Phytochemical and biological investigation of the bark of *Garcinia fusca* Pierre.

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For my father and my late mother,

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ABBREVIATIONS

AflO	Gene encoding the SAM-dependent *O*-methyltransferase in the *Aspergillus* species
AflY	Gene encoding an enzyme catalyzing the Baeyer-Villiger oxidation in the *Aspergillus* species
$[\alpha]_D$	Specific optical rotation
brs	Broad singlet
BHT	Butylated hydroxytoluene
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
C_2D_6CO	Deuterated acetone
CHS	Chalcone synthase
cm	Centimetre
COSY	Correlated spectroscopy
CPC	Centrifugal partition chromatography
δ	Chemical shift
d	Doublet
1D	One-dimensional
2D	Two-dimensional
DCM	Dichloromethane
dd	Doublet of doublet
DMAPP	Dimethylallylpyrophosphate
DMSO	Dimethylsulphoxide
DPPH	2,2-Dipheny-1-(2,4,6-trinitrophenyl)hydrazyl
EDTA	Ethylenediaminetetraacetic acid
EtOAc	Ethyl acetate
EtOH	Ethanol
FC	Flash chromatography
fr.	Fraction
FCS	Fetal calf serum
FAD	Flavin adenine dinucleotide

GPC	Gel permeation chromatography
GPP	Geranyldiphosphate
HMBC	Heteronuclear multiple bond correlation
HMEC-1	Human microvascular endothelial cell line-1
HPLC	High performance liquid chromatography
HRESI	High resolution electrospray ionization
HSQC	Heteronuclear single quantum coherence
IC_{50}	50% Inhibition concentration
J	Coupling constant
m	Multiplet
MdpB	Gene encoding dehydratase in *Aspergillus spp.*
MdpC	Gene encoding phenol reductase in *Aspergillus spp.*
MdpD	Gene encoding mono-oxygenase in *Aspergillus spp.*
MdpJ	Gene encoding glutathione-S-transferase in *Aspergillus spp.*
MdpK	Gene encoding oxidoreductase in *Aspergillus spp.*
MdpL	Gene encoding Baeyer-Villiger oxidase in *Aspergillus spp.*
MeCN	Acetonitrile
MeOH	Methanol
mg	Milligram
min	Minute
mm	Millimetre
µM	Micromolar
µl	Microliter
MEM	Minimum essential medium
MHz	Megahertz
MIC	Minimum inhibitory concentration
MS	Mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
m/*z*	Mass-to-charge ratio
n	Number
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser enhancement spectroscopy

p.a.	Pro analysis
PAL	Phenylalanine ammonia lyase
PE	Petroleum ether
ppm	Part per million
PBS	Phosphate buffered saline
R_f	Retardation factor
ROESY	Rotating-frame nuclear overhauser effect correlation spectroscopy
RP_{18}	Reversed phase C-18
s	Singlet
SAM	*S*-Adenosyl methionine
SDS	Sodium dodecyl sulphate
t	Triplet
TLC	Thin layer chromatography
UV	Ultraviolet
Ver-A	A cytochrome P450 monooxygenase in *Aspergillus* species
Ver-1	An NADH-dependent deoxygenase in *Aspergillus* species
XtpA	Gene encoding prenyl transferase in *Aspergillus spp.*
XtpB	Gene encoding prenyl transferase in *Aspergillus spp.*
XtpC	Gene encoding oxidoreductase in *Aspergillus spp.*

SUMMARY

The *Garcinia* is a large genus of the family Guttiferae or Clusiaceae, nearly consisting of 450 species. This genus has been of interest due to its therapeutic value in the folk medicine for the treatment of various kinds of diseases such as vomiting, swelling, tapeworms, dysentery, chronic diarrhoea, piles, pains and heart complaints, as well as due to the successive finding of both chemically and biologically interesting compounds (Vo et al., 2012). In Vietnam, around thirty one *Garcinia* species were found, some of which were phytochemically and biologically investigated (Pham, 1999). For further phytochemical and biological study of the genus *Garcinia* in Vietnam, *Garcinia fusca* Pierre was chosen for the investigation.

In this work, the air-dried and powdered bark of *G. fusca* Pierre (1.95 kg) was successively extracted with *n*-hexane and ethyl acetate. The NMR-guided fractionation of both the *n*-hexane and ethyl acetate extracts led to the isolation of thirty one phenolic compounds by means of various chromatographic methods such as TLC, FC, GPC, CPC and semi-preparative HPLC. The structural elucidation was carried out based on data of UV, MS, 1D and 2D NMR spectra together with the optical rotation. The compounds isolated from the *n*-hexane extract comprise a tocotrienol, a benzaldehyde derivative and twenty xanthones, five of which (**18 – 22**) are new and given trivial names fuscaxanthones (+) I, J – M. Meanwhile four biflavonoids and five xanthones were obtained from the ethyl acetate extract, three of which (**24 – 26**) are new and given trivial names fuscaxanthones N – P, respectively. All the xanthones are prenylated 1,3,6,7-tetrahydroxyxanthone derivatives. Eight new xanthones possess the basic structure of cowanol, which was previously reported from *G. cowa* (Na Pattalung et al., 1994). However the different features were observed. Fuscaxanthone J (**21**) might have been *O*-methylated at 7-OH to form cowanol. In fuscaxanthone (+) I (**19**), the double-bond at C-17/C-18 of the geranyl group is hydroxylated, whereas the double-bond of the 4-hydroxy-3-methylbut-2-enyl group is epoxylated in fuscaxanthone K (**20**). In fuscaxanthones N and O (**24** and **25**), the double-bond at C-22/C-23 of the geranyl group is hydroxylated and relocated to C-21/C-22 or C-23/C-24. Meanwhile in fuscaxanthone L and P (**18** and **26**), the geranyl group is cyclized to give a monoterpenoid substitute with a C_6 ring. To the best of our knowledge,

parvixanthone I, which was reported from *G. parvifolia*, was the only xanthone with such a substitute (Xu et al., 2001). Fuscaxanthone M (**22**) is a dimer of cowanol, which is combined by an unusual C-O-C linkage.

The new xanthones and cowanol were tested for cytotoxicity on HeLa cells to establish a preliminary basis of a structure – activity relationship. The results showed that these xanthones had cytotoxic effect with the IC_{50} values in the range of 19.1 – 45.9 μM, except that compounds **20** and **22** were inactive. The modification of the geranyl group or the 4-hydroxy-3-methylbut-2-enyl group brought about a decrease or loss of activity compared with cowanol. Moreover griffipavixanthone, which was previously isolated from *G. griffithii*, *G. pavifolia* and *G.oblongifolia* (Xu et al., 1998; Shi et al., 2014), exhibited a high cytotoxicity against HeLa cells with an IC_{50} value of 7.9 ± 0.7 μM. This bixanthone was further evaluated for anti-angiogenic activity by *in vitro* proliferation assay on HMEC-1 cell line and it showed a greatly strong anti-proliferative effect with an IC_{50} value of 0.15 ± 0.0085 μM after 72 h incubation, which indicated that griffipavixanthone is a potential anti-angiogenic agent which should be further demonstrated in vivo and in further molecular assays.

The results of this work together with those previously reported (Ito et al., 2003; Nontakham et al., 2014) revealed that *Garcinia fusca* Pierre is a rich source of tetraoxygenated xanthones and biflavonoids. The cytotoxicity of those xanthones suggested that the bark of this plant may have an anti-tumor effect, which should be further studied for use in medicine.

1. INTRODUCTION

Vietnam is situated along the eastern coast of the Indochina Peninsula in Southeast Asia and covers an area of 329,500 square kilometres. The country is bordered to the north by China, to the west by Laos and Cambodia and to the east by South China Sea (known in Vietnam as the East Sea). As a result of the warm and humid tropical climate, approximately 39% of Vietnam territory is under forest cover with around 13,000 species of vascular plants, over 8,000 of which have been identified to date (Sterling and Hurley, 2005; Queiroz et al., 2013). Early on Vietnamese people discovered pharmaceutical properties of some plants and used them as medical remedies to prevent or treat some diseases such as eating ginger to treat cold and flu, drinking the water extract of *Artemisia* as an anti-malaria agent and chewing betel to prevent tooth decay. These formed the basis of Vietnamese traditional medicine. During the thousand years of Chinese domination, Vietnamese traditional medicine was deeply influenced by Chinese traditional medicine. In the nineteenth century, Western medical practices were introduced to Vietnam with the arrival of the French (Ladinsky et al., 1987). Nowadays Vietnamese medicine is a harmonious merging of Chinese, Vietnamese and Western medicinal system, in which traditional medicine has an important role in promoting the health of Vietnamese people, especially in primary health care at the commune level and the treatment of chronic illnesses such as AIDS and cancer which seem to be increasing. The statistics of the Vietnam Ministry of Health showed that about 30% of patients annually received the treatment with traditional medicines (WHO, 2005). Vietnamese traditional medical remedies are mainly based on medicinal plants. According to a report of the Vietnam Ministry of Health in 2014, 3,948 of 10,386 identified species have been known as medicinal plants, a large number of which have not been phytochemically investigated to improve use value. Moreover pharmaceutical properties of the remaining species have not determined yet. The report also showed that many valuable and rare medicinal plants are in the risk of extinction because of deforestation and uncontrolled exploitation of medicinal sources (The Vietnam Ministry of Health, 2014).

The Guttiferae or Clusiaceae is a family of plants including about 37 genera and 1610 species of trees and shrubs. This family is mainly tropical (Gustafsson et al., 2002). In Vietnam, there are around sixty five species of six genera comprising *Calophyllum, Cratoxylum, Garcinia, Mammea, Mesua* and *Ochrocapus*. Some species are used in folk medicine to treat various diseases such as dysentery, diarrhoea, coughs, fever, scabies, burns, menstrual disturbances and ulcer; whilst pharmaceutical properties of the remaining species have not been known (Vo, 1997; Pham, 1999). Though many species of the family Guttiferae have been phytochemically and biologically investigated all over the world, approximately twenty species collected in Vietnam have been reported so far. In order to contribute to phytochemical and biological study of the family Guttiferae in Vietnam, we have investigated the species *Garcinia fusca* Pierre collected in south Vietnam. The aim of this work was the isolation of natural compounds to characterize the phytochemical profile in detail and to provide possible new structures for drug discovery whereby that helps to improve use value of this species in the traditional medicine. Moreover, that also supplies phytochemical database of this species for classification and conservation of medicinal plants in Vietnam. The results of the phytochemical and biological investigation on the *n*-hexane and ethyl acetate extracts of *G. fusca* Pierre are presented in this thesis.

2. THE GENUS *GARCINIA*

2.1. General characters

The *Garcinia* is a large genus of the family Guttiferae (Clusiaceae), comprising around 450 species of tropical trees and shrubs with yellow resin or latex, mostly native to the Old World (tropical and South Africa, Madagascar, south-eastern Asia, north-eastern Australia and western Polynesia), with a few species in the tropical regions of the Americas. Various Asiatic species are used as a cathartic or stimulant, dye or artist's pigment. Many species are important timber resources used for construction and furniture. In Vietnam there are about thirty one species (Pham, 1999).

The members of the genus are various considerably in size and form but generally have opposite leaves (occasionally whorled), entire (untoothed or lobed), which are often evergreen, often thick and leathery but occasionally papery, with prominent secondary veins. The flowers, often fragrant, commonly have four or five parts, and occur in singly or in clusters of up to 5, which may be terminal (at branch tips) or axillary (where leaf meets stem). The fruit is a berry with a thin to leathery skin and one to five seeds (or more) embedded in a fleshy or pulpy, often edible, aril. The delicious mangosteen which is produced by *G. mangostana*, has been called "Queen of fruits" (Bailey and Bailey, 1976; Xiwen et al., 2007).

2.2. Chemical constituents of *Garcinia*

The genus *Garcinia* consists of many medicinal plants containing potential therapeutic agents which have been globally used in folk medicine. Those have attracted researchers to pharmaceutical and phytochemical investigations of species from the genus. To the best of our knowledge, approximately one hundred and seven species of the genus have been phytochemically investigated so far. A variety of plant parts were studied such as latex, resin, leaves, bark, root, stems, twigs, heartwood, branches, fruits, pericarps, seeds and flowers, see **Table 1**. These investigations revealed that the genus *Garcinia* produces different types of secondary metabolites including xanthones, biflavonoids, benzophenones, acylphloroglucinols, triterpenoids, depsidones, tocotrienols and biphenyls.

Table 1. *Garcinia* species which have been phytochemically investigated to date

Species	Parts	
G. achachairu	Seeds	Dal Molin et al., 2012
G. afzelii	Stem bark	Kamdem et al., 2006
	Seeds	Lannang et al., 2010
G. amplexicaulis	Stem bark	Lavaud et al., 2013 and 2015
G. aristata	Fruits	Cuesta-Rubio et al., 2001
G. assigu	Stem bark	Ito et al., 1997 and 2003
G. atroviridis	Stem bark	Kosin et al., 1998; Tan et al., 2014
	Root	Permana et al., 2001 and 2005
	Fruits	Mackeen et al., 2002; Tan et al., 2013
G. bakeriana	Leaves	Al-Shagdari et al., 2013
G. bancana	Twigs	Rukachaisirikul et al., 2005
	Leaves	
G. benthami	Stem bark	Elya et al., 2006
	Bark	Nguyen et al., 2011
	Leaves	
G. bracteata	Leaves	Thoison et al., 2000 and 2005
	Twigs	Na et al., 2010; Li et al., 2015
	Stem bark	Niu et al., 2012
	Stems	Hu et al., 2013 and 2014
	Fruits	Na et al., 2013
G. brasiliensis	Seeds	Martins et al., 2007; Naldoni et al., 2009
	Pericarps	Martins et al., 2008; Gontijo et al., 2012
G. brevipedicellata	Stem bark	Ngoupayo et al., 2008
G. buchananii	Heartwood	Jackson et al., 1971
	Stem bark	Stark et al., 2012 and 2013
G. cambogia	Latex	Rama et al., 1980
	Root	Iinuma et al., 1998
	Fruits	Masullo et al., 2010

Table 1. (continued)

G. cantleyana	Trunk bark	Shadid et al., 2007
	Twigs	Jantan et al., 2012
G. celebica	Leaves	Elfita et al., 2009
G. chapelieri	Bark	Rambeloson et al., 2014
G. cochinchinensis	Bark	Nguyen et al., 2011; Trinh et al., 2013
G. conrauana	Bark	Hussain and Waterman, 1982
	Heartwood	
	Seeds	
	Leaves	
G. cornea	Stem bark	Elfita et al., 2009
G. cowa	Latex	Na Pattalung et al., 1994; Mahabusarakam et al., 2005
	Fruits	Panthong et al., 2006; Sriyatep et al., 2014
	Stems	Shen et al., 2007; Siridechakorn et al., 2012
	Stem bark	
G. cuneifolia	Stem bark	Ea et al., 2003
G. cymosa	Stem bark	Elfita et al., 2009
G. densivenia	Stem bark	Waterman and Crichton, 1980
G. dioica	Bark	Iinuma et al., 1996
G. dulcis	Branches	Harrison et al., 1994
	Root	Iinuma et al., 1996
	Bark	Ito et al., 1997
	Leaves	Kosela et al., 2000
	Fruits	Deachathai et al., 2005
	Flowers	Deachathai et al., 2006
	Seeds	Deachathai et al., 2008
G. echinocarpa	Bark	Bandaranayake et al., 1975
G. edulis	Root bark	Magadula, 2010
G. esculenta	Twigs	Zhu, et al., 2014
G. eugeniifolia	Heartwood	Jackson et al., 1971

Table 1. (continued)

	Stem bark	Hartati et al., 2008
G. ferrea	Bark	Bui et al., 2014
G. forbesii	Branches	Harrison et al., 1992
	Twigs	
G. fusca	Stem bark	Ito et al., 2003
	Root	Nontakham et al., 2014
G. gardneriana	Leaves	Castardo et al., 2008
	Bark	Otuki et al., 2011
	Seeds	
G. gaudichaudii	Leaves	Cao et al., 1998
	Bark	Xu et al., 2000
G. gerrardii	Root bark	Sordat-Diserens et al., 1989
G. griffithii	Stem bark	Elfita et al., 2009; Nilar et al., 2005
	Leaves	Alkadi et al., 2013
	Bark	Xu et al., 1998
G. hanburyi	Latex	Asano et al., 1996; Tao et al., 2009
	Fruits	Reutrakul et al., 2007
	Leaves	Reutrakul, 2010
	Twigs	
G. hombroniana	Pericarps	Rukachaisirikul et al., 2000
	Leaves	Rukachaisirikul et al., 2005
	Twigs	Klaiklay et al., 2013
	Bark	Jamila et al., 2014
G. huillensis	Stem bark	Bakana et al., 1987; Dibwe et al., 2012
G. humilis	Bark	Herath et al., 2005; Haase, 2011
G. indica	Seeds	Badami and Desai, 1968
	Leaves	Badami and Razdan, 1972
	Heartwood	Cotterill et al., 1977
	Fruits	Krishnamurthy et al., 1981 and 1982

Table 1. (continued)

	Stem bark	Lakshmi et al., 2002
G. intermedia	Leaves	Abe et al., 2004
G. kola	Fruits	Hussain et al., 1982
	Seeds	Iwu, 1985
	Bark	Kabangu et al., 1987
	Root	Iwu et al., 1990; Niwa et al., 1994
	Stems	Terashima et al., 1999a and 1999b
	Mesocarp	Morabandza et al., 2013
G. lateriflora	Stem bark	Kosela et al., 1999; Ren et al., 2010
G. lancilimba	Stem bark	Yang et al., 2007; Han et al., 2008
G. latissima	Bark	Ito et al., 1997
G. linii	Root	Chen et al., 2004 and 2006
G. livingstonei	Heartwood	Pelter et al., 1971
	Root bark	Sordat-Diserens et al., 1990 and 1992
	Leaves	Kaikabo et al., 2009
	Fruits	Yang et al., 2010
G. lucida	Bark	Nyemba et al., 1990
	Stem bark	Fotie et al., 2007
G. macrophylla	Leaves	Andrade et al., 2007
	Flowers	
	Twigs	Williams et al., 2003
G. madruno	Aerial	Osorio et al., 2013
G. maingayii	Stem bark	Hartati et al., 2007
G. malaccensis	Stem bark	Taher et al., 2012; Alkadi et al., 2013
G. mangostana	Fruits	Govindachari et al. 1971; Suksamrarn et al., 2006
	Leaves	Parveen et al., 1990 and 1991
	Pericarps	Sen et al., 1982; Suksamrarn et al., 2002
	Heartwood	Nilar and Harrison, 2002
	Root bark	Ea et al., 2006

Table 1. (continued)

	Stem bark	Ea et al, 2008; Han et al., 2009
G. mannii	Bark	Crichton and Water, 1979
	Heartwood	Hussain and Waterman, 1982
	Seeds	
	Leaves	
G. merguensis	Bark	Nguyen et al., 2003
	Wood	Kijjoa et al., 2008
	Twigs	Trisuwan et al., 2013
G. multiflora	Heartwood	Chen et al., 1975
	Stems	Chiang et al., 2003
	Fruits	Chen et al., 2008
	Twigs	Liu et al., 2010
	Stem bark	Jing et al., 2013
	Leaves	Jiang et al., 2014
G. myrtifolia	Bark	Spino et al., 1995
G. neglecta	Leaves	Ito et al., 2001
G. nervosa	Stem bark	Ampofo and Waterman, 1986
	Leaves	Babu et al., 1988; Ilyas et al., 1994
G. nigrolineata	Bark	Rukachaisirikul et al., 2003
	Leaves	Rukachaisirikul et al., 2003
	Twigs	Rukachaisirikul et al., 2005
G. nobilis	Stem bark	Fouotsa et al., 2012
	Twigs	Fouotsa et al., 2014
G. nujiangensis	Twigs	Tang et al., 2015
G. oblongifolia	Bark	Hamed et al., 2006
	Stems	Wu et al., 2008
	Leaves	Zhang et al., 2014
	Bark	Feng et al., 2014
G. oligantha	Stems	Gao et al., 2013; Wu et al., 2013

Table 1. (continued)

G. oliveri	Bark	Ha et al., 2009 and 2012
G. opaca	Leaves	Goh et al., 1992
	Bark	Mori et al., 2014
G. ovalifolia	Stem bark	Waterman and Crichton, 1980; Lannang et al., 2013
	Leaves	Gustafson et al., 1992
G. parvifolia	Leaves	Xu et al., 2000
	Bark	Xu et al., 2001
	Stem bark	Kardono et al., 2006; Syamsudin et al., 2013
	Twigs	Rukachaisirikul et al., 2006
G. paucinervis	Leaves	Gao et al., 2010; Wu et al., 2013
	Stem bark	Fan et al., 2012
	Stems	Hu et al., 2014
G. penangiana	Leaves	Jabit et al., 2007
G. penduculata	Heartwood	Rao et al., 1974
	Pericarps	Sahu et al., 1989
	Bark	Vo et al., 2012
G. polyantha	Stem bark	Lannang et al., 2005
	Wood	Louh et al., 2008
	Root bark	Lannang et al., 2008
	Leaves	Lannang et al., 2014
G. porrecta	Stem bark	Kardono et al., 2006
G. prainiana	Leaves	Klaiklay et al., 2011; Mawa and Said, 2012
	Twigs	Susanti et al., 2013
G. preussii	Leaves	Messi et al., 2012
G. propinqua	Twigs	Tantapakul et al., 2012
G. pseudoguttifera	Heartwood	Ali et al., 2000
G. puat	Leaves	Ito et al., 2001
G. punctata	Stem bark	Ngameni et al., 2014
G. purpurea	Pericarp	Iinuma et al., 1996

Table 1. (continued)

G. pyrifera	Stem bark	Ampofo and Waterman, 1986
	Fruits	Roux et al., 2000
G. quadrifaria	Stem bark	Waterman and Hussain, 1982
G. quaesita	Bark	Gunatilaka et al., 1984
G. rigida	Leaves	Elya et al., 2006 and 2011
G. scortechinii	Twigs	Rukachaisirikul et al., 2000
	Latex	Rukachaisirikul et al., 2003
	Stem bark	Rukachaisirikul et al., 2005
	Fruits	Sukpondma et al., 2005
G. semseii	Stem bark	Magadula et al., 2008
	Pericarps	Magadula et al., 2012
G. shomburgkiana	Bark	Vo et al., 2012
	Wood	Mungmee et al., 2013
	Stems	Ito et al., 2013
G. smeathmannii	Stem bark	Komguem et al., 2005; Kuete et al., 2007
	Root bark	Lannang et al., 2006
G. solomonensis	Stem bark	Carroll et al., 2009
G. speciosa	Bark	Vieira et al., 2004
	Trunk bark	Rukachaisirikul et al., 2003
G. spicata	Bark	Konoshima et al., 1970
	Fruit	Lyles et al., 2014
	Leaves	Gunatilaka et al., 1984
G. subelliptica	Heartwood	Fukuyama et al., 1991
	Root bark	Iinuma et al., 1995
	Wood	Fukuayama et al., 1998
	Seeds	Weng et al., 2003 and 2004
	Stem bark	Abe et al., 2003
	Fruits	Zhang et al., 2010
	Leaves	Ito et al., 2013

Table 1. (continued)

G. succifolia	Wood	Duangsrisai et al., 2014
G. staudtii	Stem bark	Waterman and Hussain, 1982
G. talboti	Root	Joshi et al., 1970
G. terpnophylla	Bark	Bandaranayake et al., 1975
	Wood	
G. tetralata	Stem bark	Wang et al., 2008; Guo et al., 2011
	Branches	Na and Xu, 2010
	Leaves	
G. tetranda	Wood	Purwaningsih and Ersam, 2007
G. thwaitesii	Bark	Gunatilaka et al., 1983
	Timber	
G. vieillardii	Stem bark	Hay et al., 2004 and 2008
G. vilersiana	Bark	Nguyen et al., 1999; Bui et al., 2012
G. virgata	Stem bark	Merza et al., 2004 and 2006
G. volkensii	Heartwood	Herbin et al., 1970
	Bark	Magadula et al., 2014
G. xanthochymus	Fruits	Karanjgoakar et al., 1973; Baggett et al., 2005
	Bark	Chen et al., 2008 and 2010
	Stem bark	Ji et al., 2012
	Twigs	Trisuwan et al., 2014
G. xipshuanbannaensis	Fruits	Shen et al., 2006
	Leaves	Na and Xu, 2010
	Twigs	Han et al., 2008; Na and Xu, 2009

2.2.1. Xanthones

The latex or resin of *Garcinia* species is commonly yellow because of the presence of xanthone derivatives. Xanthones have been widely found in all species of this genus including simple oxygenated xanthones, prenylated xanthones, caged xanthones and bisxanthones.

2.2.1.1. Simple oxygenated xanthones

These compounds can be di-, tri-, tetra- or penta-oxygenated xanthones with simple substituents such as hydroxyl, methoxyl and methyl groups (see **Figure 1**). Two xanthones, 1,5- and 1,7-dihydroxyxanthones are simple di-oxygenated xanthones found extensively in many species (Bennett and Lee, 1989). Simple tri-oxygenated compounds as 1,3,5-trihydroxyxanthone and 1,5-dihydroxy-2-methoxyxanthone were isolated from *G. xanthochymus* (Baslas, 1979). Three xanthones, 1,3,5,7-, 1,3,6,7-tetrahydroxyxanthones and 1,2-dihyroxy-5,6-dimethoxyxanthone, which were reported from *G. penduculata* and *G. subelliptica*, are examples for simple tetra-oxygenated xanthones (Rao et al., 1974; Minami et al., 1995). Simple penta-oxygenated xanthones are rarely found in the genus, whereas some were isolated from the genus *Calophyllum* (Guttiferae). Three xanthones, 1,3,4,5,8-pentahydroxyxanthone, 1,3,8-trihydroxy-4,6-dimethoxyxanthone and 1,3,7-trihydroxy-4,6-dimethoxyxanthone, are simple penta-hydroxyxanthones identified from two species *G. tetranda* and *G. hombroniana* (Purwaningsih and Ersam, 2007; Klaiklay et al., 2013).

2.2.1.2. Prenylated xanthones

Prenylated xanthones are di-, tri-, tetra- and penta-oxigenated xanthones, whose the aromatic ring system is substituted by prenyl groups such as isoprenyl, 1,1-dimethylprop-2-enyl, geranyl and farnesyl (see **Figure 2**). These groups can be cyclized with adjacent hydroxyl groups at the *ortho*-position to give tetra- or penta-cyclic xanthones with furano- or pyrano rings, respectively. A xanthone can be substituted by one, two or three prenyl groups. Natural xanthones with more than three prenyl groups have not yet been found up to date. A large number of prenylated tri- and tetra-oxygenated xanthones have been reported from the genus. Prenylated penta-

oxygenated xanthones are rare. 1,7-Dihydroxy-3-methoxy-2-(3-methylbut-2-enyl)xanthone, isocowanol and dulciol C, three xanthones from *G. mangostana, G. pyrifera* and *G. dulcis*, are three xanthones substituted by one, two and three prenyl groups (Mahabusarakam et al., 1987; Ampofo and Waterman, 1986; Iinuma et al., 1996). Garcigerrin A, a tri-oxygenated xanthone with a pyrano ring from *G. gerrardii* and rheediaxanthone B, a tetra-oxygenated xanthone with a furano ring from *G. polyantha*, are two examples of tetracyclic xanthones (Ampofo and Waterman, 1986; Sordat-Diserens et al., 1989). Subelliptenone C from *G. subelliptica* is a tetra-cyclic xanthone with a furo ring (Iinuma et al., 1995). BR-xanthone A, a xanthone from *G. mangostana* and 1,5-dihydroxy-6,6-dimethylpyrano(2,3:6,7)-4″,4″,5″-trimethylfurano(2″,3″:3,4)-2-(3-methylbut-2-enyl)xanthone, a xanthone from *G. opaca*, are representative of penta-cyclic xanthones (Balasubramanian and Rajagopalan, 1988; Goh et al., 1992). 5-Farnesyltoxyloxanthone, a tetraoxygenated xanthone isolated from *G. merguensis*, is a rare xanthone with a farnesyl group (Kijjoa et al., 2008).

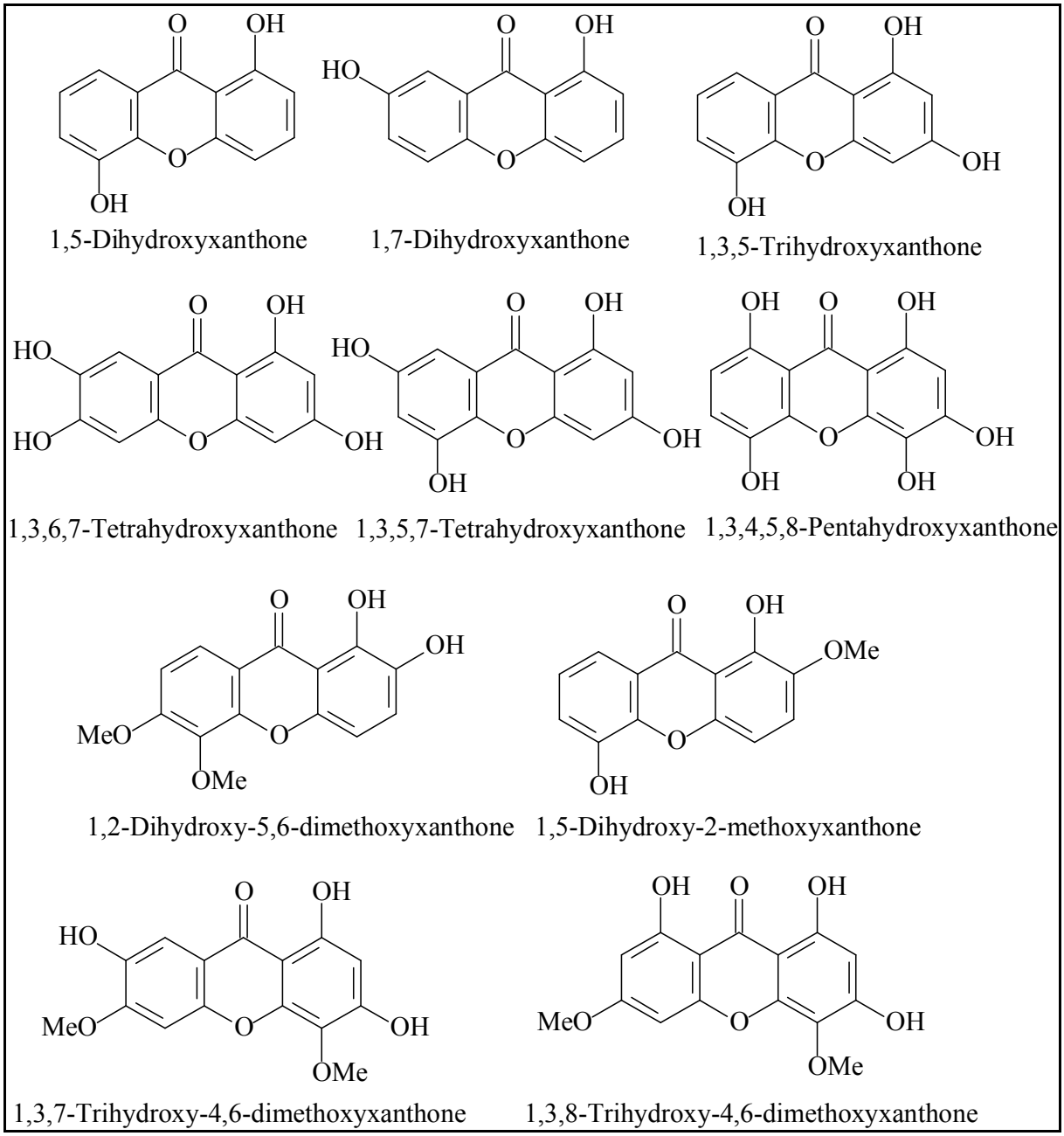


Figure 1. Simple xanthones from some *Garcinia* species

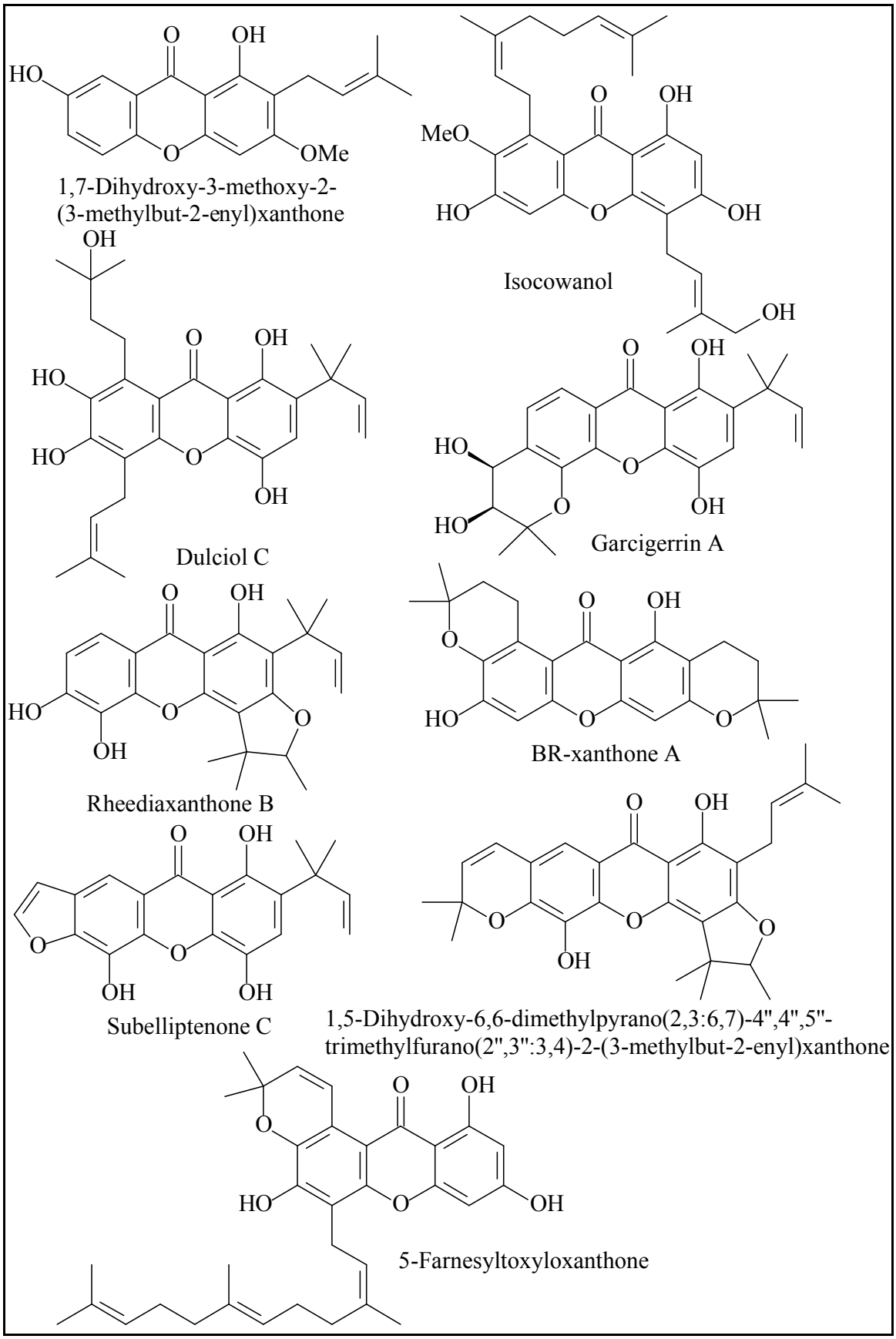


Figure 2. Prenylated xanthones from some *Garcinia* species

2.2.1.3. Caged xanthones

These compounds are polyprenylated xanthones, in which the ring B is converted into an unusual ring, 4-oxa-tricyclo[4.3.1.$0^{3,7}$]dec-2-one (caged) scaffold (Ollis et al., 1965). This motif can be further modified with various substituents on the aromatic ring A and can also be oxidized to form a wide range of compounds with interesting structures, see **Figure 3**. Caged xanthones have been restrictively found in some species of the genus *Garcinia*, especially *G. hanburyi*. Over one hundred compounds have been identified from the genus. Morellin, a caged xanthone from *G. morella*, was first reported in 1937 (Rao, 1937). Gambogellic acid, one of caged xanthones isolated from *G. hanburyi*, has a polycyclic structure (Asano et al., 1996). Although the majority of caged xanthones possess the general motif, a few compounds have alternative structures. For example, 6-O-methylneobractatin identified from *G. bracteata*, contains the neo-motif (Thoison et al., 2000). Lateriflorone isolated from *G. lateriflora,* has a spiroxalactone core by oxidation of the ring C (Kosela et al., 1999), whilst the ring B in the structure of scortechinone K, one of caged xanthones from *G. scortechinii*, undergoes ring-opening oxidation (Rukachaisirikul et al., 2003).

2.2.1.4. Bisxanthones

Bisxanthones are dimeric xanthones, which have been found in some species of the family Guttiferae. They are formed from prenylated monomeric xanthones by ring-closure coupling through side-chains, see **Figure 4**. Garcilivins A-C, which was isolated from the bark of the of roots *G. livingstonei*, have been reported for the first time from the genus. These compounds are dimerics linked *via* a 6-membered ring, which is formally derived from a Diels-Alder-type reaction between two isoprenyl units. (Sordat-Diserens et al., 1992). Bigarcinenone A from the bark of *G. xanthochymus* is a dimer linked *via* a 5-membered ring like cratoxyxanthone. A biosynthetic pathway of the latter one was proposed to involve the coupling of two xanthone radicals, see Scheme **11** (Zhong et al., 2008). Bigarcinenone B, which was also obtained from *G. xanthochymus*, is a bisxanthone that is connected *via* a 6- and 6-membered ring system (Chen et al., 2011).

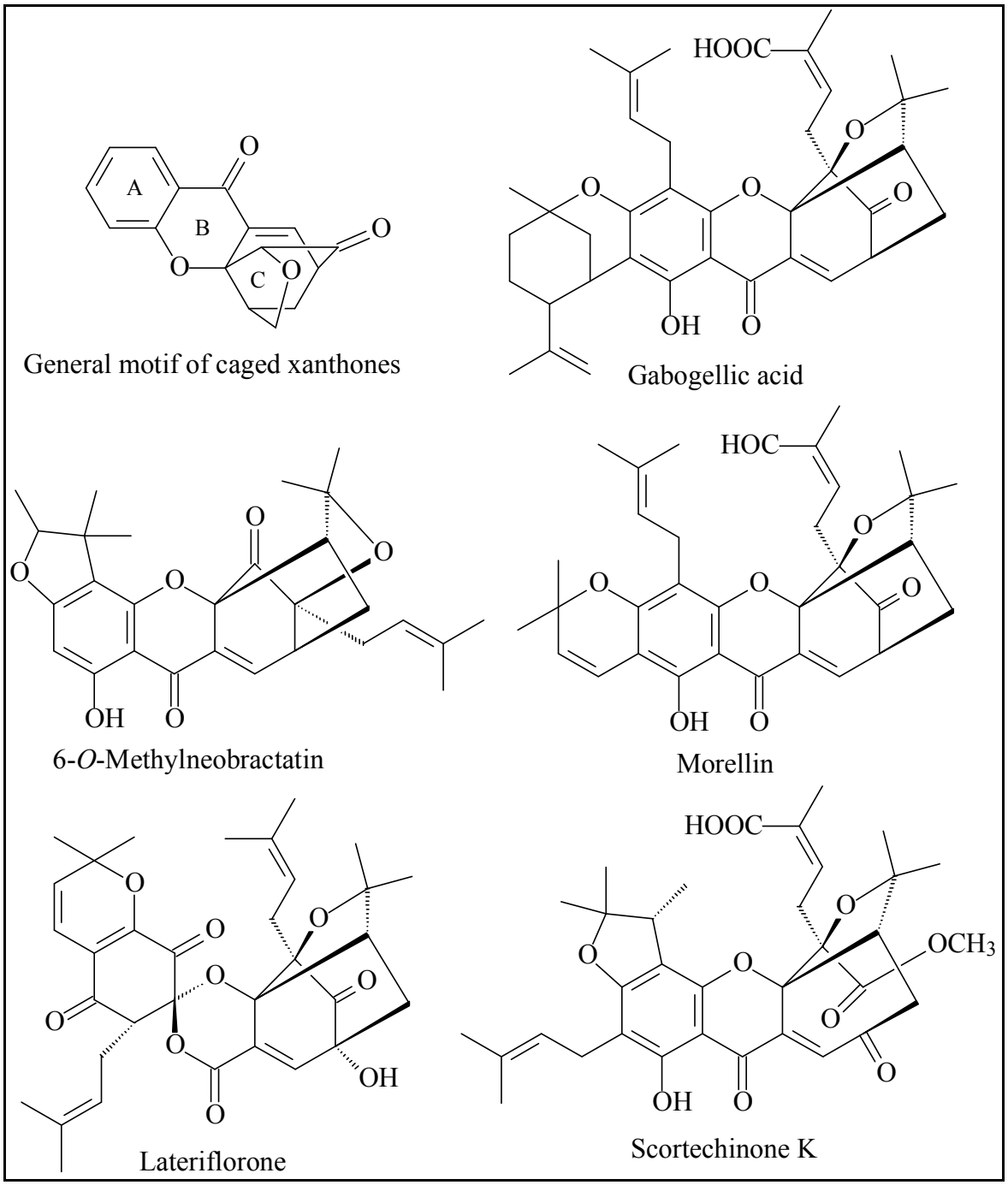


Figure 3. Caged xanthones from some *Garcinia* species

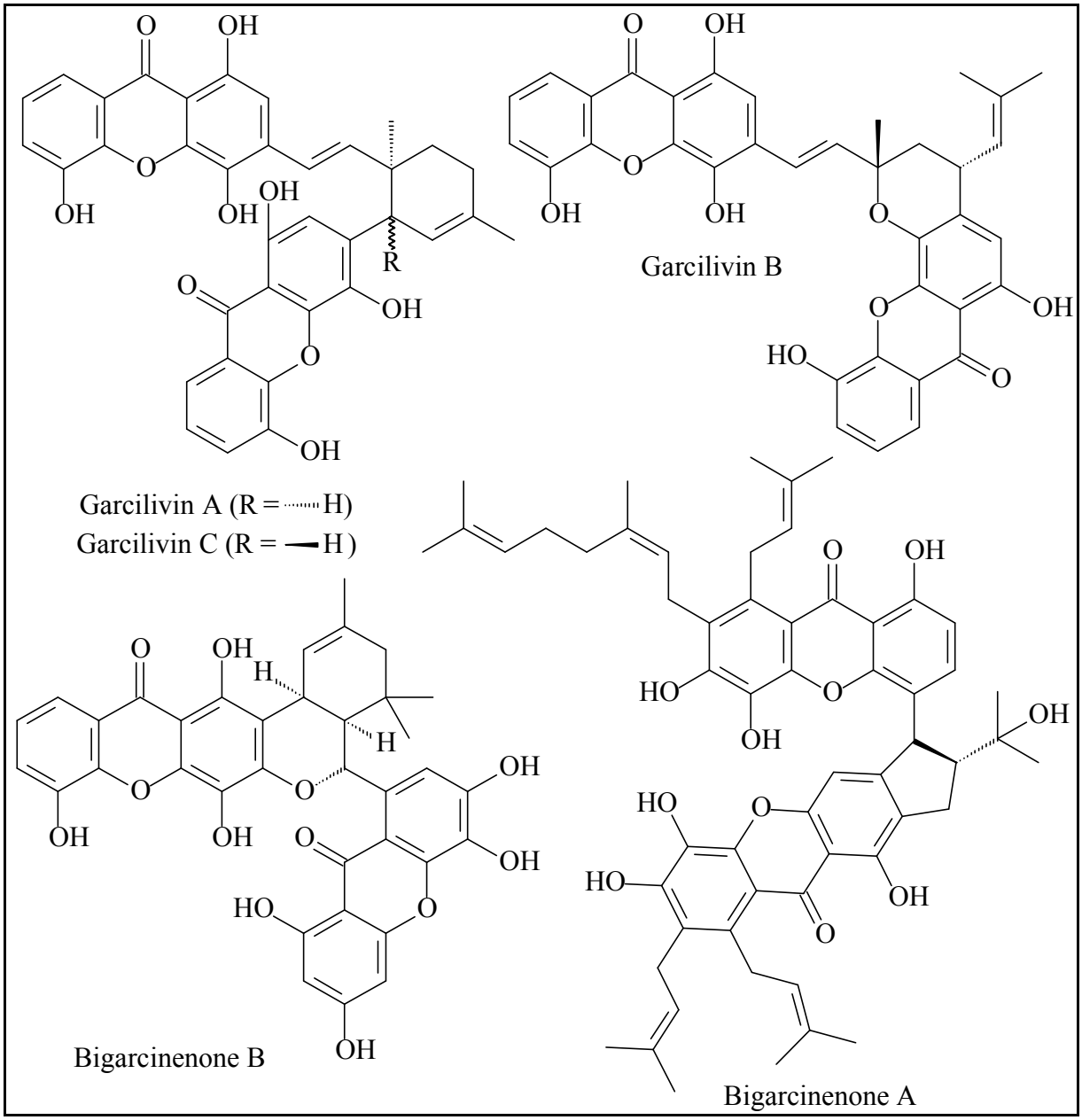


Figure 4. Bisxanthones from some *Garcinia* species

2.2.2. Biflavonoids

The genus *Garcinia* is shown to be a source of biflavonoids. To the best of our knowledge, about one hundred biflavonoids have been identified from this genus to date. The majority of these compounds are dimers of two flavanones, two flavones, between a flavanone and a flavone or a flavanonol, which are linked at the positions C-3 and C-8″ or C-3′ and C-8″. There are four main groups of *Garcinia* biflavonoids: GB-1 type with a flavanone-(3-8″)-flavanonol system, GB-1a type with a flavanone-

(3-8″)-flavanone system, morelloflavone type with a flavanone-(3-8″)-flavone system and amentoflavone type with a flavone-(3′-8″)-flavone system (Ferreira et al., 2012; Ito et al., 2013). Beside these four types, some biflavonoids have alternative structures. For instance, lateriflavone with the 6-8″ linkage has been isolated from *G. lateriflora*, whereas (2′→2‴)-biflavonol from *G. nervosa* has a rare linkage between C-2′ and C-2‴, see **Figure 5** (Parveen et al., 1994; Ren et al., 2010).

2.2.3. Benzophenones

Benzophenones from *Garcinia* comprise simple benzophenones and polyprenylated benzophenones, in which the aromatic ring A can undergo oxidation, prenylation and cyclization to form polycyclic benzophenones with a bicyclo[3.3.1]nonane-2,4,9-trione skeleton (Zhang et al., 2010). These polycyclic benzophenones are divided into three types corresponding to the position of the benzoyl group. Type A includes compounds with the benzoyl group at C-1, for example garcimultiflorone A from *G. multiflora* (Chen et al., 2009). Type B, with the benzoyl group at C-3 such as pedunculol from *G. pendunculata*, was widely found in the genus (Sahu et al., 1989). Garcinielliptone K, which was obtained from *G. subelliptica*, is representative of type C with the benzoyl group at C-5 (Weng et al., 2004). Cyclization involving the β-diketone and olefinic groups in polyprenylated compounds led to formation of interesting adamantanyl benzophenones such as garciniagifolone A from *G. oblongifolia* (Shan et al., 2012).. Additionally in some cases, the ring B can be modified into a bicyclo[3.3.2]decane-2,4,10-trione skeleton such as gambogenone reported from *G. xanthochymus* or can undergo intramolecular oxidative coupling between the enol and the aromatic ring B to form corresponding polycyclic xanthone derivatives, for examples garcinialone obtained from *G. multiflora* (Baggett et al., 2005; Chien et al., 2008). Doitunggarcinone B, which was isolated from *G. propinqua*, is an unusually rearranged benzophenone, see **Figure 6** (Tantapakul et al., 2012).

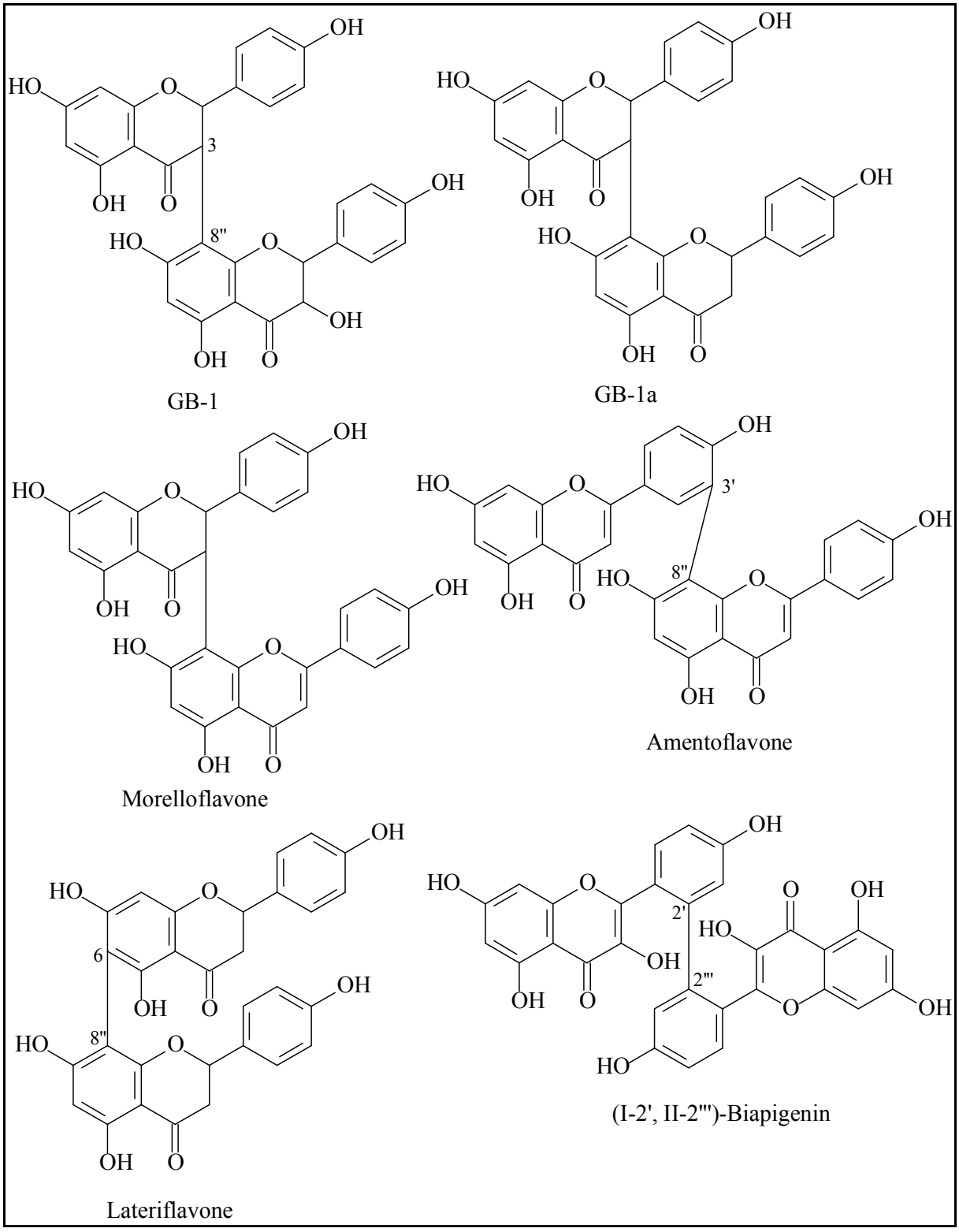


Figure 5. Biflavonoids from some *Garcinia* species

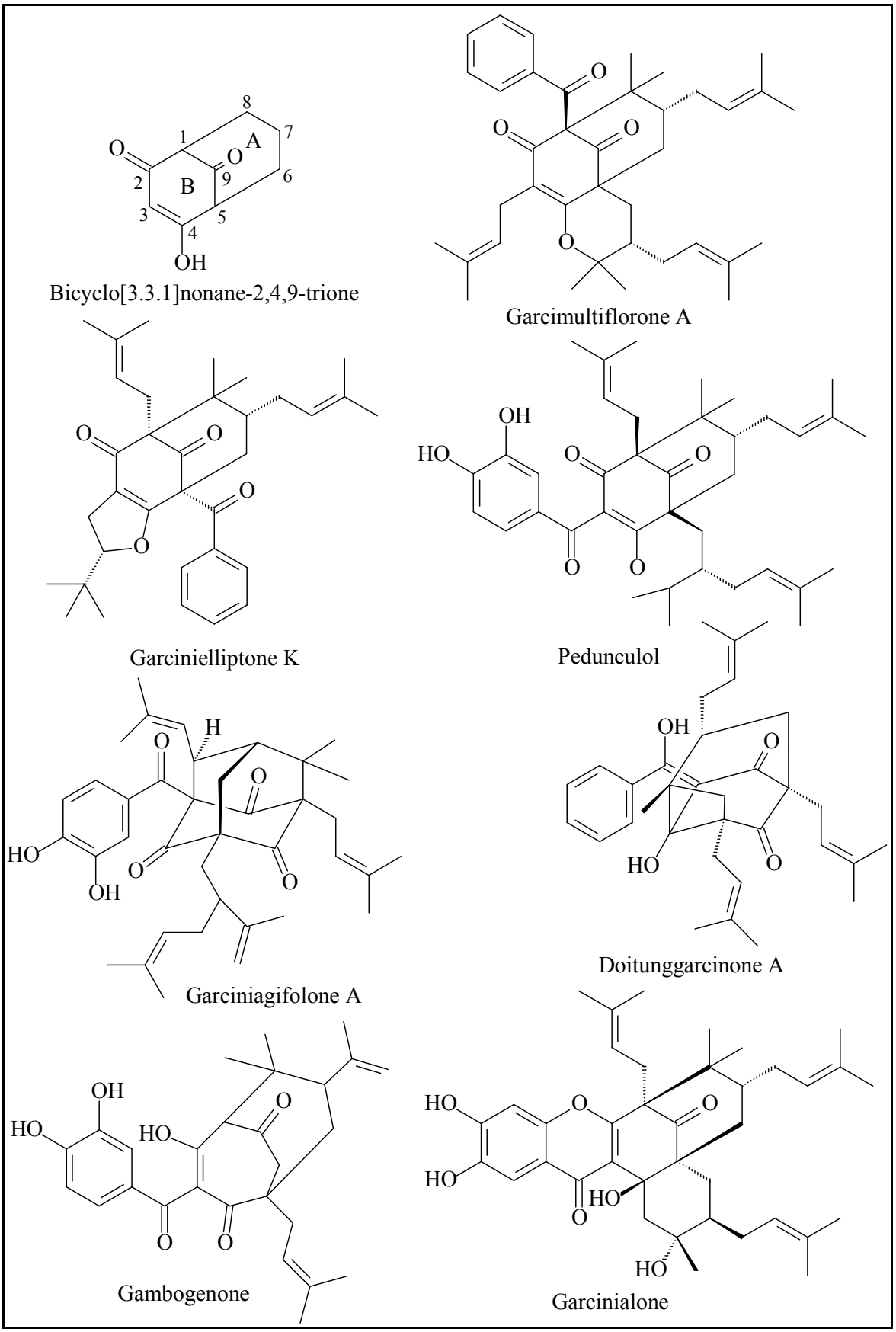


Figure 6. Benzophenones from some *Garcinia* species

2.2.4. Phloroglucinols

Phloroglucinol derivatives reported in the genus include simple phloroglucinols and complex compounds with an oxidized and polyprenylated nucleus or a bicyclo[3.3.1]nonane-1,3,9-trione skeleton. Parvifoliol A is one of simple phloroglucinols isolated from *G. parvifolia* (Rukachaisirikul et al., 2006). Garcinielliptone HB is one of seven polyprenylated phloroglucinols identified from *G. subelliptica*, whilst garcicowin A from *G. cowa* is a phloroglucinol derivative with the bicycle[3.3.1]nonane-1,3,9-trione core (Lu et al., 2008; Xu et al., 2010). Garcinielliptone HF, which was also obtained from *G. subelliptica*, is a phloroglucinol with an unprecedented skeleton, see **Figure 7** (Wu et al., 2008).

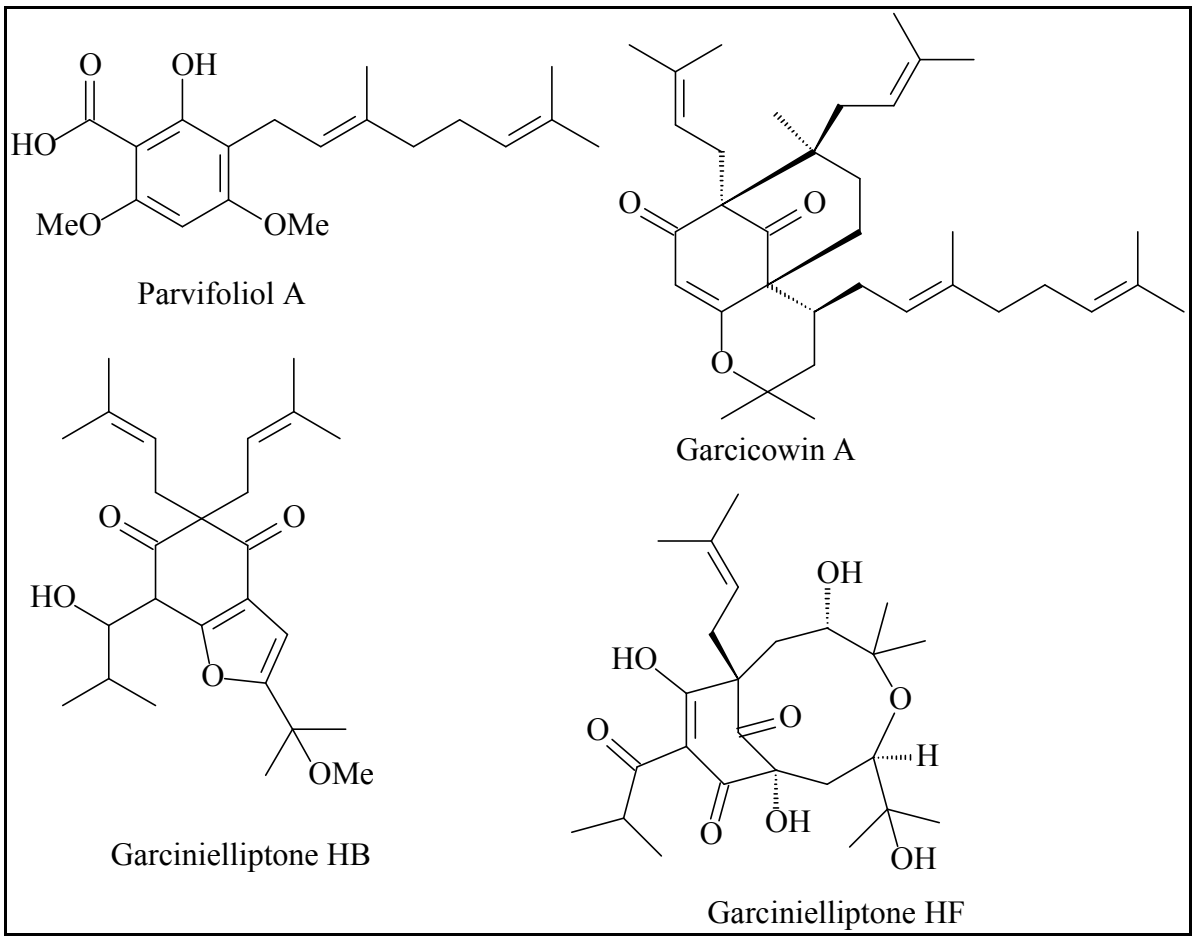


Figure 7. Phloroglucinol derivatives from some *Garcinia* species

2.2.5. Depsidones

Depsidones are polyphenolic compounds containing the 11H-dibenzo[*b,e*][1,4]-dioxepin-11-one system. These compounds were mainly found in lichens, which usually provide polyketide-derived natural products. However a substantial number of depsidones were reported from the genus *Garcinia*, which is well known as a rich source of shikimate-derived aromatic compounds. Depsidones from the genus are commonly substituted with hydroxyl, methoxyl, isoprenyl and geranyl groups. For instance, garcidepsidone A, one of four prenylated depsidones, was isolated from *G. parvifolia* (Xu et al., 2000). Prenyl side-chains can be cyclized with hydroxyl groups at the position *ortho* to give tetra- and penta-cyclic compounds such as garcinisidone B and C, a tetracyclic depsidone and a penta-cyclic depsidone, respectively, were identified from *G. neglecta*, see **Figure 8** (Ito et al., 2001).

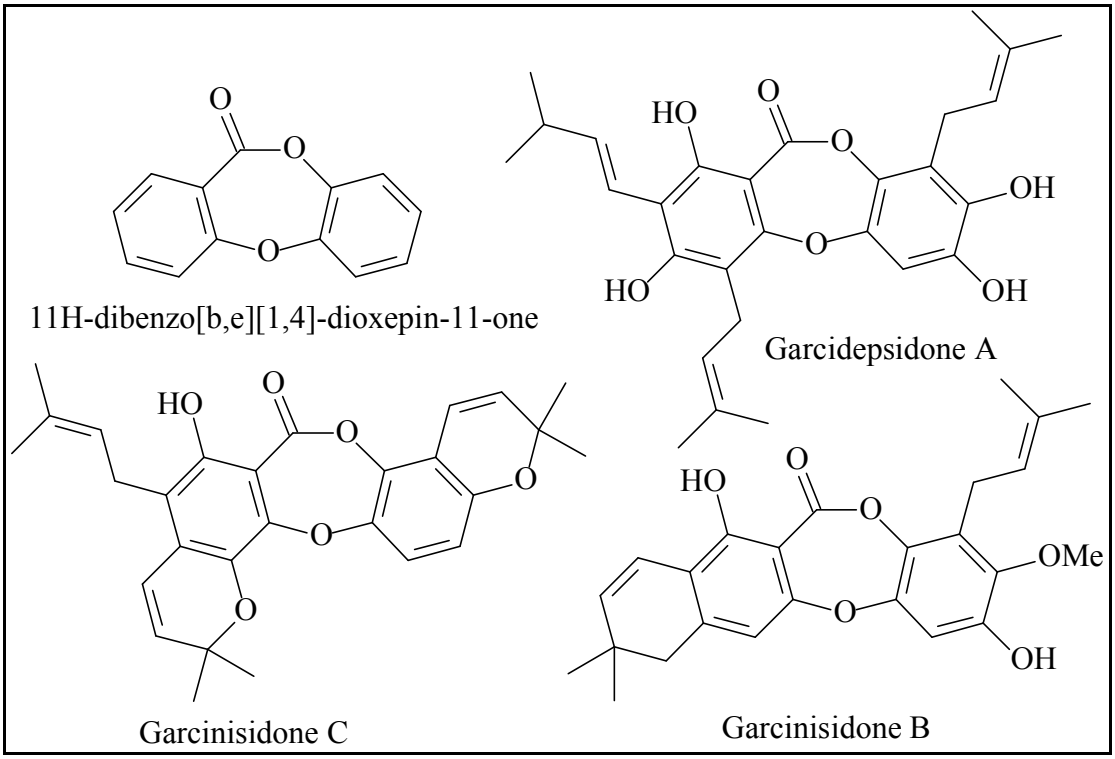


Figure 8. Depsidones from some *Garcinia* species

2.2.6. Tocotrienols

Tocotrienol derivatives were isolated from some *Garcinia* species. They have a 6-chromanol skeleton substituted by a farnesyl group at the position C-2, which can be oxidized at two terminal methyl groups. These compounds can be mono- or dimeric derivatives. For instance, 5-formyl-*δ*-tocotrienol is a mono derivative identified from *G. virgate* (Merza et al., 2004), whilst δ,γ-bi-*O*-amplexichromanol, δ,γ-biamplexichromanol and δ,δ-biamplexichromanoate are dimeric tocotrienols reported from *G. amplexicaulis*, see **Figure 9** (Lavaud et al., 2015).

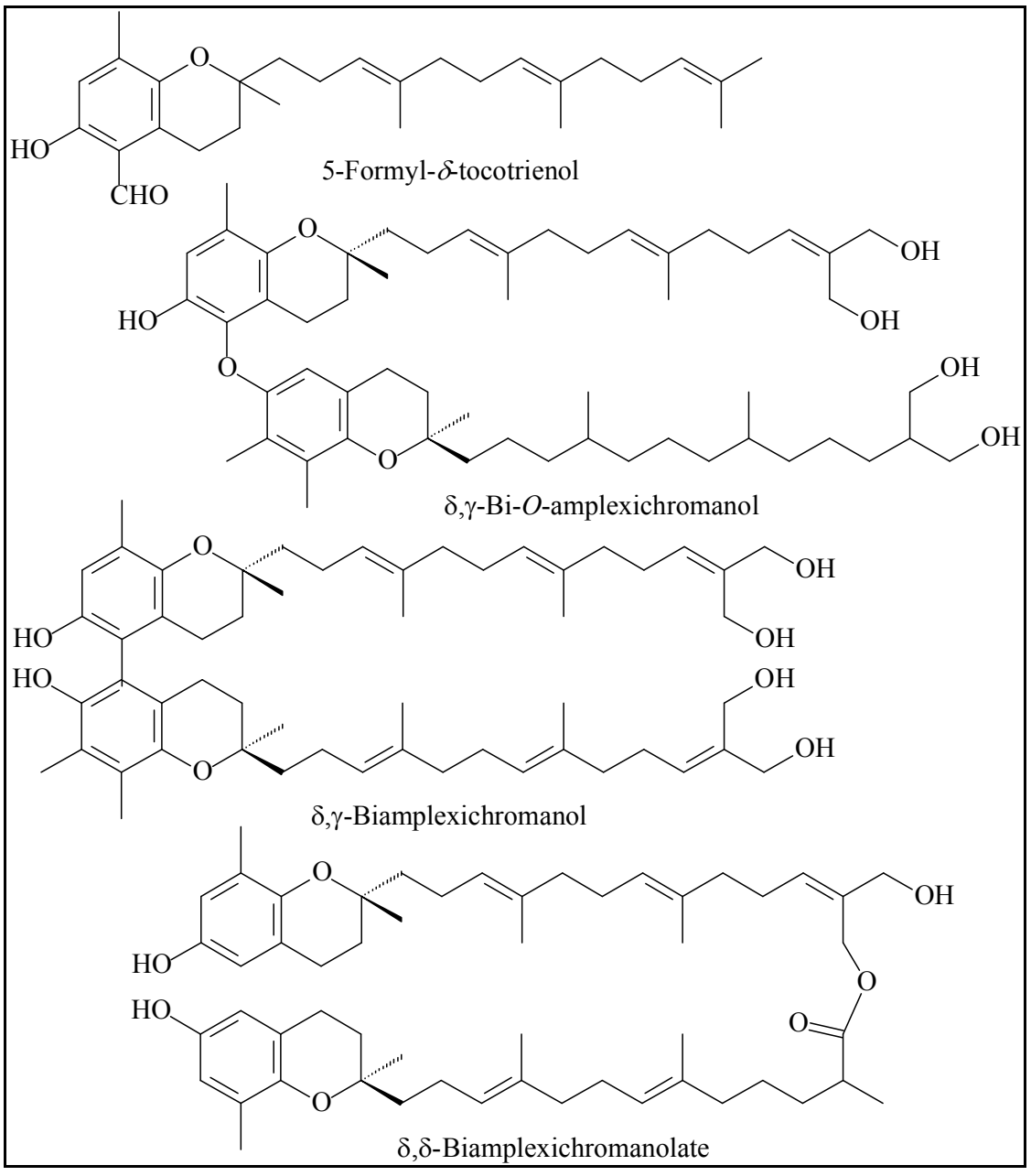


Figure 9. Tocotrienols from some *Garcinia* species

2.2.7. Biphenyls

A series of biphenyl derivatives were identified from the genus. These compounds possess a biphenyl core substituted by hydroxyl, methoxyl and isoprenyl groups such as garcibiphenyls B and C from the root of *G. linii* (Chen et al., 2006). Oblongifoliagarcinines A and C, which were reported from *G. oblongifolia*, are tri- and tetracyclic biphenyls, see **Figure 10** (Wu et al., 2008).

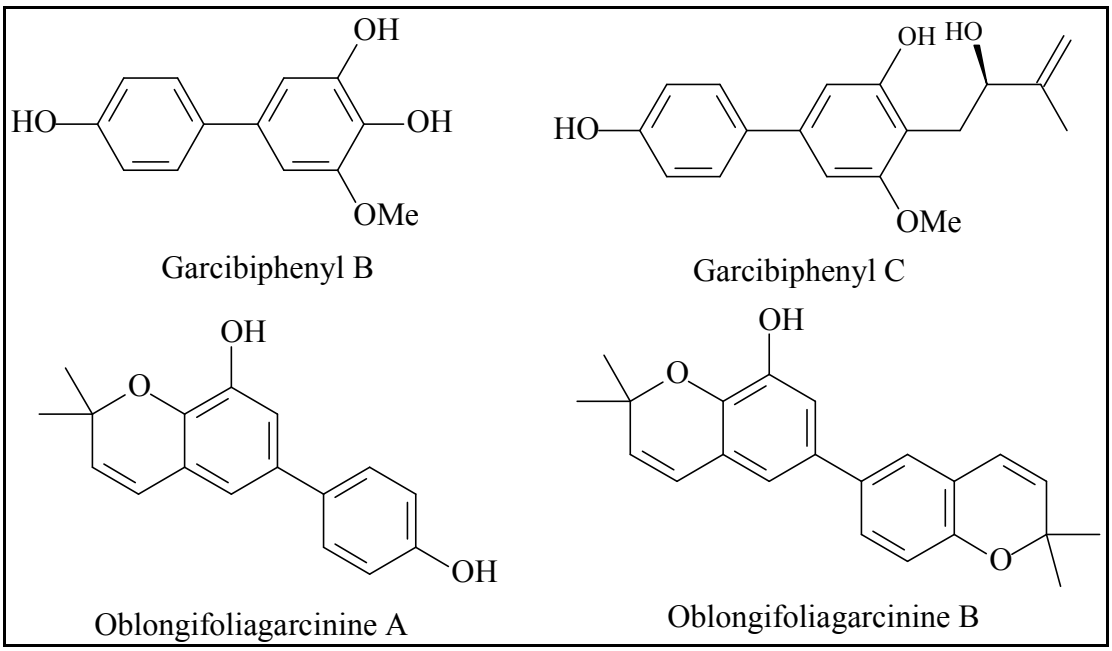


Figure 10. Biphenyl derivatives from some *Garcinia* species

2.2.8. Triterpenoids

Triterpenoids from this genus consist of lanostanes, friedolanostanes, abeolanostanes, cycloartanes, friedocycloartanes, friedelanes, protostanes, triterpenes with lup-20(29)-en-type and oleanolic acid skeletons. Garcihombronane A and D are a friedolanostane and a lanostane of ten triterpenes isolated from the leaves of *G. hombroniana* (Rukachaisirikul et al., 2000 and 2005). 14*β*,15*β*-Epoxy-3*β*-hydroxy-9-oxo-11[10-8]-abeolanostan-22-*cis*,24-*trans*-dien-28-oic acid is one of four 11(10-8)-abeolanostanes reported from *G. speciosa* (Vieira et al., 2004), whereas garciosaterpene A is one of three protostanes also isolated from this species (Rukachaisirikul et al., 2003). 2*α*-Hydroxy-3*β*-*O*-acetyllup-20(29)-en-28-oic acid, a lup-20(29)-en-type triterpene and an oleane, 3-*O*-(4′-*O*-acetyl)-*α*-L-

arabinopyranosyloleanolic acid, have been reported from the resin of *G. hanburyi* (Wang et al., 2008). (22Z,24E)-3α-Hydroxy-17,13-friedocycloarta-12,22,24-trien-26-oic acid is a friedocycloartane obtained from the bark of *G. benthami*, whilst ovalifolone A is a friedelane derivative from *G. ovalifolia*, see **Figure 11** (Nguyen et al., 2011; Lannang et al., 2013).

2.2.9. Other compounds

Hydroxycitric acid and its derivatives were isolated from the fruits of three species, *G. cambogia*, *G. indica* and *G. atroviridis* (Lewis, 1969). (-)-Hydroxycitric acid has drawn all worldwide attention because of its anti-obesity property (Krishnamurthy and Sapna, 2008). Some sesquiterpenes were identified from the genus, for instance, scortechterpenes A and B from the fruit of *G. scortechinii* (Sukpondma et al., 2005). Garcinielliptones N and O are two novel terpenoids isolated from the seed of *G. subelliptica* (Weng et al., 2004). Some unusual compounds were reported from the genus such as three benzophenone-xanthone dimers from the root of *G. dulcis*, garciduols A-C have been first identified in nature (Iinuma et al., 1996). Two flavanone-chromone dimers, preussianone and I-4′,I-5,II-5,I-7,II-7-pentahydroxyflavanone[I-3,II-8]-chromone were isolated from the leaves of *G. preussii* and *G. dulcis* (Messi et al., 2012; Ansari and Rahman, 1975). The unsubstituted chromone moiety is supposed to be derived by elimination of a phenyl ring from a biflavone. Garcinianins A and B, two new proanthocyanidins from the leaves of *G. multiflora*, have been first reported from the genus (Jiang et al., 2014). In addition, the essential oil of some species contains volatile compounds, see **Figure 12** and **13** (Macleod and Pieris, 1982).

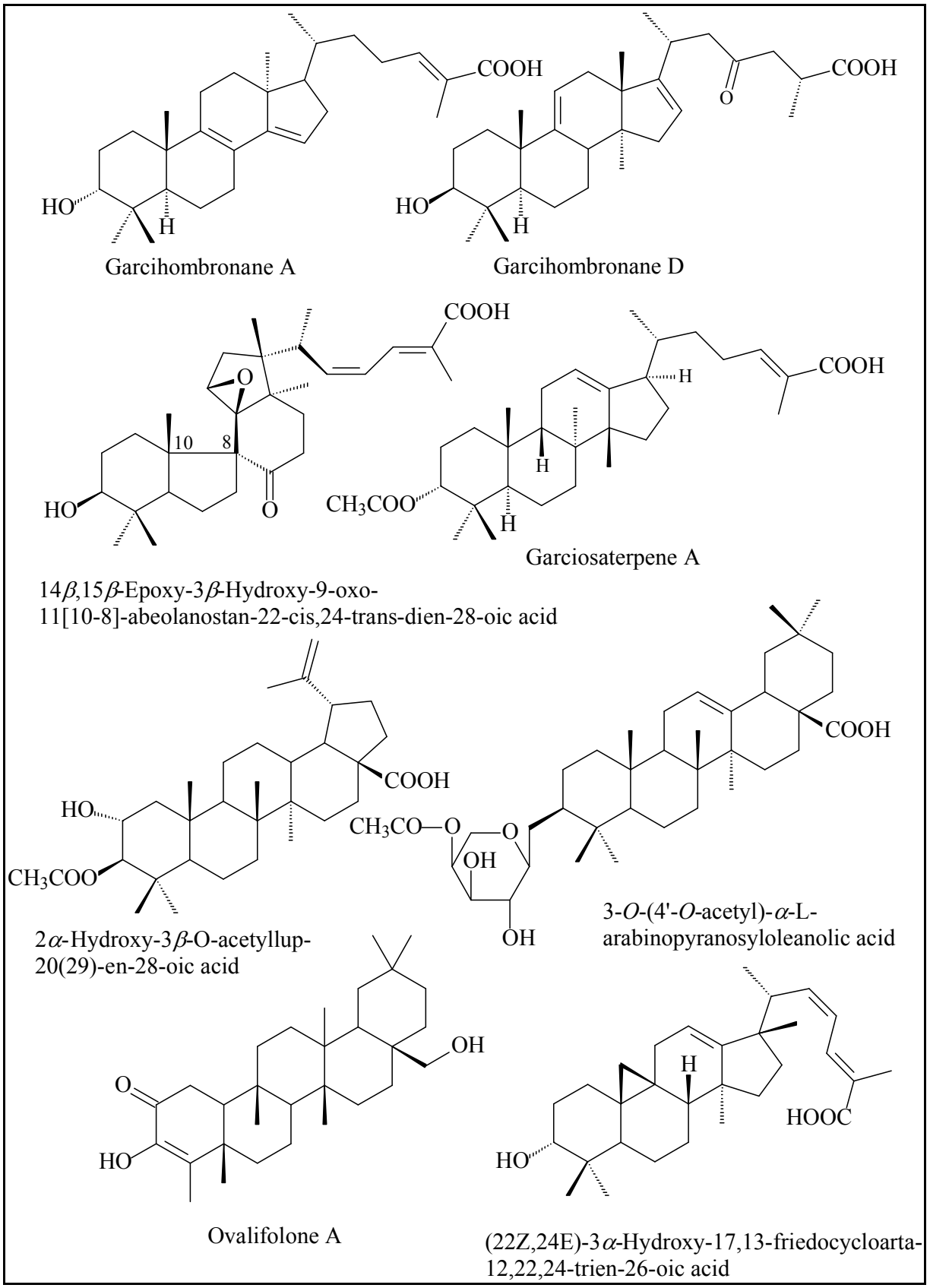


Figure 11. Triterpenoids from some *Garcinia* species

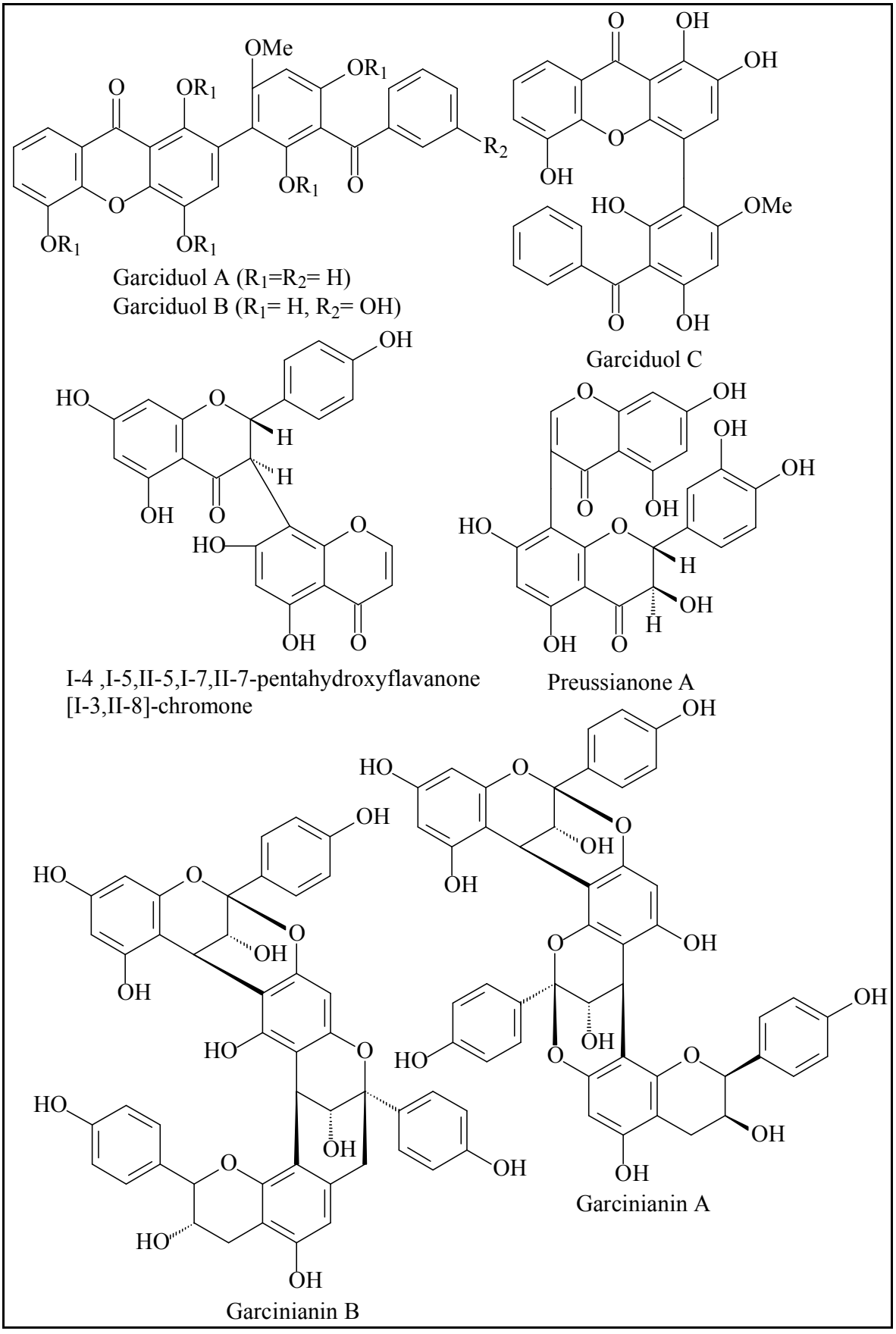


Figure 12. Some rare phenolic compounds in the genus *Garcinia*

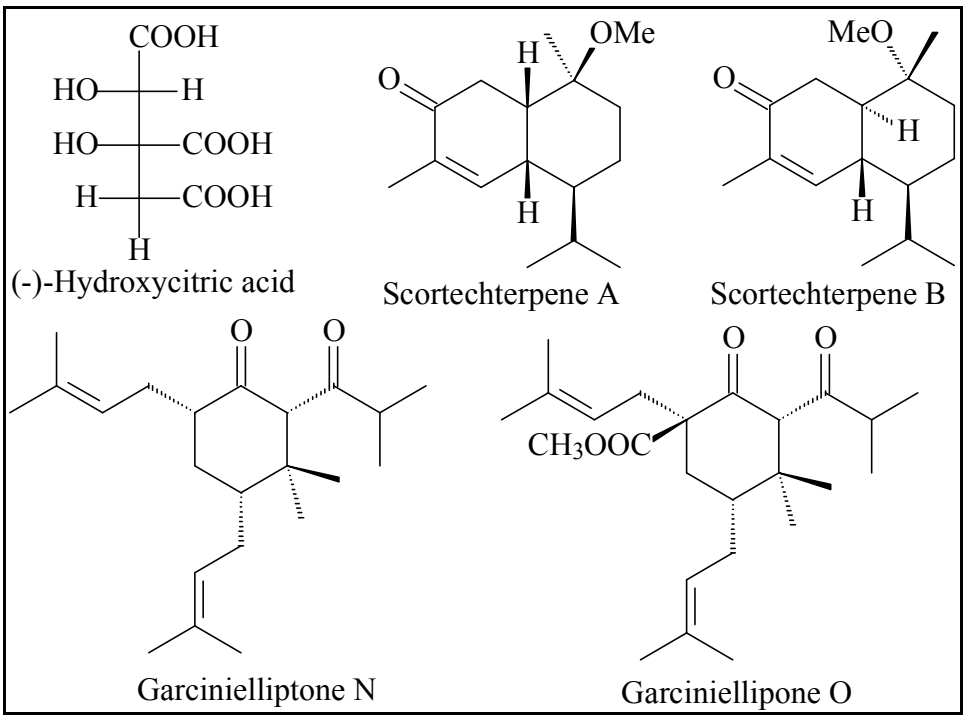


Figure 13. (-)-Hydroxycitric acid and terpenoids from some *Garcinia* species

2.3. Pharmacological and biological properties of *Garcinia*

Many species of the genus are used in traditional medicine around the world, particularly in Asia and Africa. The pericarp of *G. mangostana* is used in Southeast Asia for the treatment of skin infections, wounds, dysentery, diarrhoea, fever, arthritis and inflammation (Pedraza-Chaverri, 2008). The leaves and seeds of *G. dulcis* are used in Indonesian folk medicine to treat lymphatitis, parotitis and struma, whereas its stem bark is used in Thailand as an antiseptic and the fruit juice as an anti-scurvy and expectorant. In addition, its root extract is also used as an antipyretic and antitoxin (Kalahari and Hemi, 1986; Wuttidhammavej, 1997). The bark of *G. cowa* is used in Thai folk medicine as an antipyretic and antimicrobial agent. Its latex is also used as anti-fever agent (Na Pattalung et al., 1994). In India, the fruit of *G. indica* is anthelmintic and useful for piles, dysentery, tumours, pain and heart complaints (Jena et al., 2002). *G. cambogia* extract has been used in Indian traditional medicine to treat tumours, ulcers, haemorrhoids, diarrhoea, dysentery, fever, open sores and parasites (Duke, 2002). The gum of *G. hanburyii* is used internally in Thailand as a purgative, vermifuge and for treatment of infected wounds. It is also applied for treatment of chronic dermatitis, haemorrhoids and bedsore. In China, it was developed as an antitumor medicine (Saralamp et al., 1996; Han et al., 2006). *G. xanthochymus* is widely used in Chinese traditional medicine for dispelling worms and removing food toxin (Lin et al., 2003). *G. hombroniana*, a seashore mangosteen in Malaysia, is used as protective medicine after child birth and to cure skin allergies (Jamila, 2014). In Africa, *G. preussii* is traditionally used to treat stomach aches and its leaves are prepared as a decoction to relieve toothache (Bouquet, 1969; Visser, 1975). Extracts of *G. kola* are used in Nigerian ethnic medicine against laryngitis, cough and liver diseases. Its seeds are used in Africa as an antidote (Iwu et al., 1985 and 1987). The leaves and flowers of *G. afzelii* are used in Cameroon and Ghana for antibacterial properties (Waffo et al., 2006). In Fiji, an extract of the leaves of *G. pseudoguttifera* is mixed with coconut oil and used to relieve pain in the limbs (Cambie and Ash, 1994).

Pharmacological and biological investigations of natural products from the genus showed that some of them possess a wide range of various properties such as anti-

oxidant, antifungal, antimicrobial, anti-inflammatory, anticancer and antiviral activities (Hemshekhar et al, 2011).

2.3.1. Anti-oxidant activity

1,8-Dihydroxy-6-methoxyxanthone, a tri-oxygenated xanthone from the wood *G. subelliptica* exhibited inhibitory activities in three *in vitro* assays viz., anti-lipidperoxidation in rat brain homogenates, DPPH free radical scavenging and superoxide anion scavenging assays at 5 μg/ml (Minami et al., 1994). α-Mangostin from the pericarp of *G. mangostana* inhibited 7,12-dimethylbenz[α]anthracene-induced preneoplastic lesions in a mouse mammary organ culture assay with an IC_{50} of 1.0 μg/ml (Jung et al., 2006). Garcidepsidone B, a depsidone from the twigs of *G. parvifolia*, gave an IC_{50} of 0.13 μM equal to that of BHT in the DPPH free radical scavenging assay (Rukachaisirikul et al., 2006). The antioxidant activity of bigarcinenone A, a bisxanthone from the bark of *G. xanthochymus*, is even stronger than that of BHT in a DPPH radical scavenging test. Bigarcinenone A gave an IC_{50} of 9.2 μM, compared to the positive control, BHT with an IC_{50} of 20 μM, see **Figure 14** (Zhong et al., 2008).

2.3.2. Antifungal activity

Beside the anti-oxidant activity, α-mangostin also exhibited the inhibition towards the fungi *Alternaria solani*, *Cunninghamella echinulata* and *Candida albicans* that causes candidiasis with a MIC of 1 mg/ml. It was shown to be more efficient than the existing antifungal drugs such as clotrimazole and nystatin (Sundaram et al., 1983; Kaomonkolgit et al., 2009). Two isoprenylated tri-oxygenated xanthones, 1,4,5-trihydroxy-3-(3-methylbut-2-enyl)xanthone and 4-(3′,7′-dimethylocta-2′,6′-dienyl)-1,3,5-trihydroxyxanthone of *G. livingstonei* showed an activity against the plant pathogenic fungus *Cladosporium cucumerinum* at 0.5 and 0.2 μg, respectively, see **Figure 14** (Sordat-Diserens et al., 1992).

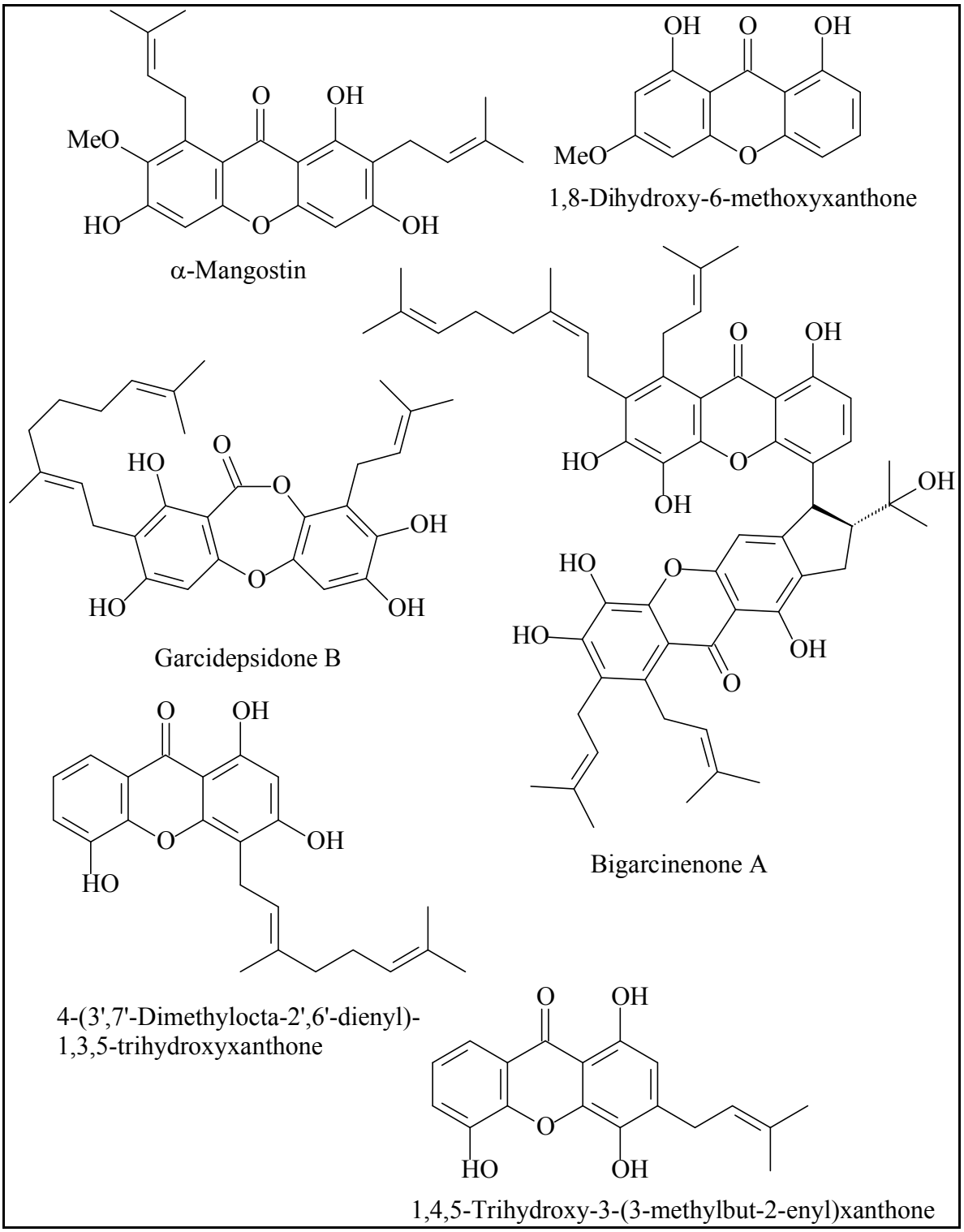


Figure 14. Some anti-oxidant and antifungal compounds from the genus *Garcinia*

2.3.3. Antimicrobial activity

Rubraxanthone isolated from *G. dioica* displayed higher activity against *Staphylococcal* strains (MIC = 0.31-1.25 µg/ml) than that of the antibiotic, vancomycin with MIC values of 3.13-6.25 µg/ml (Iinuma et al., 1996). Garcilivin A, a bisxanthone from *G. livingstonei*, showed a high anti-parasitic activity against two trypanosomes, *T. brucei brucei* and *T. cruzi* that cause the fata human diseases, sleeping sickness and chagas disease with IC_{50} values of 0.4 µM and 4.0 µM, respectively. Moreover, this compound also exhibited antiplasmodial property against *Plasmodium falciparum* with an IC_{50} of 6.7 µM (Mbwambo et al., 2006). Xanthochymol, a polyprenylated benzophenone from *G. xanthochymus* and *G. subelliptica*, was evaluated for the antibacterial property against methicillin-resistant *Staphylococcus aureus* with the lowest minimum inhibitory concentration at 3.1-12.5 µg/ml, nearly equal to that of vancomycin (Iinuma et al., 1995). Guttiferone A, a polyisoprenylated benzophenone from the fresh fruits of *G. aristata*, showed a potent antiplasmodial effect against *Plasmodium falciparum* with an IC_{50} of 0.5 µM, nearly similar to that of chloroquine (IC_{50} = 0.3 µM), a 4-aminoquinoline drug used in the treatment or prevention of malaria (Monzote et al., 2011). Amentoflavone, a biflavone from some *Garcinia* species, was reported to be more active against *Mycobacterium smegmatis* than the drug isoniazid used in the clinical treatment of tuberculosis. This compound gave a MIC of 0.6 µg/ml compared to isoniazid with a MIC of 1.3 µg/ml (Kaikabo and Eloff et al., 2011). Kolaviron, a biflavonoid complex from the seeds of *G. kola* containing GB-1, GB-2 and kolaflavanone, exhibited potent antiplasmodial activity against *Plasmodium berghei* infection in Swiss albino mice, see **Figure 15** (Oluwatosin et al., 2014).

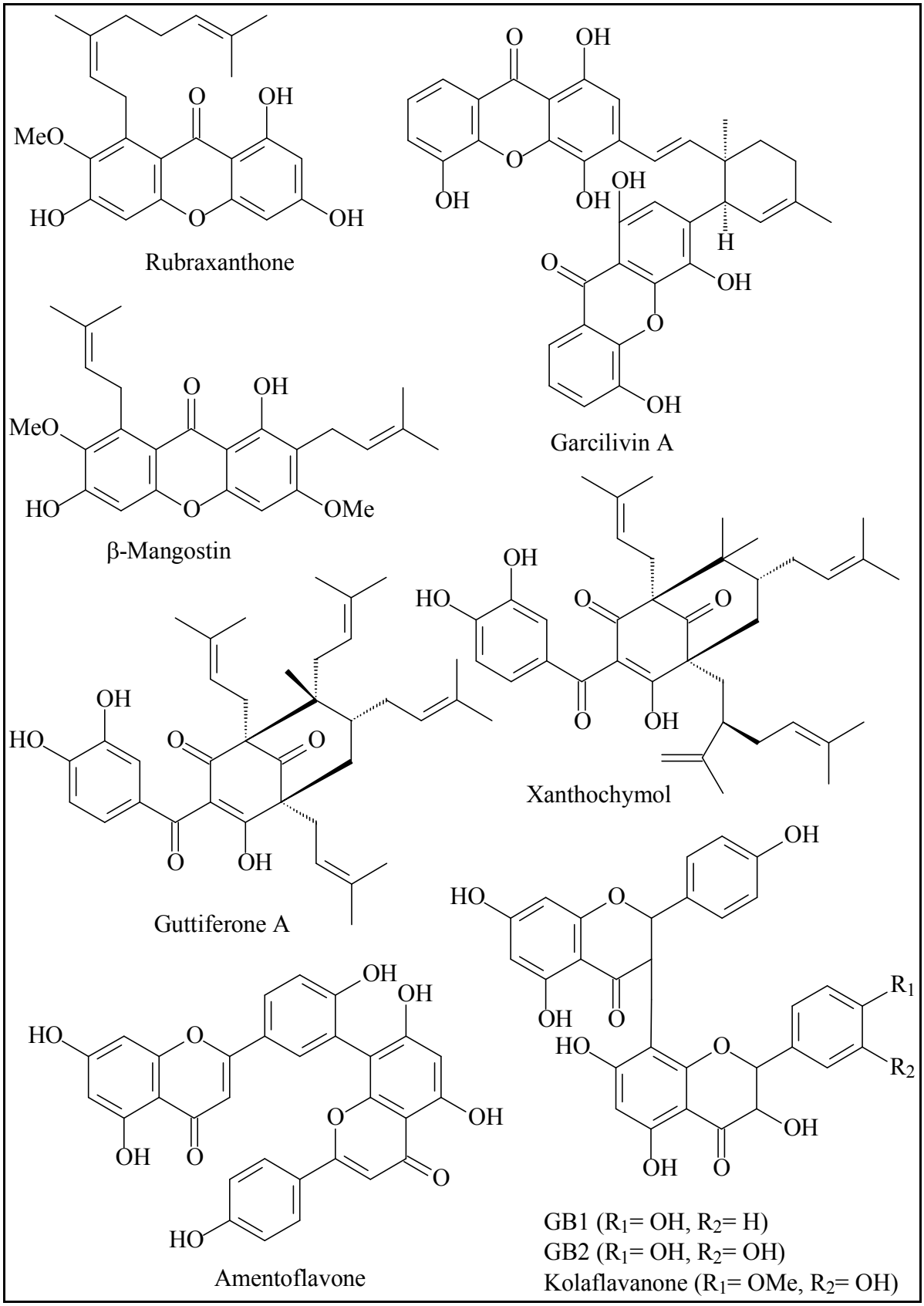


Figure 15. Some anti-microbial compounds from the genus *Garcinia*

2.3.4. Anti-inflammatory activity

Garcinielliptones L and M, two polyisoprenylated phloroglucinols from the seeds of *G. subelliptica*, showed potent inhibitory effects on the release of β-glucuronidase and on histamine from peritoneal mast cell stimulated with *p*-methoxy-*N*-methyphenethylamine in a concentration-dependent manner. They also exhibited potent activities on NO production in culture media of RAW 264.7 cells in response to lipopolysaccharide (LPS) and in culture media of N9 cells in response to LPS/interferon-γ (IFN-γ) (Weng et al., 2004). Two xanthones, α- and γ-mangostin from the pericarps of *G. mangostana*, showed significant properties in the expression decrease of TNF-α, IL-1β, IL-6, IL-8, MCP-1 (monocyte chemoattractant protein) and TLR-2 (toll-like receptor). They also potently inhibited the LPS induced NO and PEG_2 activity in RAW264.7 macrophages with IC_{50} concentration of 3.1 and 6.0 μM (Bumrungpert et al., 2009; Chen et al., 2008). In the study of the effects on neutrophil pro-inflammatory responses of benzophenones from *G. multiflora,* garcimultiflorone D potently inhibited fMLP/CB-induced superoxide anion generation and elastase release with IC_{50} values of 7.21 and 6.0 μg/ml, respectively, see **Figure 16** (Ting et al., 2012).

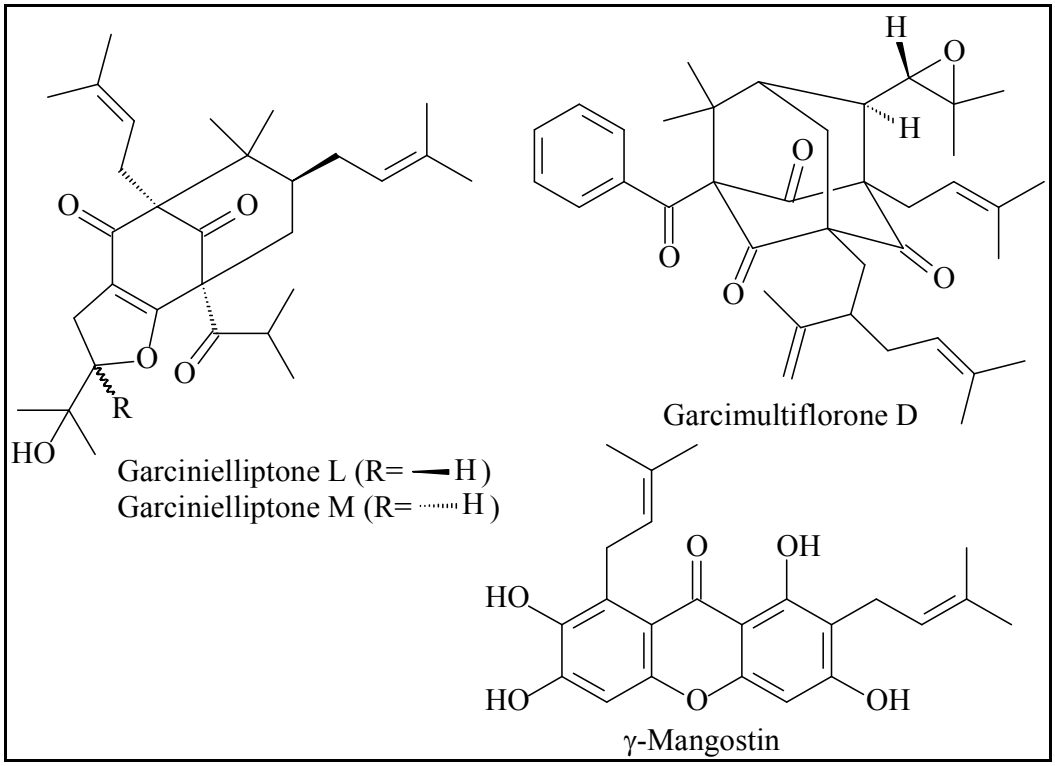


Figure 16. Some anti-inflammatory compounds from the genus *Garcinia*

2.3.5. Anticancer activity

Gambogic acid and epigambogic acid, two caged xanthones from the gamboges of *G. hanburyi*, were examined for their cytotoxicity against human leukaemia K562/S and doxorubicin-resistant K562/R cell lines. They were shown to be potent agents against both cell lines with IC_{50} values of 1.32 and 0.89 μM for gambogic acid, 1.11 and 0.86 μM for epigambogic acid, respectively (Han et al., 2005). 7-Hydroxyforbesione, a caged xanthone from the leaves of *G. cantleyana*, exhibited significant cytotoxicity against MDA-MB-231, CaOV-3, MCF-7 and HeLa cancer cell lines with IC_{50} values ranging from 0.22 - 2.17 μg/ml (Shadid et al., 2007). For 3-*O*-(4′-*O*-acetyl)-α-L-arabinopyranosyloleanolic acid, a triterpene from the resin of *G. hanburyi*, the anti-proliferative effects and the apoptosis induction abilities in four human leukaemia cell lines consisting of HL-60, NB4, U937 and K562 were determined with IC_{50} values of 2.45, 2.69, 2.42 and 4.15 μM, respectively (Wang et al., 2008). Guttiferone A, an anti-oxidant benzophenone from some *Garcinia* species, displayed strong activity against HTC-116 and HT29 cell lines with the same IC_{50} values of 5.0 μM (Yang et al. 2010). GB1, a biflavone reported from some *Garcinia* species, inhibited α-glucosidase and aromatase with IC_{50} values of 0.9 and 11.3 μM, respectively. It was discussed to be a potential dietary supplement or phytomedicine for the prevention of breast cancer and type II diabetes mellitus. Morelloflavone, another *Garcinia* biflavone, was found to inhibit proteasome at an IC_{50} concentration of 1.3 μM, see **Figure 17** (Antia et al., 2010; Ren et al., 2010).

2.3.6. Antiviral activity

Morelloflavone demonstrated potent activity against HIV-1 (strain LAV-1) in phytohemagglutinin-stimulated primary human peripheral blood mononuclear cells at an EC_{50} value of 6.9 μM and a selectivity index value of approximately 10, whilst amentoflavone exhibited significant antiviral activity against two strains of influenza A, H1N1 and H3N2 with EC_{50} values of 3.1 and 4.3 μg/ml, respectively (Lin et al., 1999). Morellic acid, gambogic acid and dihydroisomorellin, three caged xanthones from *G. hanburyi*, showed potent HIV-1 RT inhibitory property with IC_{50} values < 50 μg/ml (Reutrakul et al., 2007). Garciosaterpenes A and C, two pronostanes from the

bark of *G. speciosa*, were determined to have strong inhibitory activities against HIV-1 RT with IC_{50} values of 15.5 and 12.2 μg/ml, respectively, see **Figure 17** and **18** (Rukachaisirikul et al., 2003).

2.3.7. Other properties

(-)-Hydroxycitric acid, which was found in the fruits of three species *G. cambogia*, *G. indica* and *G. atroviridis*, exhibited the *in vitro* inhibitory effect on the conversion of lactate, acetate and glucose to fatty acids in bovine and rat adipose tissues (Hood et al., 1985). Furthermore, anti-inflammatory, anti-oxidative stress and insulin resistance properties of this acid were evaluated using obese male Zucker rats with type II diabetes associated with inflammation of the IL-6 and plasma C-reactive protein and oxidative stress makers of malondialdehyde, protein carbonyl and protein tyrosine nitration. The results showed that (-)-hydroxycitric acid reduced food-intake, body weight gain as well as decreased the inflammation, oxidative stress and insulin resistance (Asgar et al., 2007). Two biflavonoids of *G. kola* seeds (GB1 and GB2) were tested for anti-hepatotoxic activities using four experimental toxins, namely carbon tetrachloride, galactosamine, α-amanitin and phalloidin. These compounds significantly modified the action of all these hepatoxins. At 100 mg/kg orally, they reduced thiopental-induced sleep in CCl_4-poisoned rats, see **Figure 19** (Iwu et al., 1987).

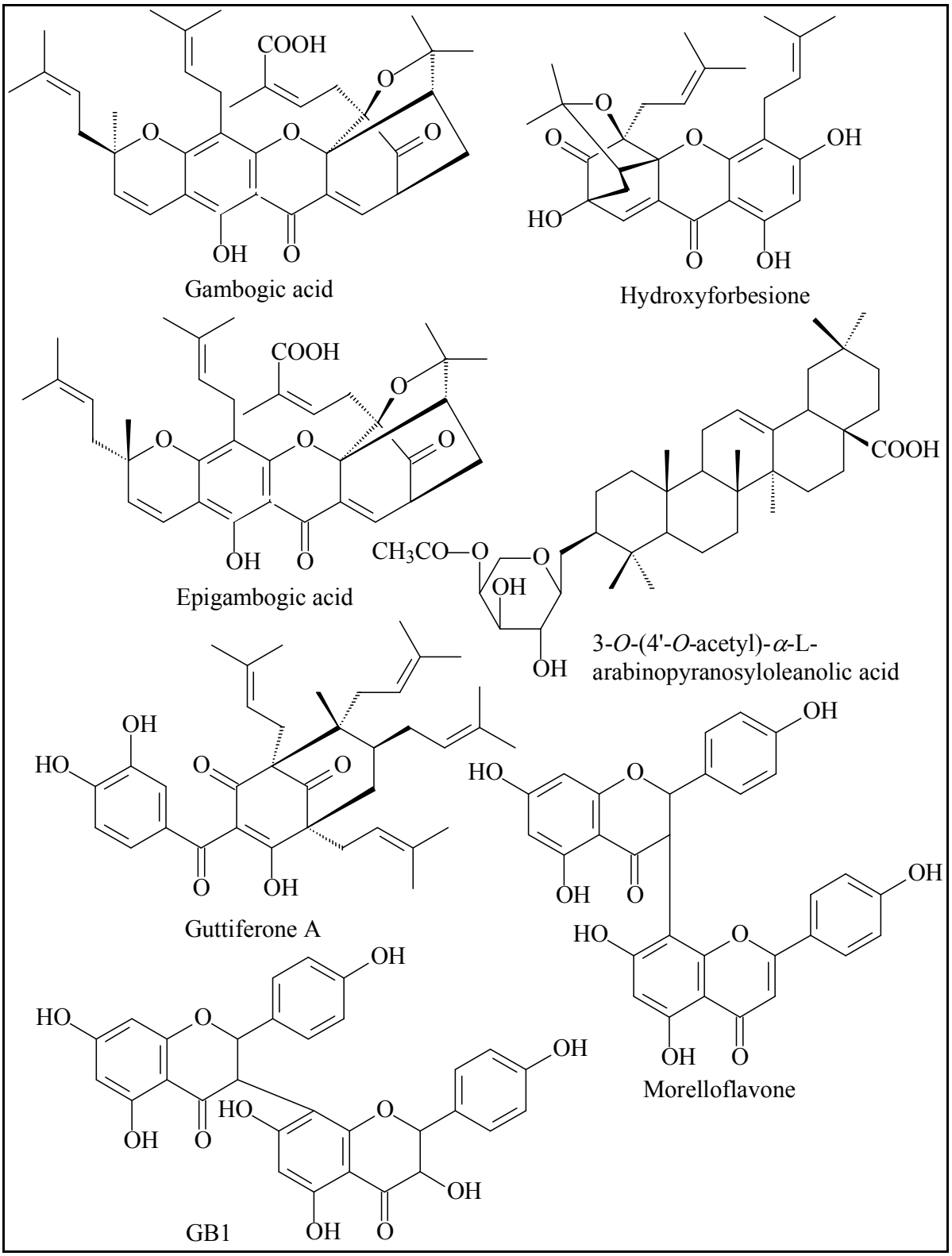


Figure 17. Some compounds from the genus *Garcinia* with cytotoxic activity

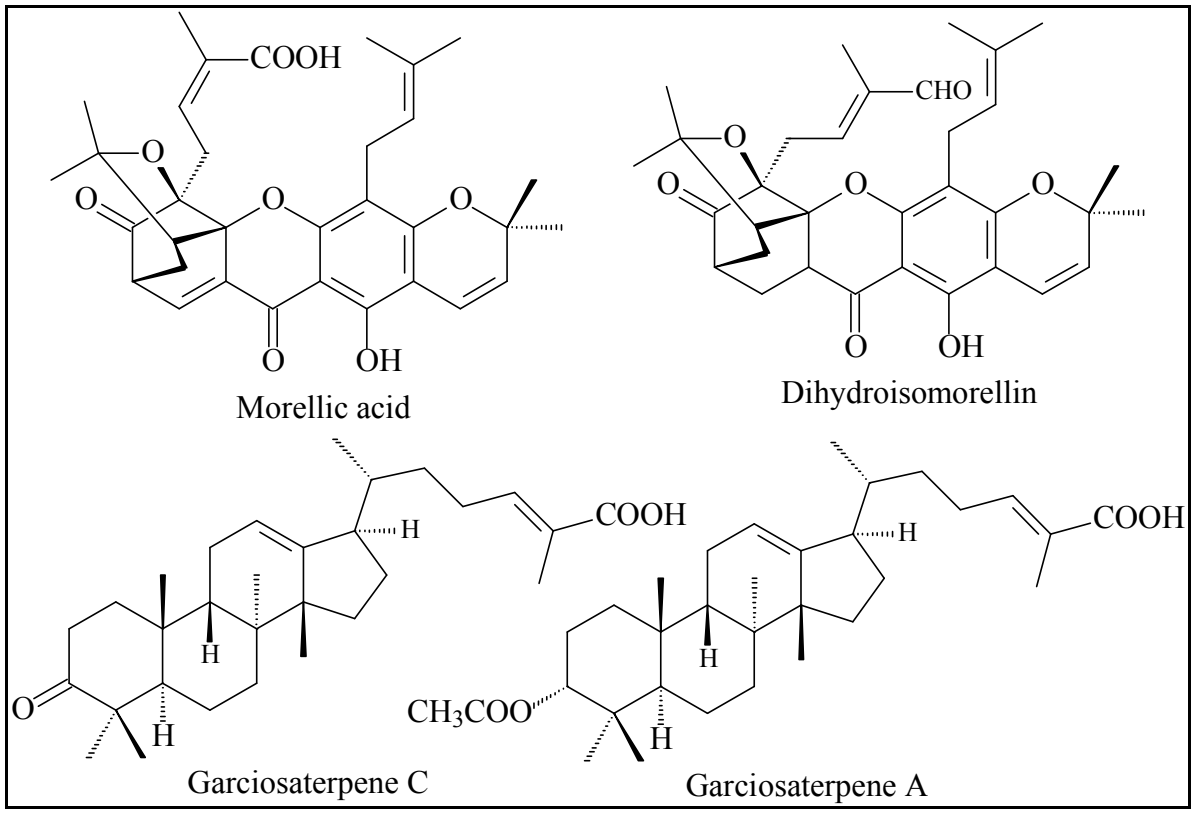


Figure 18. Some antiviral compounds from the genus *Garcinia*

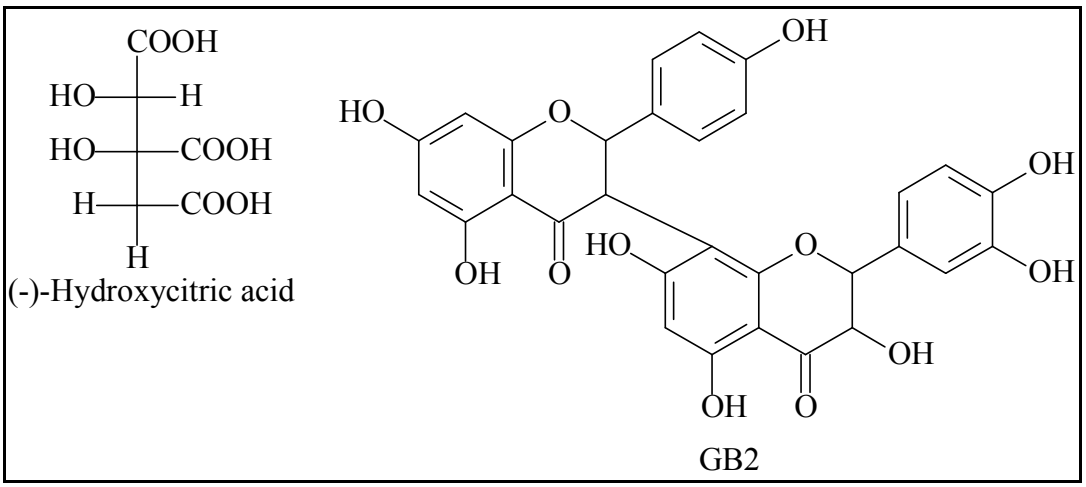


Figure 19. Compounds with other biological activities from the genus *Garcinia*

2.4. Study of the genus *Garcinia* in Vietnam

There are around thirty one species of the genus in Vietnam, see **Table 2**. They are distributed in different areas of the country, particularly in the forests. Fruits of some species are edible such as *G. harmandii*, *G. merguensis*, *G. multiflora*, *G. fusca* and especially *G. mangostana*, which is cultivated as a fruit-tree. Young leaves of some species are used as vegetables, for example, *G. cowa* and *G. oliveri*. Some species are used in traditional medicine for the treatment of various diseases. The bark of *G. cochincinensis* is used to cure allergy, itches and skin diseases, whereas the buds are useful for threatened abortion. The ground bark of *G. oliveri* is mixed with that of *G. vilersiana* to make a powder used as medicinal agent against sprains. *G. pendunculata* is used to treat constipation and digestive problems. *G. schomburgkiana* is used for the treatment of coughs and menstrual disturbances. The pericarp of *G. mangostana* is used as antibacterial and antibiotic agent as well as for the treatment of dysentery, fever and inflammation (Vo, 1997; Pham, 1999).

Twelve species, which were collected in Vietnam, have phytochemically been investigated so far, as shown in **Table 2** with bold types. New compounds including prenylated xanthones, caged xanthones, prenylated benzophenones, phloroglucinol derivatives, triterpenoids and depsidones, were reported from these species. Some of them possess biological properties such as cytotoxic, antitumor and anti-oxidant activities. Neoisobractatins A and B, two caged xanthones from the leaves of *G. bracteata*, exhibited a significant cytotoxic activity on KB cells with IC_{50} values of 0.14 and 0.16 μg/ml, respectively (Thoison, 2005). (+)-Guttiferone G, which was identified from the bark of *G. cochinchinensis*, was tested as an inhibitor of human sirtuins SIRT1 and SIRT2 (Gey et al., 2007). Guttiferone Q, a polyisoprenylated benzophenone from the pericarp of *G. cochinchinensis*, showed potent cytotoxicity against cell lines, MCF-7, HeLa and NCI-H460 with IC_{50} values in the range of 2.74-4.04 μg/ml (Nguyen et al., 2011). Oliveridepsidones A-D, four depsidones from the bark of *G. oliveri*, exhibited DPPH radical scavenging activity, see **Figure 20** (Ha et al., 2011).

Some of the remaning species collected in other countries have been phytochemically and biologically studied such as *G. cowa* (Na Pattalung et al., 1994;

Panthong et al., 2006; Mahabusarakam et al., 2005), *G. gaudichaudii* (Xu et al., 2000; Cao et al., 1998a and 1998b), *G. multiflora* (Chen et al., 1975; Chiang et al., 2003; Chen et al., 2009, Liu et al., 2010), *G. nigrolineata* (Rukachaisirikul et al., 2003a, 2003b and 2005), *G. oligantha* (Gao et al., 2013; Wu et al., 2013; Yang et al., 2014) and *G. xanthochymus* (Karanjoakar et al, 1973; Baggett et al., 2005; Chen et al., 2010; Trisuwan et al., 2014). However these species have not yet been collected in Vietnam to date. Nowadays collection and identification of *Garcinia* species in Vietnam are indeed very difficult because the majority of species was not easily found in nature any more. They are merely distributed in the thick forests throughout the country. Some species are seemingly extinct such as *G. fagraeoides* (Vietnam's Red Data Book).

Table 2. *Garcinia* species distributed in Vietnam

Species name	Plant part	New compounds
G. bassacensis Pierre		
***G. benthami* Pierre**	Bark	Two triterpenoids, a friedolanostane and a friedocycloartane, along with a benzophenone, benthaphenone
	Leaves	Two friedolanostanes (Nguyen et al., 2011)
***G. bracteata* Wu ex Li**	Bark	Two prenylated xanthones, garcibracteatone and 5-*O*-methylxanthone V_1, together with a benzophenone, xerophenone C
	Leaves	Ten prenylated xanthones including eight caged xanthones, bractatin, isobractatin, 1-*O*-methylbractatin, 1-*O*-methylisobractatin, 1-*O*-methyl-8-methoxy-8,8a-dihydrobractatin, 1-*O*-methylneobractatin, neoisobractatins A and B, bracteaxanthone I and II. (Thoison et al., 2000 and 2005)
***G. cochinchinensis* Lour**	Pericarp	Three polyisoprenylated benzophenones, guttiferones Q-S (Nguyen et al., 2011)
	Bark	Two prenylated benzophenones, guttiferone T and (+)-guttiferone G (Trinh et al., 2013; Gey et al., 2007)
G. cowa Roxb		
***G. delpyana* Pierre**	Bark	In study
***G. ferrea* Pierre**	Bark	Three triterpenoids, a protostane and two lanostanes (Bui et al., 2014)
G. fagraeoides		
G. fusca Pierre		
G. gaudichaudii Planch		
G. gracilis Pierre		
G. hainanensis Merrill		

Table 2. (continued)

G. hanburyii Hook. f.		
G. harmandii Pierre		
G. lanessanii Pierre		
G. mackeaniana Craib		
***G. mangostana* L.**	Pericarp	(Nguyen et al., 2009; Ha et al., 2009)
G. multiflora Champ. Ex		
***G. merguensis* Wight**	Bark	A xanthone, merguenone (Nguyen et al., 2003; Vo et al., 2008)
G. nigrolineata Pl. ex T.		
***G. oblongifolia* Champ. ex**	Bark	Four polyprenylated benzophenones, oblongifolins A-D (Hamed et al., 2006)
G. oligantha Merr		
***G. oliveri* Pierre**	Bark	Four depsidones, oliverixanthones A-D, together with two prenylated xanthones 6-*O*-methylcowanin and oliverixanthone (Ha, 2009 and 2012)
***G. pendunculata* Roxb**	Bark	Three isoprenylated xanthones, pendunxanthones A-C (Vo et al., 2012)
G. planchonii Pierre		
G. poilanei Gagn		
G. schefferi Pierre		
***G. schomburgkiana* Pierre**	Bark	Two tetraoxygenated xanthones, 6-*O*-demethyloliverixanthone and schomburgxanthone (Vo et al., 2012)
G. tinctoria (DC.)		
***G. vilersiana* Pierre**	Bark	A xanthone, 1-*O*-methylglobuxanthone (Nguyen and Harrison, 2000; Nguyen et al., 2009; Bui et al., 2012)
G. xanthochymus Hook. f.		

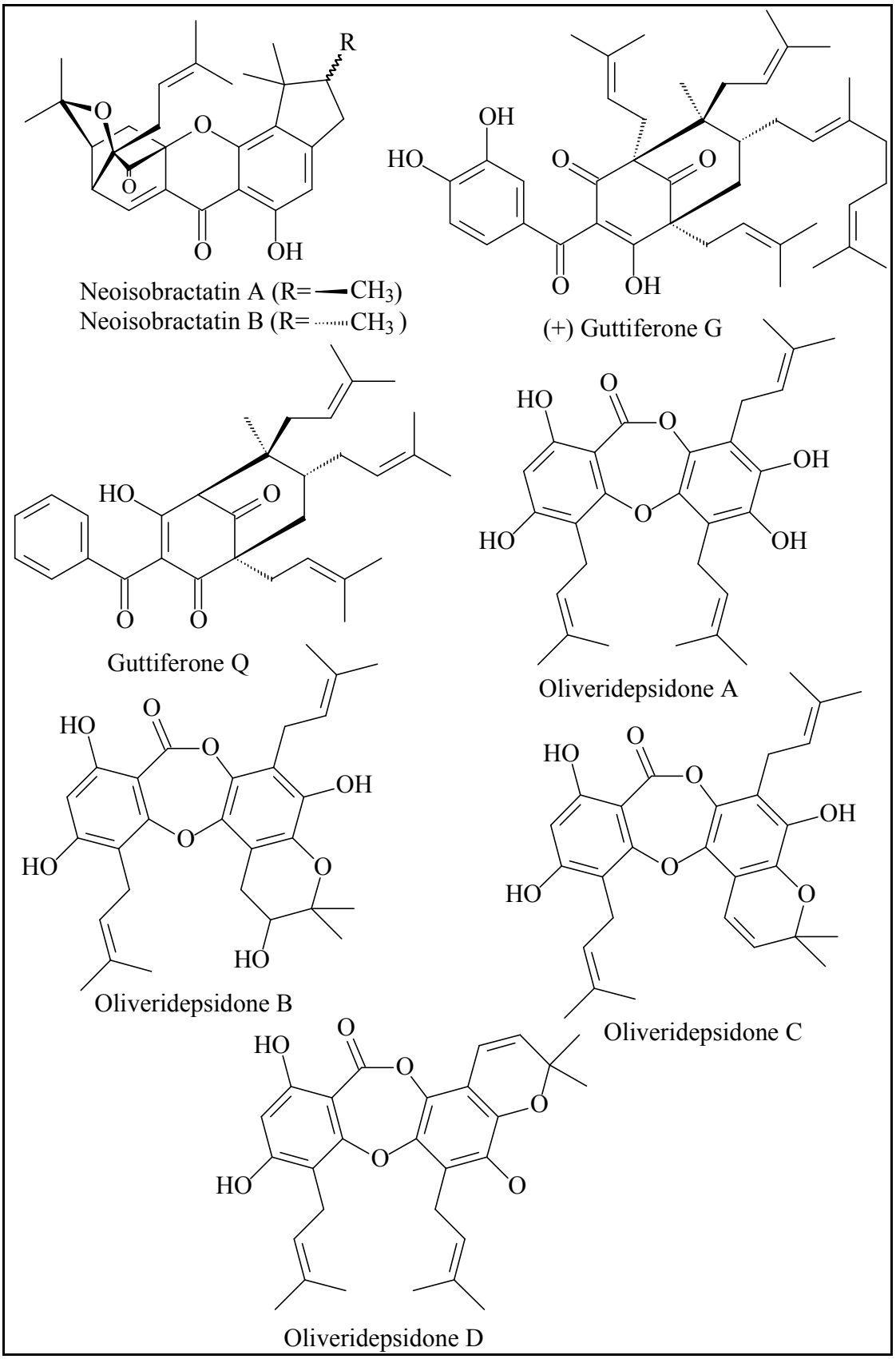


Figure 20. Compounds with different bioactivities from some *Garcinia* species collected in Vietnam

2.5. *Garcinia fusca* Pierre

2.5.1. Botanical features and uses in traditional medicine

Garcinia fusca Pierre is belonged to the genus *Garcinia* of the family Guttiferae (Clusiaceae). The trivial name of this plant in Vietnamese is Bứa lửa. It is a ca. 8 m high tree with reddish wood and dark brown bark. The leaf blade is relatively narrow and elliptic-lanceolate. Leaves are blackish on dry. Male flowers are three in cymules with four petals. Fruits are pointed and edible with segments. Seeds are 12-15 cm long. This species is distributed in table forests from the middle to the South East of Vietnam (Pham, 1999). It can be also found in some Asian countries with the tropical climate such as Thailand, Malaysia, Indonesia and India. Green fruits and leaves are commonly used in food preparation as a spice or favouring. The root, stem, leaf and fruit of this plant are ethno-medically used for improvement of blood circulation, expectorant, treatment of coughs and indigestion, laxative, and the relief of fever (Poomipamorn and Kumkong, 1997).

2.5.2. Previous chemical and biological investigations

In 2003, Ito et al. investigated the stem bark of this species collected in Thailand. The investigation resulted in the identification of eight new prenylated xanthones, fuscaxanthones A-H, along with eight known xanthones, which exhibited the inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate induced Espstein-Barr virus early antigen activation in Raji cells, see **Figure 21** (Ito et al., 2003). In 2014, Nontakham et al. carried out a chemical investigation on the roots of this genus as part of the on-going project to search for new bioactive compounds from *Garcinia* species in Thailand. A new oxygenated xanthone, fuscaxanthone I, along with thirteen known compounds consisting of nine xanthone, a biphenyl and three biflavonoids were isolated. Anti-*Helicobacter pylori* activity of these compounds was evaluated (Nontakham et al., 2014).

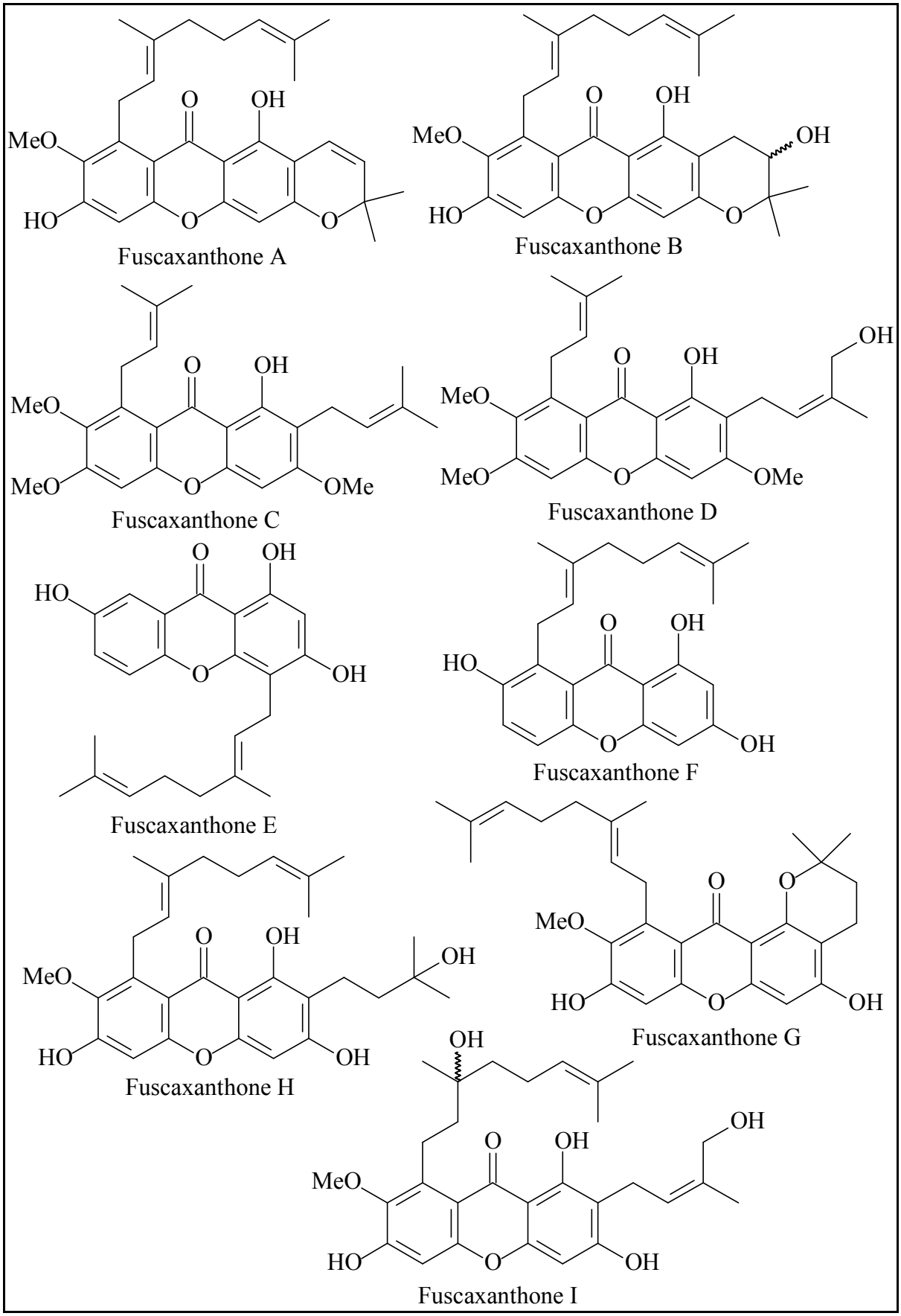


Figure 21. New xanthones, fuscaxanthones A-I, from *G. fusca* Pierre

3. BIOSYNTHESIS OF XANTHONES AND BIFLAVONOIDS

3.1. Biosynthetic pathway of xanthones

Xanthones are natural polyphenolic compounds commonly occurring in higher plants, fungi and lichens. These compounds have the symmetrical basic skeleton, 9H-xanthen-9-one as shown in **Figure 22** (Cardona et al., 1990; Peres and Nagem, 1996). The families Gentianaceae, Guttiferae and Polygalaceae are rich sources of xanthones (Negi et al., 2013). Natural xanthones can be subdivided based on the nature of substituents into six main groups: simple oxygenated xanthones, prenylated xanthones, xanthone glycosides, bisxanthone, xanthonolignoids and miscellaneous xanthones (Mandal et al., 1992).

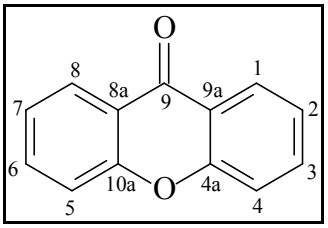


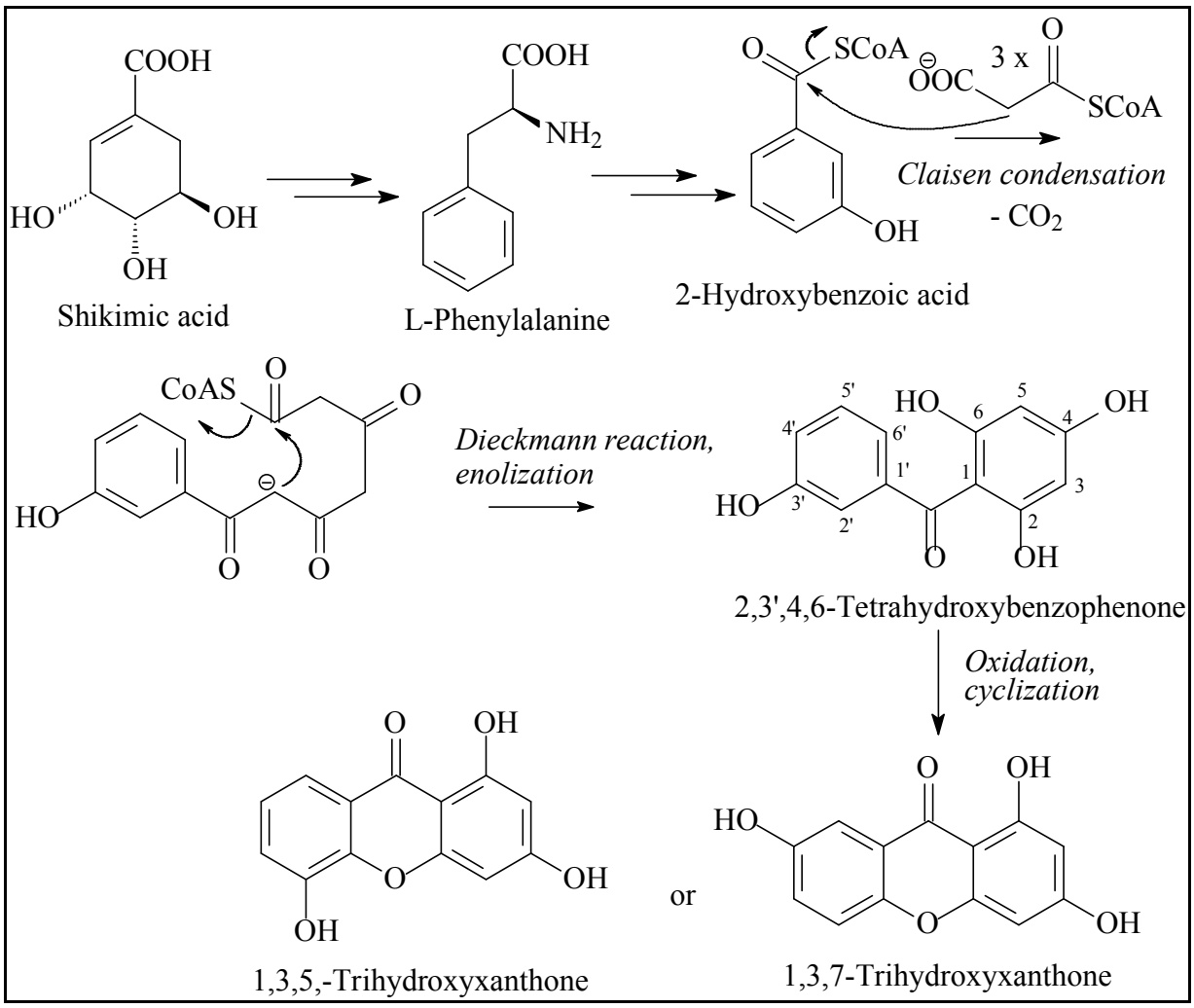
Figure 22. Basic skeleton of xanthones.

The biosynthesis of xanthones has been thoroughly investigated *in vivo* and *in vitro* for fourty years *via* radiolabeled experiments (El-Seedi et al., 2010). It has been known that compounds may be formed from polyhydroxybenzophenone intermediates through two different processes involved:

- The acetate polymalonic pathway in lower plants and fungi.
- The mixed shikimate – acetate pathway in higher plants.

In the latter one, L-phenylalanine, which is formed from shikimate, is oxidized to form *m*-hydroxybenzoic acid by losing two carbon atoms from the side chain. This acid combines with three units of malonyl coenzyme A *via* a series of Claisen condensations to yield the intermediate as shown in **Scheme 1**. The shikimate-acetate intermediate undergoes subsequent folding and ring-closure of a Dieckmann condensation and enolization to give a substituted benzophenone, which generates the central ring of the xanthone moiety *via* an oxidative phenol coupling catalysed by

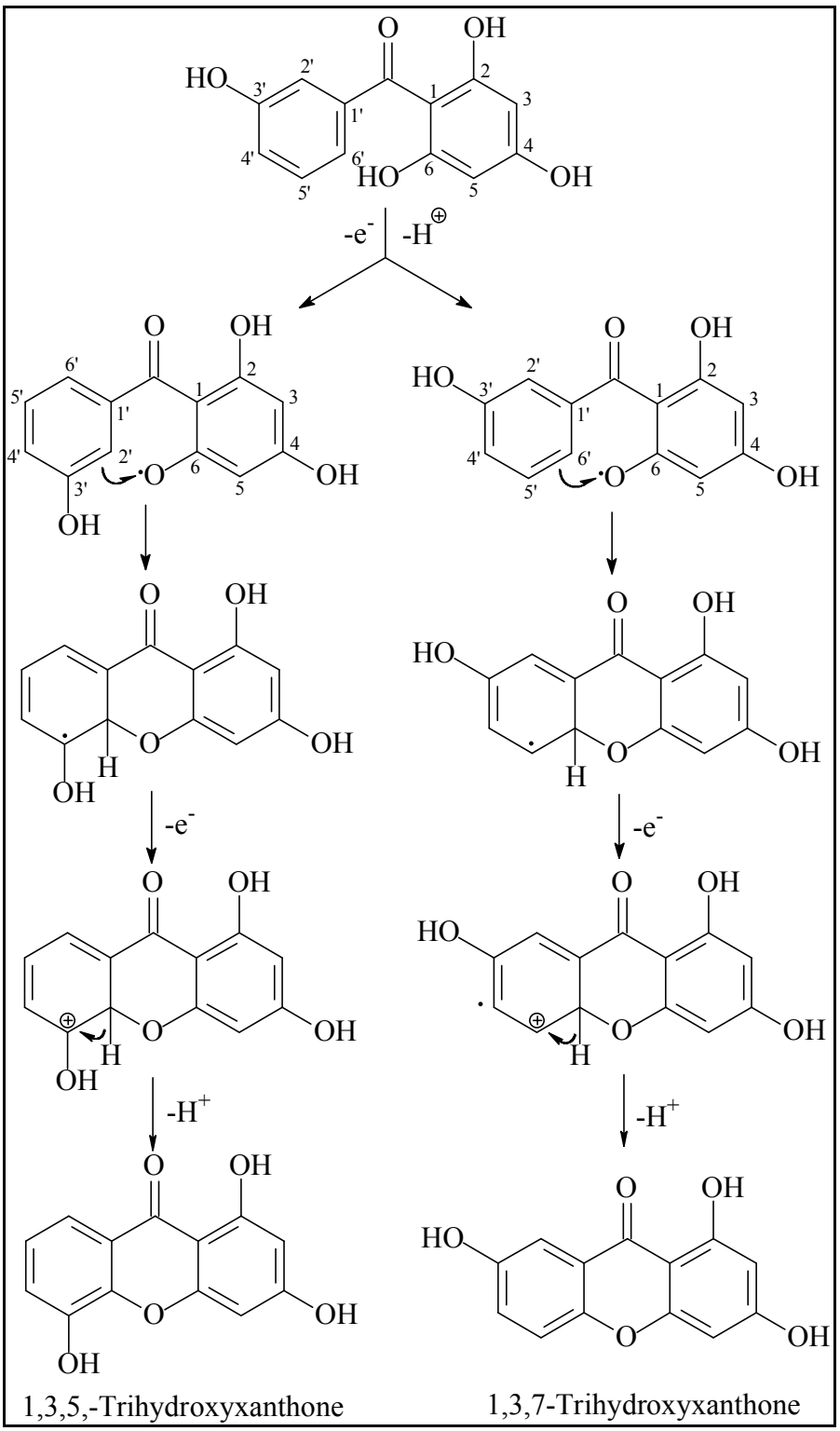
xanthone synthase. This reaction can take place in two ways depending on the folding of the bezophenone either in the *ortho* or the *para* position to the 3′-hydroxyl group to give 1,3,5- or 1,3,7-trihydroxyxanthone. These two xanthones are precursors for most xanthones in higher plants. This pathway has been proved by experiments, in which *Gentiana lutea* plant was supplied with ^{14}C-labeled phenylalanine or ^{14}C-labeled acetate (Gupta and Lewis, 1971; Fujita and Inoue, 1980; Beerhues et al., 1999).



Scheme 1. Formation of trihydroxyxanthone from shikimic acid.

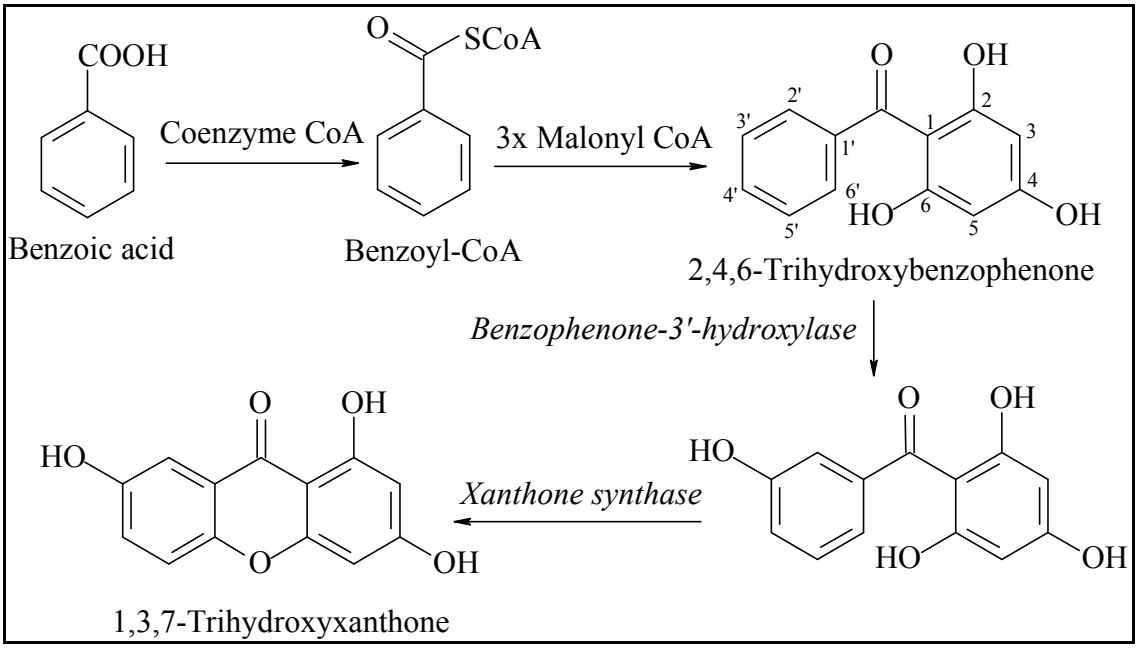
The reaction mechanism of the regioselective intermolecular cyclization of the 2,3′,4,6-tetrahydroxybenzophenone is likely to involve two one-electron oxidation steps. The first one-electron transfer and a deprotonation yield a phenoxy radical in the B-ring, which cyclizes the benzophenone by an electrophilic attack to give a hydroxyl-

cyclohexadienyl radical. This radical loses an electron and a proton to form a xanthone, as shown in **Scheme 2** (Peters et al., 1998).



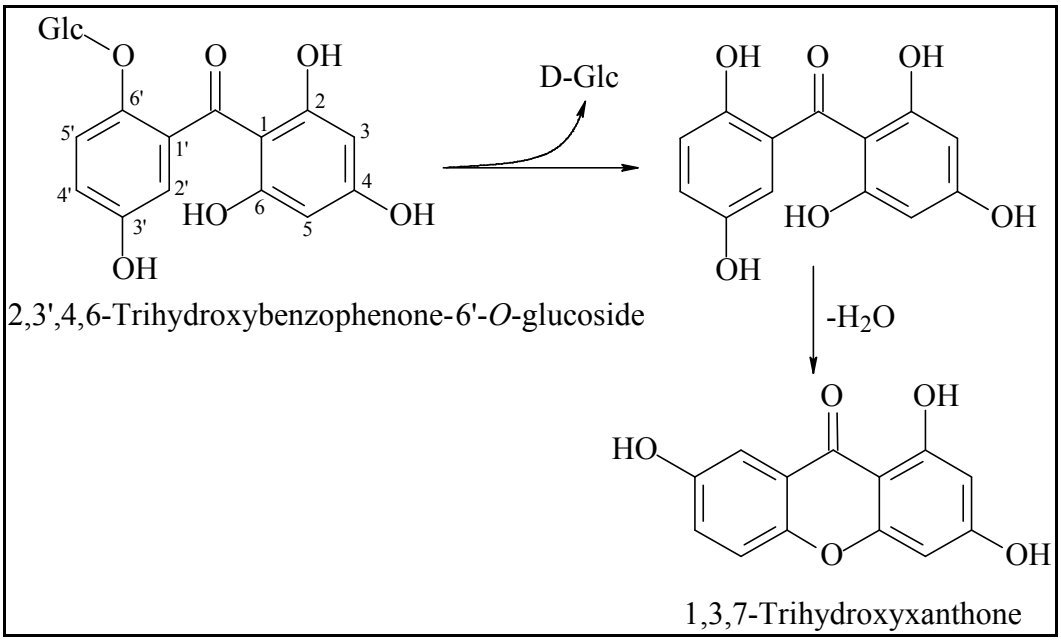
Scheme 2. The reaction mechanism of the regioselective intermolecular cyclization of the 2,3′,4,6-tetrahydroxybenzophenone.

An alternative biosynthesis of xanthone in cell cultures of *Hypericum androsaemum* has been determined. The condensation of benzoyl-CoA with three molecules of malonyl-CoA gives 2,4,6-trihydroxybenzophenone, which is hydroxylated at C-3′ by the presence of benzophenone-3′-hydroxylase to yield 2,3′,4,6-tetrahydroxybenzophenone. The 3′-hydroxy group is essential for the oxidative phenolic coupling reaction catalysed by xanthone synthase, as shown in **Scheme 3** (Schmidt and Beerhues, 1997).



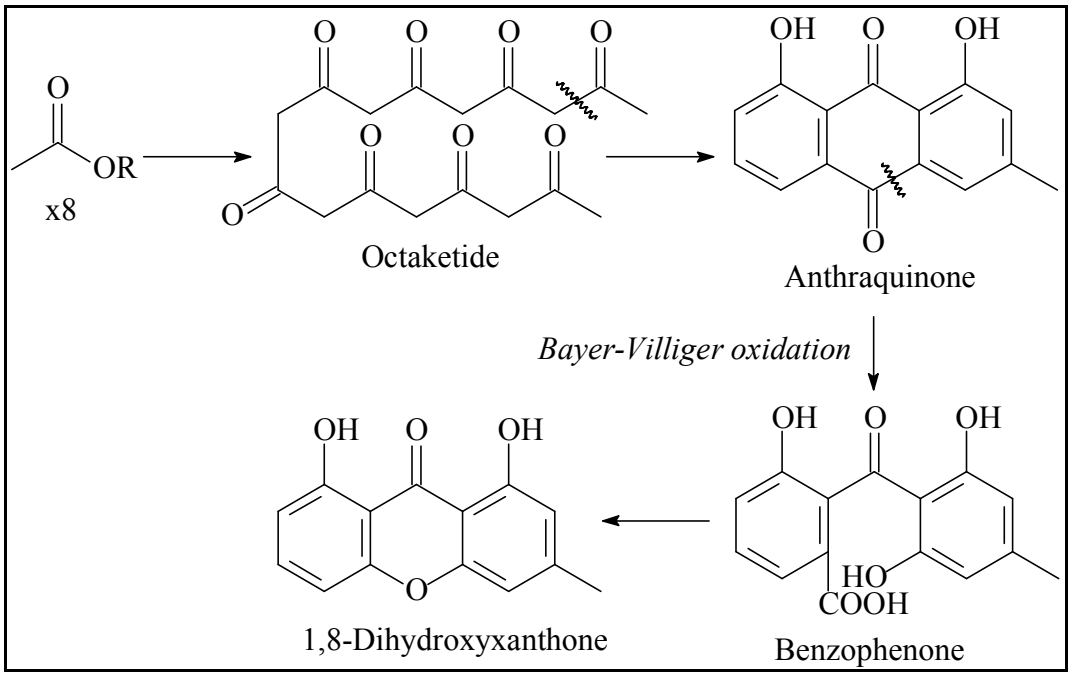
Scheme 3. Biosynthesis of 1,3,7-trihydroxyxanthone in cell cultures of *H. androsaemum*.

Another demonstrated biosynthetic pathway in the formation of plant xanthones involves intermediate glucosidation at an early stage of benzophenone biosynthesis before cyclization of the two rings. This pathway has been found in the herb *Hypericum annulatum*, in which 2,3′,4,6-tetrahydroxybenzophenone-2′-*O*-glucoside has been confirmed to be a precursor of 1,3,7-trihydroxyxanthone. The glucoside undergoes acidic or enzymatic hydrolysis and subsequent dehydration of the hydroxyl groups from phloroglucinol rings A and B in the *ortho* position to the carbonyl group to give the xanthone. This would confirm the hypothesis that some xanthones are formed in plants by dehydration of 2,2′-dihydroxybenzophenone, see **Scheme 4** (Kitanov and Nedialkov, 2001).



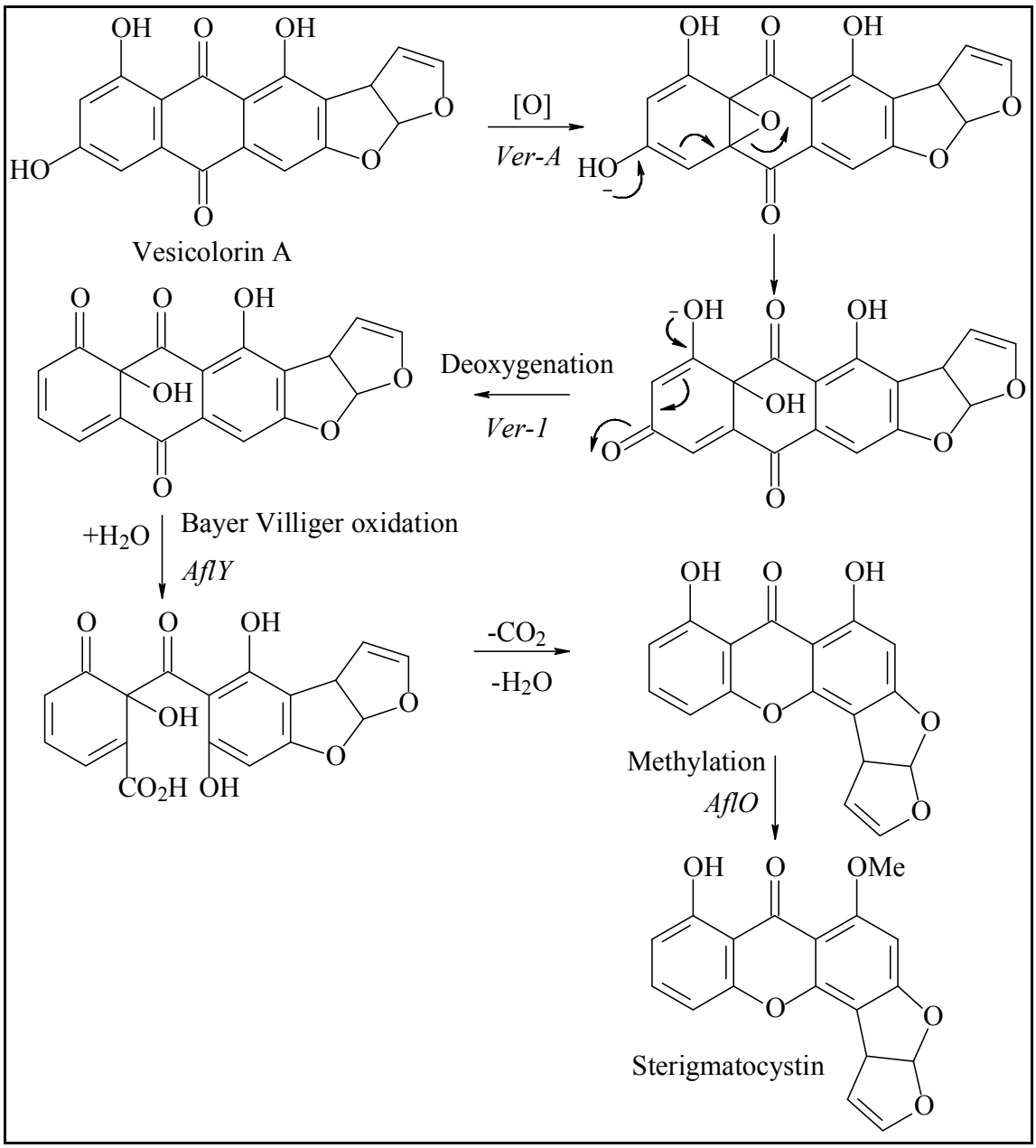
Scheme 4. Formation of plant xanthones involves intermediate glucosidation.

Xanthones occurring in fungi, such as *Aspergillus* strains, have a complex biosynthesis. They are proposed to be formed *via* an anthrone or anthraquinone intermediate derived from eight acetate units as shown in **Scheme 5** (Holker et al., 1974). In 1975, Birch et al. has proved that polyketides are the biosynthetic precursors of xanthones for fungi by feeding experiments with radiolabeled acetate.

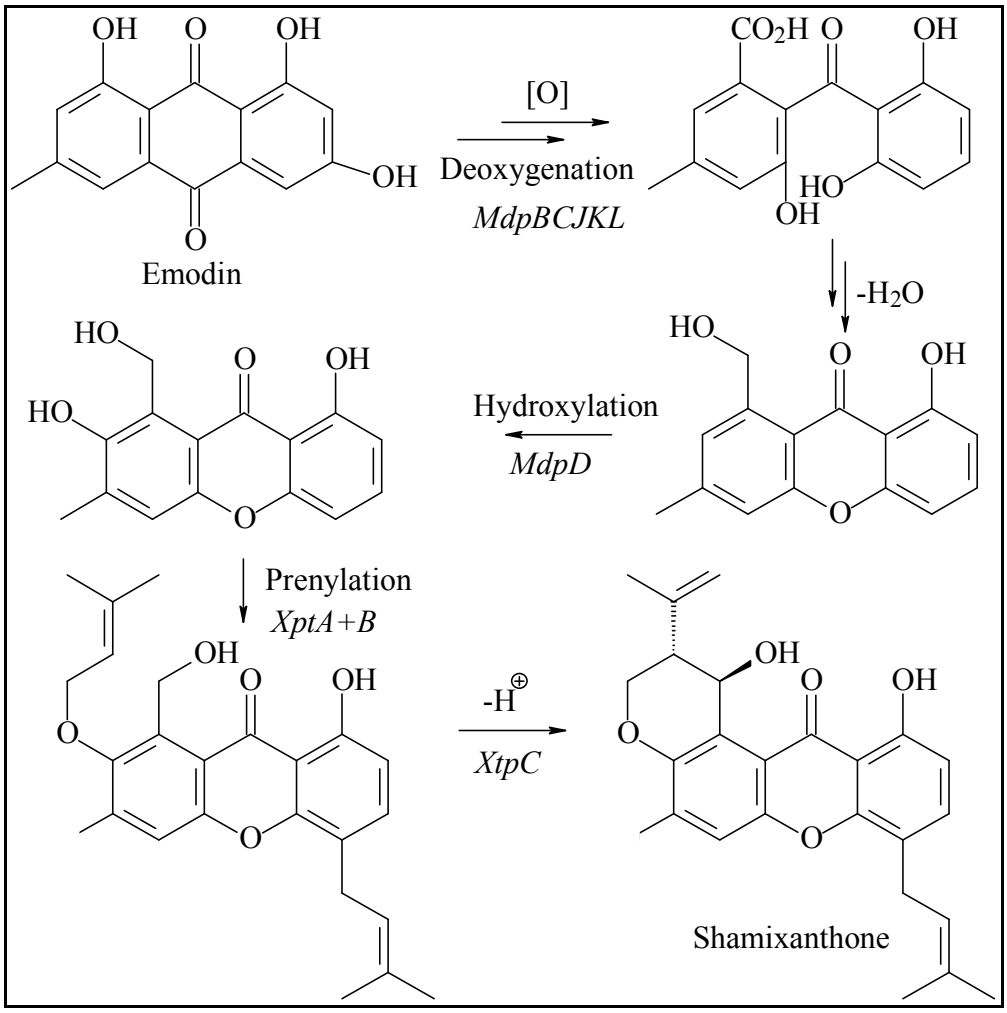


Scheme 5. Formation of xanthones from fungi.

In 2005, Henry and Townsend reported the generation of sterigmatocystin through an enzyme-catalysed sequence of epoxidation, rearrangement, deoxygenation, Bayer-Villiger oxidation and further decarboxylation of versicolorin A, an anthraquinone derivative from *Aspergillus* strains, see **Scheme 6** (Henry and Townsend, 2005). In 2012, Simpson has described the biosynthetic pathway of shamixanthone *via* key steps of oxidative ring-scission, ring-closure and reduction of emodin, which are catalysed by synthase enzymes in *Aspergillus nidulans*, see **Scheme 7** (Simpson, 2012).

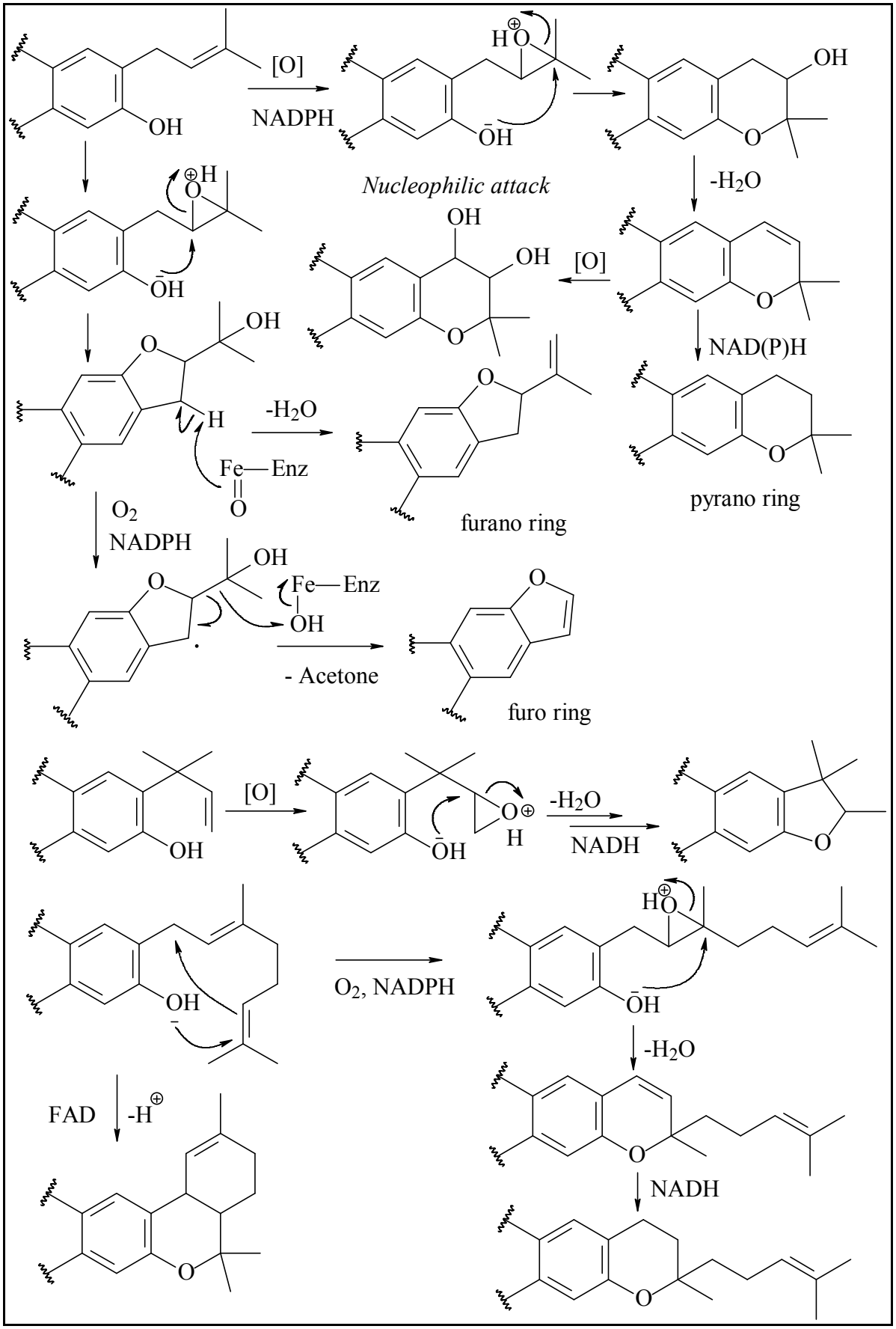


Scheme 6. Biosynthetic pathway of sterigmatocystin in *Aspergillus* strains.



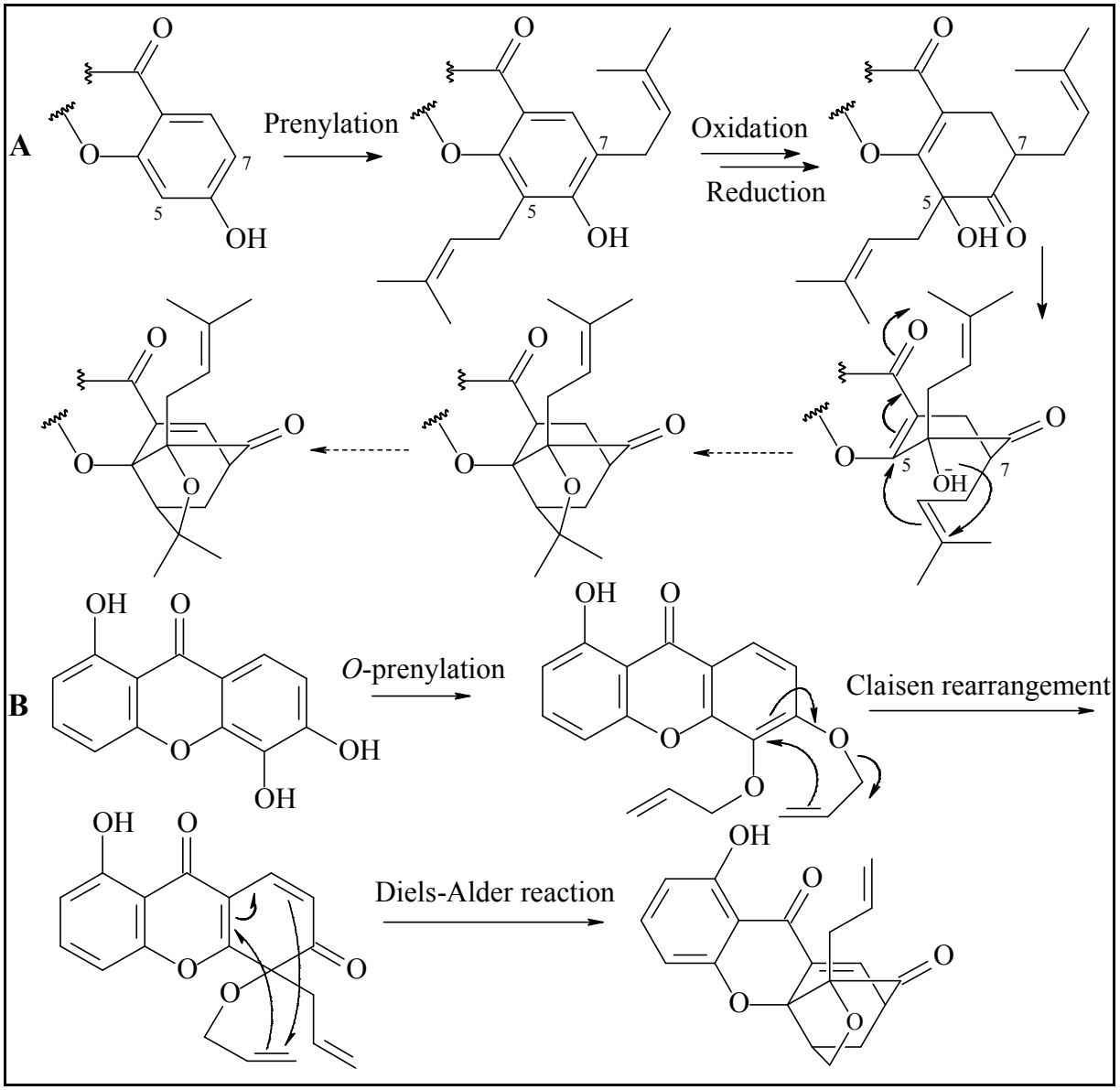
Scheme 7. Biosynthetic pathway of shamixanthone in *Aspergillus nidulans*.

As displayed above, xanthones in both plants and fungi are formed from benzophenone intermediates, which are generated from two distinct pathways. Therefore, the pattern of oxidation between them also differs. Fungal xanthones almost have hydroxylation at C-1 and C-8, whereas those in plants frequently display 1,3,5- or 1,3,7-hydroxylation (Wezeman et al, 2014). The aromatic ring system of xanthones may be substituted with a variety of prenyl, hydroxyl and methoxyl groups that form a large number of possible structures. Prenyl groups can be modified by hydroxylation, oxidation or cyclization with adjacent hydroxyl groups *via* nucleophilic attack to give tetra- or penta-cyclic xanthones, see **Scheme 8** (Dewick, 2009).



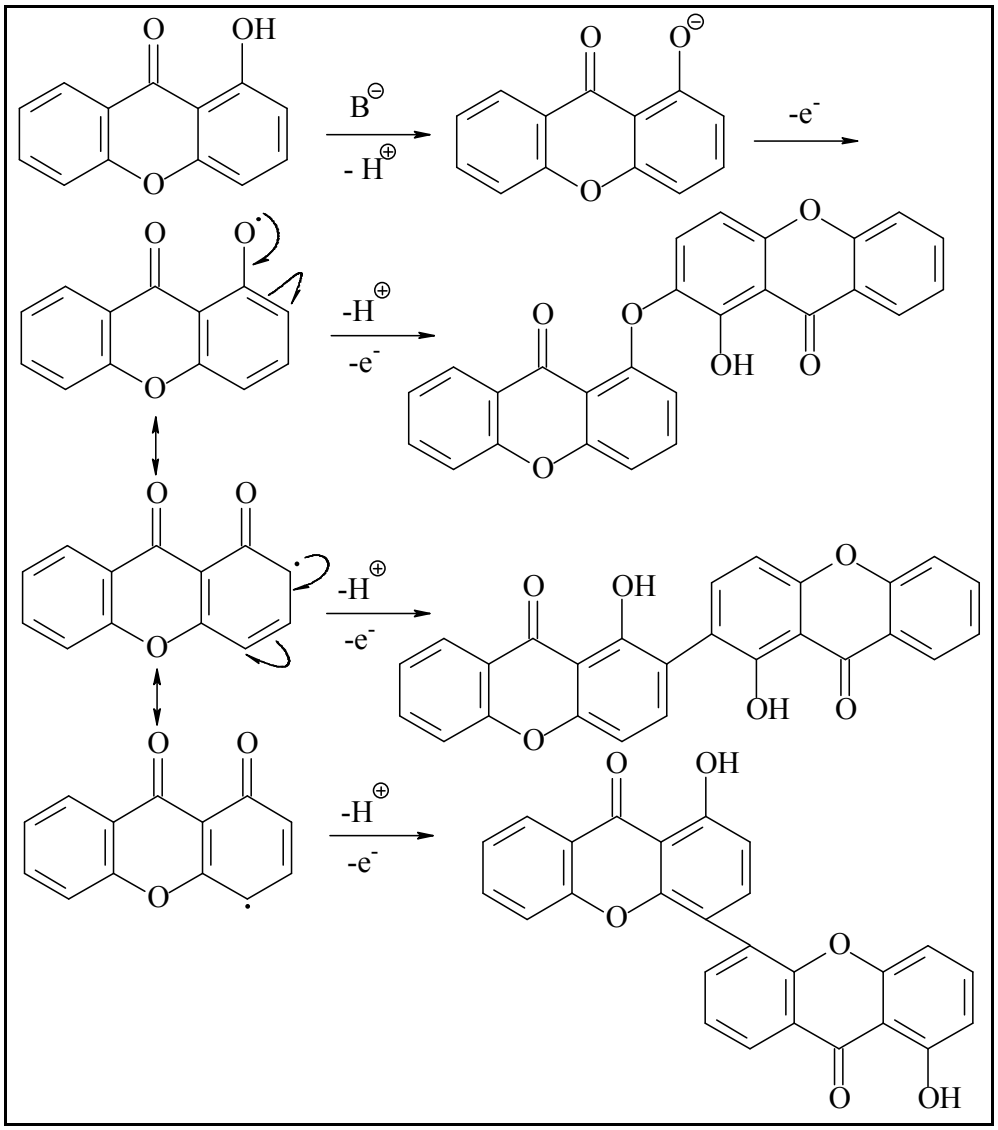
Scheme 8. Cyclization of prenyl groups with adjacent hydroxyl groups.

Different hypotheses have been proposed for the biosynthetic conversation of simpler xanthones into the more complex caged structures (Dakanali and Theosorakis, 2011). In the first one, a xanthone is prenylated at an early stage at C-5 and C-7 positions. An oxidation-reduction-oxidation sequence of the prenylated xanthone gives an intermediate, which can be cyclized by a nucleophilic attack of the hydroxyl group at C-5 on the prenyl group at C-7 to form the caged structure (Kartha et al, 1963) as showed in **Scheme 9** (A). The second one has been proposed for the generation of the caged motif *via* a Claisen rearrangement followed by a Diel-Alder reaction on the intermediate dienone as illustrated in **Scheme 9** (B, Quillinan and Scheinmann, 1971)**.**



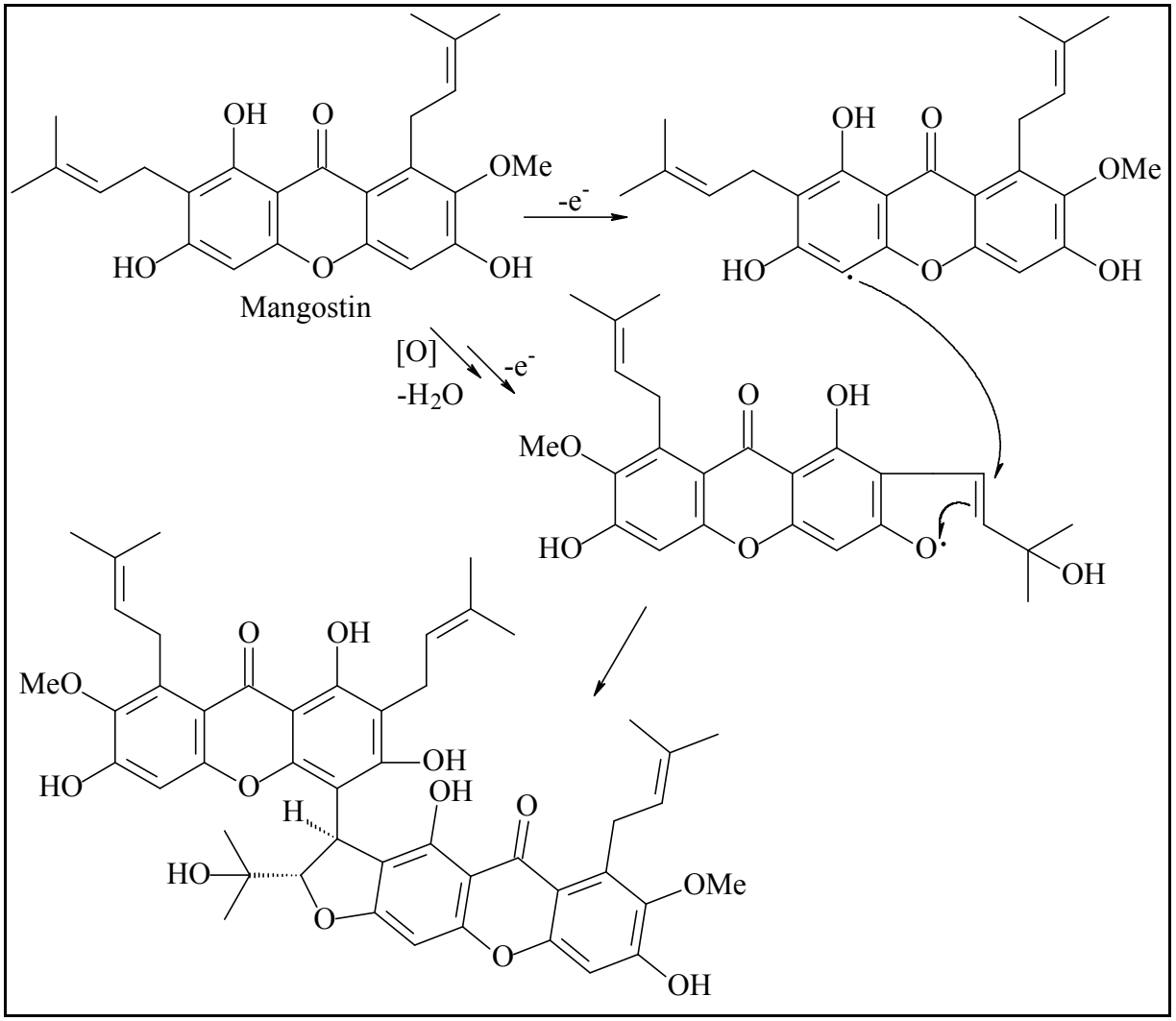
Scheme 9. Two hypotheses regarding the formation of caged xanthones.

Mono xanthones can be linked to generate dimeric or trimeric types by a variety of ways: a rotatable or atropisomeric biaryl C-C bond, a biaryl ether C-O-C linkage, aryl-O-alkyl linkages and prenyl derivative-prenyl derivative linkages. Although the biosynthesis of dimeric xanthones has been studied for some compounds, until now it is still unclear, particularly regarding the involvement of enzymes. The common way of xanthone dimerization found in plants, bacteria, lichens and fungi is the biaryl linkage as proposed in **Scheme 10**. First, a hydroxy group deprotonates to give an electron-negative intermediate. This can be easily oxidized by single-electron-transfer to a xanthonyl radical, which then forms aryl radicals by resonant effects. Radicals can couple to electron-donors at the *ortho* and *para* positions to give dimers (Wezeman et al, 2014).

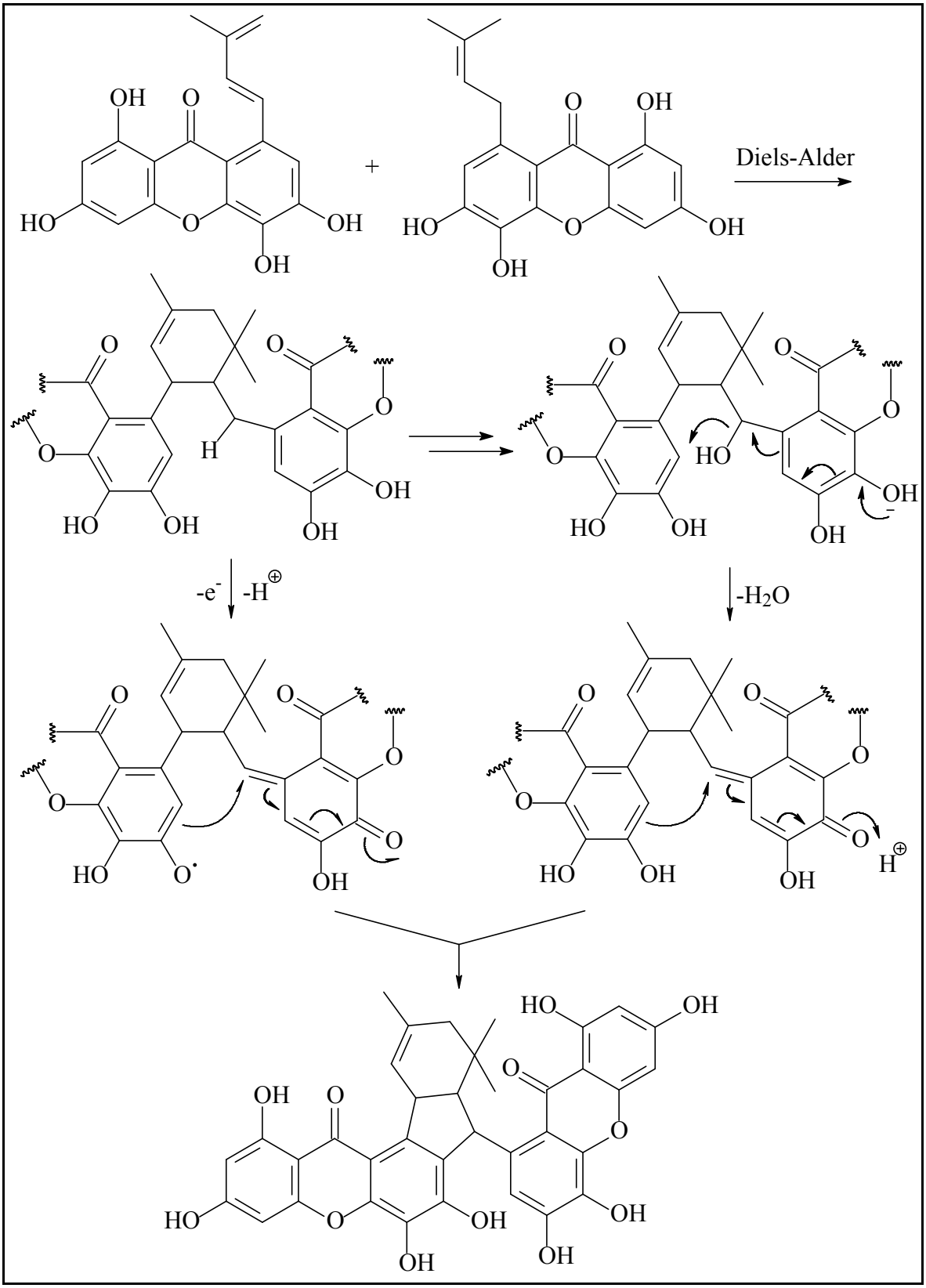


Scheme 10. Common formation of dimeric xanthones.

Bisxanthones which have been found in some plants of the family Gutttiferae (Clusiaceae) are mostly generated by aryl-*O*-alkyl or prenyl derivative-prenyl derivative linkages, for example cratoxyxanthone from *Cratoxylum cochinchinese* and griffipavixanthone from *Garcinia griffithii* (Sia et al., 1995; Xu et al., 1998). A possible biosynthetic pathway of cratoxyxanthone was proposed *via* the coupling of two mangostin-derived radicals, see **Scheme 11**. Meanwhile the biosynthesis of griffipavixanthone was suggested to involve an initial Diels-Alder reaction of two prenyl groups of the two xanthones to provide a cyclohexene derivative and this one is followed by another ionic or radical cyclization to give a fused 5-membered ring as shown in **Scheme 12**.



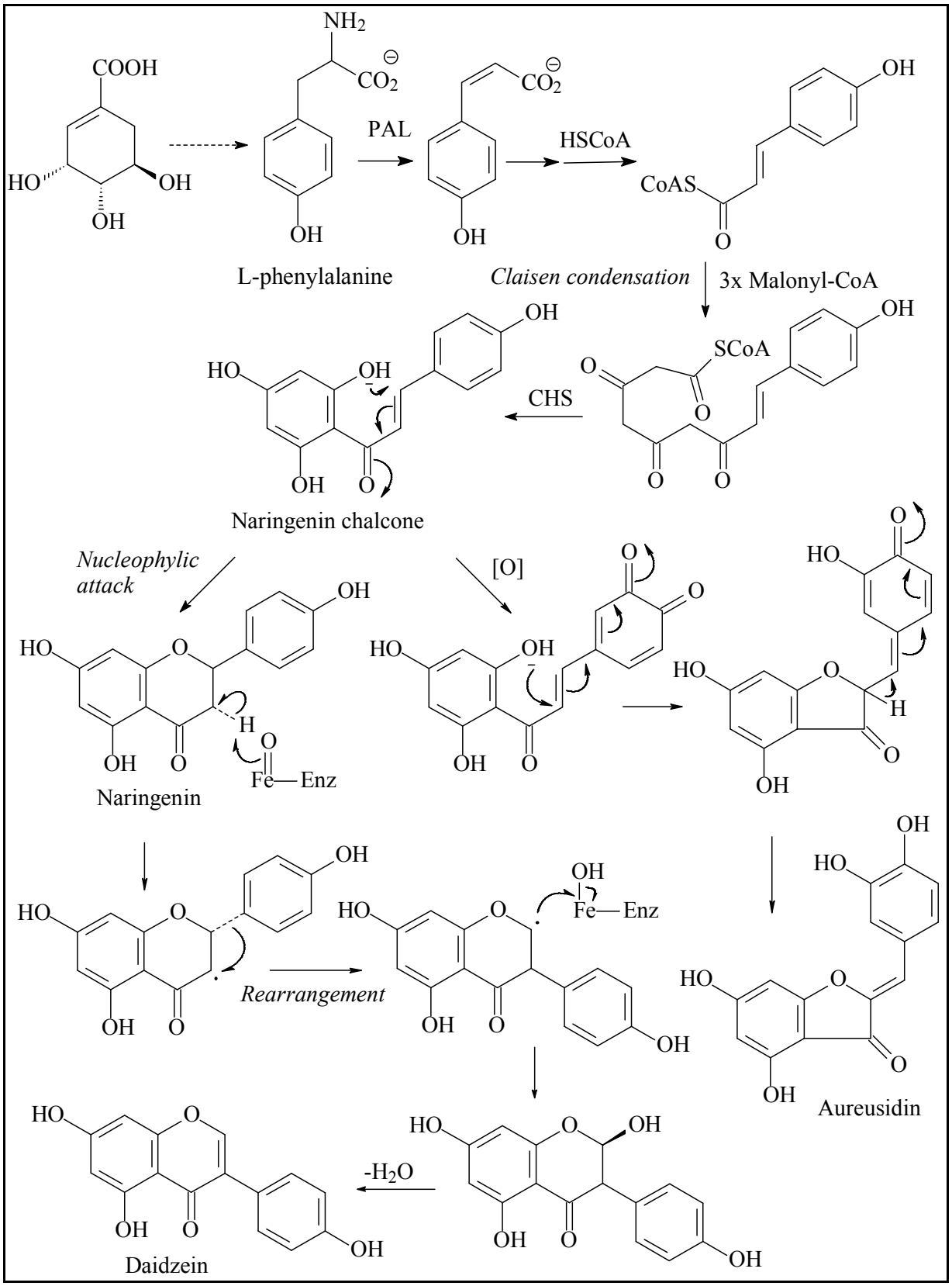
Scheme 11. Proposed biosynthesis of cratoxyxanthone.



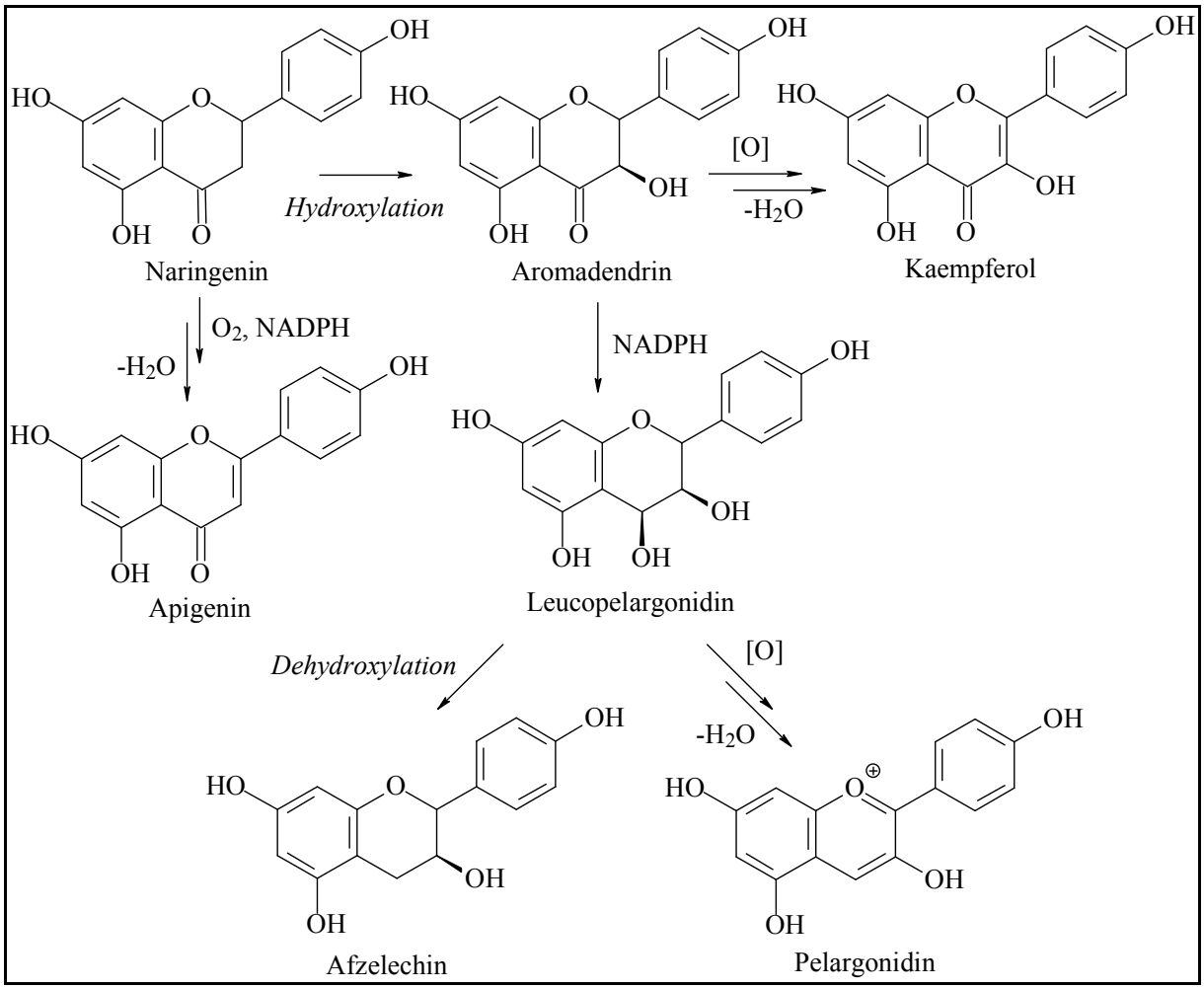
Scheme 12. Proposed biosynthesis of griffipavixanthone.

3.2. Biosynthetic pathway of biflavonoids

Biflavonoids are dimers of flavonoids linked by a C-C or C-O-C bond at different positions in flavonoid skeletons to give a large number of possible structures. Monomers can be flavans, flavones, flavonols, flavanones, flavanonols, chalcones, aurones, isoflavonoids or even neoflavonoids. Biflavonoids can be formed by two identical or non-identical units of flavonoids in a symmetrical or asymmetrical type (Mercader and Pomilio, 2012). Ginkgetin was the first biflavonoid found in nature. This compound was isolated from the leaves of *Ginkgo biloba* by Furukawa in 1929 (Baker and Simmonds, 1940). Nowadays, the number of biflavonoids continually increases. Though the biosynthetic pathway of flavonoids has been well established, the formation of natural biflavonoids has not been thoroughly identified. It was proposed to concern a radical dimerization (Geiger and Quinn, 1975). Up to date the enzymatic dimerization of the monomeric units has not been observed yet. Chalcones derived from the shikimate pathway are showed to be precursors of different classes of flavonoids, see **Scheme 13**. L-Phenylalanine, which is generated from shikimate, is deaminated by phenylalanine deaminase to yield (*E*)-cinnamic acid. This acid is subsequently transformed into 4-coumaroyl-CoA by enzyme-catalysed hydroxylation and reduction of HSCoA. The chain extension of 4-coumaroyl-CoA with three malonyl-CoA *via* Claisen condensation forms a polyketide intermediate which can be folded to give naringenin chalcone (chalcones). This chalcone can undergo oxidation and nucleophilic attack to afford aureusidin (aurones). An internal Michael-type nucleophilic attack of naringenin chalcone also produces naringenin (flavanones). This key flavanone may undergo radical oxidation, rearrangement and dehydration to give daidzein (isoflavonoids). Moreover naringenin may be transformed into apigenin (flavones) by oxidation (hydroxylation) and dehydration at C-2/C-3 or hydroxylated at C-3 to yield aromadendrin (flavanonols) and further oxidized to form kaempferol (flavonols) as shown in **Scheme 14**. Leucopelargonidin (flavandiols) can be generated by ketone-elimination of aromadendrin. This flavandiol may be transformed into afzelechin (flavanols) by dehydroxylation at C-4 or undergo a series of oxidation and dehydration to yield pelargonidin (anthocyanidins) (Harborne, 1998; Velisek et al., 2008; Dewick, 2009).

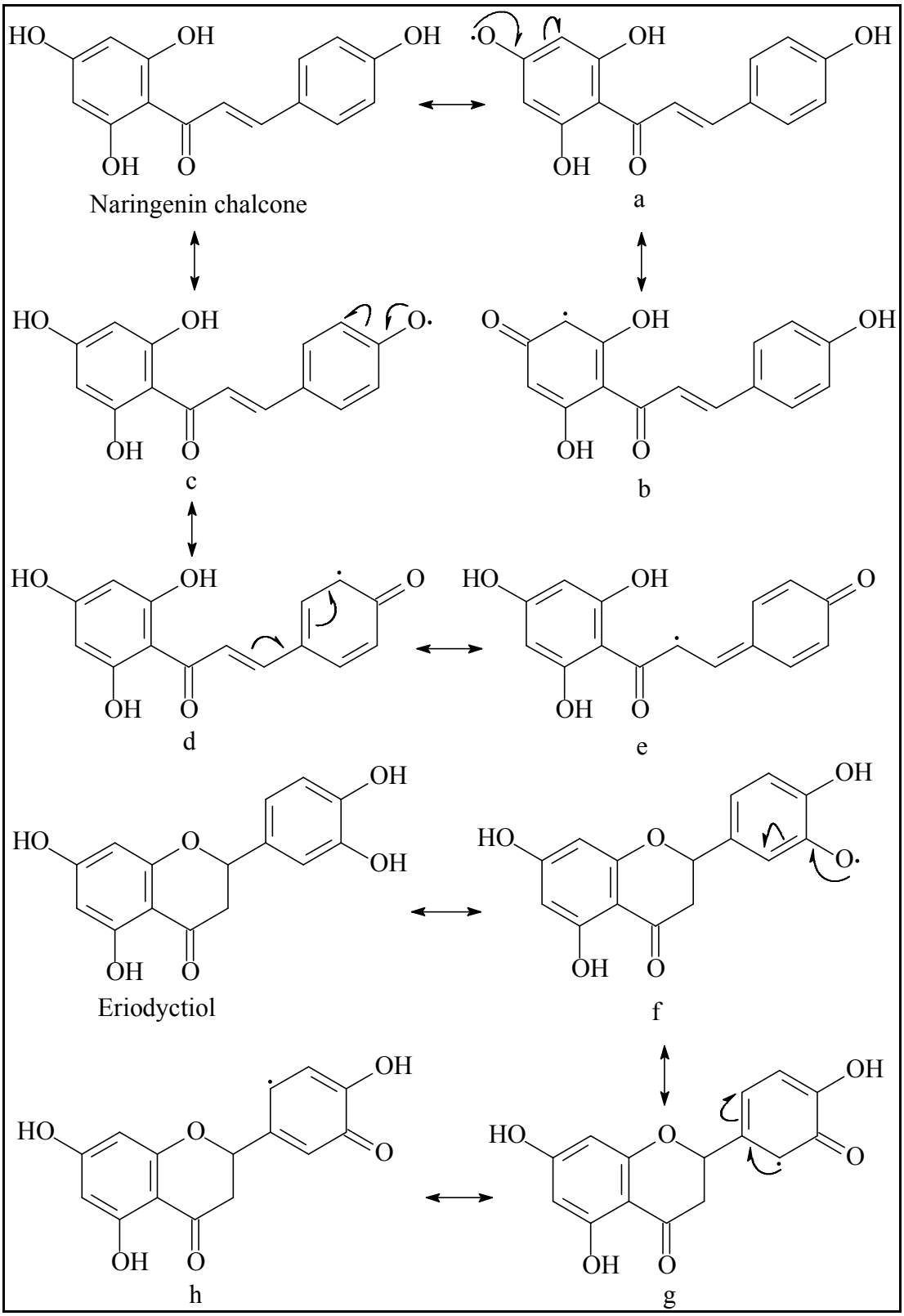


Scheme 13. Formation of chalcones, flavanones, aurones and isoflavonoids.



Scheme 14. Formation of flavanonols, flavones, flavonols, flavandiols, flavanols and anthocyanins.

Biflavonoids was proposed to be formed from the radical dimerization of monomeric flavonoids. Because flavonoids are phenolic compounds, they are susceptible to one-electron oxidation to generate radicals. Theoretically, one-electron oxidation can occur in any type of flavonoids. Geiger and Quinn suggested that chalcones, the precursors of flavonoids, undergo one-electron oxidation to afford a series of appropriate radicals which may couple to form biflavonoids as illustrated in **Scheme 15** (Geiger and Quinn, 1975). The removal of a phenol proton brings about an oxygen free radical, which can be stabilized by delocalization. This key radical in turn generates radicals (a-e). In principle, those radicals can freely couple to produce biflavonoids. However not all dimers have been found to exist in nature so far. There are around twenty four types of simple biflavonoids identified to date, see **Table 3** and **Figures 23-26** (Mercader and Pomilio, 2012).



Scheme 15. Formation of flavonoid radicals.

Table 3. Combination of flavonoid radicals to form simple biflavonoids

Combination of radicals	Type of linkage	Example
e + e	I-3, II-3″	Chamaejasmenin derivatives from the roots of *Stellera chamaejasme* (Yang et al, 2005)
e + b	I-3, II-6″	Stephaflavones from *Stephania tetrandra* and Ridiculaflavones from *Aristolochia ridicula* (Si et al., 2001; Machado and Lopes, 2005)
	I-3, II-8″	*Garcinia* biflavones (Jackson et al., 1971)
e + d	I-3, II-3‴	Taiwaniaflavones from the leaves of *Taiwania cryptomeriodides* (Gadek and Quinn, 1985)
b + b	I-6, II-6″	Succedaneaflavones from the drupes of *Rhus succedea* (Lin et al., 1997)
	I-6, II-8″	Agathisflavones from the genus *Agathis* (Ofman et al., 1995)
	I-8, II-8″	Cupressusflavones from the genus *Cupressus* (Gardek and Quinn, 1985)
g + b	I-2′, II-6″	Dicranolomins from the gametophytes of *Dicranoloma robustum* (Markham et al., 1988)
	I-2′, II-8″	Philonotisflavones from the gametophytes of *Philonotis fontana* (Geiger and Bokel, 1989)
h + h (g + g)	I-2′, II-2‴	(2′→2‴)-Biapigenin from *Garcinia nervosa* (Parveen et al., 2004)
d + b	I-3′, II-6″	Robustaflavones from the genus *Selaginella* (Lin and Chou, 2000)
	I-3′, II-8″	Amentoflavones from the family Guttiferae (Abe et al., 2004)
	I-3′, II-7″	Lophirone M from the leaves of *Lophira alata* (Murakami et al., 1992)

Table 3. (continued)

h + b	I-6′, II-6″	Hegoflavones from the fronds of *Alsophila spinulosa* (Wada et al., 1985)
h + h	I-5′, II-5‴	(I-5′,II-5′)-Bisdihydroquercetin from the bark of *Pseudotsuga menziesii* (Lai et al., 1992)
e + c	I-3 ,*O*, II-4‴	Delicaflavone from the aerial parts of the *Selaginella delicatula* (Lin and Chou, 2000)
b + a	I-6 ,*O*, II-7″	Masazinoflavanone from the leaves of *Rhus tripartitum* (Mahjoub et al., 2005)
	I-6 ,*O*, II-8″	(I-6,*O*,II-8)-Biapigenin from the leaves of *Viburnum cotinifolium* (Muhaisen et al., 2002)
d + c	I-3′, *O*, II-4‴	Ochnaflavones from the genus *Ochna* (Reddy et al., 2008)
d + a	I-3′, *O*, II-7″	Lophirone L from the leaves of *Lophira alata* (Tih et al., 1992)
c + b	I-4′ ,*O*, II-6″	Hinokiflavone from *Thuja javanica* (Roy et al., 1985)
	I-4′, *O*, II-8″	Lanaroflavone from the whole plant of *Lanaria lanata* (Dora and Edwards, 1991)
	I-4′, *O*, II-4‴	Loniflavone from *Lonicera japonica* (Kang, 2005)
	I-8,CH_2, II-8″	Pentagrammetin from *Pentagramma triangularis* (Roitman et al., 1993)

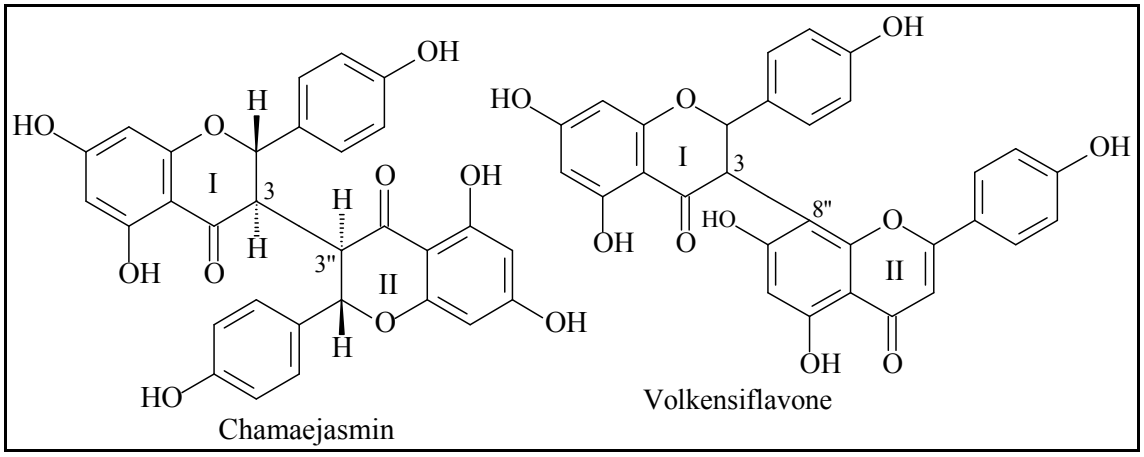


Figure 23. Examples of different types of simple biflavonoids.

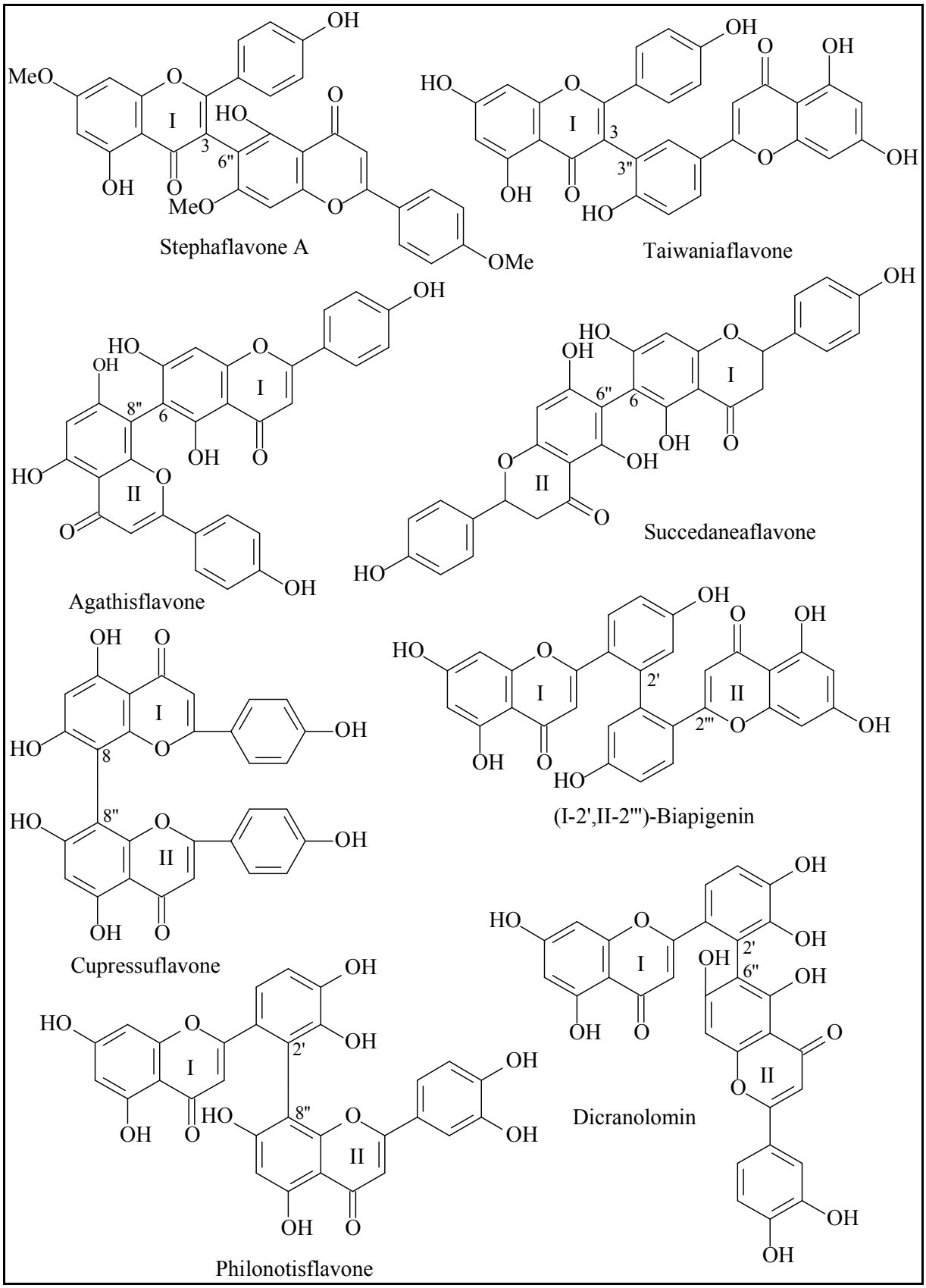


Figure 24. Examples of different types of simple biflavonoids (*continued*)

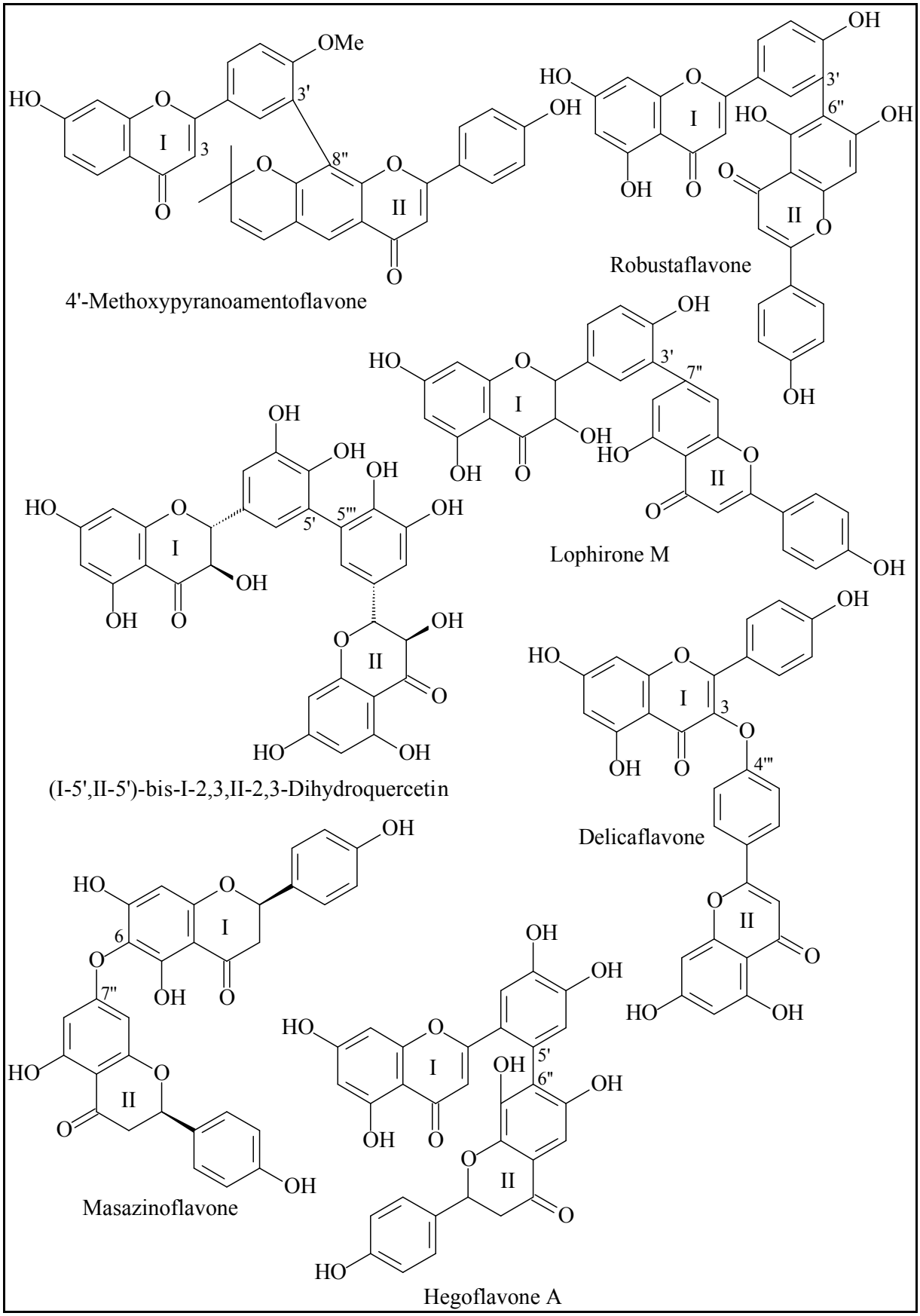


Figure 25. Examples of different types of simple biflavonoids (*continued*)

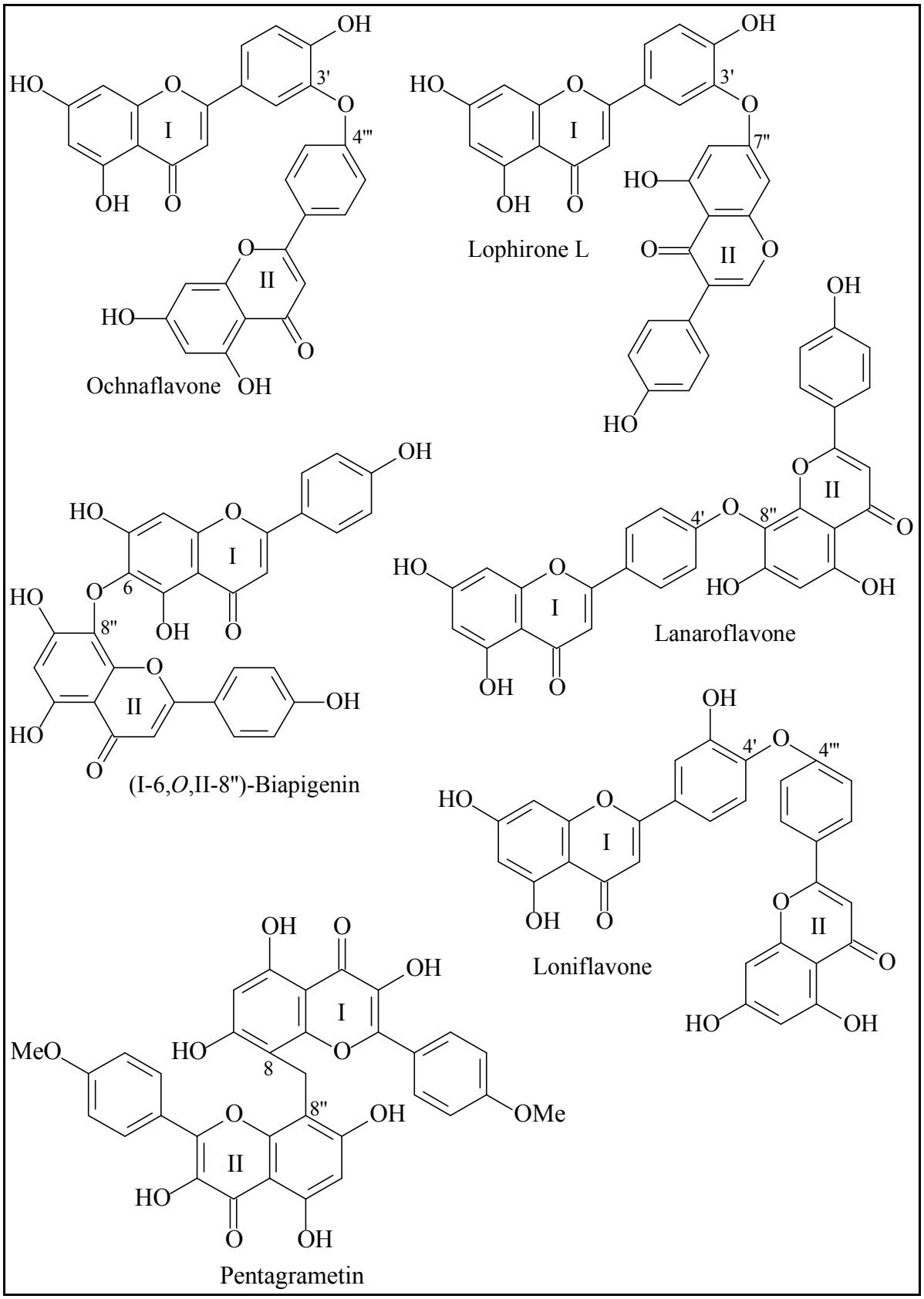


Figure 26. Examples of different types of simple biflavonoids (*continued*)

4. MATERIAL AND METHODS

4.1. General experimental procedures

Extraction was carried out using a sohxlet system of 1000 ml (Ducan). Thin layer chromatography (TLC) was performed using silica gel 60 F_{254} and RP_{18} F_{254} pre-coated aluminium sheets (Merck, 0.2 and 2.0 mm). TLC plates were visualized by UV light (CAMAG) at 254 nm and 366 nm after sprayed with anisaldehyde/sulphuric acid reagent (anisaldehyde, 2 ml; H_2SO_4 98%, 10 ml, AcOH, 16 ml and MeOH, 170 ml) and heated on a thermoplate (Desaga). The solution of $FeCl_3$ in methanol was used for detecting phenolic compounds. Open column chromatography (CC) was carried out on silica gel (Merck, 63 – 200 μm) using glass columns of various dimensions. Gel permeation chromatography (GPC) was performed on Sephadex LH-20 (GE Healthcare) with MeOH or MeOH – $CHCl_3$ 3:7 as eluent. Flash chromatography (FC) was performed on an Armen Instrument Spot System with columns: SVF D26 (26 x 110 mm) and SVP D40 (4- x 160 mm) on silica gel 60 (Merck, 63 – 200 μm). Centrifugal partition chromatography (CPC) was carried out using an Armen Spot Centrifugal Partition Chromatography SCPC-250 with the solvent system HEMWat (*n*-hexane/ethyl acetate/methanol/water) as eluent. All solvents used for TLC, CC, GPC, FC and CPC were p.a. (pro analysis, Merck). Semi-preparative HPLC separations were performed on a Varian ProStar 210 solvent delivery module equipped with a Varian Prostar 335 photodiode array detector. Two prepacked columns, Varian Dynamax Pursuit XRs C18 of 21.4 x 250 mm and Eclipse XDB-C18 of 9.4 x 250 mm (HP), were used for RP_{18} HPLC. All solvents used were HPLC grade (Merck).

1D and 2D NMR spectra were measured using a Bruker ADVANCE 600 spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) or 400 spectrometer (400 MHz for ^{1}H and 115 MHz for ^{13}C) with tetramethylsilane (TMS) as an internal standard and $CDCl_3$ or acetone-d_6 (Deutero GmbH) as the solvent. The spectra were processed using the NMR softwares: MestReNOva 6.0.2-5475 (Mestrelab Research) and Topspin 3.1 (Brucker). High-resolution mass spectra were measured on a Finnigan MAT SSQ 710 A spectrometer at 70 eV (HRESIMS, positive mode) or recorded on an Agilent 6540 UHD (HRESIMS, positive and negative mode). UV spectra were

obtained by the Varian Prostar 335 photodiode array detector of the HPLC system. Optical rotation was recorded using an UniPol L1000 polarimeter (Schmidt & Haensch).

The MEM-Earle (0.85 g/l $NaHCO_3$, Biochrom) supplemented with 1% of L-glutamine (200 mM), 10% of fetal bovine serum and 1 % of non-essential amino (Biochrom) were used for culture of HeLa cells (ATCC CCL17). The medium ECGM (Porvitro GmbH) with supplement mix and antibiotics (Porvitro GmbH) were used for HMEC-1 cells (CDC). The incubation was carried out in a NU-5500E incubator (NUAIRE). Cell-counting was performed using a neubauer improved haemocytometer (Brand) and a CK X 41 SF microscope (Olympus). A Megafuge 1.0 centrifugal (Heraeus Sepatech) was used for centrifugation. Autoclaving was carried out using an Autoklav 23 (Melag) or a Varioklav 500 (H+P). All the pipettes used were from Eppendorf. Tris(4-dimethylaminophenyl)methyliumchlorid (crystal violet) was obtained from Merck. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) and 3,3′-Dimethyl-4,4′-bis(5-amino-4-hydroxy-2,7-disulfonaphtyl-3-azo)-[1,1′-biphenyl] dye were from Sigma-Aldrich. The optical density was measured at 560 nm using a SpectraFlour Plus microplate reader (Tecan). Cytotoxicity and proliferation assays were performed in 96 well plates (TPP).

4.2. Extraction, fractionation and isolation

4.2.1. Material of plant

The bark of *Garcinia fusca* Pierre was collected in Binh Phuoc Province, south Vietnam in January 2011 and was identified by Mr Dang Van Son, Institute of Tropical Biology, Ho Chi Minh City. A voucher specimen (Hieu-GF-01/2011) is deposited in the Natural Product and Medicinal Chemistry Lab, Ho Chi Minh City University of Sciences. The bark was air-dried and ground into powder.

4.2.2. Extraction of plant material

The powder of the bark of *G. fusca* Pierre (1.95 kg) was first exhaustively extracted with 3.0 L of *n*-hexane by using a soxhlet system (1000 ml), a form of solid-liquid

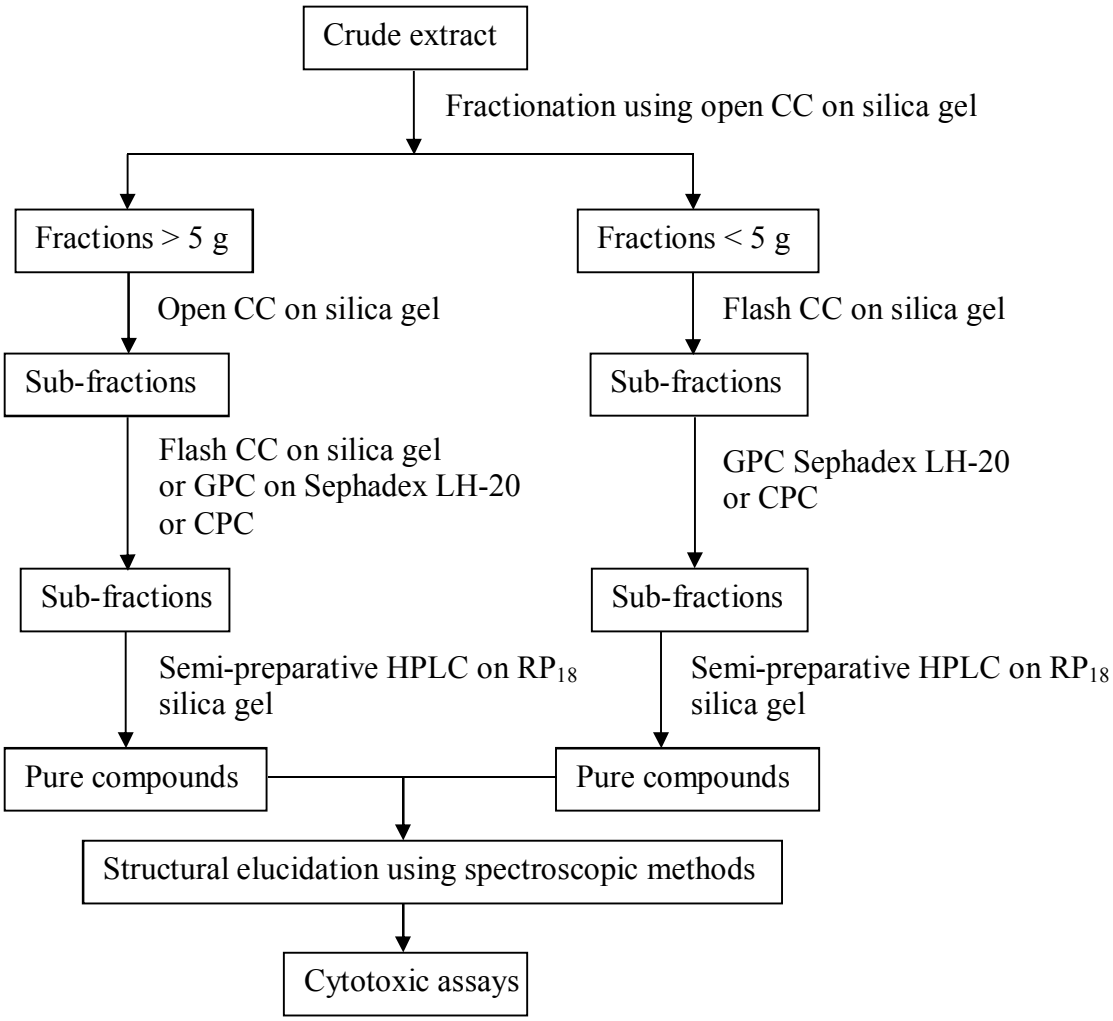
extraction. The solvent was heated to reflux and successively percolated the material. The extraction of *n*-hexane was done until the liquid passing through the material was colourless. The residue of material was subsequently extracted with the same volume of ethyl acetate. After extraction the solvents were removed by a rotary evaporator to yield the crude extracts of *n*-hexane and ethyl acetate, which were shortly named as GFH (*G. fusca n*-hexane extract) and GFE (*G. fusca* ethyl acetate extract), respectively.

4.2.3. Fractionation of crude extracts

The *n*-hexane extract (GFH, 90.5 g) was divided into two portions. Each portion was subjected to an open CC using a column of 10 x 40 cm dimension, 800 g of silica gel as stationary phase and a solvent system of acetone and petroleum ether (0-100%) as mobile phase to give 30 fractions (500 ml of each). On the basis of TLC, identical fractions were combined to afford 8 main fractions which were shortly named GFH-1 - 8. The ethyl acetate extract (GFE, 112.3 g) was also split into two portions which were in turn applied to CC with the same column and amount of silica gel using a gradient elution of acetone and petroleum ether (0-100%) to produce 35 fractions (500 ml of each). The combination of similar fractions was also based on TLC to give 8 main fractions (GFE-1 - 8).

4.2.4. Isolation of substances from fractions

Chromatography is one of the powerful methods for the separation and purification of natural products. It is based on the distribution of a component between two phases - a moving one (mobile phase) that is passed over an immobile phase (stationary phase). Components will be released by the mobile phase at various rates because of their different affinities with the stationary phase. A variety of chromatography methods are widely used for the isolation of natural products such as adsorption, partition, ion-exchange and permeation chromatography. Mobile phase can be either gas or liquid. For the isolation of compounds from *G. fusca* Pierre, the adsorption, partition and permeation chromatography were used. The general strategy of the isolation and structural elucidation of substances is shown in **Scheme 16**.



Scheme 16. The general strategy of the isolation and structural elucidation of substances from *G. fusca* Pierre

4.2.4.1. Thin layer chromatography (TLC)

TLC was used to select appropriate solvent systems for the CC of fractions based on their R_f (retardation factor) values. The R_f value refers to the distance that a compound moves in chromatography relative to the solvent front. It can be calculated by the following formula (Braithwaite and Smith, 1996).

Formula 1: Calculation of retardation factor (R_f)

$$R_f = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent front}}$$

A suitable solvent system should give useful R_f values of between 0.3 and 0.7, i.e., a compound can be separated from other components without staying at or so close to the original point or with the solvent front. The separation between two compounds is possible with an ΔR_f value (the difference between two R_f values) of at least 0.2-0.3 (Braithwaite and Smith, 1996). TLC is also used to determine two identical compounds relying on their R_f values in the same solvent system or for the combination of similar fractions. It is even used to show the relative number of components existing in a given fraction or if an isolated substance was pure. Moreover TLC is also applied to identify types of compounds in extracts or fractions based on their physical and chemical properties with ultraviolet (UV) and typical reagents (Gibbons, 2006).

In the present work, aluminium sheets coated by silica gel or reverse phase silica C_{18} of 0.2 mm thickness with fluorescent indicator (Merck) were used for TLC. Samples were applied on the plate *via* a micropipette with a volume of 0.5 µl and a concentration of 5 mg/ml. For the selection of suitable eluent of CC, the plates were developed with different solvent systems. After the development, the location of compounds was detected using UV or chemical reagents. Coloured substances are easily visible to naked-eyes as separate spots, whilst colourless ones require chemical or physical methods. Substances having an enone or a benzene ring are detectable with UV. Therefore they can be visualized by UV when irradiated at 254 nm. The plates contain a fluorescent indicator which absorbs light at 254 nm and re-emits at the green end of the spectrum, thus if a substance absorbs at 254 nm, it will be detectable as a dark spot against the green background. Methanolic ferric chloride ($FeCl_3$/MeOH) reagent was used to detect phenolic compounds as dark green spots. Compounds which are not detectable with UV could be visualized by spraying the plate with anisaldehyde-H_2SO_4 reagent or sulphuric acid 10%. The plate was heated after spraying at 100-105ºC until the colours have totally occurred. Depending on the structure of compounds, the colours of spots changed from pink to dark brown. After heated, substances were also detected by UV at 366 nm as light spots against the dark violet background.

4.2.4.2. Preparative TLC

The practice of this technique is similar to that of qualitative analysis but differs in the thickness of plates. They are principally coated by 1-5 mm of adsorbent for using in the preparation of large sample quantities (10-100 mg). Pre-coated glass plates of 2 mm thickness (Merck) and 20 x 20 cm dimension with fluorescence indicator were used for the present work. The adsorbent was silica gel 60 (63-200 μm). The sample was dissolved in acetone (5 mg/ml) and applied onto the plate as a streak by a pasteur pipette. The chromatography was carried out in a glass tank of 22 x 22 cm with 100 ml of a suitable solvent system. A filter paper of 20 x 20 cm was soaked with the solvent and put into the tank to keep the internal atmosphere saturated with solvent vapour. After the development was complete, the plate was dried and the bands of substances were detected by UV at 254 nm. The bands were then scraped off with a spatula and the substance was washed out of the adsorbent by acetone. The pure substance obtained after the removal of acetone was structurally determined by spectroscopic techniques.

4.2.4.3. Open column chromatography

Open CC using silica gel as an adsorbent is one of traditional forms of adsorption chromatography. This is a simple and low cost CC technique. Fractions were subjected to open CC by using simple glass columns of various dimensions depending on the amount of sample. The stationary phase was silica gel 60 (Merck, 63-200 μm) and the mobile phase was the appropriate solvent systems selected by TLC. The column was packed by wet-column method, in which silica gel was soaked in the solvent with the ratio of 0.5 mg/ml before poured into the column. The quantity of silica gel used was fifty times of the sample amount. The packed column was equilibrated by at least two bed volumes of the solvent before the sample adsorbed on silica gel was applied onto the top of the packed column. Afterwards the ready column was eluted by a gradient elution. The solvent runs through the column under the influence of gravity. The chromatography was carried out within 3-7 hours and the eluent containing separated components was collected in test tubes or conical flasks. Collected fractions were subsequently applied to TLC for combination.

4.2.4.4. Flash chromatography

The standard method of flash chromatography (FC) is based on the report of Still in 1978 (Still et al., 1978). This is a rapid column chromatography technique using a narrower packing than classical CC and a slightly increased over-pressure to achieve adequate flow-rates. The technique is much efficient for the separation of components with ΔR_f values of less than 0.2. It is suitable for sample loads ranging from 10 mg to 10 g depending on the difference of R_f values (Braithwaite and Smith, 1996). The flash chromatography system used in the present work is Spot Liquid Chromatography Flash (Armen Instrument). Plastic columns of 26 x 110 mm (SVF D26-Si60) or 40 x 160 mm (SVP D40-Si60) were tightly packed with silica gel 60 (63-200 µm) without solvent. The packed column was subsequently eluted with solvent by a mini pressure of maximum 7 bar until it was homogeneous without air-bubbles. The sample adsorbed on silica gel was then applied onto the top of the column. The column SVF D26-Si60 was used for the sample quantity of 0.3-1.5 g, whilst the column SVP D40-Si60 was suitable for 0.9-4.5 g of the sample. The elution was a gradient with the flow-rate of 10-30 ml/min and the eluent was automatically collected in test tubes. The chromatography was controlled by the built-in UV detector at 290 nm. The combination of collected fractions was based on the result of TLC.

4.2.4.5. Size-exclusion chromatography

Size-exclusion chromatography or gel filtration is one of the permeation chromatographic methods, in which gels or polymers are used as stationary phase and the separation of uncharged compounds is based on the relative size or mass of molecules, i.e., large components leave the column first followed by smaller ones in order of their decreasing size. This technique was first performed in 1954 by Mould and Synge and the systematic use of the principle was introduced in 1959 by Porath and Flodin (Braithwaite and Smith, 1996). In this work, Sephadex LH-20 (GE Healthcare) was used as the stationary phase and the mobile phase was methanol or a mixture of methanol and chloroform of 3:7 by volume. Sephadex LH-20 is a hydroxypropylated polysaccharide developed for gel filtration and CC of natural products in the range of 100-5000 molecular weight. In the experiment, the gel was

suspended in the solvent of 4.0 ml/g before poured into an appropriate glass column depending on the amount of sample. The packed column was stationary in at least three hours for full swelling of the gel and afterwards equilibrated with three bed volumes of the solvent. The sample was dissolved in the solvent before applied onto the packed column in such a way that the sample volume was 5% of the total bed volume. The ready column was then eluted with a flow rate of 0.5 ml/min. The solvent flows through the column under the influence of gravity. The eluent was manually collected in test tubes. The fractions were then combined into sub-fractions based on TLC.

4.2.4.6. Centrifugal partition chromatography

Centrifugal partition chromatography (CPC) is a liquid-liquid chromatographic technique originally pioneered by Ito in 1964 (Ito, 1970). The principle of this technique is based on the different partitions of components between two immiscible liquid phases. Each one has a specific partition (distribution) coefficient (K_d) defined by the following formula (Kumar et al., 2014).

Formula 2: Calculation of partition coefficient (K_d)

$$K_d = \frac{[X]_{upper}}{[X]_{lower}}$$

For a given compound X, at the equilibrium of two liquid phases and for a given temperature:

$[X]_{upper}$ = the concentration of X in the upper phase

$[X]_{lower}$ = the concentration of X in the lower phase

CPC functions with one of two liquid phases used as stationary phase and the other one as mobile phase. The column system consists of a series of channels linked in cascade by ducts and aligned in cartridges or disks in a circle around a rotor. The liquid stationary phase is fed into the column and held in channels by the centrifugal force generated by the spinning rotor. The liquid mobile phase containing the

components of the sample is then pumped through the column. The separation is achieved as a function of the specific partition coefficient (K_d) of each component. For the ideal separation of the components, the solvent system should result in a partition coefficient of 1. However based on practical experiments, $0.2 < K_d < 5$ can be used without excessive elution and broadening band (Wilson, 2012). Many strategies for selection of solvent system have been reported in literature. Foucault suggested that there should be a solvent that is soluble in the other solvents of the solvent system and this solvent should completely dissolve the sample (Foucault, 1995)

For this research, the CPC system used is Spot Centrifugal Partition Chromatography SCPC-250 (Armen). The solvent system of HEMWat (*n*-hexane/ethyl acetate/methanol/water) was selected with an appropriate ratio so that the partition coefficient is between 0.2 and 5. Two modes of the CPC used for the separation are descending mode in which the lower phase is the mobile phase and ascending mode in which the upper phase is the mobile phase. For the chromatography, the sample amount of 1 g was dissolved in 10 ml of a mixture containing 50% of mobile phase and stationary phase. The speed of the rotor was 1000 U min^{-1} and the flow rate of elution was 5 ml/m. The eluent containing separated components was automatically collected in test tubes. At first, the column was fed with the lower phase. The upper phase was subsequently pumped through the column until the equilibration was established. The sample volume of 10 ml was then ejected into the equilibrated column. The upper phase passes through the column and elutes separated components. After 60 minutes of the chromatography, the ascending mode was switched into descending mode, i.e., the lower phase was at that moment used as the mobile phase. The chromatography was carried out within at least two hours. Collected fractions were subsequently applied to TLC for combination.

4.2.4.7. Semi-preparative high performance liquid chromatography

High performance liquid chromatography (HPLC) is a column chromatographic technique using a small particle size packing and a large pressure elution in order to get a high performance of separation. In 1967, Horvath et al. set up one of the first practical HPLC systems for their research on nucleotides (Braithwaite and Smith,

1996). Nowadays HPLC is widely used for qualitative and quantitative analysis as well as preparation of natural or synthetic compounds. In the present work, semi-preparative HPLC was used for the isolation and purification of substances is a ProStar system (Varian) consisting of an auto-sampler, two pumps with maximum pressure of 400 bar, a diode array detector (DAD) and a fraction collector. Two pre-packed columns were used for the preparation are Dynamax Pursuit XRs C18 of 21.4 x 250 mm and Eclipse XDB-C18 of 9.4 x 250 mm. Both columns were packed with RP_{18} silica of 5 µm. The former was used for the sample amount of 10-20 mg and the flow rate of maximum 20 ml/m, whilst the later was used for that less than 10 mg of the sample and the flow rate of maximum 6 ml/min. The solvent system of water and acetonitrile was selected for the elution. The sample was dissolved in acetonitrile with the ratio of 10 mg/ml before injected into the column. The preparation time was around 30-40 minutes and the separation was monitored by UV. The purified substances were then structurally identified using spectroscopic techniques such as 1D and 2D NMR, UV and mass spectrometry.

4.3. Structural elucidation of isolated compounds

Spectroscopic and spectrometric methods are considered as effective and useful tools for the structural identification of natural products. These include ultraviolet - visible (UV-vis), infrared (IR), nuclear magnetic resonance (NMR), X-ray, circular dichroism (CD) and mass spectrometry. A molecular structure including the relative and the absolute configurations can be completely determined by the combination of these methods. In this work, the structural determination of the new natural compounds was mainly performed by one (1D) and two dimensional (2D) NMR spectroscopy associated with UV and mass spectroscopy as well as optical rotation. The steps of the structural elucidation was executed in succession as follows

- Identification of the class of substance based on ^{1}H NMR and UV spectra.
- Calculation of unsaturation degree or hydrogen deficiency index and establishment of the molecular formula from data of ^{13}C NMR and mass spectra.
- Analysis of skeletal connectivity to accomplish the covalent structure relying on 2D NMR spectra such as HMQC, HMBC and COSY.
- Determination of the relative stereochemistry using NOESY or ROESY spectroscopy and optical rotation.

4.3.1. Ultraviolet-visible spectroscopy

As the electromagnetic radiation in the ultraviolet and visible region (190-800 nm) passes through a transparent substance, a portion of the radiation may be absorbed in a form of energy which can make electrons in molecules move from a state of low energy (ground state) to a state of higher energy (excited state). The absorbed energy is exactly equal to the energy difference between these two states. Once that happens, the residual radiation is recorded by a detector to give a spectrum which is called an absorption spectrum. The possible transitions of electrons are from the π or nonbonding (n) orbitals to unoccupied or anti-bonding orbitals (π^* and σ^*). The UV-vis spectrum is basically recorded as a plot of absorbance (A, mAU) *versus* wavelength (λ, nm). The absorbance obeying the Beer-Lambert Law can be calculated for a given wavelength by the following formula (Pavia et al., 2009)

Formula 3: Calculation of absorbance (A)

$$A = \log(I_0/I) = \varepsilon.c.l$$

A = absorbance

I_0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

c = molar concentration of solute

l = length of sample cell (cm)

ε = molar absorptivity

The appearance of several peaks of absorbed maximum wavelengths (λ_{max}) in the UV-vis spectrum is caused by conjugated systems and electronic transitions between the different vibrational and rotational energy levels for each electronic state. The λ_{max} values are useful for the structural elucidation and those of conjugated systems such as diens or enones can be predicted by Woodward's rules for the $\pi \rightarrow \pi^*$ transition. Each type of compound gives specific absorption bands. For examples, carotenoids commonly show three distinct peaks between 400 and 500 nm in methanol, whilst biflavonoids perform two peaks at 260-290 nm and 320-340 nm. Xanthones give four different peaks at 230-245, 250-265, 305-330 and 340-400 nm (Harborne, 1998). In the present work, the UV spectra of the isolated compounds were measured in the 200-420 nm region by the diode array spectrophotometer of Prostar HPLC system.

4.3.2. Mass spectrometry (MS)

A substance can be transformed into gas phase ions by various ionization methods. These ions are then accelerated by an electromagnetic field, separated by their mass-to-charge (m/z) ratio and counted by a detector. The signal is recorded and output as a graph of the number of ions detected *versus* their m/z ratio, called a mass spectrum. Common ionization methods can be divided into three main groups, viz gas-phase, desorption and evaporative ionization. The gas-phase ionization including electron impact (EI) and chemical ionization (CI) methods is suitable for volatile and small organic molecules. The desorption ionization consists of three methods: secondary ion

mass spectrometry (SIMS), fast atom bombardment (FAB) and matrix-assisted laser desorption ionization (MALDI). The evaporative ionization comprising thermo-spray (TS) and electro-spray (ES) methods, along with desorption, are appropriate for non-volatile and large molecules, especially peptides and proteins (Pavia et al., 2009). There are two significant categories of mass spectrometers, low resolution and high resolution. High resolution mass spectrum (HRMS) can determine an extremely precise *m*/*z* value up to four or five decimal numbers, i.e., only one rational molecular formula will fit the data especially in combination with ^{1}H and ^{13}C NMR data. In the present work, the molecular weight and the molecular formula of new compounds were obtained from electrospray ionization- high resolution spectra (ESI-HRMS) which were measured by the instrument Q-TOF 6540 UHD (Agilent). In ESI, the sample in solution is sprayed out from a capillary into a heated chamber at nearly atmospheric pressure. The tip of the capillary is maintained a high voltage potential of 2-5 keV to expel the sample molecules in charged droplets into the ionization chamber. The charged droplets are subjected to a counter-flow of a nebulizing gas that evaporates solvent molecules from the droplets until solvent-free sample ions are released in the vapour phase. These ions will then pass through the high resolution mass spectrometer to yield a HRESI-MS. With this ionization method $[M+H]^+$, $[M+Na]^+$ or $[M+K]^+$ ions are produced but fragmentation is rare (Silverstein et al., 2005). For a compound of general formula $C_aH_bN_cO_dX_e$ in which X is a halogen atom, the degree of unsaturation can be calculated by the following formula

Formula 4: Calculation of degree of unsaturation

$$\text{Degree of unsaturation} = \frac{(2 + 2a - b + c - e)}{2}$$

a = number of carbon atoms b = number of hydrogen atoms

c = number of nitrogen atoms e = number of halogen atoms

4.3.3. Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) has been shown to be a powerful spectroscopic method for the structural determination of natural products, especially novel compounds sometimes possessing unusual or unprecedented structures. It has become the main choice for the establishment of chemical structures, particularly when X-ray crystallography is inapplicable. Nowadays modern NMR spectroscopy can be performed by high magnetic spectrometers of up to 900 MHz which allow to accomplish multi-pulsed and multidimensional experiments with a small sample amount of under 0.5 mg. Nuclei have an angular momentum described by a spin quantum number I, which gives $2I+1$ of allowed spin states with integral differences ranging from $+I$ to $-I$. Each spin state possesses a corresponding energy level when a nucleus with $I \neq 0$ is immersed in a magnetic field of strength B_0. A spectroscopic transition may occur between these levels by the absorption of electromagnetic radiation of appropriate frequency in accordance with the following equation (Pavia et al., 2009)

Formula 5: The equation of energy difference of nuclei in a magnetic field

$$\Delta E = \gamma(h/2\pi)B_0 = h\nu$$
$$\Leftrightarrow \nu = \gamma B_0/2\pi$$

ΔE = the energy difference between the possible spin states

ν = the frequency of the absorbed electromagnetic radiation which is usually in the radiofrequency region of *ca*. 1-1000MHz

γ = magnetogyric ratio, a constant for each nucleus

Natural products commonly contain nuclei having spin number I of ½ such as ^{1}H, ^{13}C, ^{15}N and ^{31}P. Among these, ^{1}H and ^{13}C are the most important nuclei because they constitute the skeleton of substances. The proton ^{1}H is quite sensitive with NMR because of its high natural abundance (99.98 %) and therefore it is first investigated for the structural elucidation of substances (Neri and Tringali, 2001). In the present work, the 1D and 2D NMR spectra of isolated compounds were obtained using NMR

spectrometers of AVANCE 300, 400 and 600 Kryo (Bruker). The sample was dissolved in 0.6 ml of deuterated chloroform ($CDCl_3$) or deuterated acetone (C_2D_6CO) and the solution was then dispensed into a 5 mm diameter NMR tube. The spectra were measured at the room temperature or 393ºK using the Fourier transform (FT) technique. The NMR-active nuclei are simultaneously excited by an irradiation pulse. When the pulse is switched off, the excited nuclei slowly return to their original spin state and emit electromagnetic radiation of different frequencies. This is so-called relaxation process. The signal is recorded in the form of the free induction decay (FID) (Mitchell and Costisella, 2007). Tetramethylsilane (TMS), $(CH_3)_4Si$, was used as the internal reference compound.

4.3.3.1. One dimensional (1D) NMR spectra

1D NMR spectra are typically displayed as an absorption spectra, the axes of which are the frequency (chemical shift) and the intensity. Many functional groups or types of hydrogens or carbons contained in a molecule can be identified by the characteristic chemical shift values in ^{1}H and ^{13}C NMR spectra.

4.3.3.1.1. ^{1}H NMR spectra

Protons in a molecule have resonances at various frequencies because of their different chemical environment. Therefore, each type of proton gives a resonance peak in a limited range of chemical shifts. The peak of a nucleus can be split into a multiplet with (n + 1) components as a result of the spin-spin coupling between this nucleus and adjacent equivalent nuclei, where n is the number of neighbouring equivalent protons with the same coupling constant. If the nuclei are not equivalent, the peak will appear as a multiple-multiplet of 2^n components (Neri and Tringali, 2001). The relative intensity ratios of components in multiplets follow entries in Pascal's triangle. ^{1}H NMR spectra reveal information about types of hydrogens and the number of each in a molecule based on their chemical shifts, integration values and coupling constants. For instance, aromatic protons have resonance in the chemical shift range of 6-8 ppm, whereas protons bonded with sp^3 carbons have resonance in between 0 to 3 ppm. The proton of aldehyde group has resonance near 9 to 10 ppm and that of acid group is in

the 10-12 ppm region. In alkenes, protons that are *cis* to each other have ^{3}J coupling constants of 6-12 Hz, whilst those of *trans* protons are between 11 and 18 Hz. In aromatic compounds, *ortho* protons (^{3}J = 7-10 Hz) couple stronger than *meta* ones (^{3}J = 2-3 Hz) and *para* ones (^{3}J = 0-1 Hz) (Pavia et al., 2009).

4.3.3.1.2. Proton-decoupled ^{13}C NMR spectra

Spin-spin coupling between ^{13}C atoms are rarely observed but the spin-spin interaction of protons bonded directly to ^{13}C atoms can split the carbon signal responding to the n + 1 Rule. However proton-coupled ^{13}C NMR spectra of large molecules are too complicated to interpret because of overlap of the multiplets from different carbons. This is caused by the ^{13}C-H coupling constants being often larger than the chemical shift differences of the carbons. In proton-decoupled ^{13}C NMR spectra, overlapping multiplets are transformed into singlets and therefore, the spectra are easier to interpret. ^{13}C NMR spectra show information about the number and types of carbons and functional groups. For example, the carbonyl carbons of ketones often have resonance at low field of 180-220 ppm, whereas those of aldehydes and carboxylic acids have resonance near 170 to 190 ppm. The chemical shifts of aromatic and unsaturated carbons are in the 90-170 ppm region and those of saturated carbons are between 10 and 90 ppm (Pavia et al., 2009).

4.3.3.2. Two dimensional (2D) NMR spectra

2D NMR spectra are obtained by recording resonance signals as a function of two time variables and carrying out two Fourier transformations on a matrix of data. Therefore both of the horizontal and vertical axes in 2D NMR spectra are two chemical shift (frequency) axes. The 2D spectra give cross peaks showing correlations between the two axes and the data are displayed as a series of contours (Mitchell and Costisella, 2007).

4.3.3.2.1. ^{1}H-^{1}H COrrelation SpectroscopY (COSY)

A COSY spectrum shows homonuclear correlations between coupling protons in a molecule. The ^{1}H chemical shifts are thus plotted on both axes. The off-diagonal peaks

in the spectrum are the important peaks, whilst the diagonal peaks are only considered as reference points. The spectrum provides information on which proton couples with which one. It also indicates *H-H* connectivities, *gemical, vicinal* or long range couplings. Correlations can be observed for all protons except the hydroxylic proton, which is often rapidly exchanged in protonic solvents (Breitmaier, 2002).

4.3.3.2.2. Heteronuclear Single Quantum Correlation (HSQC) spectra

A HSQC spectrum displays the heteronuclear correlations of protons with ^{13}C atoms to which they are directly attached. Therefore the ^{1}H chemical shifts are plotted on an axe and the ^{13}C chemical shifts on the other one. All C-H single bonds of the molecule can be determined by the HSQC spectrum and hence it provides information on which protons are bonded to which carbons. Multiplets of protons in large molecules frequently overlap in ^{1}H NMR spectra and are difficult to be identified. HSQC spectra can help to determine these protons (Breitmaier, 2002).

4.3.3.2.3. Heteronuclear Multiple Bond Coherence (HMBC) spectra

The HSQC spectrum shows the ^{1}H-^{13}C correlations through a single bond ($^{1}J_{CH}$) and thereby is only applied to ^{13}C atoms which are attached by protons. The HMBC technique is the modification of HSQC technique. A HMBC spectrum is applicable to non-protonated or quaternary ^{13}C atoms to provide $^{2}J_{CH}$, $^{3}J_{CH}$ and $^{4}J_{CH}$ correlations. The HMBC spectrum is very useful to identify quaternary carbons which can not be observed in the ^{13}C NMR spectrum in some cases because of their low signal intensity (Breitmaier, 2002). The combination of data of COSY, HSQC and HMBC spectra produces essential information of skeletal connectivity on which structural fragments can be determined and then reassembled to accomplish the overall covalent structure of the substance.

4.3.3.2.4. Nuclear Overhauser Effect SpectroscopY (NOESY) and Rotating-frame Overhauser Effect SpectroscopY (ROESY) spectra

NOESY and ROESY spectra display the correlations of protons that are close to each other in space with the common distance of 4.5 Å or less. Spatial interactions

between protons can be obtained *via* many bonds. In these spectra, the ^{1}H chemical shifts are plotted on both axes like a COSY spectrum. NOESY and ROESY spectra are extremely helpful for the determination of relative stereochemistry in molecular structures (Silverstein et al., 2005).

4.3.4. Optical rotation

Optical activity is the ability of chiral substances to rotate the plane of polarisation of polarised light. Optical rotation is considered to be positive (+) for dextrorotatory substances, i.e. those that rotate the plane of polarisation in a clockwise direction and negative (-) for laevorotatory substances. The specific rotation of a chiral compound can be calculated from the observed angle of rotation according to the expression (Carey, 2001).

Formula 6: The calculation of specific rotation

$$[\alpha]_D^t = \frac{100.\alpha}{c.l}$$

$[\alpha]$ = specific rotation

α = angle of rotation observed by a polarimeter

c = concentration of the substance in g/100 ml

l = length of the polarimeter tube in decimetres

t = temperature of measurement (ºC)

D = D-line of sodium (λ = 589.3 nm)

In the present work, the optical rotations of new substances were obtained using a polarimeter of UniPol L1000 (Schmidt and Bonaduz). The samples were prepared for a concentration of 1 mg/ml in methanol and then dispensed in a polarimeter tube of 5 cm. The angle of rotation was observed at the wavelength of the D-line of sodium at 22 ± 0.5 ºC. Measurements were repeated at least five times and the final angle of rotations was obtained as an average value.

4.4. Biological assays

The cytotoxicity of new compounds was tested on HeLa cell line using MTT assay. Moreover anti-angiogenic activity was carried out with a bixanthone using proliferation assay on human microvascular endothelial cell line-1 (HMEC-1)

4.4.1. Preparation of samples

The samples were prepared as stock solutions with a concentration of 100 mM in dimethyl sulfoxide (DMSO) and stored at -20 ºC. For the assays, the final concentration of DMSO per well was not more than 1%.

4.4.2. Cytotoxicity assay on HeLa cells

The HeLa cell line was originated by Gey et al. in 1952 from cervical cells taken from Henrietta Lacks, a young cancer patient. These cells were the first human cells continuously grown in culture and widely used for the research of anti-cancer activity of natural or synthesis substances (Heneen, 1976). The cytotoxicity of new compounds was evaluated by the MTT assay on HeLa cells as described by Mosman (1983) and modified according to Heilmann et. al (2001).

4.4.2.1. Cell culture

HeLa cells were cultured in Minimum Essential Medium (MEM-Earle with 0.85 g/l $NaHCO_3$ of Biochrom) supplemented with 1% of L-glutamine (200 mM), 10% of fetal bovine serum and 1 % of non-essential amino acids at 37 ºC in a humidified 5 % CO_2 environment. For the passage of the cells, the medium was removed through a pasteur-pipette and the cells were washed with 5 ml of phosphate buffered saline (PBS). The cells were then incubated with 2 ml of trypsin/EDTA for 5 minutes to release from the bottom of flask. The cell suspension was centrifuged with 8 ml of the medium in a 15 ml centrifuge tube at a speed of 700 U/min at 20 ºC for 3 minutes. The upper solution was removed and the cells were homogenised with 7 ml of the medium. Afterwards 350 µl of the cell suspension was dispersed into a 50 ml culture flask containing 20 ml of the medium for growth. After 2-3 days, the old medium was changed by the same amount of fresh medium. The cells were split after 4-5 days.

4.4.2.2. Cell counting

The counting of cells was carried out using a haemocytometer of nine 1 x 1 mm squares. The cell suspension (10 µl) was mixed with 10 µl of Trypan Blue solution and then a volume of 10 µl of the mixture was applied to the chamber of haemocytometer. Trypan Blue is used to staining dead cells. The number of unstained cells in the four large corner squares was counted using a microscope. The number of cells per ml can be calculated as the following equation.

Formula 7: The calculation of the number of cells per ml

$$\textit{Cells per ml} = \textit{The average of cells per large square} \times \textit{Dilution factor} \times 10^4$$

In this study, the dilution factor is 2. Because the volume of each large square is 0.1 µl, the average of cells per large square must be multiplied with 10^4 to give the number of cells per millilitre.

4.4.2.3. MTT assay

The assays were carried out in 96-well plates and the number of cells per well was 9000 cells. Nine different concentrations of the samples were prepared in the medium with a volume of 75 µl per well and 75 µl of the cell suspension was then added to each well so that the total volume per well was 150 µl. The plates were incubated at 37 ºC in a humidified 5 % CO_2 environment for 72 hours. After incubation, 15 µl of an aqueous solution of methylthiazoyl tetrazolium bromide (MTT) was added into each well containing the cells and incubated for next 4 hours. MTT is reduced to a purple formazan by NADH of living cells. After incubation, the solution was removed and 150 µl of sodium dodecylsulfate (SDS) solution (10%) was added to each well. The plates were subsequently placed in the dark for 24 hours at the room temperature. The optical density was measured at 560 nm using a microplate reader (Tecan SpectraFlour Plus). For the determination of the IC_{50} values, the optical density was plotted against the log scale of the concentration. Each concentration was performed in hexaplicates and the assays have been repeated at least three times. Maximal observed standard deviation was about 15% (absolute).

4.4.3. Proliferation assay on HMEC-1

Vascular endothelial cells line the inside of blood vessel walls throughout the circulatory system to compose an interface between flowing blood and the vessel wall. They are involved in the formation of new blood vessels called angiogenesis. Angiogenesis has been shown as a target for the fight against diseases characterized by either poor vascularisation or abnormal vasculature. Compounds that inhibit or induce the formation of new blood vessels (angiogenesis inhibitors) can help to combat such diseases (Birbrair et al., 2014). The proliferation assay on human microvascular endothelial cell line-1 (HMEC-1) is (among others) an in vitro method to identify the anti-angiogenic activity of a substance. The HMEC-1 is derived from human foreskin and transfected by a gene of simian virus 40. These transfected cells have been created by Dr. Thomas Lawley and his team including members from the University of Emory and the Centers for Disease Control and Prevention (CDC), USA. They possess all of the characteristic makers of endothelial cells and can last from 25 to 27 generations without losing their genetic characteristics (Ades et al., 1992). The proliferation assay was carried out as described by Schmidt et al. (2012).

4.4.3.1. Cell culture

HMEC-1 was cultured in Endothelial Cell Growth Medium (ECGM, Provitro) supplemented with 10% of FCS and antibiotics at 37 ºC in a humidified 5 % CO2 environment. For the passage of the cells, the medium was removed through a pasteur-pipette and the cells were washed twice with 10 ml of phosphate buffered saline (PBS). The cells were then released by incubation with 4 ml of trypsin/EDTA for 5 min. The cell suspension was centrifuged with 6 ml of the medium in a 15 ml centrifuge tube at a speed of 1000 U/min at 20 ºC for 5 minutes. The upper solution was removed and the cells were homogenised with 10 ml of the medium. Afterwards 1 ml of the cell suspension was dispersed into a 50 ml culture flask containing 20 ml of the medium (ECGM) for growth.

4.4.3.2. Proliferation assay

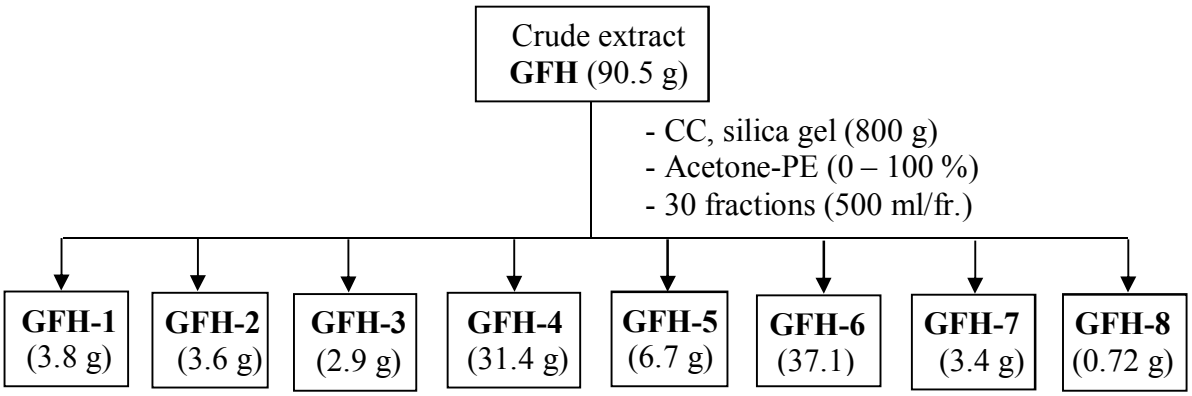
The assays were carried out in 96-well plates. The HMEC-1 cells were seeded in the plates coated with collagen (100 µl of the cell suspension per well) and the number of cells in each well was 1500 cells. The plates were incubated at 37 ºC in a 5% CO_2 atmosphere with constant humidity. After 24 hours, the medium in a reference plate was removed and the cells in this plate were stained with a crystal violet solution for 10 minutes in the dark at the room temperature to serve for the baseline. The cells in other plates were treated with 100 µl of the samples with various concentrations in the culture medium. After 72 h incubation, the solution was removed and the cells were stained with the crystal violet solution for 10 minutes in the dark at the room temperature. The plates were then washed with distilled water and 100 µl of 0.1 M sodium nitrate solution was added in each well. The optical density was measured at 560 nm using a microplate reader (Tecan SpectraFlour Plus). For the determination of the IC_{50} values, the optical density was plotted against the log concentration. Every test was performed at least in hexaplicates and the assays have been repeated at least three times. The maximal observed standard deviation was about 15% (absolute). Xanthohumol was used as positive control and pure solvent (0.1 % DMSO) was used as negative control.

5. RESULTS AND DISCUSSION

5.1. Experiments

5.1.1. Fractionation and isolation of the *n*-hexane extract

The *n*-hexane extract of the bark of *Garcinia fusca* (GFH, 90.5 g) was divided into two portions which were in turn applied to open column chromatography (CC) using a column of 10 x 40 cm dimension, 800 g of silica gel as stationary phase and a solvent system of acetone and petroleum ether (0-100%) as mobile phase to give 30 sub-fractions (500 ml of each). Identical fractions were combined on the basis of TLC to afford 8 main fractions which were shortly named GFH-1 - 8 as shown in **Scheme 17**.

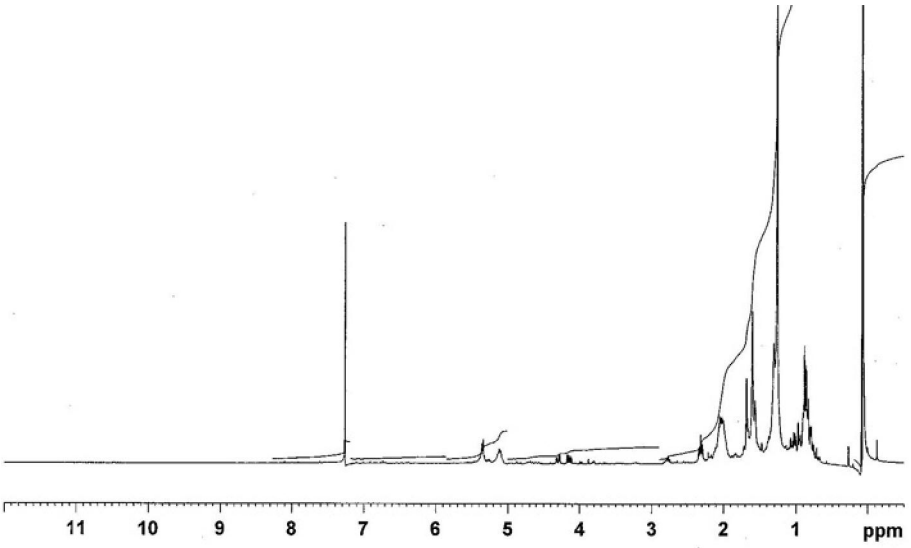


Scheme 17. Fractionation of the *n*-hexane extract (GFH)

The ^{1}H NMR spectra of the fractions (see **Figure 27 - 29**) revealed that the fractions from GFH-3 to GFH-7 contained prenylated xanthone derivatives based on typical signals at 13 - 14 ppm (hydrogen-bonded hydroxyl groups), 6 – 7.5 ppm (aromatic protons), 5 – 5.5 ppm (olefinic protons of prenyl groups), 4 – 4.2 ppm (methylene protons of prenyl substitutes at the *peri* position, C-8), 3.7 – 4 ppm (methoxyl groups and protons of oxygenated sp^3 carbons) and 1 – 2 ppm (vinyl methyls in prenyl chains). The fractions GFH-4 and GFH-5 comprised xanthones with *peri* prenyl groups because their spectra showed resonance peaks at 4 – 4.2 ppm. On the contrary, the absence of this signal in the spectrum of the fraction GFH-6 indicated that this fraction did not contain xanthones with prenyl groups at the *peri* position (C-8).

Moreover in the spectrum of the fraction GFH-7, there was a signal at 4.3 ppm due to the resonance of a deshielded proton of an oxygenated sp^3 carbon. On the basis of those spectral data and TLC, we chose fractions GFH-2, GFH-4, GFH-6 and GFH-7 for phytochemical investigation.

GFH-1



GFH-2

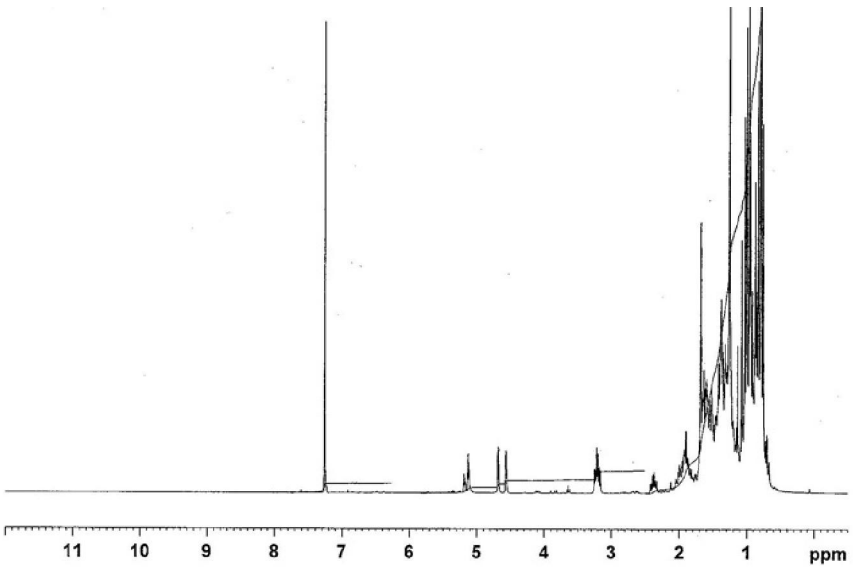


Figure 27. ^{1}H NMR spectra of fractions GFH-1 and GFH-2

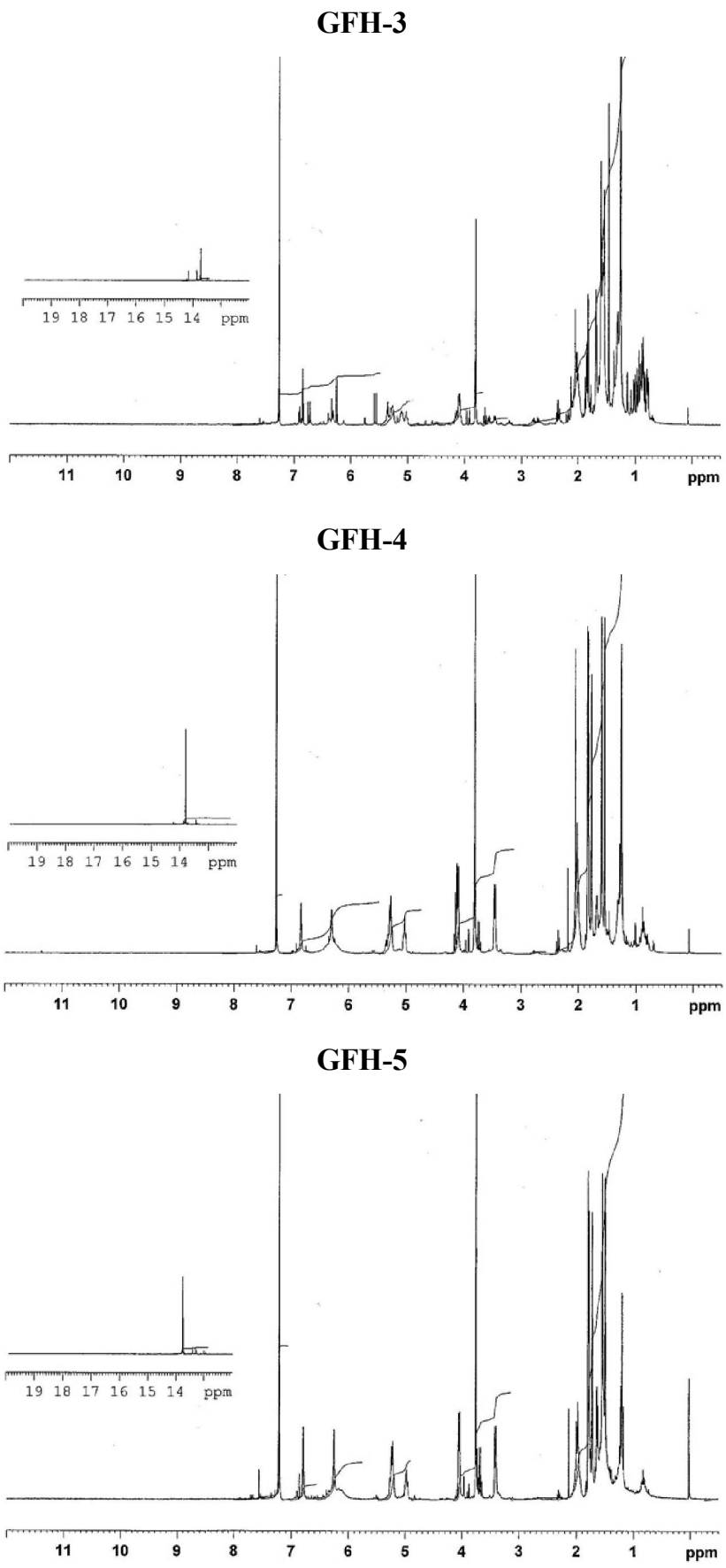
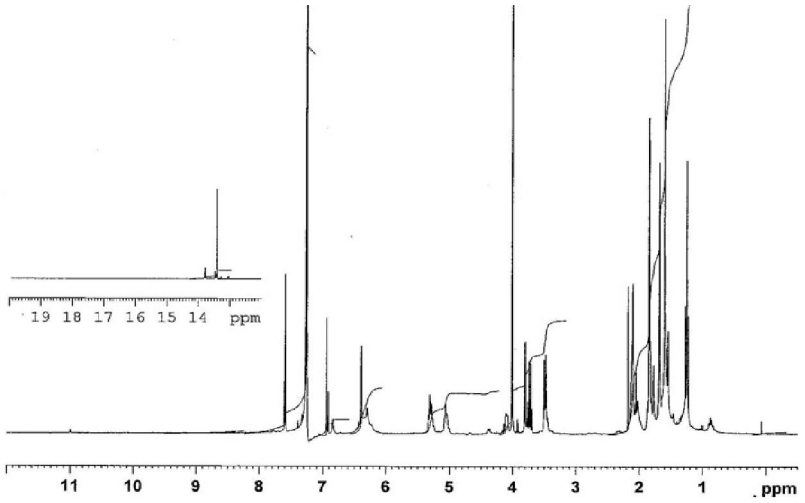
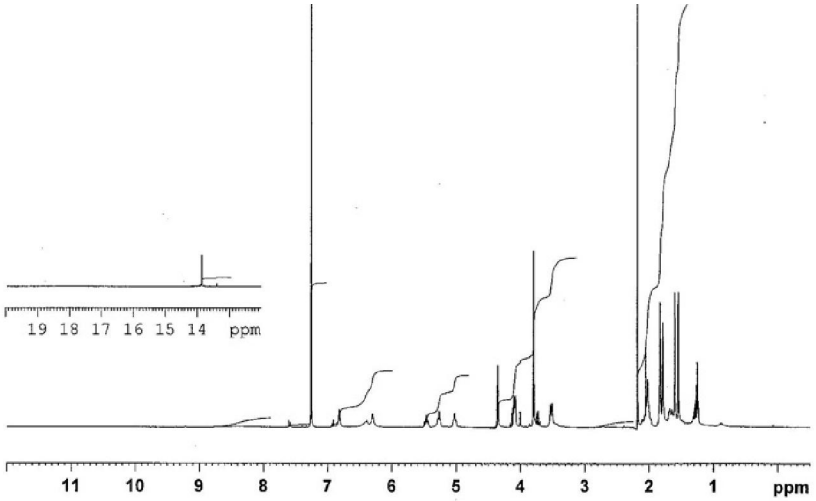


Figure 28. ^{1}H NMR spectra of fractions GFH-3 – 5

GFH-6



GFH-7



GFH-8

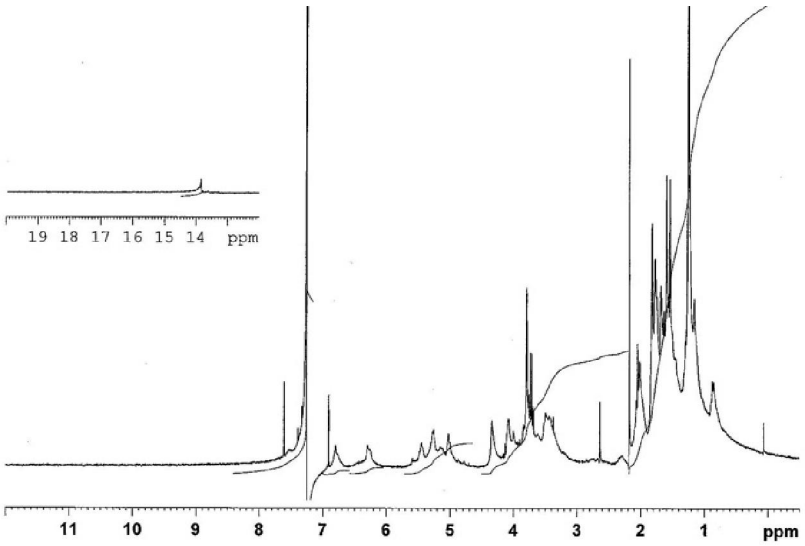
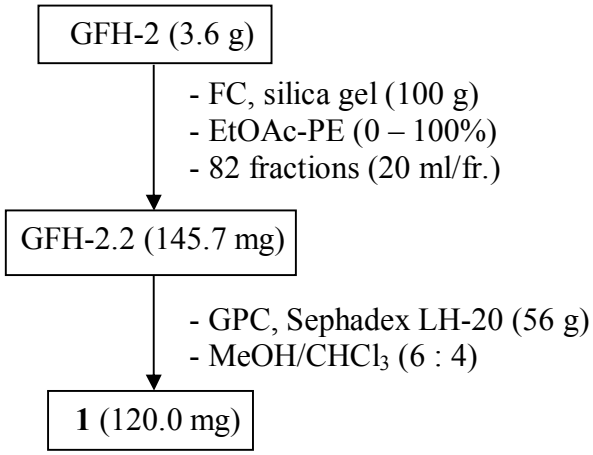


Figure 29. ^{1}H NMR spectra of fractions GFH-6 - 8

5.1.1.1. Isolation from fraction GFH-2

The fraction GFH-2 (3.6 g) was applied to FC (40 x 160 mm, 100 g of silica gel, 0 – 100 % EtOAc-PE, 30 ml/min) to yield 82 fractions (20 ml/fraction) which were combined on the basis of TLC to give 5 fractions (GFH-2.1 – 5). The fraction GFH-2.2 (145.7 mg) was then purified by GPC (2 x 70 cm, 23 g of Sephadex LH-20, MeOH/$CHCl_3$ 6 : 4) to give **1** (120.0 mg), see **Scheme 18**.

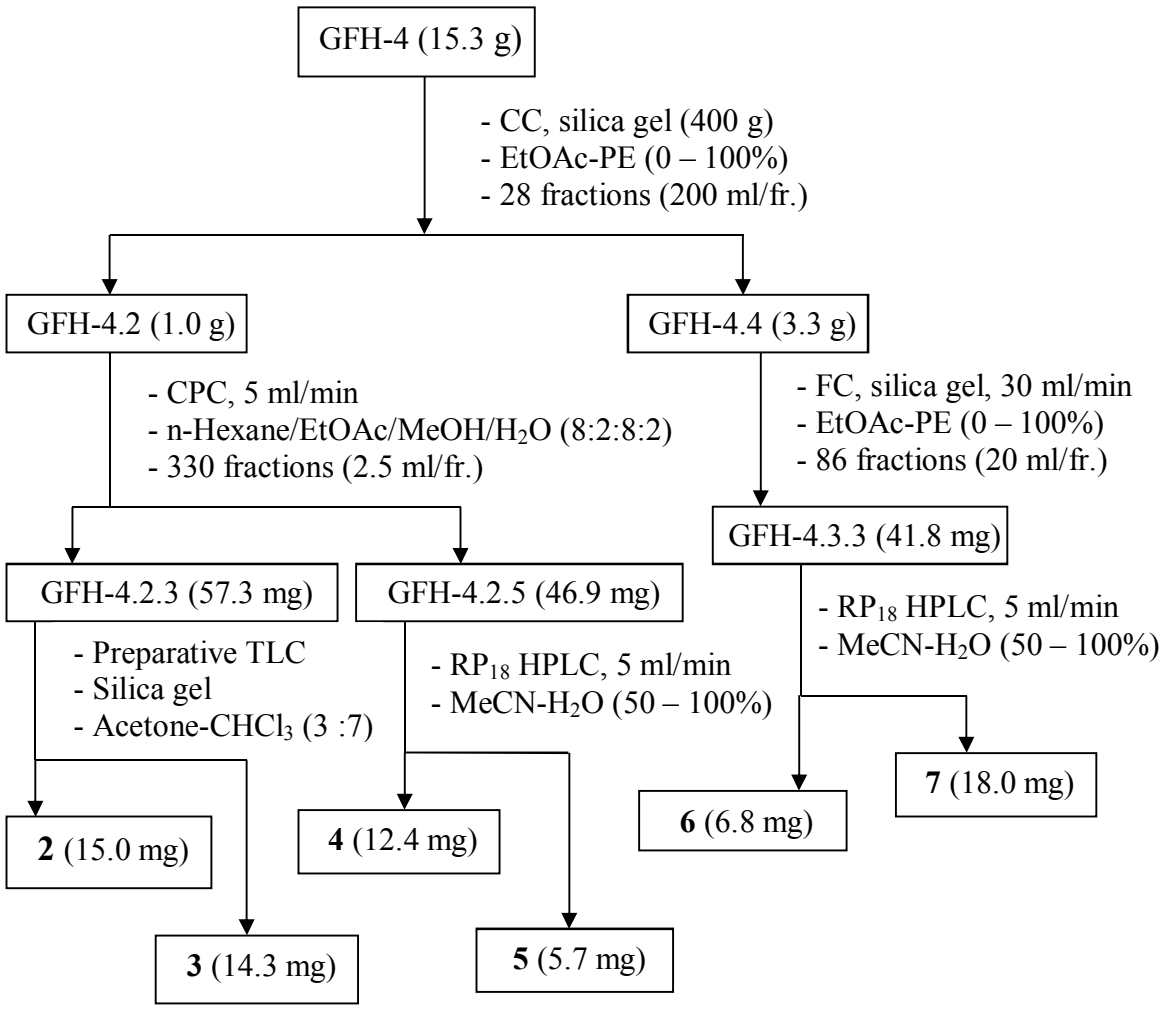


Scheme 18. Isolation of compound **1**

5.1.1.2. Isolation of xanthones from fraction GFH-4

The half of the fraction GFH-4 (15.3 g) was subjected to CC (5 x 50 cm, 400 g of silica gel, 0-100 % EtOAc-PE) to yield 28 fractions (250 ml/fraction) combined on the basis of TLC to give 4 fractions (GFH-4.1 - 5), see **Scheme 19**. An amount of 1.0 g of the fraction GFH-4.2 was further applied to CPC (*n*-hexane/EtOAc/MeOH/H_2O 8:2:8:2, 5 ml/min) to afford 330 fractions (2.5 ml/fraction) combined by relying on TLC to give 7 fractions (GFH-4.2.1 - 7). The fraction GFH-4.2.3 (57.3 mg) was then separated by preparative TLC (2 mm, silica gel, acetone/$CHCl_3$ 3 : 7) to yield **2** (15.0 mg) and **3** (14.3 mg). The fraction GFH-4.2.5 (46.9 mg) was similarly applied to semi-preparative HPLC to produce **4** (12.4 mg) and **5** (5.7 mg). The fraction GFH-4.4 (3.3 g) was further chromatographed by FC (40 x 160 mm, 100 g of silica gel, 0 – 100 % EtOAc-PE, 30 ml/min) to afford 86 fractions (20 ml/fraction) combined on the basis of TLC to give 4 fractions (GFH-4.3.1 - 4). Semi-preparative HPLC (21.4 x 250 mm, RP-

$_{18}$ silica, 50 - 100% MeCN-H_2O, 10 ml/min) of GFH-4.3.2 (41.8 mg) furnished **6** (6.8 mg) and **7** (18.0 mg).

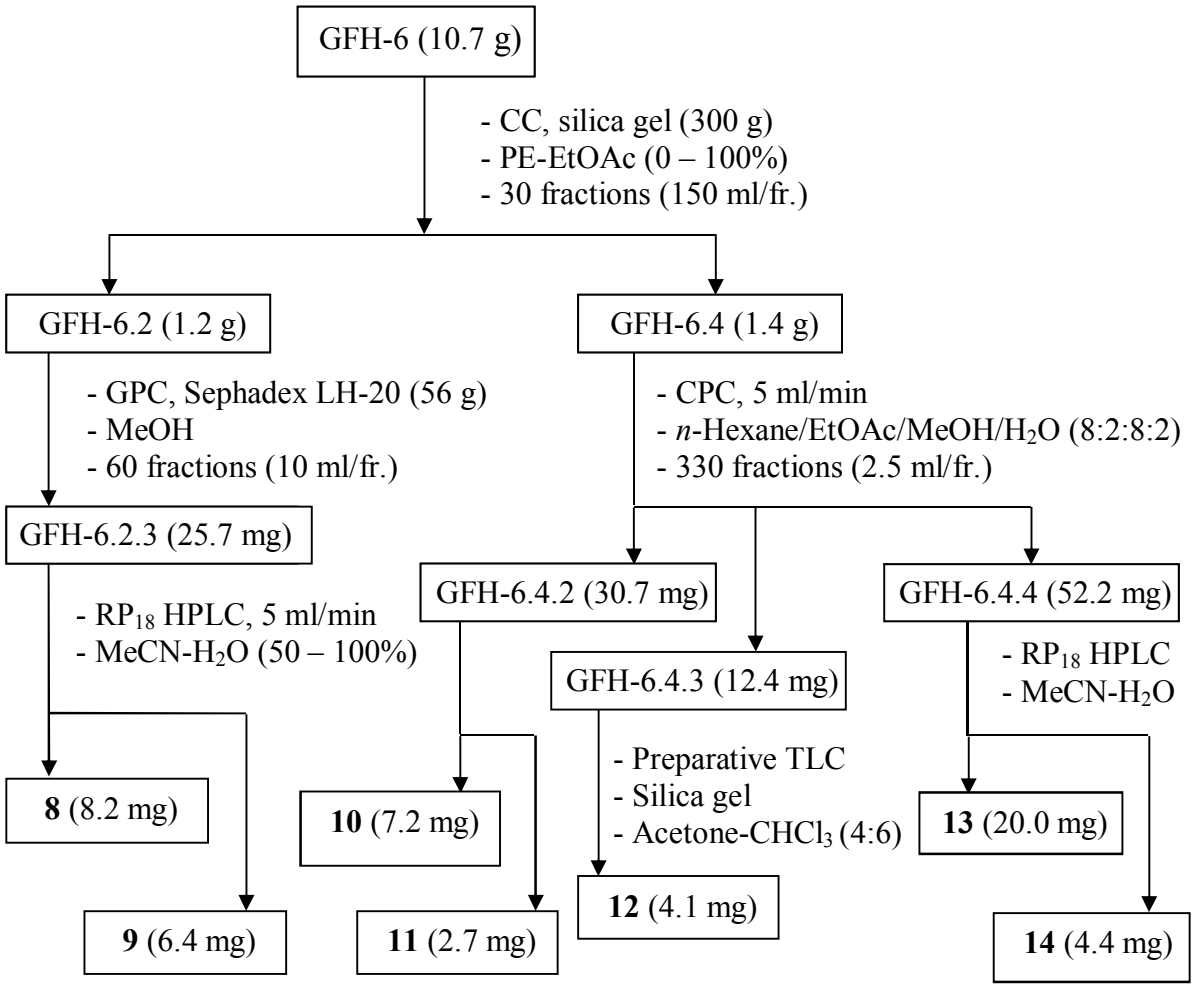


Scheme 19. Isolation of xanthones from fraction GFH-4

5.1.1.3. Isolation from fraction GFH-6

An amount of 10.7 g of GFH-6 was fractionated by CC (5 x 50 cm, 300 g of silica gel, 0-100 % acetone-PE) to produce 36 fractions (150 ml/fraction) combined on the basis of TLC similarities to give 6 fractions (GFH-6.1 – 6). Fraction GFH-6.2 (1.2 g) was purified by GPC (5 x 70 cm, 56 g of Sephadex LH-20, MeOH/$CHCl_3$ 6 : 4) and further subjected to semi-preparative HPLC (21.4 x 250 mm, RP_{18} silica, 50 - 100% MeCN-H_2O, 10 ml/min) to give **8** (8.2 mg) and **9** (6.4 mg). Besides the fraction GFH-6.4 (1.4 g) was chromatographed by CPC (*n*-hexane/EtOAc/MeOH/H_2O 8:2:8:2, 5

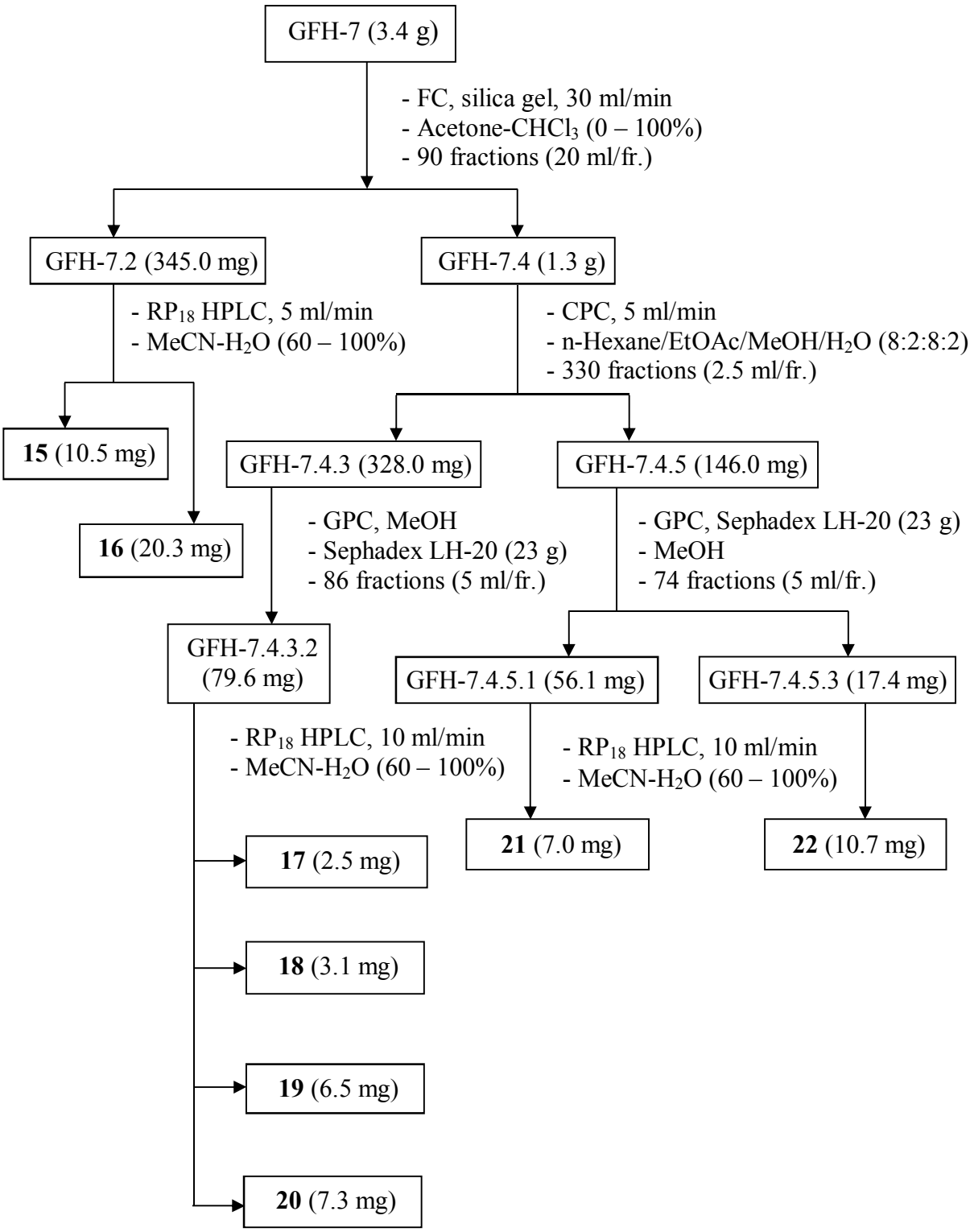
ml/min) to afford 330 fractions (2.5 ml/fraction) combined on the basis of TLC similarities to give 5 fractions (GFH-6.4.1 – 5). The separation of fraction GFH-6.4.3 (12.4 mg) using preparative TLC (2 mm, silica gel, acetone/$CHCl_3$ 4:6) obtained **10** (4.1 mg). The fraction GFH-6.4.2 (30.7 mg) was purified by semi-preparative HPLC (21.4 x 250 mm, RP_{18} silica, 50 - 100% MeCN-H_2O, 10 ml/min) to furnish **11** (7.2 mg) and **12** (2.7 mg). Similarly, semi-preparative HPLC of GFH-6.4.4 (52.2 mg) yielded **13** (20.0 mg) and **14** (4.4 mg), see **Scheme 20**.



Scheme 20. Isolation of compounds from fraction GFH-6

5.1.1.4. Isolation of xanthones from fraction GFH-7

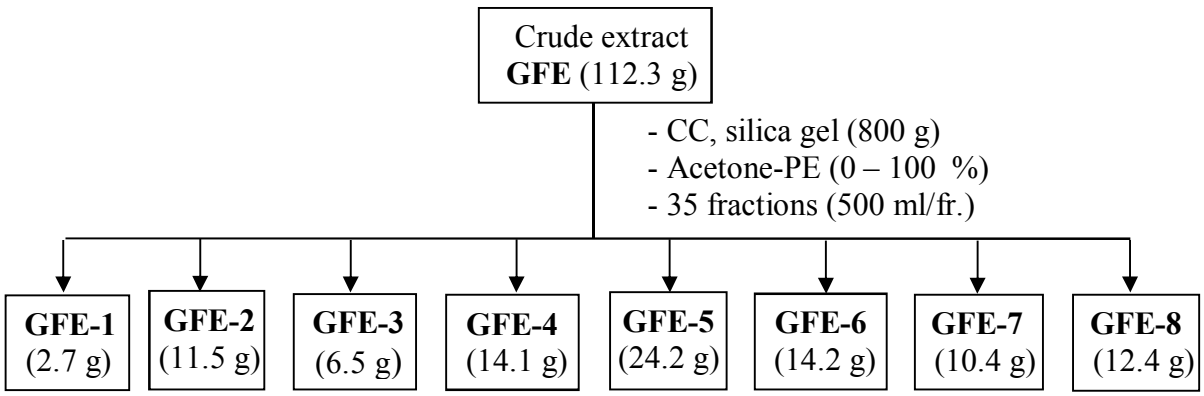
FC (40 x 160 mm, 100 g of silica gel, 0 – 100% acetone-$CHCl_3$, 30 ml/min) of the fraction GFH-7 (3.4 g) produced 90 fractions (20 ml/fraction) combined by relying on TLC to give 5 fractions (GFH-7.1 - 5). The fraction GFH-7.2 (345.0 mg) was purified by semi-preparative HPLC (21.4 x 250 mm, RP_{18} silica, 60 - 100% MeCN-H_2O, 10 ml/min) to yield **15** (10.5 mg) and **16** (20.3 mg). The fraction GFH-7.4 (1.3 g) was further chromatographed by CPC (*n*-hexane/EtOAc/MeOH/H_2O 8:2:8:2, 5 ml/min) to afford 330 fractions (2.5 ml/fraction) combined on the basis of TLC to give 6 fractions (GFH-7.4.1 – 6). GPC (2 x 70 cm, 23 g of Sephadex LH-20, MeOH) of GFH-7.4.3 (328.0 mg) produced 86 fractions (5 ml/fraction) combined by relying on TLC to give 3 fractions (GFH-7.4.3.1 – 3). Semi-preparative HPLC (21.4 x 250 mm, RP_{18} silica, 60 - 100% MeCN-H_2O, 10 ml/min) of GFH-7.4.3.2 (79.6 mg) to furnish **17** (2.5 mg), **18** (3.1 mg), **19** (6.5 mg) and **20** (7.3 mg). The fraction GFH-7.4.5 (146.0 mg) was separated by GPC (2 x 70 cm, 23 g of Sephadex LH-20, MeOH) to produce 74 fractions (5 ml/fraction) combined on the basis of TLC to give 3 fractions (GFH-7.4.5.1 - 3). The purification of GFH-7.4.5.1 (56.1 mg) using semi-preparative HPLC (21.4 x 250 mm, RP_{18} silica, 60 - 100% MeCN-H_2O, 10 ml/min) yielded **21** (7.0 mg), whereas GFH-7.4.5.3 (17.4 mg) was similarly purified by semi-preparative HPLC to obtain **22** (10.7 mg), see **Scheme 21**.



Scheme 21. Isolation of xanthones from fraction GFH-7

5.1.2. Fractionation of the ethyl acetate extract and further isolation

The ethyl acetate extract of *Garcinia fusca* (GFE, 112.3 g) was split into two portions which was in turn applied to CC (10 x 40 cm, 800 g of silica gel, 0 – 100% acetone-PE) to produce 35 sub-fractions (500 ml of each). The combination of identical fractions was based on TLC to give 8 main fractions (GFE1 - 8) (**Scheme 22**).



Scheme 22. Fractionation of the ethyl acetate extract (GFE)

The ^{1}H NMR spectra of fractions (see **Figure 30 - 31**) indicated that the fractions GFE-3 and GFE-4 contained prenylated xanthone derivatives based on typical signals at 13.9 ppm (hydrogen-bonded hydroxyl groups), 6 – 7.5 ppm (aromatic protons), 5 – 5.5 ppm (olefinic protons of prenyl groups), 4.1 ppm (methylene protons of prenyl substitutes at the *peri* position, C-8), 3.8 ppm (methoxyl groups and protons of oxygenated sp^3 carbons) and 1 – 2 ppm (tertiary methyls in prenyl chains). On the other hand, the spectra of fraction GFE-4 also showed a signal at 4.3 ppm due to the resonance of deshielded proton of an oxygenated sp^3 carbon. The spectra of the fractions from GFE-5 to GFE-7 showed the characteristic signals of biflavonoids at 11.5 – 12.0 ppm and 12.5 – 14.0 ppm (hydroxyl groups in hydrogen bonds), 6 - 8 ppm (aromatic and olefinic protons), 5.5 – 6. ppm (deshielded protons of oxygenated sp^3 carbons) and 4.5 -5.2 (deshielded protons of tertiary carbons). On the basis of those spectral data and TLC, we chose fractions GFE-4 and GFE-6 for phytochemical investigation.

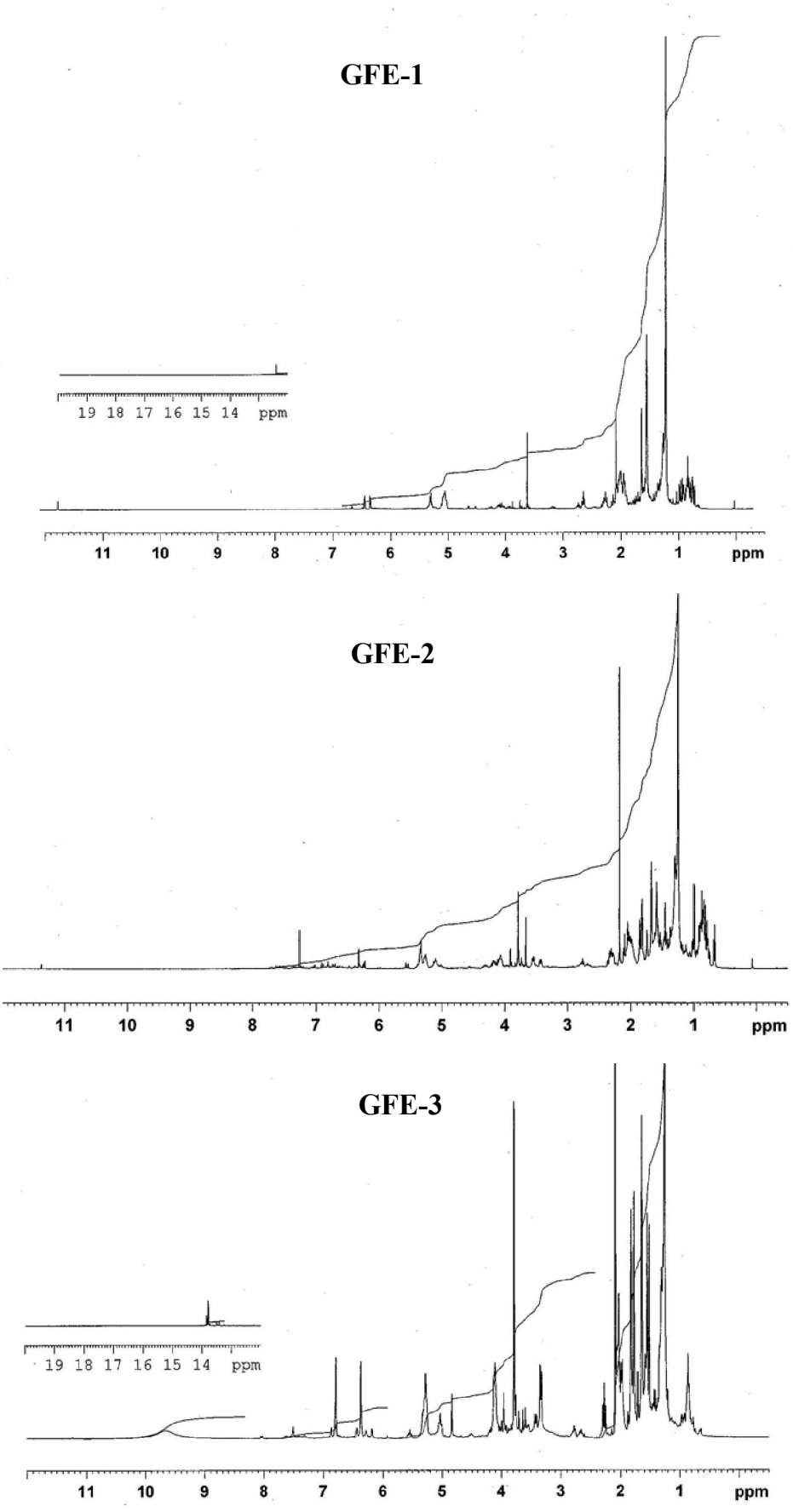


Figure 30. ^{1}H NMR spectra of fractions GFE-1 – 3

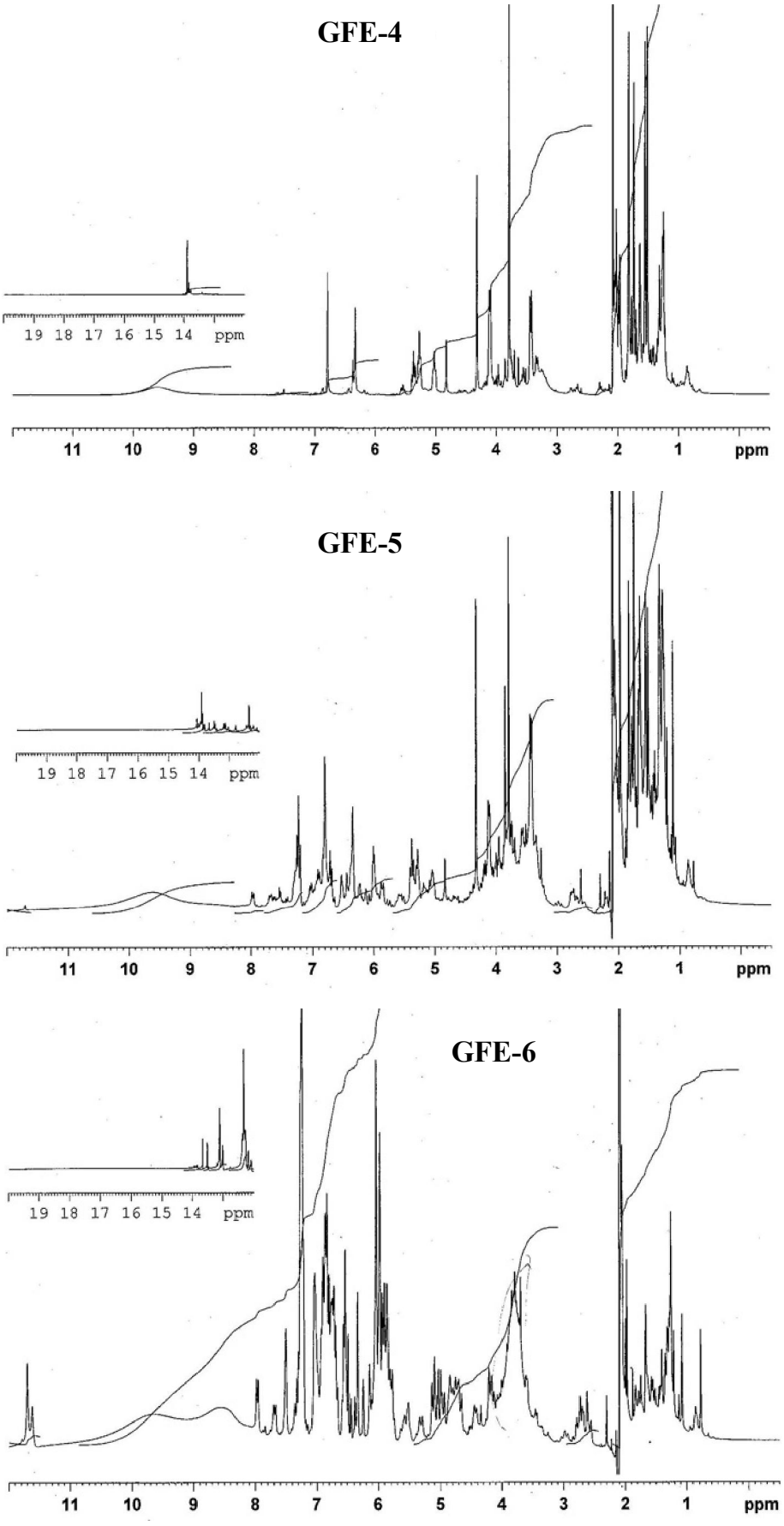


Figure 31. ^{1}H NMR spectra of fractions GFE-4 – 6

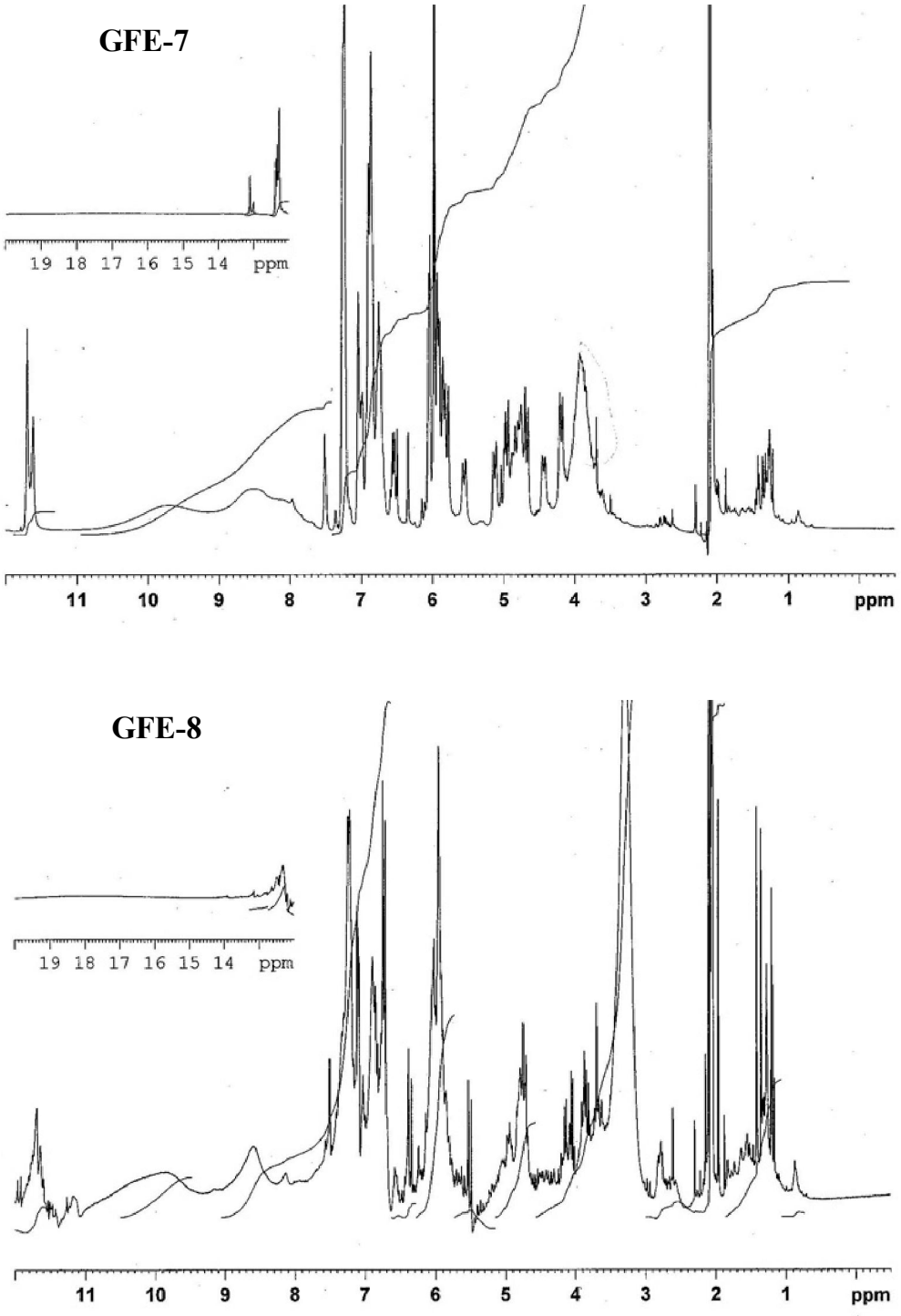
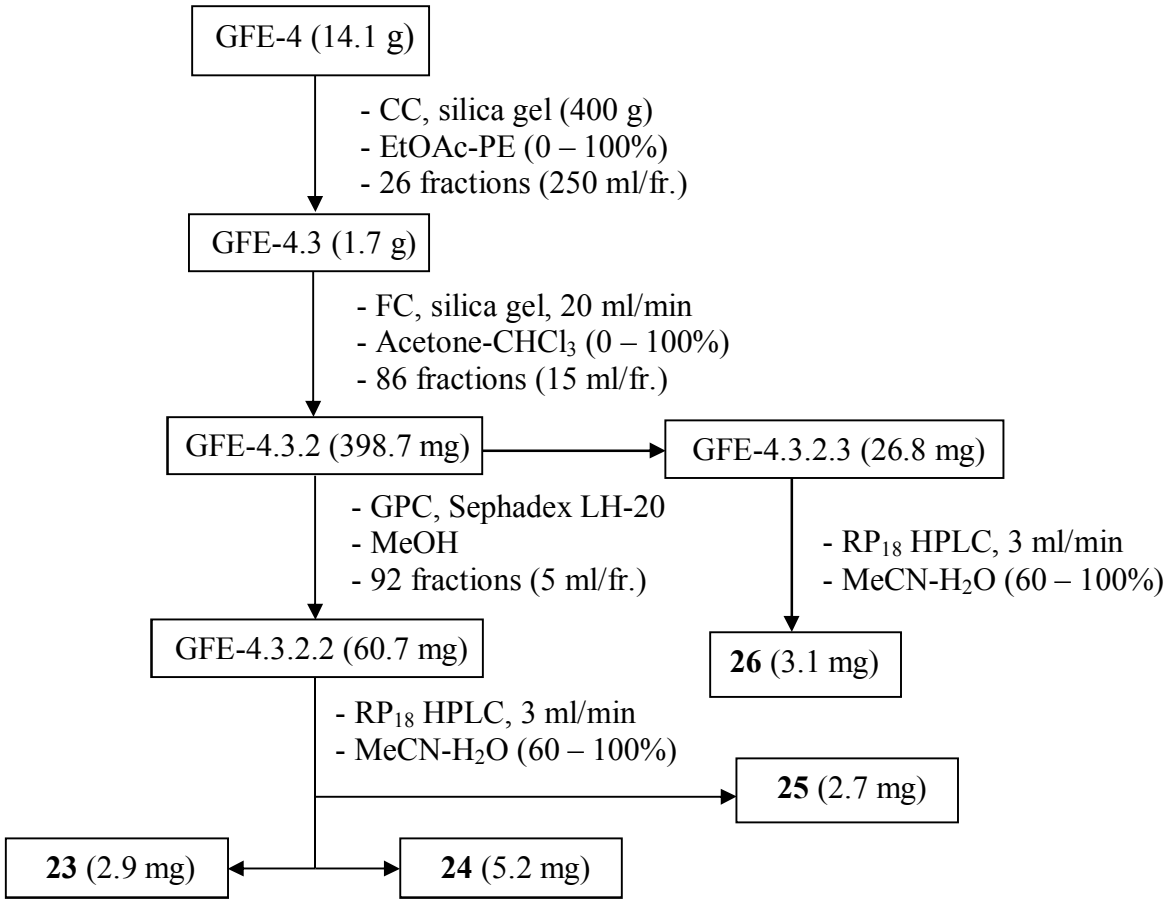


Figure 32. ^{1}H NMR spectra of fractions GFE-7 and GFE-8

5.1.2.1. Isolation of xanthones from fraction GFE-4

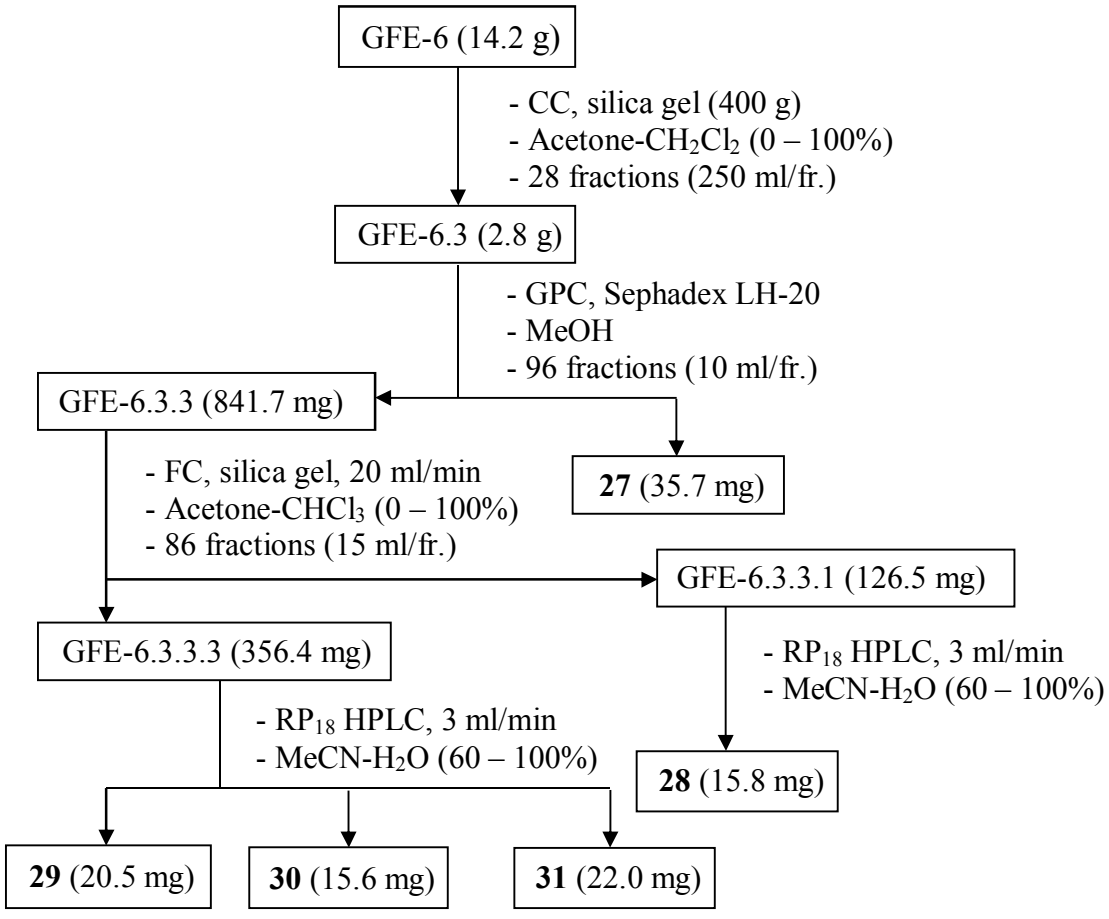
CC (5 x 50 cm, 400 g of silica gel, 0-100% acetone-PE) of the fraction GFE-4 (14.1 g) produced 26 fractions (250 ml/fraction) combined by relying on TLC to give 6 fractions (GFE-4.1 – 6). The fraction GFE-4.2 (1.7 g) was further applied to FC (40 x 160 mm, 80 g of silica gel, 0 – 100% acetone-$CHCl_3$, 20 ml/min) to afford 86 fractions (15 ml/fraction) combined on the basis of TLC to give 5 fractions (GFE-4.3.1 - 5). GPC (2 x 70 cm, 23 g of Sephadex LH-20, MeOH) of GFE-4.3.2 (398.7 mg) yielded 92 fractions (5 ml/fraction) combined by relying on TLC to give 3 fractions (GFE-4.3.2.1 – 3). The repeated purification of fraction GFE-4.3.2.2 (60.7 mg) using semi-preparative HPLC (9.4 x 250 mm, RP18 silica, 60 - 100% MeCN-H_2O, 3 ml/min) furnished **23** (2.9 mg), **24** (5.2 mg)) and **25** (2.7 mg). Moreover 26.8 mg of the fraction GFE-4.3.2.3 was purified by semi-preparative HPLC (9.4 x 250 mm, RP18 silica, 60 - 100% MeCN-H_2O, 3 ml/min) to obtain **26** (3.1 mg), see **Scheme 23**.



Scheme 23. Isolation of xanthones from fraction GFE-4

5.1.2.2. Isolation from fraction GFE-6

The fraction GFE-6 (14.2) was applied to CC (5 x 50 cm, 400 g of silica gel, 0-100% acetone-CH_2Cl_2) of the fraction GFE-4 (14.1 g) to afford 28 fractions (250 ml/fraction) combined on the basis TLC to give 5 fractions (GFE-6.1 – 5). GPC (5 x 70 cm, 56 g of Sephadex LH-20, MeOH) of the fraction GFE-6.3 (2.8 g) produced 96 fractions (10 ml/fraction) combined by relying on TLC to give 6 fractions (GFE-6.3.1 – 6). The fraction GFE-6.3.4 was **27** (35.7 mg). the fraction GFE-6.3.3 (841.7 mg) was further chromatographed by FC (40 x 160 mm, 35 g silica gel, 10 ml/min) to yield 60 fractions (10 ml/fraction) combined on the basis of TLC to give 3 fractions (GFE-6.3.3.1 – 3). GFE-6.3.3.1 (126.5 mg) was purified using semi-preparative HPLC to obtain **28** (15.8 mg). The repeated purification of the fraction GFE-6.3.3.3 (356.4 mg) using semi-preparative HPLC (9.4 x 250 mm, RP_{18} silica, 60 - 100% MeCN-H_2O, 3 ml/min) furnished **29** (20.5), **30** (15.6) and **31** (22.0), see **Scheme 24**.



Scheme 24. Isolation of compounds from fraction GFE-6

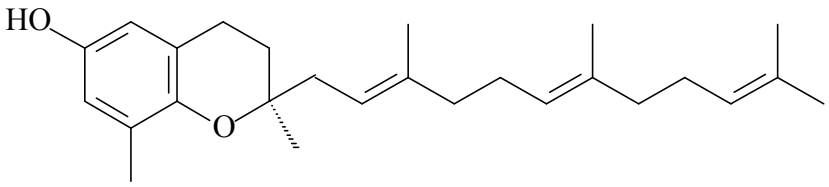
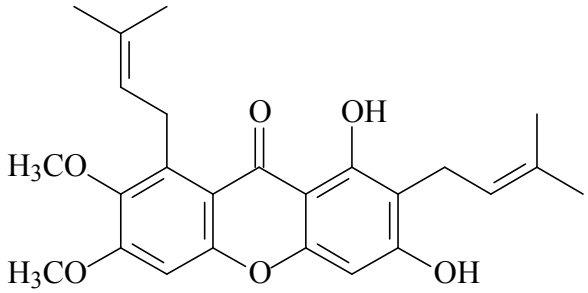
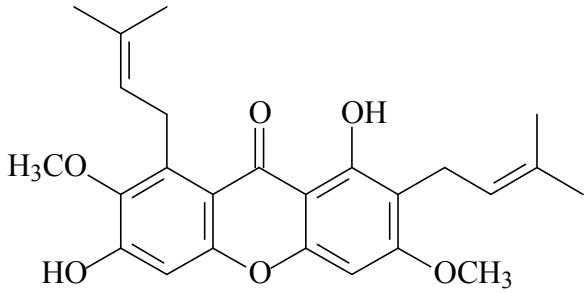
5.2. Structural elucidation of isolated substances

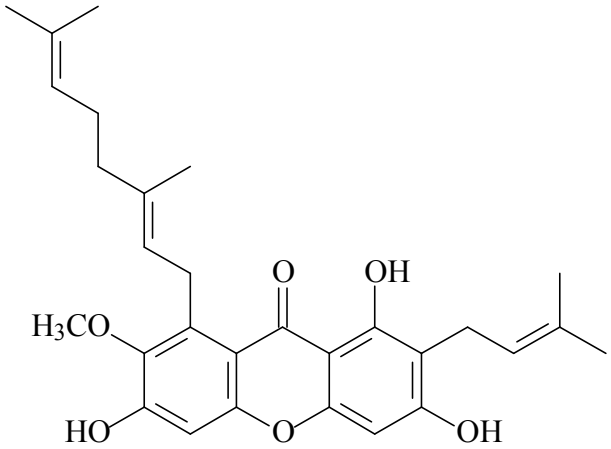
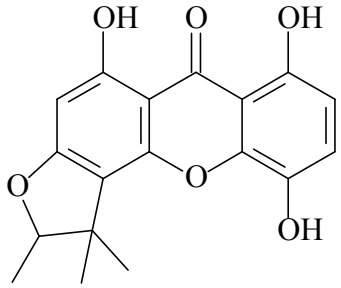
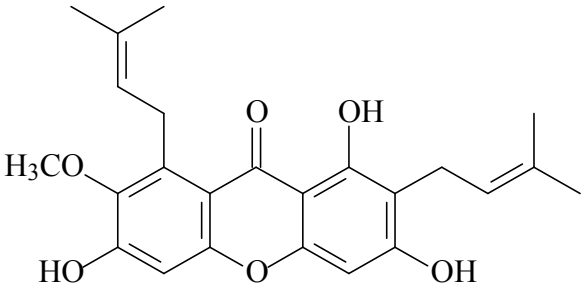
5.2.1. Structural identification of substances from the *n*-hexane extract

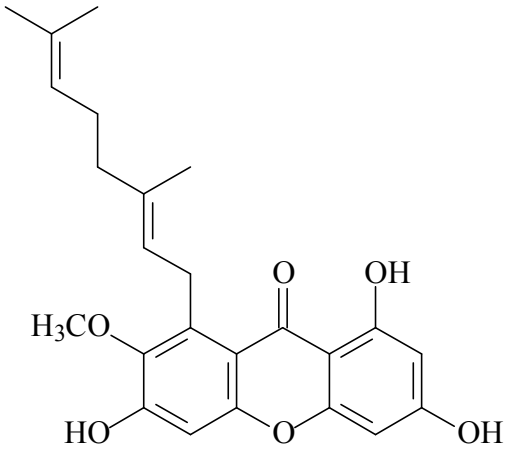
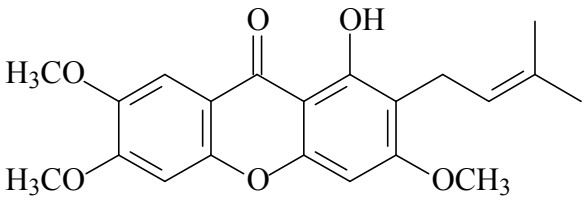
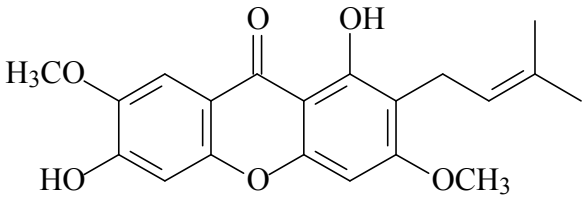
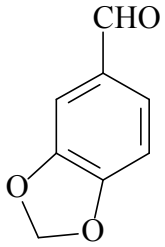
Thin layer and column chromatography as well as semi-preparative HPLC of the *n*-hexane extract resulted in the isolation of twenty two substances. On the basis of NMR spectra data (see **Appendices 1 – 17**) and comparison with those of literatures, the structures of seventeen known compounds were determined as shown in **Table 4**. Compound **8** was previously reported as a semi-synthetic xanthone. Five other substances (**18 – 22**) have been first isolated and their structures were elucidated by relying on the data of UV, 1D and 2D NMR, mass spectrometry and optical rotation.

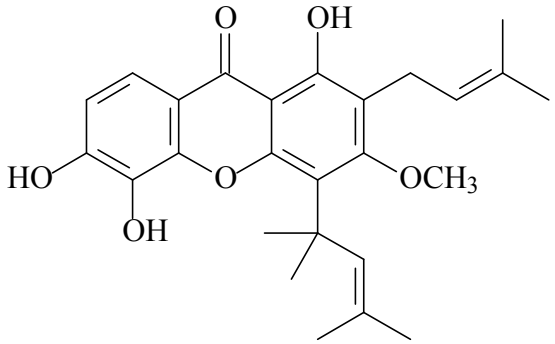
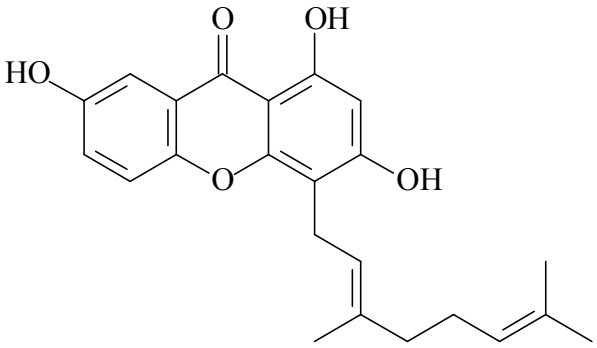
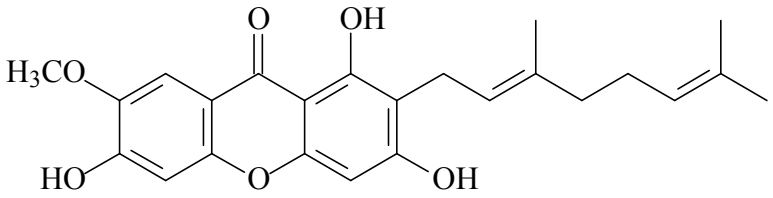
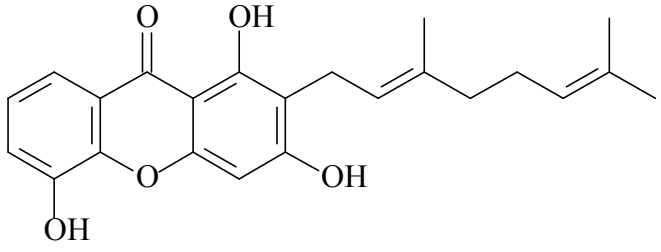
In general, all the new compounds (**18 - 22**) gave positive test with $FeCl_3$ in methanol, which revealed their phenolic nature. The UV spectra of these compounds displayed four maxima at 213 - 220, 242 - 244, 314 - 318 and 356 - 360 nm, which are similar to those of 1,3,6,7-tetraoxygenated xanthones (Iinuma et al., 1996; Nilar and Harrison, 2002). The ^{1}H and ^{13}C NMR spectra of these compounds indicated that they are prenylated xanthone derivatives containing a hydrogen-bonded hydroxyl group at δ_H 13.72 – 14.08 with the appropriate interacting carbonyl carbon at δ_C 181.7 – 183.0, two isolated aromatic protons at δ_H 6.79 – 6.83 and 6.23 – 6.36 and a methoxyl group at δ_H 3.78 – 3.83 (except for **21**). Besides the ^{1}H and ^{13}C NMR spectra also showed the presence of geranyl and isoprenyl side chains, whose benzylic methylene protons are deshielded by the adjacent carbonyl group. This allowed the placement of these side chains to the *peri* position (C-8) (Xu et al., 2001; Nilar and Harrison, 2002).

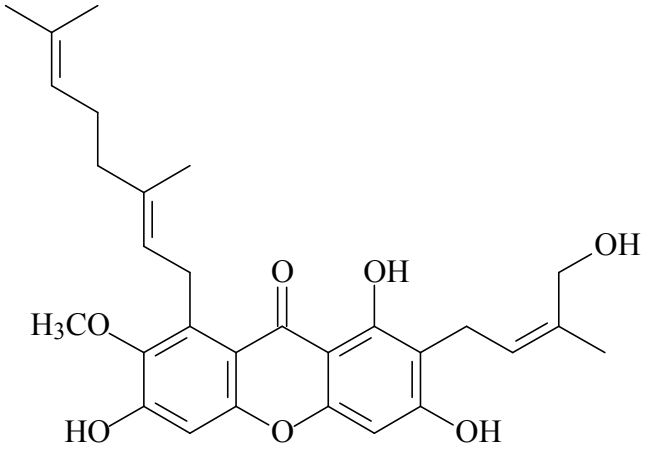
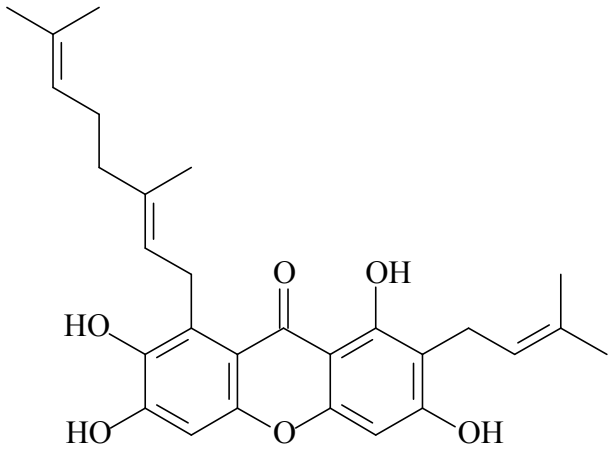
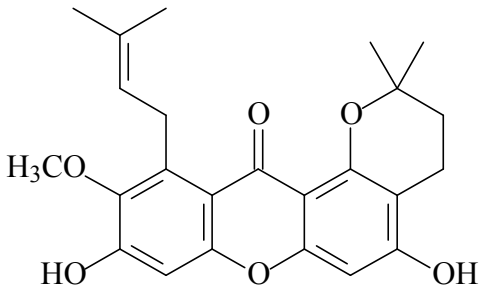
Table 4. The structure of substances isolated from the *n*-hexane extract

Isolated compounds	Structure
1	 δ-Tocotrienol (Ohnmacht et al., 2007)
2	 1,3-Dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone (Pattanaprateeb et al., 2004)
3	 β-Mangostin (Mahaburarakam et al., 1987)

4	 Cowanin (Na Pattalung et al., 1994)
5	 Garbogiol (Iinuma et al., 1997)
6	 α-Mangostin (Mahaburarakam et al., 1987)

7	 Rubraxanthone (Ampofo and Waterman, 1986)
8	 1-Hydroxy-3,6,7-trimethoxy-2-(3-methyl-but-2-enyl)-xanthone (Nilar and Harrison, 2002)
9	 1,6-Dihydroxy-3,7-dimethoxy-2-(3-methyl-but-2-enyl)-xanthone (Nilar and Harrison, 2002)
10	 Piperaldehyde

11	 Nigrolineaxanthone E (Rukachaisirikul et al., 2003)
12	 Fuscaxanthone E (Ito et al., 2003)
13	 Cowaxanthone (Na Pattalung et al., 1994)
14	 Mangostinone (Asai et al., 1995)

15	 Cowanol (Na Pattalung et al., 1994)
16	 Norcowanin (Na Pattalung et al., 1994)
17	 1-Isomangostin (Mahabusarakam et al., 1987)

5.2.1.1. Compound 21 (fuscaxanthone J)

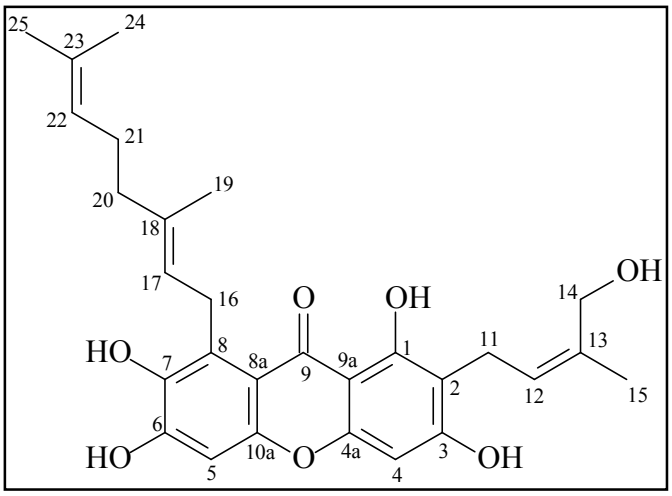


Figure 33. Structure of fuscaxanthone J (**21**)

Compound **21** was isolated as a yellow oil. The molecular formula was established as $C_{28}H_{32}O_7$ by HRESIMS (*m/z* 481.2217 $[M+H]^+$). The ^1H and ^{13}C NMR spectra of **21** (see **Figure 34**; **Table 5** and **6**) contained signals for a hydrogen-bonded hydroxyl group [δ_H 14.08 (1H, s, 1-OH)] with the interacting carbonyl carbon [δ_C 183.0 (C-9)], two isolated aromatic protons [δ_H 6.81 (1H, s, H-5) and 6.35 (1H, s, H-4); δ_C 101.0 (C-5) and 93.3 (C-4)], a geranyl group [δ_H 5.34 (1H, m like t, J = 8.0 Hz, H-22), 5.06 (1H, m like t, J = 6.8 Hz, H-17), 4.21 (2H, d, J = 6.8 Hz, H_2-16), 2.05 (2H, m, H_2-21), 1.97 (2H, m, H_2-20), 1.86 (3H, d, J = 0.8 Hz, H_3-19), 1.57 (3H, d, J = 0.8 Hz, H_3-24) and 1.53 (3H, s, H_3-25); δ_C 135.0 (C-18), 131.4 (C-23), 125.2 (C-22), 124.4 (C-17), 40.6 (C-20), 27.4 (C-21), 26.2 (C-16), 25.7 (C-24), 17.6 (C-25) and 16.6 (C-19)] and an isoprenyl group [δ_H 5.37 (1H, m like t, J = 8.0 Hz, H-12), 4.30 (2H, s, H_2-14), 3.45 (2H, d, J = 8.0 Hz, H_2-11) and 1.73 (3H, d, J = 1.2 Hz, H_3-15); δ_C 135.7 (C-13), 125.4 (C-12), 61.7 (C-14), 22.0 (C-15) and 21.7 (C-11)], in which one of two vinyl methyl groups is hydroxylated to form a 4-hydroxy-3-methylbut-2-enyl group. This was confirmed by the HMBC correlations of the protons of the oxygenated vinyl methylene group at δ_H 4.30 with two olefinic carbons (δ_H 135.7, C-13 and 125.4, C-14) and the vinyl methyl carbon (δ_C 22.0, C-15). The ^{13}C NMR spectra also showed resonances due to ten substituted aromatic carbons, six of which are oxygenated (δ_C 152.6 – 162.7). The ^1H and ^{13}C NMR data of **21** are the same as those of cowanol,

which has previously reported from *G. cowa* (Na Pattalung et al., 1994). However there is a difference regarding the absence of the signal for a methoxyl group.

In the HMBC spectrum, the chelated hydroxyl proton (δ_H 14.08, 1-OH) correlated with an oxygenated aromatic carbon (δ_C 161.2) and two substituted aromatic carbons (δ_C 109.7 and 103.7). The isolated aromatic proton (δ_H 6.35) gave cross-peaks with two latter carbons and two oxygenated aromatic carbons (δ_C 162.7 and 155.9). On the other hand, the benzylic methylene protons (δ_H 3.45) had correlations with a substituted aromatic carbon at δ_C 109.7 and two oxygenated aromatic carbons (δ_C 161.2 and 162.7). These HMBC data revealed that the oxygenated aromatic carbon at δ_C 161.2 is C-1, C-3 is oxygenated (δ_C 162.7), C-4a is the carbon at δ_C 155.9, C-4 is protonated (δ_C 93.3) and the 4-hydroxy-3-methylbut-2-enyl group is attached to C-2 (δ_C 109.7). In the xanthone B ring, two benzylic methylene protons at δ_H 4.21 (δ_C 26.2) exhibited a less shielding by the adjacent carbonyl group, i.e., the geranyl group is placed at C-8. Furthermore these protons gave HMBC correlations with an oxygenated aromatic carbon (δ_C 141.7) and two substituted aromatic carbons (δ_C 128.9 and 111.9). Meanwhile, the isolated aromatic proton at δ_H 6.81 (δ_C 101.0) showed cross-peaks to a substituted aromatic carbon at δ_C 111.9 and three oxygenated aromatic carbons (δ_C 153.6, 152.6 and 141.7). Thus, the substituted aromatic carbon at δ_C 128.9 is C-8, bearing the geranyl group and the other one is C-8a. Three oxygenated aromatic carbons are C-6, C-10a and C-7, respectively. The isolated aromatic proton is bonded to C-5. The NOESY correlations between H-14/H-11 and H-15/H-12 indicated that the double bond of the isoprenyl group has the *Z*-configuration. The compound **21** was identified as 1,2,6,7-tetrahydroxy-2-(*Z*)-(4-hydroxy-3-methylbut-2-enyl)-8-(3,7-dimethylocta-2,6-dienyl)xanthen-9-one and was given the trivial name fuscaxanthone J.

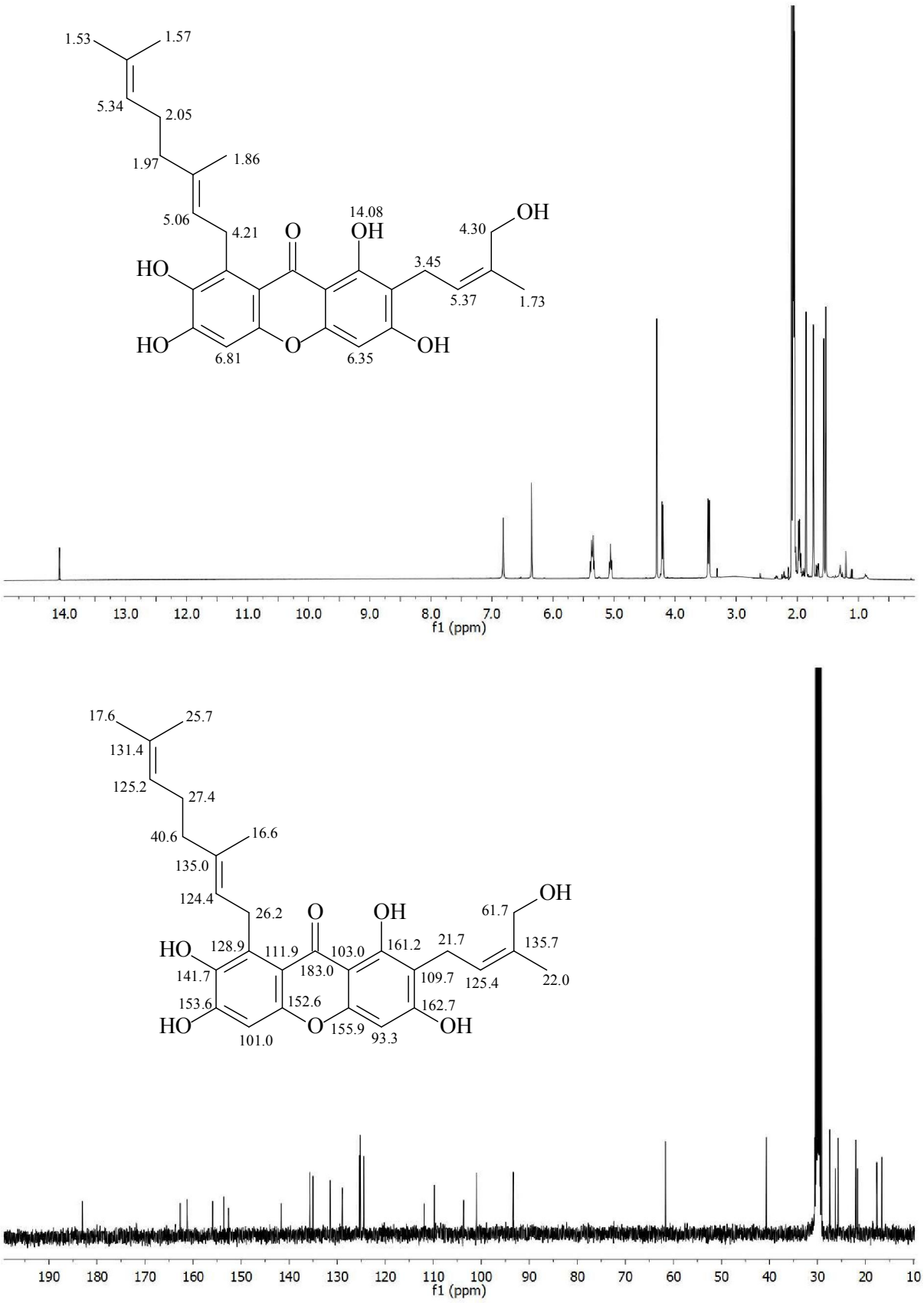


Figure 34. ^{1}H and ^{13}C NMR spectra of fuscaxanthone J (**21**)

5.2.1.2. Compound 20 (fuscaxanthone K)

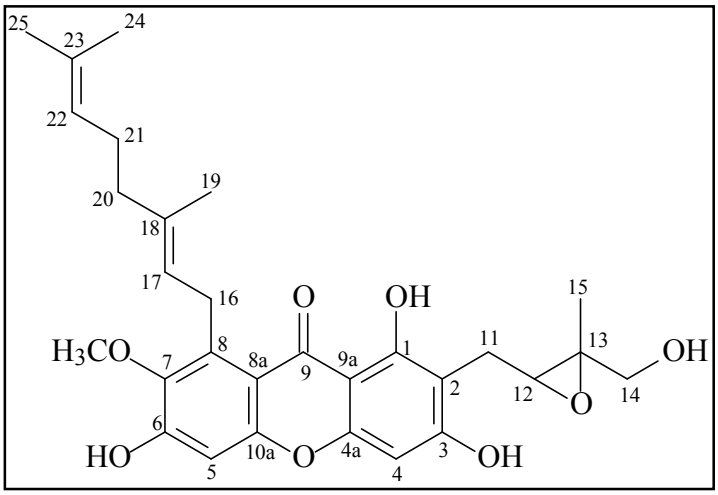


Figure 35. Structure of fuscaxanthone K (**20**)

Compound **20** was obtained as a yellow oil and given the molecular formula $C_{29}H_{34}O_8$ by HRESIMS (*m/z* 511.2334 $[M + H]^+$). In the ^1H NMR spectrum (see **Figure 38**; **Table 5** and **6**), the presence of a chelated hydroxyl group [δ_H 13.72 (1H, s, 1-OH)], two isolated aromatic protons [δ_H 6.83 (1H, s, H-5) and 6.23 (1H, s, H-4)], a methoxyl group [δ_H 3.79 (3H, s, 7-OCH_3)] and a geranyl group [δ_H 5.28 (1H, m like t, J = 6.6 Hz, H-17), 5.04 (1H, m, H-22), 4.12 (2H, d, J = 6.6 Hz, H-16), 2.05 (2H, m, H_2-21), 1.98 (2H, m, H_2-20), 1.82 (3H, s, H_3-19), 1.55 (3H, s, H_3-24) and 1.51 (3H, s, H_3-25)] were observed together with a benzylic methylene group [δ_H 3.22 and 3.12 (2H, dd, J = 8.1 and 15.6 Hz, H_2-11)], a deshielded methine proton [δ_H 5.03 (1H, m, H-12)], an oxygenated methylene group [δ_H 3.69 and 3.57 (2H, d, J = 9.6 Hz, H_2-14)] and a vinyl methyl group [δ_H 1.19 (3H, s, H_3-15)]. The ^{13}C NMR spectrum (see **Figure 38**; **Table 5** and **6**) contained signals of twenty nine carbons including a conjugated carbonyl carbon (δ_C 182.2), twelve aromatic carbons and four oxygenated sp^3 carbons (δ_C 60.8 – 89.1). Of twelve aromatic carbons, six are oxygenated (δ_C 157.5 – 167.4). These spectral data supported **20** to be a tetra-oxygenated xanthone derivative carrying a hydrogen-bonded hydroxyl group, a geranyl group, a methoxyl group and a C_5 unit.

In the HMBC spectrum, two protons of the oxygenated methylene group (δ_H 3.69 and 3.57; δ_C 67.2) correlated with an oxygenated tertiary carbon (δ_C 73.6), an oxygenated secondary carbon (δ_H 5.03; δ_C 89.1) and the secondary carbon (δ_H 1.19; δ_C 20.2). The benzylic methylene protons (δ_H 3.22 and 3.12, δ_C 26.1) also showed cross-peaks to the oxygenated tertiary carbon and the oxygenated secondary carbon. Moreover these protons gave the COSY correlation with the deshielded methine proton (δ_H 5.03). These data suggested that the C_5 unit is an isoprenyl group, in which the double bond is oxidized and a vinyl methyl group is hydroxylated to form the 2,3-epoxy-4-hydroxy-3-methylbutyl group. Besides the chelated hydroxyl group (δ_H 13.72) correlated with C-1 (δ_C 158.0) and C-9a (δ_C 103.7) and a substituted aromatic carbon (δ_C 108.5), whereas the benzylic methylene protons of the 2,3-epoxy-4-hydroxy-3-methylbutyl side chain also exhibited correlations with C-1, the substituted aromatic carbon and another oxygenated aromatic carbon (δ_C 167.4). The side chain is therefore bonded to C-2 (δ_C 108.5) and C-3 is oxygenated (δ_C 167.4). The isolated aromatic proton at δ_H 6.23 is attached to C-4 because it gave cross-peaks to C-2, C-3 and another oxygenated aromatic carbon (δ_C 157.4), which is C-4a. In the xanthone B ring, the downfield shift (δ_H 4.12) of the benzylic methylene protons of the geranyl group revealed that this group is located at C-8 as in the case of compound **21**. These methylene protons (H_2-16) and the methoxyl protons (δ_H 3.79) showed correlations to an oxygenated aromatic carbon (δ_C 144.2), indicating that this carbon is C-7, which carries the methoxyl group. This was confirmed by the HMBC correlation between H_2-16 with C-7, C-8a (δ_C 111.2) and a substituted aromatic carbon (δ_C 137.4) which has to be C-8. Meanwhile the remaining isolated aromatic proton (δ_H 6.83) gave cross-peaks to C-7, C-8a and two oxygenated aromatic carbons (δ_H 157.4 and 155.9). This proton is therefore bonded to C-5 and two oxygenated aromatic carbons are C-6 and C-10a.

In the ROESY spectrum (**Figure 36**), the protons H_2-14 gave an interaction with the proton H-12, whereas they did not show any interaction with H_2-11. This indicated that the oxygenated methyl group is *trans* to the benzylic methylene group in the 2,3-epoxy-4-hydroxy-3-methylbutyl side chain. Therefore, the configuration of two chiral

carbons C-12 and C-13 was suggested as *R* by comparison of the optical rotation of **20** with that of (2*S*-*trans*)-2-methyl-3-phenyloxiranemethanol, see **Figure 37** (Gao et al., 1987). Two chiral carbons of this compound possess *S* configuration corresponding to the optical rotation $[\alpha]^{25}_{D}$ – 16.9º, whereas that of **20** is + 76.3º (measured at 22 ºC). Hence **20** was determined as *12R*,*13R*-1,3,5-trihydroxy-2-(2,3-epoxy-4-hydroxy-3-methylbutyl)-7-methoxyxanthen-9-one and given the trivial name fuscaxanthone K.

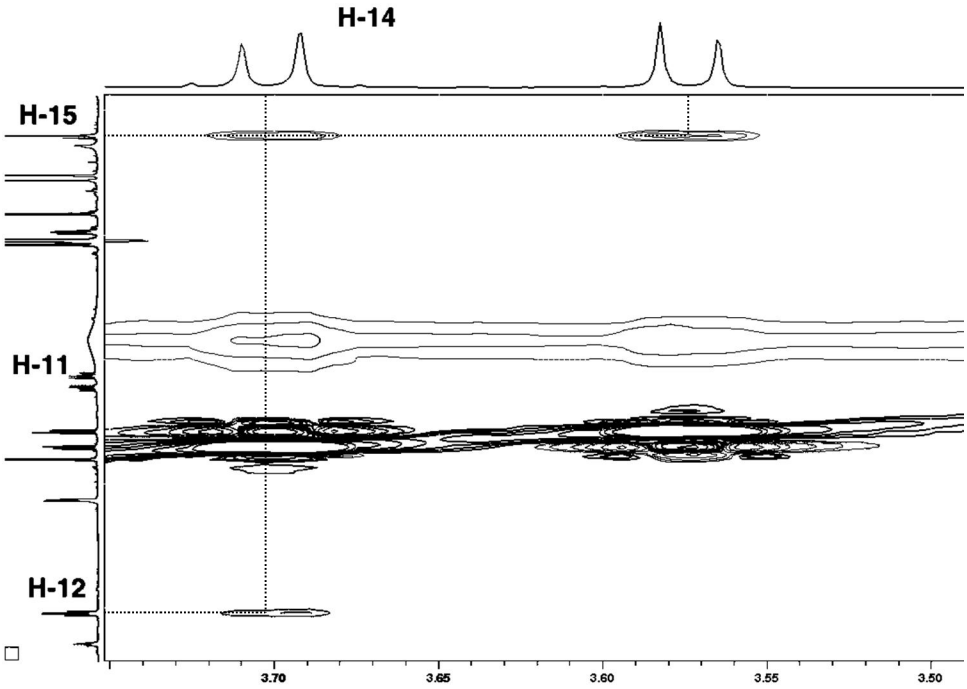


Figure 36. The ROESY interaction of H_2-14

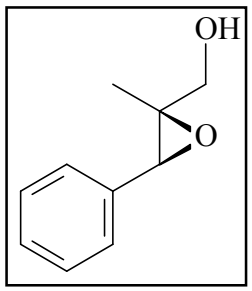


Figure 37. (2*S*-*trans*)-2-methyl-3-phenyloxiranemethanol

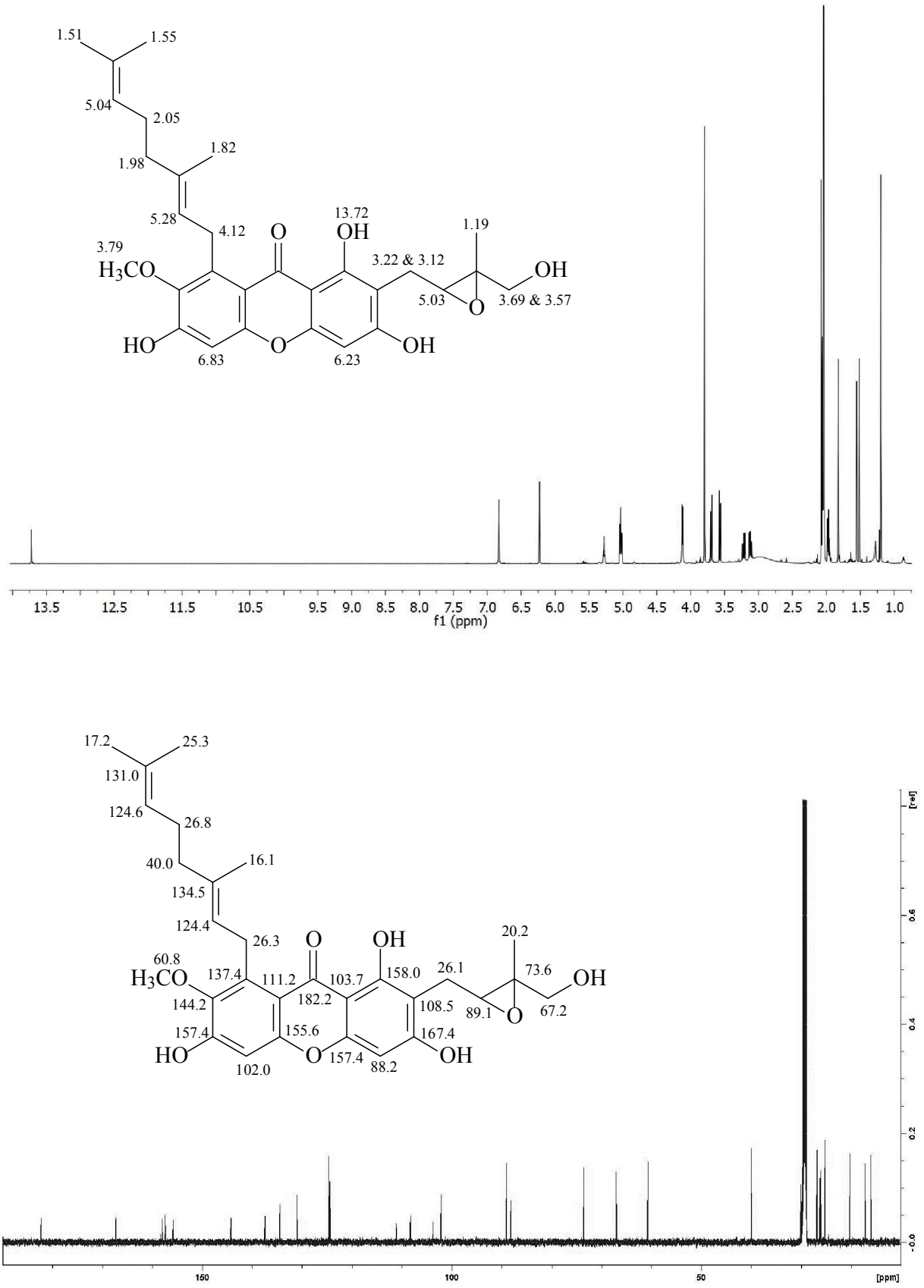


Figure 38. ^{1}H and ^{13}C NMR spectra of fuscaxanthone K (**20**)

5.2.1.3. Compound 19 ((+)-fuscaxanthone I)

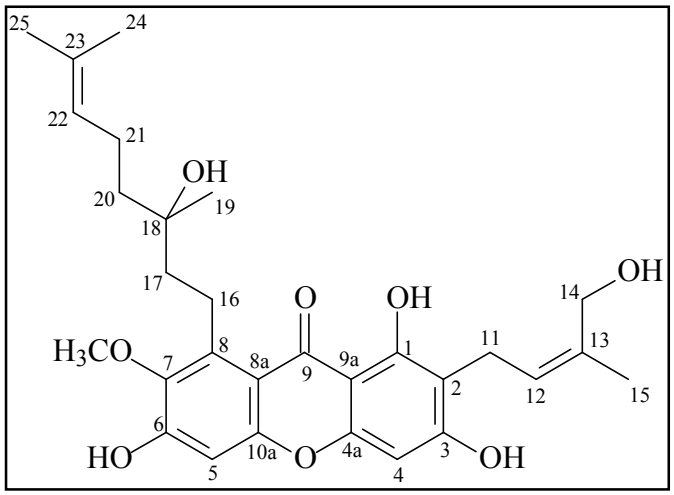


Figure 39. Structure of (+)-fuscaxanthone I (**19**)

Compound **19** was isolated as a yellow amorphous powder and showed a $[M+H]^+$ ion peak at *m/z* 513.2478 in HRESIMS, corresponding to the molecular formula $C_{29}H_{36}O_8$. The ^1H and ^{13}C NMR spectrum of **19** (see **Figure 40**; **Table 5** and **6**) showed resonances for a hydrogen-bonded hydroxyl group [δ_H 13.89 (1H, s, 1-OH)] corresponding to the chelated carbonyl carbon [δ_C 181.7 (C-9)], two isolated aromatic protons [δ_H 6.79 (1H, s, H-5) and 6.34 (1H, s, H-4); δ_C 101.6 (C-5) and 92.5 (C-4)], a methoxyl group [δ_H 3.83 (3H, s, 7-OCH_3), δ_C 60.5 (CH_3O-7)], a 4-hydroxy-3-methylbut-2-enyl side chain [δ_H 5.35 (1H, t, J = 7.4 Hz, H-12), 4.28 (2H, s, H_2-14), 3.42 (2H, d, J = 7.4 Hz, H_2-11), 1.71 (3H, s, H-15); δ_C 134.7 (C-13), 124.4 (C-12), 60.7 (C-14), 21.0 (C-15) and 20.7 (C-11)], an isoprenyl group [δ_H 5.17 (1H, t, J = 7.2 Hz, H-22), 2.18 (2H, m, H_2-21), 1.66 (3H, s, H_3-24) and 1.63 (3H, s, H_3-25); δ_C 130.3 (C-23), 125.2 (C-22), 24.8 (C-24), 22.4 (C-21) and 16.7 (C-25)], a benzylic methylene group [δ_H 3.42 (2H, m, H_2-16), δ_C 21.9 (C-16)], two methylene groups [δ_H 1.75 (2H, m, H_2-17) and 1.57 (2H, m, H_2-20); δ_C 42.5 (C-17) and 41.6 (C-20)] and a methyl group [δ_H 1.28 (3H, s, H_3-19); δ_C 26.4 (C-19)]. The ^1H and ^{13}C NMR data of **19** quite resembled to those of fuscaxanthone I, which was previously reported from *G. fusca* collected in Thailand (Nontakham et al., 2014). In this xanthone, the double bond at C-17/C-18 of the geranyl group is hydroxylated to produce the 3-hydroxy-3,7-dimethyl-oct-6-enyl group. This group was confirmed by the HMBC and COSY correlations.

The HMBC correlations between the shielded benzylic methylene protons (δ_H 3.22) with the secondary carbon (δ_C 42.5) and an oxygenated quaternary carbon (δ_C 71.3), in addition to the COSY interaction with the methylene protons (δ_H 1.75) showed that the secondary carbon is C-17 and C-18 is the oxygenated quaternary carbon. The HMBC cross-peaks of a methyl group (δ_H 1.28) with C-17, C-18 and a secondary carbon (δ_C 41.6) indicated that this methyl group is located at C-18 and the secondary carbon is C-20. The COSY interaction between H-20 and the vinyl methylene protons of the isoprenyl group pointed out the connectivity of C-20 with the isoprenyl group. Moreover the HMBC correlations of the benzyl methylene protons H_2-16 with two aromatic substituted carbons at δ_C 138.9 (C-8) and 110.8 (C-8a) and an oxygenated aromatic carbon at δ_C 143.5 (C-7) as well as the ROESY interaction with the methoxyl protons allowed the placement of the hydroxylated geranyl group at C-8. On the other hand, the benzylic methylene protons of the 4-hydroxy-3-methylbut-2-enyl side chain gave the HMBC correlations with C-1 (δ_C 160.6), the substituted aromatic carbon at δ_C 109.7 (C-2) and C-3 (δ_C 162.3), i.e. this side chain is obviously attached to C-2. Thus, **19** was identified as 1,3,6-trihydroxy-2-(4-hydroxy-3-methylbut-2-enyl)-8-(3-hydroxy-3,7-dimethyl-oct-6-enyl)-7-methoxyxanthen-9-one. However the optical rotation of **19** ($[\alpha]^{22}_D$ + 36.0º) is different from that of fuscaxanthone I ($[\alpha]^{26}_D$ – 9.5º), in which the configuration of the chiral carbon C-18 could not be resolved. Comparison of the optical rotation of **19** with that of (3'*S*)-4-hydroxy-3-(3'-hydroxy-3',7'-dimethyloct-6'-enoyl)benzaldehyde ($[\alpha]^{25}_D$ – 12.0º) (see **Figure 41**) (Chakor et al., 2011) suggested that the chiral carbon C-18 of **19** has *R* configuration and the compound was given trivial name (+)-fuscaxanthone I.

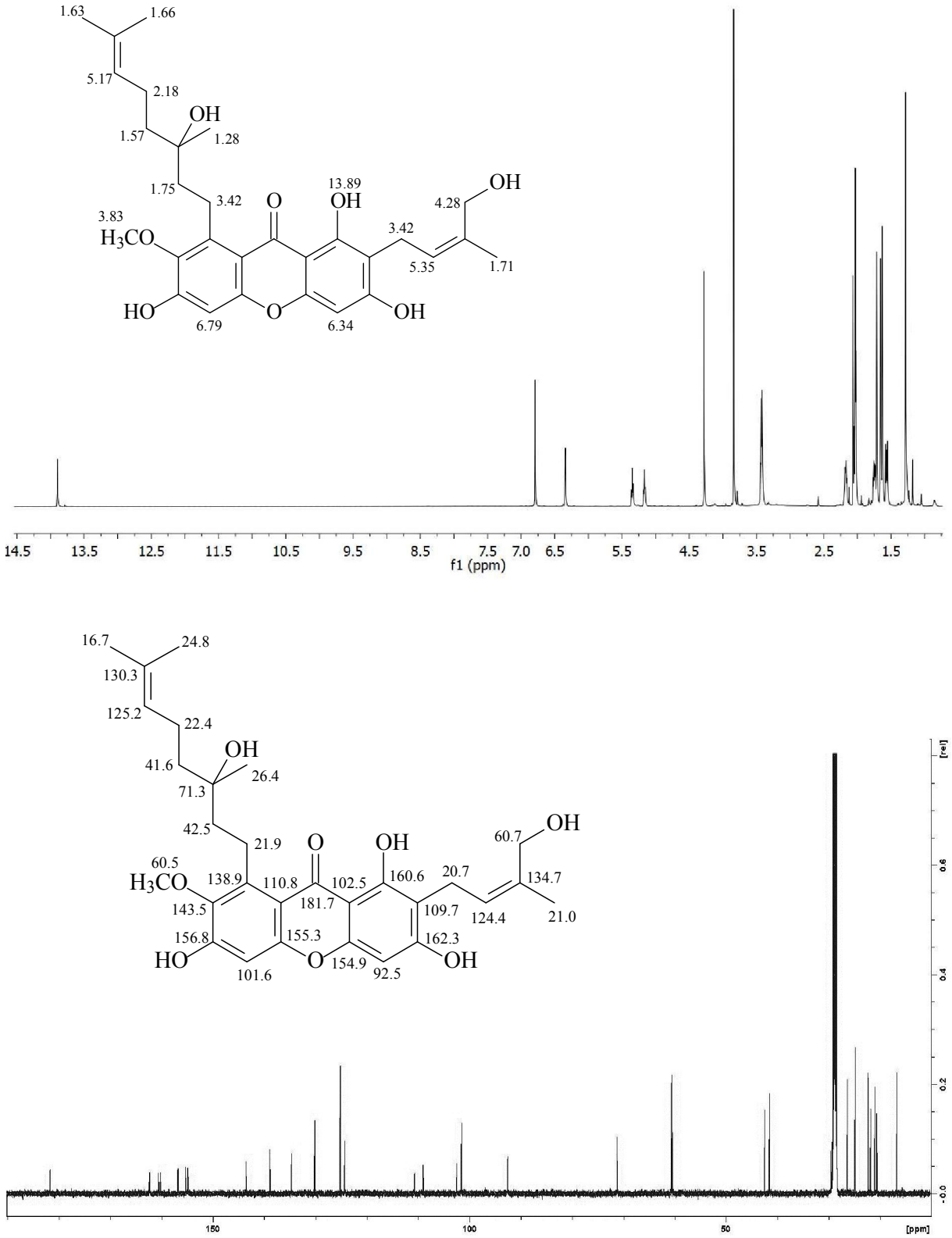


Figure 40. ^{1}H and ^{13}H NMR spectra of (+)-fuscaxanthone I (**19**)

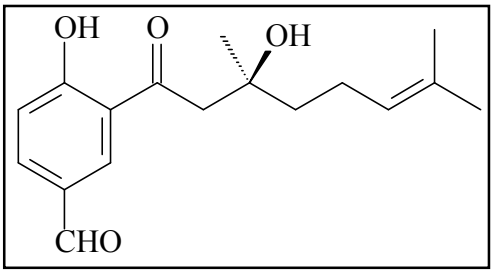


Figure 41. (3'*S*)-4-Hydroxy-3-(3'-hydroxy-3',7'-dimethyloct-6'-enoyl)benzaldehyde

Table 5. ^{1}H NMR data of **21, 19** and **20** in acetone-d_6

Position	**21**	**19**	**20**
1-OH	14.08 s	13.89 s	13.72 s
4	6.35 s	6.34 s	6.23 s
5	6.81 s	6.79 s	6.83 s
11	3.45 d (8.0 Hz)	3.42 d (7.4 Hz)	3.22 & 3.12 dd (8.1 & 15.6 Hz)
12	5.37 t (8.0 Hz)	5.35 t (7.4 Hz)	5.03 m
14	4.20 s	4.28 s	3.96 & 3.57 d (9.6 Hz)
15	1.73 s	1.71 s	1.19 s
16	4.21 d (6.8 Hz)	3.42 overlap with H-11	4.12 d (6.6 Hz)
17	5.06 t (6.8 Hz)	1.75 m	5.28 t (6.6 Hz)
19	1.86 d (0.8 Hz)	1.28 s	1.82 s
20	1.97 m	1.57 m	1.98 m
21	2.05 m	2.18 m	2.05 m
22	5.34 t (8.0 Hz)	5.17 t (7.2 Hz)	5.04 m
24	1.57 s	1.66 s	1.55 s
25	1.53 s	1.63 s	1.51 s
7-OCH_3		3.83 s	3.79 s

600 MHz (J in Hz and δ in ppm) at 298ºK.

Table 6. ^{13}C NMR data of **21, 19** and **20** in acetone-d_6

Position	**21**	**19**	**20**
1	161.2	160.6	158.0
2	109.7	109.7	108.5
3	162.7	162.3	164.7
4	93.3	92.5	88.2
4a	155.9	154.9	157.4
5	101.0	101.6	102.0
6	153.6[a]	156.8[a]	157.4[a]
7	141.7	143.5	144.2
8	128.9	138.9	137.4
8a	111.9	110.8	111.2
9	183.0	181.7	182.2
9a	103.0	102.5	103.7
10a	152.6[a]	155.3[a]	155.6[a]
11	21.7	20.7	26.1
12	125.4	124.4	89.1
13	135.7	134.7	73.6
14	61.7	60.7	67.2
15	22.0	21.0	20.2
16	26.2	21.9	26.3
17	124.4	42.5	124.4
18	135.0	71.3	134.5
19	16.6	26.4	16.1
20	40.6	41.6	40.0
21	27.4	22.4	26.8
22	125.2	125.2	124.6
23	131.4	130.3	131.0
24	25.7	24.8	25.3
25	17.6	16.7	17.2
7-OCH_3		60.5	60.8

[a] Interchangeable in the same column, 150 MHz (δ in ppm) at 298ºK

5.2.1.4. Compound 18 (fuscaxanthone L)

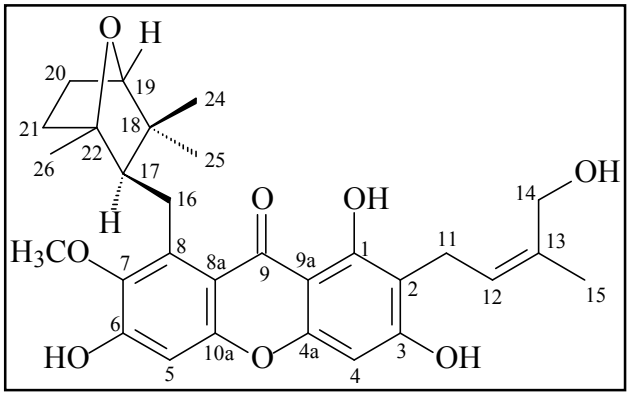


Figure 42. Structure of fuscaxanthone L (**18**)

Compound **18** was obtained as yellow oil. The ^{1}H and ^{13}C NMR spectrum (see **Figure 45**; **Table 7**) of this compound showed similarities to those of **19**, corresponding to a 1,3,6-trihydroxy-7-methoxyxanthone nucleus [δ_H 13.88 (1H, s, 1-OH), 6.80 (1H, s, H-5), 6.36 (1H, s, H-4) and 3.78 (3H, s, 7-OCH_3); δ_C 182.4 (C-9), 163.5 (C-3), 161.5 (C-1), 157.6 (C-6), 156.9 (C-10a), 155.8 (C-4a), 145.0 (C-7), 139.9 (C-8), 112.1 (C-8a), 110.0 (C-2), 103.5 (C-9a), 102.4 (C-5) and 93.5 (C-4)], a 4-hydroxy-3-methylbut-2-enyl group at C-2 [δ_H 5.36 (1H, t, J = 6.9 Hz, H-12), 4.29 (2H, s, H_2-14), 3.44 (2H, d, J = 6.9 Hz, H_2-11) and 1.72 (3H, s, H_3-15); δ_C 135.8 (C-13), 125.3 (C-12), 61.8 (C-14), 22.0 (C-15) and 21.7 (C-11)] and a C_{10} unit, in which the benzylic methylene protons at C-8 [δ_H 3.40 and 3.26, 2H, dd, J = 6.6 and 13.2 Hz, H_2-16, δ_C 25.9 (C-16)] were highly shielded. The absence of signals for two double-bonds indicated that the C_{10} unit is saturated. The molecular formula of **18** was determined as $C_{29}H_{34}O_8$ by HRESIMS (m/z 511.2332 $[M + H]^+$), and the degrees of unsaturation are thirteen. Apart from eleven degrees of unsaturation in the xanthone nucleus and the 4-hydroxy-3-methylbut-2-enyl group, the C_{10} unit certainly contained two degrees of unsaturation, i.e. there is a two-ring system in this unit.

The shielded benzylic methylene protons (δ_H 3.40 and 3.26) showed the HMBC correlations with an oxygenated quaternary carbon (δ_C 87.5), a tertiary carbon (δ_C 58.0) and a quaternary carbon (δ_C 46.6). On the other hand, these protons also exhibited the COSY interaction with the methine proton [δ_H 1.86 (1H, t, J = 6.6 Hz)].

Thus, the tertiary carbon is C-17, the quaternary carbon is C-18 and C-22 is the oxygenated quaternary carbon. The HMBC correlations of the protons of two methyl carbons (δ_H 1.09 and 0.89; δ_C 25.7 and 24.5) with C-17, C-18 and an oxygenated tertiary carbon [δ_H 3.63 (1H, d, J = 5.4 Hz); δ_C 86.4] revealed that these two methyl groups are bonded to C-18 and the oxygenated tertiary carbon is C-19. The COSY interactions between the methylene protons [δ_H 1.82 and 1.51 (2H, m); δ_C 26.5] with H-19 and the other methylene protons [δ_H 1.42 and 1.24 (2H, m); δ_C 40.1] showed that the secondary carbon at δ_H 26.5 is C-20 and C-21 is the other carbon (δ_C 40.1). The HMBC cross-peaks of the methyl protons [δ_H 1.27 (3H, s); δ_C 18.9] with C-17, C-21 and C-22 confirmed the connectivity of C-21 with C-22 as well as the position of this methyl group at C-22. Hence the C_{10} unit was established as the 1,3,3-trimethyl-7-oxabicyclo[2.2.1]hept-2-ylmethyl group comprising three chiral carbons at C-17, C-19 and C-22. This was supported by comparison of the spectral data of **18** with those of parvixanthone I, which was previously isolated from *G. parvifolia* (Xu et al., 2001). This compound also contains a 1,3,3-trimethyl-7-oxabicyclo[2.2.1]hept-2-ylmethyl group at C-8 but the carbon C-2 is bonded with a proton instead of the 4-hydroxy-3-methylbut-2-enyl side chain as in the case of **18**. Due to the boat conformation of the 7-oxabicyclo[2.2.1] system with the *oxo*-bridge, the direction of the proton H-19 and the methyl group at C-22 is required to be *endo*. In the NOESY spectrum, H-19 gave the interaction with the protons of the methyl group (δ_H 1.09, H_3-24) and a proton (δ_H 1.82) of H_2-20, i.e. the direction of this methyl group and this proton is consequently *exo* (**Figure 43**). Meanwhile the direction of H-17 (δ_H 1.86) and a proton (δ_H 1.42) of H_2-21 were determined as *endo* based on the NOESY interaction with the methyl protons H_3-25 (see **Figure 44**) Furthermore, the HMBC cross-peaks of the benzylic methylene protons (δ_H 3.40 and 3.26, C-16) with C-7 (δ_C 145.0), C-8 (δ_C 139.9) and C-8a (δ_C 112.1) pointed out the location of the 1,3,3-trimethyl-7-oxabicyclo[2.2.1]hept-2-ylmethyl group at C-8. Thus, compound **18** was identified as 1,3,6-trihydroxy-2-(4-hydroxy-3-methylbut-2-enyl)-7-methoxy-8-(1,3,3-trimethyl-7-oxabicyclo[2.2.1]hept-2-ylmethyl)xanthen-9-one and was given a trivial name

fuscaxanthone L. However the absolute configuration of the chiral carbons could not be determined.

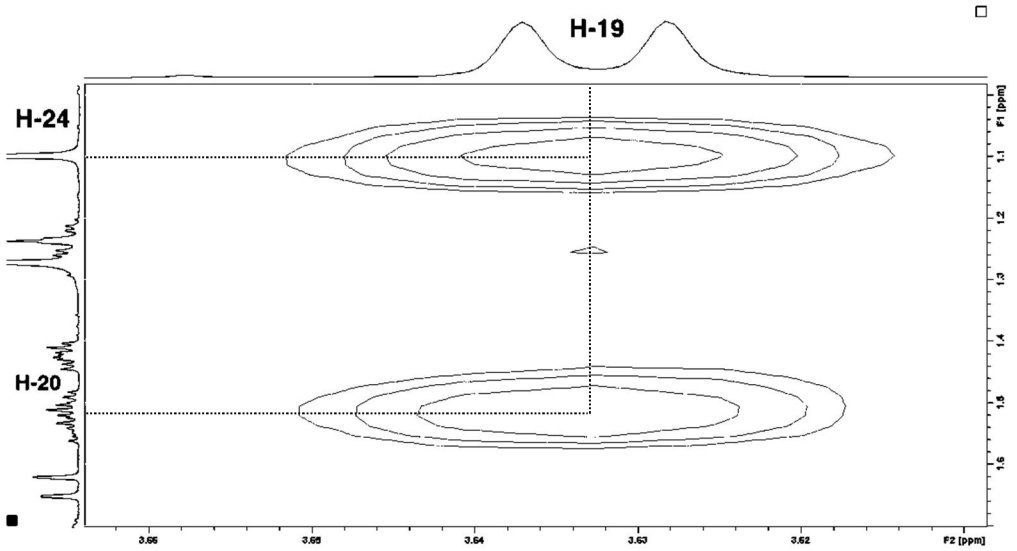


Figure 43. The NOESY interaction between H_3-24, H_α-20 and H-19

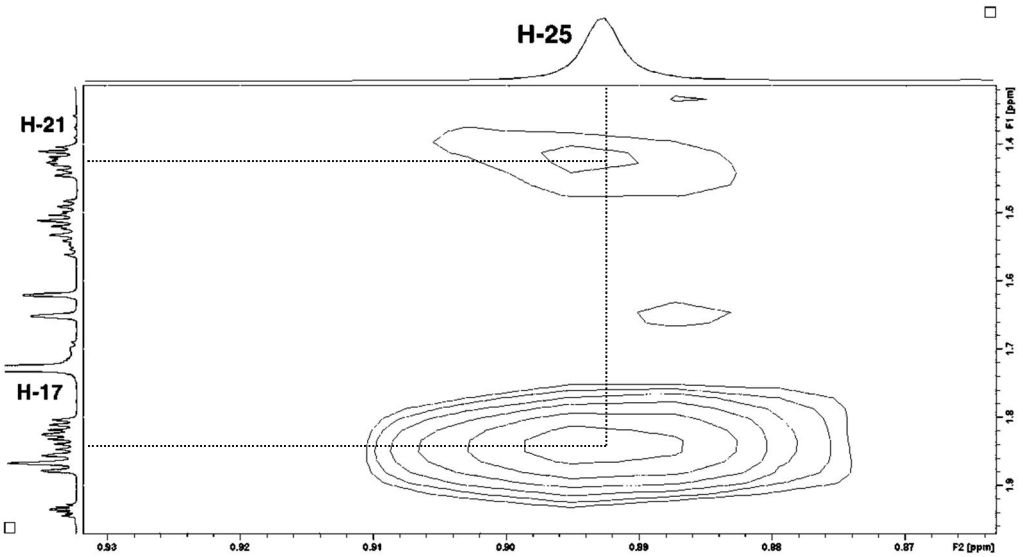


Figure 44. The NOESY interaction between H_β-21, H-17 and H_3-25

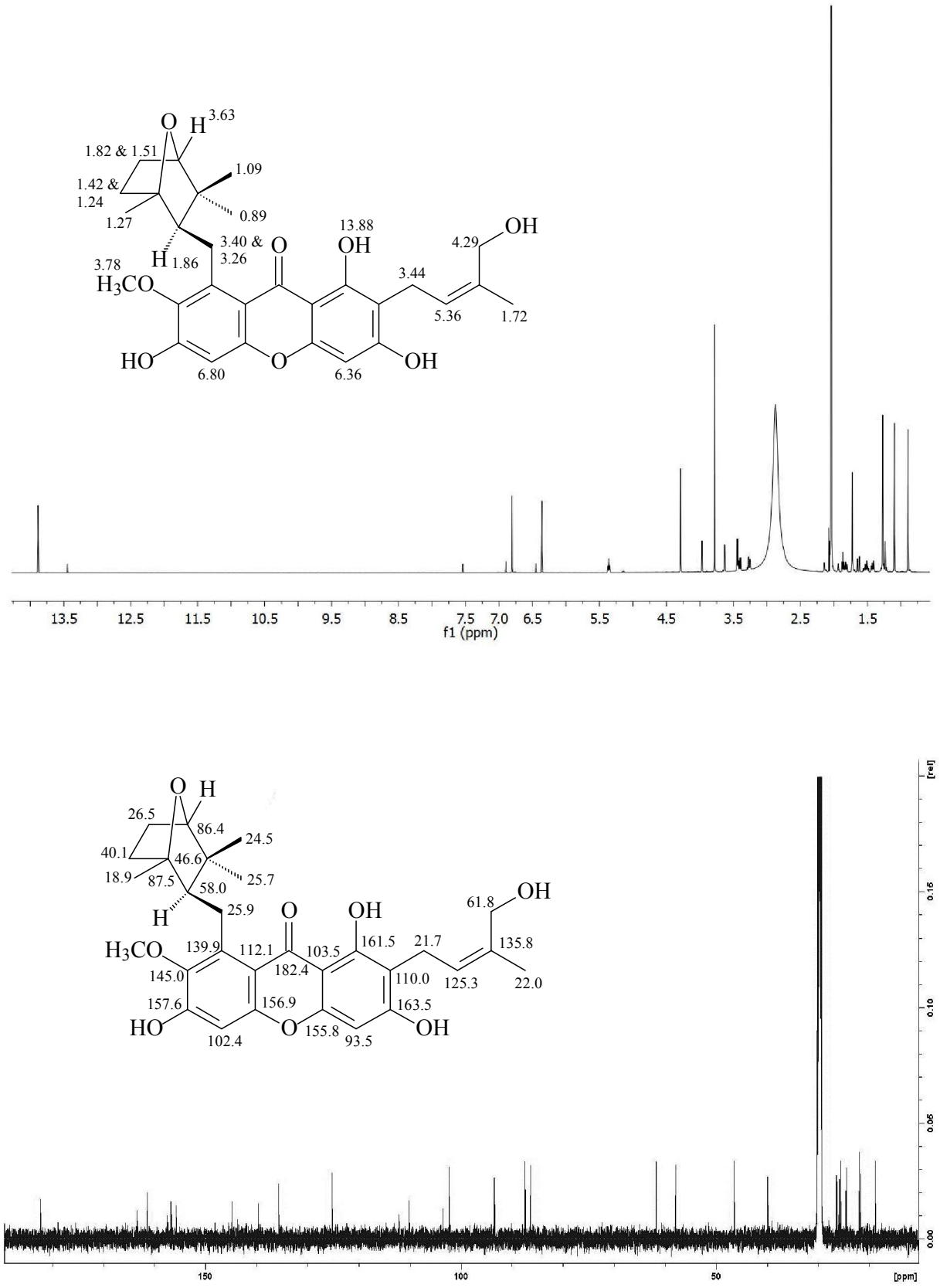


Figure 45. ^{1}H and ^{13}C NMR spectra of fuscaxanthone L (**18**)

Table 7. ^{1}H and ^{13}C NMR data of **18** in acetone-d_6

Position	δ_H	δ_C
1		161.5
2		110.0
3		163.5
4	6.36 s	93.5
4a		155.8
5	6.80 s	102.4
6		157.6[a]
7		145.0
8		139.9
8a		112.1
9		182.4
9a		103.5
10a		156.9[a]
11	3.44 d (6.9 Hz)	21.7
12	5.36 t (6.9 Hz)	125.3
13		135.8
14	4.29 s	61.8
15	1.72 s	22.0
16	3.40 dd (6.6 & 13.2 Hz)	25.9
	3.26 dd (6.6 & 13.2 Hz)	
17	1.86 t (6.6 Hz)	58.0
18		46.6
19	3.63 d (5.4 Hz)	86.4
20	1.82 m	26.5
	1.51 m	
21	1.42 m	40.1
	1.24 m	
22		87.5
23		
24	1.09 s	24.5
25	0.89 s	25.7
26	1.27 s	18.9
1-OH	13.88 s	
7-OCH_3	3.78 s	60.5

[a] Interchangeable, 600 MHz for ^{1}H and 150 MHz for ^{13}C (J in Hz and δ in ppm) at 298ºK

5.2.1.5. Compound 22 (fuscaxanthone M)

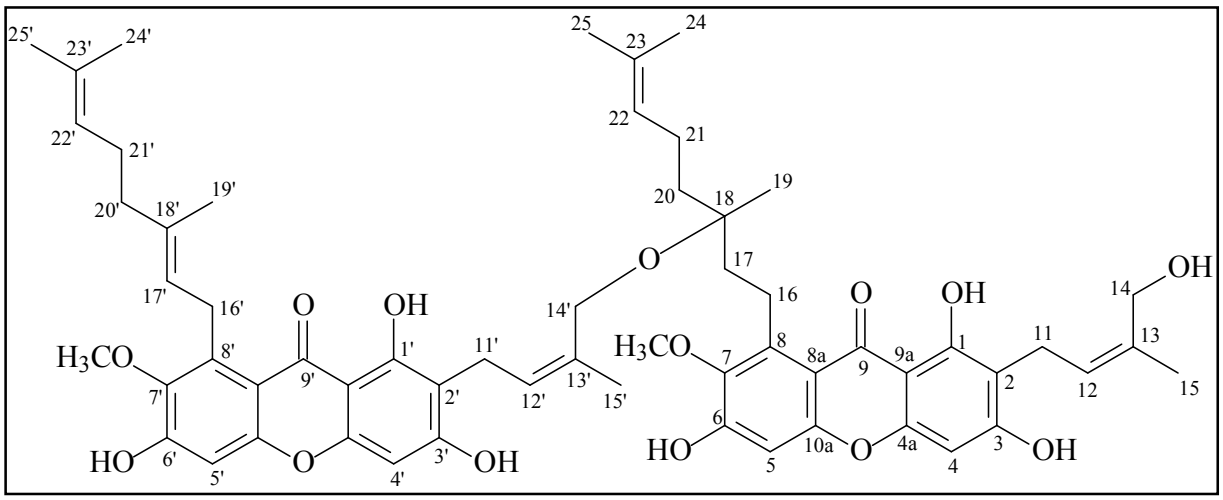


Figure 46. Structure of fuscaxanthone M (**22**)

Compound **22** was isolated as yellow oil. The ^{1}H and ^{13}C NMR spectra (see **Figure 49** and **Table 8**) indicated that this compound contains two prenylated xanthone fragments. The fragment **B** is cowanol (Na Pattalung et al., 1994), comprising a hydrogen-bonded hydroxyl group [δ_H 13.80 (1H, s, H-1')] corresponding with a chelated carbonyl carbon [δ_C 181.8 (C-8')], two isolated aromatic protons [δ_H 6.82 (1H, s, H-5') and 6.29 (1H, s, H-4'); δ_C 101.6 (C-5') and 93.7 (C-4')], a methoxyl group [δ_H 3.82 (3H, s, 7'-OCH_3); δ_C 62.1 (CH_3O-7')], a 4-hydroxy-3-methyl-but-2-enyl group [δ_H 5.36 (1H, t, J = 7.7 Hz, H-12'), 4.23 (1H, d, J = 10.8 Hz, H_A-14'), 4.16 (1H, d, J = 10.8 Hz, H_B-14'), 3.66 (2H, d, J = 7.7 Hz, H_2-11') and 1.82 (3H, s, H-15'); δ_C 131.9 (C-13'), 126.6 (C-12'), 61.8 (C-14'), 23.6 (C-15') and 21.4 (C-11')] and a geranyl group [δ_H 5.27 (1H, t, J = 6.0 Hz, H-17'), 5.16 (1H, t, J = 7.2 Hz, H-22'), 4.10 (2H, d, J = 6.0 Hz, H_2-16'), 2.04 (2H, m, H_2-21'), 1.99 (2H, m, H_2-20'), 1.82 (3H, s, H_3-19), 1.60 (3H, s, H_3-24') and 1.54 (3H, s, H_3-25'); δ_C 135.5 (C-18'), 131.3 (C-23'), 124.3 (C-22'), 123.4 (C-17'), 39.8 (C-20'), 26.6 (C-16'), 26.5 (C-21'), 25.5 (C-24'), 17.7 (C-25') and 16.5 (C-19')]. The fragment **A** resembled **19**, including a hydroxyl group [δ_H 13.92 (1H, s, H-1)] chelated with a conjugated carbonyl carbon [δ_C 181.9 (C-8)], two isolated aromatic protons [δ_H 6.81 (1H, s, H-5) and 6.28 (1H, s, H-4); δ_C 101.8 (C-5) and 93.9 (C-4)], a methoxyl group [δ_H 3.80 (3H, s, 7-OCH_3); δ_C 62.0

(CH_3O-7)], a 4-hydroxy-3-methyl-but-2-enyl group [δ_H 5.47 (1H, t, J = 6.8 Hz, H-12), 4.36 (2H, s, H_2-14), 3.52 (2H, d, J = 6.8 Hz, H-11) and 1.78 (3H, s, H-15); δ_C 133.1 (C-13), 127.0 (C-12), 63.0 (C-14), 21.6 (C-11) and 23.0 (C-15)] and a hydroxylated geranyl [δ_H 5.02 (1H, t, J = 6.9 Hz, H-22), 3.42 (2H, m, H_2-16), 2.15 (2H, m, H_2-21), 1.92 (1H, m, H_A-20), 1.82 (1H, m, H_A-17), 1.76 (1H, m, H_B-17), 1.70 (3H, s, H_3-24), 1.67 (1H, m, H_B-20), 1.64 (3H, s, H_3-25) and 1.45 (3H, s, H_3-19); δ_C 131.6 (C-23), 124.4 (C-22), 79.3 (C-18), 37.9 (C-20), 37.8 (C-17), 25.7 (C-24), 22.9 (C-19), 22.4 (C-21), 21.9 (C-16) and 17.7 (C-25)].

In the HMBC spectrum, the benzyl methylene protons H-16 (δ_H 3.42) correlated with the secondary carbon C-17 and the oxygenated quaternary carbon C-18. The methylene protons H-17 gave cross-peaks with C-16 and C-18. These data together with the COSY interaction between H-16 and H-17 indicated that the double bond at C-17/C-18 of the geranyl group is hydroxylated. Furthermore the HMBC correlations of the proton H-16 with C-8, C-7 and C-8a, in addition to the NOESY interaction with the methoxyl protons 7-OCH_3 allowed the placement of the hydroxylated geranyl group at C-8. The molecular formula of **22** was calculated to be $C_{58}H_{68}O_{14}$ with 25 degrees of unsaturation based on HRESIMS (*m/z* 1011.4503 $[M + Na]^+$). Because the cowanol fragment has seven oxygen atoms, the fragment **A** therefore contains seven oxygen atoms. Due to six oxygenated aromatic carbons of the xanthone nucleus and an oxygenated methyl carbon of the 4-hydroxy-3-methyl-but-2-enyl group, the oxygenated quaternary carbon C-18 of the hydroxylated geranyl group has to be combined with the cowanol fragment by a C-O-C linkage. This was confirmed by the HMBC correlation of H-14' with C-18 (**Figure 47**), in addition to the NOESY interaction between the vinyl methyl protons H_3-15' and the methylene protons H_2-17, see **Figure 48**. Hence compound **22** was determined as 8-{3-[1',3',6',-trihydroxy-2'-(4-hydroxy-3-methylbut-2-enyl)-7'-methoxy-8'-(3,7-dimethylocta-2,6-dienyl)xanthen-9-one-14'-oxyl]-3,7-dimethyloct-2,6-dienyl}-1,3,6-trihydroxy-2-(4-hydroxy-3-methylbut-2-enyl)-7-methoxy-8-(3,7-dimethylocta-6-enyl)bixanthone and was given trivial name fuscaxanthone M. The configuration of the chiral carbon C-18 was suggested to be *R* by comparison of the optical rotation of **22** ($[\alpha]^{22}_D$ + 54.6º) with

that of (3'*S*)-4-hydroxy-3-(3'-hydroxy-3',7'-dimethyloct-6'-enoyl)benzaldehyde ($[\alpha]^{25}_{D}$ – 12.0º) (Chakor et al., 2011).

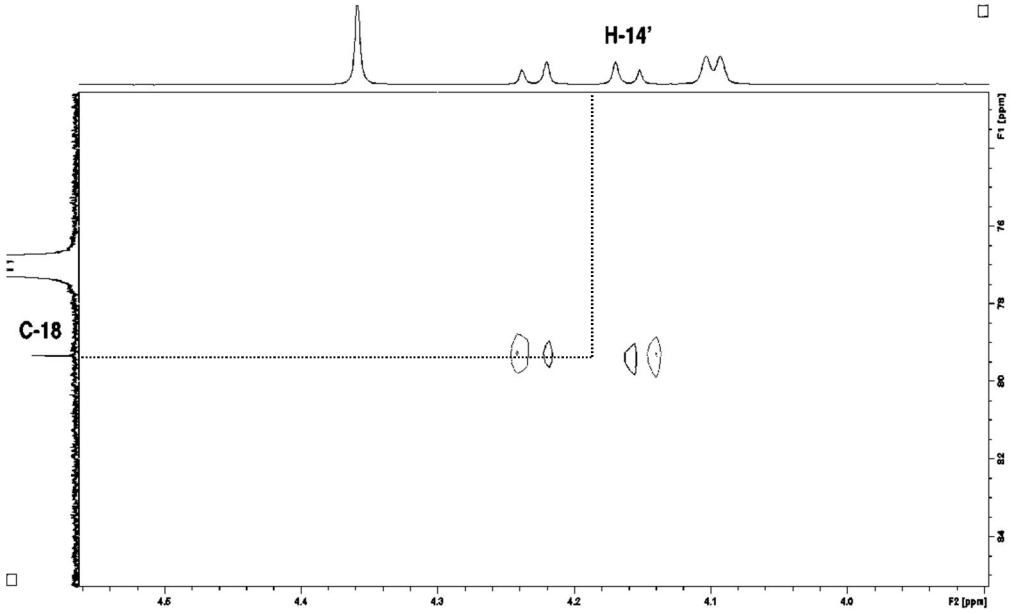


Figure 47. The HMBC correlation of H-14' with C-18

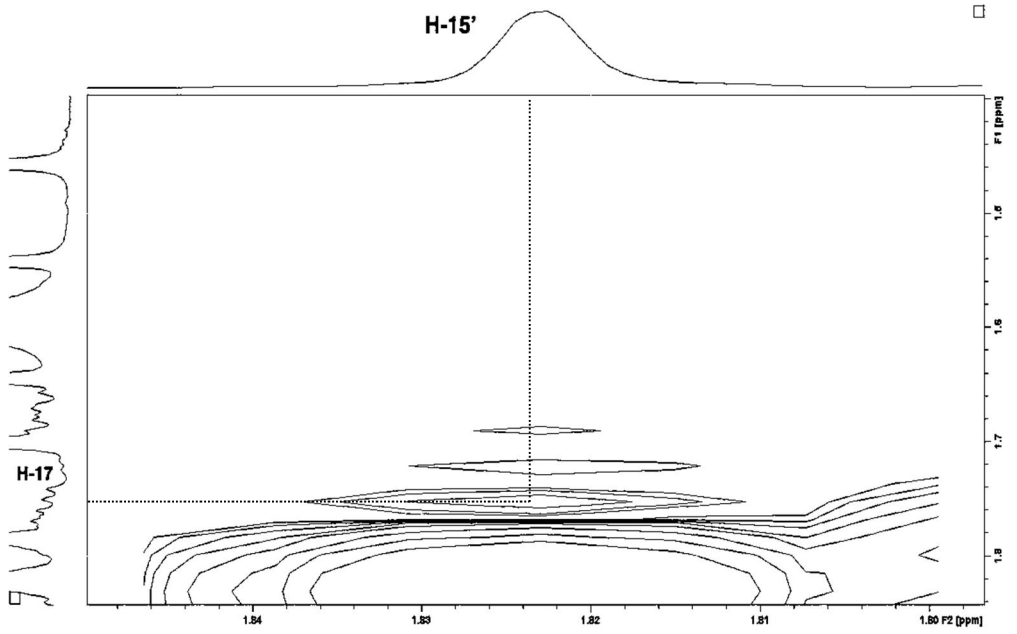


Figure 48. The NOESY interaction between H-15' and H-17

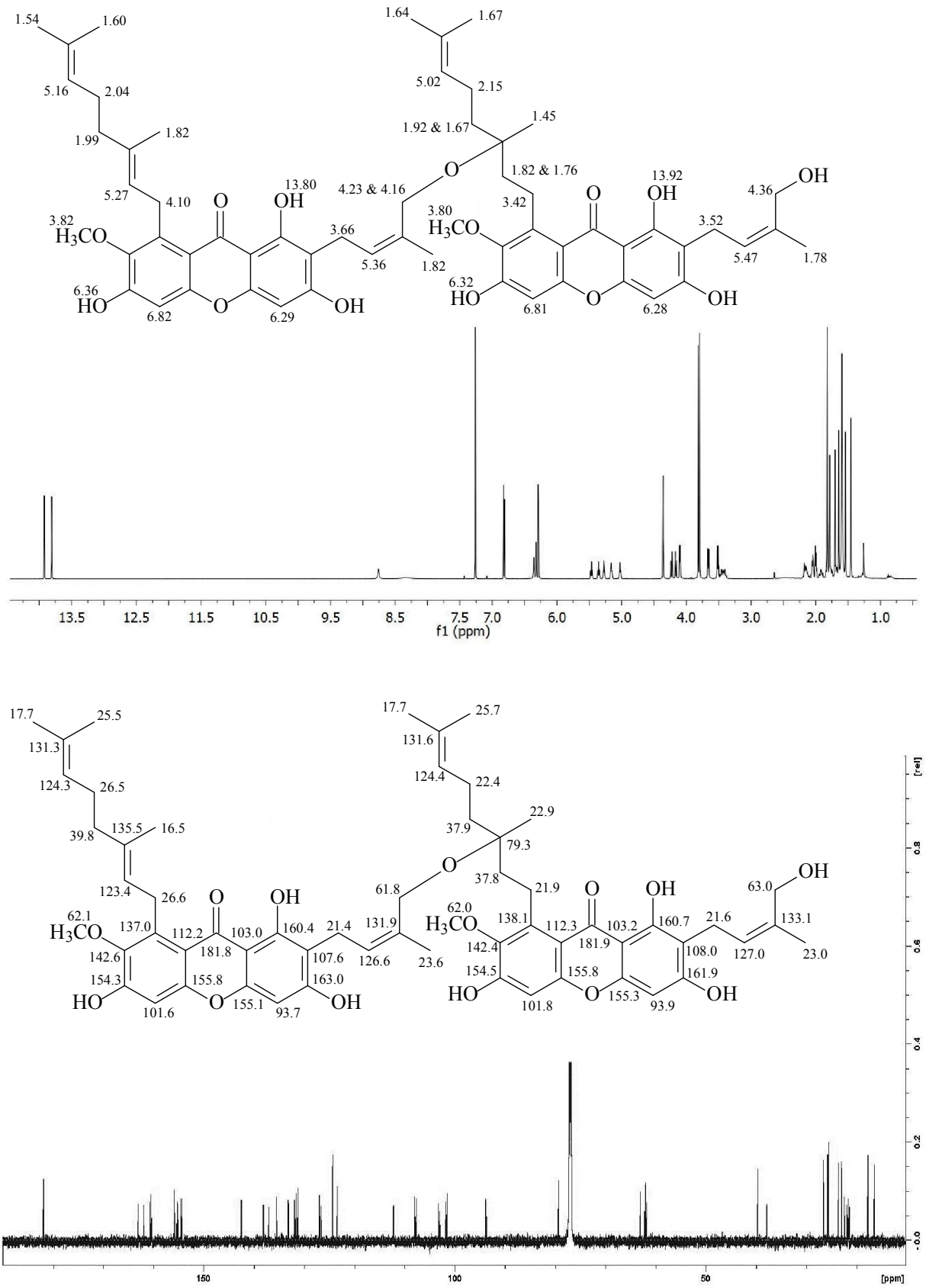


Figure 49. ^{1}H and ^{13}C NMR spectra of fuscaxanthone M (**22**)

Table 8. ^{1}H and ^{13}C NMR data of **22** in acetone-d_6

	Fragment **A**			Fragment **B**	
	δ_H	δ_C		δ_H	δ_C
1		160.7	1'		160.4
2		108.0	2'		107.6
3		161.9	3'		163.0
4	6.28 s	93.9	4'	6.29 s	93.7
4a		155.3	4'a		155.1
5	6.81 s	101.8	5'	6.82 s	101.6
6		154.5[a]	6'		154.3[a]
7		142.4	7'		142.6
8		138.1	8'		137.0
8a		112.3	8'a		112.2
9		181.9	9'		181.8
9a		103.2	9'a		103.0
10a		155.8[a]	10'a		155.8[a]
11	3.52 d (6.8 Hz)	21.6	11'	3.66 d (7.7 Hz)	21.4
12	5.47 t (6.8 Hz)	127.0	12'	5.36 t (7.7 Hz)	126.6
13		133.1	13'		131.9
14	4.36 s	63.0	14'	4.23 d (10.8 Hz)	61.8
				4.16 d (10.8 Hz)	
15	1.78 s	23.0	15'	1.82 s	23.5
16	3.42 m	21.9	16'	4.10 d (6.0 Hz)	26.6
17	1.82 m	37.8	17'	5.27 t (6.0 Hz)	123.4
	1.76 m				
18		79.3	18'		135.5
19	1.45 s	22.9	19'	1.82 s	16.5
20	1.92 m	37.9	20'	1.99 m	39.8
	1.67 m				
21	2.15 m	22.4	21'	2.04 m	26.5
22	5.05 t (6.9 Hz)	124.4	22'	5.16 t (7.2 Hz)	124.3
23		131.6	23'		131.3
24	1.70 s	25.7	24'	1.60 s	25.5
25	1.64 s	17.7	25'	1.54 s	17.7
1-OH	13.92 s		1'-OH	13.88 s	
7-OCH_3	3.80 s	62.0	7'-OCH_3	3.78 s	62.1

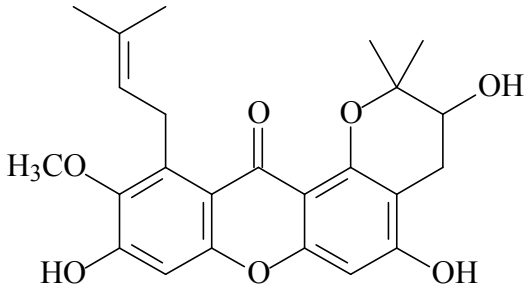
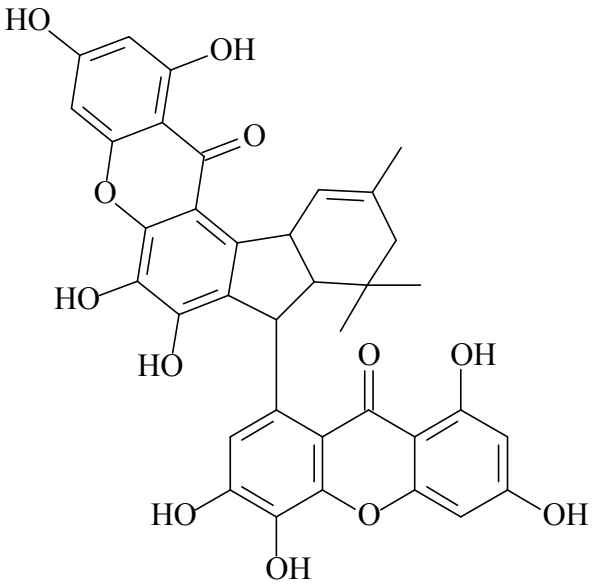
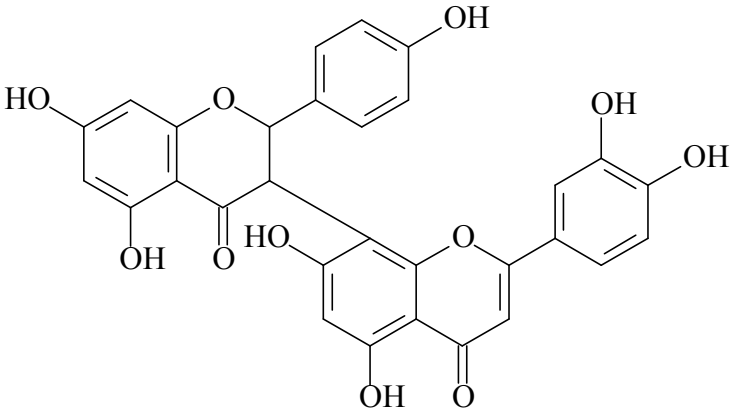
[a] Interexchangeable, 600 MHz for ^{1}H and 150 MHz for ^{13}C (J in Hz and δ in ppm) at 298ºK

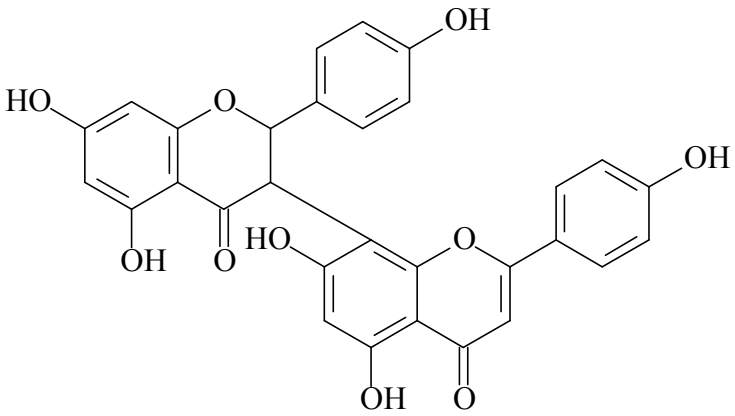
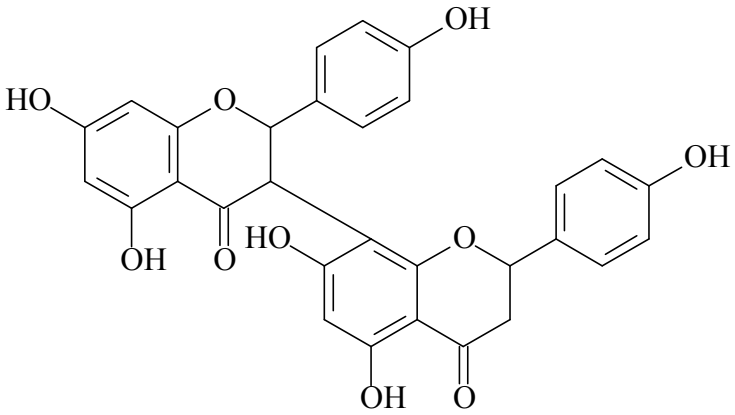
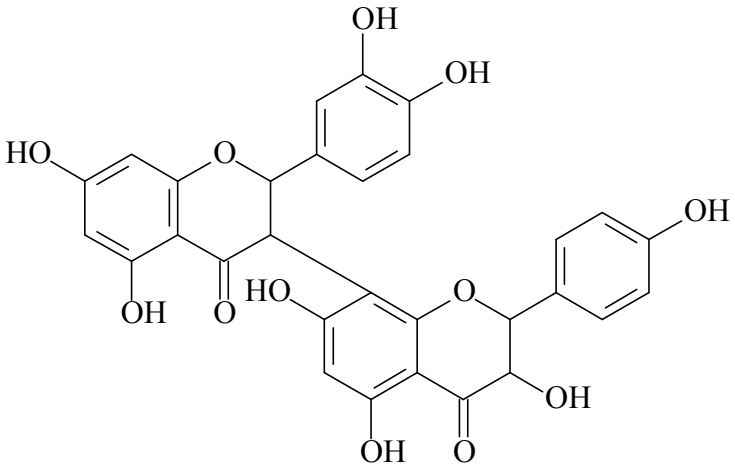
5.2.2. Structure identification of substances from the ethyl acetate extract

Thin layer and column chromatography together with semi-preparative HPLC of the ethyl acetate extract led to the isolation of nine substances. Analysis of NMR spectra (see **Appendix 18 – 23**) and comparison with those of previous reports, the structures of six known compounds were elucidated as shown in **Table 9**. Meanwhile the structures of three new substances were identified by relying on data of UV, 1D and 2D NMR, MS spectra and optical rotation.

Generally, all three new compounds (**24 - 26**) reacted positively with $FeCl_3$ in methanol, which indicated their phenolic feature. The UV spectra of these compounds are similar to those of 1,3,6,7-tetraoxygenated xanthones, comprising four maxima at 208 - 213, 243 - 245, 315 - 317 and 355 - 360 nm (Iinuma et al., 1996; Nilar and Harrison, 2002). The ^{1}H and ^{13}C NMR spectra of these compounds resembled those of the new compounds from the *n*-hexane extract, which revealed that they were prenylated xanthone derivatives containing a hydrogen-bonded hydroxyl group at δ_H 13.88 – 13.99, a conjugated carbonyl carbon at δ_C 182.8 – 183.1, two isolated aromatic protons at δ_H 6.81 – 6.83 and 6.34 – 6.36 and a methoxyl group at δ_H 3.77 – 3.79. Moreover the ^{1}H and ^{13}C NMR spectra also showed the presence of hydroxylated geranyl and isoprenyl groups.

Table 9. The structure of substances isolated from the ethyl acetate extract

Isolated compounds	Structure
23	 11-Hydroxy-1-isomangostin (Sia et al., 1995)
27	 Griffipavixanthone (Xu et al., 1998)
28	 Morelloflavone (Waterman and Crichton, 1980)

29	 Volkinflavone (Chen et al., 1975)
30	 GB-1a (Jackson et al., 1971)
31	 GB-2 (Jackson et al., 1971)

5.2.2.1. Compound 24 (fuscaxanthone N)

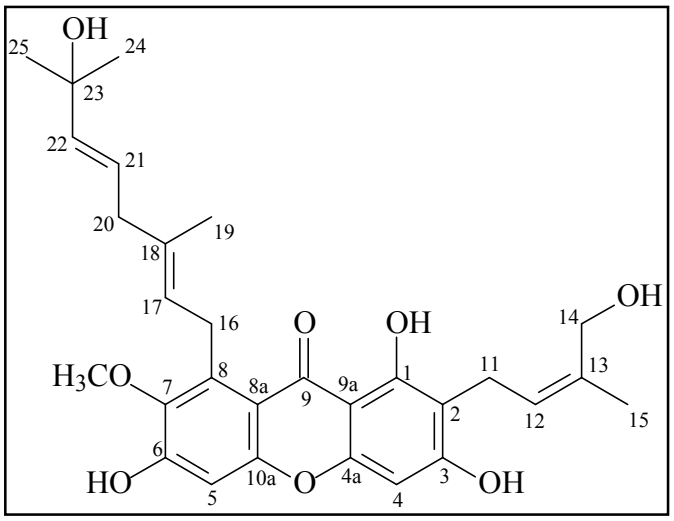


Figure 50. Structure of fuscaxanthone N (**24**)

Compound **24** was obtained as a yellow oil and the molecular formula was deduced to be $C_{29}H_{34}O_8$ from the HRESIMS (*m/z* 509.2169 $[M - H]^-$). In the ^1H and ^{13}C NMR spectrum (see **Figure 53** and **Table 10**), the presence of a hydrogen-bonded hydroxyl group [δ_H 13.88 (1H, s, 1-OH)] corresponding with a chelated carbonyl carbon [δ_C 182.8 (C-9)], two separated aromatic protons [δ_H 6.83 (1H, s, H-5) and 6.36 (1H, s, H-4); δ_C 102.8 (C-5) and 93.5 (C-4)], a methoxyl group [δ_H 3.79 (3H, s, 7-OCH_3); δ_C 61.4 (CH_3O-7)] and a 4-hydroxy-3-methylbut-2-enyl group [δ_H 5.34 (1H, t, J = 7.8 Hz, H-12), 4.29 (1H, s, H-14), 3.44 (2H, d, J = 7.8 Hz, H_2-11) and 1.72 (3H, s, H_3-15); δ_C 135.7 (C-13), 125.3 (C-12), 61.8 (C-14), 22.0 (C-15) and 21.7 (C-11)] were observed together with three vinyl protons [δ_H 5.58 (1H, m, H-22), 5.55 (1H, m, H-21) and 5.35 (1H, t, J = 6.9 Hz, H-17); δ_C 136.9 (C-22), 128.6 (C-21) and 125.5 (C-17)], a benzylic methylene group [δ_H 4.13 (2H, t, J = 6.9 Hz, H_2-16); δ_C 26.8 (C-16)], an vinyl methylene group [δ_H 2.67 (2H, d, J = 6.0 Hz, H_2-20), δ_C 43.5 (C-20)] and three methyl groups [δ_H 1.81 (3H, s, H_3-19), 1.29 (3H, s, H_3-24) and 1.21 (3H, s, H_3-25); δ_C 24.9 (C-24 and C-25) and 16.5 (C-19)]. The ^{13}C NMR spectrum also showed signals for six oxygenated aromatic carbons (δ_C 144.5 – 163.2) and an oxygenated quaternary carbon [δ_C 81.4 (C-23)]. These spectral data indicated that **24** is a prenylated 1,3,6,7-tetrahydroxyxanthone like the new xanthones isolated from the *n*-hexane extract,

containing the 4-hydroxy-3-methylbut-2-enyl group and a C_{10} side chain with two double-bonds. These results were further supported by the data of the HMBC and COSY spectra.

The HMBC correlations of the benzylic methylene protons (δ_H 4.13) with a fully substituted olefinic carbon (δ_C 134.1) and a protonated olefinic carbon (δ_C 125.5) as well as the COSY interaction between these protons and the vinyl proton (δ_C 5.35) showed that the fully substituted olefinic carbon is C-18 and the protonated olefinic carbon is C-17. The HMBC cross-peaks of the methyl protons at δ_H 1.81 and the allylic protons (δ_H 2.67) with C-17 and C-18 revealed that the methyl group and the vinyl methylene group are bonded to C-18. The protons H_2-20 gave the HMBC correlations with two protonated olefinic carbons (δ_C 136.9 and 128.6) and the COSY interaction with the vinyl proton (δ_H 5.55). This indicated that the olefinic carbon (δ_C 128.6) is C-21 and the other one is C-22. Moreover the HMBC correlations of the vinyl proton (δ_H 5.58) with the oxygenated quaternary carbon (δ_C 81.4) and two methyl carbons (δ_C 24.9) showed that the oxygenated quaternary carbon is C-23 and two methyl groups are attached to C-23. In the ROESY spectrum (see **Figures 51** and **52**), the interactions of H-20/H-22 and H-21/H-24 showed that the configuration of the double bond at C-21/C-22 is *trans*. Thus, the C_{10} side chain is a 7-hydroxy-3,7-dimethylocta-2,5-dienyl group. This was supported by comparison of the spectral data with those of 1,3,6-trihydroxy-8-(7-hydroxy-3,7-dimethyl-2,5-octadienyl)-7-methoxyxanthone, which was previously reported from *G. dioica* (Iinuma et al., 1996). Furthermore the benzylic methylene protons of this side chain gave the resonance in a low field (δ_H 4.13) together with the HMBC correlations with C-7 (δ_C 144.5), C-8 (137.9) and C-8a (111.9), i.e. they are deshielded by the adjacent carbonyl group and the C_{10} unit is located at C-8. On the other hand, the benzylic methylene protons (δ_H 5.34) of the 4-hydroxy-3-methylbut-2-enyl group exhibited the HMBC cross-peaks with two oxygenated aromatic carbons [δ_C 163.2 (C-3) and 161.6 (C-1)] and the substituted aromatic carbon [δ_C 110.1 (C-2)]. This allowed the placement of the group at C-2. Hence, compound **24** was determined as 1,3,6-trihydroxy-2-(4-hydroxy-3-

methylbut-2-enyl)-8-(7-hydroxy-3,7-dimethylocta-2,5-dienyl)-7-methoxyxanthone and was given a trivial name fuscaxanthone N.

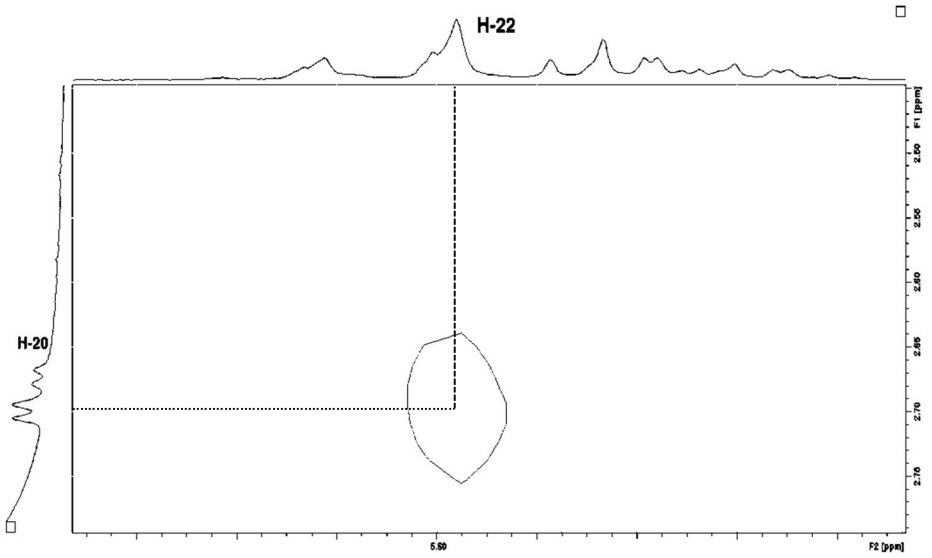


Figure 51. The NOESY interaction between H-22 and H_2-20

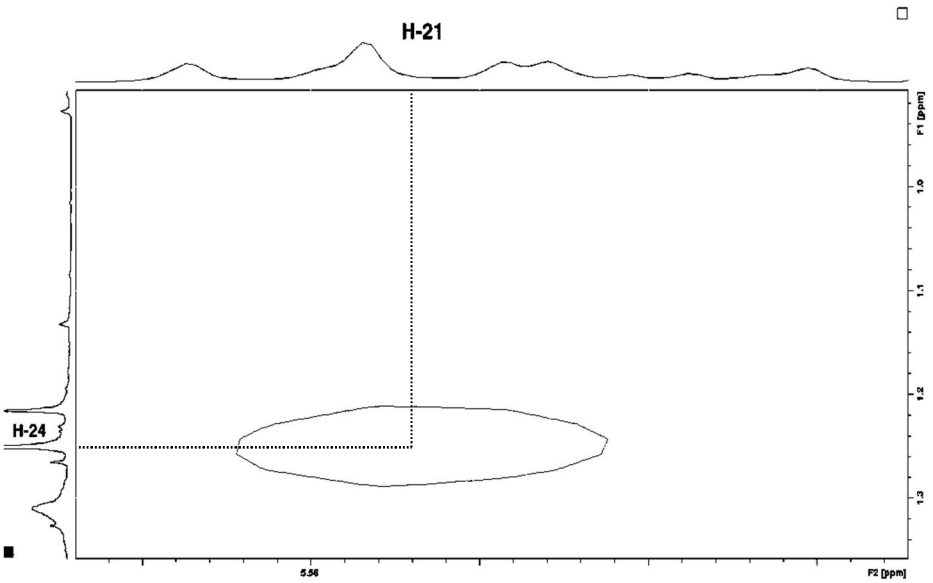


Figure 52. The NOESY interaction between H-21 and H_3-24

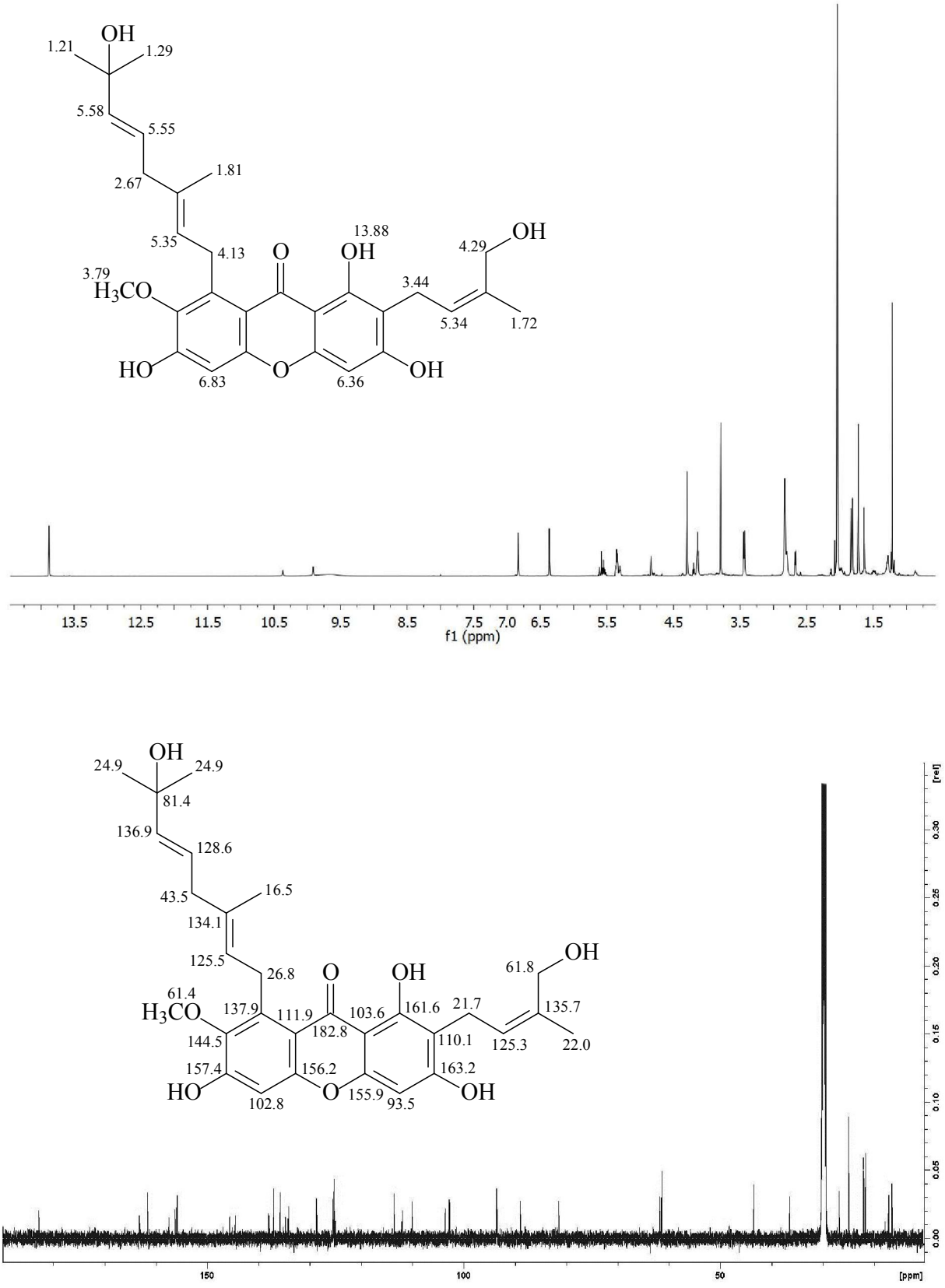


Figure 53. ^{1}H and ^{13}C NMR spectra of fuscaxanthone N (**24**)

5.2.2.2. Compound 25 (fuscaxanthone O)

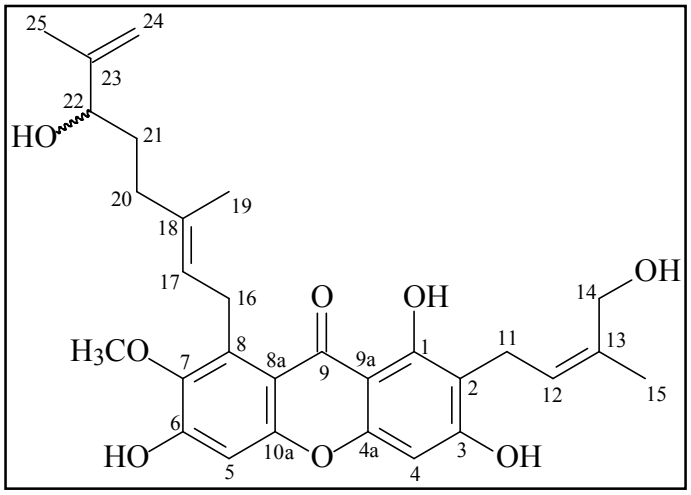


Figure 54. Structure of fuscaxanthone O (**25**)

Compound **25** was isolated as a yellow oil and the molecular formula was established as $C_{29}H_{34}O_8$ based on the HRESIMS (*m/z* 511.2322 $[M + H]^+$). The ^1H and ^{13}C NMR spectra (**Figure 56** and **Table 10**) were similar to those of compound **24**, comprising the signals for a 1,3,6,7-tetrahydroxyxanthone nucleus [δ_H 13.91 (1H, s, 1-OH), 6.81 (1H, s, H-5), 6.35 (1H, s, H-4); δ_C 181.8 (C-9), 163.3 (C-3), 161.6 (C-1), 157.6 (C6/C-10a), 156.3 (C10a/C-6), 155.9 (C-4a), 144.6 (C-7), 138.1 (C-8), 111.8 (C-8a), 110.1 (C-2), 103.5 (C-9a), 102.7 (C-5) and 93.5 (C-4)], a methoxyl group [δ_H 3.79 (3H, s, 7-OCH_3); δ_C 61.4 (CH_3O-7)], a 4-hydroxy-3-methylbut-2-enyl group [δ_H 5.35 (1H, t, J = 7.8 Hz, H-12), 4.29 (2H, s, H_2-14), 3.43 (2H, d, J = 7.8 Hz, H_2-11) and 1.72 (3H, s, H_3-15); δ_C 135.7 (C-13), 125.3 (C-12), 61.8 (C-14), 22.0 (C-15) and 21.7 (C-11)] and a modified geranyl group [δ_C 5.30 (1H, t, J = 6.6 Hz, H-17), 4.82 (1H, br s, H_Z-24), 4.67 (1H, br s, H_E-24), 4.13 (2H, d, J = 6.6 Hz, H_2-16), 3.94 (1H, t, J = 6.6 Hz, H-22), 2.05 (1H, m, H_A-20), 1.98 (1H, m, H_B-20), 1.84 (3H, s, H_3-19), 1.63 (3H, s, H_3-25) and 1.58 (2H, m, H_2-21); δ_C 149.2 (C-23), 135.2 (C-18), 124.7 (C-17), 110.3 (C-24), 75.2 (C-22), 36.6 (C-20), 34.5 (C-21), 26.8 (C-16), 17.8 (C-25) and 16.7 (C-19)].

The benzylic methylene protons of the geranyl group gave the less shielded signal at δ_H 4.13 and the HMBC correlations with C-7, C-8 and C-8a, i.e. this group is bonded to C-8. The COSY interaction between two allylic protons (δ_H 2.05 and 1.98) and two methylene protons at δ_H 1.58 indicated the connectivity of C-20 and C-21.

The protons H_2-21 exhibited the HMBC cross-peaks with a fully substituted olefinic carbon (δ_C 149.2) and an oxygenated tertiary carbon (δ_C 75.2), in addition to the COSY interaction with the proton of the oxygenated tertiary carbon (δ_H 3.94), which revealed that the oxygenated quaternary carbon is C-22 and C-23 is the fully substituted olefinic carbon. The proton H-22 gave the HMBC correlations to an unsubstituted olefinic carbon (δ_C 110.3) and a vinyl methyl group (δ_C 17.8), i.e. the unsubstituted olefinic carbon is C-24 and the methyl group is C-25. The vinyl proton (δ_H 4.82) is H_Z due to the NOESY interaction with the protons of the vinyl methyl group and the other one (δ_H 4.67) is H_E, corresponding to the NOESY interaction with the proton H-22 (see **Figure 55**). Hence the geranyl group is modified into the 6-hydroxy-3,7-dimethyloct-2,7-dienyl group. This was further confirmed by comparison of the spectral data of this group with those of parvixanthone C, which was previously isolated from *G. parvifolia* (Xu et al., 2001). Compound **25** was therefore identified as 1,3,6-trihydroxy-2-(4-hydroxy-3-methylbut-2-enyl)-8-(6-hydroxy-3,7-dimethyloct-2,7-dienyl)-7-methoxyxanthen-9-one and was given a trivial name fuscaxanthone O. However the configuration of the chiral carbon C-22 could not be determined.

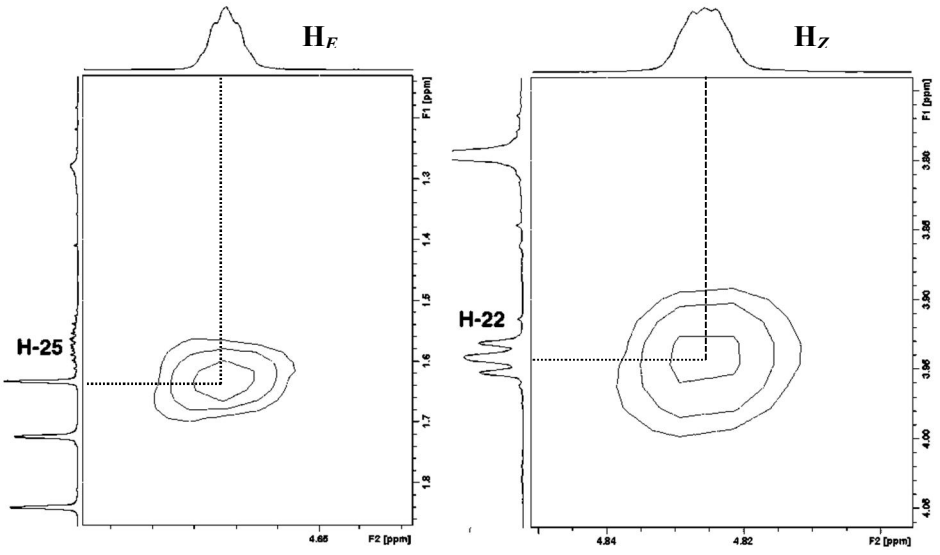


Figure 55. The NOESY interaction between H_2-24 and H-22 and H_3-25

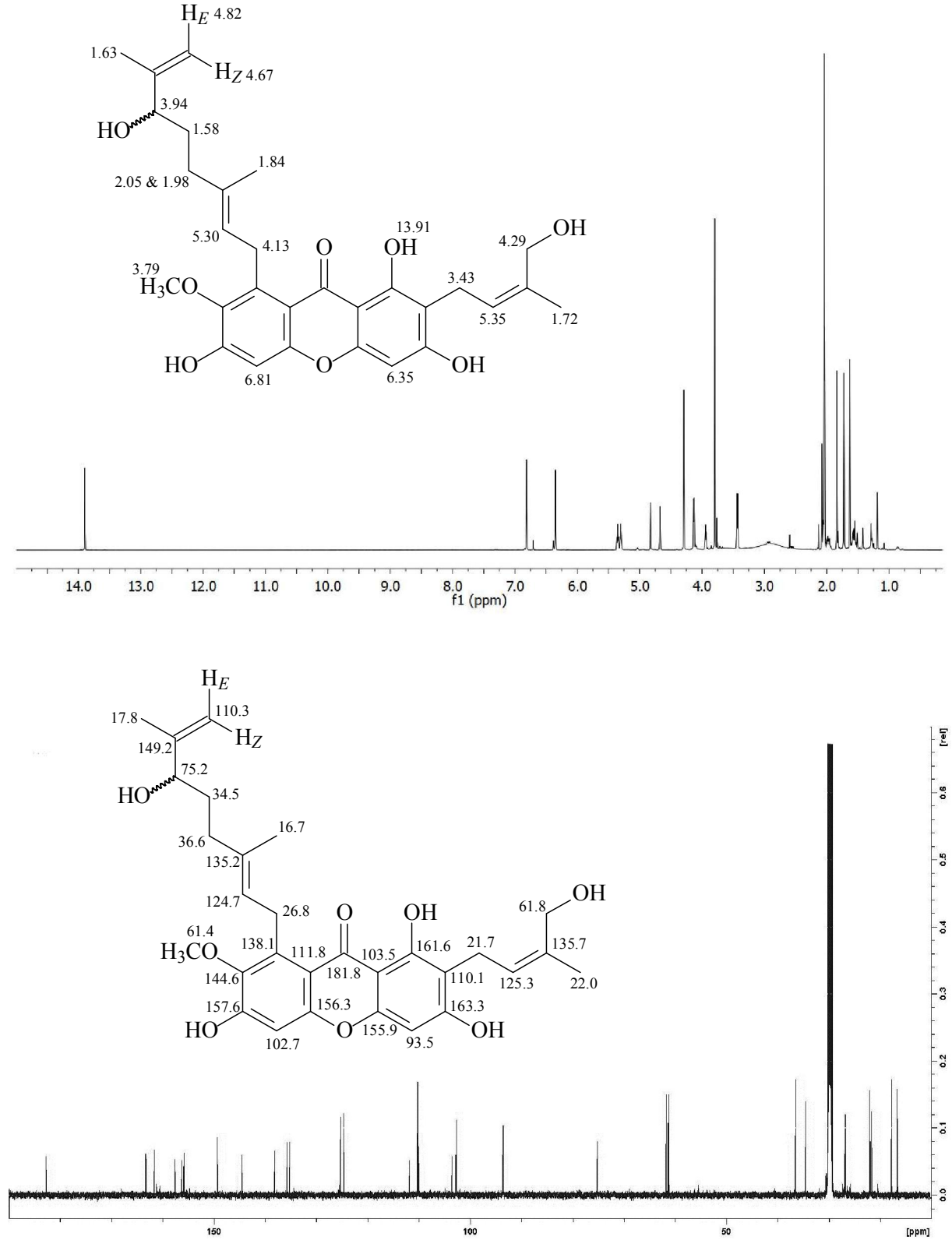


Figure 56. ^{1}H and ^{13}C NMR spectra of fuscaxanthone O (**25**)

Table 10. ^{1}H and ^{13}C NMR data of **24** and **25** in acetone-d_6

Position	**24**		**25**	
	δ_H	δ_C	δ_H	δ_C
1		161.6		161.6
2		108.0		110.1
3		163.2		163.3
4	6.36 s	93.5	6.35 s	93.5
4a		155.9		155.9
5	6.83 s	102.8	6.81 s	102.7
6		157.4[a]		157.6[a]
7		144.5		144.6
8		137.8		138.1
8a		111.9		111.8
9		182.8		181.8
9a		103.6		103.5
10a		156.2[a]		156.3[a]
11	3.44 d (7.8 Hz)	21.7	3.43 d (7.8 Hz)	21.7
12	5.34 t (7.8 Hz)	125.3	5.35 t (7.8 Hz)	125.3
13		135.7		135.7
14	4.29 s	61.8	4.29 s	61.8
15	1.72 s	22.0	1.72 s	22.0
16	4.13 t (6.9 Hz)	26.8	4.13 d (6.6 Hz)	26.8
17	5.35 t (6.9 Hz)	125.5	5.30 t (6.6 Hz)	124.7
18		134.1		135.2
19	1.81 s	16.5	1.84 s	16.7
20	2.67 d (6.0 Hz)	43.5	2.05 m	36.6
			1.98 m	
21	5.55 m	128.6	1.58 m	34.5
22	5.58 m	136.9	3.94 t (6.6 Hz)	75.2
23		81.4		149.2
24	1.29 s	24.9	4.82 brs H_Z	110.3
			4.67 brs H_E	
25	1.21 s	24.9	1.63 s	17.8
1-OH	13.88 s		13.91 s	
7-OCH_3	3.79 s	61.4	3.79 s	62.1

[a] Interexchangeable, 600 MHz for ^{1}H and 150 MHz for ^{13}C (J in Hz and δ in ppm) at 298ºK

5.2.2.3. Compound 26 (fuscaxanthone P)

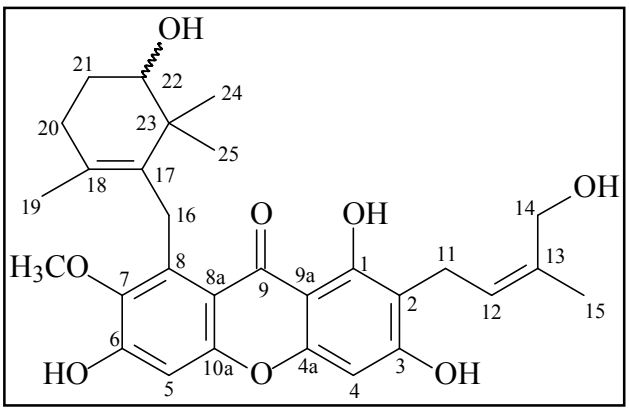
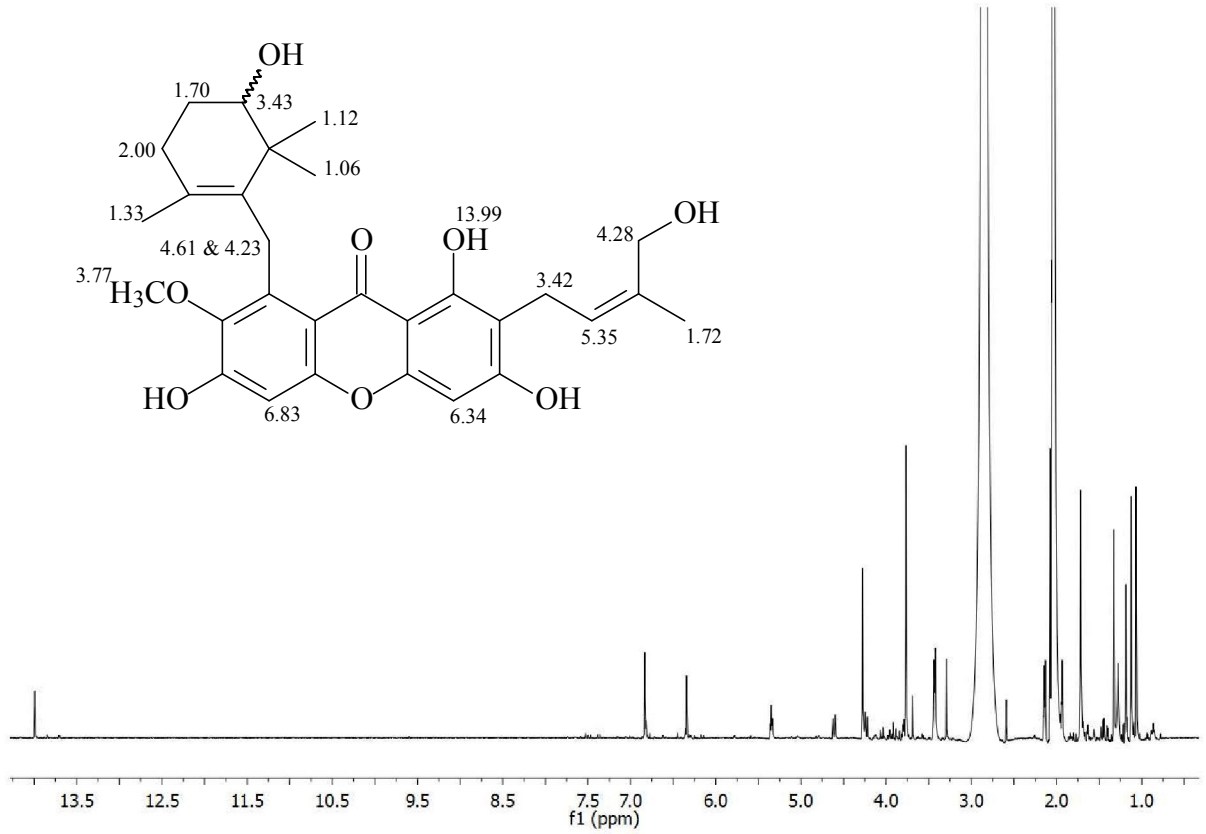


Figure 57. Structure of fuscaxanthone P (**26**)

Compound **26** was isolated as a yellow oil and was given the molecular formula as $C_{29}H_{34}O_8$ with thirteen degrees of unsaturation on the basis of HRESIMS (*m*/*z* 511.2330 $[M + H]^+$). The ^1H and ^{13}C NMR spectra (see **Figure 58** and **Table 11**) indicated that this compound is a 1,3,6,7-tetrahydroxyxanthone including a hydrogen-bonded hydroxyl group [δ_H 13.99 (1H, s, 1-OH)], two separated aromatic protons [δ_H 6.83 (1H, s, H-5) and 6.34 (1H, s, H-4); δ_C 102.8 (C-5) and 93.5 (C-4)], a methoxyl group [δ_H 3.77 (3H, s, 7-OCH_3); δ_C 60.3 (CH_3O-7)] and a 4-hydroxy-3-methylbut-2-enyl group [δ_H 5.35 (1H, t, J = 7.6 Hz, H-12), 4.28 (2H, s, H_2-14), 3.43 (2H, d, J = 7.6 Hz, H_2-11) and 1.72 (3H, s, H_3-15); δ_C 135.6 (C-13), 125.3 (C-12), 61.7 (C-14), 22.2 (C-15) and 21.7 (C-11)]. The ^1H and ^{13}C spectra also displayed the resonances for a C_{10} side chain consisting of a benzylic methylene group [δ_H 4.61 (1H, d, J = 15.6 Hz, H_A-16) and 4.23 (1H, d, J = 15.6 Hz, H_B-16); δ_C 28.7 (C-16)], a oxygenated methine group [δ_H 3.43 (1H, overlapped with H-11, H-22); δ_C 76.0 (C-22)], an vinyl methylene group [δ_H 2.00 (2H, overlapped, H_2-20); δ_C 32.8 (C-20)], another methylene group [δ_H 1.70 (2H, m, H_2-21); δ_C 27.9 (C-21)], a vinyl methyl group [δ_H 1.33 (3H, s, H_3-19); δ_C 20.7 (C-19)] and two other methyl groups [δ_H 1.12 (3H, s, H_3-24/25), 1.06 (3H, s, H_3-25/24); δ_C 26.6 (C-24/25) and 21.8 (C-25/24)]. Due to thirteen degrees of unsaturation in the molecule, except for ten degrees of the xanthone nucleus and one of the 4-hydroxy-3-methylbut-2-enyl group, the C_{10} side chain therefore has two degrees of

unsaturation, i.e. this group contains a double-bond and a ring, which was further confirmed by the HMBC and COSY spectra.

The benzylic methylene protons (δ_H 4.61) showed the HMBC correlations with two fully substituted olefinic carbons (δ_C 135.8 and 125.6) and a quaternary carbon (δ_C 41.7). Besides the protons of two methyl groups (δ_H 1.12 and 1.06) gave the HMBC cross-peaks to the fully substituted carbon (δ_C 135.8) and the quaternary carbon as well as the ROESY interactions with the benzylic methylene protons. This revealed that the fully substituted carbon at δ_C 135.8 is C-17, the other one is C-18, C-23 is the quaternary carbon and two methyl groups are attached to C-23. The HMBC correlations of the proton of the oxygenated methine group (δ_H 3.43; δ_C 76.0) with C-23 and the two methyl groups pointed out the connectivity of this oxygenated methine group with C-23 and the oxygenated tertiary carbon is C-22. The COSY interaction between the methylene proton (δ_H 1.70) and H-22 indicated that this methylene group is linked with C-22. The allylic protons (δ_H 2.00) gave the HMBC correlations to C-17, C-18, C-21 and C-22, which revealed that these protons are located at C-20. Moreover the vinyl methyl protons (δ_H 1.33) exhibited the HMBC cross-peaks with C-17, C-18 and C-20, i.e. this methyl group is bonded to C-18. Hence the C_{10} side chain was determined as the 5-hydroxy-2,6,6-trimethylcyclohexenylmethyl group. The HMBC correlation of H-16 with C-7 (δ_C 145.7), C-8 (δ_C 139.4) and C-8a (112.5), in addition to the ROESY interaction with the methoxyl protons (7-OCH_3) allowed the placement of the 5-hydroxy-2,6,6-trimethylcyclohexenylmethyl group at C-8. Compound **26** was identified as 1,3,6-trihydroxy-2-(4-hydroxy-3-methylbut-2-enyl)-8-(5-hydroxy-2,6,6-trimethylcyclohexenylmethyl)-7-methoxyxanthone and was given a trivial name fuscaxanthone P. However the configuration of the chiral carbon C-23 could not be resolved.



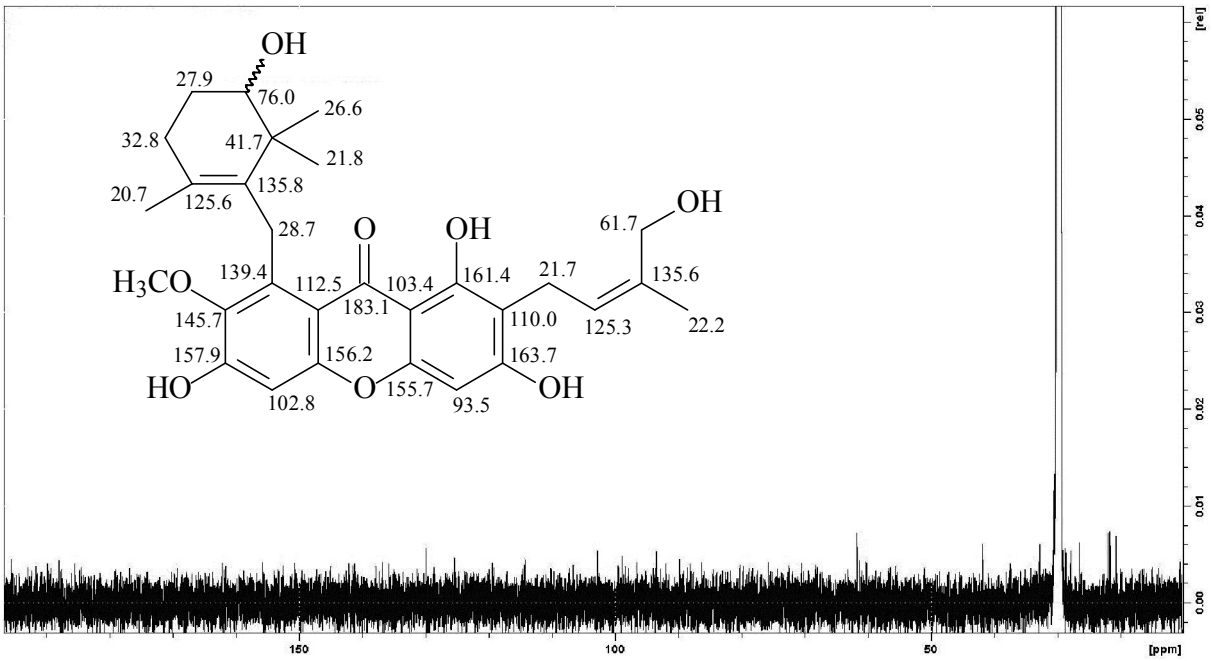


Figure 58. ^{1}H and ^{13}C NMR spectra of fuscaxanthone P (**26**)

Table 11. ^{1}H and ^{13}C NMR data of **26** in acetone-d_6

Position	δ_H	δ_C
1		161.4
2		110.0
3		163.7
4	6.34 s	93.5
4a		155.7
5	6.83 s	102.8
6		157.9[a]
7		145.7
8		139.4
8a		112.5
9		183.1
9a		103.4
10a		156.2[a]
11	3.43 d (7.6 Hz)	21.7
12	5.35 t (7.6 Hz)	125.3
13		135.6
14	4.28 s	61.7
15	1.72 s	22.2
16	4.61 d (15.6 Hz)	28.7
	4.23 d (15.6 Hz)	
17		135.8
18		125.6
19	1.33 d (5.4 Hz)	20.7
20	2.00 overlap with H-20	32.8
21	1.70 m	27.9
22	3.43 overlap with H-11	76.0
23		41.7
24	1.12[a] s	26.6
25	1.06[a] s	21.8
1-OH	13.99 s	
7-OCH_3	3.77 s	60.3

[a] Interchangeable, 600 MHz for ^{1}H and 150 MHz for ^{13}C (J in Hz and δ in ppm) at 298ºK

5.3. Cytotoxicity of new substances

Eight new xanthones, cowanol and griffpavixanthone were tested for the cytotoxicity on HeLa cells using a modified MTT assay according to Mosman (1983). As shown in **Table 12**, griffpavixanthone possessed relatively high activity with IC_{50} value of 7.9 ± 0.7 μM, whereas cowanol and (+)-fuscaxanthone I (**19**) showed moderate cytotoxicity with IC_{50} values of 19.1 ± 1.0 and 19.9 ± 0.9 μM, respectively. Five compounds fuscaxanthone L (**18**), fuscaxanthone J (**21**), fuscaxanthone N (**24**), fuscaxanthone O (**25**) and fuscaxanthone P (**26**) exhibited a weak inhibitory effect with the range of IC_{50} values of 32.5 – 45.9 μM. Meanwhile the remaining compounds fuscaxanthone K (**20**) and fuscaxanthone M (**22**) were not cytotoxic. The new xanthones possess the basic structure of cowanol with modification of the geranyl or 4-hydroxy-3-methylbut-2-enyl group. Demethylation of the methoxyl group in **21** or modification of the double-bond at C-22/C-23 as in fuscaxanthone N and O (**24** and **25**) or ring-closure of the geranyl group in fuscaxanthone L and P (**18** and **26**) resultes in a decrease of the activity compared to that of cowanol. The hydroxylation of the double-bond at C-17/C-18 in (+)-fuscaxanthone I (**19**) has no effect on the cytotoxicity, whereas the epoxylation of the double-bond at C-12/C-13 in fuscaxanthone K (**20**) brings about the losing of activity. Compound **22** is derived from two cowanol units through a C-O-C linkage. Though cowanol showed moderate activity, the C-O-C linkage in fuscaxanthone M (**22**) resultes in the loss of cytotoxicity.

Griffipavixanthone is a dimeric xanthone which was previously isolated from three species of the *Garcinia* genus, *G. griffithii*, *G. pavifolia* and *G.oblongifolia* (Xu et al., 1998; Shi et al., 2014). This xanthone was reported to exhibit a high *in vitro* cytotoxicity against three cancer cell lines P388, LL/2 and Wehil64 with ED_{50} values of 3.4, 6.8 and 4.6 μg/ml, respectively (Xu et al., 1998). In 2014, Shi et al. investigated the effect of griffipavixanthone on cell proliferation of several human non-small cell lung cancer cell lines (H520, H549, H1299 and A-549), human breast cancer cells (MCF-7 and MDA-231), human prostate cells (DU145, PC3 and LNcaP) and human colon cancer cells (HCT-116, HT-29 and SW-480). The investigation showed significant anti-proliferation activity of griffipavixanthone on all of these cells with the

IC_{50} values in the range of 3.03 – 29.33 μM. Furthermore this xanthone showed low cytotoxic effects on non-tumorigenic LO2 cells indicating that it may be selectively cytotoxic for human cancer cells (Shi et al., 2014). Because of such interesting biological properties, in the present work, griffipavixanthone was additionally tested for anti-angiogenic activity by *in vitro* proliferation assay on HMEC-1 cell line as described by Schmidt et al. (2012). It exhibited a very potent anti-proliferative effect on HMEC-1 cells with an IC_{50} value of 0.15 ± 0.0085 μM after 72 h incubation. The result revealed that griffipavixanthone is a promising anti-angiogenic agent which should be further demonstrated in vivo.

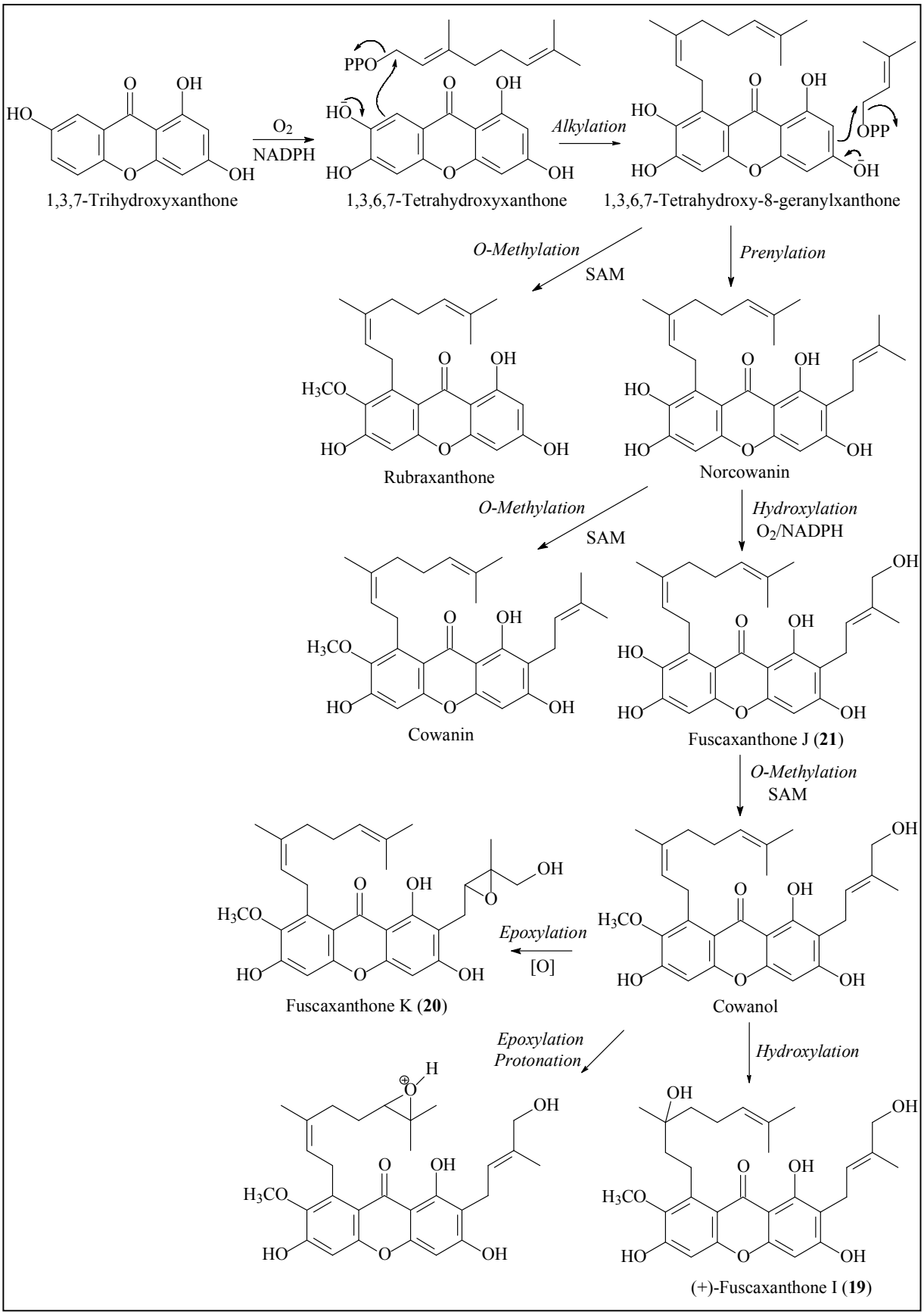
Table 12. Cytotoxicity of **18** – **22**, **24** – **26**, cowanol and griffipavixanthone against HeLa cells.

Compound	IC_{50} ± SD (μM)[a]
Cowanol	19.1 ± 1.0
Fuscaxanthone L (**18**)	41.5 ± 0.4
(+)-Fuscaxanthone I (**19**)	19.9 ± 0.9
Fuscaxanthone K (**20**)	inactive
Fuscaxanthone J (**21**)	32.5 ± 2.8
Fuscaxanthone M (**22**)	inactive
Fuscaxanthone N (**24**)	44.3 ± 2.5
Fuscaxanthone O (**25**)	35.4 ± 1.7
Fuscaxanthone P (**26**)	45.9 ± 1.9
Griffipavixanthone	7.9 ± 0.4

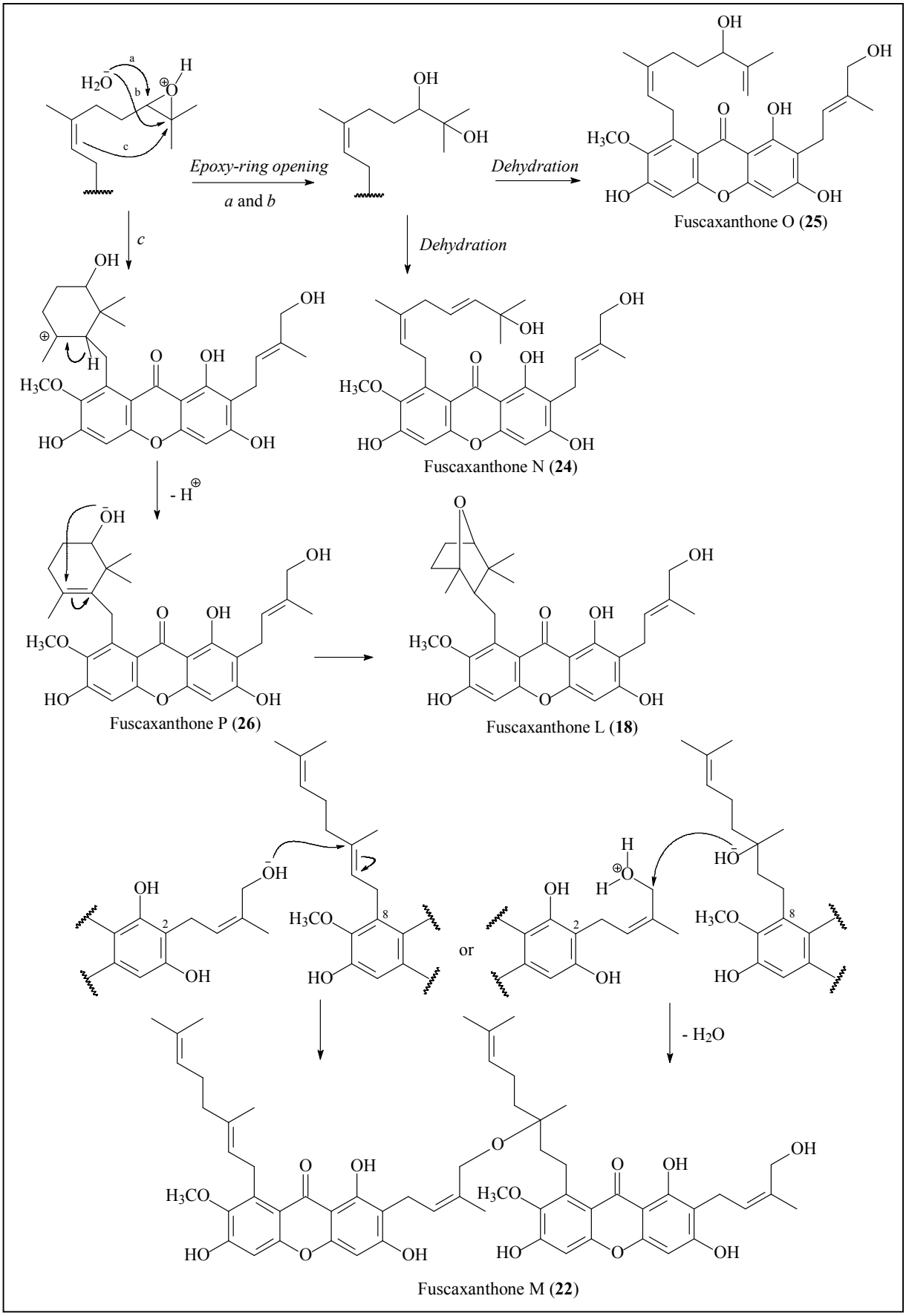
[a] Mean of six measurements

5.4. Proposed biosynthesis of the new xanthones in *Garcinia fusca* Pierre

On the basis of the structure of eight new xanthones as well as the presence of rubraxanthone, norcowanin, cowanin and cowanol, a biosynthetic route (**Scheme 25** and **26**) is suggested for the new xanthones from 1,3,7-trihydroxyxanthone, which together with 1,3,5-trihydroxyxanthone were shown as the precursors for most of xanthones in plants (see section **3.1**, page 48). This xanthone is hydroxylated at C-6 by O_2/NADPH to form 1,3,6,7-tetrahydroxyxanthone, which will be subsequently alkylated at C-8 by a geranyl diphosphate (GPP) to give 1,3,6,7-tetrahydroxy-8-geranylxanthone. The hydroxyl group at C-7 of this xanthone can be methylated by SAM to give rubraxanthone. Meanwhile prenylation of this xanthone at C-2 by a dimethylallyl diphosphate (DMAPP) yielded norcowanin, which is further *O*-methylated at 7-OH by SAM to produce cowanin. On the other hand, a vinyl methyl of isoprenyl group in norcowanin is hydroxylated by O_2/NADPH to form fuscaxanthone J (**21**), which can be subsequently *O*-methylated at 7-OH by SAM to give cowanol. Epoxylation of the double-bond of the isoprenyl group in cowanol yield fuscaxanthone K (**20**), whilst hydroxylation of the double-bond at C-17/C-18 of the geranyl group will produce (+)-fuscaxanthone I (**19**). Moreover the double-bond at C-22/C-23 of the geranyl group in cowanol can be epoxylated by O_2/NADPH and then the epoxy ring can be protonated and opened by a nucleophilic attack of water to generate a diol derivative which is dehydrated to form fuscaxanthone N and O (**24** and **25**). Besides the epoxy ring can be opened by the nucleophilic attack of the double-bond at C-17/C-18 to yield the 5-hydroxy-2,6,6-trimethylcyclohexenylmethyl group as in fuscaxanthone P (**26**). The double-bond of the 5-hydroxy-2,6,6-trimethylcyclohexenylmethyl group could further undergo the nucleophilic attack of the hydroxyl group to generate an oxo-bridge as in fuscaxanthone L (**18**). Similarly, compound **22** (fuscaxanthone M) is derived from two cowanol units *via* the C-O-C linkage, which can be formed either by the nucleophilic attack of the hydroxyl group of the 4-hydroxy-3-methylbut-2-enyl side chain to the double-bond at C-17/C-18 of the geranyl group or by condensation between cowanol and (+)-fuscaxanthone I (**19**).



Scheme 25. Proposed biosynthesis of the new xanthones in *G. fusca*



Scheme 26. Proposed biosynthesis of the new xanthones in *G. fusca* (*continued*)

6. CONCLUSION

The phytochemical investigation of the *n*-hexane extract (GFH) and the ethyl acetate extract (GFE) of the bark of *Garcinia fusca* collected in south Vietnam resulted in the isolation of thirty one natural compounds using various chromatographic methods such as TLC, CC, FC, CPC, GPC and semi-preparative HPLC on silica gel, RP_{18} and Sephadex LH-20. The structures of isolated compounds were determined on the basis of spectral data such as UV, 1D and 2D NMR and MS spectra, as well as the optical rotation. These compounds include a tocotrienol, a benzaldehyde derivative, four biflavonoids and twenty five prenylated xanthones, nine of which have been first found in nature. The cytotoxicity of the new xanthones was tested on HeLa cells using MTT assay. The results indicated that five of them exhibited cytotoxic effect with the IC_{50} values in the range of 19.9 – 45.9 μM. Furthermore, the anti-angiogenic activity of a bixanthone, griffipavixanthone was evaluated in a proliferation assay using HMEC-1 cell line. This bixanthone showed strong anti-proliferation activity with the IC_{50} of 0.15 ± 0.0085 μM, which revealed that it is a potential anti-angiogenic agent and should be further investigated in vivo.

Because of the limited time, some fractions have not been studied yet. However the present work showed the difference of the chemical composition of the bark between this species and the species collected in Thailand (Ito et al., 2003; Nontakham et al., 2014). For further work, the remaining fractions will be investigated to isolate more compounds and new compounds will be biologically studied for various properties such as anti-oxidant, antibacterial and anticancer activities.

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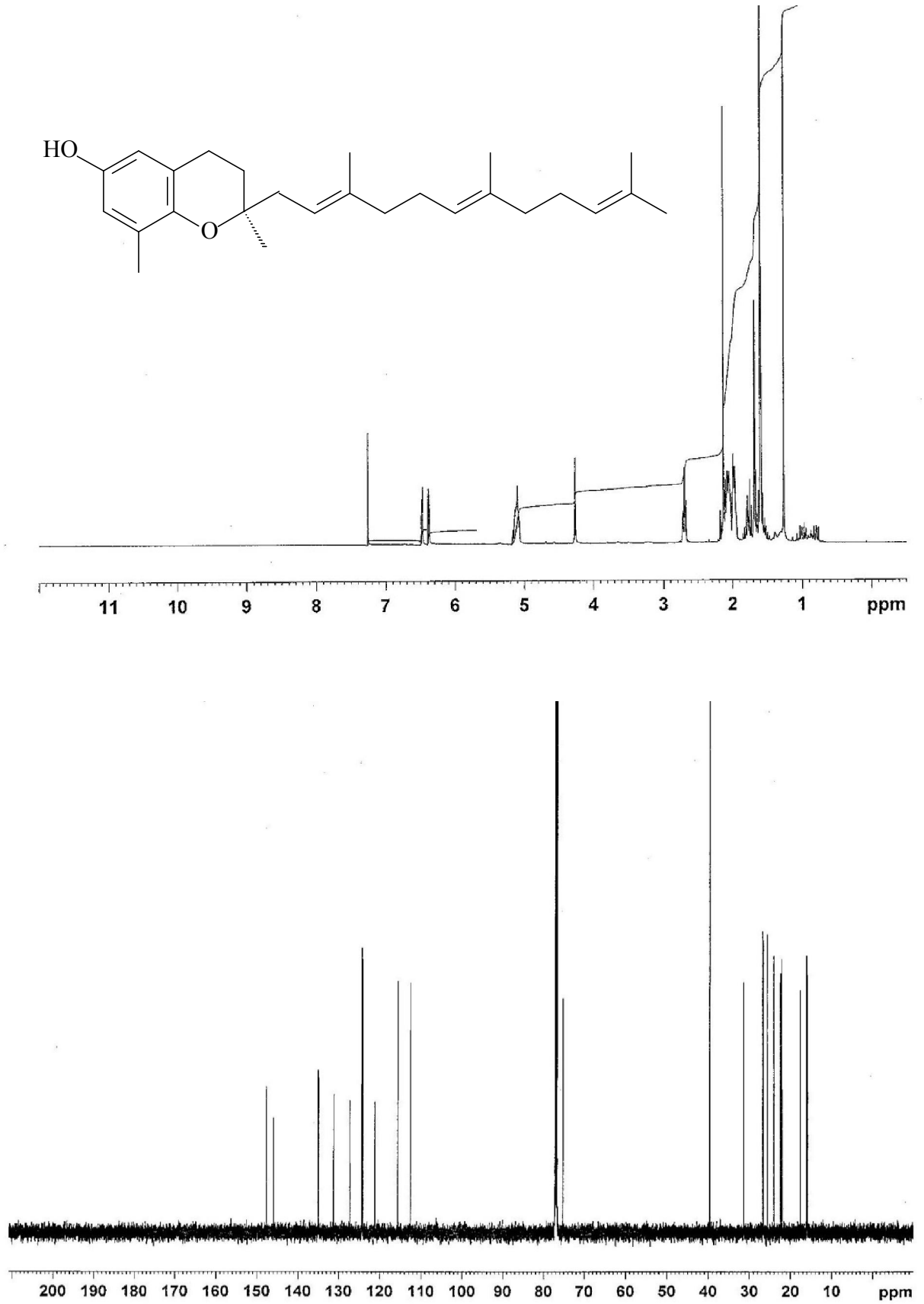
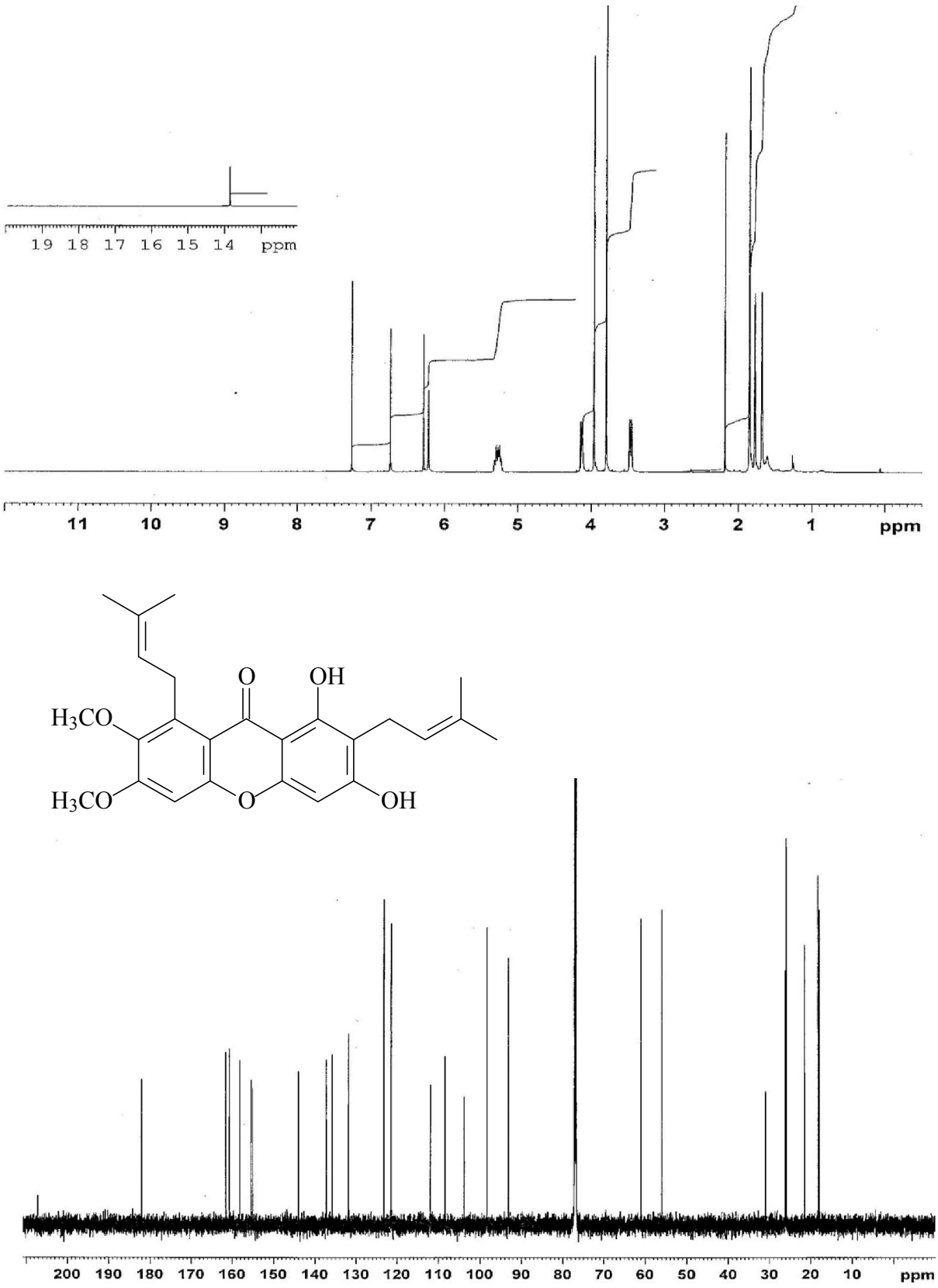
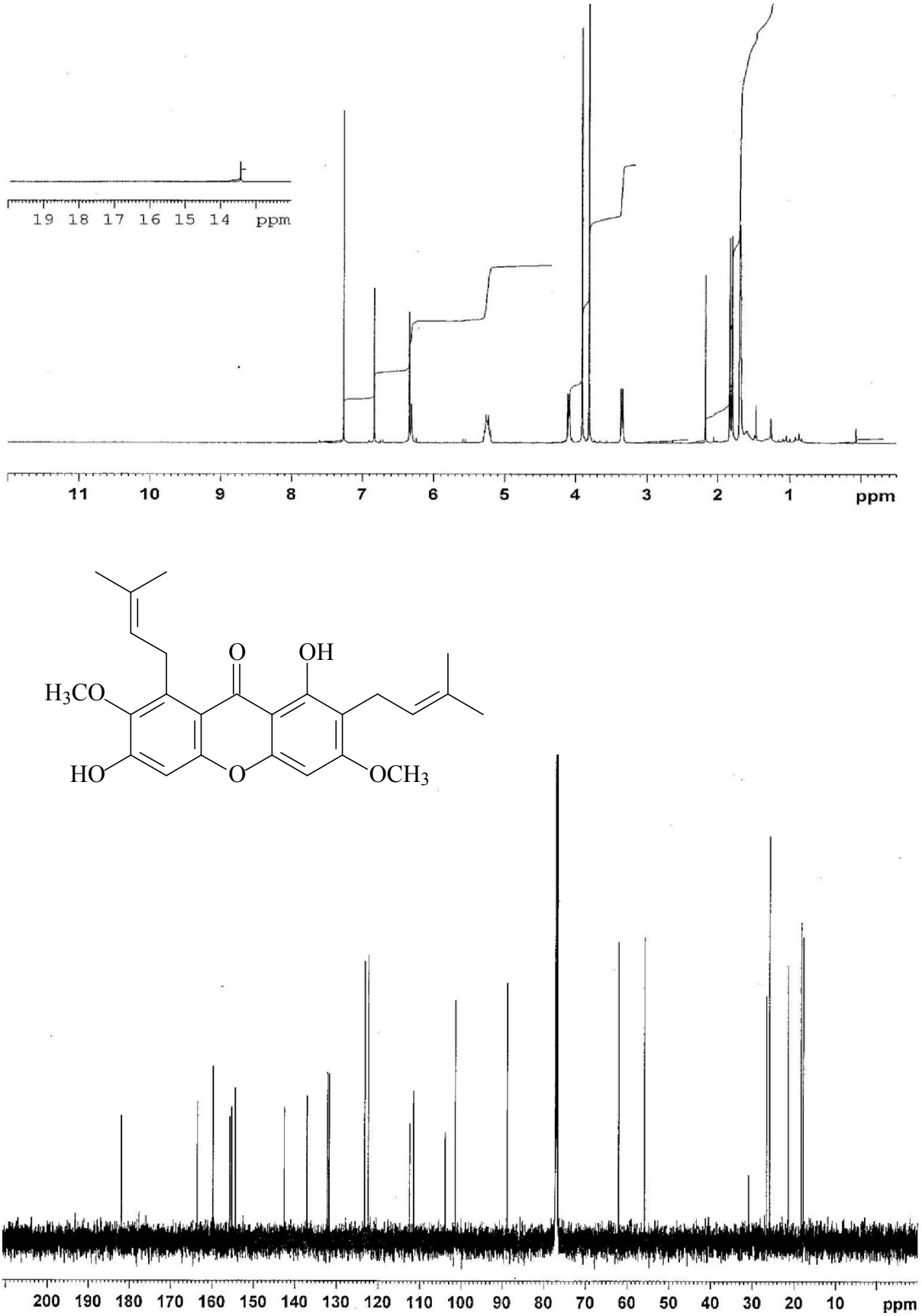


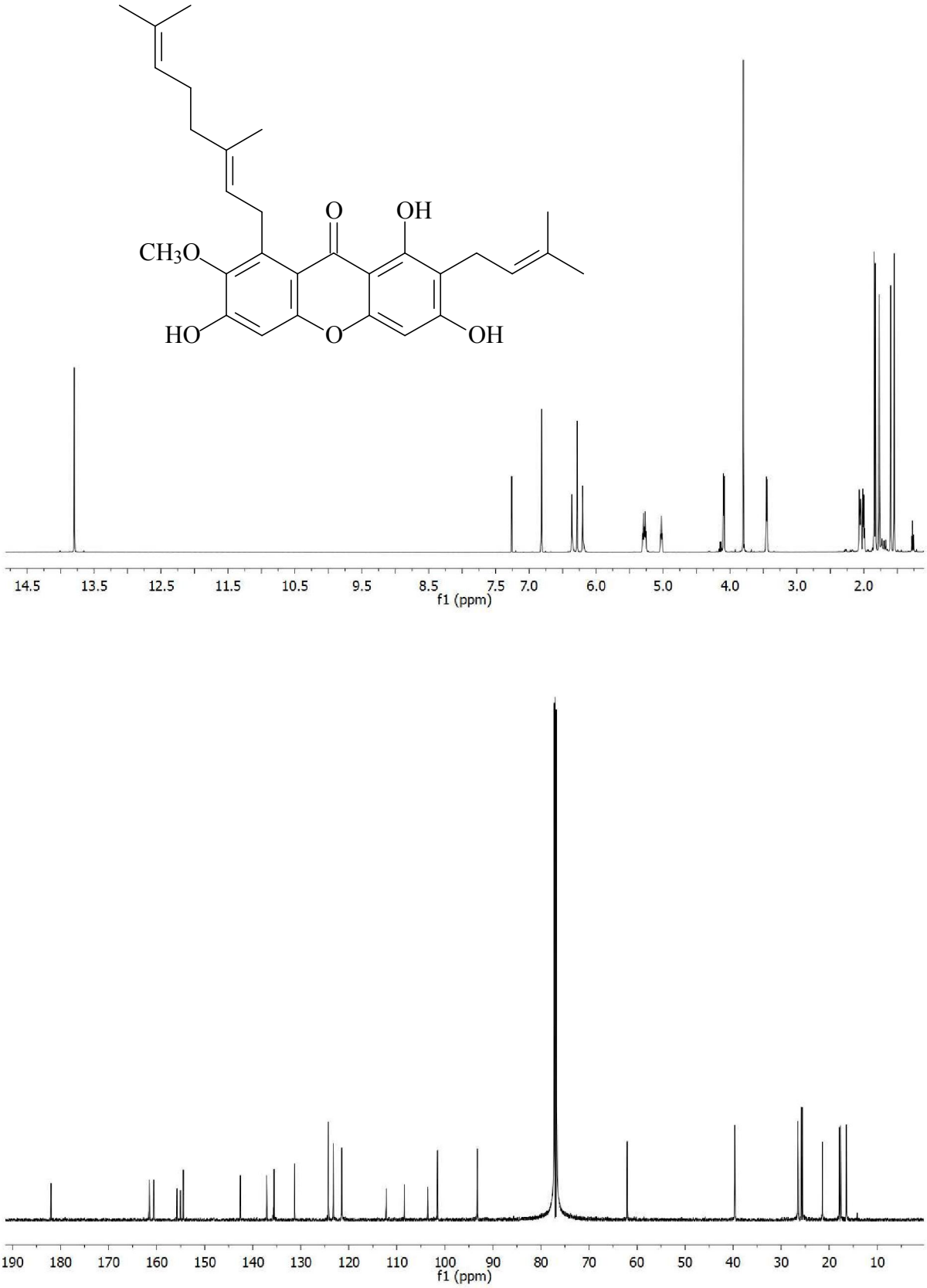
Appendix 1. ^{1}H and ^{13}C NMR spectra of δ-tocotrienol (**1**)



Appendix 2. ^{1}H and ^{13}C NMR spectra of 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone (**2**)



Appendix 3. ^{1}H and ^{13}C NMR spectra of β-mangostin (**3**)



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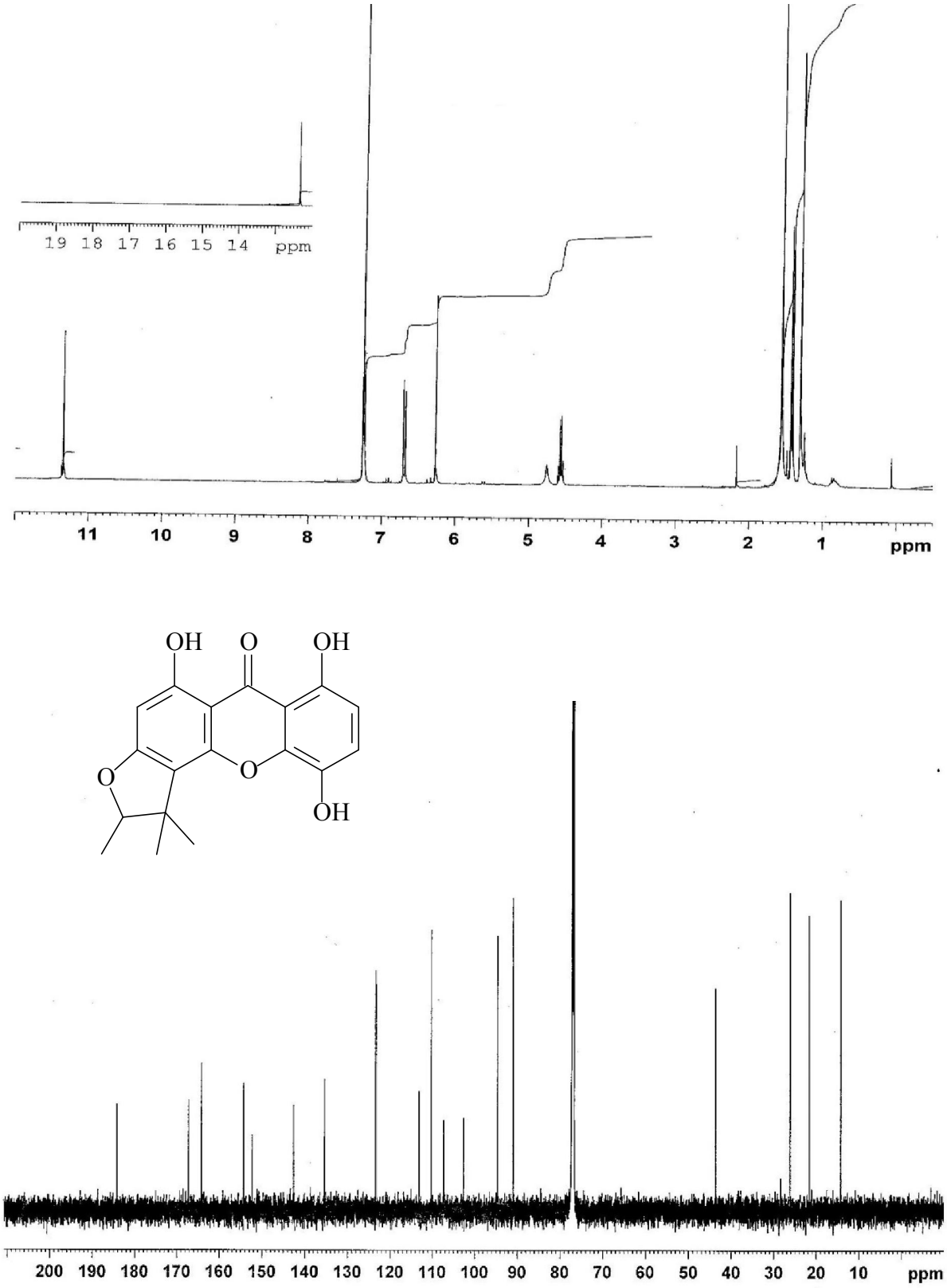

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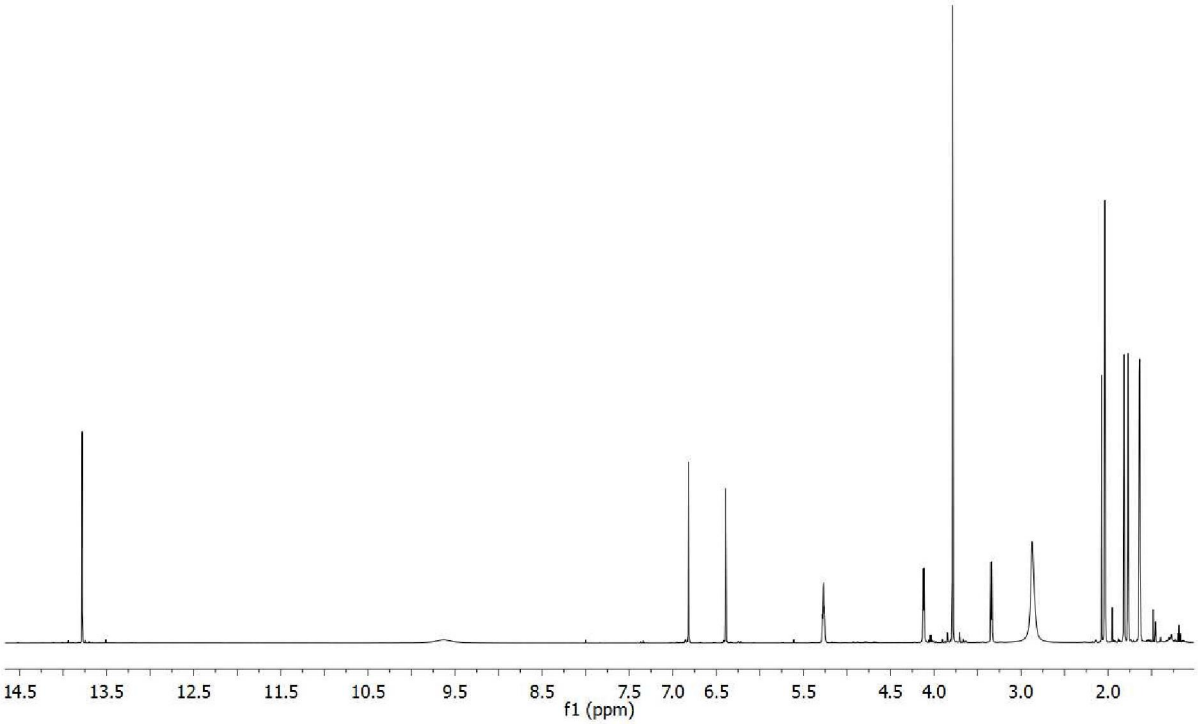
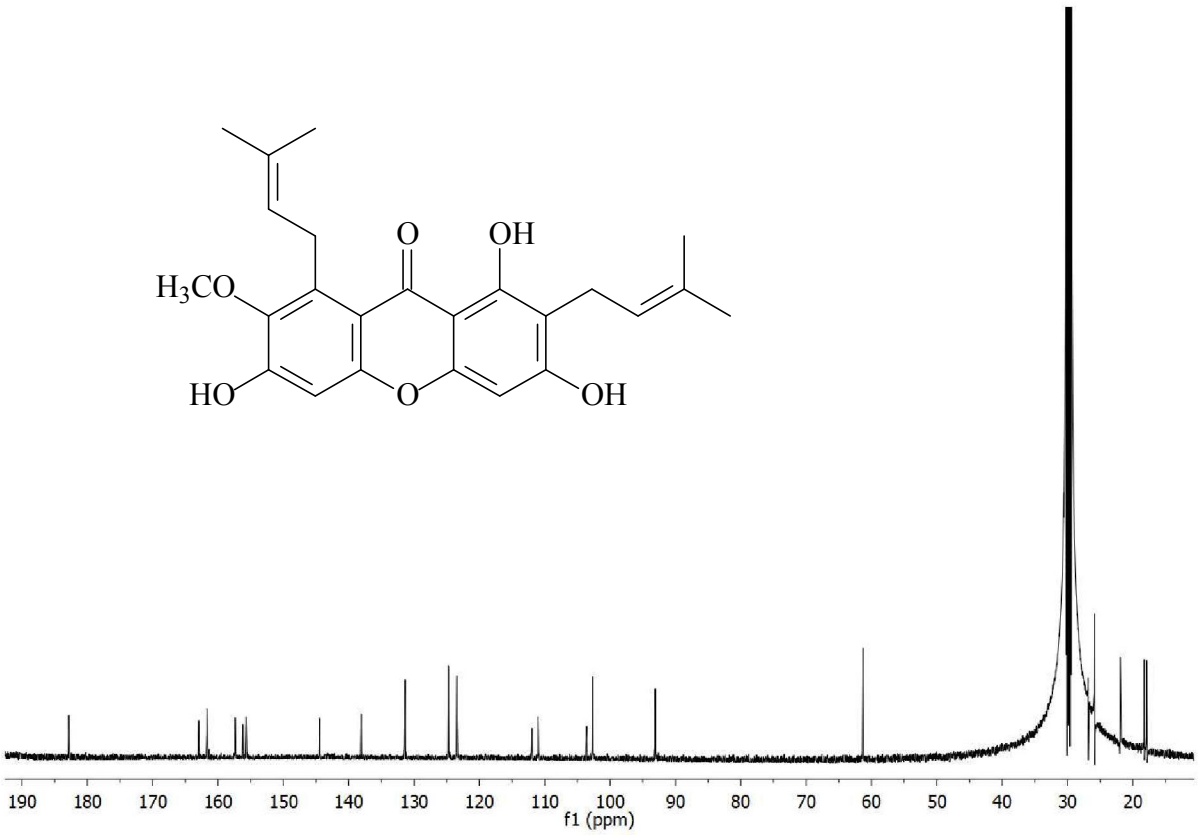
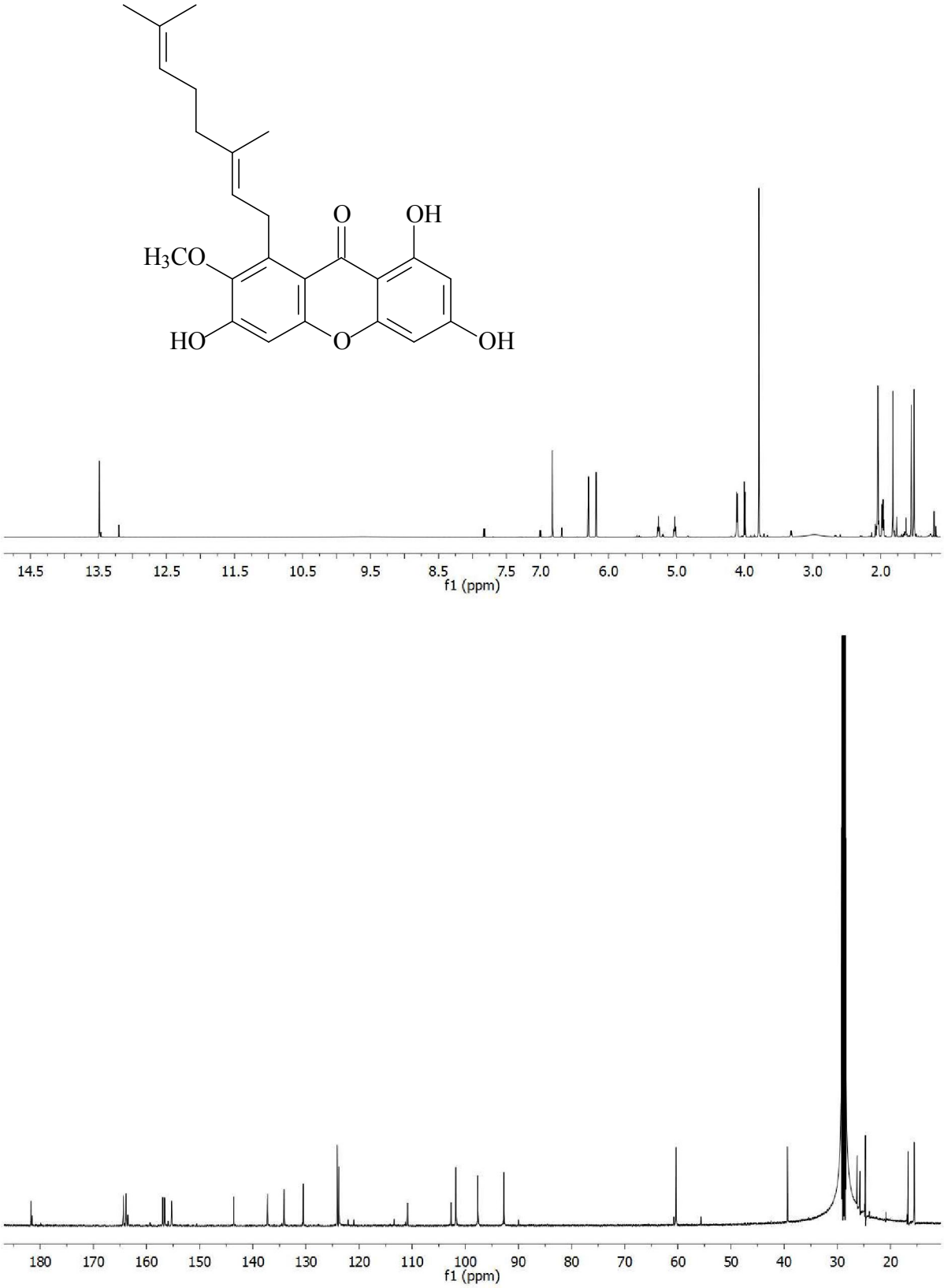




Appendix 6. ^{1}H and ^{13}C NMR spectra of α-mangostin (**6**)



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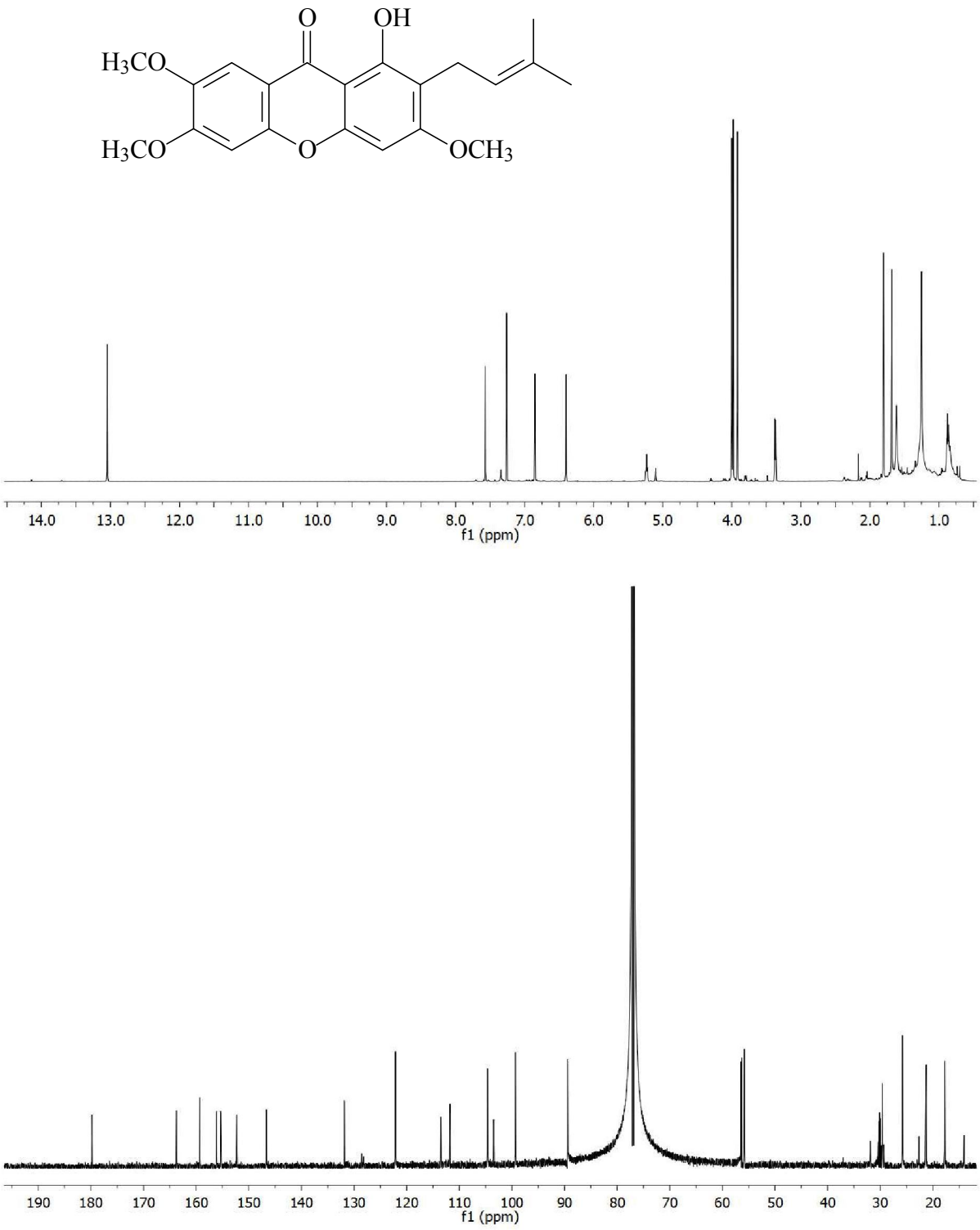
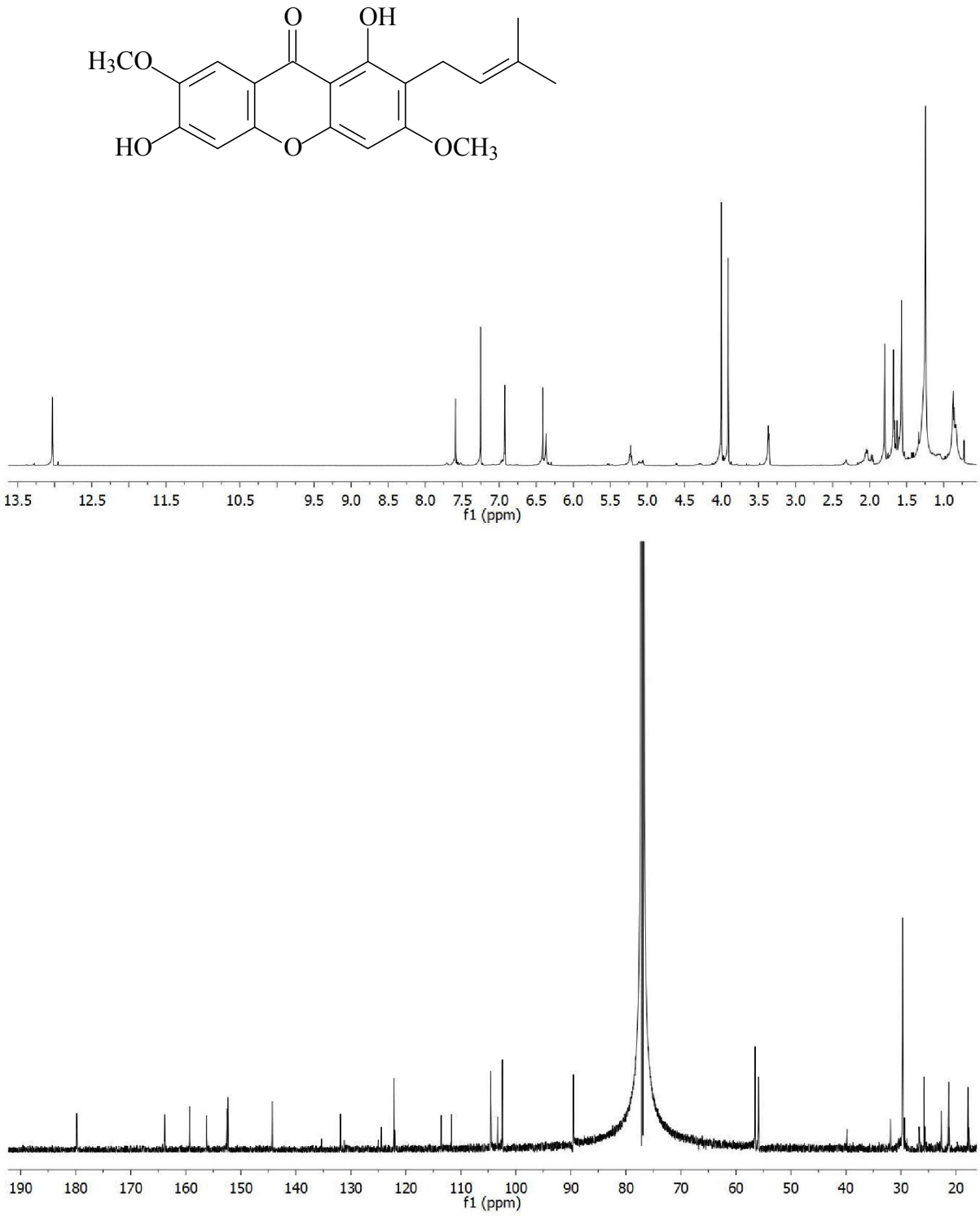


Appendix 8. ^{1}H and ^{13}C NMR spectra of 1-hydroxy-3,6,7-trimethoxy-2-(3-methyl-but-2-enyl)-xanthone (**8**)



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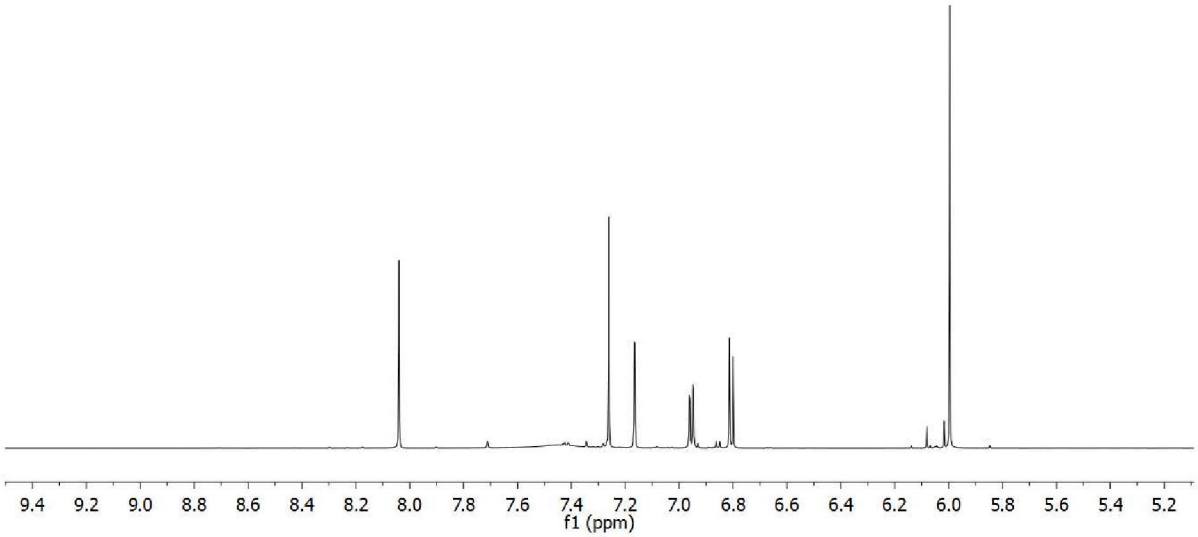

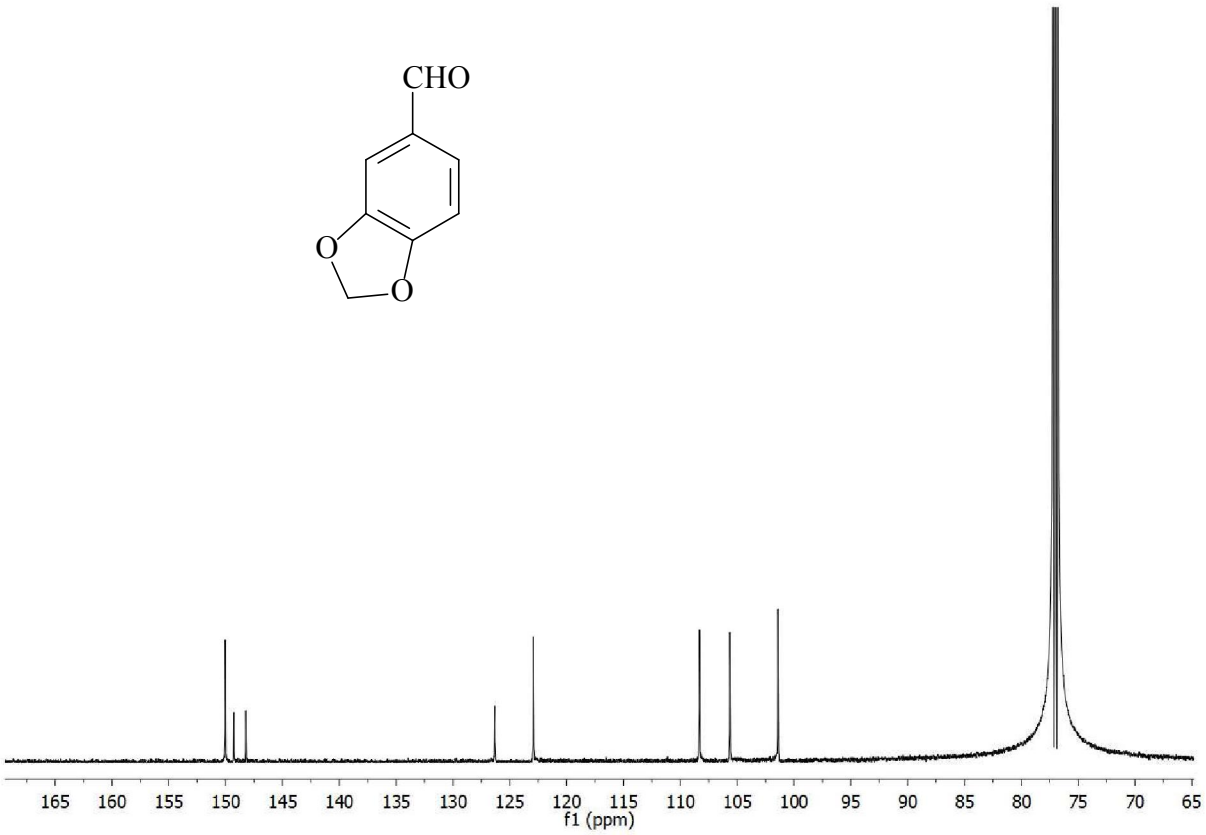

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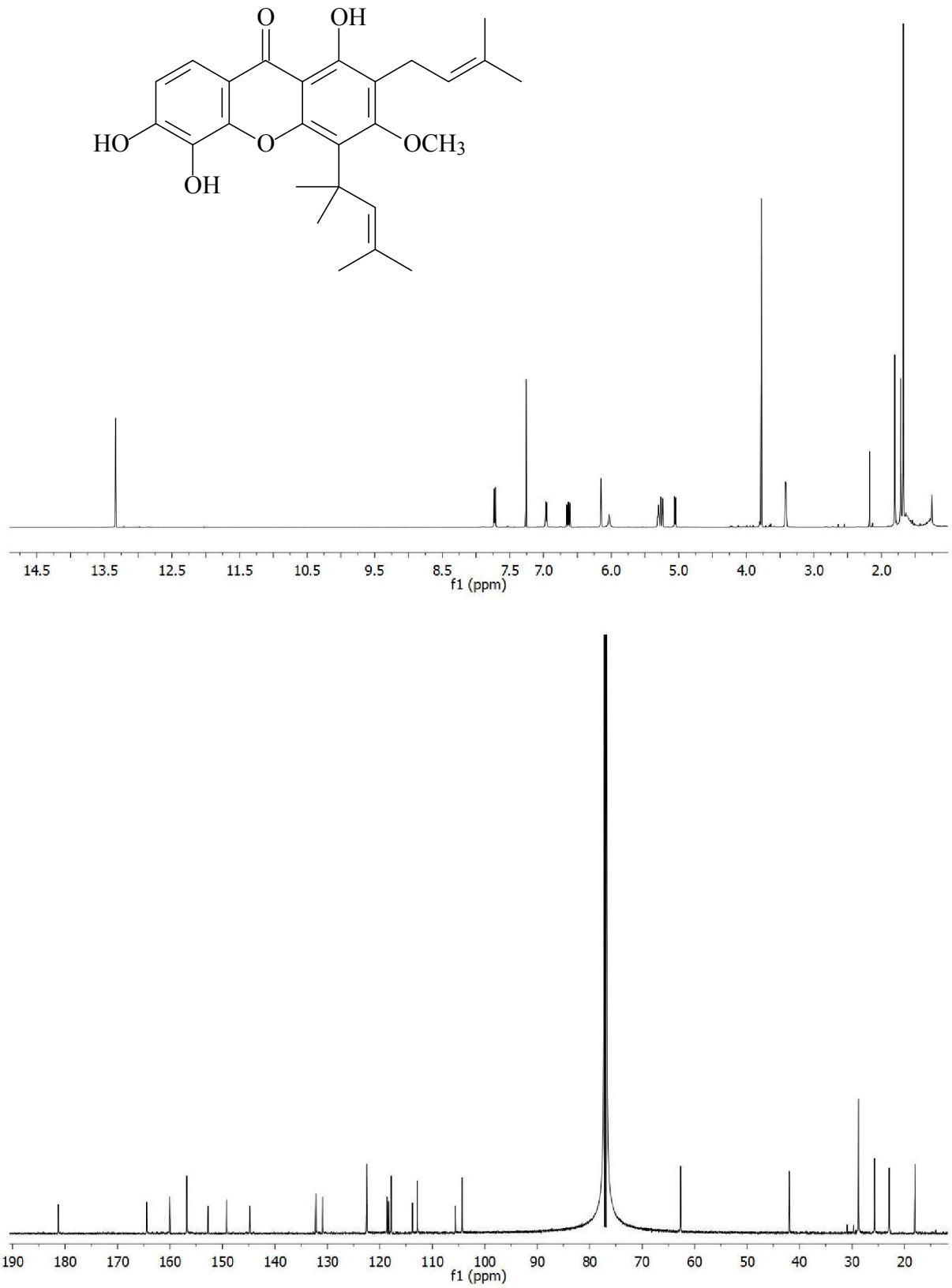

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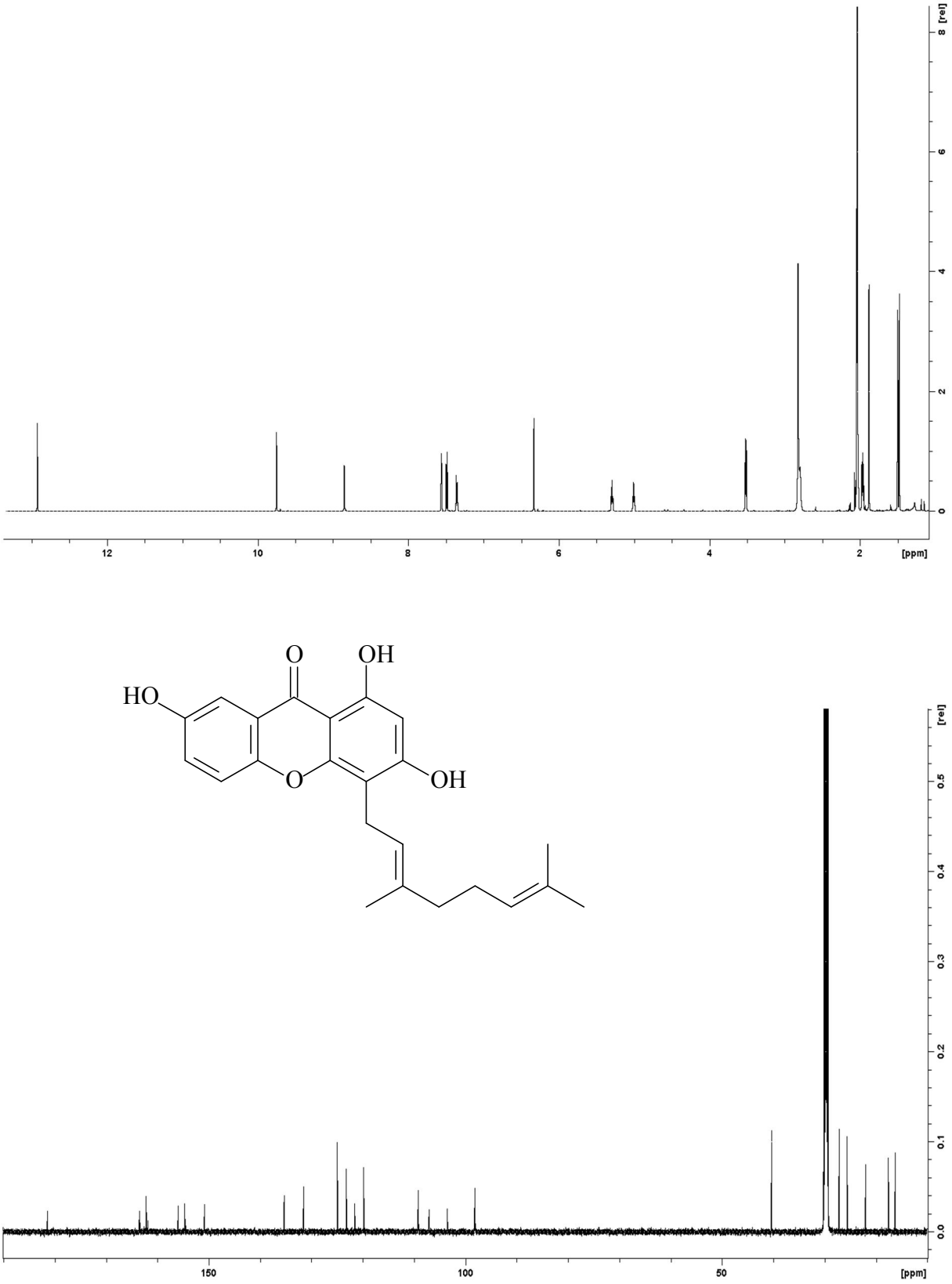

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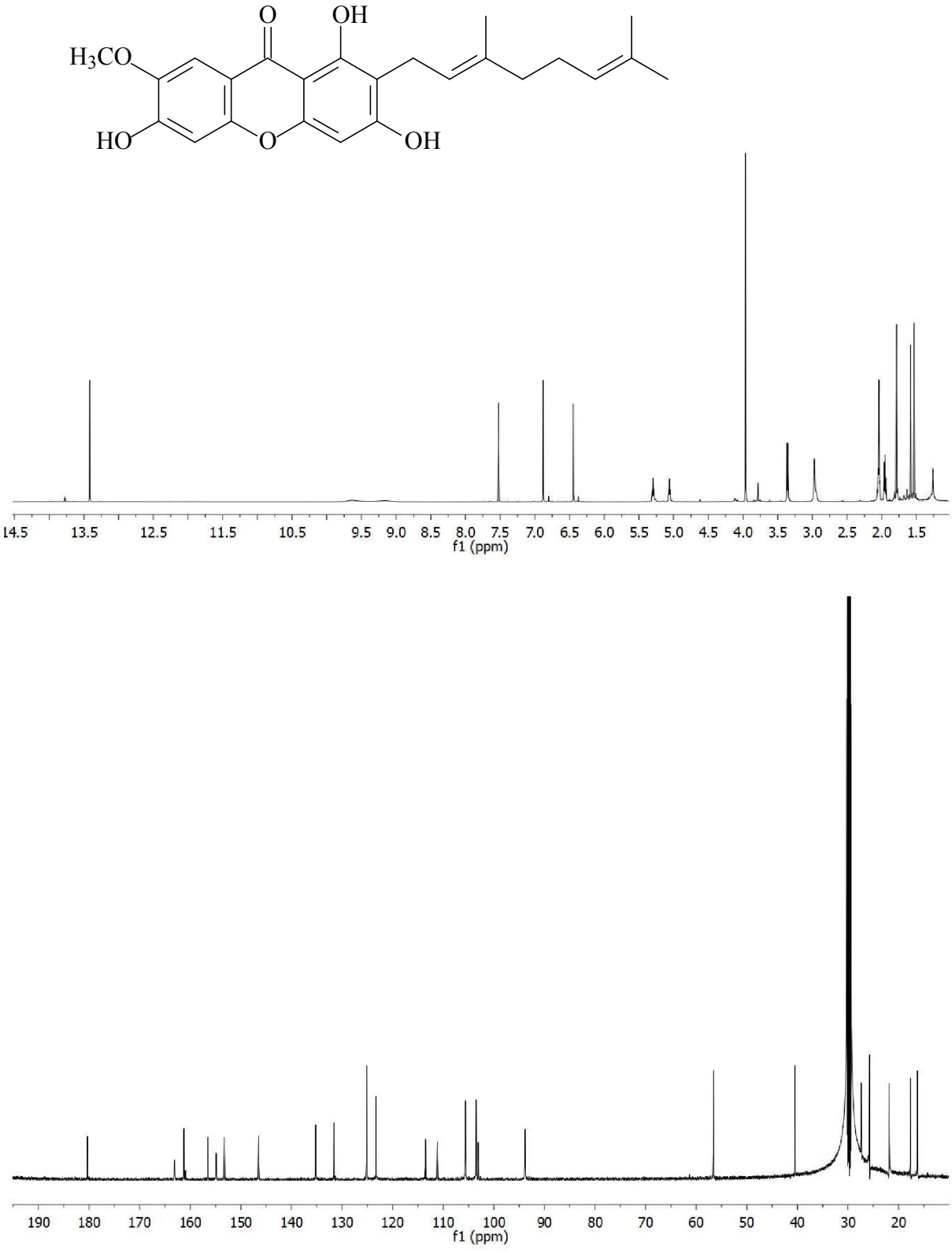

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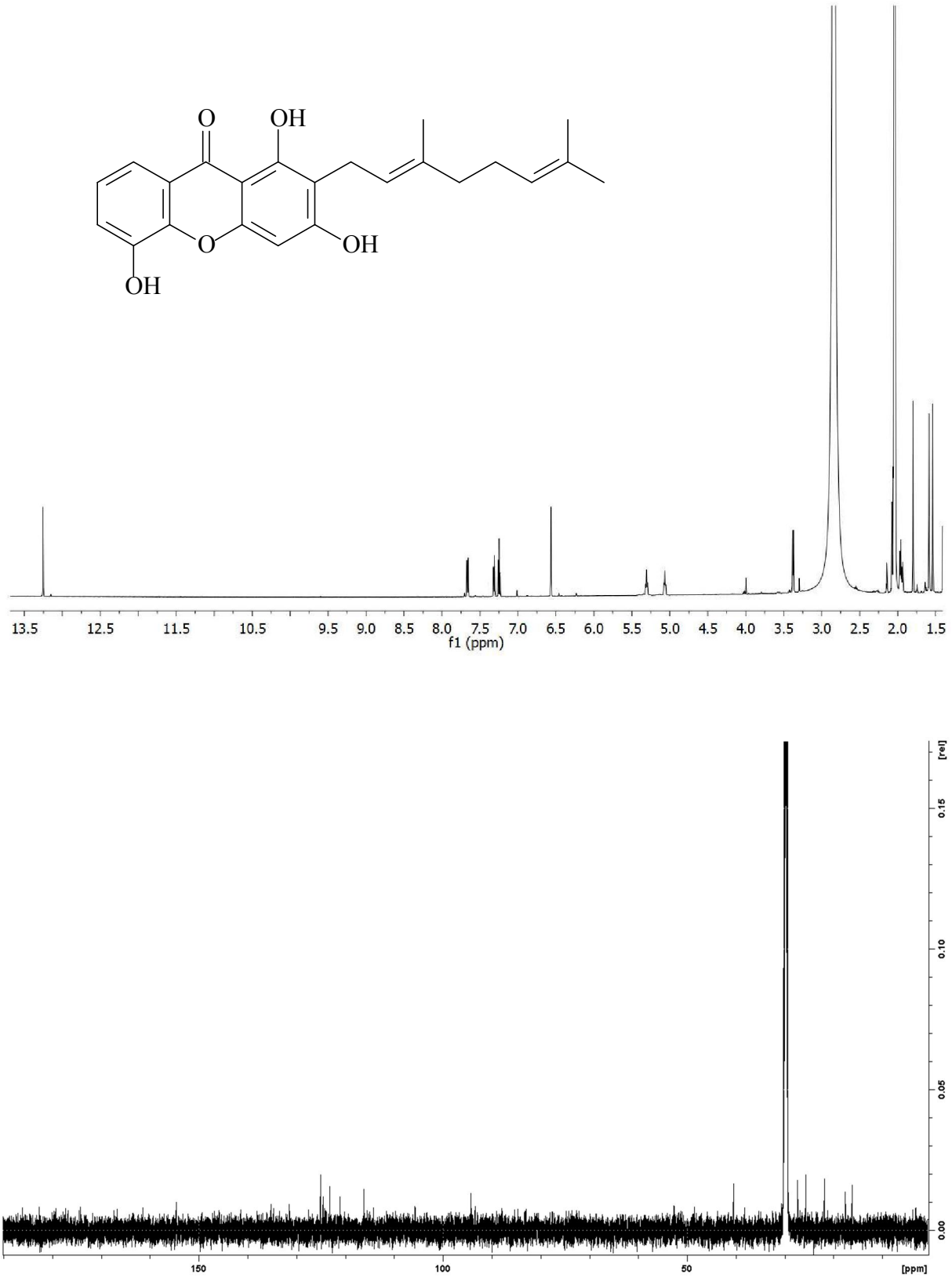

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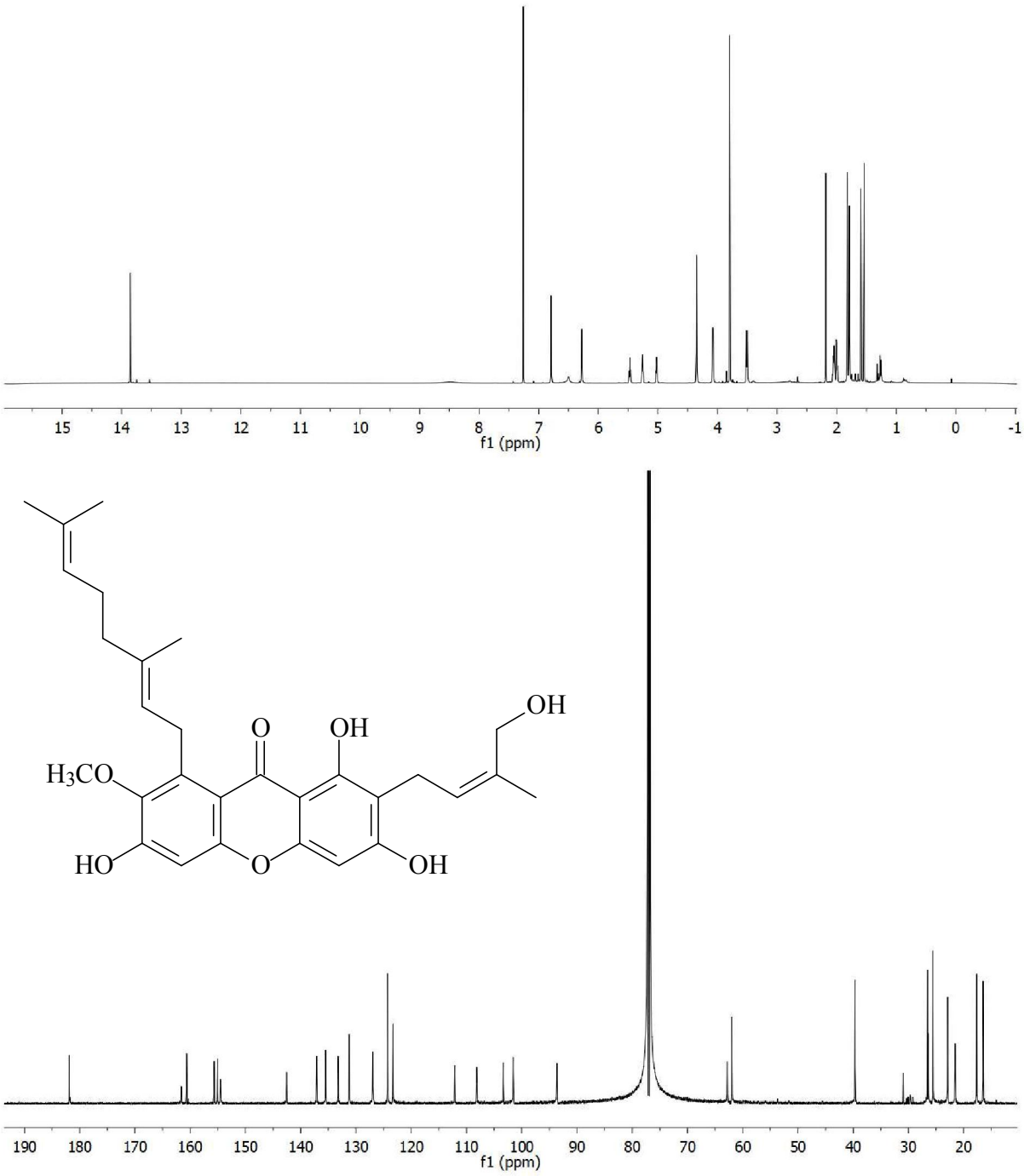

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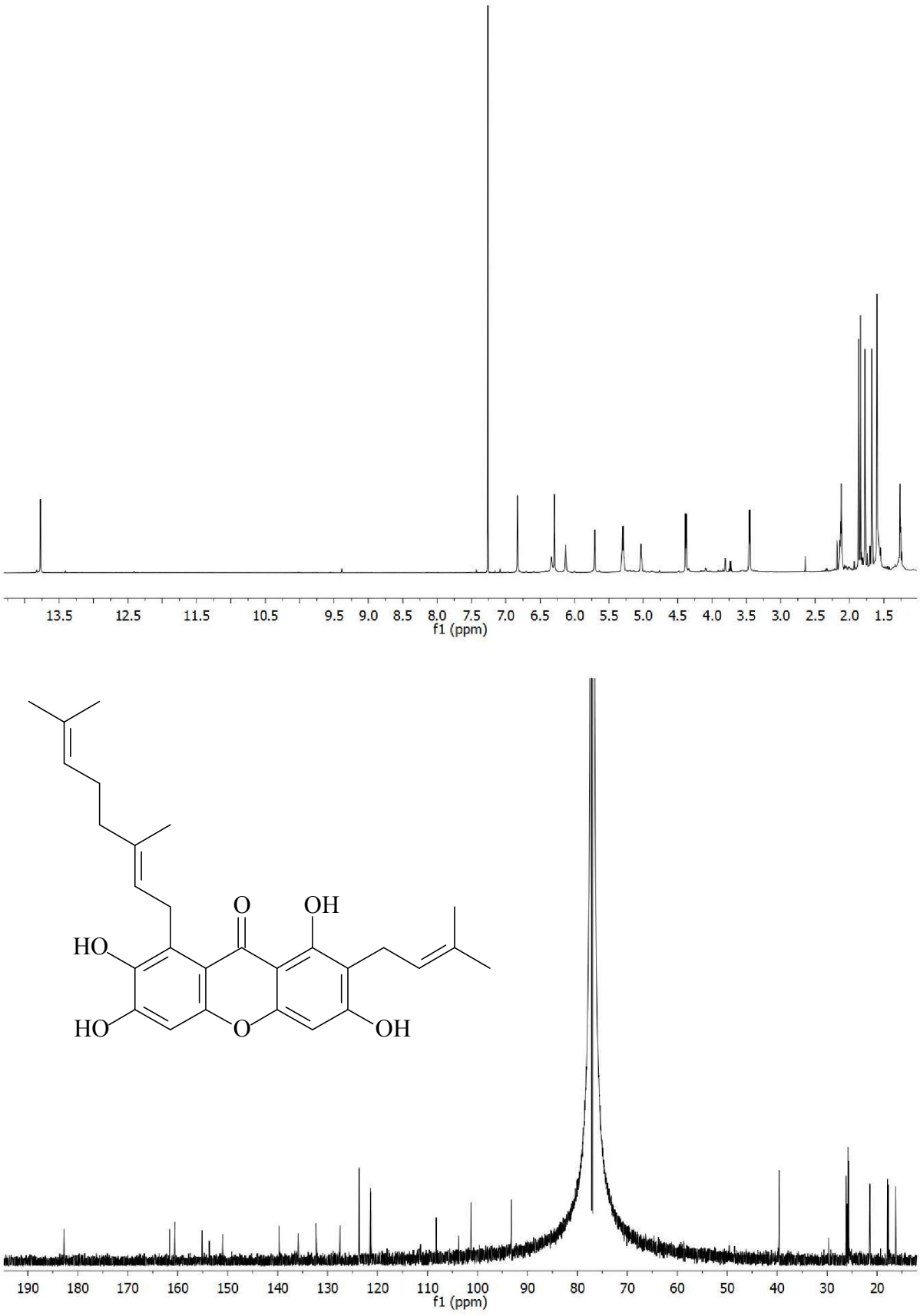

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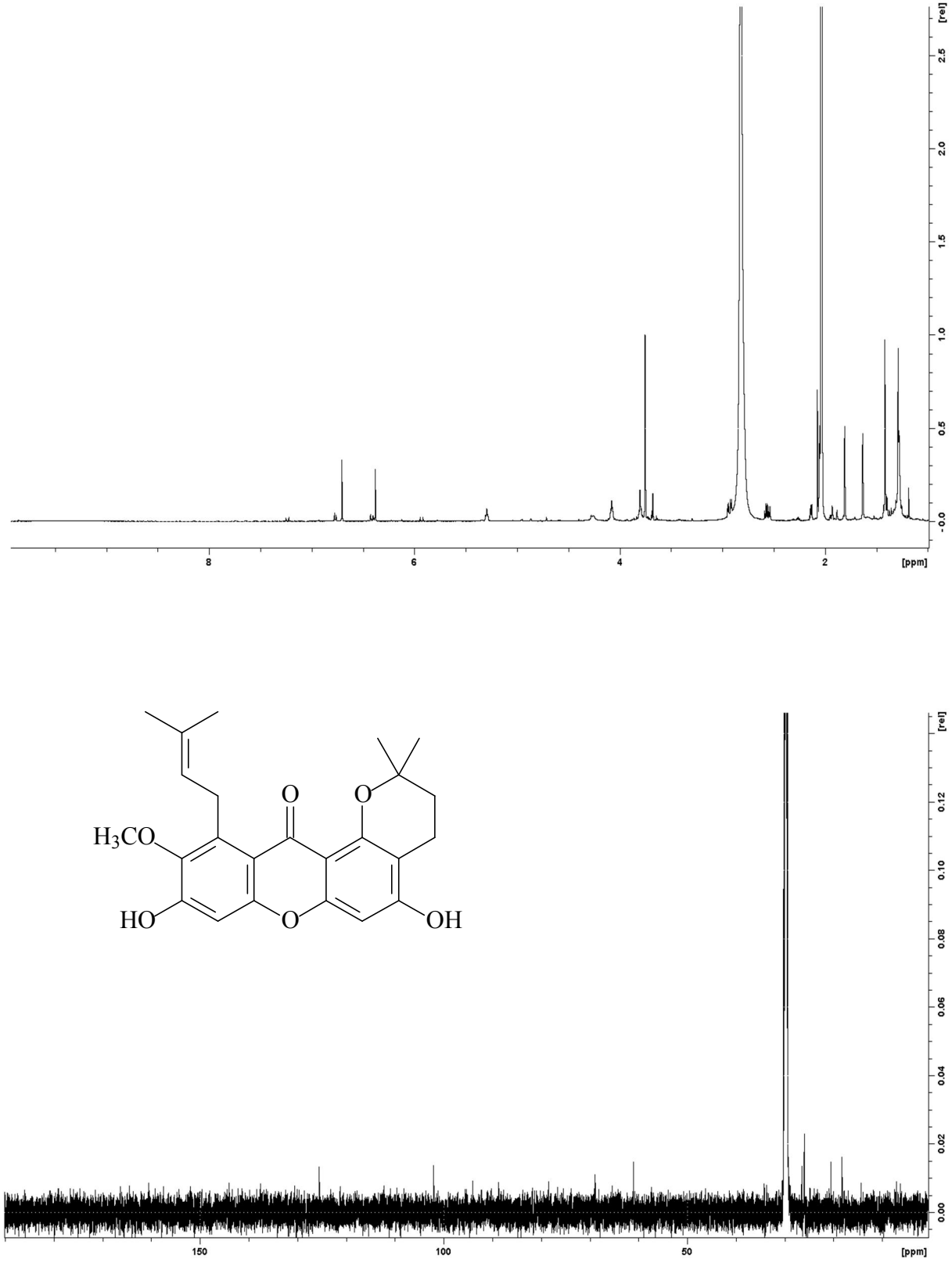

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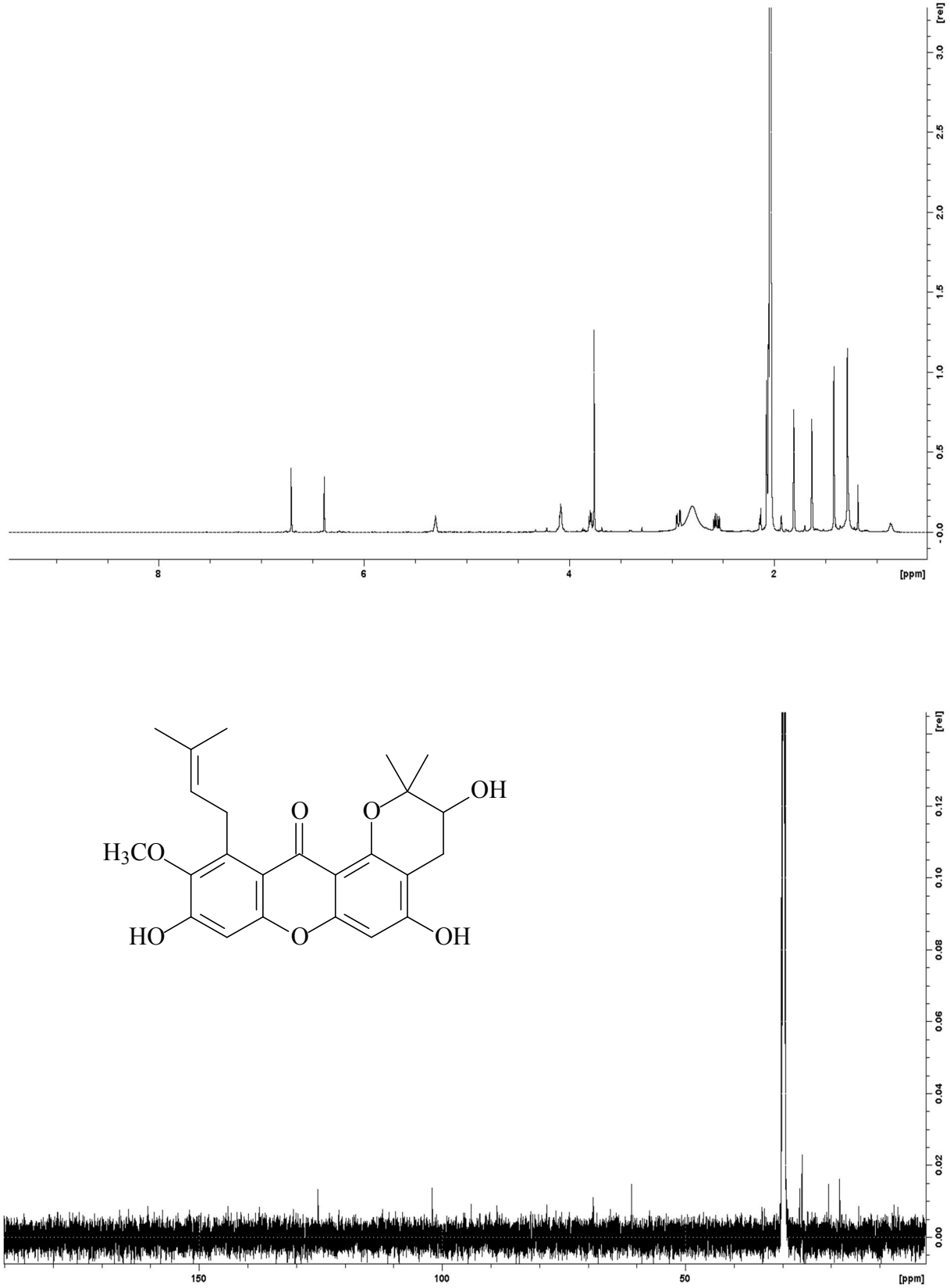

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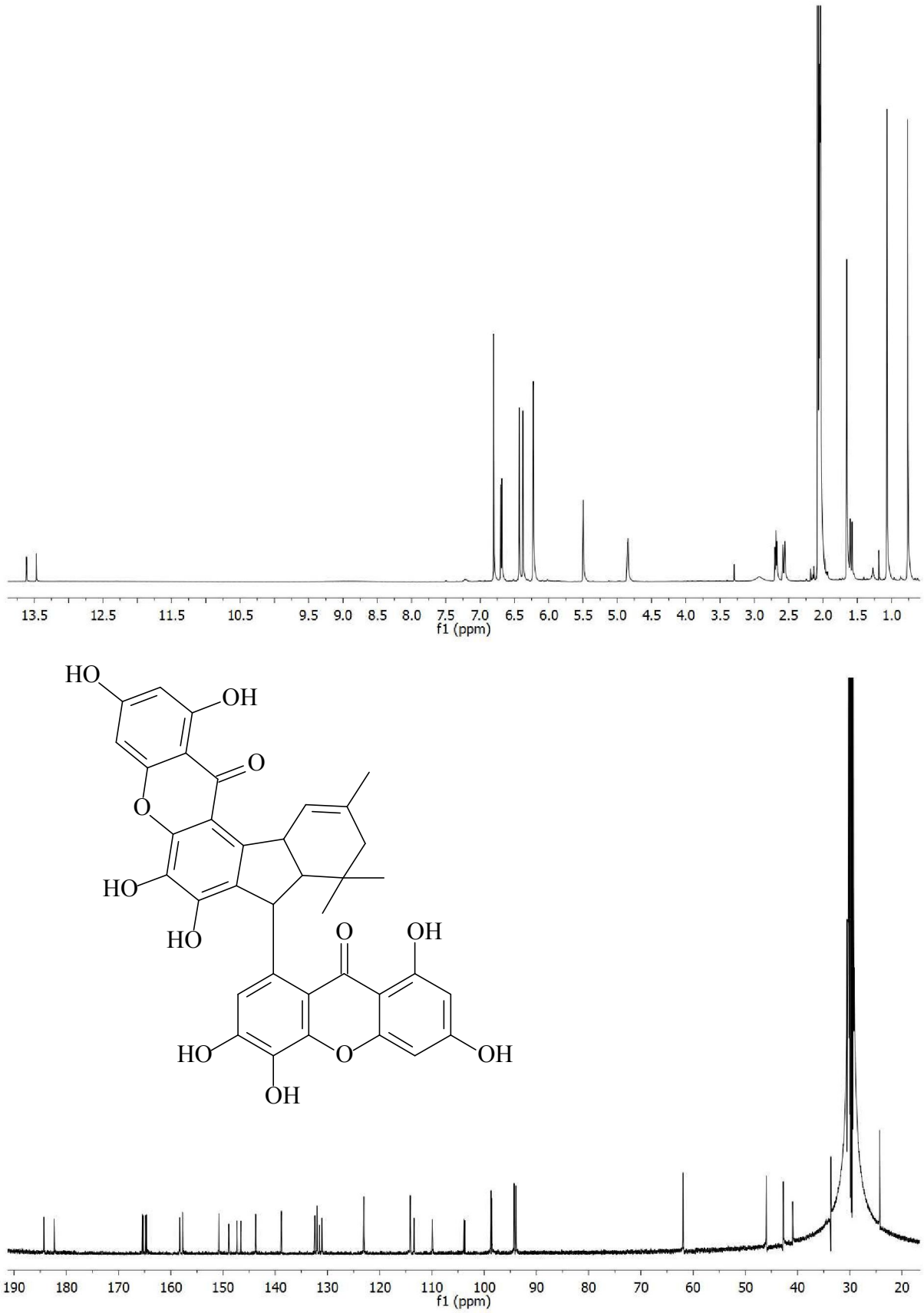

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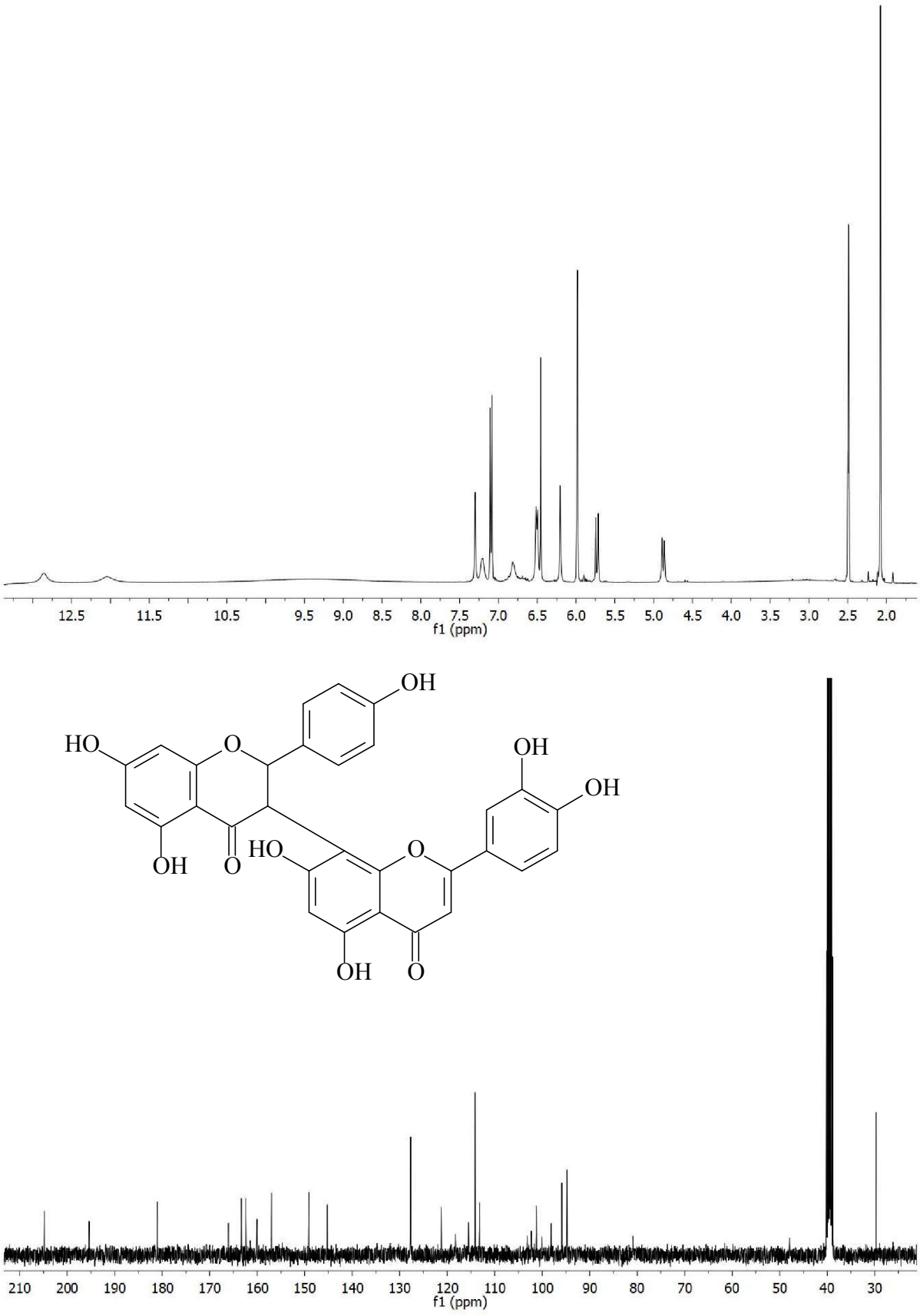

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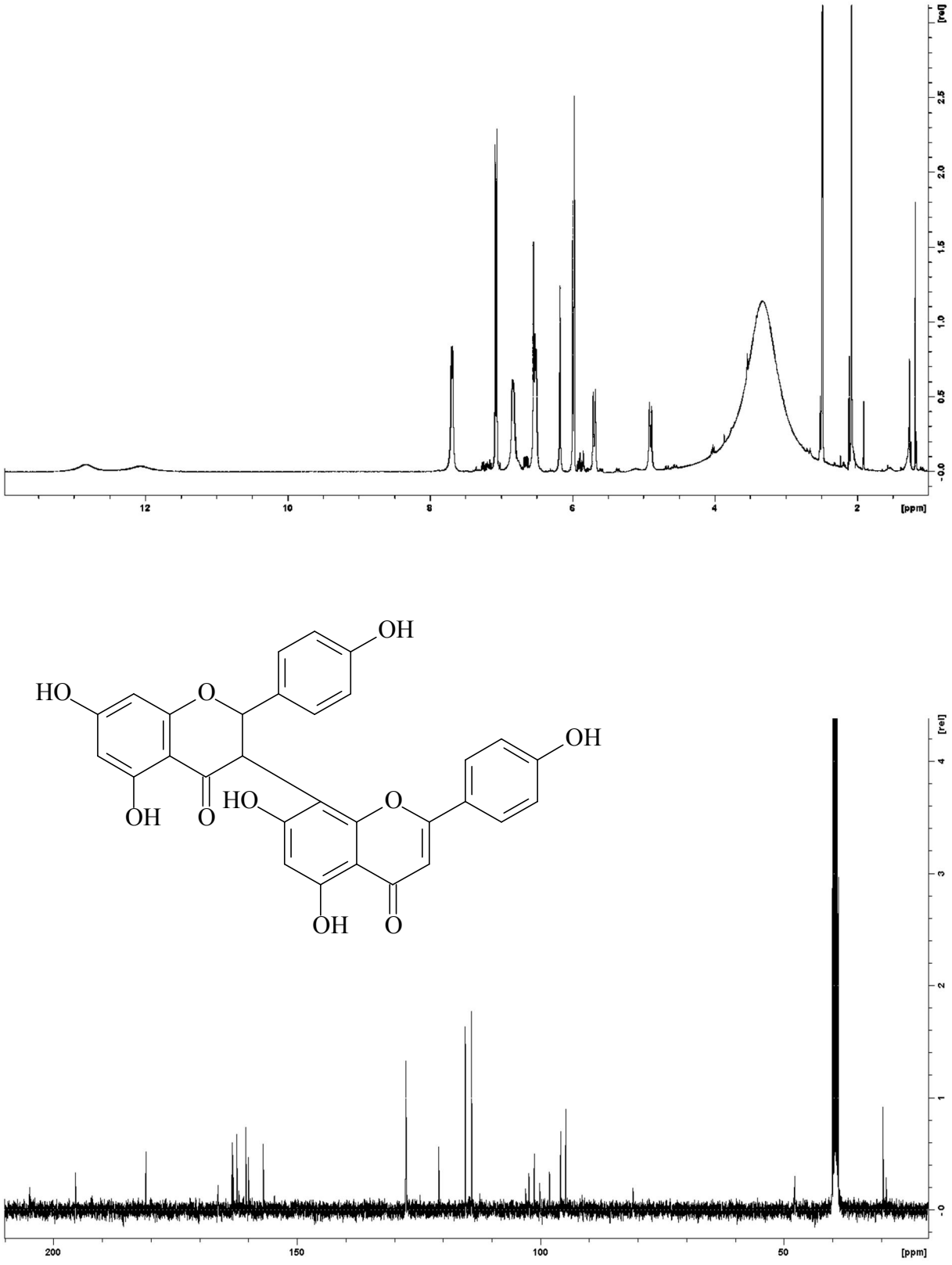

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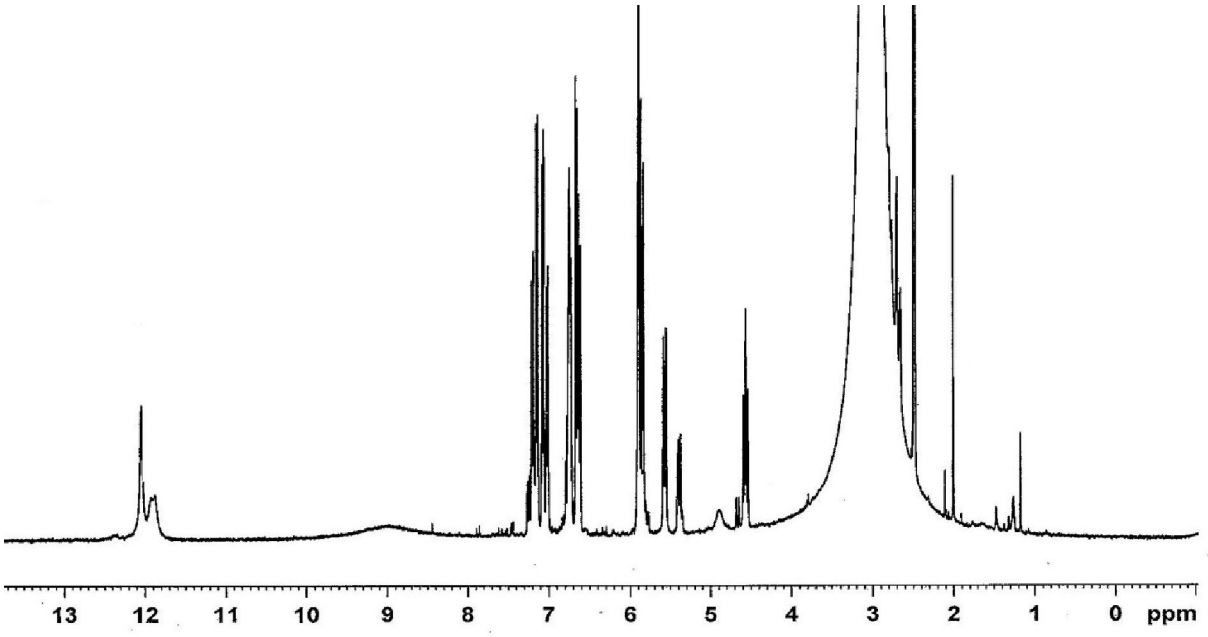
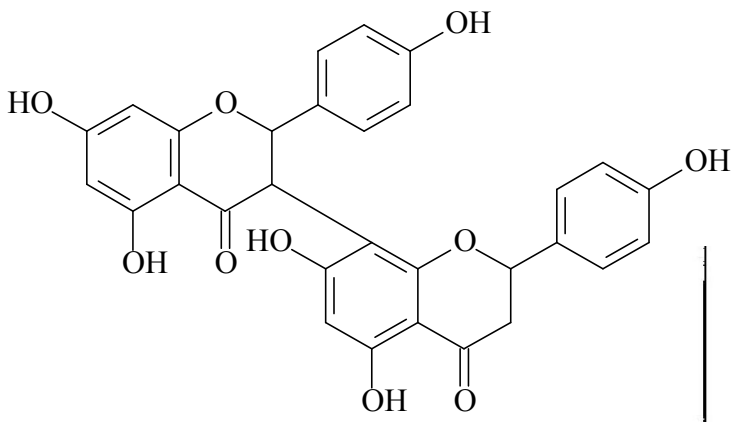
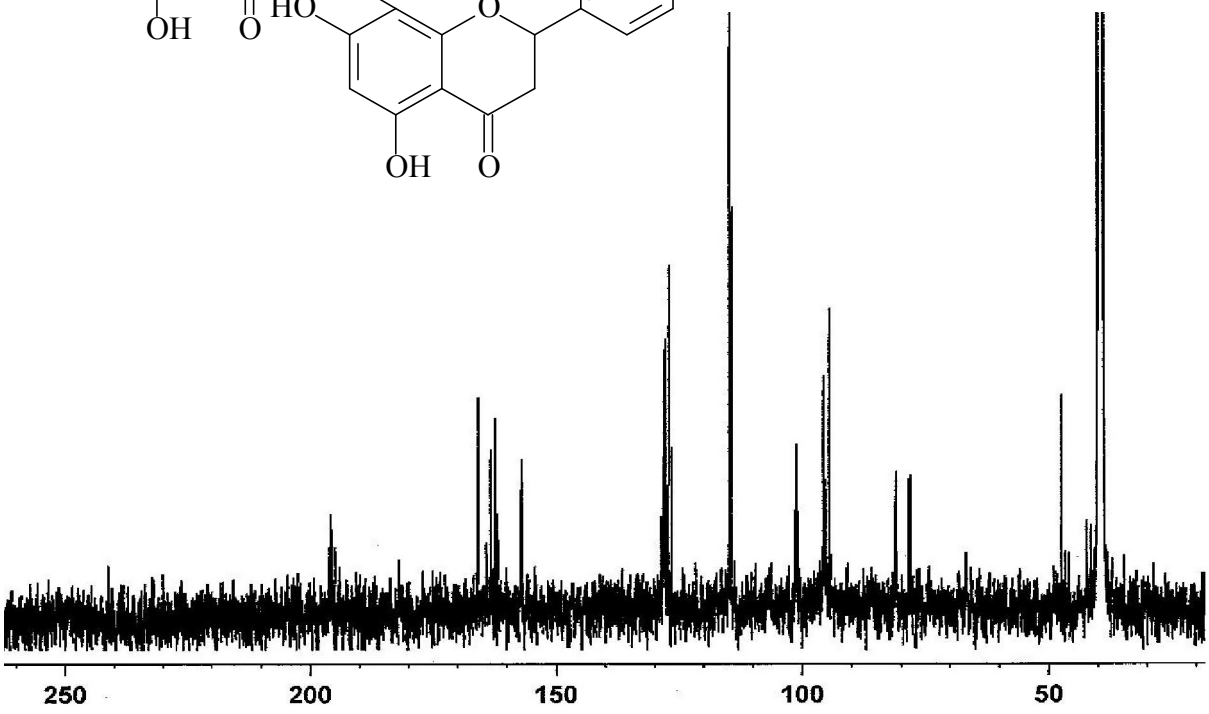
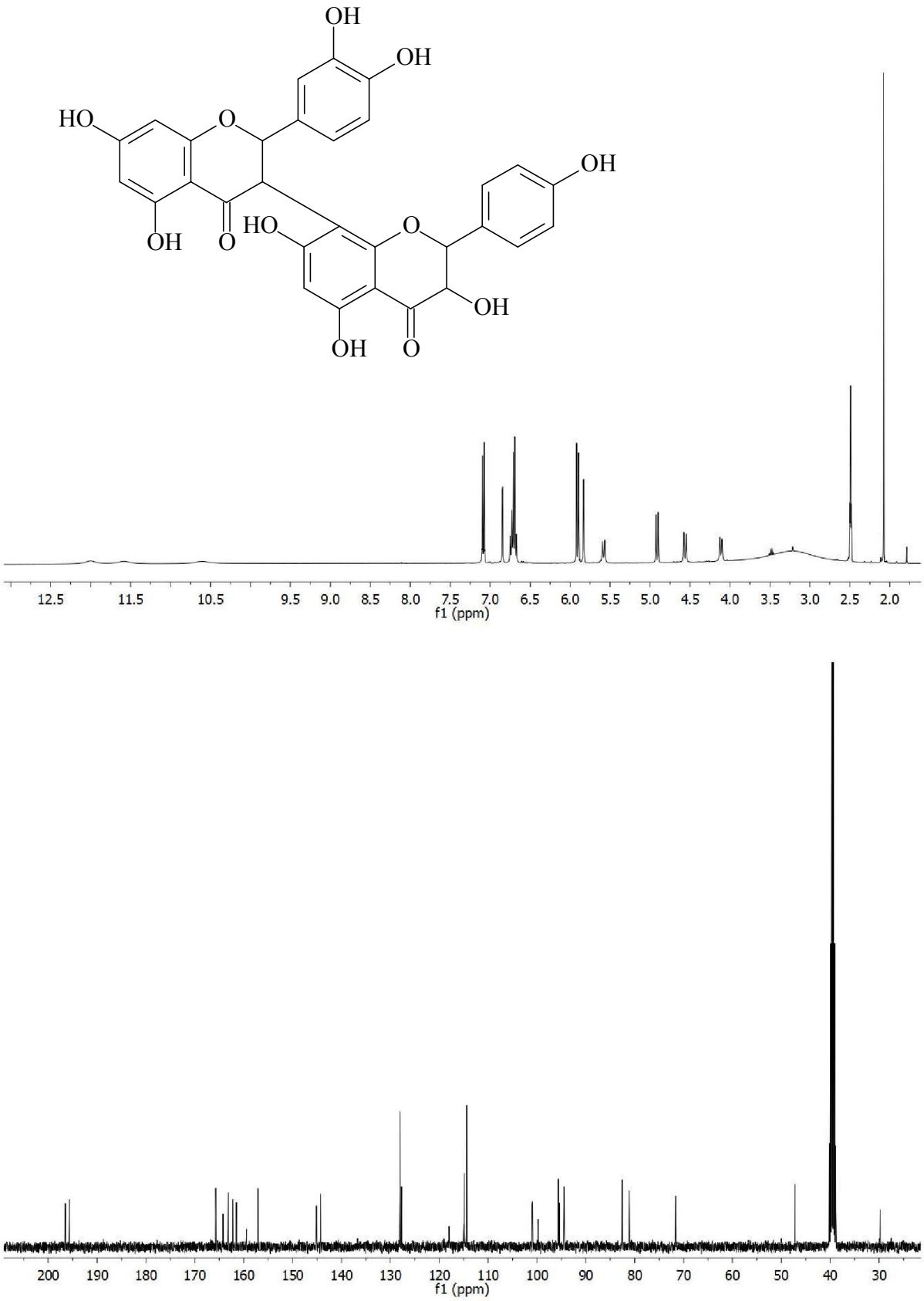






Appendix 22. ^{1}H and ^{13}C NMR spectra of GB-1a (**30**)



Appendix 23. ^{1}H and ^{13}C NMR spectra of GB-2 (**31**)

CURRICULUM VITAE

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