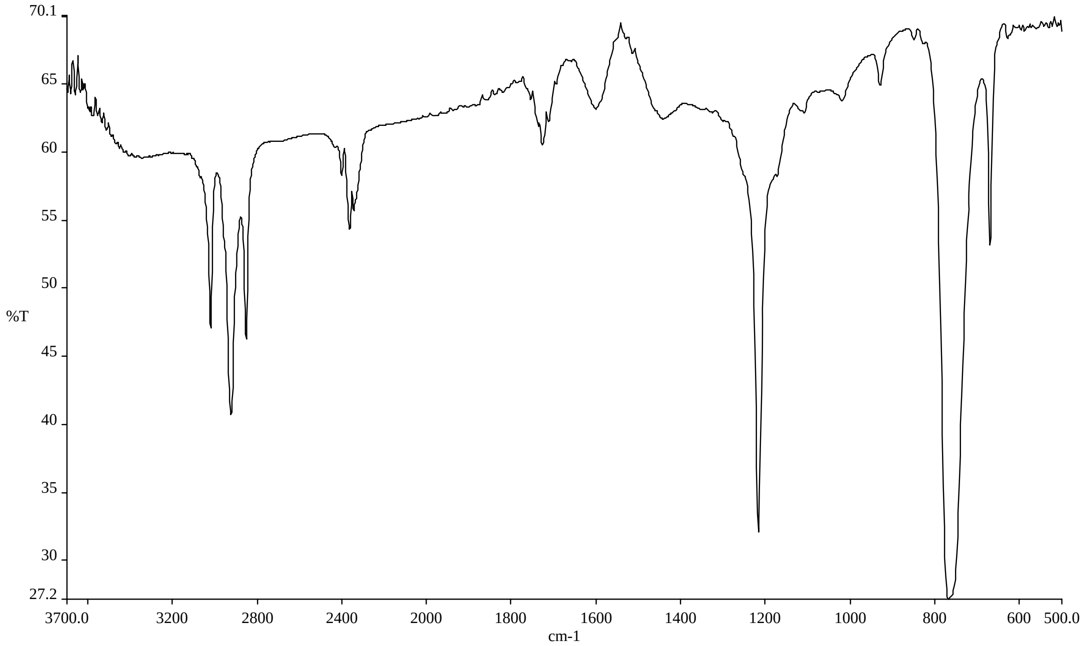


Figure A-69 FT-IR (Neat) spectrum of **SM13**

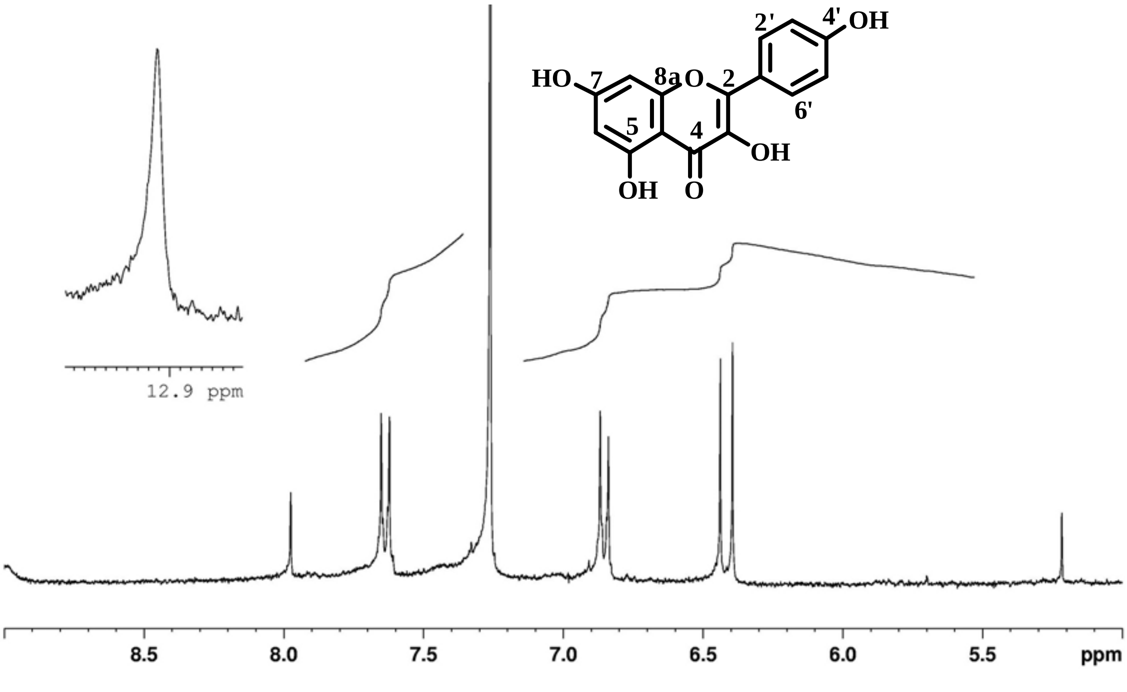


Figure A-70 ^{1}H-NMR (300 MHz) ($CDCl_3$+DMSO-d_6) spectrum of **SM13**

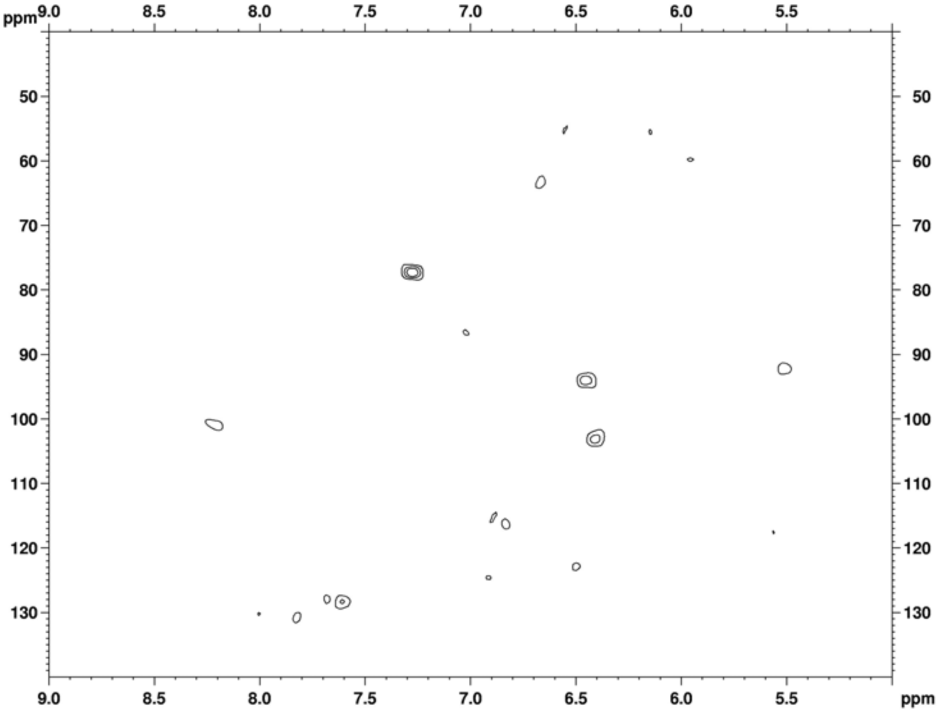


Figure A-71 2D HMQC spectrum of **SM13**

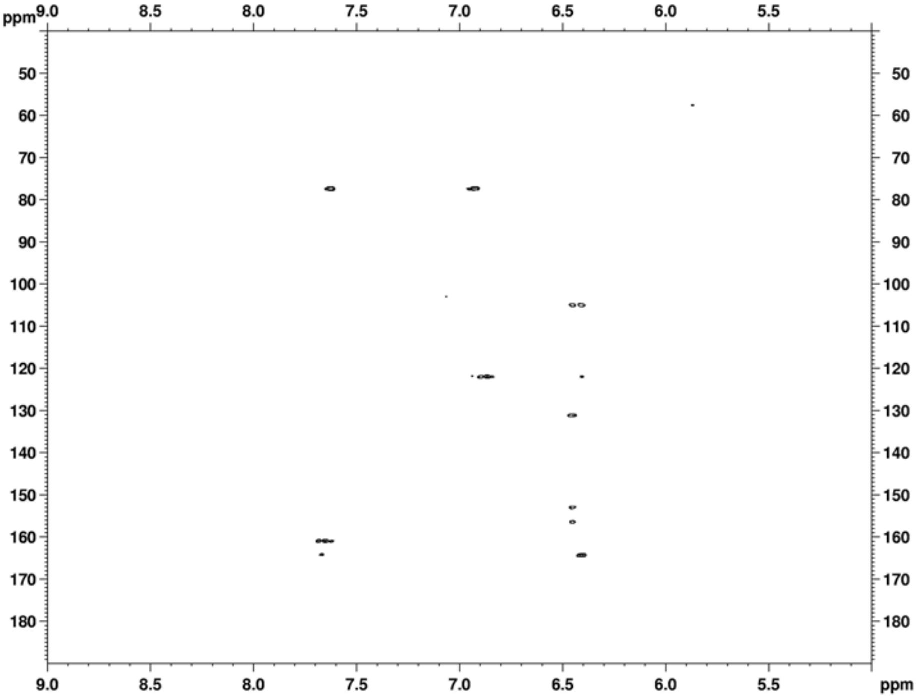


Figure A-72 2D HMBC spectrum of **SM13**

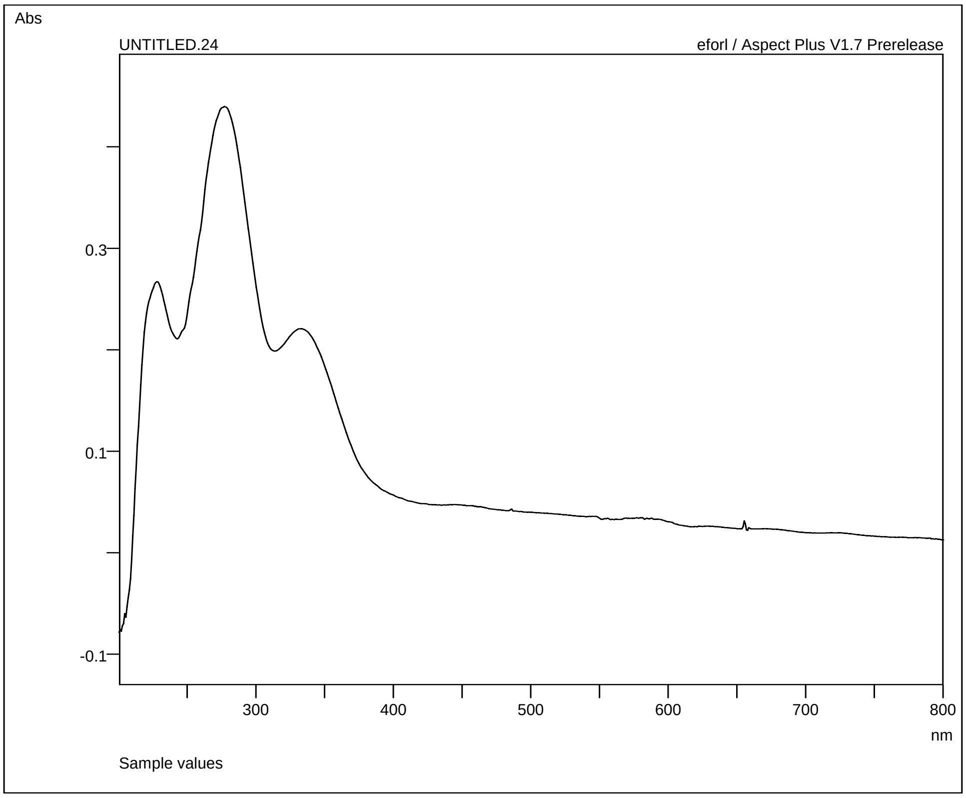


Figure A-73 UV (CH_3OH) spectrum of **SM14**

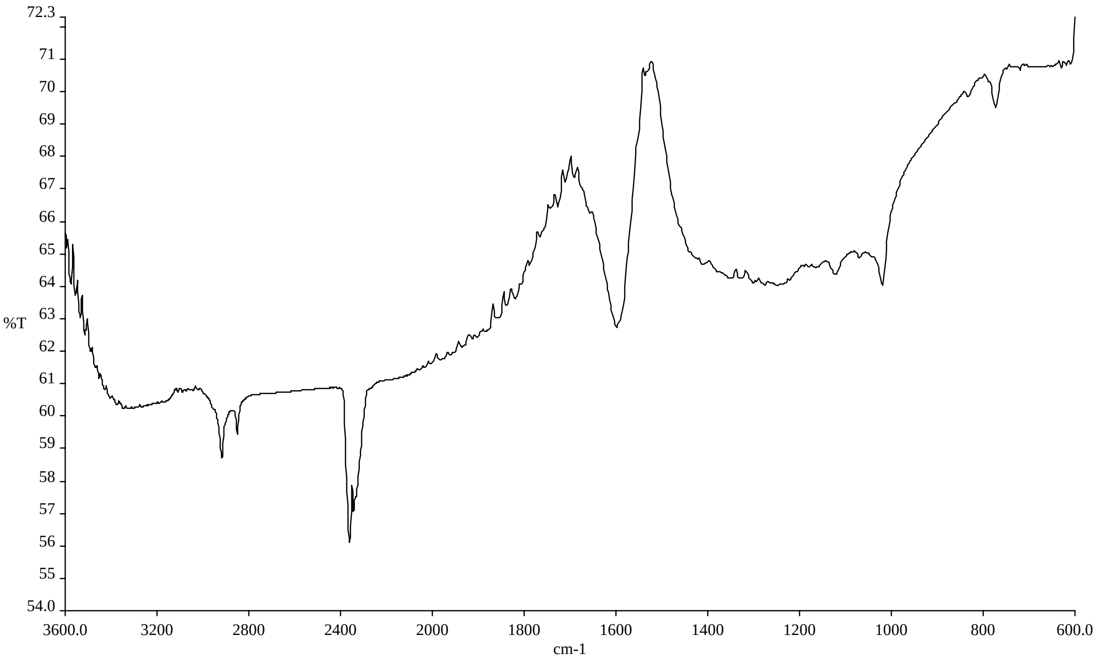
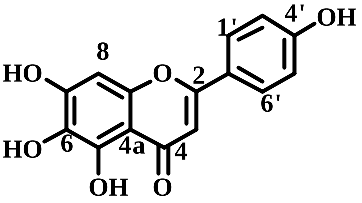


Figure A-74 FT-IR (Neat) spectrum of **SM14**



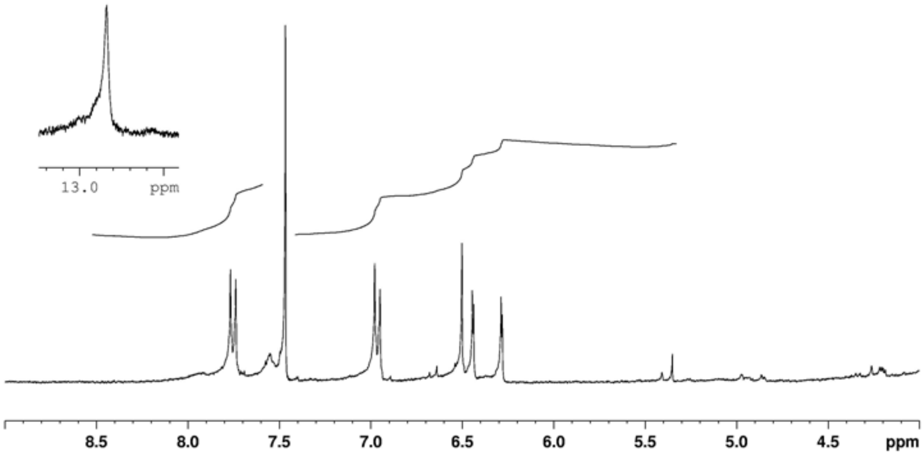


Figure A-75 ^{1}H-NMR (300 MHz) ($CDCl_3$+DMSO-d_6) spectrum of **SM14**

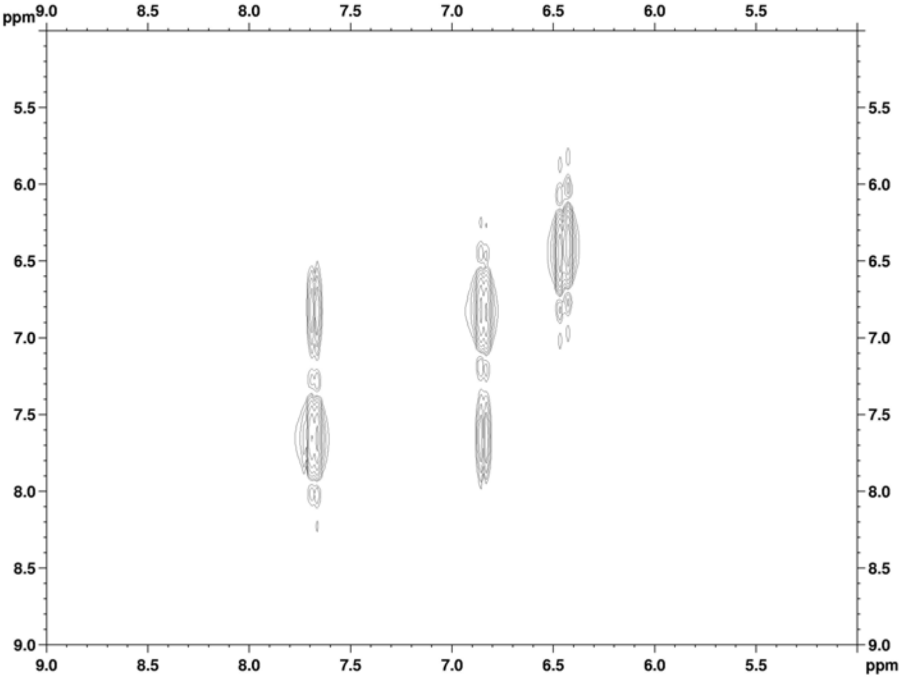


Figure A-76 ^{1}H-^{1}H COSY spectrum of **SM14**

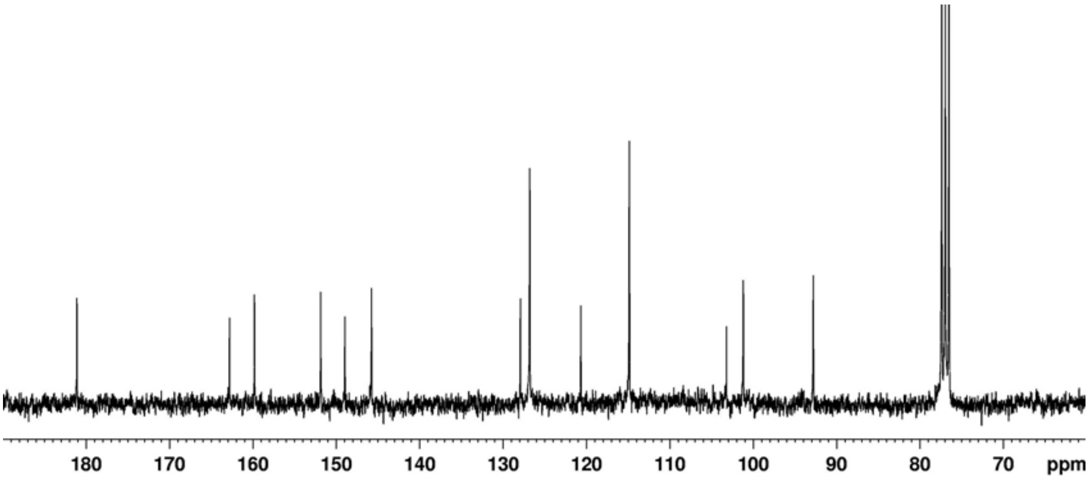


Figure A-77 ^{13}C-NMR (75 MHz) ($CDCl_3$+DMSO-d_6) spectrum of **SM14**

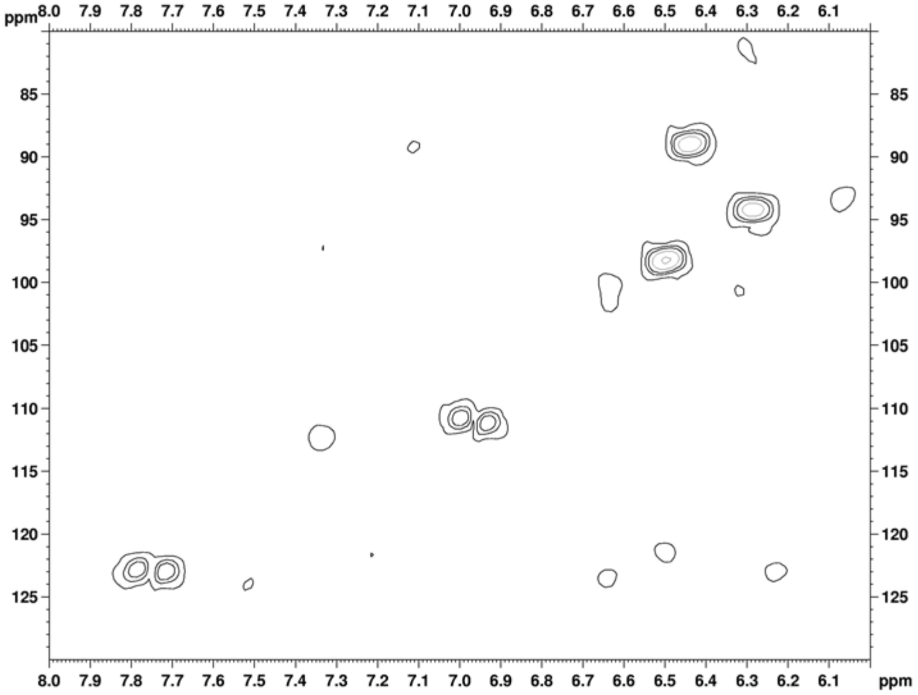


Figure A-78 2D HMQC spectrum of **SM14**

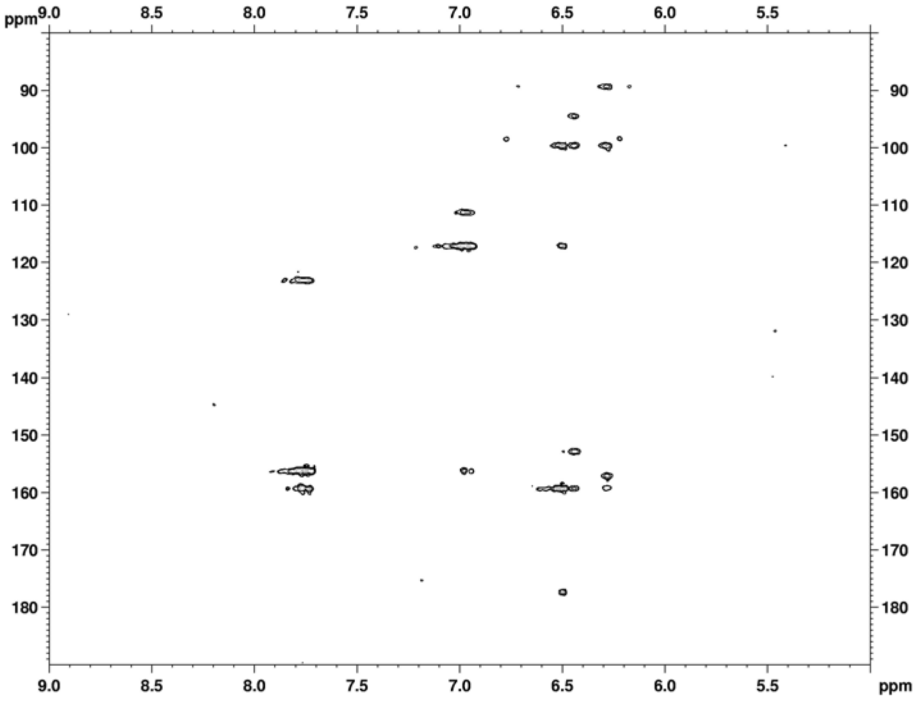


Figure A-79 2D HMBC spectrum of **SM14**

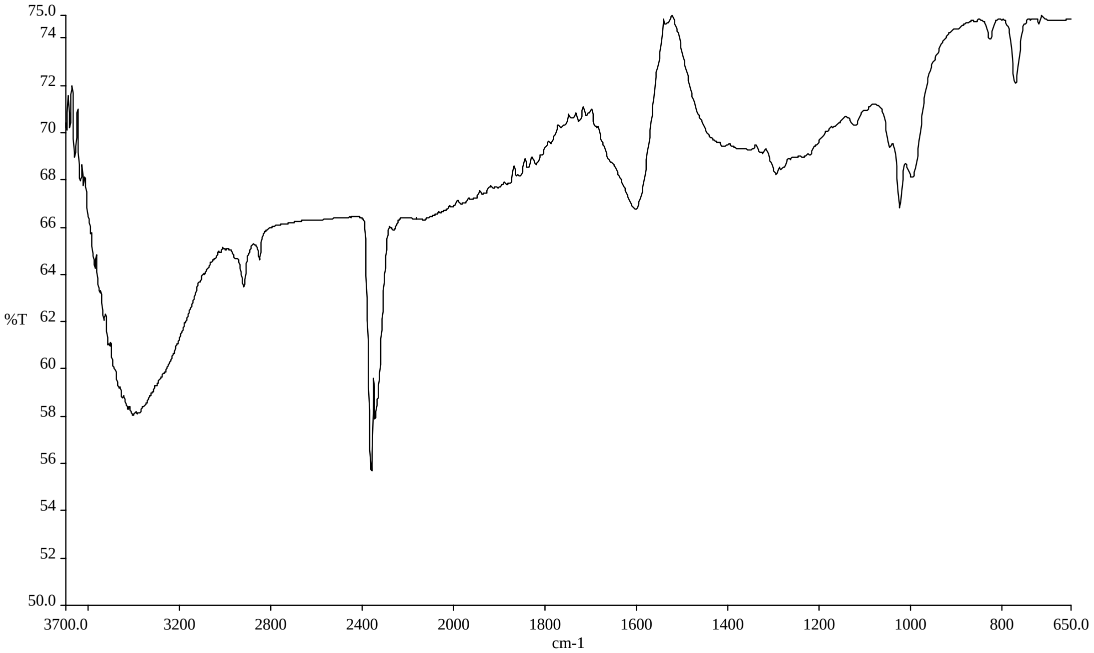
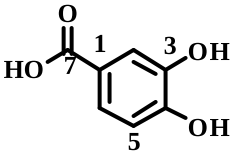


Figure A-80 FT-IR (Neat) spectrum of **SM15**



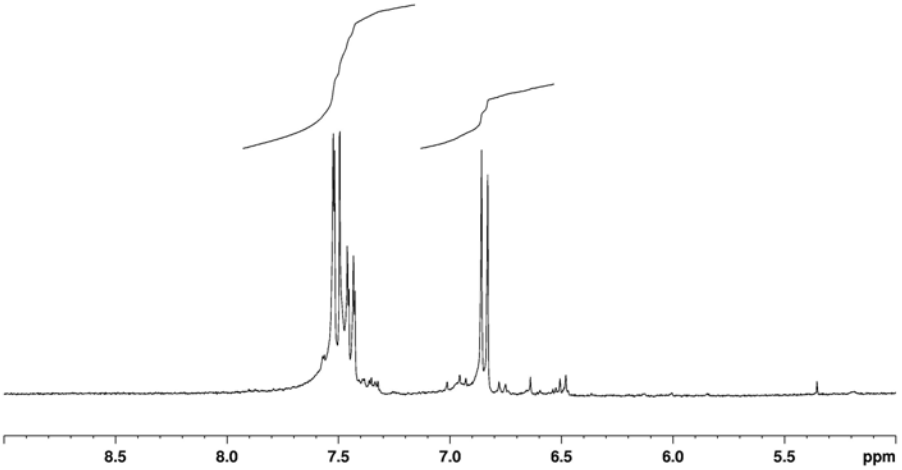


Figure A-81 ^{1}H-NMR (300 MHz) ($CDCl_3$+DMSO-d_6) spectrum of **SM15**

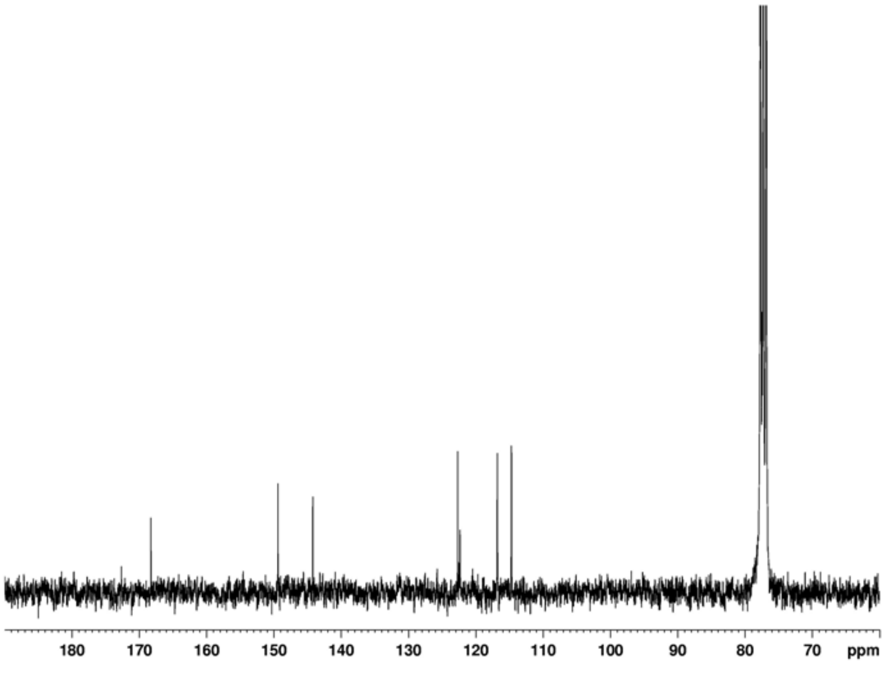


Figure A-82 ^{13}C-NMR (75 MHz) ($CDCl_3$+DMSO-d_6) spectrum of **SM15**

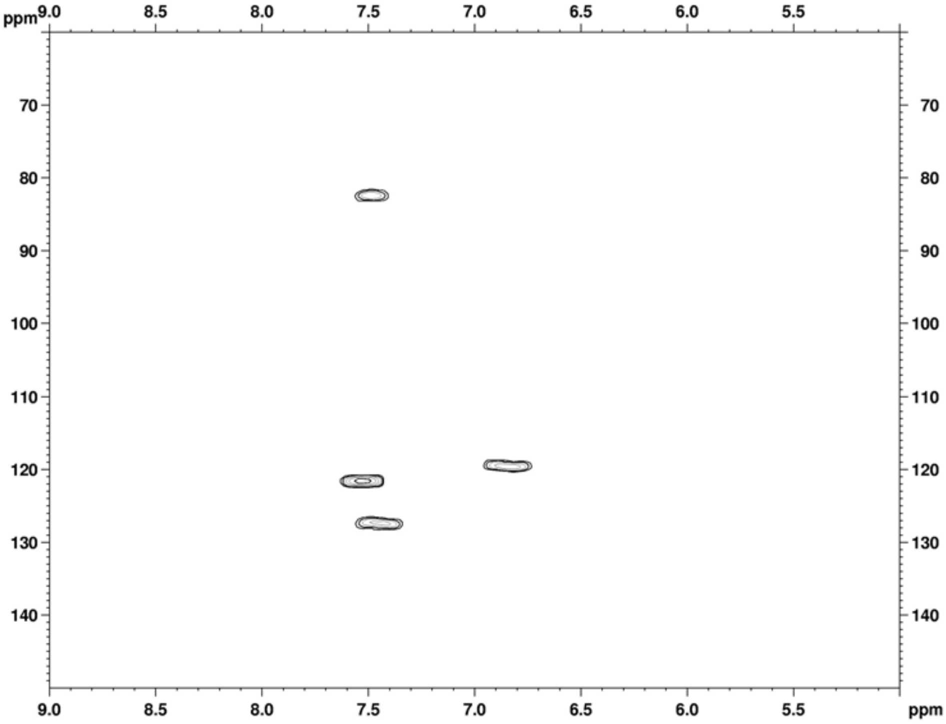


Figure A-83 2D HMQC spectrum of **SM15**

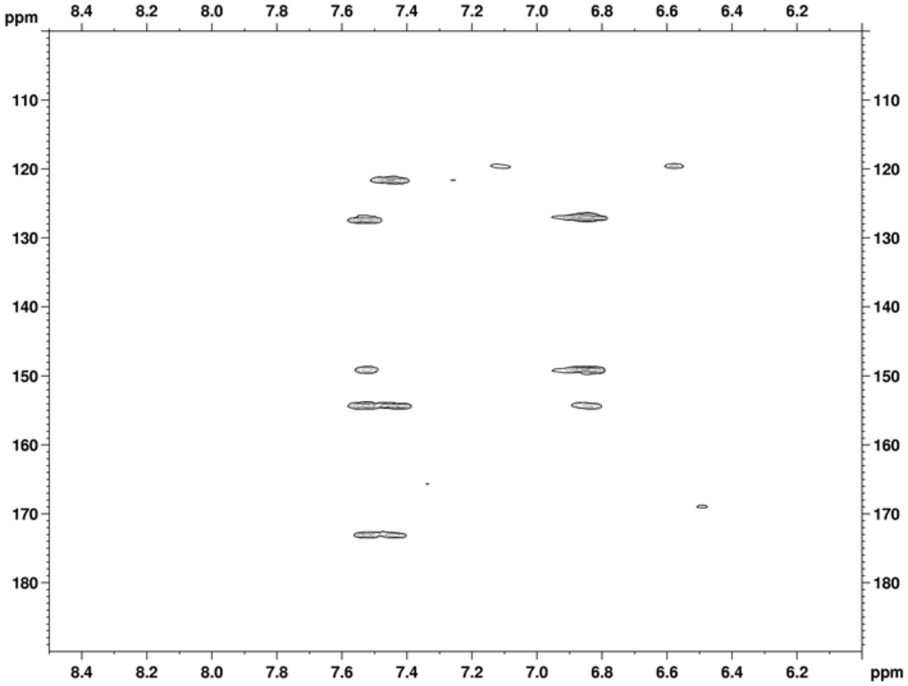


Figure A-84 2D HMBC spectrum of **SM15**

2. ^{1}H-NMR and ^{13}C-NMR spectral data of known compounds from literatures

Table A-1 ^{13}C NMR spectral data of friedelin (Ahad *et al*., 1991)

Position	δ_C/ppm	Position	δ_C/ppm
1	22.3 (CH_2)	16	36.0 (CH_2)
2	41.5 (CH_2)	17	30.0 (C)
3	213.2 (C)	18	42.8 (CH)
4	58.2 (CH)	19	35.4 (CH_2)
5	42.2 (C)	20	28.1 (C)
6	41.3 (CH_2)	21	39.3 (CH_2)
7	18.2 (CH_2)	22	32.8 (CH_2)
8	53.1 (CH)	23	6.8 (CH_3)
9	37.5 (C)	24	14.7 (CH_3)
10	59.5 (CH)	25	18.0 (CH_3)
11	35.6 (CH_2)	26	18.7 (CH_3)
12	30.5 (CH_2)	27	20.3 (CH_3)
13	39.7 (C)	28	32.1 (CH_3)
14	38.3 (C)	29	31.8 (CH_3)
15	32.4 (CH_2)	30	35.0 (CH_3)

Table A-2^{1}H, ^{13}C NMR spectral data of betulinic acid (Macias *et al*., 1994)

Position	**δ_H/ppm (multiplicity, *J*/Hz)**	**δ_C (C-Type)**	**Position**	**δ_H/ppm (multiplicity, *J*/Hz)**	**δ_C (C-Type)**
1	0.95 (*m*), 1.70 (*m*)	38.5 (CH_2)	16	1.43 (*m*), 2.23 (*m*)	32.8 (CH_2)
2	1.57 (*m*), 1.62 (*m*)	28.2 (CH)	17	-	56.6 (C)
3	3.19 (*dd*, 10.8, 5.4)	78.1 (C)	18	1.63 (*m*)	49.7 (CH)
4	-	39.4 (CH)	19	3.02 (*m*)	47.7 (CH)
5	0.71 (*m*)	55.9 (CH_2)	20	-	151.4 (C)
6	1.45 (*m*), 1.55 (*m*)	18.7 (CH_2)	21	1.40 (*m*), 1.93 (*m*)	31.4 (CH_2)
7	1.42 (*m*)	34.7 (C)	22	1.43 (*m*), 1.91 (*m*)	37.4 (CH_2)
8	-	41.0 (CH)	23	0.95 (*s*)	28.5 (CH_3)
9	1.33 (*m*)	50.9 (C)	24	0.75 (*s*)	16.2 (CH_3)
10	-	37.5 (CH_2)	25	0.86 (*s*)	16.3 (CH_3)
11	1.25 (*m*), 1.45 (*m*)	21.1 (CH_2)	26	0.97 (*s*)	16.2 (CH_3)
12	1.07 (*m*), 1.73 (*m*)	26.0 (CH)	27	1.01 (*s*)	14.8 (CH_3)
13	2.30 (*m*)	39.2 (C)	28	-	179.0 (C)
14	-	42.8 (CH_2)	29	4.59 (*dd*, 2.2, 1.0)	110.0 (CH_2)
15	1.18 (*m*), 1.53 (*m*)	30.2 (CH_2)		4.71 (*d*, 2.2)	
			30	1.69 (*d*, 1.0)	19.4 (CH_3)

Table A-3 ^{1}H, ^{13}C NMR spectral data of *R*-(-)-mellein in $CDCl_3$ (Dimitriadis *et al*., 1997)

Position	δ_H **(multiplicity, *J*/Hz)**	δ_C **(C-Type)**
1	-	169.9 (C=O)
3	4.73 (*m*)	76.1 (CH)
4	2.93 (*d*, 7.3)	34.6 (CH_2)
4a	-	139.3 (C)
5	6.89 (*d*, 8.3)	117.9 (CH)
6	7.41 (*m*)	136.1 (CH)
7	6.69 (*d*, 7.3)	116.2 (CH)
8	-	162.1 (C)
8a	-	108.2 (C)
9	1.53 (*d*, 6.3)	20.8(CH_3)
8-OH	11.3 (*s*)	-

Table A-4 ^{1}H, ^{13}C NMR NMR spectral data of tectochrysin in $CDCl_3$ (Sutthanut *et al*., 2007)

Position	**δ_H (multiplicity, *J*/Hz)**	**δ_C (C-Type)**
2	-	163.8 (C)
3	6.64(*s*)	105.8 (CH)
4	-	182.4 (C=O)
4a	-	105.6 (C)
5	-	162.1 (C)
6	6.36 (*d*, 2.0)	98.1 (CH)
7	-	165.5 (C)
8	6.48 (*d*, 2.0)	92.7 (CH)
8a	-	158.0 (C)
1'	-	131.4 (C)
2',6'	7.87 (*m*)	129.0 (CH)
3', 5'	7.52 (*m*)	126.2 (CH)
4'	7.52 (*m*)	131.8 (CH)
5-OH	12.73 (*s*)	-
7-OMe	3.89 (*s*)	55.7 (CH_3)

Table A-5 ^{1}H NMR spectral data of luteolin in $CDCl_3$ (Herrera *et al*., 1996)

Position	δ_H **(multiplicity, *J*/Hz)**
2	-
3	6.57 (*s*)
4	-
4a	-
5	-
6	6.34 (*d*, 2.3)
7	-
8	6.47 (*d*, 2.3)
8a	-
1'	-
2'	7.31 (*d*, 2.1)
5'	6.95 (*d*, 8.5)
6'	7.50 (*dd*, 8.5, 2.1)
5-OH	12.84 (*s*)
7-OMe	3.86 (*s*)
3'-OMe	3.94 (*s*)
4'-OMe	3.96 (*s*)

Table A-6 ^{1}H, ^{13}C NMR spectral data of (+)-rengyolone in $CDCl_3$ (Tutiwachwuttikul *et al.*, 2003)

Position	**δ_H (multiplicity, *J*/Hz)**	**δ_C (C-Type)**
1	-	75.3 (C)
2	4.24 (*ddd*, 1.5 ,4.8, 5.8)	81.4 (CH)
3_α	2.78 (*dd*, 16.9, 4.8)	40.2 (CH_2)
3_β	2.61 (*ddd*, 16.9, 5.8, 0.5)	
4	-	197.1 (C=O)
5	6.01 (*d*, 10.2)	128.4 (CH)
6	6.76 (*dd*, 10.2, 1.5)	148.4 (CH)
7_a	2.33 (*ddd*, 8.4, 6.3, 13.0)	39.5 (CH_2)
7_b	2.22 (*br ddd*, 8.1, 6.5, 13.0)	
8_a	4.07 (*ddd*, 6.5, 8.1 ,8.7)	66.2 (CH_2)
8_b	3.95 (*ddd*, 6.3, 8.4, 8.7)	

Table A-7 ^{1}H, ^{13}C NMR spectral data of chrysin in tetramethylsilane (Chen *et al.*, 2003)

Position	**δ_H (multiplicity, *J*/Hz)**	**δ_C (C-Type)**
2	-	164.5 (C)
3	6.4 (*s*)	104.9 (CH)
4	-	182.7 (C=O)
4a	-	104.3 (C)
5	-	165.6 (C)
6	6.1 (*d*, 2.1)	99.3 (CH)
7	-	158.4(C)
8	6.5 (*d*, 2.1)	95.5 (CH)
8a	-	158.4 (C)
2',6'	7.9 (*dd*, 7.5, 1.8)	126.3 (CH)
3', 5'	7.5 (*m*)	129.1 (CH)
4'	7.5 (*m*)	131.9 (CH)

Table A-8 ^{1}H, ^{13}C NMR spectral data of galangin in (pyridine-d_5) (Facundo *et al*., 2003)

Position	**δ_H (multiplicity, *J*/Hz)**	**δ_C (C-Type)**
2	-	146.1 (C)
3	-	139.5 (C)
4	-	177.9 (C=O)
4a	-	104.9 (C)
5	-	162.7 (C)
6	6.78 (*d*, 2.1)	99.7 (CH)
7	-	166.2 (C)
8	6.79 (*d*, 2.1)	94.7 (CH)
8a	-	157.9 (C)
1'	-	132.7 (C)
2', 6'	8.52 (*dd*, 8.0, 1.5)	129.1 (CH)
3',5'	7.43-7.60 (*m*)	128.6 (CH)
4'	7.43-7.60 (*m*)	130.2 (CH)
3-OH	-	-
5-OH	13.18 (*s*)	-
7-OH	10.12 (*s*)	-

Table A-9 ^{1}H, ^{13}C NMR spectral data of apigenin in DMSO-d_6 (Nakasugi *et al*., 2000)

Position	**δ_H (multiplicity, *J*/Hz)**	**δ_C (C-Type)**
2	-	163.5 (C)
3	6.73 (*s*)	102.7 (CH)
4	-	181.5 (C=O)
4a	-	103.3 (C)
5	-	161.3 (C)
6	6.17 (*d*, 2.0)	98.9 (CH)
7	-	164.8(C)
8	6.45 (*d*, 2.0)	93.9 (CH)
8a	-	157.3 (C)
1'	-	121.1 (C)
2', 6'	7.91 (*d*, 9.0)	128.3 (CH)
3', 5'	6.92 (*d*, 9.0)	115.9 (CH)
4'	-	161.1 (C)
5-OH	12.93 (*s*)	-

Table A-10 ^{1}H, ^{13}C NMR spectral data of kaempferol in (Acetone-d_6) (Liu *et al*., 2008)

Position	**δ_H (multiplicity, *J*/Hz)**	**δ_C (C-Type)**
2	-	146.1 (C)
3	-	135.5 (C)
4	-	175.6 (C=O)
4a	-	103.0 (C)
5	-	156.8 (C)
6	6.24 (*d*, 2.0)	98.1 (CH)
7	-	164.1 (C)
8	6.50 (*d*, 2.0)	93.5 (CH)
8a	-	161.0 (C)
1'	-	122.1 (C)
2', 6'	8.10 (*d*, 9.0)	129.4 (CH)
3', 5'	6.97 (*d*, 9.0)	115.3 (CH)
4'	-	159.3 (C)

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List of Publication and Proceedings

Saowanee Maungjunburee and Wilawan Mahabusarakam. "Flavonoids from the stem bark of *Oroxylum indicum* (L.) Benth. ex Kurz." The 7th IMT-GT UNINET and The 3rd Joint International PSU-UNS Conferences 2010, Prince of Songkla University, Hat-Yai, Songkhla, Thailand. 7-8 October 2010. (Poster presentation)