Anti-tyrosinase and anti-microbial activities of Thai medicinal plants

Imron Meechai

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Pharmacy in Pharmaceutical Sciences

Prince of Songkla University

2010

Thesis Title	Anti-tyrosinase and anti-microbial activities of Thai medicinal plants
Author	Mr. Imron Meechai
Major Program	Pharmaceutical Sciences

Major Advisor:

……

(Dr. Sukanya Dej-adisai)

Co-advisor:

……

(Asst. Prof. Dr. Jindaporn Puripattanavong)

Examining Committee:

……Chairperson

(Assoc. Prof. Dr. Sunibhond Pummangura)

……

(Asst. Prof. Dr. Sanae Kaewnopparat)

……

(Asst. Prof. Dr. Supreeya Yuenyongsawad)

……

(Asst. Prof. Dr. Jindaporn Puripattanavong)

……

(Dr. Sukanya Dej-adisai)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Pharmacy Degree in Pharmaceutical Sciences.

……

(Prof. Dr. Amornrat Phongdara)

Dean of Graduate School

ชื่อวิทยานิพนธ์	ฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสและฤทธิ์ต้านเชื้อจุลินทรีย์จากสมุนไพรไทย
ผู้เขียน	นายอิมรอน มีชัย
สาขาวิชา	เภสัชศาสตร์
ปีการศึกษา	2553

บทคัดย่อ

ทดสอบฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสจากสารสกัดหยาบชั้นเอทานอลของสมุนไพรไทยจำนวน 77 ตัวอย่าง พบว่ามี 11 ตัวอย่างที่มีฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสที่ดี (ยับยั้งมากกว่า 50%) และเมื่อนำตัวอย่างดังกล่าวทั้ง 11 ตัว มาทดสอบฤทธิ์ต้านเชื้อจุลินทรีย์ พบว่าสารสกัดหยาบของรากจำปาดะมีฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสและฤทธิ์ต้านเชื้อจุลินทรีย์ที่ดี นำสารสกัดจากรากจำปาดะมาสกัดแยกองค์ประกอบทางเคมี โดยใช้เทคนิคทางโครมาโตกราฟี พบสารบริสุทธิ์ 5 ชนิด และสารผสม 1 ชนิด ได้แก่ Artocarpanone **(1)**, Artocarpin **(2)**, Cudraflavone C **(3)**, Alkene **(4)**, Lanosterol **(5)** และสารผสมของ β-Sitosterol และ Stigmasterol **(6)** และพบว่า **(1)** มีฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสที่ดี สำหรับ **(2)** และ **(3)** มีฤทธิ์ต้านเชื้อจุลินทรีย์ที่ดี ดังนั้น **(1), (2)** และ **(3)** จึงน่าสนใจที่จะศึกษาเพิ่มเติมเพื่อพัฒนาไปเป็นผลิตภัณฑ์เพื่อผิวขาวและยารักษาสิวต่อไป

Thesis Title	Anti-tyrosinase and anti-microbial activities of Thai medicinal plants
Author	Mr. Imron Meechai
Major Program	Pharmaceutical Sciences
Academic Year	2010

ABTRACT

Seventy seven samples of ethanol crude extract from Thai medicinal plants were examined of anti-tyrosinase activity. Eleven samples showed the potential of anti-tyrosinase activity (more than 50% of tyrosinase inhibition). Anti-microbial activity of these samples also examined. The ethanol root extract of ***Artocarpus integer*** showed the potential of anti-tyrosinase and anti-microbial activities. The chemical constituents of ***A. integer*** root extracts were isolated by chromatographic techniques. Five pure compounds and one mixture compound were isolated as Artocarpanone **(1)**, Artocarpin **(2)**, Cudraflavone C **(3)**, Alkene **(4)**, Lanosterol **(5)** and mixture of β-Sitosterol and Stigmasterol **(6)**. And **(1)** exhibited anti-tyrosinase effect, **(2)** and **(3)** also showed the potential of anti-microbial activity. **(1)**, **(2)** and **(3)** are interesting for further study in order to provide possibilities for the development of new whitening and anti-acne agents from ***A. integer***.

ACKNOWLEDGMENTS

This thesis could not successfully completed without the help of many persons. I would like to express my gratefulness to my advisor, Dr. Sukanya Dej-adisai and my co-advisor Asst. Prof. Dr. Jindaporn Puripattanavong to listen, gave good advice, was guidance of this thesis since start until successful, gave appreciate suggestion, checked and corrected the fault of this thesis, a good guidance for experiment and identification of pure compounds.

I would like to give special thanks to all grants supported by Graduate School and the Faculty of Pharmaceutical Sciences, Prince of Songkla University.

I would like to give thanks to my friends in Department of Pharmacognosy and Pharmaceutical Botany for spirit and everything.

I would like to give my deep gratitude to the officers of the Faculty of Pharmaceutical Sciences, Department of Pharmacognosy and Pharmaceutical Botany, Department of Pharmaceutical Chemistry, the Pharmaceutical Laboratory Service Center, Prince of Songkla University, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pharmaceutical Research Intrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University for their support in scientific equipments.

My sincere thanks are expressed to Ms. Sopa Kummee for their advice and help in microbiological laboratory technique and the staffs of Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University for their kindness and helps.

Finally, my graduation would not be achieved without best wish from my parents, and my family who help me for everything and always give me greatest love, willpower and financial support until this study completion.

Imron Meechai

CONTENTS

CONTENTS (CONTINUED)

CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF TABLES (CONTINUED)

LIST OF FIGURES

LIST OF FIGURES (CONTINUED)

LIST OF SCHEMES

LIST OF ABBREVIATIONS AND SYMBOLS

α	=	Alpha
β	=	Beta
cm	=	Centimeter
δ	=	Chemical shift
CFU	=	Colony forming unit
COSY	=	Correlation spectroscopy
J	=	Coupling constant
cAMP	=	cyclic adenosine monophosphate
^{o}C	=	Degree Celsius
Chloroform-d	=	Deuterated chloroform
DMSO-d_6	=	Deuterated dimethylsulfoxide
Methanol-d_4	=	Deuterated methanol
Na_2HPO_4	=	Disodium hydrogen phosphate
DEPT	=	Distortionless enhancement by polarization transfer
d	=	Doublet (for NMR spectra)
dd	=	Doublet of doublets (for NMR spectra)
$\approx$	=	Estimate
g	=	Gram
γ	=	Gramma
Hz	=	Hertz
hr	=	Hour
HMBC	=	^{1}H-detected heteronuclear multiple bond coherence
HMQC	=	^{1}H-detected heteronuclear multiple quantum coherence
kg	=	Kilogram
m/z	=	Mass to charge ratio
IC_{50}	=	Median inhibitory concentration
MHz	=	Megahertz

LIST OF ABBREVIATIONS (CONTINUED)

MSH	=	Melanocyte stimulating hormone
mg	=	Milligram
mL	=	Milliliter
mm	=	Millimeter
μg	=	Microgram
μL	=	Microliter
μM	=	Micromolar
m	=	Multiplet (for NMR spectra)
nm	=	Nanometer
s	=	Singlet (for NMR spectra)
NaCl	=	Sodium chloride
$NaH_2PO_2.2H_2O$	=	Sodium dihydrogen phosphate anhydrate
spp.	=	In the plural in place of the specific epithet
ppm	=	Part per million
%	=	Percentage
t	=	Triplet (for NMR spectra)

CHAPTER 1

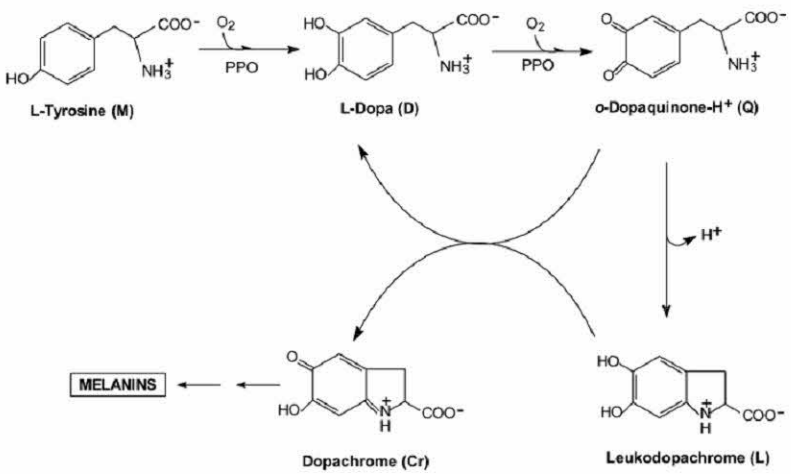
INTRODUCTION

1.1 Background

The people living in the tropical zone have the dark-colored skin and amount of melanin more than the people living in the other area (Jablonski and Chaplin, 2002). Asia is one in the tropical zone and the women in this area hopeful to have a white skin tone. The major to a white skin is against the ultraviolet (UV) radiation by covering or filtering agent, because the UV radiation is effect to skin dark-colored by indirection (Department of medical Sciences, 2001; Tengamnuay ***et al.***, 2006). Some of cosmetic products for whitening and depigmentation, addition substances for accelerate to skin white such as hydroquinone, corticosteroids and mercury, but they are harm to health of skin, such as irreversible cutaneous damage, ochronosis, accumulation of mercury in the body and poisoning (Karioti ***et al.***, 2007). These agents effect to melanogenesis, such as hydroquinone, it inhibits tyrosinase enzyme activity, which effects to the transformation of tyrosine to L-Dopa. Thus the melanogenesis and skin will be inhibition and whiteness, respectively. But effect of hydroquinone for a while, because it irritates to skin when using for a long time or more than 6 months, the skin is redness and burning (Department of medical Sciences, 2001).

Melanins are polyphenolic pigments and cause to dark-coloured (López-Serrano ***et al.***, 2004). This pigment shows brown and black colour (National Science Museum, 2007). Melanin is distributed in the living things of the natural and has many different properties. In mammals, it is found in the eye, hair and skin (Hoogduijn ***et al.***, 2004). The function of melanin is to defend the skin from UV radiation damage and removing reactive oxygen species (ROS). Various dermatological disorders result in the accumulation of an excessive level of melanin, such as melasma, age spots and sites of actinic damage (Kim and Uyama, 2005).

Tyrosinase, also known as polyphenoloxidase (Pênalver *et al.*, 2005) is an enzyme copper-containing (Gąsowska *et al.*, 2006), which is ubiquitously distributed in nature. Tyrosinase of mammals can be found in retina and skin. Tyrosinase is important to catalyst in the melanogenesis and change the other procession, such as in the vertebrates and fruits, this enzyme is important to the browning of fruits and vegetables (Clausa and Decker, 2006; García-Molina *et al.*, 2005). In the melanogenesis, this enzyme catalyses two distinct reactions as hydroxylation and oxidation. The enzyme in this reaction may call monophenolase and diphenolase, respectively. Monophenolase will change L-Tyrosine (M) to L-Dopa (D) and diphenolase will change L-Dopa (D) to *o*-Dopaquinone-H^+ (Q) and pass the intermediate finally to melanins (scheme 1.1) (García-Molina *et al.*, 2005).



Scheme 1.1 Mechanism of melanogenesis

(García-Molina *et al.*, 2005)

Acne is one dermal disease, which causes hurt social and psychological effects on sufferers. ***Propionibacterium acnes*** is anaerobic pathogen which cause to acne vulgaris or acne inflammation. However, ***Staphylococcus epidermidis*** and *S. aureus* are aerobic organism which cause abscess, usually involve to acne inflammation (Kumar *et al.*, 2007; Niyomkam, 2007 and Athikomkulchai *et al.*, 2008). Some mediators of inflammation, such as prostaglandin E2 (PGE2), it can be stimulate the melanogenesis (Petit and Piérard, 2003).

Screening of tyrosinase inhibition that is important to decrease melanin and may be developing into new drug to treatment for hyperpigmentation (Okunji *et al.*, 2007) and useful

in cosmetology (Kiken and Cohen, 2002). Tyrosinase inhibitors from natural products might help to solve the problem that concerns with the addition of the agent which is harmful to skin's health in cosmetic products. Therefore, studies of anti-tyrosinase and anti-microbial activities will be useful for development the product, which for whitening and treatment of skin infection, especially acne that usually find in the patient with hyperpigmentation (Kongcharoensuntorn ***et al.***, 2005 and Chaisawadi ***et al.***, 2008).

In this study, started by screening of anti-tyrosinase activity of 77 crude extracts from Thai medicinal plants by used kojic acid and water extract of ***Artocarpus lakoocha*** as positive controls, these agents are accepted to be the standard because they have high potential of tyrosinase inhibition activity. The crude extracts which showed more than 50% of tyrosinase inhibition would be selected for anti-microbial test. ***Artocarpus integer*** root was selected for phytochemical investigation, because it showed high potential of anti-tyrosinase and anti-microbial activities. Then this study was concerned with the isolation, purification and structure determination of chemical compounds from ***A. integer***.

1.2 Objects

The main objectives in this investigation are as follows:

1. Preliminary screening of anti-tyrosinase activity from Thai medicinal plant extracts.

2. Preliminary screening of anti-microbial activity from Thai medicinal plant extracts which show high potential of tyrosinase inhibitory activity.

3. Isolation of the chemical constituents from the selected Thai medicinal plant extract.

4. Determination of anti-tyrosinase and anti-microbial activities of isolated pure compounds.

CHAPTER 2

HISTORICAL

2.1. Melanin in mammalian

Melanins are extensively distributed pigments and are found in bacteria, fungi, plants and animals. Melanins of mammalian are divided into two types as eumelanin is brown or black and pheomelanin is red or yellow in color (Kim and Uyama, 2005). They are a heterogeneous polyphenol-like biopolymer with a complex structure where are synthesized by melanocyte cell in the basal layer of dermis, which contain specific enzyme controlling the produce of the melanins known as tyrosinase. Melanins synthesis takes place in specific organelles known as melanosomes. Melanosomes are transferred from the melanocyte cells into surrounding keratinocyte cells to protect the skin from UV radiation damage and removing reactive oxygen species (ROS) (Hoogduijn, 2004; Kim and Uyama, 2005 and Petit and Piérard, 2003).

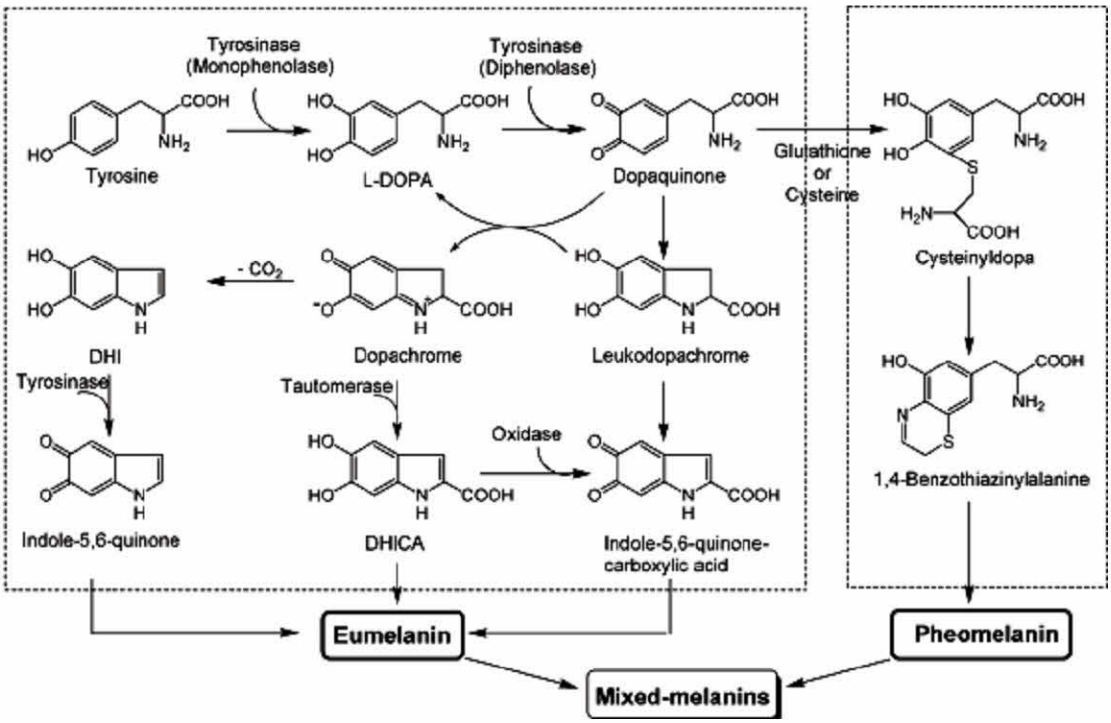
2.1.1 Melanogenesis

Melanogenesis is started with the first step of L-tyrosine hydroxylation into L-3, 4-dihydroxylphenylalanine (L-Doap) and L-Dopa oxidation into *o*-dopaquinone catalyzed by tyrosinase. This first step is the rate-limiting step in melanogenesis. Dopaquinone can undergo two different type reactions (see in scheme 2.1) (Kim and Uyama, 2005; Petit and Piérard, 2003).

The first reaction is *o*-dopaquinone cyclization, started by amino group undergoes an intramolecular 1, 4-addition to the benzene ring into leukodopachrome and quickly oxidize to dopachrome. The dopachrome formation is lead to synthesis eumelanin which can undergo two difference type reactions as (1) decarboxylation of dopachrome into 5, 6-dihydroxyindole (DHI), its following oxidation into indole-5, 6-quinone and (2) enzymatically transformed into 5,6-dihydroxyindole-2-carboxylic acid (DHICA) by dopachrome tautomerase and DHICA can be oxidation into indole-5, 6-quinone carboxylic acid. Indole-5, 6-

quinone, indole-5, 6-quinone carboxylic acid and DHICA are subsequent polymerization into eumelanin (Kim and Uyama, 2005; Petit and Piérard, 2003).

The second reaction is the group of sulfhydryl compounds such as glutathione and cysteine nucleophilically attacks *o*-dopaquinone to create cysteinyldopa or glutathionyldopa. The cysteinyldopa or glutathionyldopa are subsequent cyclization and polymerization into pheomelanin (Kim and Uyama, 2005; Petit and Piérard, 2003). The interaction between the eumelanin and pheomelanin compounds gives increase to a heterogeneous merge of mixed-type melanins (Kim and Uyama, 2005).



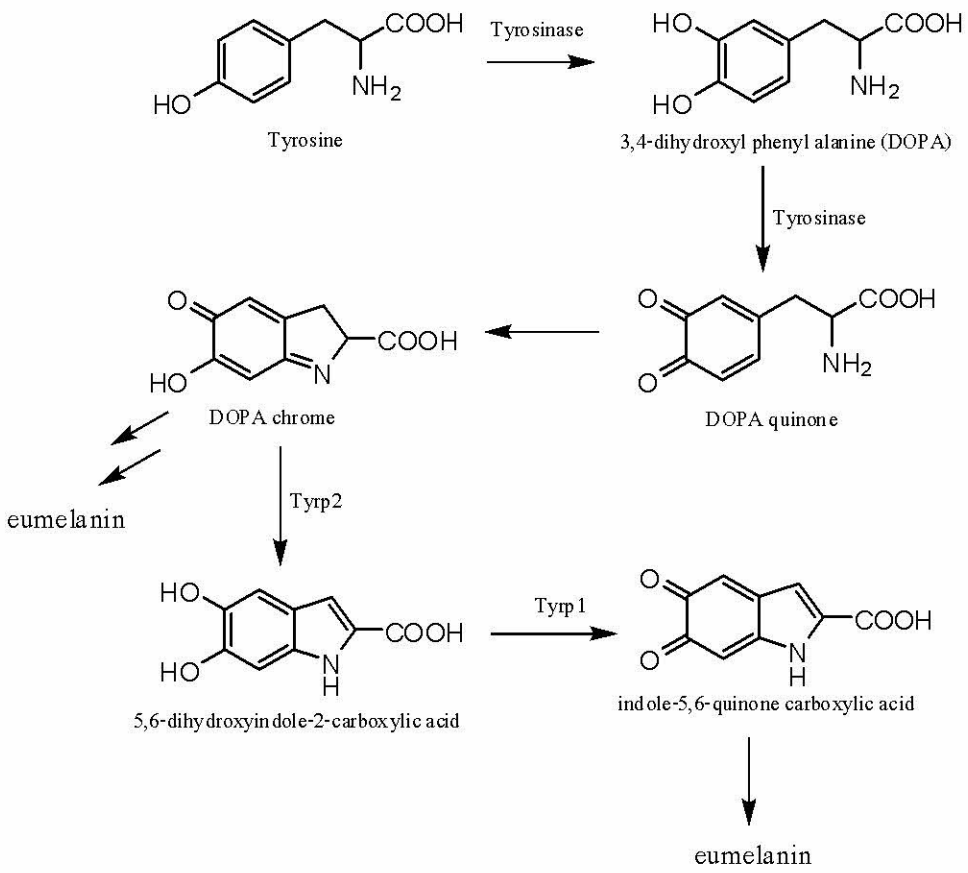
Scheme 2.1 Step of melanogenesis in human's skin

(Kim and Uyama, 2005)

The initiation of the melanogenesis procedure may be involved with ultraviolet radiation, free radical, reactive oxygen species, inflammatory mediators and hormone (Petit and Piérard, 2003). These factors may act in combination which response to make the skin disorder, such as age spot, melasma, freckles and other forms of melanin hyperpigmentation (Kim and Uyama, 2005; Petit and Piérard, 2003).

2.1.2 Anti-tyrosinase and tyrosinase inhibitor

Melanogenesis is a highly cooperative process carried out by tyrosinase family proteins, which include tyrosinase, tyrosinase-related protein 1 (tyrp1) and tyrosinase-related protein 2 (tyrp2) (see in scheme 2.2) (Wang and Hebert, 2006). On the contrary, if one step of the melanogenesis fails, melanins are disappear on the skin. However, tyrosinases are the first and rate-limiting step of melanogenesis (see in scheme 2.2) (Clausa and Decker, 2006). Therefore, tyrosinase inhibitors have become increasingly important for depigmentation and whitening products that may be used to preclude or treat hyperpigmentation (Kim and Uyama, 2005).



Scheme 2.2 Melanogenesis pathway regulated by tyrosinase, tyrosinase family proteins Tyrp1 and Tyrp2 (Wang and Hebert, 2006)

2.1.2.1 Enzyme tyrosinase

Tyrosinase (1.14.18.1) also known as polyphenoloxidase (PPO) is the copper-containing enzyme. It uses its molecular oxygen of binuclear copper center to catalyze two different enzymatic reactions (Claus and Decker, 2006; Wang and Hebert, 2006), the hydroxylation of L-tyrosine to L-3, 4-dihydroxylphenylalanine (L-Dopa) and the subsequent oxidation of L-Dopa to DOPAquinone (see scheme 2.2). Tyrosinase of human is a type I membrane glycoprotein that contains 529 amino acid (Wang and Hebert, 2006).

2.1.2.2 Tyrosinase inhibitors

The commercials of tyrosinase inhibitors are contain in cosmetic and whitening products, such as kojic acid, arbutin and azelaic acid (Kim and Uyama, 2005; Petit and Piérard, 2003). However, many previously reports are studied for tyrosinase inhibition from medicinal plants, which development for whitening and anti-browning agents. Some of tyrosinase inhibitors from previously reports are showed in table 2.1

2.1.3 Anti-tyrosinase assay by enzymetic and cell culture methods

The ***in vitro*** mushroom tyrosinase inhibition assay in basic step evaluate the direct consequence of a given skin whitener on tyrosinase activity. The substrate of this enzyme is L-tyrosine and the reaction involve the occurrence of the co-substrate is L-Dopa (see in scheme 2.1). The activity of tyrosinase and tyrosinase inhibitor are quantified following the detection of dopachrome at 475 (Petit and Piérard, 2003) or 492 nm by spectrometer, this method as known dopachrome method (Sritularak, 1998a; Sritularak, 1998b). Other cell-free enzymatic analyze can be performed, for order to investigation, Tyrp-1 and tyrp-2 activities (Petit and Piérard, 2003).

Table 2.1 Anti-tyrosinase activity from medicinal plants

Medicinal plants	**Compound/ Crude extract**	**IC_{50}**	**Reference**
Camellia sinensis	Epigallocatechin-3-*O*-gallate (EGCG)	34.58 μM	No *et al.*,1999
Camellia sinensis	Gallocatechin-3-*O*-gallate (GCG)	17.34 μM	No *et al.*,1999
Camellia sinensis	Epicatechin-3-*O*-gallate (ECG)	34.10 μM	No *et al.*,1999
Morus alba (leaves)	Mulberroside F	0.49 μM	Lee *et al.*, 2002
Citrus sp. (peel)	Nobiletin	42.6 μM	Sasaki and Yoshizaki, 2002
Pharbitis nil (seed)	Ethanol extract	24.9 μg/mL	Wang *et al.*, 2006
Sophora japonica (flower)	Ethanol extract	95.6 μg/mL	Wang *et al.*, 2006
Spatholobus suberectus (stem)	Ethanol extract	83.9 μg/mL	Wang *et al.*, 2006
Morus alba (leaves)	Ethanol extract	78.3 μg/mL	Wang *et al.*, 2006

2.2 Acne

Acne expands in follicular pilosebaceous unit. These units are largest on the face, neck and back. The developments of acne cause from the four major pathogenic factors are increased sebum production, disorders of the microflora, cornification of the pilosebaceous duct and inflammation. The microorganisms are commensally of normal skin as ***Propionibacterium acnes***, ***Staphylococcus aureus*** and *S. epidermidis*, proliferate rapidly during teenager and often involved in the development of acnes (Niyomkan, 2008).

2.2.1 Acne vulgaris

Acne vulgaris is the majority general skin disease, a chronic inflammatory disorder in teenager consist pilosebaceous follicles. *P. **acnes*** is anaerobic microorganism, it is cause to acne vulgaris by metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils. *S. **aureus*** is aerobic microorganism, usually involves in superficial infections within the sebaceous unit (Traidej-Chomnawang, 2005).

2.2.2 Acne treatments and anti-bacterial activity from medicinal plants

Variety of the most suitable therapy should be based on an inclusive assessment of the patient and pharmacological profile of the agents. The options of treatment for acne are as follows (Niyomkan, 2008).

(1) Physical therapy: There are many treatments including acne surgery, laser and phototherapy and using intralesional corticosteroid.

(2) Systemic therapy: The two major systemic modalities used in acne are anti-biotics and estrogenic hormone. Both represents major step forward in the therapy of this disease.

(3) Local therapy: The general therapy including tropical agents' tretinoin, azelaic acid, salicylic acid, benzoyl peroxide and anti-bacterials (clindamycin, erythromycin).

In previously reports, many medicinal plants were examined for antimicrobial activity against microorganisms frequently involved in acne inflammation such as ***Propionibacterium acnes***, ***Staphylococcus epidermidis*** and *S. **aureus***. Anti-bacterial activity from some medicinal plants is against *P. **acne**, S. **aureus*** and *S. **epidermidis*** shown in table 2.2.

Table 2.2 Anti-bacterial activity from some medicinal plants

Medicinal plants	Microorganism	Compound/ Crude extract	MIC (mg/mL)	MBC (mg/mL)	Reference
Alpinia galanga	*P. acnes*	1'-acetoxychavicol acetate	0.062	0.250	Niyomkamn *et al.*, 2007
Coscinium fenestratum	*P. acnes*	Ethanol extract	0.049	0.049	Kumar *et al.*, 2007
	S. epidermidis	Ethanol extract	0.049	0.165	
Curcuma aromatica	*P. acnes*	Ethanol extract	5	-	Giwanon *et al.*, 2006
	S. epidermidis	Ethanol extract	5-10	-	
	S. aureus	Ethanol extract	5-10	-	

- = not detect

2.3 Phytochemical investigation techniques

The generally phytochemical investigation techniques are chromatographic and spectroscopic techniques. These techniques are useful for isolation and identification of compound from the mixture compounds.

2.3.1 Chromatographic techniques

Chromatography is a method of separation in which the components to be separated are scattered in the middle of two phases, one of which is stationary phase while the mobile phase moves in an accurate direction. The purpose of chromatography is to separate the components of a mixture for purification. The chromatographic techniques for isolation of compound are as follow (Ettre, 1993; http://en.wikipedia.org/ wiki/Chromatography):

(1) Column chromatography is a method used to purify compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms up to kilograms. The classical preparative chromatography column is a glass tube with a diameter from 5 mm to 50 mm and a height of 50 cm to 1 m with a tap at the bottom. Two methods are normally used to prepare a column; the dry method and the wet method. The stationary phase in column chromatography is a solid. The most common stationary phase for column chromatography is silica gel. The mobile phase is either a pure solvent or a mixture of dissimilar solvents and has also been selected so that the different compounds can be separated successfully (http://en.wikipedia.org/wiki/Column_chromatography).

(2) Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. It is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel and aluminium oxide. Different compounds in the sample mixture move at dissimilar rates suitable to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent (Stoddard, 2007; http://en.wikipedia.org/wiki/Thin_layer_chromatography).

2.3.2 Spectroscopic technique

The majority important methods for identify compound are the nuclear magnetic resonant spectroscopy (^{1}H and ^{13}C NMR spectroscopy), the mass spectrometry, the infrared and the UV/Visible spectroscopy. These methods are the main tool of modern chemistry for the identification of molecular structures and used to determine and confirm molecular structures. (www.oc-praktikum.de/en/articles/pdf/Spectroscopy_en.pdf). These techniques were used in this study.

2.3.3 Chemical constituents of *Artocarpus* spp.

In this study, we have been selecting ***Artocarpus integer*** for phytochemical investigation. ***A. integer*** is the tree in genus ***Artocarpus***. ***Artocarpus*** belongs to the Moraceae family. The chemical constituents of plants in the genus ***Artocarpus*** can be classified into five

groups, namely flavonoids, triterpenoids, steroids, stilbenes and miscellaneous substances. The chemical constituents which were isolated from ***Artocarpus*** spp. from previously reports are showed in table 2.3 and the chemical structure of isolated compounds are showed in figure 2.1 (except the structure numbers 42, 43, 44, 119, 136, 137 and 138)

Table 2.3 Chemical constituents of *Artocarpus* spp.

Compound	Plant part	Scientific name	Reference
Acetylcholine [1]	Seed	***A. heterophyllus***	Pereira ***et al.***, 1962
Afzelechin-(4β⟶8)-chatechin [2]	Leaf	***A. heterophyllus***	An ***et al.***, 1992
Albanin A [3]	Root Stem	***A. gomezianus*** ***A. heterophyllus***	Likhiwitayawuid ***et al.***, 2000 Arung ***et al.***, 2006
γ-Amimobutyric acid [4]	Leaf	***A. altilis***	Durand ***et al.***, 1962
α-Amyrin [5]	Latex	***A. altilis***	Ultee, 1949
α-Amyrin acetate [6]	Fruit	***A. altilis***	Altman and Zito, 1976
Andalasin A [7]	Root	***A. gomezianus***	Likhitwitayawuid and Sritularak, 2001
Apigenin [8]	Heartwood	***A. altilis***	Shimizu ***et al.***, 1998
Arbutin [9]	Leaf	***A. gomezianus***	Kingroungpet, 1994
Artelasticin [10]	Heartwood	***A. elasticus*** ***A. lanceifolius***	Kijjoa ***et al.***, 1996 Syah ***et al.***, 2001
Artelasticinol [11]	Root bark	***A. elasticus***	Ko ***et al.***, 2005
Artelastin [12]	Heartwood	***A. elasticus***	Kijjoa ***et al.***, 1996
Artelastinin [13]	Heartwood	***A. elasticus***	Kijjoa ***et al.***, 1998

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Artelastofuran [14]	Heartwood	*A. elasticus*	Kijjoa *et al.*, 1998
	Heartwood	*A. lanceifolius*	Syah *et al.*, 2001
Artelastoheterol [15]	Root bark	*A. elasticus*	Ko *et al.*, 2005
Artelastoxanthone [16]	Root bark	*A. elasticus*	Ko *et al.*, 2005
Artocarbene [19]	Root bark	*A. heterophyllus*	Lu and Lin, 1994
	Heartwood	*A. incisus*	Shimizu *et al.*, 1997
Artocarpanone [20]	Heartwood	*A. heterophyllus*	Radhakrishnan *et al.*, 1965
	Heartwood	*A. hirsuta*	Venkataraman, 1972
	Heartwood	*A. integer*	Pendse *et al.*, 1976
Artocarpanone A [21]	Root bark	*A. heterophyllus*	Lin *et al.*, 1995
Artocarpesin [22]	Heartwood	*A. altilis*	Shimizu *et al.*, 1998
	Heartwood	*A. chaplacha*	Rao *et al.*, 1972
			Kijjoa *et al.*, 1996
	Heartwood	*A. elasticus*	Venkataraman, 1972
	Heartwood	*A. gomezianus*	
	Heartwood	*A. heterophyllus*	Radhakrishnan *et al.*, 1965
	Heartwood	*A. hirsuta*	Venkataraman, 1972
	Heartwood	*A. integer*	Pendse *et al.*, 1976
Artocarpetin [23]	Heartwood	*A. heterophyllus*	Venkataraman, 1972
	Heartwood	*A. hirsuta*	Venkataraman, 1972
	Heartwood	*A. integer*	Pendse *et al.*, 1976

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Artocarpetin A [24]	Root	*A. heterophyllus*	Lin *et al.*, 1995
Artocarpetin B [25]	Root	*A. heterophyllus*	Chung *et al.*, 1995
Artocarpin [26]	Heartwood	*A. altilis*	Venkataraman, 1972
	Root	*A. chama*	Wang *et al.*, 2004
	Heartwood	*A. communis*	Han *et al.*, 2006
	Heartwood	*A. champeden*	Euis *et al.*, 2005
	Heartwood	*A. chaplacha*	Rao *et al.*, 1972
	Heartwood	*A. elasticus*	Kijjoa *et al.*, 1996
	Heartwood	*A. gomezianus*	Venkataraman, 1972
	Heartwood	*A. heterophyllus*	Radhakrishnan *et al.*, 1965
	Heartwood	*A. hirsuta*	Venkataraman, 1972
	Heartwood	*A. lakoocha*	Venkataraman, 1972
Artocarpol A [27]	Root bark	*A. rigida*	Chung *et al.*, 2000
Artocarpol B [28]	Root bark	*A. rigida*	Ko *et al.*, 2000
Artocarpol C [29]	Root bark	*A. rigida*	Ko *et al.*, 2000
Artocarpol D [30]	Root bark	*A. rigida*	Ko *et al.*, 2000
Artocarpol E [31]	Root bark	*A. rigida*	Ko *et al.*, 2000
Artocarpol F [32]	Root bark	*A. rigida*	Ko *et al.*, 2000
Artocarpol G [33]	Root bark	*A. rigida*	Lu *et al.*, 2002
Artocarpol H [34]	Root bark	*A. rigida*	Lu *et al.*, 2002
Artocarpol I [35]	Root	*A. rigida*	Lu *et al.*, 2003
Artocarpone A [36]	Bark	*A. champeden*	Widyawaruyanti *et al.*, 2007

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Artocarpone B [37]	Bark	*A. champeden*	Widyawaruyanti *et al.*, 2007
Artocarpus chalcone AC-3-1 [38]	Leaf	*A. altilis*	Wang *et al.*, 2007b
Artocarpus chalcone AC-5-1 [39]	Leaf	*A. altilis*	Wang *et al.*, 2007b
Artocarpus flavone KB-2 [40]	Bark	*A. communis*	Fujimoto *et al.*, 1990
Artocarpus flavone KB-3 (Artonin E) [41]	Root	*A. chama*	Wang *et al.*, 2004
	Bark	*A. communis*	Fujimoto *et al.*, 1990
	Bark	*A. kemando*	Seo *et al.*, 2003
	Trunk	*A. lanceifolius*	Cao *et al.*, 2003
	Bark	*A. rigida*	Hano *et al.*, 1990b
Artocarpus integra α-D-Galactose specific lectin [42]	Seed	*A. heterophyllus*	Suresh *et al.*, 1982
Artocarpus lactin C [43]	Seed	*A. integer*	Hashim *et al.*, 1992
Artocarpus lectin CE-A-I [44]	Seed	*A. integrifolia*	Ferreia *et al.*, 1992
Artochamin A (Artoindonesianin D) [45]	Root	*A. chama*	Wang *et al.*, 2004
	Root	*A. kemando*	Hakim *et al.*, 2006
Artochamin B [46]	Root	*A. chama*	Wang *et al.*, 2004
Artochamin C [47]	Root	*A. chama*	Wang *et al.*, 2004
Artochamin D [48]	Root	*A. chama*	Wang *et al.*, 2004
Artochamin E [49]	Root	*A. chama*	Wang *et al.*, 2004
Artocommunol CA [50]	Root	*A. communis*	Chan *et al.*, 2003
Artocommunol CB [51]	Root	*A. communis*	Chan *et al.*, 2003
Artocommunol CC [52]	Root	*A. communis*	Chan *et al.*, 2003

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Artocommunol CD [53]	Root	*A. communis*	Chan *et al.*, 2003
Artocommunol CE [54]	Root	*A. communis*	Chan *et al.*, 2003
Artoflavanone [55]	Root	*A. heterophyllus*	Dayal and Seshadri, 1974
Artogomezianol [56]	Root	*A. gomezianus*	Likhitwitayawuid and Sritularak., 2001
Artogomezianone [57]	Heartwood	*A. gomezianus*	Likhitwitayawuid *e t al.,* 2006
Artoindonesianin A [58]	Root	*A. champeden*	Hakim *et al.*, 1999
Artoindonesianin A-2 [59]	Heartwood	*A. champeden*	Syah *et al.*, 2006b
Artoindonesianin A-3 [60]	Heartwood	*A. champeden*	Syah *et al.*, 2006b
Artoindonesianin B [61]	Root Root	*A. champeden* *A. kemando*	Hakim *et al.*, 1999 Hakim *et al.*, 2006
Artoindonesianin E [62]	Bark	*A. champeden*	Widyawaruyanti *et al.*, 2007
Artoindonesianin G [63]	Heartwood	*A. lanceifolius*	Syah *et al.*, 2001
Artoindonesianin H [64]	Heartwood	*A. lanceifolius*	Syah *et al.*, 2001
Artoindonesianin I [65]	Heartwood	*A. lanceifolius*	Syah *et al.*, 2001
Artoindonesianin J [66]	Root and bark	*A. bracteata*	Ersam *et al.*, 2002
Artoindonesianin L [67]	Root bark	*A. rotunda*	Suhartati *et al.*, 2001
Artoindonesianin M [68]	Heartwood	*A. champeden*	Euis *et al.*, 2005
Artoindonesianin N [69]	Bark	*A. gomezianus*	Hakim *et al.*, 2002b
Artoindonesianin O [70]	Bark	*A. gomezianus*	Hakim *et al.*, 2002b
Artoindonesianin P [71]	Bark	*A. lanceifolius*	Hakim *et al.*, 2002a
Artoindonesianin Q [72]	Bark	*A. champeden*	Syah *et al.*, 2002b

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Artoindonesianin R [73]	Bark	*A. champeden*	Syah *et al.*, 2002b; Widyawaruyanti *et al.*, 2007
Artoindonesianin S [74]	Heartwood	*A. champeden*	Syah *et al.*, 2002b
Artoindonesianin T [75]	Heartwood	*A. champeden*	Syah *et al.*, 2002b
Artoindonesianin U [76]	Heartwood	*A. champeden*	Syah *et al.*, 2004
Artoindonesianin V [77]	Heartwood	*A. champeden*	Syah *et al.*, 2004
Artoindonesianin X [78]	Root bark	*A. fretessi*	Soekamto *et al.*, 2003
Artoindonesianin Y [79]	Root bark	*A. fretessi*	Soekamto *et al.*, 2003
Artoindonesianin Z-1 [80]	Bark	*A. lanceifolius*	Syah *et al.*, 2006a
Artoindonesianin Z-2 [81]	Bark	*A. lanceifolius*	Syah *et al.*, 2006a
Artoindonesianin Z-3 [82]	Bark	*A. lanceifolius*	Hakim *et al.*, 2006
Artolastochromene [83]	Heartwood	*A. elasticus*	Kijjoa *et al.*, 1996
Artolastofuran [84]	Heartwood	*A. elasticus*	Kijjoa *et al.*, 1998
Artomunoflavanone [85]	Root	*A. communis*	Weng et al., 2006
Artomunoxanthentrione [86]	Root bark	*A. communis*	Shieh and Lin, 1992
Artomunoxanthone [87]	Root bark	*A. communis*	Shieh and Lin, 1992
Artomunoxanthotrione epoxide [88]	Root bark	*A. communis*	Lin *et* al., 1992
Artonin A [89]	Root Root Bark Root bark Bark	*A. chama* *A. champeden* *A. heterophyllus* *A. fretessi*	Wang *et al.*, 2004 Hakim *et al.*, 1999 Widyawaruyanti *et al.*, 2007 Hano *et al.*, 1989 Soekamto *et al.*, 2003
Artonin B [90]	Root bark	*A. heterophyllus*	Hano *et al.*, 1990a
Artonin C [91]	Root bark	*A. heterophyllus*	Hano *et al.*, 1990a
Artonin D [92]	Root bark	*A. heterophyllus*	Hano *et al.*, 1990a

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Artonin F [93]	Bark	*A. communis*	Hano *et al.*, 1990c
	Root bark	*A. elasticus*	Ko *et al.*, 2005
Artonin G [94]	Bark	*A. rigida*	Hano *et al.*, 1990b
Artonin H [95]	Bark	*A. rigida*	Hano *et* al., 1990b
Artonin I [96]	Root bark	*A. heterophyllus*	Hano *et al.*,1990a
Artonin J [97]	Root bark	*A. heterophyllus*	Aida *et al.*, 1993
	Root bark	*A. teysmanii*	Makmur *et al.*, 2000
Artonin K [98]	Bark	*A. altilis*	Aida *et al.*, 1997
	Root bark	*A. heterophyllus*	Aida *et al.*, 1993
Artonin L [99]	Root bark	*A. heterophyllus*	Aida *et al.*, 1993
Artonin M [100]	Bark	*A. rigida*	Hano *et* al., 1993
Artonin N [101]	Bark	*A. rigida*	Hano *et* al., 1993
Artonin O [102]	Bark	*A. rigida*	Hano *et* al., 1993
Artonin P [103]	Bark	*A. rigida*	Hano *et* al., 1993
Artonin Q [104]	Bark	*A. heterophyllus*	Aida *et al.*, 1994
Artonin R [105]	Bark	*A. heterophyllus*	Aida *et al.*, 1994
Artonin S [106]	Heartwood	*A. elasticus*	Cidade *et al.*, 2001
	Bark	*A. heterophyllus*	Aida *et al.*, 1994
Artonin T [107]	Bark	*A. heterophyllus*	Aida *et al.*, 1994
Artonin U [108]	Bark	*A. heterophyllus*	Aida *et al.*, 1994
Artonin V [109]	Root bark	*A. altilis*	Hano *et al.*, 1994
Artonin X [110]	Bark	*A. heterophyllus*	Shinomiya *et al.*, 1995
Artonol A [111]	Bark	*A. communis*	Aida *et al.*, 1997
Artonol B [112]	Bark	*A. communis*	Aida *et al.*, 1997

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Artonol C [113]	Bark	*A. communis*	Aida *et al.*, 1997
Artonol D [114]	Bark	*A. communis*	Aida *et al.*, 1997
Artonol E [115]	Bark	*A. communis*	Aida *et al.*, 1997
Artostilbene A [116]	Wood	*A. chama*	Wang *et al.*, 2007a
Artostilbene B [117]	Wood	*A. chama*	Wang *et al.*, 2007a
Aurantiamide acetate [118]	Seed	*A. heterophyllus*	Chakraborty and Mandal, 1981
β-Amyrin acetate [119]	Latex	*A. altilis*	Ultee, 1949
	Latex	*A. elasticus*	Ultee, 1949
	Bark	*A. lakoocha*	Kapel and Joshi, 1960
Betulin [120]	Root bark	*A. heterophyllus*	Lu and Lin, 1994
Betulinic acid [121]	Root	*A. heterophyllus*	Dayal and Seshadri, 1974
	Root bark		Lu and Lin, 1994
Butyosoermol [122]	Fruit	*A. heterophyllus*	Barton, 1951
Carpachromene [123]	Root	*A. bracteata*	Ersam *et al.*, 2002
	Bark		
Carpelastofuran [124]	Heartwood	*A. elasticus*	Cidade *et al.*, 2001
Chaplashin [125]	Heartwood	*A. chaplacha*	Rao *et al.*, 1972
	Heartwood	*A. integer*	Pendse *et al.*, 1976
Chromanoartobilochromen A [126]	Trunk bark	*A. nobilis*	Kumar *et al.*, 1977
Chromanoartobilochromen B [127]	Trunk bark	*A. nobilis*	Pavanasasivum *et al.*,1974; Kumar *et al.*, 1977

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Cudraflavone A	Stem	***A. altilis***	Chen ***et al.***, 1993
(Isocyclomorusin) [128]	Root	***A. chama***	Wang ***et al.***, 2004
	Root bark	***A. communis***	Shieh and Lin, 1992
	Heartwood		
	Root	***A. gomezianus***	Likhitwitayawuid ***et al.***, 2000
	Heartwood		Likhitwitayawuid ***et al.***, 2000
	Root bark	***A. heterophyllus***	Lin ***et al.***, 1995
Cudraflavone C [129]	Heartwood	***A. champeden***	Euis ***et al.***, 2005
	Heartwood	***A. communis***	Han ***et al.***, 2006
	Root bark	***A. glaucus***	Hakim ***et al.***, 2006
	Root	***A. gomezianus***	Likhitwitayawuid ***et al.***, 2000
	Stem	***A. heterophyllus***	Arung ***et al.***, 2006
Cyanomaclurin [130]	Heartwood	***A. heterophyllus***	Radhakrishnan ***et al.***, 1965
	Heartwood	***A. hirsuta***	Venkataraman, 1972
	Heartwood	***A. integer***	Pendse ***et al.***, 1976
Cycloaltilisin [131]	Stem	***A. altilis***	Chen ***et al.***, 1993
Cycloart-23-ene-3β-25-diol [132]	Fruit	***A. altilis***	Altman and Zito, 1976
	Fruit	***A. heterophyllus***	Kielland and Malterud, 1994

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Cycloart-24-ene-3β-ol (Cycloartenol) [133]	Fruit	*A. altilis*	Altman and Zito, 1976; Pavanasasivam and Sultanbawa, 1973 Barton, 1951
	Fruit Wood	*A. heterophyllus*	Nogueira and Correia, 1958
	Bark		Pavanasasivam and Sultanbawa, 1973; Barik *et al.*, 1994
	Latex	*A. lakoocha*	Pavanasasivam and Sultanbawa, 1973
	Bark	*A. nobilis*	Pavanasasivam and Sultanbawa, 1973
	Heartwood		Pavanasasivam and Sultanbawa, 1973
Cycloart-25-ene-3β-24-diol [134]	Fruit	*A. altilis*	Altman and Zito, 1976
	Fruit	*A. heterophyllus*	Kielland and Malterud , 1994
Cycloartelastoxanthendiol [135]	Root bark	*A. elasticus*	Ko *et al.*, 2005
Cycloartelastoxanthone [136]	Root bark	*A. elasticus*	Ko *et al.*, 2005

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Cycloartenone [137]	Bark	***A. altilis***	Pavanasasivam and Sultsnbswa, 1973
	Fruit	***A. heterophyllus***	Barton, 1951
	Bark		Pavanasasivam and Sultanbawa, 1973
	Root		Dayal and Seshadri, 1974
	Latex		Pant and Chaturvedi, 1989
	Bark	***A. lakoocha***	Barik ***et al.***, 1994
			Pavanasasivam and Sultanbawa, 1973
	Bark	***A. nobilis***	Pavanasasivam and Sultsnbswa, 1973
	Heartwood		Pavanasasivam and Sultsnbswa, 1973
Cycloartenyl actate [138]	Bark	***A. altilis***	Pavanasasivam and Sultsnbswa, 1973
	Stem bark	***A. chaplacha***	Mahato, Banerjee and Chakravarti, 1971
	Bark	***A. heterophyllus***	Pavanasasivam and Sultsnbswa, 1973
	Bark	***A. nobilis***	Pavanasasivam and Sultsnbswa, 1973
	Heartwood		Pavanasasivam and Sultsnbswa, 1973

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Cycloartenyl acetate [139]	Bark	*A. altilis*	Pavanasasivam and Sultsnbswa, 1973
	Stem bark	*A. chaplacha*	Mahato, Banerjee and Chakravarti, 1971
	Bark	*A. heterophyllus*	Pavanasasivam and Sultsnbswa, 1973
	Bark	*A. nobilis*	Pavanasasivam and Sultsnbswa, 1973
	Heartwood		Pavanasasivam and Sultsnbswa, 1973
Cycloartobiloxanthone [140]	Root	*A. chama*	Wang *et al.*, 2004
	Bark	*A. communis*	Hano *et al.*, 1990c
	Root bark	*A. elasticus*	Ko *et al.*, 2005
	Bark	*A. nobilis*	Sultanbawa and Surendrakumar, 1989
	Bark	*A. rigida*	Hano *et al.*, 1990b
Cycloartocarpesin [141]	Heartwood	*A. chaplacha*	Rao *et al.*, 1972
	Heartwood	*A. heterophyllus*	Parthasarathy *et al.*, 1969
	Heartwood	*A. hirsutus*	Venkataraman, 1972
	Heartwood	*A. integer*	Pendse *et al.*, 1976

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compounds	Plant part	Scientific name	Reference
Cycloartocarpin [142]	Heartwood	*A. altilis*	Venkataraman, 1972
	Heartwood	*A. communis*	Han *et al.*, 2006
	Heartwood	*A. chaplacha*	Rao *et al.*,1972
	Heartwood	*A. elasticus*	Pense *et al.*, 1976
	Heartwood	*A. gomezianus*	Venkataraman, 1972
	Heartwood	*A. heterophyllus*	Venkataraman, 1972
	Heartwood	*A. hirsuta*	Venkataraman, 1972
	Heartwood	*A. integer*	Pendse *et al.*, 1976
	Root	*A. kemando*	Hakim *et al.*, 2006
	Heartwood	*A. lakoocha*	Venkataraman, 1972
Cycloartocarpin A [143]	Root	*A. chama*	Wang *et al.*, 2004
	Root bark	*A. heterophyllus*	Lu and Lin, 1994
	Bark	*A. maingayii*	Hakim *et al.*, 2006
Cycloartomunin [144]	Root bark	*A. communis*	Lin and Shieh, 1991
Cycloartomunoxanthone [145]	Root bark	*A. communis*	Lin and Shieh, 1991
Cyclochampedol [146]	Bark	*A. champeden*	Achmad *et al.*, 1996
Cyclocommunin	Stem	*A. altilis*	Chen *et al.*, 1993
(Isocyclomulberrin) [147]	Root bark	*A. communis*	Lin and Shieh, 1991
Cyclocommunol [148]	Root bark	*A. communis*	Lin and Shieh, 1991
Cyclocommunomethonol [149]	Root	*A. communis*	Weng *et al.*, 2006
Cycloheterophyllin [150]	Bark	*A. heterophyllus*	Rao *et al.*, 1971
	Root bark		Hano *et al.*, 1989
	Bark	*A. champeden*	Widyawaruyanti *et al.*, 2007
Cyclointegrin [151]	Heartwood	*A. integer*	Pendse *et al.*, 1976
9,19-cyclolanost-25-ene-3β,24-diol (24R, 24S) [152]	Latex	*A. heterophyllus*	Barik *et al* ., 1997

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
9,19-cyclolanost-3-one-24,25-diol (24R, 24S) [153]	Latex	*A. heterophyllus*	Barik *et al.*, 1994
Cyclomorusin [154]	Stem	*A. altilis*	Chen *et al.*, 1993
	Root bark	*A. communis*	Lin and Shieh, 1991
Cyclomulberrin [155]	Stem	*A. altilis*	Chen *et al.*, 1993
	Root bark	*A. communis*	Lin and Shieh, 1991
Dihydroartomunoxanthone [156]	Root	*A. communis*	Weng *et al.*, 2006
Dihydrocycloartomunin [157]	Root bark	*A. communis*	Lin and Shieh, 1991
(-)-Dihydrofuranoartobilochromen A [158]	Trunk bark	*A. nobilis*	Kumar *et al.*, 1977
(-)-Dihydrofuranoartobilochromen B-1 [159]	Trunk bark	*A. nobilis*	Kumar *et al.*, 1977
(-)-Dihydrofuranoartobilochromen B-2 [160]	Trunk bark	*A. nobilis*	Kumar *et al.*, 1977
Dihydroisocycloartomunin [161]	Root bark	*A. altilis*	Lin and Shieh, 1992
(+)-Dihydromorin [162]	Heartwood	*A. altilis*	Shimizu *et al.*, 1998
	Root bark	*A. communis*	Su *et al.*, 2002
	Heartwood	*A. heterophyllus*	Venkataraman, 1972
	Heartwood	*A. hirsuta*	Venkataraman, 1972
	Heartwood	*A. integer*	Pendse *et al.*, 1976
5,7-Dihydroxyflavone-3-O-α-L-rhamnoside [163]	Root bark	*A. lakoocha*	Chauhan and Kumari, 1979
1-Dotriacontanol [164]	Leaf	*A. gomezianus*	Kingroungpet, 1994
Engeletin [165]	Stem	*A. altilis*	Chen *et al.*, 1993
Furanoartobilochromen A [166]	Bark	*A. nobilis*	Pavanasasivum *et al.*, 1974

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Furanoartobilochromen B-1 [167]	Bark	*A. nobilis*	Pavanasasivum *et al.*, 1974
Furanoartobilochromen B-2 [168]	Bark	*A. nobilis*	Pavanasasivum *et al.*, 1974
Galangin-3-O-α-L-(-)-rhamnopyranoside [169]	Root bark	*A. lakoocha*	Chauhan and Kumari, 1979
Galangin-3-O-β-D-galactopyranosyl-(1⟶4)-α-L-rhamnopyranoside [170]	Root bark	*A. lakoocha*	Chauhan and Kumari, 1979
3'-Geranyl-2',3,4,4'-tetrahydroxychalcone [171]	Leaf Leaf Fruit	*A. incisus* *A. nobilis*	Shimizu *et al.*, 2000 Jayasinghe *et al.*, 2004 Jayasinghe *et al.*, 2006
8-Geranyl-3',4',7-trihydroxyflavanone [172]	Fruit	*A. nobilis*	Jayasinghe *et al.*, 2006
8-Geranyl-4',5,7-trihydroxyflavanone [173]	Fruit	*A. nobilis*	Jayasinghe *et al.*, 2006
8-Geranyl-4',7-dihydroxyflavanone [174]	Fruit	*A. nobilis*	Jayasinghe *et al.*, 2006
Gemichalcone B [175]	Heartwood	*A. communis*	Han *et al.*, 2006
Gemichalcone C [176]	Heartwood	*A. communis*	Han *et al.*, 2006
Heteroartonin A [177]	Root	*A. heterophyllus*	Chung *et al.*, 1995
Heteroflavanone A [178]	Root bark	*A. heterophyllus*	Lu and Lin, 1993
Heteroflavanone B [179]	Root bark	*A. heterophyllus*	Lu and Lin, 1993
Heteroflavanone C [180]	Root bark	*A. heterophyllus* *A. champeden*	Lu and Lin, 1994 Widyawaruyanti *et al.*, 2007

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Heterophyllin [181]	Root bark Bark	*A. heterophyllus* *A. champeden*	Hano *et al.*, 1989 Widyawarutanti *et al.*, 2007
Heterophyllol [182]	Root bark	*A. heterophyllus*	Lu and Lin, 1993
5'-Hydroxycudraflavone A [183]	Heartwood Bark	*A. champeden* *A. scortechinii*	Syah *et al.*, 2004 Hakim *et al.*, 2006
5-Hydroxy-7-2'-4'-trimethoxyflavone [184]	Stem	*A. lakoocha*	Pavaro and Reutrakul, 1976
9-Hydroxytridecyldocasanoate [185]	Root bark	*A. heterophyllus*	Lu and Lin, 1994
4- Hydroxytridecyldocasanoate [186]	Latex	*A. heterophyllus*	Pant and Chaturvedi, 1989
Integrin [187]	Heartwood Heartwood	*A. elasticus* *A. integer*	Pense *et al.*, 1976 Pendse *et al.*, 1976
Isoartocarpetin [188]	Heartwood	*A. incisus*	Shimizu *et al.*, 1998
Isobacachalcone [189]	Heartwood	*A. communis*	Han *et al.*, 2006
Isocycloartenyl acetate [190]	Bark	*A. chaplacha*	Mahato *et al.*, 1971
Isocycloheterophyllin [191]	Bark	*A. heterophyllus*	Rao *et al.*, 1973
Isonymphaeol-B [192]	Fruit	*A. nobilis*	Jayasinghe *et al.*, 2006
Jacalin [193]	Seed	*A. heterophyllus*	Hagieara *et al.*, 1988; Ferreia *et al.*, 1992
Kaempferol-3-O-β-D-xylanopyranoside [194]	Root bark	*A. lakoocha*	Chauhan *et al.*, 1982
Kozonol C [195]	Root and bark	*A. bracteata*	Ersam *et al.*, 2002
Kuwanon C [196]	Stem	*A. heterophyllus*	Arung *et al.*, 2006
Kuwanon R [197]	Root bark	*A. heterophyllus*	Shinomiya *et al.*, 1995
Kuwanon T [198]	Root bark	*A. heterophyllus*	Shinomiya *et al.*, 1995

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Lakoochin A [199]	Root	*A. lakoocha*	Puntumchai *et al.*, 2004
Lakoochin B [200]	Root	*A. lakoocha*	Puntumchai *et al.*, 2004
Lespeol [201]	Fruit	*A. nobilis*	Jayasinghe *et al.*, 2006
Lupeol [202]	Root bark	*A. lakoocha*	Chauhan and Kumari, 1979
Lupeol acetate [203]	Root bark	*A. altilis*	Shieh and Lin, 1992
	Stem bark	*A. chaplacha*	Mahato *et al.*, 1971
	Latex	*A. elasticus*	Utee, 1949
	Leaf	*A. gomezianus*	Kingroungpet, 1994
	Bark	*A. heterophyllus*	Kapil and Joshi, 1960
	Bark	*A. lakoocha*	Kapil and Joshi, 1960
Lymphoagglutinin [204]	Seed	*A. heterophyllus*	Arora *et al.*, 1987
	Seed	*A. hirsuta*	Arora *et al.*, 1987
	Seed	*A. lakoocha*	Arora *et al.*, 1987
Mesoerythritol [205]	Leaf	*A. gomezianus*	Venkataraman, 1972
Morachalcone A [206]	Heartwood	*A. communis*	Han *et al.*, 2006
Morin [207]	Heartwood	*A. altilis*	Venkataraman, 1972
	Heartwood	*A. gomezianus*	Venkataraman, 1972
	Heartwood	*A. heterophyllus*	Radhakrishnan *et al.*, 1965;
	Heartwood	*A. hirsuta*	Parthasarathy *et al.*, 1969;
	Heartwood	*A. integer*	Venkataraman, 1972
			Pendse *et al.*, 1976

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Morusin [208]	Bark	*A. communis*	Fujimoto *et al.*, 1990
Mulberrin [209]	Bark	*A. fretessi*	Soekamto *et al.*, 2003
Mulberrochromene [210]	Bark	*A. fretessi*	Soekamto *et al.*, 2003
(+)-Norartocarpanone [211]	Heartwood	*A. incisus*	Shimizu *et al.*, 1998
Norartocarpetin [212]	Heartwood	*A. altilis*	Venkataraman, 1972
	Heartwood	*A. dadah*	Su *et al.*, 2002
	Root bark	*A. fretessi*	Soekamto *et al* ., 2003
	Heartwood	*A. gomezianus*	Venkataraman, 1972
	Heartwood	*A. heterophyllus*	Radhakrishnan *et al.*, 1965
	Heartwood	*A. hirsuta*	Venkataraman, 1972
	Heartwood	*A. integer*	Pendse *et al.*, 1976
	Root bark	*A. kemando*	Hakim *et al.*, 2006
Norartocarpin [213]	Heartwood	*A. elasticus*	Pense *et al.*, 1976
	Heartwood	*A. heterophyllus*	Venkataraman, 1972
	Heartwood	*A. lakoocha*	Venkataraman, 1972
Norcycloartocarpin [214]	Heartwood	*A. lakoocha*	Venkataraman, 1972
Oxydihydroartocarpesin [215]	Heartwood	*A. heterophyllus*	Pathasarathy *et al.*, 1969
	Heartwood	*A. hirsuta*	Venkataraman, 1972
	Heartwood	*A. integer*	Pendse *et al.*, 1976
Oxydihydromorusin [216]	Bark	*A. rigida*	Hano, Inami and Nomura, 1990b
Oxyisocyclointegrin [217]	Heartwood	*A. integer*	Pendse *et al.*, 1976

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
Oxyresveratrol [218]	Heartwood	*A. chaplacha*	Rao *et al.*, 1972
	Bark	*A. gomezianus*	Hakim *et al.*, 2002b
	Heartwood		Likhitwitayawuid *et al.*, 2000
	Heartwood	*A. lakoocha*	Venkataraman, 1972; Mongolsuk, Robertson and Towers, 1957
4-Prenyloxyresceratrol [219]	Heartwood	*A. incisus*	Shimizu *et al.*, 1997
Procyanidin B-3 [220]	Leaf	*A. heterophyllus*	An *et al.*,1992
Procyanindin C-1 [221]	Leaf	*A. heterophyllus*	An *et al.*,1992
Querectin-3-O-α-L-rhamnopyranoside [222]	Root bark	*A. lakoocha*	Chauhan *et al.*, 1982
Recinoleic acid [223]	Seed oil	*A. heterophyllus*	Daulatabad and Mirajkar, 1989
Resorcinol [224]	Heartwood	*A. chaplacha*	Rao *et al.*, 1972
	Root	*A. gomezianus*	Sritularrak, 1998
	Heartwood	*A. lakoocha*	Venkataraman, 1972
β-Resorcyaldehyde [225]	Heartwood	*A. chaplacha*	Rao *et al.*, 1972
Resveratrol [226]	Heartwood	*A. chaplacha*	Rao *et al.*, 1972
	Root	*A. gomezianus*	Likhitwitayawuid *et al.*, 2000
	Heartwood	*A. lakoocha*	Venkataraman, 1972
Rubraflavone C [227]	Root bark	*A. rigida*	Lu *et al.*, 2002
Simiarenol [228]	Leaf	*A. gomezianus*	Kingroungpet, 1994
Stigmasterol [229]	Root	*A. gomezianus*	Sritularak, 1998
	Heartwood	*A. lakoocha*	Wetwitayaklung, 1994

Table 2.3 Chemical constituents of *Artocarpus* spp. (continued)

Compound	Plant part	Scientific name	Reference
β-sitosterol [230]	Root bark	***A. altilis***	Shieh and Lin, 1992
	Stem bark	***A. chaplacha***	Mahato ***et al.***, 1971
	Heartwood	***A. elasticus***	Pendse ***et al.***, 1976
	Leaf	***A. gomezianus***	Kingroungpet, 1994
	Heartwood	***A. heterophyllus***	Pathasarathy ***et al.***, 1969
	Root		Dayal and Seshadri, 1974
	Root bark		Lu and Lin, 1994
	Heartwood	***A. integer***	Pendse ***et al.***, 1976
	Root bark	***A. lakoocha***	Chauhan and Kumari, 1979
Ursolic acid [231]	Root	***A. heterophyllus***	Dayal and Seshadri, 1974
	Root bark		Lu and Lin, 1994
Xanthoangelol [232]	Leaf	***A. nobilis***	Jayasinghe ***et al.***, 2004
	Fruit		Jayasinghe ***et al.***, 2006
Xanthoangelol B [233]	Leaf	***A. nobilis***	Jayasinghe ***et al.***, 2004
	Fruit		Jayasinghe ***et al.***, 2006

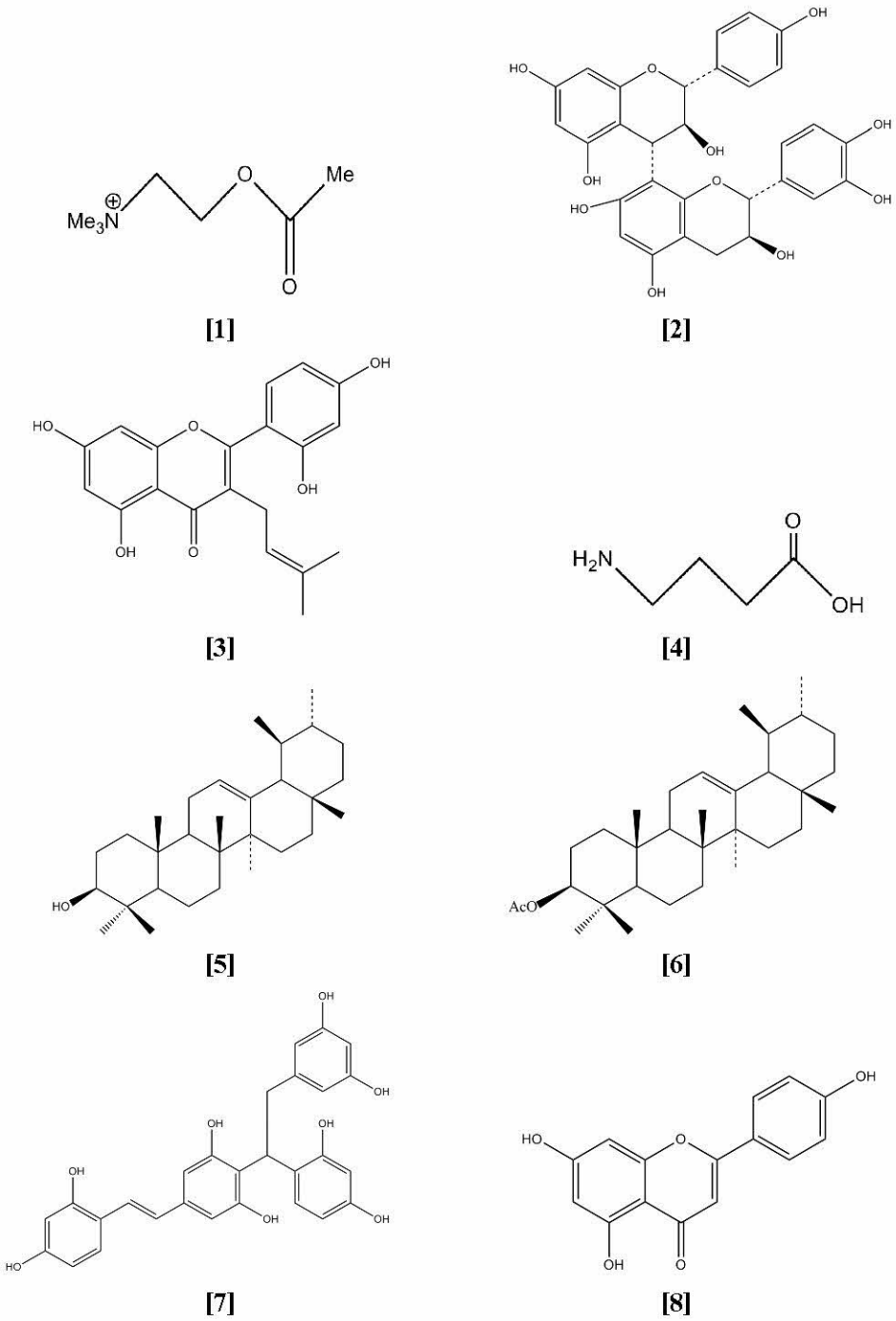


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3

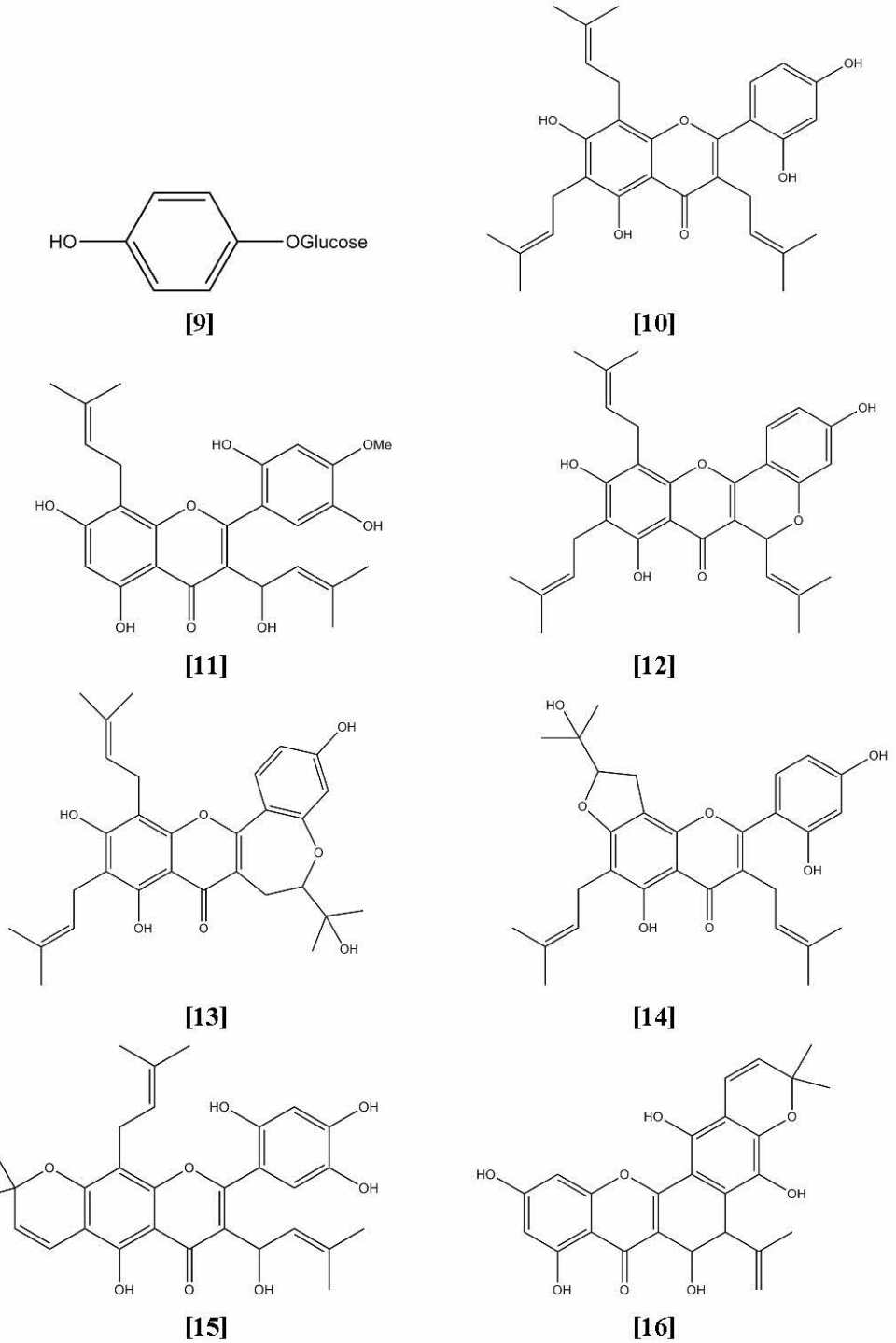


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

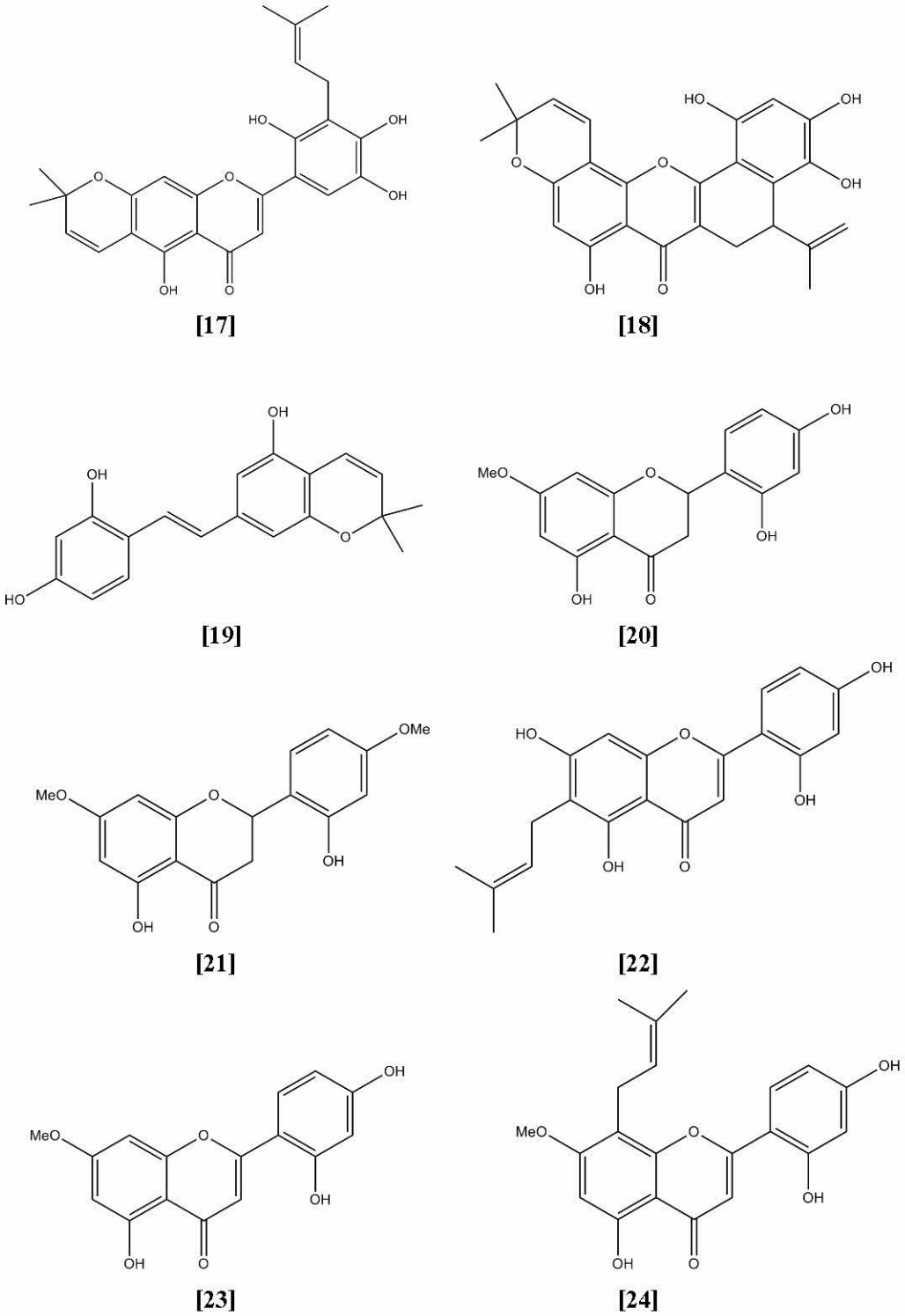
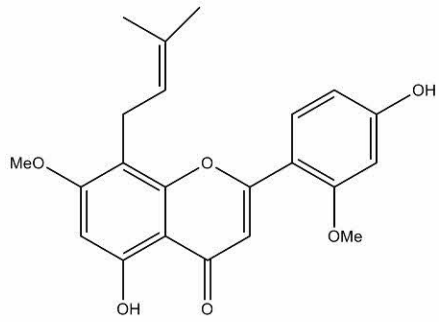
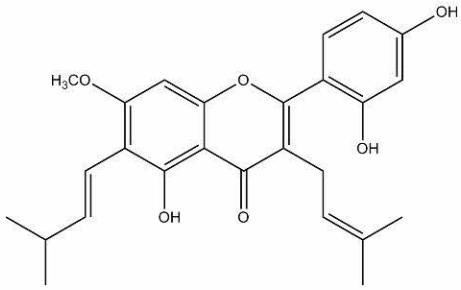


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

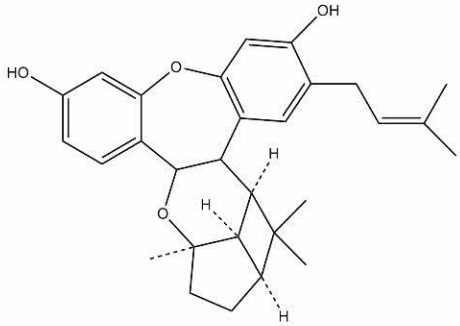
Numbers of these structures are related to table 2.3 (continued)



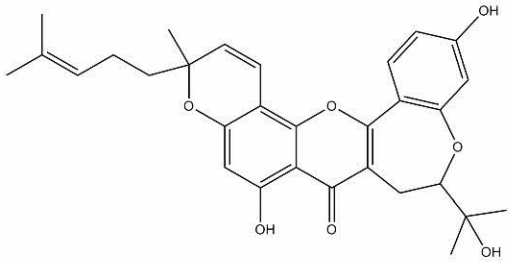
[25]



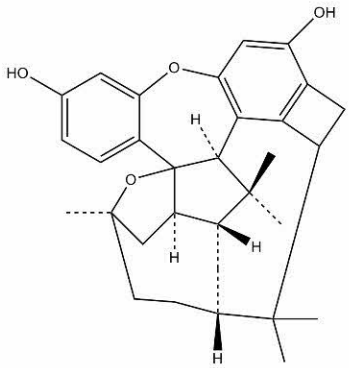
[26]



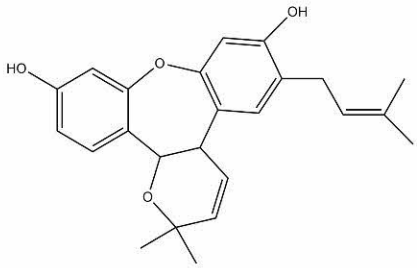
[27]



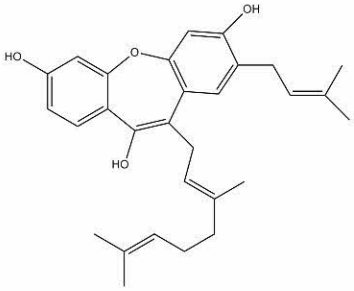
[28]



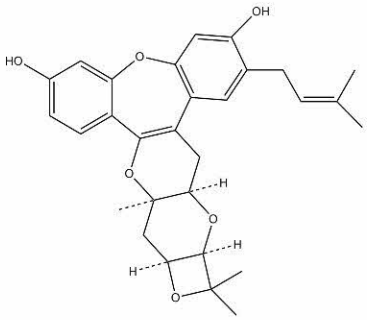
[29]



[30]



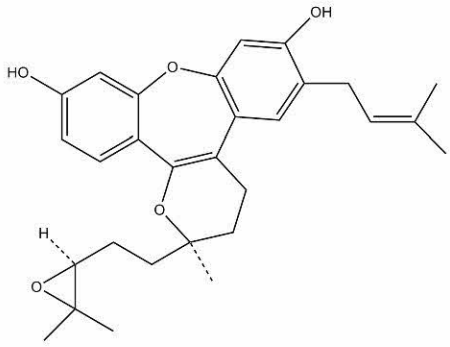
[31]



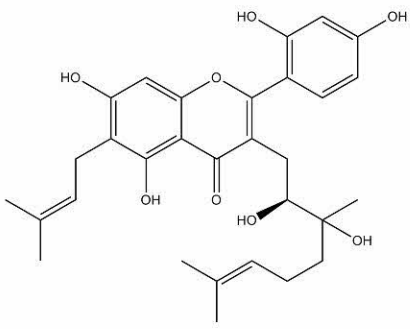
[32]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

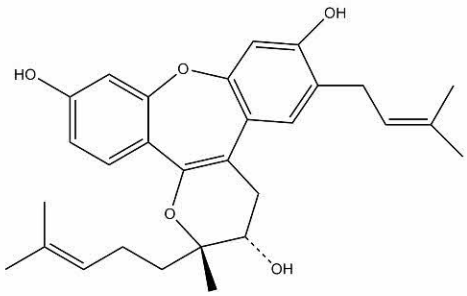
Numbers of these structures are related to table 2.3 (continued)



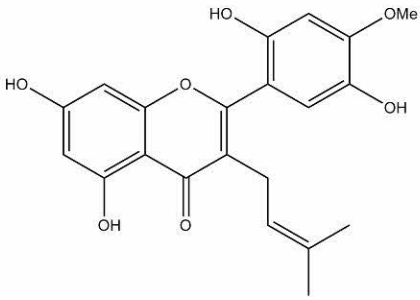
[33]



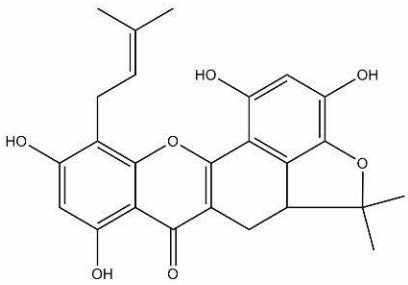
[34]



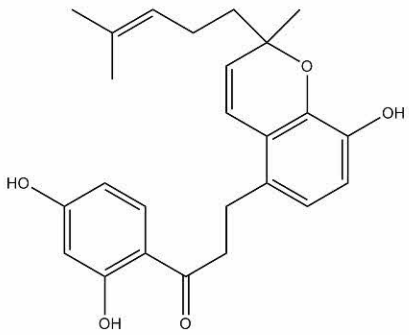
[35]



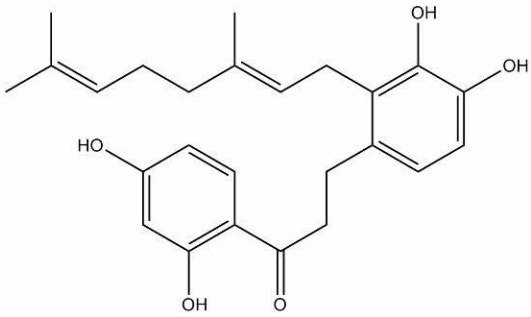
[36]



[37]



[38]



[39]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

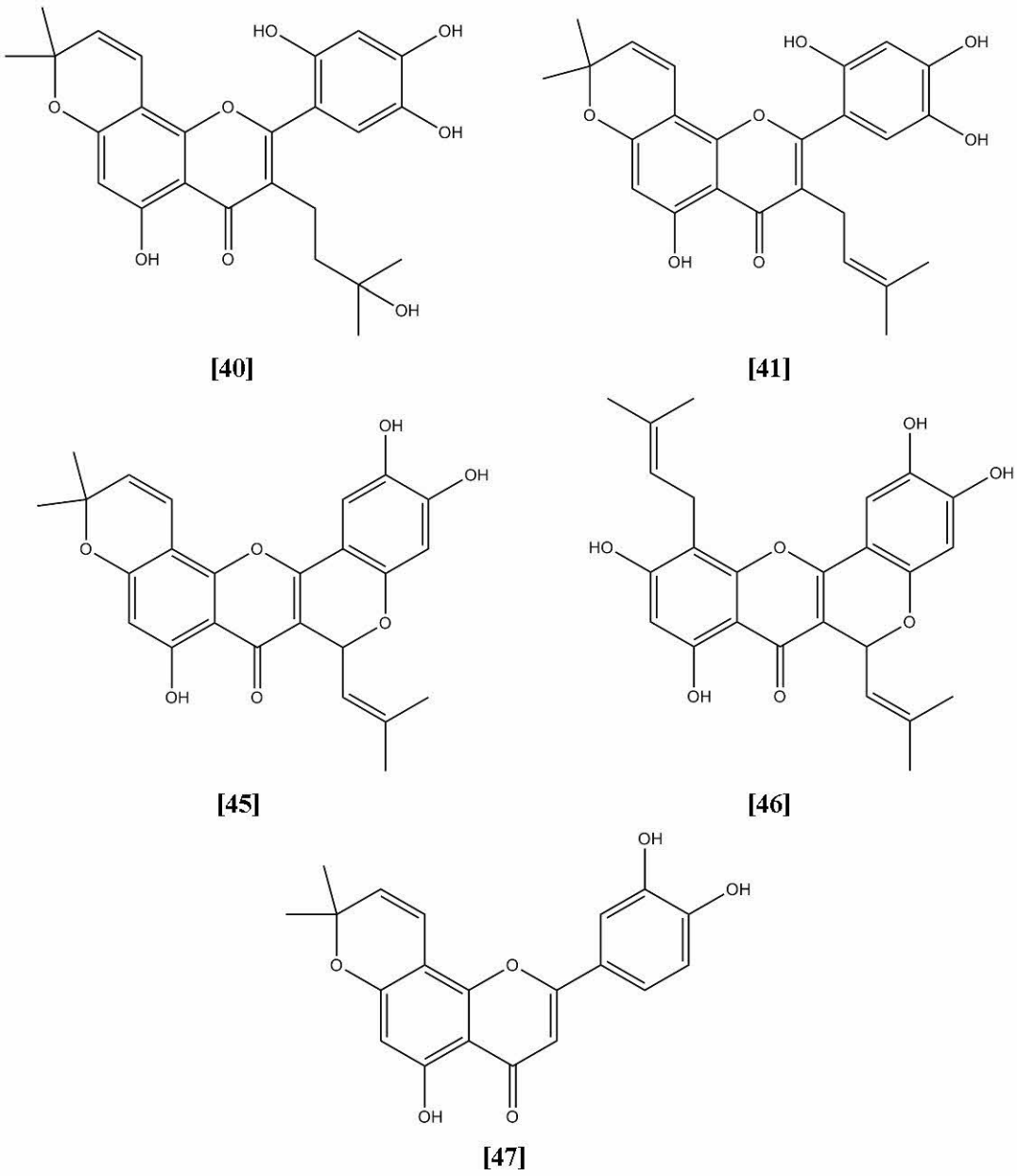


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

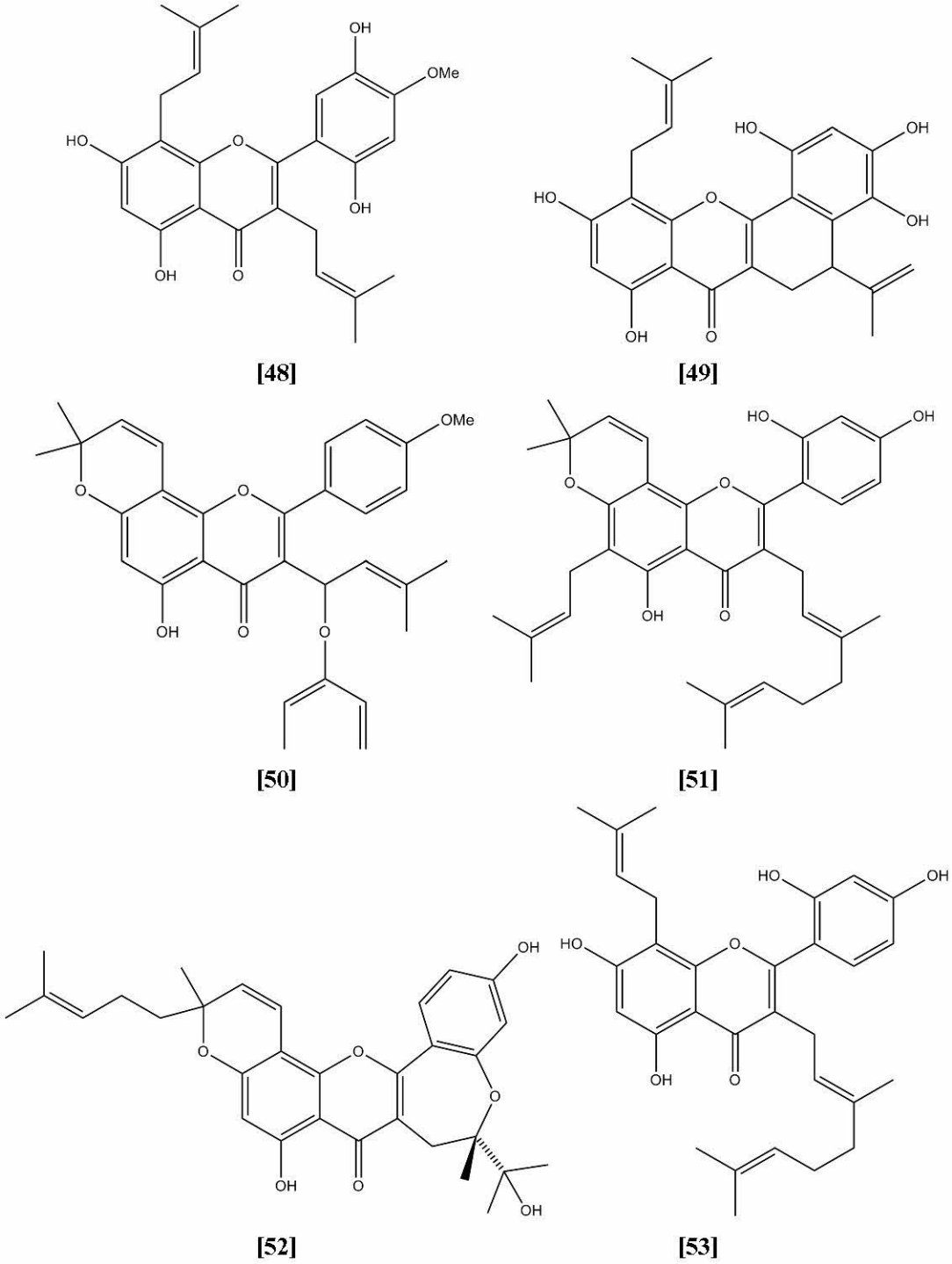


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

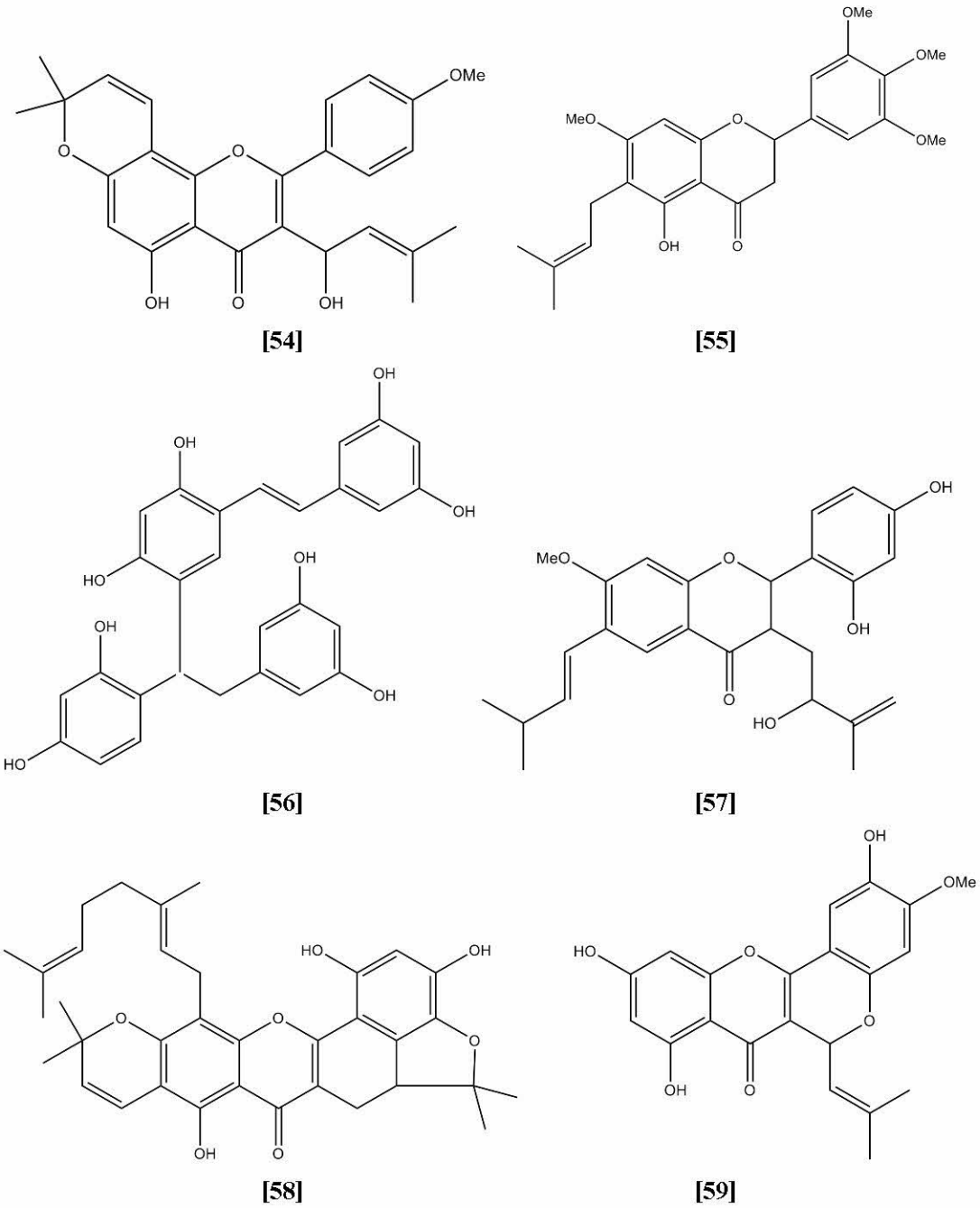
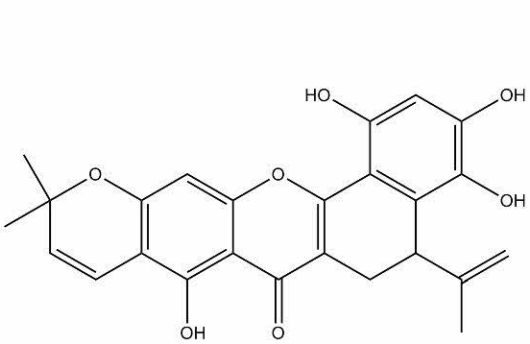
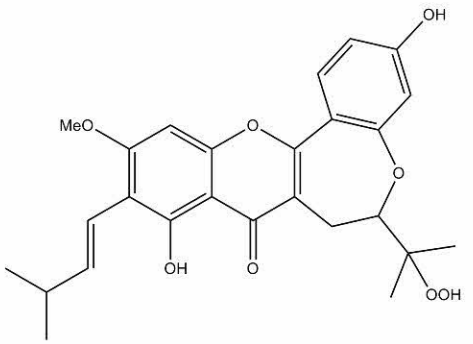


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

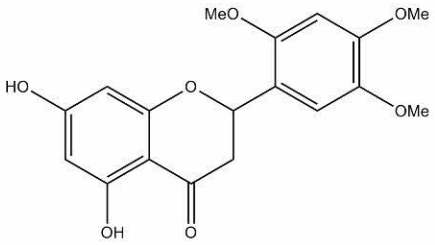
Numbers of these structures are related to table 2.3 (continued)



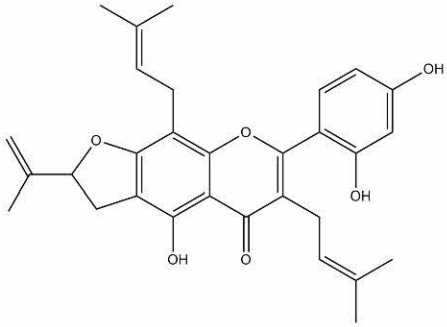
[60]



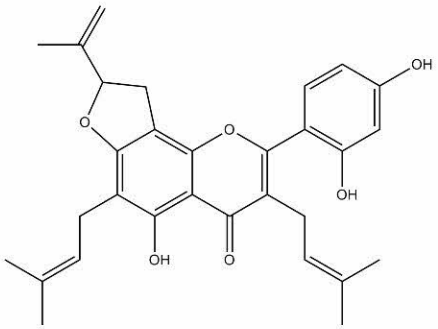
[61]



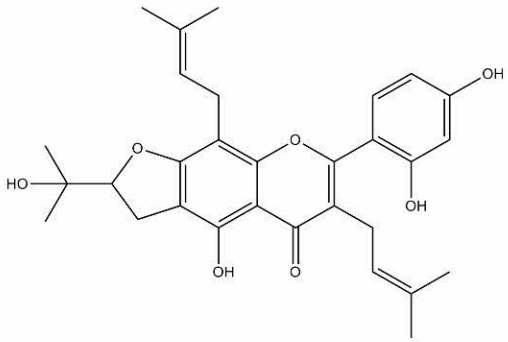
[62]



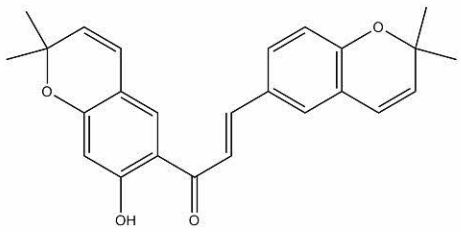
[63]



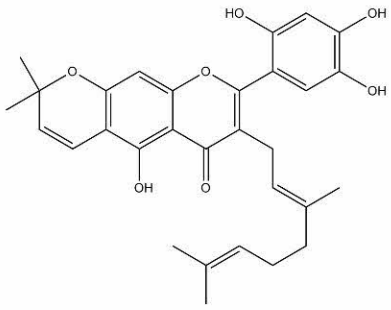
[64]



[65]



[66]



[67]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

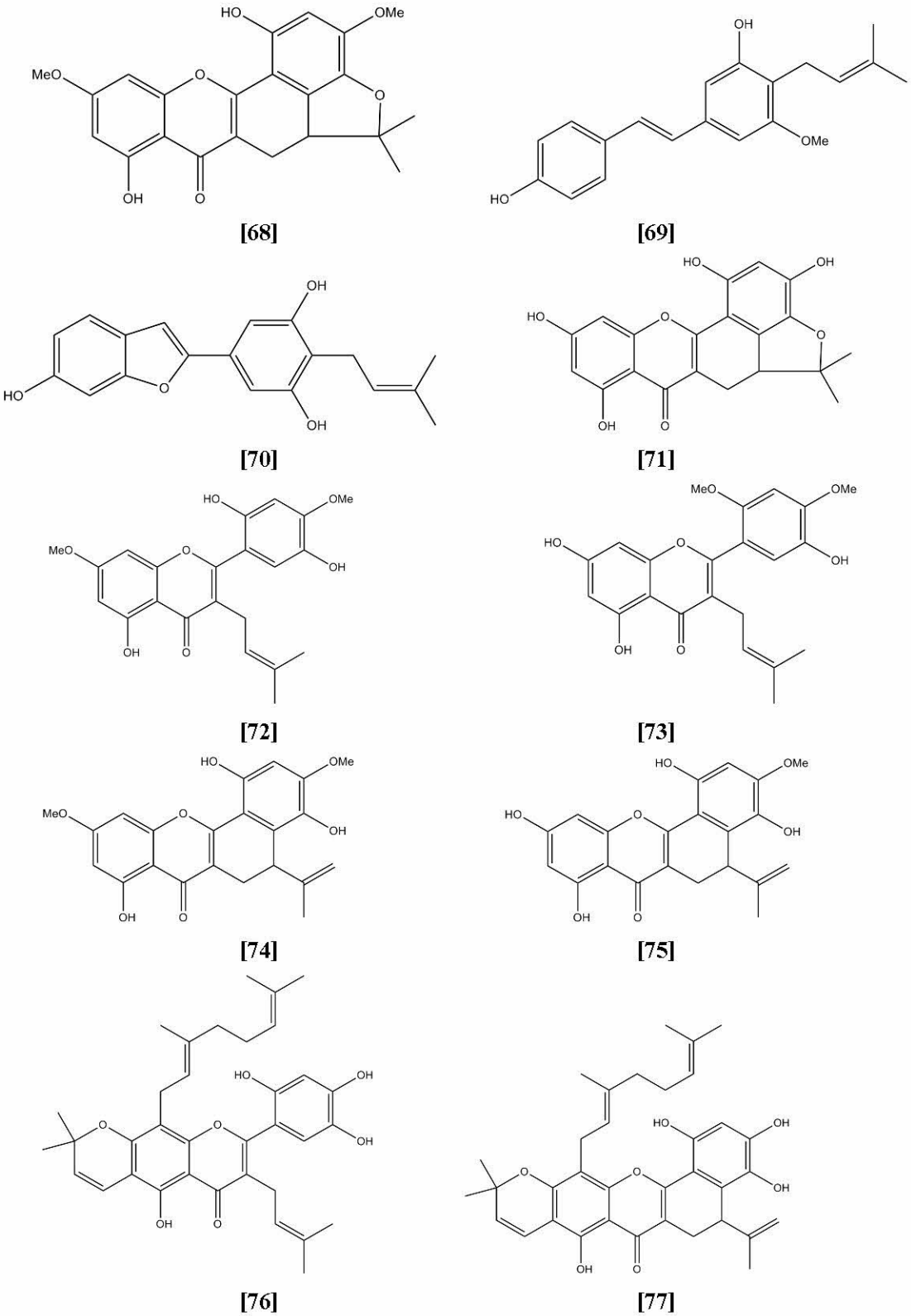


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

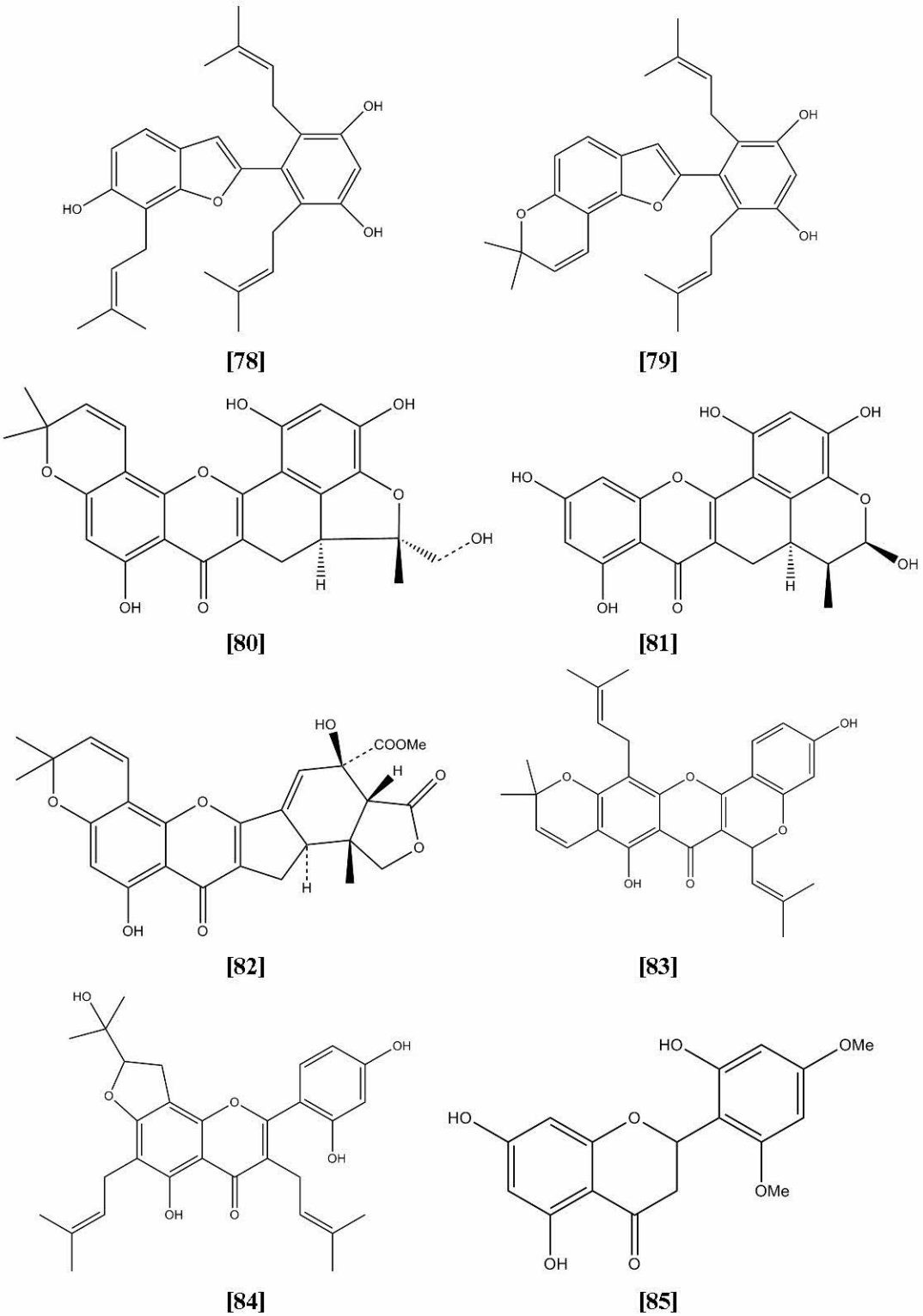


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

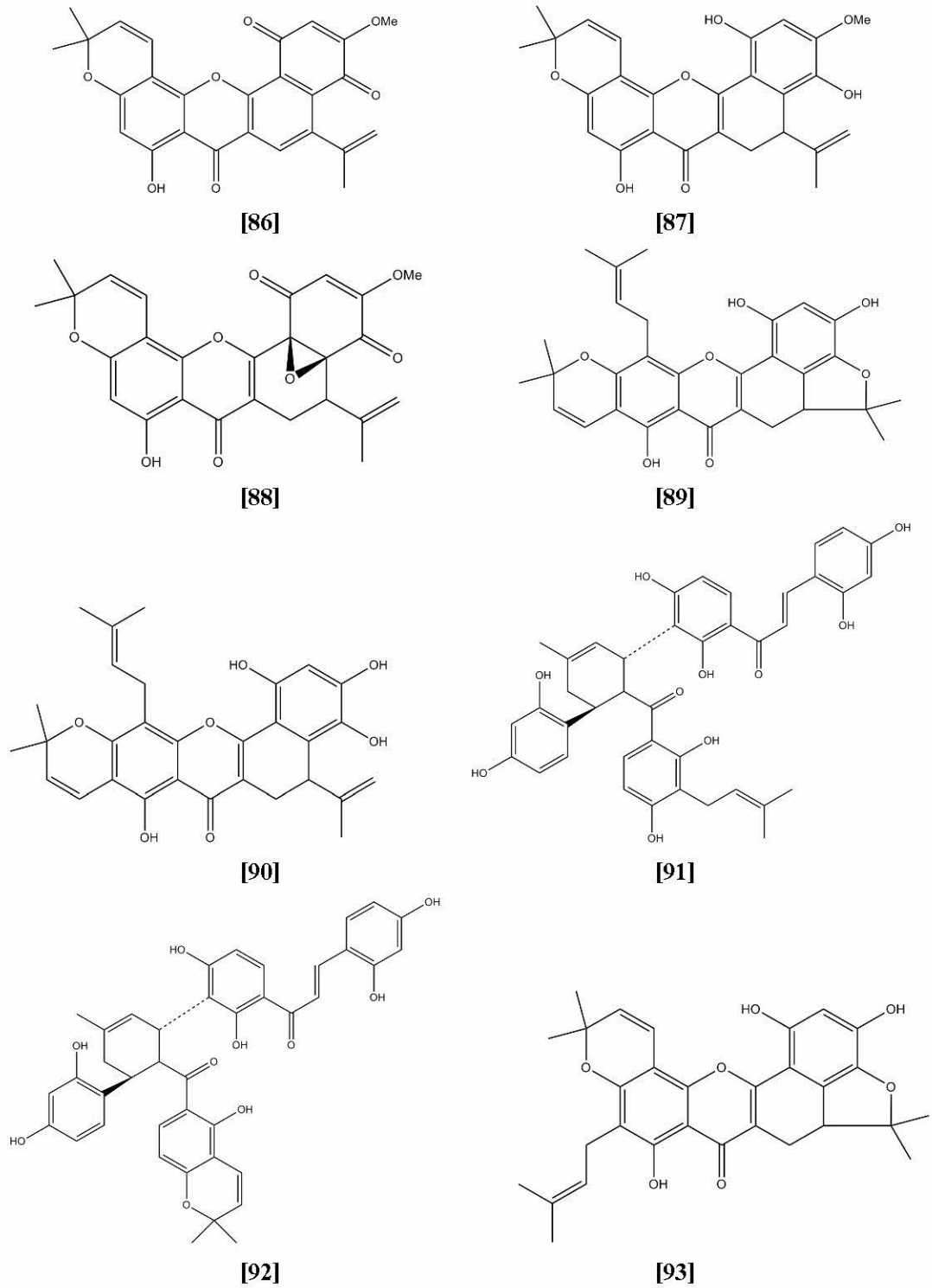


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

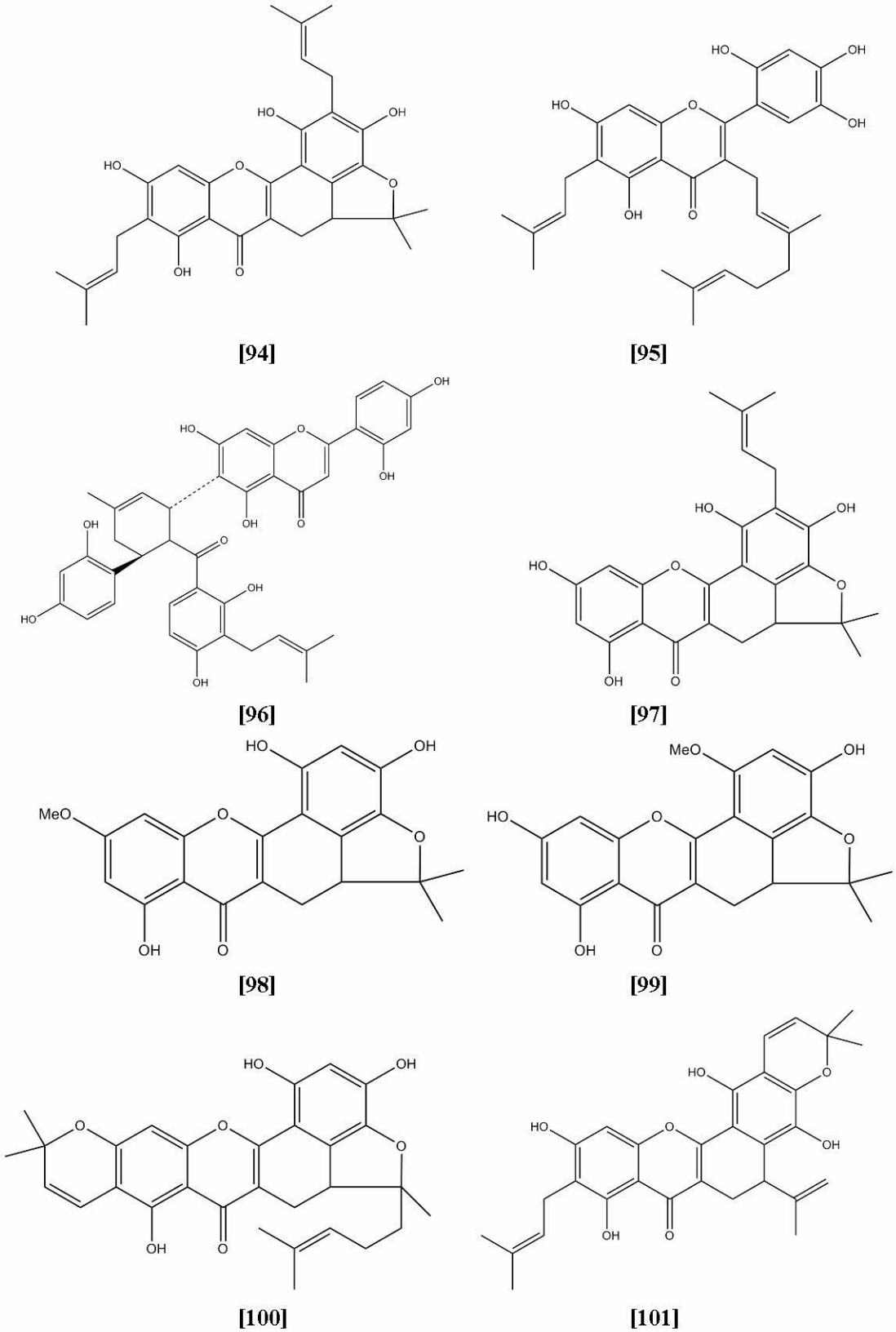
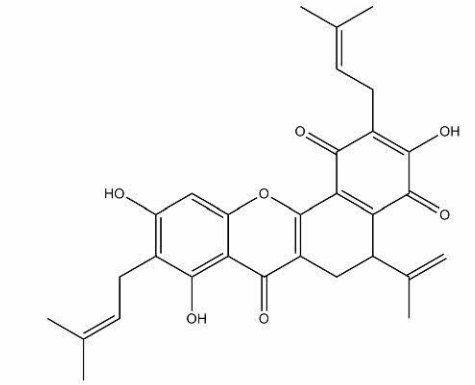
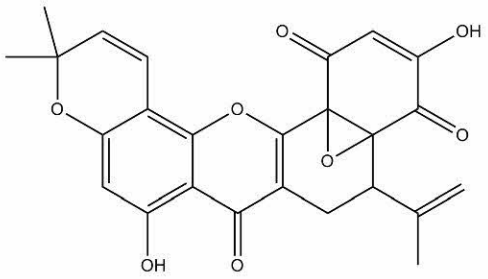


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

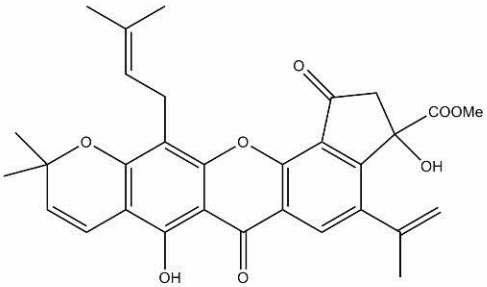
Numbers of these structures are related to table 2.3 (continued)



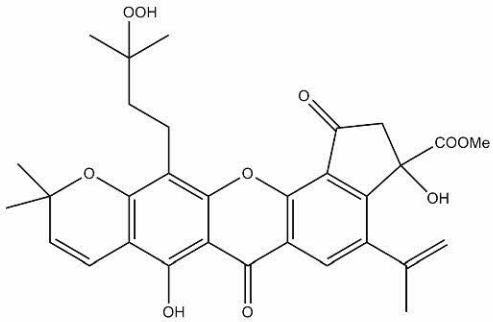
[102]



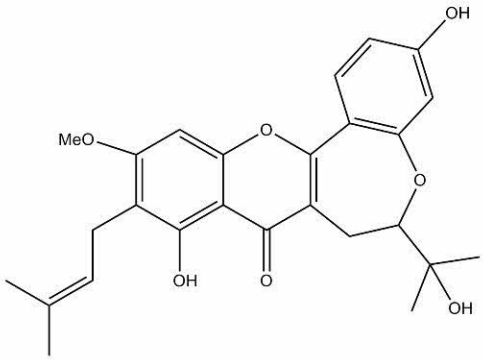
[103]



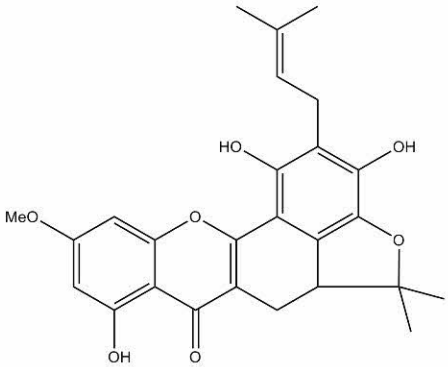
[104]



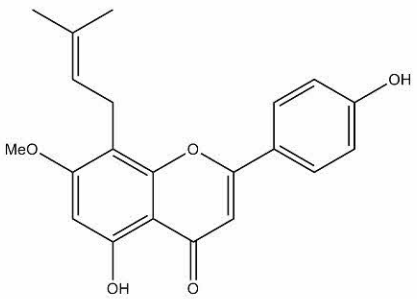
[105]



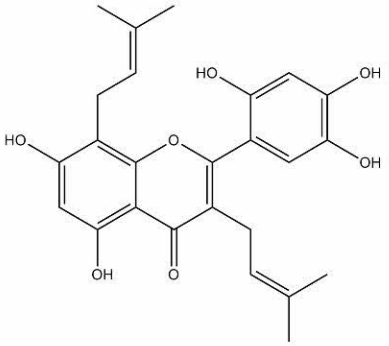
[106]



[107]



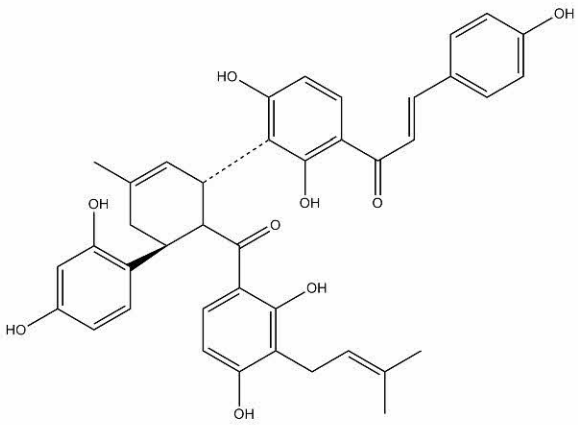
[108]



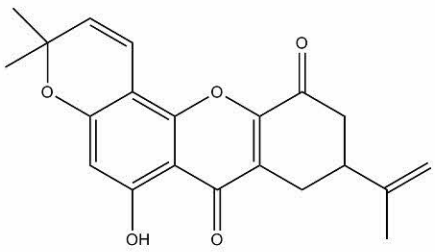
[109]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

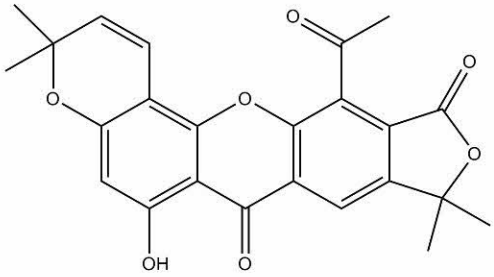
Numbers of these structures are related to table 2.3 (continued)



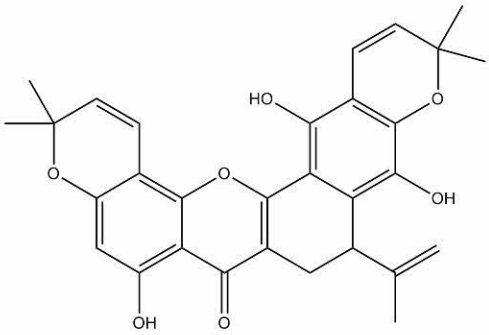
[110]



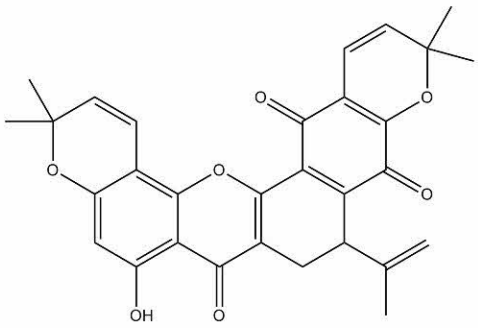
[111]



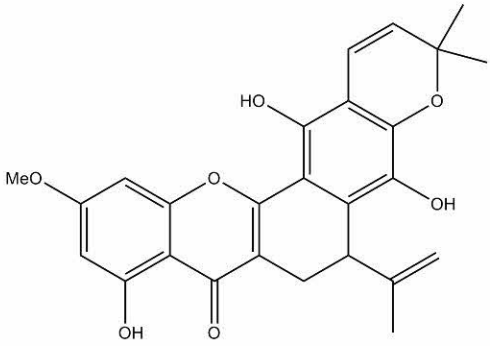
[112]



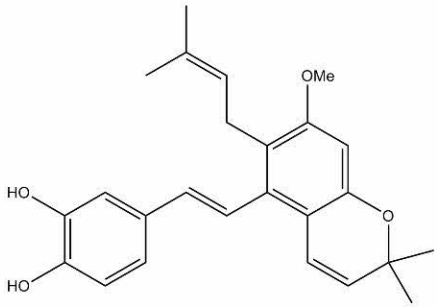
[113]



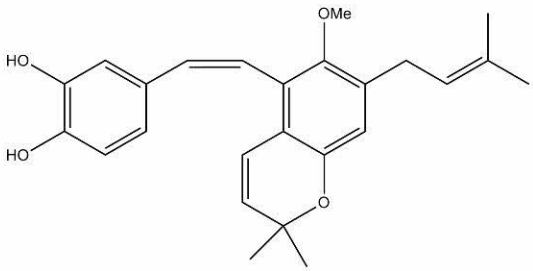
[114]



[115]



[116]



[117]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

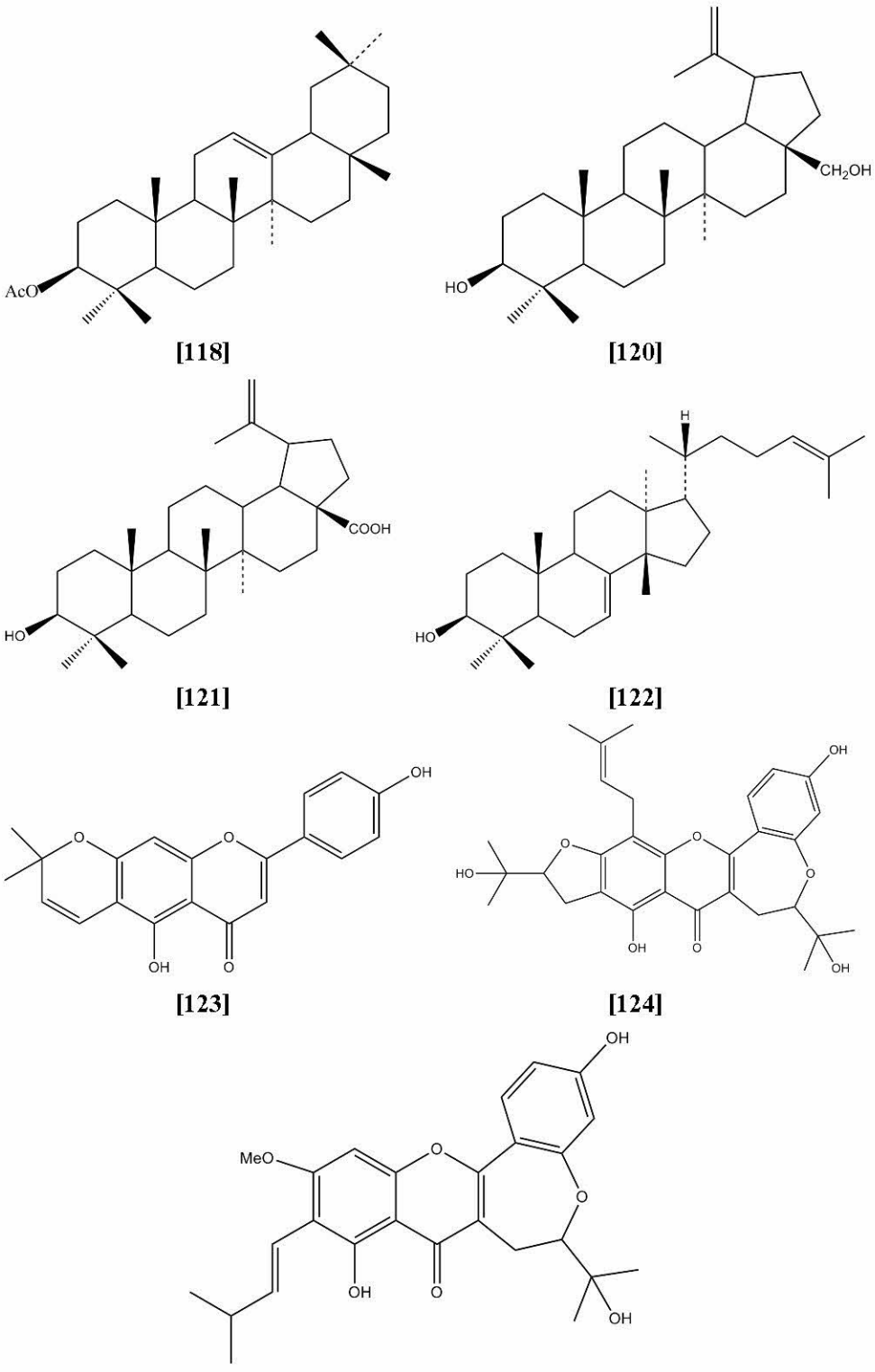


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

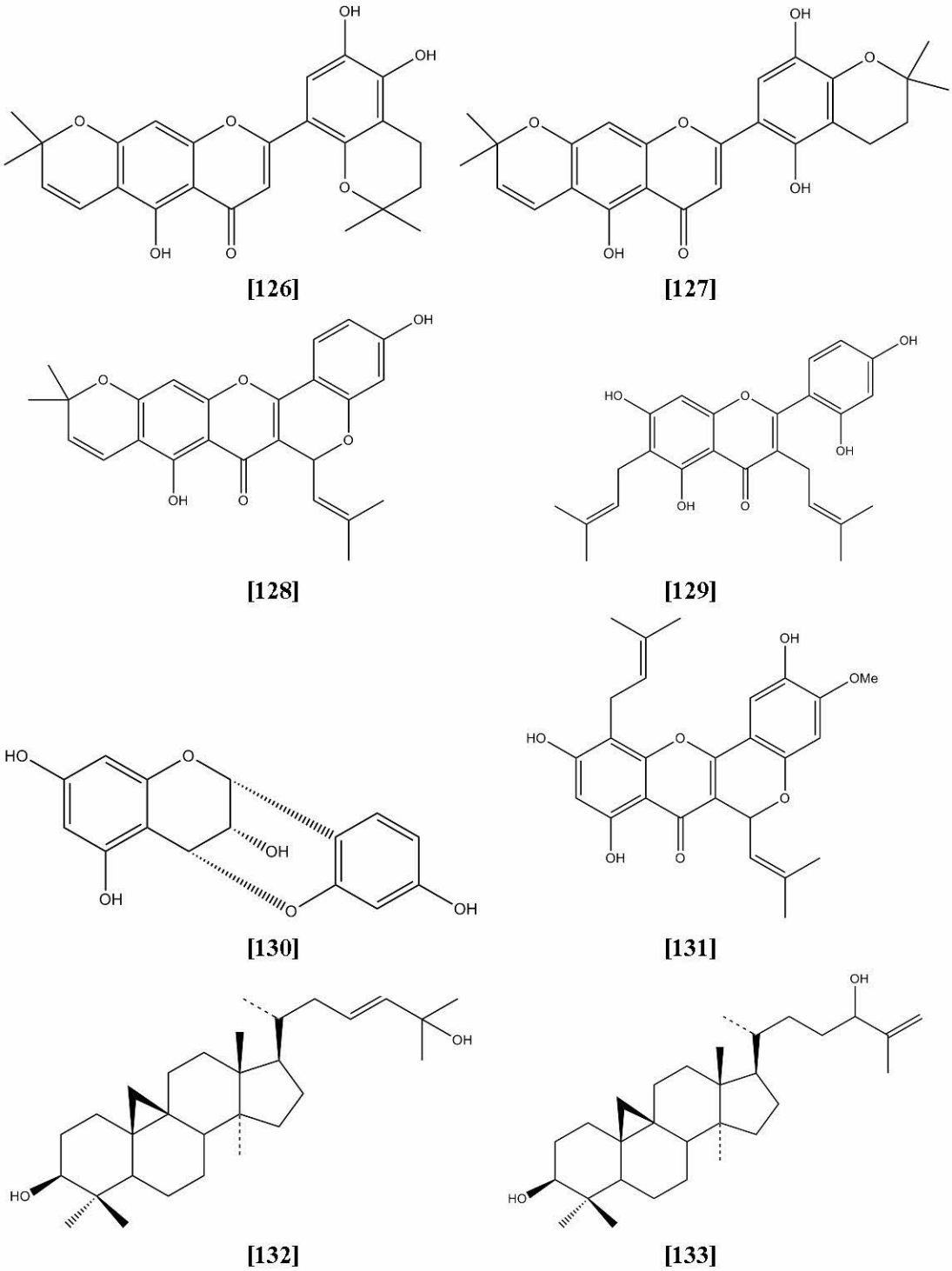


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

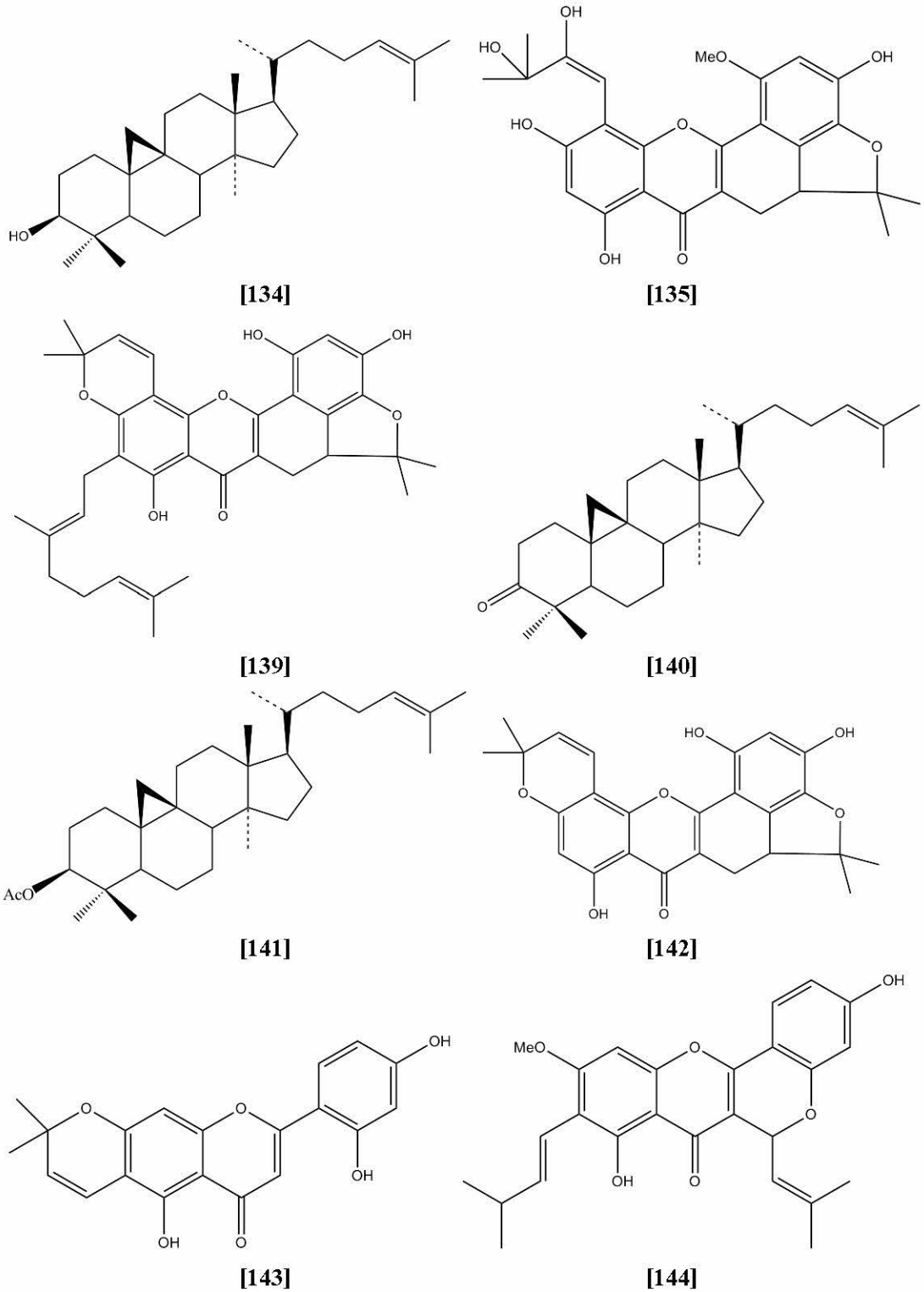


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

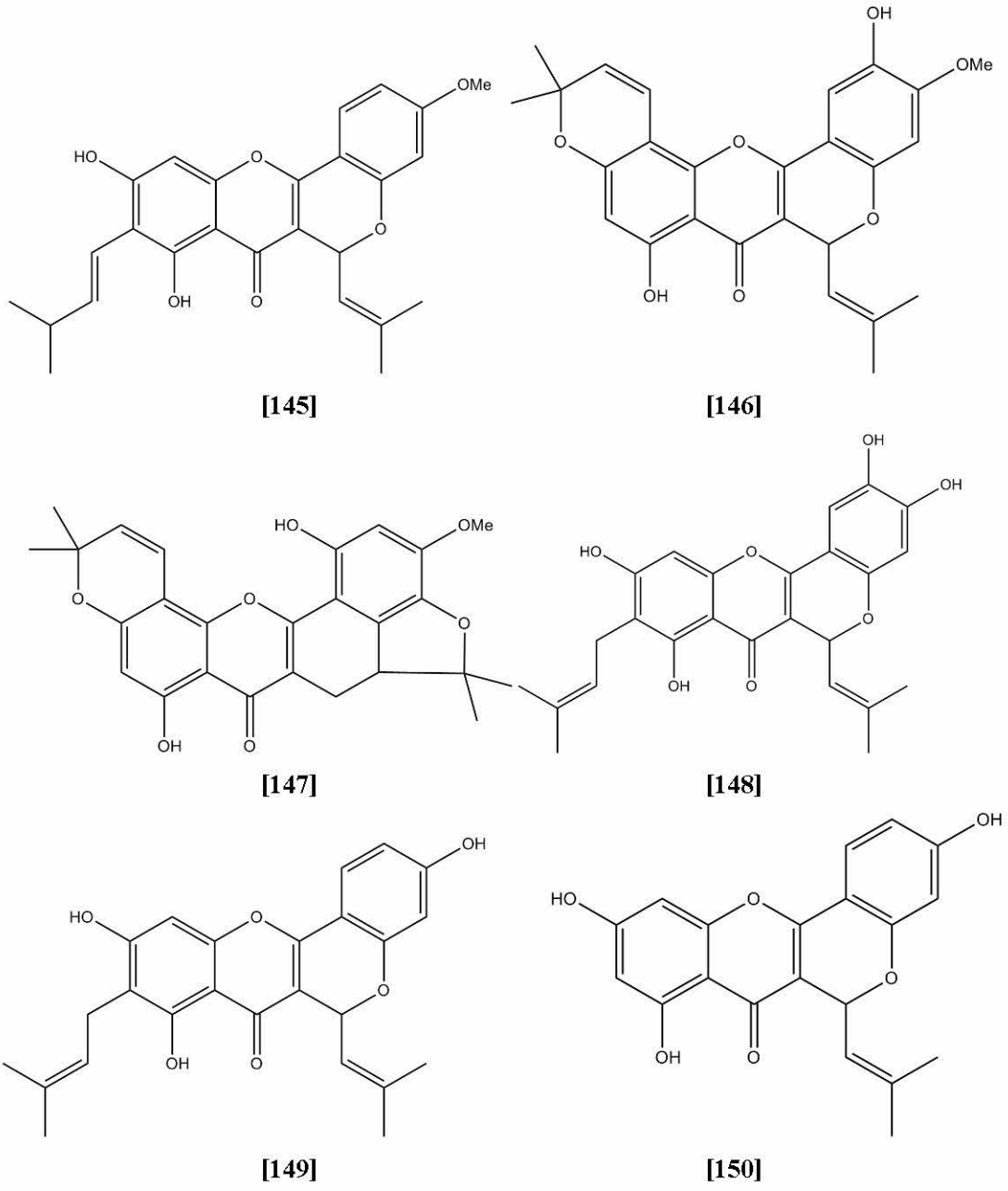


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

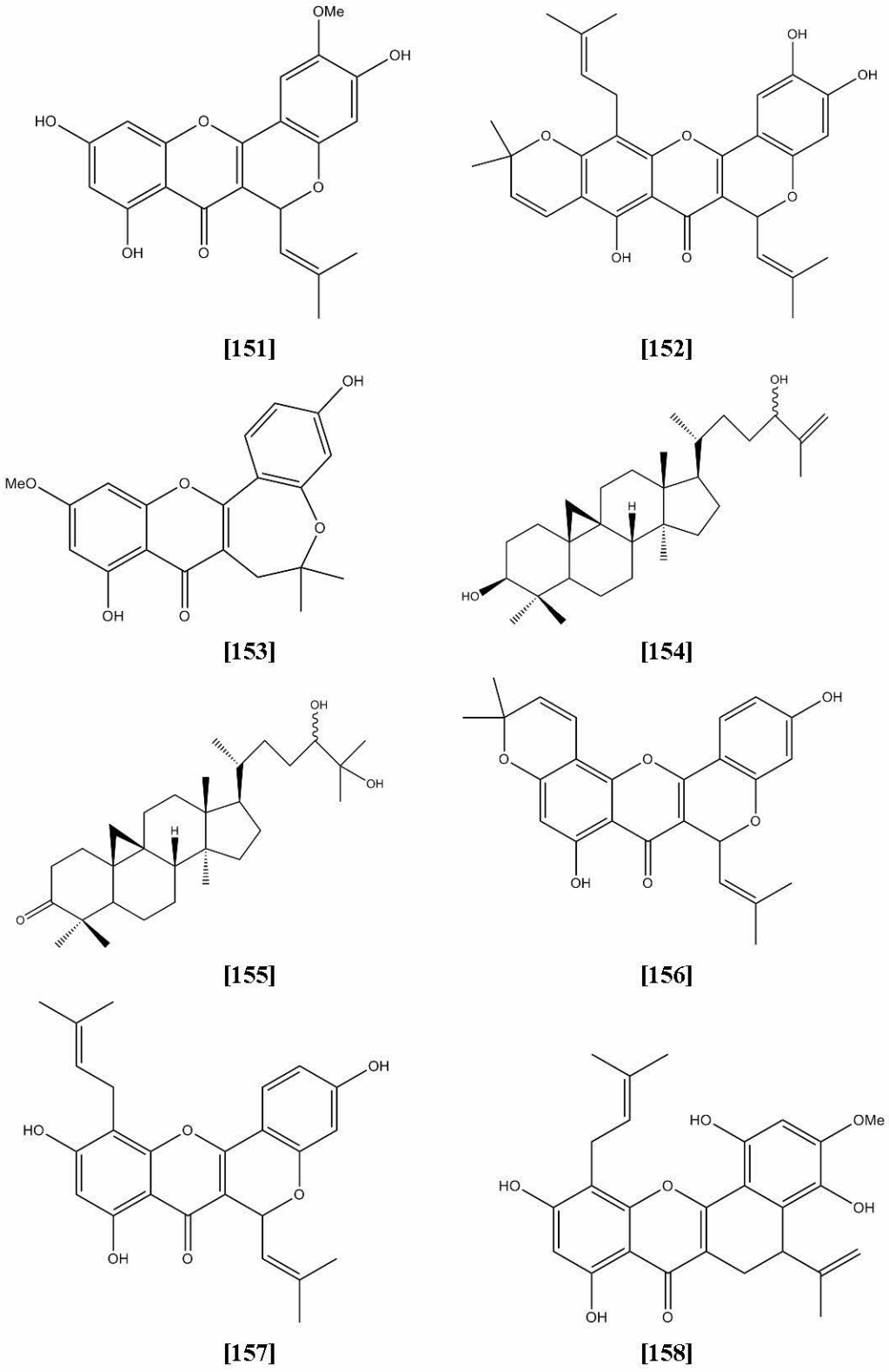
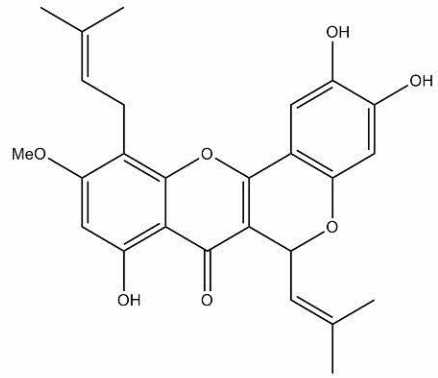
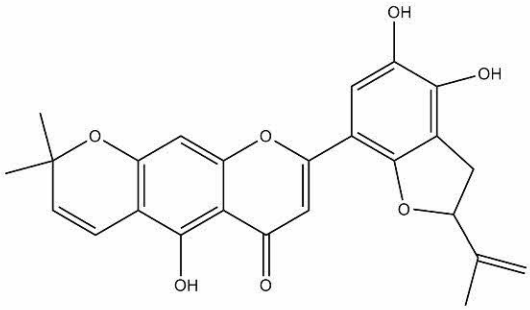


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

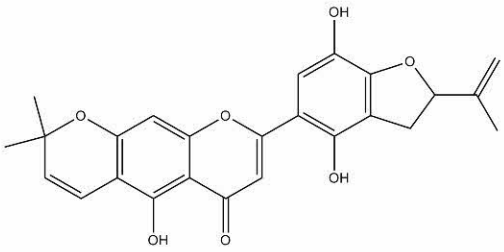
Numbers of these structures are related to table 2.3 (continued)



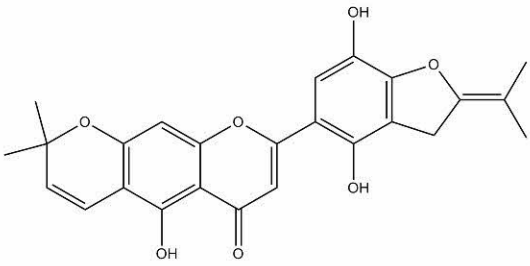
[159]



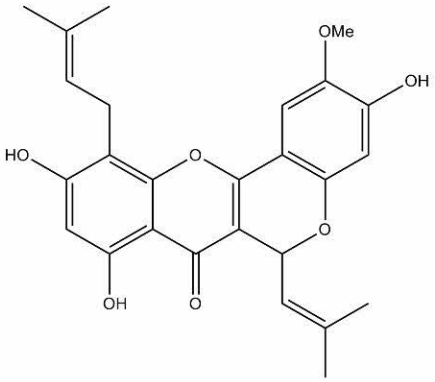
[160]



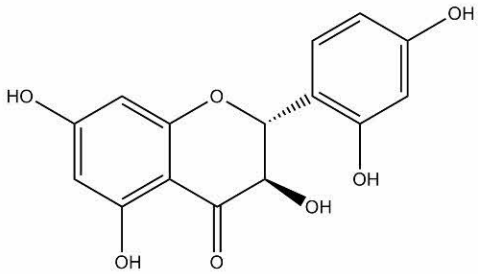
[161]



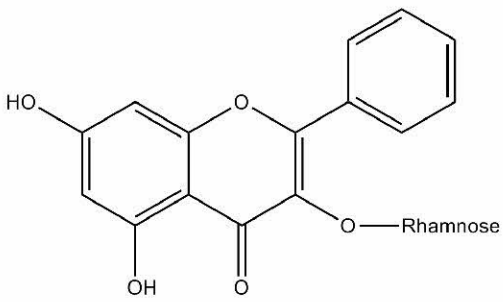
[162]



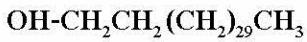
[163]



[164]



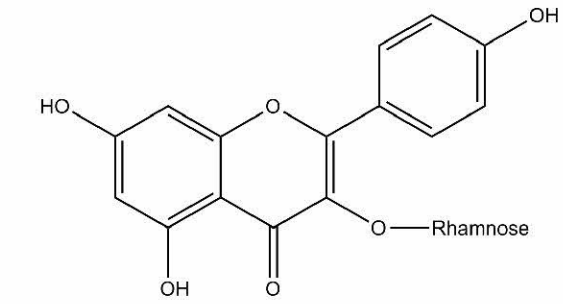
[165]



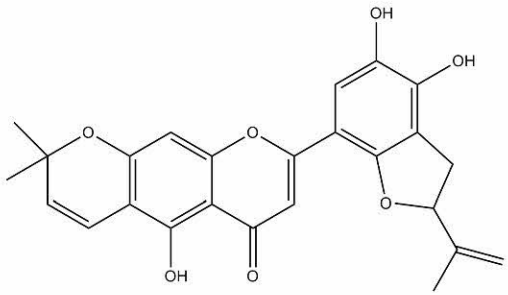
[166]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

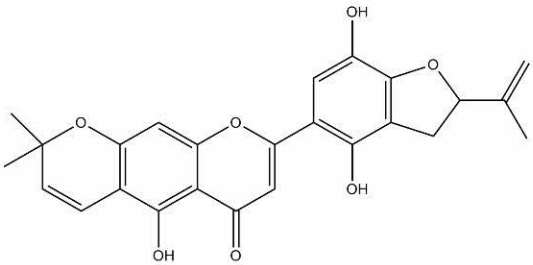
Numbers of these structures are related to table 2.3 (continued)



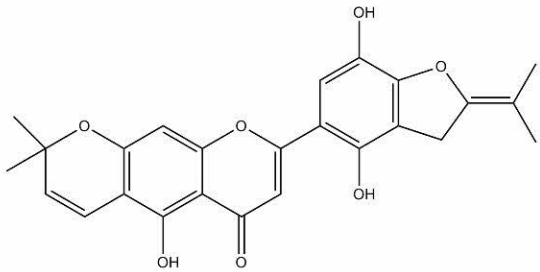
[167]



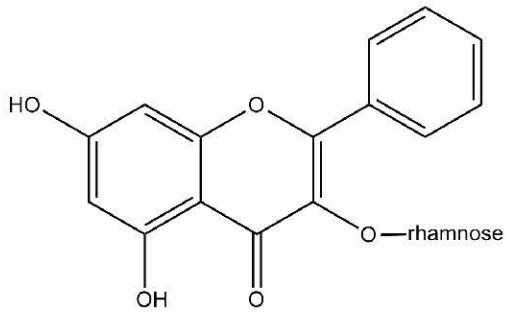
[168]



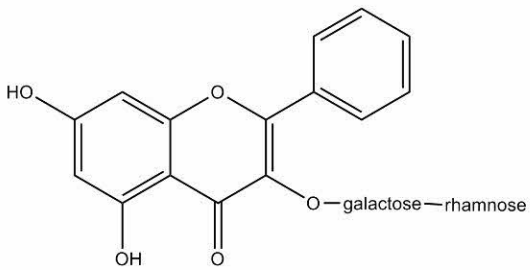
[169]



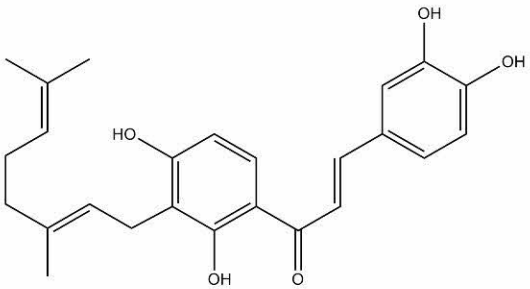
[170]



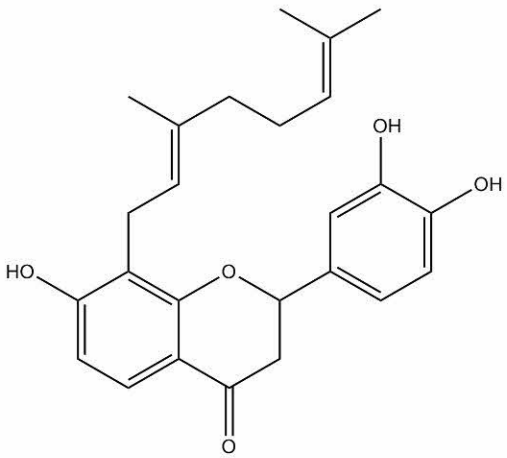
[171]



[172]



[173]



[174]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

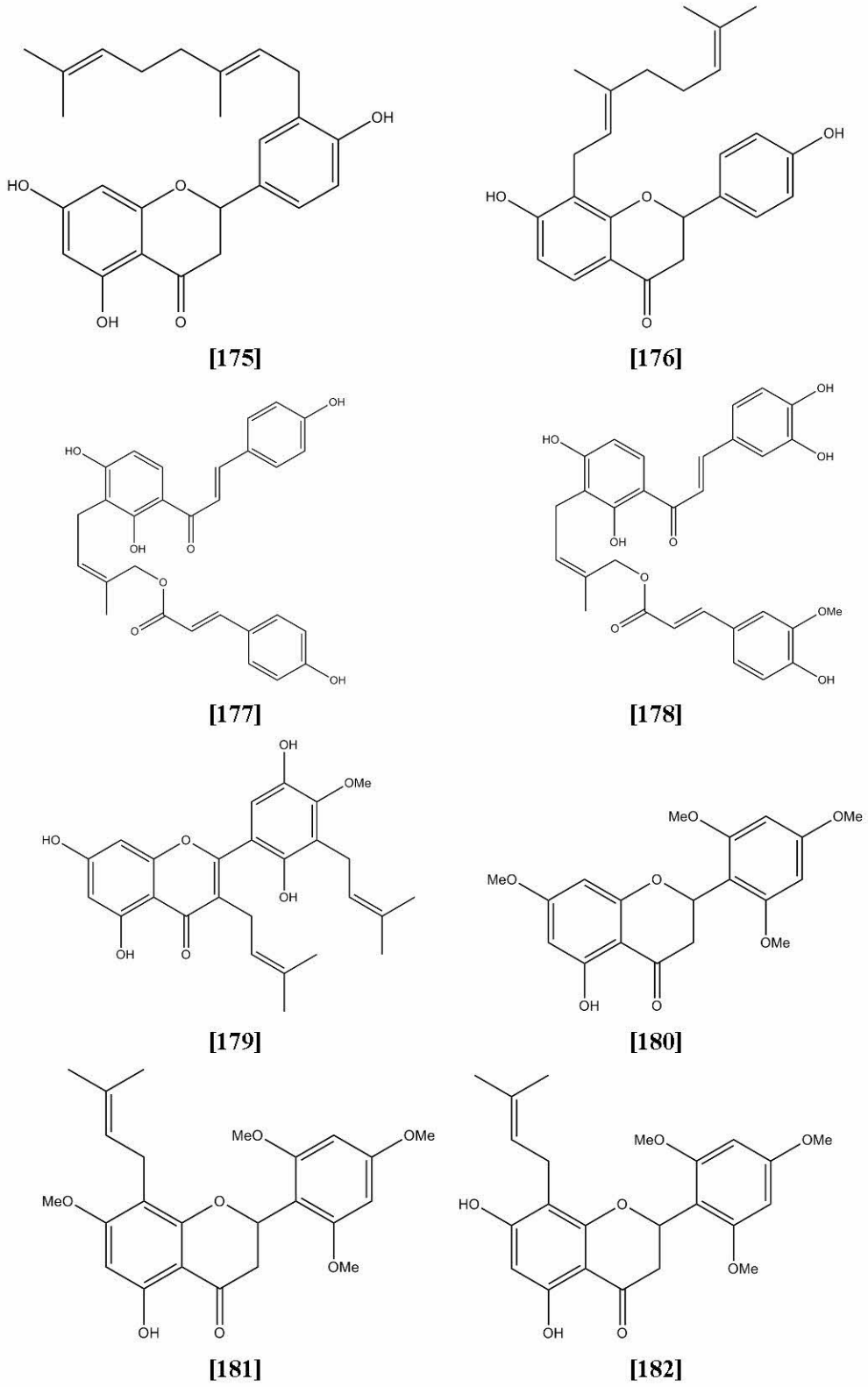
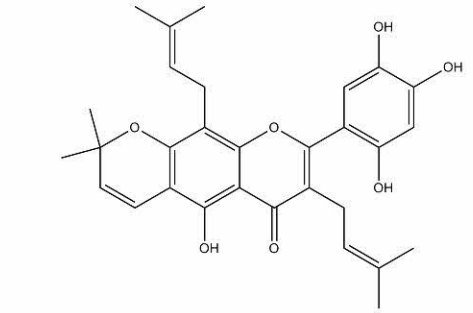
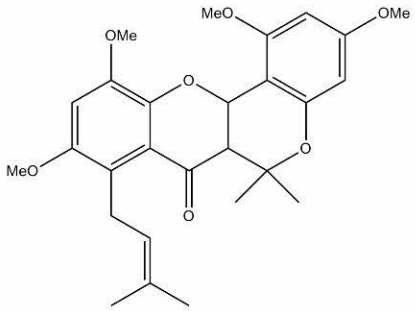


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

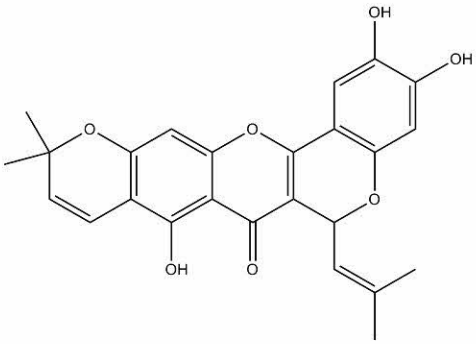
Numbers of these structures are related to table 2.3 (continued)



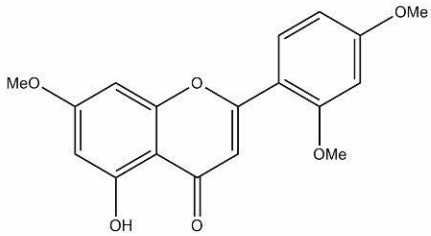
[183]



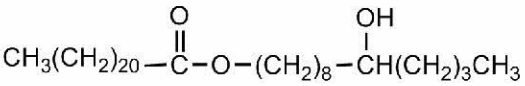
[184]



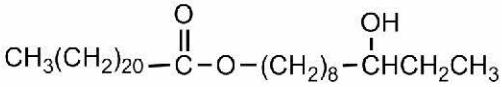
[185]



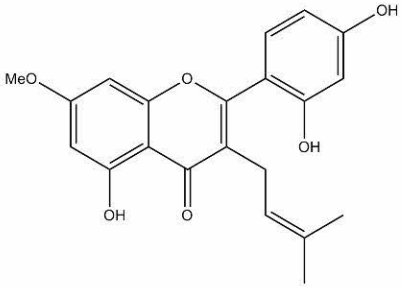
[186]



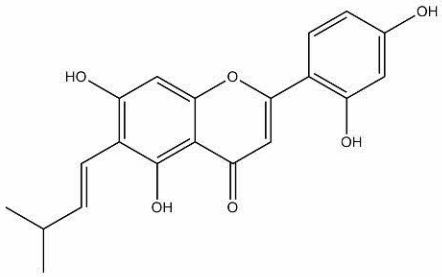
[187]



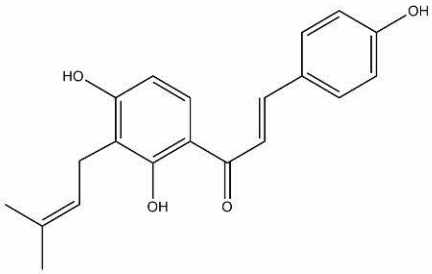
[188]



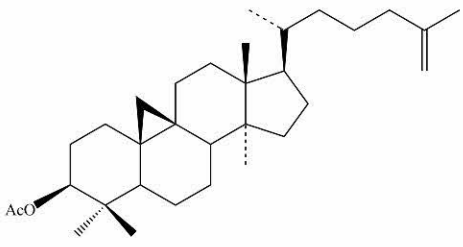
[189]



[190]



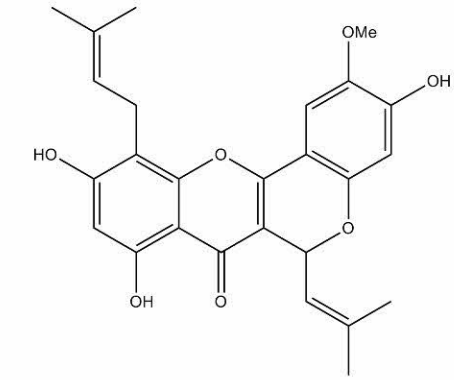
[191]



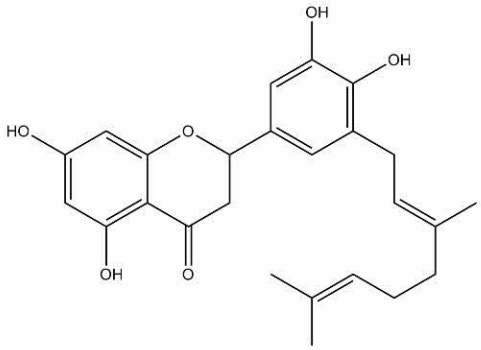
[192]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

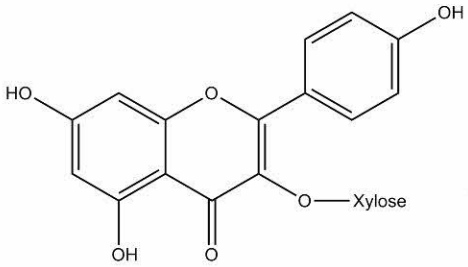
Numbers of these structures are related to table 2.3 (continued)



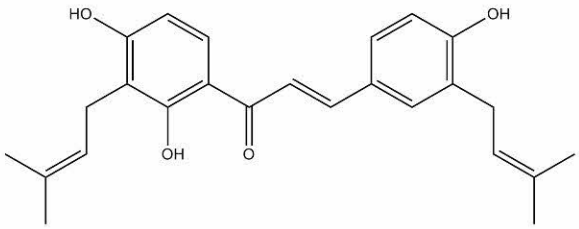
[193]



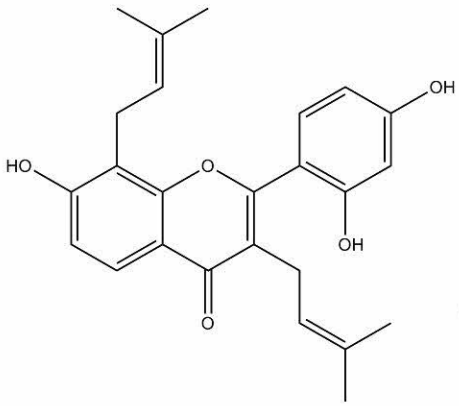
[194]



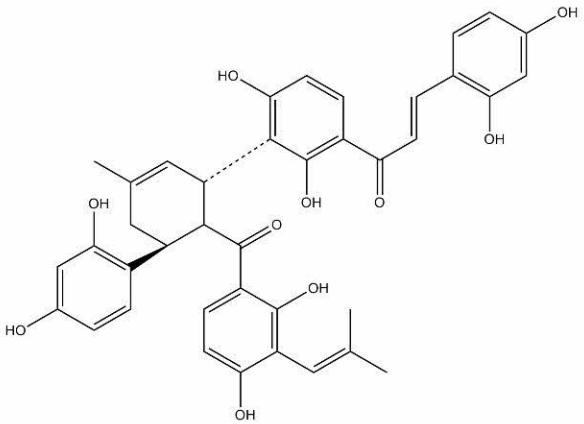
[195]



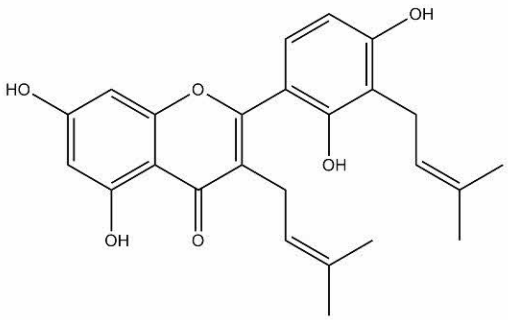
[196]



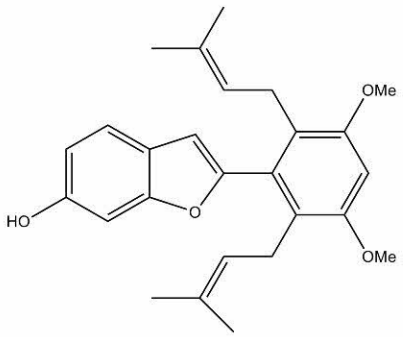
[197]



[198]



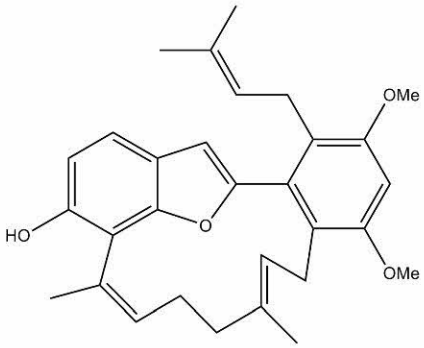
[199]



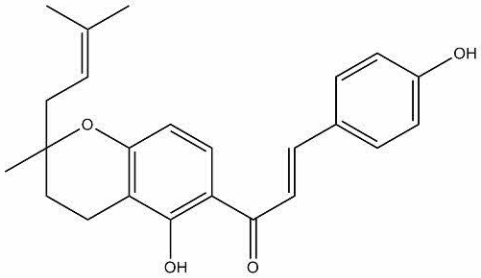
[200]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

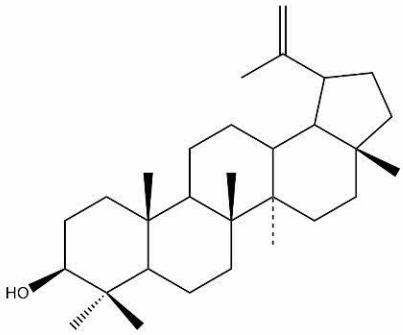
Numbers of these structures are related to table 2.3 (continued)



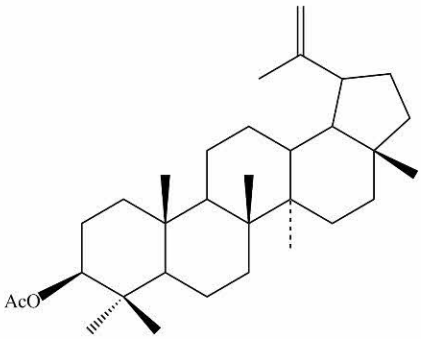
[201]



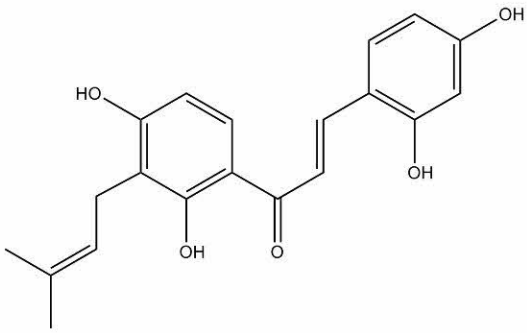
[202]



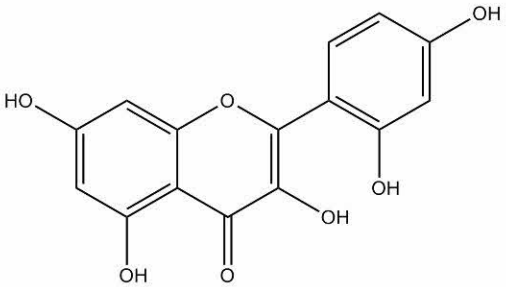
[203]



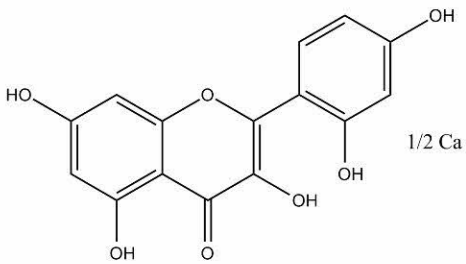
[204]



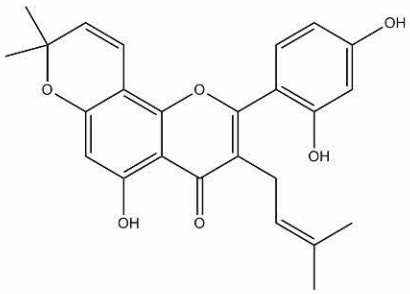
[205]



[206]



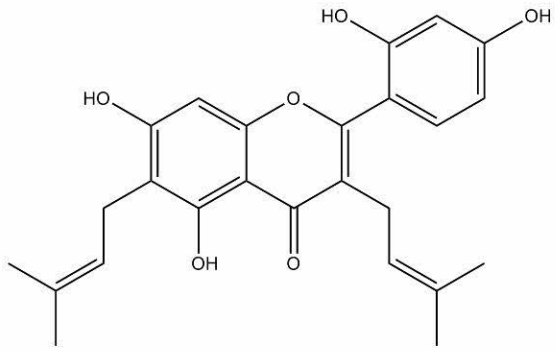
[207]



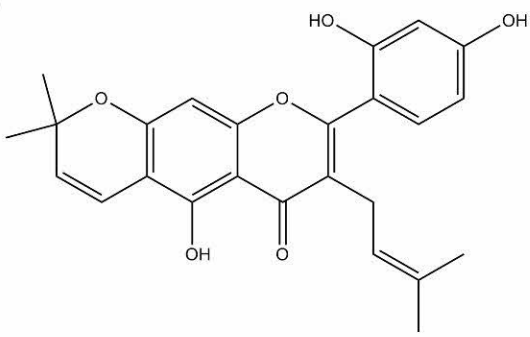
[208]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

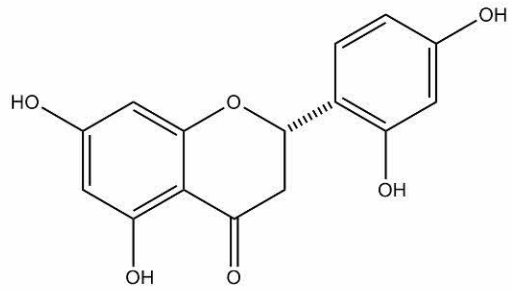
Numbers of these structures are related to table 2.3 (continued)



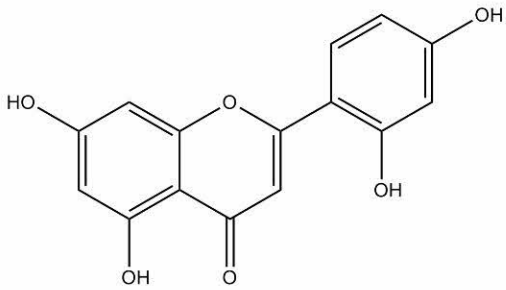
[209]



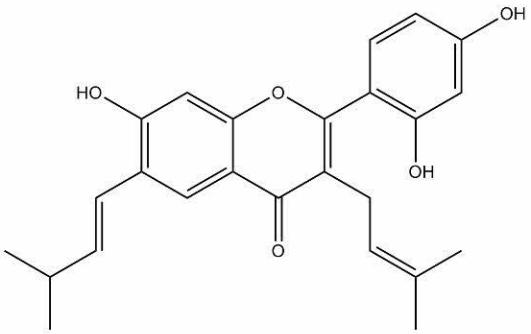
[210]



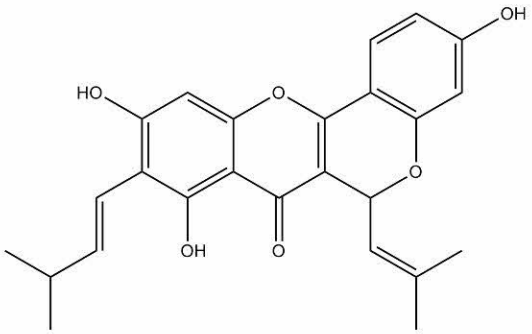
[211]



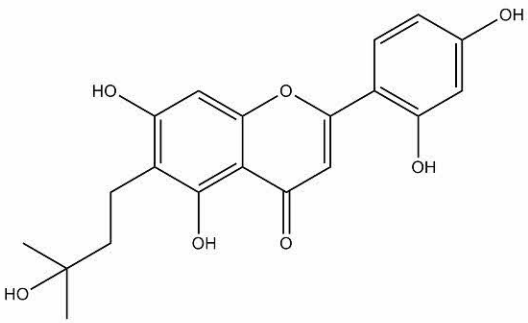
[212]



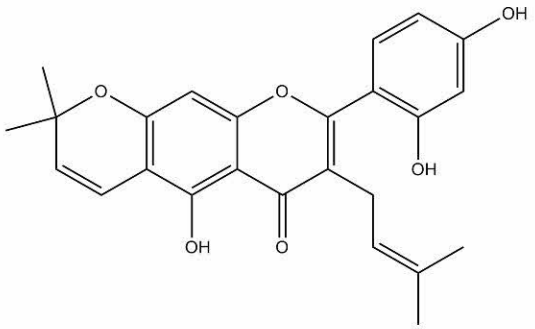
[213]



[214]



[215]



[216]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

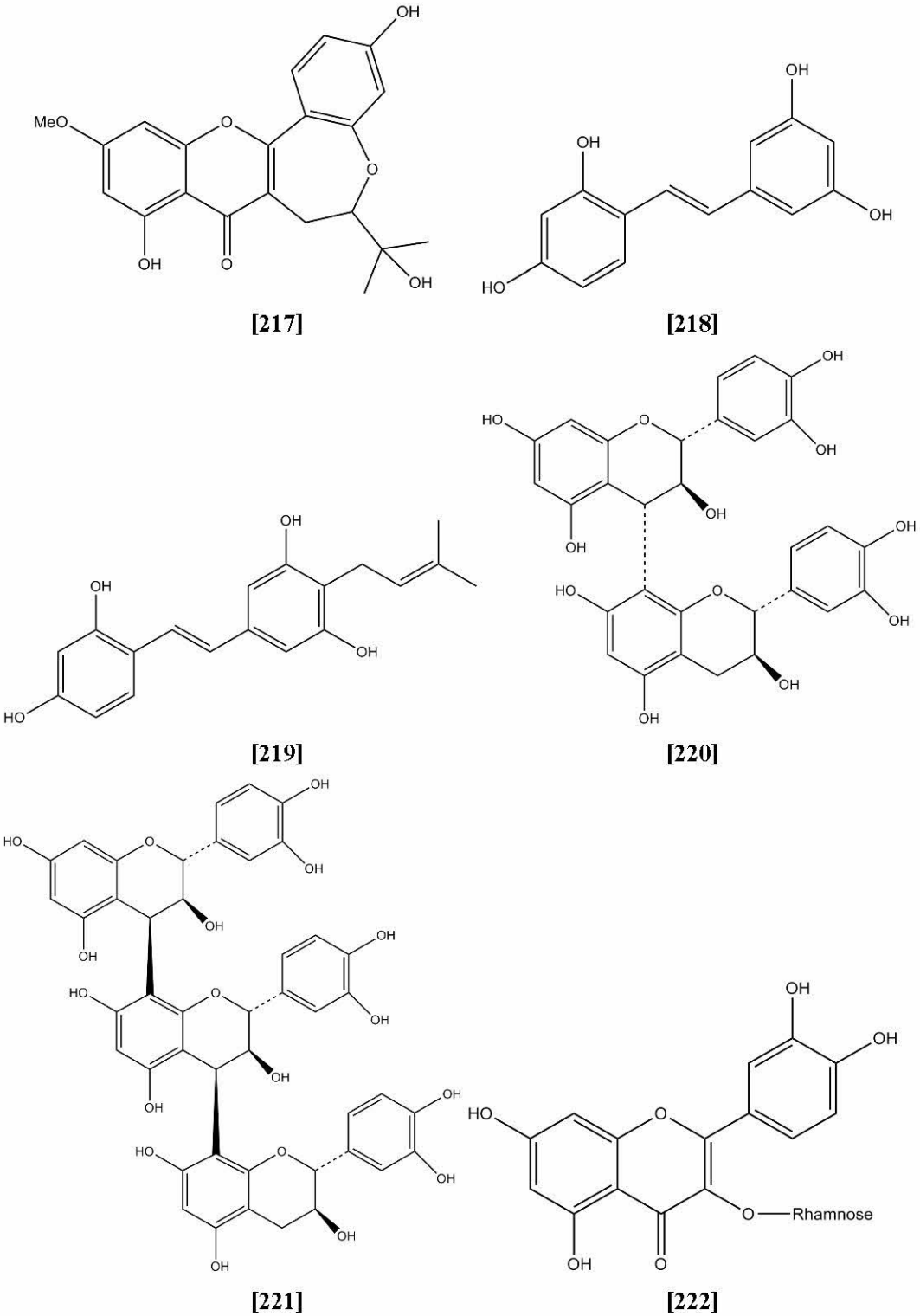


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

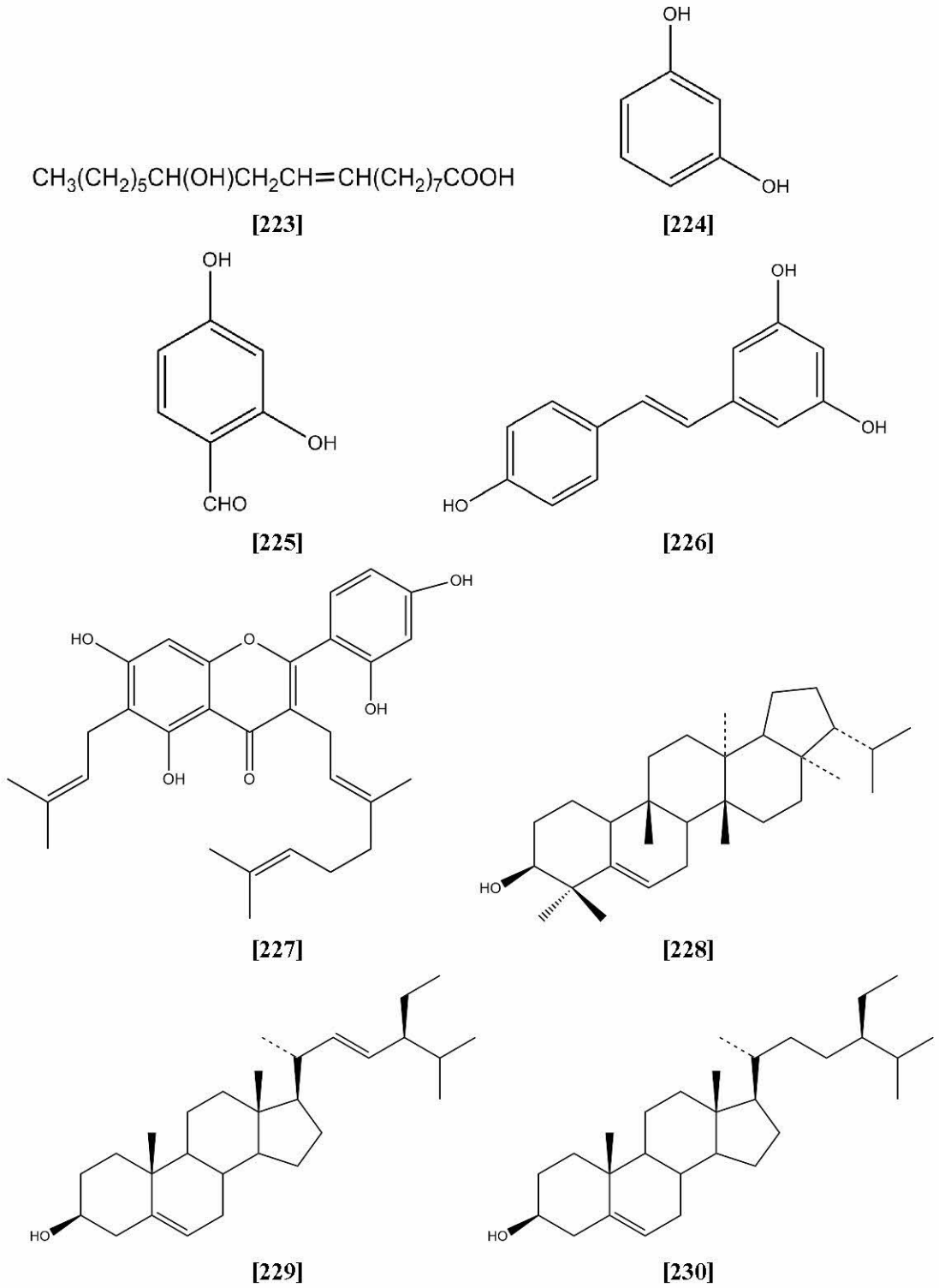
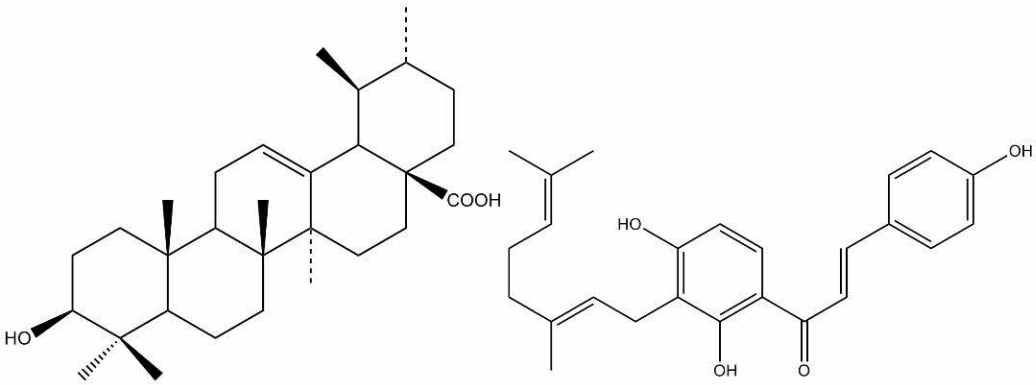
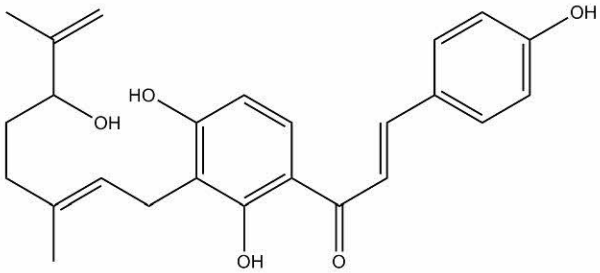


Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)



[231] [232]



[233]

Figure 2.1 Structure of compounds previously isolated from *Artocarpus* spp.

Numbers of these structures are related to table 2.3 (continued)

CHAPTER 3

EXPERIMENTAL

3.1 Material

3.1.1 Plant material

Seventy seven samples of Thai medicinal plants were selected in order to prepare the crude extract for screening of anti-tyrosinase activity as shown in table 3.1. 21 samples are from Moraceae family because many previously reports of the plants in this family have shown high potential anti-tyrosinase activity (Sritularak ***et al.***, 1998a, Likhitwitayawuid and Sritularak, 2001, Lee ***et al.***, 2002, Arung ***et al.***, 2006).

Table 3.1 Seventy seven samples from Thai medicinal plants tested for anti-tyroisnase and anti-microbial activities determination

No.	Family	Scientific name	Thai name	Plant part
1	Acanthaceae	***Barleria lupulina***	เสลดพังพอน	Leaf
2	Acanthaceae	***Andrographis paniculata***	ฟ้าทะลายโจร	Leaf
3	Acanthaceae	***Clinacanthus nutans***	พญาปล้องทอง	Leaf
4	Acanthaceae	***Thunbergia laurifolia***	รางจืด	Leaf
5	Anacardiaceae	***Spondias cytherea***	มะกอกฝรั่ง	Leaf
6	Annonaceae	***Desmos chinensis***	สายหยุด	Stem bark
7	Annonaceae	***Desmos chinensis***	สายหยุด	Leaf
8	Averrhoaceae	***Averrhoa bilimbi***	ตะลิงปลิง	Fruit
9	Averrhoaceae	***Averrhoa bilimbi***	ตะลิงปลิง	Juice
10	Capparidaceae	***Crateva magna***	กุ่มน้ำ	Branch
11	Capparidaceae	***Cleoma viscose***	ผักเสี้ยนผี	Leaf

Table 3.1 Seventy seven samples from Thai medicinal plants tested for anti-tyroisnase and anti-microbial activities determination (continued)

No.	Family	Scientific name	Thai name	Plant part
12	Caesalpiniaceae	*Caesalpinia sappan*	ฝาง	Heartwood
13	Compositae	*Chromolaena odoratum*	สาบเสือ	Leaf
14	Compositae	*Saussurea lappa*	โกฐกระดูก	Rhizome
15	Combretaceae	*Combretum quadrangulare*	สะแกนา	Leaf
16	Euphorbiaceae	*Antidesma sp.*	มะเม่า	Leaf
17	Euphorbiaceae	*Bridelia ovata*	มะกา	Leaf
18	Euphorbiaceae	*Baccaurea macrophylla*	ลังแข	Fruit
19	Euphorbiaceae	*Euphorbia tirucalli*	พญาไร้ใบ	Aerial
20	Euphorbiaceae	*Phyllanthus acidus*	มะยม	Fruit
21	Euphorbiaceae	*Phyllanthus acidus*	มะยม	Root
22	Euphorbiaceae	*Sauropus changiana*	ลิ้นมังกร	Leaf
23	Guttiferae	*Garcinia cowa*	ชะมวง	Leaf
24	Guttiferae	*Garcinia atroviridis*	ส้มแขก	Leaf
25	Lauraceae	*Cinnamomum iners*	อบเชย	Branch
26	Labiatae	*Ocimum tenuiflorum*	กระเพรา	Leaf
27	Labiatae	*Ocimum americanum*	แมงลัก	Leaf
28	Labiatae	*Ocimum basilicum*	โหระพา	Leaf
29	Leguminosae	*Acacia concinna*	ส้มป่อย	Leaf
30	Lythraceae	*Punica granatum*	ทับทิม	Peel (young)
31	Lythraceae	*Punica granatum*	ทับทิม	Peel (old)
32	Meliaceae	*Melia toosenden*	เลี่ยน	Stem bark
33	Meliaceae	*Aglaia andamanica*	สังกะโต้ง	Leaf
34	Melastomataceae	*Melastoma malabathricum*	โคลงเคลง	Leaf
35	Moraceae	*Artocarpus heterophyllus*	ขนุน	Leaf
36	Moraceae	*Artocarpus altitis*	สาเก	Stem bark
37	Moraceae	*Artocarpus altitis*	สาเก	Leaf

Table 3.1 Seventy seven samples from Thai medicinal plants tested for anti-tyroisnase and anti-microbial activities determination (continued)

No.	Family	Scientific name	Thai name	Plant part
38	Moraceae	*Artocarpus altitis*	สาเก	Heartwood
39	Moraceae	*Artocarpus integer*	จำปาดะ	Root
40	Moraceae	*Artocarpus integer*	จำปาดะ	Wood
41	Moraceae	*Artocarpus integer*	จำปาดะ	Root bark
42	Moraceae	*Artocarpus integer*	จำปาดะ	Bark
43	Moraceae	*Streblus asper*	ข่อย	Leaf
44	Moraceae	*Streblus asper*	ข่อย	Branch
45	Moraceae	*Streblus asper*	ข่อย	Heartwood
46	Moraceae	*Streblus asper*	ข่อย	Stem bark
47	Moraceae	*Ficus benjamina*	ไทร	Leaf
48	Moraceae	*Ficus benjamina*	ไทร	Branch
49	Moraceae	*Ficus benjamina*	ไทร	Wood
50	Moraceae	*Ficus racemosa*	มะเดื่ออุทุมพร	Wood
51	Moraceae	*Ficus religiosa*	โพธิ์	Leaf
52	Moraceae	*Ficus religiosa*	โพธิ์	Branch
53	Moraceae	*Ficus religiosa*	โพธิ์	Stem bark
54	Moraceae	*Ficus religiosa*	โพธิ์	Wood
55	Moraceae	*Cudrania javanensis*	แกแล	Wood
56	Malvaceae	*Gossypium arboretum*	ฝ้ายแดง	Aerial
57	Mimosaceae	*Albizia procera*	ทิ้งถ่อน	Stem bark
58	Mimosaceae	*Mimosa* sp.	ไมยราบ (ลำต้นแดง)	Aerial
59	Mimosaceae	*Mimosa* sp.	ไมยราบ (ลำต้นแดง)	Flower
60	Piperaceae	*Piper nigrum*	พริกไท	Leaf
61	Piperaceae	*Piper chaba*	ดีปลี	Leaf
62	Rubiaceae	*Hydnophytum formicarum*	หัวร้อยรู	Rhizome
63	Rubiaceae	*Hydnophytum formicarum*	หัวร้อยรู	Rhizome

Table 3.1 Seventy seven samples from Thai medicinal plants tested for anti-tyroisnase and anti-microbial activities determination (continued)

No.	Family	Scientific name	Thai name	Plant part
64	Rutaceae	*Citrus ichanqensis*	มะส้าน	Peel
65	Saururaceae	*Houttuynia cordata*	พลูคาว	Aerial
66	Sapindaceae	*Nephelium lappaceum*	เงาะ (โรงเรียน)	Peel
67	Sapindaceae	*Cardiospermum halicacabum*	โคกกะออม	Stem
68	Salvadoraceae	*Azima sarmentosa*	หนามพุงดอ	Root
69	Solanaceae	*Solanum ferox*	มะอึก	Branch
70	Solanaceae	*Capsicum frutescens*	พริก	Fruit
71	Simaroubaceae	*Eurycoma longifolia*	ปลาไหลเผือก	Root
72	Taccaceae	*Tacca leontopetaloides*	เท้ายายม่อม	Stem
73	Verbenaceae	*Vitex trifolia*	คนทีสอ	Fruit
74	Verbenaceae	*Vitex trifolia*	คนทีสอ	Leaf
75	Verbenaceae	*Vitex* sp.	คนทีสอ (แดง)	Leaf
76	Umbelliferae	*Conioselinum univittatum*	โกฐหัวบัว	Rhizome
77	Zingiberaceae	*Languas galangal*	ข่า	Rhizome

3.1.2 Microbial, Media, Antibiotic, Enzyme and Chemical

(1) Microbial

Microbial	Source
- *Staphylococcus aureus* ATCC 25923	- Department of Pathology, Faculty of Medicine, Prince of Songkla University
- *Staphylococcus epidermidis* TISTR 517	- Thailand Institute of Scientific and Technology Research
- *Candida albicans* TISTR 5779	- Thailand Institute of Scientific and Technology Research

- *Propionibacterium acnes* DMST 14916	- Department of Medical Science, Ministry of Public Health, Thailand
- *Trichophyton mentagophytes*	- Department of Medical Science, Ministry of Public Health, Thailand
- *Trichophyton rubrum*	- Department of Medical Science, Ministry of Public Health, Thailand

(2) Media

Media	Source
- Mueller Hinton Agar (MHA)	- Difco, Bacto Dickinson and Company, Spark USA.
- Mueller Hinton Broth (MHB)	- Difco, Bacto Dickinson and Company, Spark USA.
- Brain Heart Infusion Broth (BHIB)	- Difco, Bacto Dickinson and Company, Spark USA.
- Sabouraud's Dextrose Agar (SDA)	- Difco, Bacto Dickinson and Company, Spark USA.
- Tryptic Soy Broth (TSB)	- Difco, Bacto Dickinson and Company, Spark USA.

(3) Antibiotic

Antibiotic	Source
- Oxacillin paper disc	- Oxoid Limited, England.
- Oxacillin sodium salt	- Fluka, Sigma-Aldrich, China
- Amphotericin B	- Sigma, Sigma-Aldrich, Germany
- Ketoconazole	- Sigma, Sigma-Aldrich, Germany

(4) Enzyme

Enzyme	Source
- Tyrosinase enzyme	- Sigma, Sigma-Aldrich, Germany

(5) Chemical

Chemical

- $NaH_2PO_2.2H_2O$

- Na_2HPO_4

- Kojic acid
- Dimethyl sulfoxide (DMSO)

Source

- MAY & BAKER Limited Dagenham England
- MAY & BAKER Limited Dagenham England
- Sigma-Aldrich, Germany
- Sigma-Aldrich, Germany

All solvents for extraction and isolation of compound were commercial grade and redistilled prior to use.

3.1.4 General techniques and equipments

Analytical Thin-Layer Chromatography (TLC)

Technique	:	One dimension, ascending
Absorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	7x5* cm (*= depend on amount of samples)
Detection	:	Ultraviolet light at the wavelength of 254 and 365 nm

Quick and Flash Column Chromatography

Adsorbent	:	Silica gel 60 particle size 0.040-0.063 nm (230-400 mesh ASTM) (Merck, Germany) and Flash Silica gel particle size 0.040-0.063 nm (Silicycle chemical division, Canada)
Packing method	:	Dry and Wet Packing
Sample loading	:	The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, dried and then placed gently on surface of the adsorbent on column for dry packing and the sample was dissolved in a small amount of eluent and then applied gently on surface of adsorbent in column for wet packing.
Detection	:	Fractions were examined by TLC observing under Ultraviolet light at the wavelengths of 254 and 365 nm.

Ultraviolet (UV) Absorption Spectra

Isolated compound was dissolved in methanol or chloroform before measured Ultraviolet absorption. UV spectra were obtained on a Spectronic Genesys 6 UV-Visible Spectrophotometer, Thermo Scientific, Thermo Electron Corporation (Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University).

Infrared (IR) Absorption Spectra

IR (KBr disc) spectra were obtained from a Perkin Elmer FT-IR Spectrum One spectrometer (Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University).

Mass Spectra (MS)

Electron Impact Mass Spectra (EIMS) were measured on a Thermofinnigan MAT 95 XL mass spectrometer, low resolution electron ionization mass spectrometry (Scientific Equipment Center, Prince of Songkla University).

Proton-1 and Carbon-13 Nuclear Magnetic Resonance (^{1}H and ^{13}C-NMR) Spectra

^{1}H and ^{13}C spectra were obtained with a Fourier Transform NMR Spectrometer (^{1}H-NMR 500 MHz and ^{13}C-NMR 125 MHz), model UNITY INNOVA, Varian (Scientific Equipment Center, Prince of Songkla University) was used for identification of M-1, M-2 and M-3, and Bruker Avance DPX-300 FT-NMR spectrometer (^{1}H-NMR 300 MHz and ^{13}C-NMR 75 MHz) (Faculty of Pharmaceutical Sciences, Chulalongkorn University) was used for identification of H-1, H-2 and H-3.

Absorbance of Anti-tyrosinase activity

Absorbance at 492 nm was performed on a DTX 880 Microplate Reader (Multimode Detector) (Pharmaceutical Laboratory Service Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University).

Melting Point

Melting points were obtained on a BUCIH MIA-21 melting point apparatus (Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University).

Optical rotation

Optical rotations were measured on a Perkin Elmer 341 polarimeter (Pharmaceutical Research Intrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

3.2 Methods

3.2.1 Plant extraction

Plant materials were chopped and blended into small pieces. They were extracted repeatedly with ethanol at room temperature 3 days (x3 times). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40 °C to yield the ethanol extract and were kept at 4 °C until examination.

3.2.2 Anti-tyrosinase activity

Anti-tyrosinase activity was performed by using Dopachrome method (Sritularak, 1998a and 1998b). Dopachrome is one intermediate in melanogenesis, this method used the L-Dopa as a substrate. The oxidation of L-Dopa can be to dopachrome, which showed red and detected by visible light at 492 nm.

- Preparation of sample and positive control

1 mg of sample or positive control dissolved in 5 mL of DMSO (concentration is 200 μg/mL), kojic acid and water extract of ***Artocarpus lakoocha*** wood were used to positive control.

- Preparation of phosphate buffer pH 6.8

156 mg of $NaH_2PO_2.2H_2O$ and 142 mg of Na_2HPO_4 were mixed in 100 mL water distillation.

- Preparation of 0.85 mM L-Dopa and 203.3 unit/mL tyrosinase

0.85 mg of L-Dopa dissolved in 5 mL of phosphate buffer pH 6.8.

0.5 mg of tyrosinase enzyme 2033 unit/mg dissolved in 5 mL of phosphate buffer pH 6.8.

This method was tested in 96 well plate and detected with microplate reader at 492 nm. Each well of 96 well plate are including control, blank control, test sample and blank sample.

A. Control	20 μL tyrosinase solution (203.3 unit/mL)
	140 μL phosphate buffer pH 6.8
	20 μL dimethylsulfoxide
B. Blank control	160 μL phosphate buffer pH 6.8
	20 μL dimethylsulfoxide
C. Test sample	20 μL tyrosinase solution (203.3 unit/mL)
	140 μL phosphate buffer pH 6.8
	20 μL sample or standard solution
D. Blank sample	160 μL phosphate buffer pH 6.8
	20 μL sample or standard solution

The solution was mixed and then pre-incubated at 25 °C, 10 min, and 20 μL of 0.85 mM L-Dopa was added in all well then optical density (OD) was detected with microplate reader at 492 nm and incubated at 25 °C, 20 min and OD was detected with microplate reader after that.

The % of tyrosinase inhibition was calculated, from equation as below, the absorbance value before incubation will be subtracted from the absorbance value after incubation then all values instead of equation.

$$\% \text{ tyrosinase inhibitory} = [((A-B) - (C-D)) / (A-B)] \times 100$$

3.2.3 Screening of anti-microbial activity of Thai medicinal plant extracts (follow to Lorian (2005) and Kummee and Intaraksa (2008)) by Agar Disc Diffusion method

3.2.3.1 Preparation of sample and antibiotic

The plant extracts which showed % tyrosinase inhibition more than 50 % were selected for screening of anti-microbial activity. A sterile paper disc (diameter 6 mm) was impregnated with sample (10 µL). The concentration of each sample was 200 mg/mL (2 mg/disc), by dissolving in DMSO. Control disc was similarly prepared by using DMSO as a solvent control. The positive controls (antibiotics) were including 1 µg/disc oxacillin for testing of *S. **aureus**, S. **epidermidis*** and *P. **acnes***, 25 µg/disc Amphotericin B for testing of *C. **albicans*** and 25 µg/disc ketoconazole for testing of *T. **rubrum*** and *T. **mentagrophytes***.

3.2.3.2 Preparation of microbial and testing

Table 3.2 Condition for culturing and testing of each microorganism

Microbial	Temp. (°C)	Time (hr.)	Media
Bacteria			
- *S. aureus*	35-37	18-24	Mueller Hinton Agar (MHA) or Broth (MHB)
- *S. epidermidis*	35-37	18-24	Mueller Hinton Agar (MHA) or Broth (MHB)
- *P. acnes*[A]	35-37	72	Brain Heart Infusion Agar (BHIA) or Broth (BHIB)
Yeast			
- *C. albicans*	35-37	48	Sabouraud's Dextrose Agar (SDA)
Fungi			
- *T. rubrum*	30	7-10 days	Sabouraud's Dextrose Agar (SDA)
- *T. mentagrophytes*	30	7-10 days	Sabouraud's Dextrose Agar (SDA)

A = culturing in anaerobe condition

A bacterium or yeast from stock was streaked and incubated follow under condition in table 3.2. A colony from culture agar plate was taken to suspend in 0.85% NaCl solution. The cell suspension was dilution with 0.85% NaCl solution to achieve optical density (OD) with 0.08-0.1 by spectrophotometer at 625 nm (approximately to 10^8 CFU/mL) for bacterial and 0.11-0.13 by spectrophotometer at 530 nm (approximately to 10^6 CFU/mL) for yeast. A sterile cotton swab was dipped in the inoculum and excess was removed by rotation the swab several times against the inside wall of the tube above the fluid level. The surface of medium agar plate was inoculated by streaking the swab over the surface. Streaking was repeated three times, and each time the plate was rotated 60°. This ensured an even distribution of inoculum. The paper discs of sample, positive control and solvent were placed on the surface of bacterium inoculate follow under condition in table 3.2.

A fungus from stock was cultured and incubated follow under condition in table 3.2. A colony from culture agar slant was taken to suspend in 0.85% NaCl solution. The cell suspension was dilution with 0.85% NaCl solution to achieve optical density (OD) with 0.11-0.13 by spectrophotometer at 530 nm (approximately to 10^6 CFU/mL). The agar medium is maintained

in molten state at 45° C. One mL of inoculum was mixed with medium and added to sterile plate, cool agar medium. The contents are thoroughly mixed and allowed to solidify. The paper discs of sample, positive control and solvent were placed on the surface of fungi inoculate follow under condition in table 3.2.

3.2.3.3 Measurement of the result

The inhibition zone or clear zone diameters were then measured by venires caliper.

3.2.4 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was defined the lowest of compound to inhibit the growth of microorganisms. The samples shown clear zone were selected for determination of minimum inhibitory concentration by Broth micro-dilution method follow to Lorian, 2005, Phongpaichit ***et al.***, 2006 and Niyomkam, 2007.

3.2.4.1 Preparation of microbial for testing

The inoculum was prepared and adjusted, as noted 3.2.3.2, to contain 10^8 CFU/mL, by adjusting the turbidity of 0.85% NaCl to match OD with 0.08-0.1 of spectrophotometer at 625 nm. It was then diluted 1: 100 in MHB for aerobic bacteria and BHIB for anaerobic bacteria to contain 10^6 CFU/mL.

3.2.4.2 Preparation of sample, controls and testing

The sample was dissolved in DMSO and diluted with MHB to the concentration of 4 mg/mL, Oxacillin sodium salt as a positive control, was diluted in sterile water distillation

(water was filtered through 0.45 micron sterile filter paper) to a concentration 256 μg/mL. The growth control is 2% DMSO in media and contamination control is only media.

The test was performed in 96-well plate two-fold dilutions were prepared directly in wells, as follow: 50 μL of the working solution of positive control or sample was added to well 1 and 2 of the dilution series. To each remaining well, 50 μL of MHB or BHIB is added. With a sterile pipette, 50 μL was transferred from well 2 to well 3. After thorough mixing, 50 μL was transferred (with a separate pipette for this and each succeeding transfer) to well 4. This process was continued to the next well until to the last well, from which 50 μL was removed and discarded. The solvent control was added with 50 μL of 2% DMSO. The contamination control and growth control were added with 100 and 50 μL MHB or BHIB, respectively. The stock solution of oxacillin sodium salt was diluted with MHB or BHIB to give the concentration of 128 to 0.0625 μg/mL. The stock solution of the sample and pure compound were diluted with MHB or BHIB to give the concentration of 2000 to 0.9765 μg/mL and 256 to 0.125 μg/mL, respectively. The 50 μL of the adjusted inoculum was added to positive control, sample, and growth control wells. The final concentration of bacteria in each well was 5×10^5 CFU/mL. The cultures were then incubated follow under condition in table 3.2.

3.2.4.3 Measurement of the result

The lowest concentration that turbidity was not observed of microbial was the taken as the MIC, and confirmed with colorimetric method using Alamar blue as indicator, started by adding 5 μL of 1% Alamar blue in sterile water distillation every well and incubated 5-10 hr. The pink color showed growth of microbial, while that still blue color did not show any growth of microbial.

3.2.5 Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was defined as the lowest concentration of compound to kill microorganisms. The incubation mixture that showed positive result of

inhibitory effect (MIC) were streaked on Tryptic Soy Agar (TSA) for aerobic bacteria and BHIA for anaerobic bacterial then incubated follow under condition in table 3.2. The lowest concentration that did not show any growth was taken as the MBC.

3.3 Isolation of pure compounds

Thai medicinal plant, which showed high potential activities of anti-tyrosinase and anti-microbial, was selected for phytochemical investigation. The root of ***Artocarpus integer*** was selected. The dried roots of ***Artocarpus integer*** (2.88 kg) were chopped and blended into small pieces. They were extracted with hexane three times (3 days, each). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40 $^{\circ}$C to yield a hexane extract (9 g, 0.332 % base on dried weight of root).

The marc (after extracted with hexane) was extracted three times with dichloromethane (3 days, each). Removal of organic solvent gave a dichloromethane extract (53.95 g, 1.873 % base on dried weight of root).

The marc (after extracted with hexane and dichloromethane, respectively) was extracted three times with ethyl acetate (3 days, each). Removal of organic solvent gave an ethyl acetate extract (31.80 g, 1.104 % base on dried weight of root).

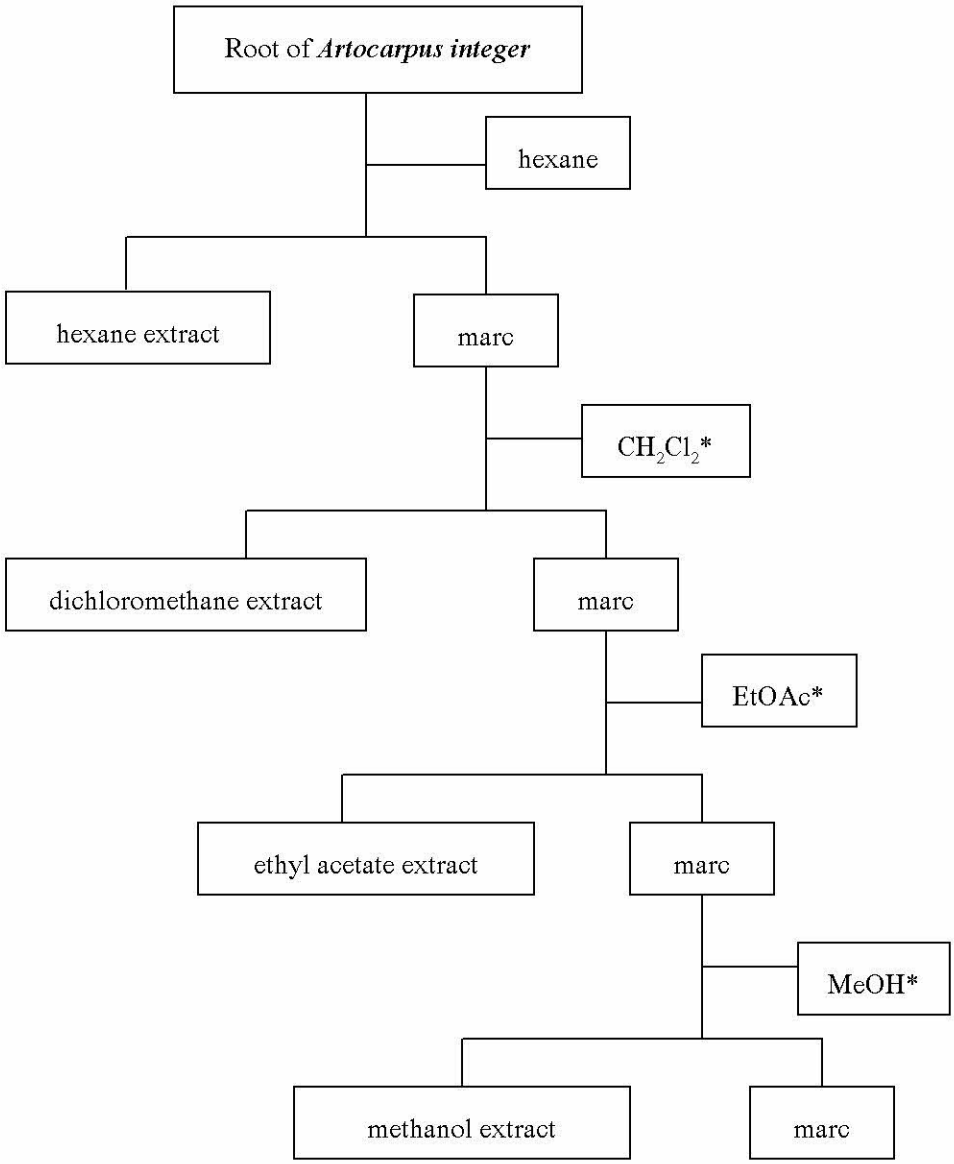
The marc (after extracted with hexane, dichloromethane and ethyl acetate, respectively) was extracted three times with methanol (3 days, each). Removal of organic solvent gave a methanol extract (86.21 g, 2.993 % base on dried weight of root). The separation of ***Artocarpus integer*** shown on scheme 3.1

These crude extracts were tested anti-tyrosinase activity (follow item 3.2.2) and TLC pattern was checked, the methanol extract showed high potential activity (see table 4.5) and the hexane extract showed different TLC pattern. Therefore, we have been isolated of the chemical constituents from methanol and hexane extracts.

3.3.1 Isolation of chemical compound from methanol extract

The methanol extract (10 g) was dissolved in a small amount of methanol, triturated with silica gel 60 (Merck) and dried under the vacuum. It was then fractionated by quick column chromatographic technique using a sintered glass-filter column (size 500 mL) of silica gel 60 (Merck) (length 15 cm; ≈ 200 g). Elution was performed in a polarity gradient manner with dichloromethane, ethyl acetate and methanol as the solvents. The ratio and volume of solvents used in this column chromatography are summarized in Table 3.3. The isolation of pure compounds from methanol extract, are summarized in scheme 3.2.

The eluates were examined by TLC and fractions with similar chromatographic pattern were combined to 11 fractions, as shown in table 3.4.



Scheme 3.1 Solvent extraction of *Artocarpus integer* root

* (CH_2Cl_2 = dichloromethane; EtOAc = ethyl acetate and MeOH = methanol)

Table 3.3 Ratio and volume of solvents in fourteen portions of quick column chromatography for isolation pure compound from methanol extract

Portion	Ratio (%) of CH_3Cl_2*: EtOAc*: MeOH*	Volume of solvents (mL)		
		CH_2Cl_2*	EtOAc*	MeOH*
1	100: 0: 0	500	0	0
2	90: 10: 0	225	25	0
3	80: 20: 0	200	50	0
4	70: 30: 0	175	75	0
5	60: 40: 0	150	100	0
6	50: 50: 0	125	125	0
7	40: 60: 0	100	150	0
8	30: 70: 0	75	175	0
9	20: 80: 0	50	200	0
10	10: 90: 0	25	225	0
11	0: 100: 0	0	250	0
12	0: 90: 10	0	225	25
13	0: 80: 20	0	200	50
14	0: 70: 30	0	525	225

* (CH_2Cl_2 = dichloromethane; EtOAc = ethyl acetate and MeOH = methanol)

Table 3.4 Combination of fractions from quick column chromatography of methanol extract (10 g)

Portion of quick coloumn	Fractions	Weight (g)
1	A	0.0459
2	B	0.0412
3-4	C	0.4400
5	D	0.3500
6	E	0.7000
7	F	0.5200
8	G	1.4400
9	H	1.2700
10-12	I	1.7300
13	J	1.9100
14	K	0.8300

3.3.1.1 Isolation of compound M-1

The fraction C (440 mg) was further purified by wet column chromatography. A 5 cm diameter of glass column was filled 30 cm length of Silica gel 60. The column was eluted with a solvent isocratic of hexane: dichloromethane: ethyl acetate in ratio 2.5:1:1, given to 5 fractions are including C-1, C-2, C-3, C-4 and C-5.

The fraction C-3 (246 mg) was further purified by wet column chromatography. A 3 cm diameter of glass column was filled 30 cm length silica gel 60. The column was eluted

with a solvent isocratic of dichloromethane 100%, given to 6 fractions are including C-3-1, C-3-2, C-3-3, C-3-4, C-3-5 and C-3-6.

The fraction C-3-3 (M-1) yielded a pure compound was obtained as yellow crystal amount 85 mg (0.00295 % based on dried weight of root). It was subsequently identified as Artocarpin.

3.3.1.2 Isolation of compounds M-2 and M-3

The fraction D (350 mg) was further purified by wet column chromatography. A 3 cm diameter of glass column was filled 30 cm length of silica gel 60. The column was eluted with a solvent isocratic of hexane: dichloromethane: ethyl acetate in ratio 2.5:1:1, given to 8 fractions are including D-1, D-2, D-3, D-4, D-5, D-6, D-7 and D-8.

The fraction D-3 (246 mg) was further purified by wet column chromatography. A 1 cm diameter of glass column was filled 20 cm length silica gel 60. The column was eluted with a solvent isocratic of hexane: dichloromethane: ethyl acetate in ratio1:4:3, given to 3 fractions are including D-3-1, D-3-2 and D-3-3.

The fraction D-3-1 (87.7 mg) was further purified by wet column chromatography. A 1 cm diameter of glass column was filled 15 cm length silica gel 60. The coloumn was eluted with a solvent isocratic of hexane: dichloromethane: ethyl acetate in ratio 1:4:3, given to 2 fractions are including D-3-1a, D-3-1b.

The fraction D-3-1a (M-2) yielded a pure compound was obtained as yellow powder amount 22 mg (0.00076 % based on dried weight of root). It was subsequently identified as Cudraflavone C.

The fraction D-5 (M-3) yielded a pure compound was obtained as white crystal amount 23 mg (0.00079 % based on dried weight of root). It was subsequently identified as Artocarpanone.

3.3.2 Isolation of chemical compound from hexane extract

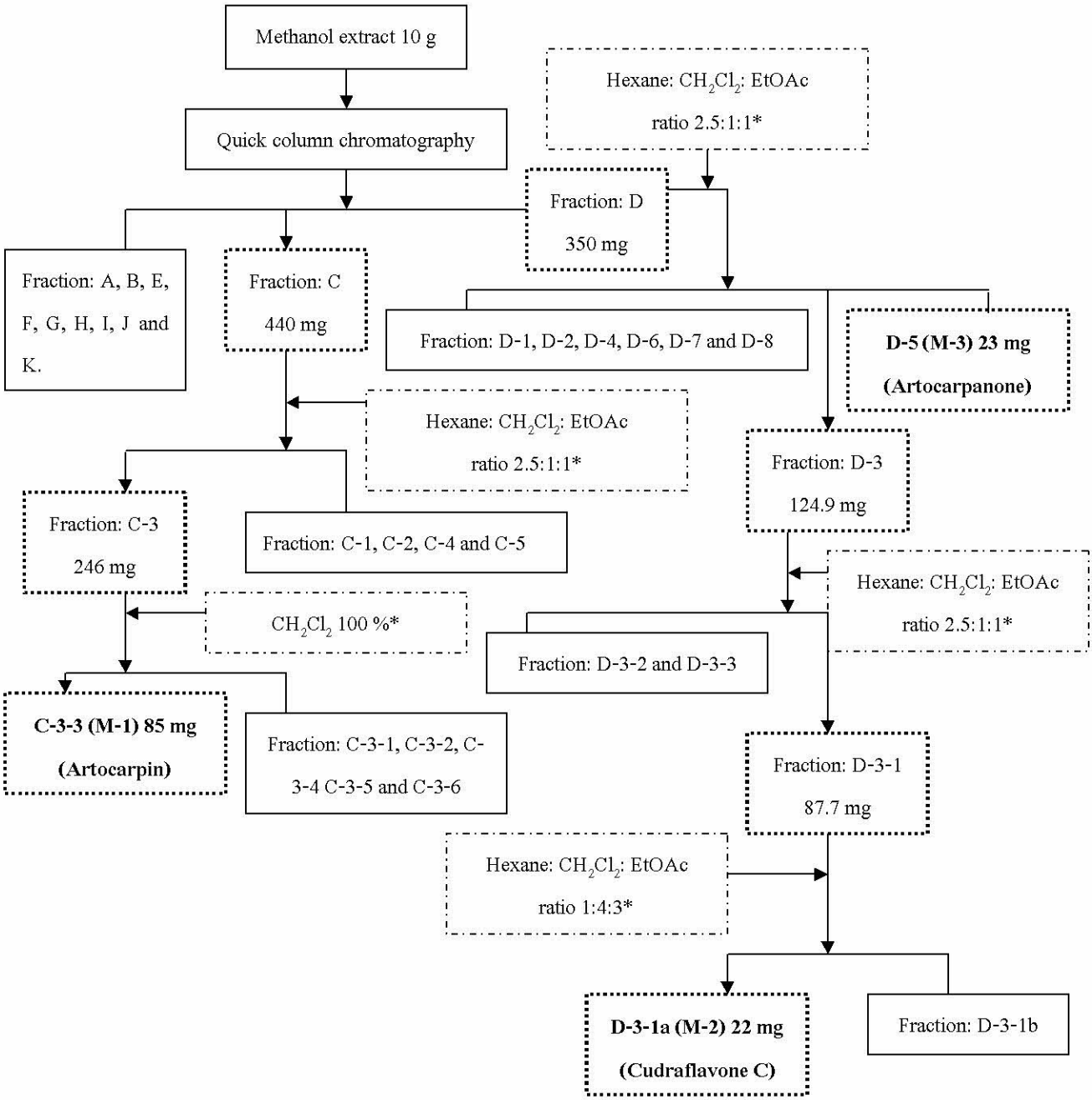
3.3.2.1 Isolation of compounds H-1, H-2 and mixture H-3

The hexane extract (2.0.14 g) was further purified by dry column chromatography. A 3 cm diameter of glass column was filled 20 cm length of flash silica gel. The column was eluted with a solvent isocratic of hexane: dichloromethane in ratio 6:4, given to 10 fractions are including CH-1, CH-2, CH-3, CH-4, CH-5, CH-6, CH-7, CH-8, CH-9 and CH-10. The isolation of pure and mixture compounds from hexane extract are summarized in scheme 3.3.

The fraction CH-1 (H-1) yielded a pure compound was obtained as colorless wax amount 13.4 mg (0.00046. % based on dried weight of root). It was subsequently identified as long chain alkene.

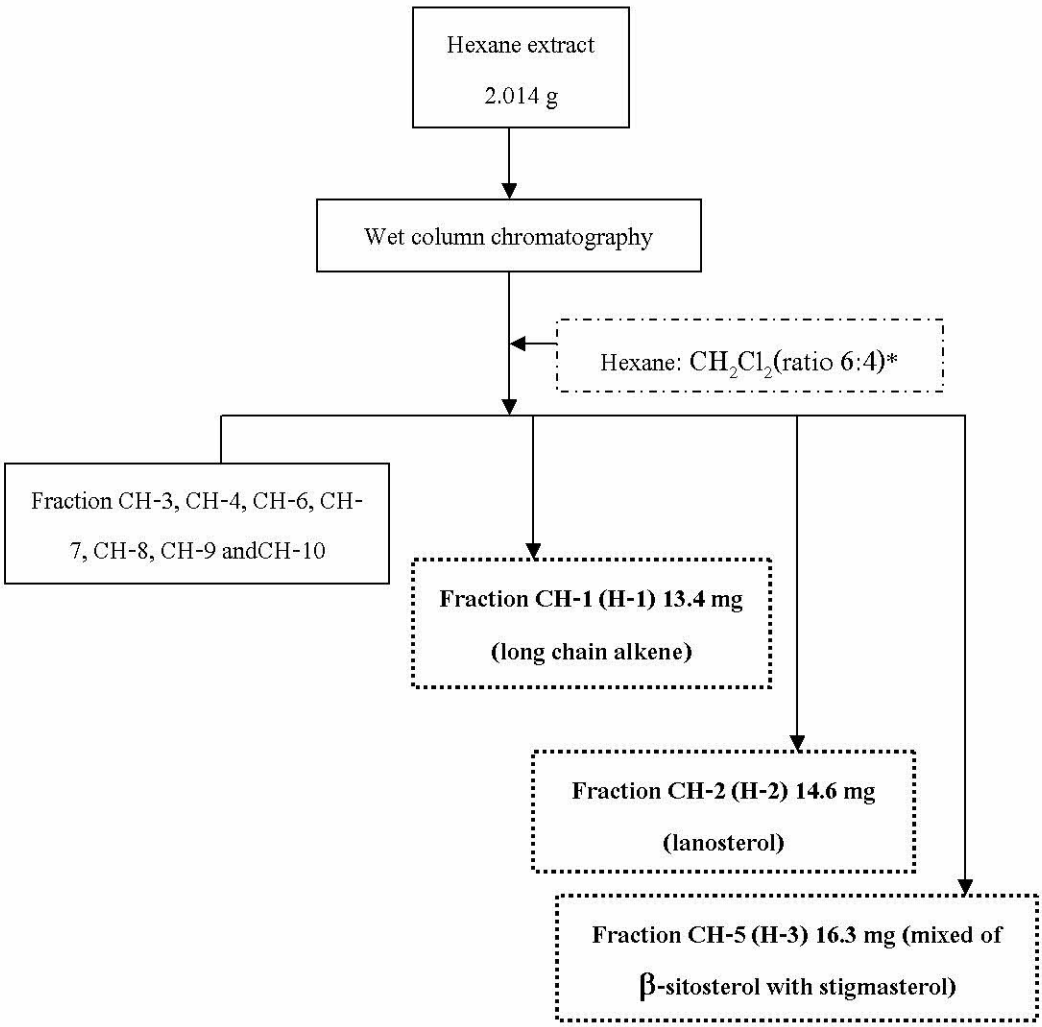
The fraction CH-2 (H-2) yielded a pure compound was obtained as colorless needles amount 14.6 mg (0.00050 % based on dried weight of root). It was subsequently identified as Lanosterol.

The fraction CH-5 (H-3) yielded a mixture compounds was obtained as colorless needles amount 16.3 mg (0.00056 % based on dried weight of root). It was subsequently identified as the mixture of β-sitosterol and stigmasterol.


Methanol extract 10 g
Quick column chromatography
Hexane: CH_2Cl_2: EtOAc
ratio 2.5:1:1*
Fraction: D
350 mg
Fraction: A, B, E, F, G, H, I, J and K.
Fraction: C
440 mg
Fraction: D-1, D-2, D-4, D-6, D-7 and D-8
D-5 (M-3) 23 mg
(Artocarpanone)
Hexane: CH_2Cl_2: EtOAc
ratio 2.5:1:1*
Fraction: D-3
124.9 mg
Fraction: C-3
246 mg
Fraction: C-1, C-2, C-4 and C-5
Hexane: CH_2Cl_2: EtOAc
ratio 2.5:1:1*
CH_2Cl_2 100 %*
Fraction: D-3-2 and D-3-3
C-3-3 (M-1) 85 mg
(Artocarpin)
Fraction: C-3-1, C-3-2, C-3-4 C-3-5 and C-3-6
Fraction: D-3-1
87.7 mg
Hexane: CH_2Cl_2: EtOAc
ratio 1:4:3*
D-3-1a (M-2) 22 mg
(Cudraflavone C)
Fraction: D-3-1b

Scheme 3.2 Isolation of pure compounds from methanol extract

* (CH_2Cl_2 = dichloromethane; EtOAc = ethyl acetate and MeOH = methanol)



Scheme 3.3 Isolation of pure and mixture compounds from hexane extract

* (CH_2Cl_2 = dichloromethane)

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Screening of Anti-tyrosinase and Anti-microbial activities from Thai medicinal plants

Among 77 samples of Thai medicinal plant extract (as shown in Table 4.1a) were investigated for anti-tyrosinase activity, 11 samples showed the tyrosinase inhibition more than 50 %, including ***Averrhoa bilimbi*** (juice), ***Sauropus changiana*** (leaves), ***Ficus racemosa*** (wood), ***Cudrania javanensis*** (wood), ***Hydnophytum formicarum*** (rhizome[W]), ***Solanum ferox*** (branch), ***Artocarpus integer*** (stem bark), ***Artocarpus integer*** (wood), ***Artocarpus integer*** (root bark), ***Artocarpus integer*** (root). The root extract of ***A. integer*** showed the highest tyrosinase inhibition (as shown in Table 4.1b). Therefore, these samples were further screening for anti-microbial activity by agar disc diffusion method. The samples showed inhibition zone, were selected in order to testing the minimum inhibitory concentration (MIC) by broth micro-dilution method and minimum bactericidal concentration (MBC) as following by item 3.2.3, 3.2.4 and 3.2.5, respectively.

The results of disc diffusion test (Table 4.2a and 4.2b) showed that the extract from ***C. javanensis*** was the most active against five microbes, as ***Staphylococcus aureus***. ***S. epidermidis***, ***P. acnes***, ***Trichophyton rubrum*** and ***T. mentagrophytes***. All extracts were inactive against ***Candida albicans***. The MIC and MBC (Table 4.3 and 4.4) were examined by testing with ***S. aureus***, ***S. epidermidis***, and ***P. acnes***. The results showed that the extracts of root and wood of ***A. integer*** were active against these microbes more than other samples. MIC values of root and wood extract against ***S. aureus***, ***S. epidermidis*** and ***P. acnes*** were 3.91, 1.95 and 0.98 μg/mL, respectively. The root extract showed anti-microbial activity with MBC values of 1000, 3.91 and 62.5 μg/mL, respectively and the wood extract showed MBC values of 250, 2000 and 62.5 μg/mL, respectively against three microbes mentioned above.

The root of ***A. integer*** showed the potential of anti-tyrosinase and anti-microbial activities. Thus, this plant was selected for phytochemical investigation.

Table 4.1a Percentage of tyrosinase inhibition of 77 samples from Thai medicinal plants

No.	Family	Scientific name	Plant part	% tyrosinase inhibition ± SD at 200 μL/mL
1	Acanthaceae	*Barlerbia lupulina*	Leaf	22.1±1.42
2	Acanthaceae	*Andrographis paniculata*	Leaf	17.15 ± 0.42
3	Acanthaceae	*Clinacanthus nutans*	Leaf	22.89 ± 2.81
4	Acanthaceae	*Thunbergia laurifolia*	Leaf	6.96 ± 2.67
5	Anacardiaceae	*Spondias cytherea*	Leaf	10.17 ± 1.48
6	Annonaceae	*Desmos chinesis*	Stem bark	34.3 ± 0.84
7	Annonaceae	*Desmos chinesis*	Leaf	-1.96 ± 3.4
8	Averrhoaceae	*Averrhoa bilimbi*	Fruit	35.63 ± 3.98
9	**Averrhoaceae**	*Averrhoa bilimbi*	**Juice**	**61.23 ± 1.55**
10	Capparidaceae	*Crateva nurvala*	Branch	5.24 ± 1.37
11	Capparidaceae	*Cleoma viscose*	Leaf	31.47 ± 4.87
12	Caesalpiniaceae	*Caesalpinia sappan*	Wood	20.27 ± 2.48
13	Compositae	*Chromolaena odoratum*	Leaf	8.63 ± 2.38
14	Compositae	*Saussurea lappa*	Rhizome	-20.48±4.76
15	Combretaceae	*Combretum quadrangulare*	Leaf	6.72±0.17
16	Euphorbiaceae	*Antidesma* sp.	Leaf	39.37±1.10
17	Euphorbiaceae	*Bridelia ovata*	Leaf	8.83±0.23
18	Euphorbiaceae	*Baccaurea macrophylla*	Fruit	29.01±4.95
19	Euphorbiaceae	*Euphorbia tirucalli*	Aerial	13.95±2.03
20	Euphorbiaceae	*Phyllanthus acidus*	Fruit	46.43±4.89
21	Euphorbiaceae	*Phyllanthus acidus*	Root	42.48±0.92
22	**Euphorbiaceae**	*Sauropus changiana*	**Leaf**	**55.79±3.87**
23	Guttiferae	*Garcinia cowa*	Leaf	17.24±0.00
24	Guttiferae	*Garcinia atroviridis*	Leaf	20.69±0.00
25	Lauraceae	*Cinnamomum iners*	Branch	22.13±4.21
26	Labiatae	*Ocimum tenuiflorum*	Leaf	-10.59±1.02

Table 4.1a Percentage of tyrosinase inhibition of 77 samples from Thai medicinal plants (continued)

No.	Family	Scientific name	Plant part	% tyrosinase inhibition ± SD at 200 μL/mL
27	Labiatae	*Ocimum americanum*	Leaf	21.18±2.04
28[W]	Labiatae	*Ocimum basilicum*	Leaf	-3.17±5.50
29	Leguminosae	*Acacia concinna*	Leaf	4.41±0.11
30	Lythraceae	*Punica granatum*	Peel (young)	32.77±2.98
31	Lythraceae	*Punica granatum*	Peel (old)	27.08±2.08
32	Meliaceae	*Melia toosenden*	Stem bark	19.29±8.29
33	Meliaceae	*Aglaia andamanica*	Leaf	0.00±0.00
34	Melastomataceae	*Melastoma malabathricum*	Leaf	11.58±8.1
35	Moraceae	*Atrocarpus heterophyllus*	Leaf	35.41±5.6
36	Moraceae	*Artocarpus altitis*	Stem bark	3.75±1.37
37	Moraceae	*Artocarpus altitis*	Leaf	8.23±1.48
38	Moraceae	*Artocarpus altitis*	Wood	20.13±1.80
39	**Moraceae**	***Artocarpus integer***	**Stem bark**	**50.01±1.79**
40	**Moraceae**	***Artocarpus integer***	**Wood**	**80.02±3.22**
41	**Moraceae**	***Artocarpus integer***	**Root bark**	**82.60±0.76**
42	**Moraceae**	***Artocarpus integer***	**Root**	**90.57±2.93**
43	Moraceae	*Streblus asper*	Leaf	17.49±1.63
44	Moraceae	*Streblus asper*	Branch	13.85±0.37
45	Moraceae	*Streblus asper*	Wood	12.34±2.83
46	Moraceae	*Streblus asper*	Stem bark	41.56±1.12
47	Moraceae	*Ficus benjamina*	Leaf	18.47 ± 0.50
48	Moraceae	*Ficus benjamina*	Branch	12.64 ± 2.31
49	Moraceae	*Ficus benjamina*	Wood	30.13 ± 1.63
50	**Moraceae**	***Ficus racemosa***	**Wood**	**56.41 ± 2.22**
51	Moraceae	*Ficus religiosa*	Leaf	-12.03 ± 6.90

Table 4.1a Percentage of tyrosinase inhibition of 77 samples from Thai medicinal plants (continued)

No.	Family	Scientific name	Plant part	% tyrosinase inhibition ± SD at 200 μL/mL
52	Moraceae	*Ficus religiosa*	Branch	3.10 ± 2.69
53	Moraceae	*Ficus religiosa*	Stem bark	1.67 ± 2.89
54	Moraceae	*Ficus religiosa*	Wood	6.44 ± 3.09
55[m]	**Moraceae**	***Cudrania javanensis***	**Wood**	**77.86 ± 2.41**
56	Malvaceae	*Gossypium arboretum*	Aerial	-17.10 ± 0.84
57	Mimosaceae	*Albizia procera*	Stem bark	40.73 ± 3.98
58	Mimosaceae	*Mimosa* sp.	Aerial	39.28 ± 1.07
59	Mimosaceae	*Mimosa* sp.	Flower	22.1 ± 1.42
60	Piperaceae	*Piper nigrum*	Leaf	2.84 ± 2.46
61	Piperaceae	*Piper chaba*	Leaf	8.57 ± 0.21
62	Rubiaceae	*Hydnophytum formicarum*	Rhizome	45.24 ± 4.12
63[w]	**Rubiaceae**	***Hydnophytum formicarum***	**Rhizome**	**53.71 ± 1.78**
64	**Rutaceae**	***Citrus ichanqensis***	**Peel**	**52.46 ± 2.50**
65	Saururaceae	*Houttuynia cordata*	Aerial	-25.24 ± 1.82
66	Sapindaceae	*Nephelium lappaceum*	Peel	23.48 ± 3.35
67	Sapindaceae	*Cardiospermum halicacabum*	Stem	-14.32 ± 4.26
68	Salvadoraceae	*Azima sarmentosa*	Root	8.83 ± 0.23
69	**Solanaceae**	***Solanum ferox***	**Branch**	**50.87 ± 3.77**
70	Solanaceae	*Capsicum frutescens*	Fruit	15.44 ± 3.12
71	Simaroubaceae	*Eurycoma longifolia*	Root	37.17 ± 4.79
72	Taccaceae	*Tacca leontopetaloides*	Stem	25.75 ± 4.58
73	Verbenaceae	*Vitex trifolia*	Fruit	32.64 ± 1.20
74	Verbenaceae	*Vitex trifolia*	Leaf	37.35 ± 1.81
75	Verbenaceae	*Vitex* sp.	Leaf	21.74 ± 4.04
76	Umbelliferae	*Conioselinum univittatum*	Rhizome	-25.20 ± 0.71
77	Zingiberaceae	*Languas galangal*	Rhizome	19.23 ± 3.85

Table 4.1b Percentage of tyrosinase inhibition of Kojic acid and *Artocarpus lakoocha* extract

Positive control	% tyrosinase inhibition± SD at 200 μL/mL
Kojic acid	89.57 ± 2.15
Artocarpus lakoocha extract[w]	90.14 ± 1.46

Table 4.1a and 4.1b; [w] = water extract; [m] = methanol extract

Table 4.2a Anti-bacterial activity of Thai medicinal plants by disc diffusion method

Sample	Part	Inhibition zone [mm] ± SD		
		S. aureus ATCC 25923	*S. epidermidis* TISTR 517	*P. acnes* DMST 14916
Ficus racemosa	Wood	-	-	13.23±0.46
Averrhoa bilimbi	Juice	7.70±0.00	-	17.00±0.72
Hydnophytum formicarum	Root	8.10±0.00	8.55±0.15	16.88±0.60
Citrus ichanqensis	Peel	-	-	8.77±0.40
Solanum ferox	Branch	7.60±0.00	-	11.23±0.70
Sauropus changiana	Leaf	-	-	-
Artocarpus integer	Stem bark	8.87±0.12	9.60±0.00	15.67±0.42
Artocarpus integer	Wood	8.13±0.15	10.60±0.00	15.95±0.68
Artocarpus integer	Root bark	11.57±0.40	13.83±0.12	15.97±0.85
Artocarpus integer	Root	9.10±0.00	10.67±0.09	15.25±0.05
Cudrania javanensis	Wood	13.37±0.40	12.67±0.12	21.55±0.64
DMSO		-	-	-
Oxacillin		20.13±0.12	21.80±0.00	36.12±0.97

- = Did not show inhibition zone;

Positive control = Oxacillin;

Negative control = DMSO

Table 4.2b Anti-fungal activity of Thai medicinal plants by disc diffusion method

Sample	Part	Inhibition zone [mm] ± SD		
		C. albicans TISTR 5779	*T. mentagrophytes*	*T. rubrum*
Ficus racemosa	Wood	-	-	-
Averrhoa bilimbi	Juice	-	-	-
Hydnophytum formicarum	Root	-	-	-
Citrus ichanqensis	Peel	-	-	8.40±0.00
Solanum ferox	Branch	-	-	-
Sauropus changiana	Leaf	-	-	-
Artocarpus integer	Stem bark	-	-	-
Artocarpus integer	Wood	-	6.83±0.20	-
Artocarpus integer	Root bark	-	-	-
Artocarpus integer	Root	-	6.60±0.17	-
Cudrania javanensis	Wood	-	7.82±0.32	8.60±0.52
DMSO		-	-	-
Amphotericin B		18.13±0.32	NT	NT
Ketoconazole		NT	38.63±0.86	30.47±0.81

- = Did not show inhibition zone;

Positive control = Amphotericin B and Ketoconazole;

Negative control = DMSO

NT = No Test

Table 4.3 MIC of Thai medicinal plants against *S. aureus*, *S. epidermidis* and *P. acnes*

Sample	Part	MIC (μg/mL)		
		S. aureus ATCC 25923	*S. epidermidis* TISTR 517	*P. acnes* DMST 14916
Ficus racemosa	Wood	NT	NT	250
Averrhoa bilimbi	Juice	1000	NT	500
Hydnophytum formicarum	Root	1000	250	250
Citrus ichanqensis	Peel	NT	NT	500
Solanum ferox	Branch	1000	NT	250
Artocarpus integer	Stem bark	1000	250	62.50
Artocarpus integer	Wood	3.91	1.95	0.98
Artocarpus integer	Root bark	62.50	31.25	31.25
Artocarpus integer	Root	3.91	1.95	0.98
Cudrania javanensis	Wood	1000	500	250
Oxacillin		0.5	0.125	0.0625

NT = No Test

Table 4.4 MBC of Thai medicinal plants against *S. aureus*, *S. epidermidis* and *P. acnes*

Sample	Part	MBC (μg/mL)		
		S. aureus ATCC 25923	*S.epidermidis* TISTR 517	*P. acnes* DMST 14916
Ficus racemosa	Wood	NT	NT	1000
Averrhoa bilimbi	Juice	2000	NT	1000
Hydnophytum formicarum	Root	2000	2000	1000
Citrus ichanqensis	Peel	NT	NT	1000
Solanum ferox	Branch	2000	NT	1000
Artocarpus integer	Stem bark	2000	2000	1000
Artocarpus integer	Wood	250	2000	62.50
Artocarpus integer	Root bark	1000	62.50	1000
Artocarpus integer	Root	1000	3.91	62.50
Cudrania javanensis	Wood	2000	2000	1000

NT = No Test

4.2 Extraction of root from *Artocarpus integer*

The dried roots of *A. integer* (2.88 kg) were extracted with hexane, dichloromethane, ethyl acetate and methanol. The dry weight, % yield and % tyrosinase inhibition of these crude extracts as shown in Table 4.5. Methanol and hexane extracts were selected for isolation of the chemical constituents.

Table 4.5 Dry weight, % yield, % tyrosinase inhibition of *A. integer* crude extracts

Crude extract	Dry weight (g)	% yield	% tyrosinase inhibition at 200 μg/mL
Hexane	9.55	0.332	14.86
Dichloromethane	53.95	1.873	53.25
Ethyl acetate	31.8	1.104	85.68
Methanol	86.21	2.993	88.77

4.3 Structure determination of isolated compounds

Three pure compounds were isolated from methanol extract and were identified as M-1 (Artocarpin), M-2 (Cudraflavone C) and M-3 (Artocarpanone). The other two pure compounds and one mixture compound were isolated from hexane extract and were identified as H-1 (long chain alkene), H-2 (Lanosterol) and H-3 (mixture of β-Sitosterol and Stigmasterol). These structures of the isolated compounds were identified by physical properties (such as melting point) and spectroscopic data including UV, IR, NMR and MS data and confirmed by comparison with previously reports.

4.3.1 Structure determination of compound M-1

The compound M-1 was obtained as a yellow crystal. The UV-visible spectrum was found maximum wavelengths (λ_{man}) at 278 and 322 nm (Figure A1) (279 and 316 nm (Cunha and Socorro, 1994)). The melting point and optical rotation showed at 136-140 °C (137-141 °C (Sritularak, 1998a)) and $[\alpha]_D^{28}$ (0.06 g/100 mL, methanol) -6.45, respectively. In IR-Spectrum (KBr) ν_{max} (Figure A2) showed at 3436 (OH), 1643 (C=O), 1542-1620 (C=C, aromatic ring) cm^{-1}, respectively (3417, 1647, 1615 and 1563 cm^{-1} (Sritularak, 1998a)). The EI mass spectrum (Figure A3) showed as molecular ion peak at ***m/z*** 436 corresponding to $C_{26}H_{28}O_6$.

The ^1H NMR spectra data showed total 25 protons, including 5 proton groups from 14 signals, as aromatic protons 4 signal (δ 6.33 (s), 6.48 (s), 6.50 (dd, J = 2.2 Hz) and 7.19 (d, J = 9.03 Hz)), olefin proton 3 signals (δ 5.14 (m), 6.69 (dd, J = 7.07, 16.23), 6.55 (dd, J = 1.22, 16.35), methyl proton 3 signals (δ 1.09 (S), 1.44 (S) and 1.62 (S)) methane proton 2 signals (δ 3.11 (d, J = 6.59 Hz), 2.46 (m), a chelated hydroxyl proton to carbonyl group 1 signal (δ 13.50) and proton of methoxyl group (δ 3.86), respectively.

The ^{13}C NMR spectra data showed total 26 carbons, including 6 carbon groups from 26 signals, as carbonyl 1 signal (δ 182.2), aromatic carbon 14 signals (δ 89.4, 103.8, 104.9, 108.3, 109.7, 112.5, 120.8, 131.5, 155.1, 156.0, 158.6, 158.9, 159.3 and 162.9), methane carbon 2 signals (δ 24.3, 33.0), olefin carbon 4 signals (δ 115.5, 121.5, 133.3 and 142.6), methyl carbon 3 signals (δ 17.6, 22.6 and 25.6) and methoxyl carbon 1 signal (δ 55.9), respectively.

From the spectra, ^1H NMR (Figure A4), ^{13}C NMR (Figure A5), DEPT (Figure A6.1 and A6.2), COSY (Figure A7), HMBC (Figure A8) and HMQC (Figure A9), physical property data and comparision with previously reports (Sritularak, 1998a; Cunha and Socorro, 1994), compound M-1 was identified as Artocarpin.

The complete ^1H and ^{13}C NMR assignments and the HMBC correlation are summarized in Table 4.6

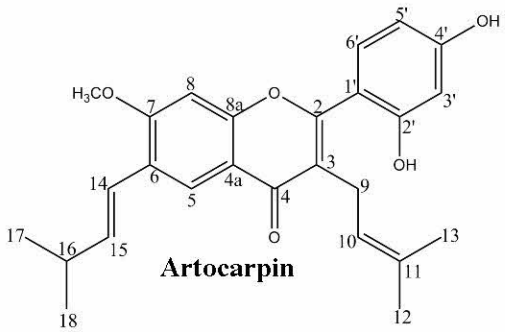


Table 4.6 ^{1}H and ^{13}C NMR spectral data of Compound M-1 (in Chloroform-*d*; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)) and Artocarpin (in DMSO-d_6; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)) with long-range correlations in HMBC spectrum

Position	Compound M-1		Artocarpin (Sritularak, 1998a)		Correlation with proton
	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	
2	159.3	-	162.9	-	H-9
3	120.8	-	121.1	-	-
4	182.2	-	182.7	-	H-8 and H-9
4a	104.9	-	105.0	-	5-OH and H-8
5	158.6	-	158.9	-	5-OH, H-8 and H-14
6	109.7	-	109.1	-	5-OH and H-8
7	162.9	-	163.4	-	7- OCH_3,H-8 and H-14
8	89.4	6.33 (s)	91.0	6.65 (s)	-
8a	156.0	-	158.6	-	-
9	24.3	3.11 (d, 6.59)	24.5	3.02 (d, 6.7)	H-10
10	121.5	5.14 (t)	122.3	5.05 (t)	H-9, H-12 and H-13
11	133.3	-	132.1	-	-
12	17.6	1.44 (s)	18.2	1.38 (s)	H-10 and H-13
13	25.6	1.62 (s)	26.3	1.55 (s)	H-10 and H-12
14	115.5	6.55 (dd, 1.22, 16.25)	116.7	6.50 (d, 16.5)	H-15
15	142.6	6.69 (dd, 7.07, 16.23)	142.0	6.64 (dd, 6.9, 16.5)	H-14, H-16,H-17 and H-18
					H-15, H-17 and H-18
16	33.0	2.46 (m)	33.4	2.44 (m)	H-15, H-16, H-17 and H-18
17	22.6	1.09 (d, 6.84)	23.5	1.06 (d, 6.6)	H-15, H-16, H-17 and H-18
					H-3'
18	22.6	1.09 (d, 6.84)	23.5	1.04 (d, 6.6)	H-6'
					H-5'
1'	112.5	-	111.7	-	H-6'
2'	155.1	-	157.3	-	H-5'
3'	103.8	6.48 (s)	103.5	6.45 (d ,1.8)	-
4'	158.9	-	161.3	-	-

Table 4.6 ^{1}H and ^{13}C NMR spectral data of Compound M-1 (in Chloroform-*d*; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)) and Artocarpin (in DMSO-d_6; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)) with long-range correlations in HMBC spectrum (continued)

Position	Compound M-1		Artocarpin (Sritularak, 1998a)		Correlation with proton
	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	
5'	108.3	6.50 (d, 2.2)	107.6	6.36 (dd, 8.1, 2.1)	-
6'	131.5	7.19 (d, 9.03)	132.9	7.12 (d, 8.4)	-
5-OH	-	13.50 (s)	-	13.89 (s)	-
7-OCH_3	55.9	3.86 (s)	56.2	3.89 (s)	-

4.3.2 Structure determination of compound M-2

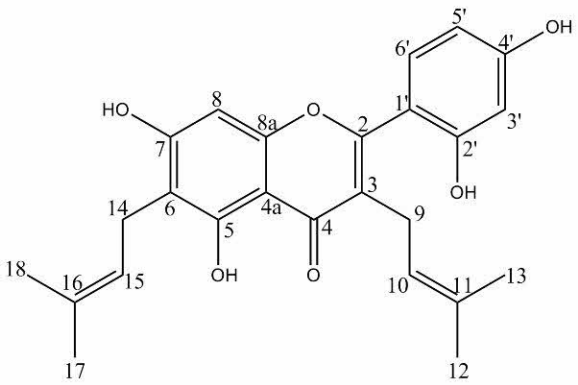
The compound M-2 was obtained as a yellow powder. The UV-visible spectrum was found maximum wavelengths (λ_{max}) at 262 and 315 nm (Figure B1) (274 and 306 nm (Sritularak, 1998a)). The optical rotation showed at $[\alpha]_D^{28}$ (0.02 g/100 mL) +30.00. In IR-Spectrum (KBr) ν_{max} (Figure B2) showed at 3401 (OH), 1643 (C=O), 1566-1623 (C=C, aromatic ring) cm^{-1}, respectively (3321, 1651, 1626 and 1575 cm^{-1} (Sritularak, 1998a)). The EI mass spectrum (Figure B3) showed as molecular ion peak at ***m/z*** 422 corresponding to $C_{25}H_{26}O_6$.

The ^{1}H NMR spectra data showed total 22 proton, including 6 proton groups from 12 signals, as aromatic proton 4 signals (δ 6.29 (s), 6.37 (dd, *J* = 8.30, 2.19 Hz), 6.39 (dd, *J* = 2.2 Hz) and 7.04 (d, *J* = 8.06 Hz)), olefin proton 2 signals (δ5.07 (m), 5.23 (m)), methane proton 2 signals (δ 3.06 (d, *J* = 2.84 Hz), 3.30 (m) and methyl proton 4 singles (δ 1.37 (S), 1.56 (S), 1.65 (S) and 1.76 (S)), respectively.

The ^{13}C NMR spectra data showed total 25 carbons, including 6 carbon groups from 25 signals, as carbonyl 1 signal (δ183.65), aromatic carbon 14 signals (δ 93.67, 103.77, 105.20, 107.95, 112.39, 113.54, 121.70, 132.36, 157.64, 157.68, 159.88, 161.73, 163.17 and 163.25), olefin carbon 4 signals (δ 122.94, 123.64, 131.88 and 132.55), methane carbon 2 signals (δ 22.27 and 24.88) and methyl carbon 4 signals (δ 17.61, 17.89, 25.81 and 25.93), respectively.

From the spectra, ^{1}H NMR (Figure B4), ^{13}C NMR (Figure B5), DEPT (Figure B6.1 and B 6.2), COSY (Figure B7), HMBC (Figure B8) and HMQC (Figure B9), physical property data and comparing with previously reports (Sritularak, 1998a), compound M-2 was identified as Cudraflavone C.

The complete ^{1}H and ^{13}C NMR assignments and the HMBC correlation are summarized in Table 4.7



Cudraflavone C

Table 4.7 ^{1}H and ^{13}C NMR spectral data of Compound M-2 (in Methanol-d_4; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)) and Cudraflavone C (in DMSO-d_6; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)) with long-range correlations in HMBC spectrum

Position	Compound M-2		Cudraflavones C (Sritularak, 1998a)		Correlation with proton
	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	
2	163.17	-	162.3	-	H-9 and H-6'
3	121.70	-	120.5	-	-
4	183.65	-	182.3	-	-
4a	105.20	-	104.1	-	H-9
5	159.88	-	159.1	-	H-14
6	112.39	-	111.3	-	-
7	163.25	-	162.6	-	H-14
8	93.67	6.29 (s)	93.5	6.34 (s)	-

Table 4.7 ^{1}H and ^{13}C NMR spectral data of Compound M-2 (in Methanol-d_4; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)) and Cudraflavone C (in DMSO-d_6; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)) with long-range correlations in HMBC spectrum (continued)

Position	Compound M-2		Cudraflavones C (Sritularak, 1998a)		Correlation with proton
	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	
8a	157.64	-	156.3	-	H-8
9	24.88	3.06 (d, 2.84)	24.5	2.97 (d, 6.6)	H-10
10	122.94	5.07 (t)	122.5	5.02 (t)	H-9, H-10, H-12 and H-13
11	132.55	-	131.9	-	H-12 and H-13
12	17.89	1.37 (s)	18.2	1.36 (s)	H-13
13	25.81	1.56 (s)	26.3	1.54 (s)	H-12
14	22.27	3.30 (m)	21.9	3.22 (d, 6.9)	H-15
15	123.64	5.23 (t)	123.2	5.17 (t)	H-14, H-15, H-17 And H-18
16	131.88	-	131.4	-	H-14, H-17 and H-18
17	17.61	1.76 (s)	18.6	1.73 (s)	H-18
18	25.93	1.65 (s)	26.3	1.62 (s)	H-17
1'	113.54	-	111.9	-	H-3' and H-5'
2'	157.68	-	157.2	-	H-3' and H-6'
3'	103.77	6.39 (d ,2.2)	103.5	6.42 (d ,2.1)	H-5'
4'	161.73	-	161.2	-	H-3'
5'	107.95	6.37 (dd, 8.30, 2.19)	107.6	6.34 (m)	H-3' and H-6'
6'	132.36	7.04 (d, 8.06)	132.0	7.06 (d, 8.04)	-
5-OH	-	-	-	13.31 (s)	-

4.3.3 Structure determination of compound M-3

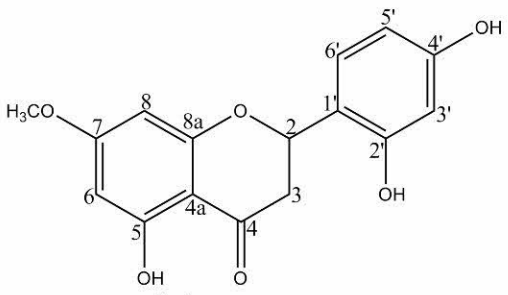
The compound M-3 was obtained as a white crystal. The UV-visible spectrum was found maximum wavelengths (λ_{max}) at 204 and 286 nm (Figure C1). The melting point and optical rotation showed at 156-160 °C (220-212 °C (Wei *et al*, 2005)) and $[\alpha]_D^{28}$ -23.58 (0.02 g/100 mL, methanol). In IR-Spectrum (KBr) ν_{max} (Figure C2) showed at 3436 (OH), 1626 (C=O), 1468-1525 (C=C, aromatic ring) cm^{-1}, respectively (3450, 1640, and 1615 cm^{-1} (Wei *et al*, 2005)). The EI mass spectrum (Figure C3) showed as molecular ion peak at ***m/z*** 302 corresponding to $C_{16}H_{14}O_6$.

The ^1H NMR spectra data showed total 11 protons, including 3 proton groups from 9 signals, as aromatic proton 5 signals (δ 6.01 (d, *J* = 2.29 Hz), 6.04 (d, *J* = 2.29 Hz), 6.32 (dd, *J* = 2.29 Hz), 6.33 (dd, *J* = 8.23, 2.29 Hz), and 7.22 (d, *J* = 8.01 Hz)), methane proton 3 signals (δ 2.71(dd, *J* = 2.57, 17.14 Hz), 3.09 (dd, *J* = 13.4, 17.15 Hz) and 5.62 (dd, *J* = 2.97, 13.04 Hz), and proton of methoxyl group (δ 3.79), respectively.

The ^{13}C NMR spectra data showed total 16 carbons, including 6 carbon groups from 16 signals, as carbonyl 1 signal (δ198.92), aromatic carbon 14 signals (δ 94.87, 95.67, 103.48, 104.04, 107.81, 117.77, 128.90, 157.68, 159.88, 165.22, 165.25 and 169.46), olefin carbon 4 signals (δ 122.94, 123.64, 131.88 and132.55), methane carbon 2 signals (δ43.15 and 76.15) and methoxyl carbon 1 signals (δ56.23), respectively.

From the spectra ^1H NMR (Figure C4), ^{13}C NMR (Figure C5), DEPT (Figure C6.1 and C6.2), COSY (Figure C7), HMQC (Figure C8) and HMBC (Figure C9)), physical property data and comparing with previously reports (Wei *et al*, 2005), compound M-3 was identified as Artocarpanone.

The complete ^1H and ^{13}C NMR assignments and the HMBC correlation are summarized in Table 4.8



Artocarpanone

Table 4.8 ^{1}H and ^{13}C NMR spectral data of Compound M-3 (in Methanol-d_4; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)) and Artocarpanone (in Acetone-d_6; ^{1}H and ^{13}C NMR (400 and 100 MHz, respectively)) with long-range correlations in HMBC spectrum

Position	Compound M-3		Artocarpanone (Wei *et al*, 2005)		Correlation with proton
	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	
2	76.15	5.62 (dd, 13.04, 2.97)	75.9	5.75 (dd, 14.0, 3.0)	H-2
3	43.15	3.09 (dd, 17.15, 13.04)	43.0	3.21 (dd, 17.0, 3.0)	H-3
		2.71 (dd, 17.14, 2.51)		2.76 (dd, 17.0, 3.0)	H-3
4	198.92	-	198.5	-	-
4a	104.04	-	104.1	-	-
5	159.88	-	160.0	-	-
6	95.67	6.01 (d, 2.29)	95.7	6.02 (d, 2.2)	H-6
7	169.46	-	169.1	-	-
8	94.87	6.04 (d, 2.29)	94.8	6.05 (d, 2.2)	H-8
8a	165.22	-	165.1	-	-
1'	117.77	-	117.1	-	H-3'
2'	157.68	-	156.7	-	-
3'	103.48	6.33 (d, 2.29)	103.9	6.47 (dd , 2.0)	-
4'	165.25	-	165.4	-	-
5'	107.81	6.32 (dd, 8.23, 2.29)	108.3	6.43 (dd, 8.0, 2.0)	H-5'
6'	128.90	7.22 (d, 8.01)	129.4	7.32 (d, 8.0)	H-6'
5-OH	-	-	-	12.17	-
7-OCH_3	56.23	3.79	56.6	3.85	

4.2.4 Structure determination of compound H-1

The compound H-1 was obtained as wax. The UV-visible spectrum was maximum wavelength (λ_{max}) at 240 nm (Figure D1). The optical rotation showed at $[\alpha]_D^{28}$ -28.57 (0.04 g/100 mL). In IR-Spectrum (KBr) ν_{max} (Figure D2) showed at 1374, 828 and 719 cm^{-1} (tri-substituted of methine group), other group as 2971, 2849 and 1462 cm^{-1} (a methylene group of alkane).

The ^{1}H NMR 300 MHz (Figure D4) showed 3 proton groups, as olefin protons (δ 5.14 (dd)), methylene and methyl protons (δ 2.06-0.86). This may suggestion that the compound H-1 is a long chain alkene.

The ^{13}C NMR 75 MHz (Figure D5) showed 3 carbon groups, as olefin carbon 2 signals (δ 124.3 and 134.3 ppm), methane and methyl 6 signals (δ 14.0-39.7 ppm).

The EI mass spectrum (Figure D3) occurred fragmentation also with alkene (Field, 1968) showed straight-chain 1-olefin.

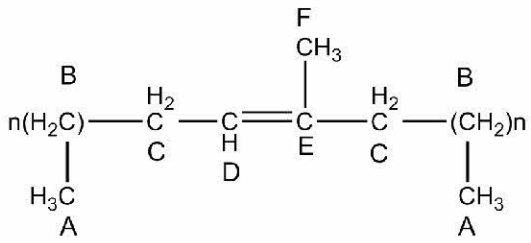


Figure 4.1 The estimation of structure H-1. (related to table 4.9)

Table 4.9 The estimation of ^{1}H- and ^{13}C-NMR. (related figure 4.1)

Protons	δ ppm	Carbons	δ ppm
A	0.88-0.92	A	14.04
B	1.28	B	22-31
C	2.06	C	22-31
D	5.14	D	124.33
E	-	E	135.00
F	1.58	F	22-31

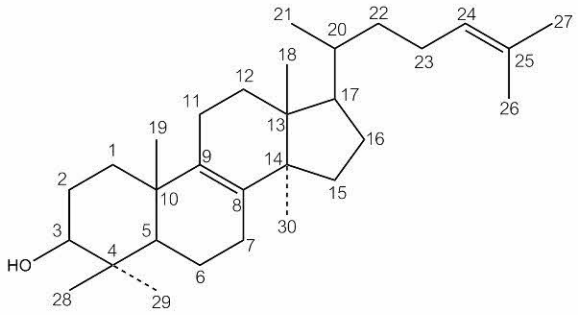
4.2.5 Structure determination of compound H-2

The compound H-2 was obtained as colorless needles. The UV-visible spectrum was found maximum wavelengths at 222, 240 and 322 nm (Figure E1). The melting point showed 130-133 °C (138-140 °C (Marienthal and Franklin, 1995)). In IR-Spectrum (KBr) ν_{max} (Figure E2) showed at 3436 cm^{-1} (OH). The EI mass spectrum (Figure E3) showed as molecular ion peak at ***m/z*** 426 corresponding to $C_{30}H_{50}O$.

The ^{1}H NMR spectra data (Figure E4) showed 4 proton groups as olefin protons (δ 5.12 (t)), methane proton (δ 3.25), methane and methyl protons (δ 0.77-2.19).

The ^{13}C NMR spectra data (Figure E5) showed with 30 signals, including 3 carbon groups, as olefin carbon 3 signals (δ125.25, 130.83 and 134.05), methane and methyl carbon (δ15.43, 15.67, 17.65, 18.15, 18.57, 18.95, 21.44, 24.77, 24.97, 25.70, 26.41, 27.94, 28.04, 28.10, 30.91, 30.97, 35.44, 35.78, 35.85, 37.21, 38.93, 44.13, 49.66, 50.65 and 79.00).

From the NMR spectroscopic data, chromatograms seem to be Lanosterol. The ^{1}H and ^{13}C NMR spectrum were compared with previously reports (Emmons, 1989; Sawai ***et al***, 2006). The complete ^{1}H and ^{13}C NMR assignments are summarized in Table 4.10.



Lanosterol

Table 4.10 ^{1}H and ^{13}C NMR spectral data of Compound H-2 (in $CDCl_3$-*d*; ^{1}H and ^{13}C NMR (300 and 75 MHz, respectively)) and Lanosterol (in $CDCl_3$-*d*; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively)

Position	Compound H-2		Lanosterol (Emmons, 1989)	
	δ_C (ppm)	δ_H (ppm) (multiplicity)	δ_C (ppm)	δ_H (ppm) (multiplicity)
1	35.44	-	35.55	-
2	27.94	-	27.81	-
3	79.00	3.25 (dd)	78.96	3.24 (dd)
4	38.93	-	38.85	-
5	50.65	1.15 (m)	50.35	1.05 (m)
6	18.15	1.53 (m)	18.22	1.63 (m)
7	26.41	-	26.46	-
8	134.05	-	134.35	-
9	134.05	-	134.35	-
10	37.21	-	36.97	-
11	21.44	-	20.97	-
12	30.97	-	30.94	-
13	44.13	-	44.43	-
14	49.66	-	49.76	-
15	30.91	-	30.82	-
16	28.10	-	28.18	-
17	50.65	1.49 (m)	50.35	1.48 (m)
18	15.67	0.77 (s)	15.71	0.68 (s)
19	18.95	0.97 (s)	19.11	0.98 (s)
20	35.78	1.38 (m)	36.24	1.39 (m)
21	18.57	0.87 (d)	18.61	0.91 (d)
22	35.85	-	36.33	-
23	24.97	-	24.89	-
24	125.25	5.12 (t)	125.22	5.10 (t)

Table 4.10 ^{1}H and ^{13}C NMR spectral data of Compound H-2 (in $CDCl_3$-*d*; ^{1}H and ^{13}C NMR (300 and 75 MHz, respectively)) and Lanosterol (in $CDCl_3$-*d*; ^{1}H and ^{13}C NMR (500 and 125 MHz, respectively) (continued)

Position	Compound H-2		Lanosterol (Emmons, 1989)	
	δ_C (ppm)	δ_H (ppm) (multiplicity)	δ_C (ppm)	δ_H (ppm) (multiplicity)
25	130.83	-	130.91	-
26	25.70	1.70 (s)	25.72	1.68 (d or s)
27	17.65	1.62 (s)	17.61	1.60 (d or s)
28	28.04	1.02 (s)	27.93	1.00 (s)
29	15.43	0.81 (s)	15.40	0.81 (s)
30	24.77	0.86 (s)	24.23	0.87 (s)

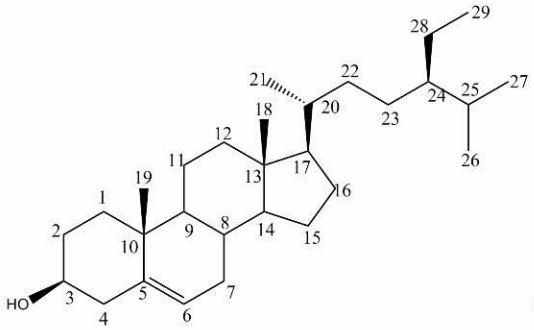
4.2.6 Structure determination of mixture H-3

The mixture H-3 was obtained as colorless needles. The UV-visible spectrum was found maximum wavelengths at 278, 298 and 326 nm (Figure G). In IR-Spectrum (KBr) ν_{max} (Figure F3) showed at 3435 cm^{-1} (OH).

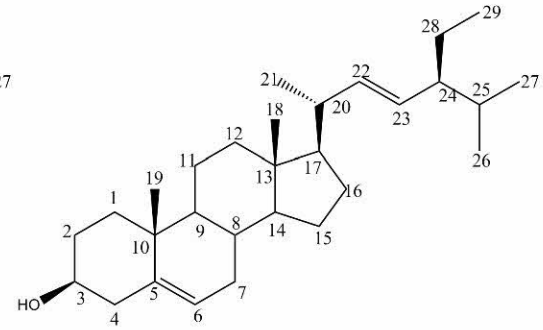
The ^{1}H NMR (Figure F1) showed olefin proton 3 signals (δ5.36 (m,), 5.17 (dd), 5.02 (dd)), methine proton (3.54 (m)), methane and methyl protons (the remaining proton signals were 0.70-2.30). The integration steps of H-6, H-22 and H-23 were approximately in ratio of 0.893:0.217:0.217. Thus, H-3 was a mixture of β-sitosterol and stigmasterol in ratio of 7.5:2.5.

The ^{13}C NMR spectrum (Figure F2) showed with 34 signals, including 3 carbon groups, as olefin carbon 4 signals (δ 121.68, 129.38, 138.28 and 140.77), methane and methyl carbon (δ11.97, 12.21, 19.79, 21.04, 21.09, 21.20, 23.10, 24.29, 24.36, 28.88, 29.21, 31.68, 31.86, 31.92, 33.98, 36.14, 36.51, 37.27, 39.70, 39.80, 40.43, 42.32, 45.88, 50.17, 51.23, 56.00, 56.10, 56.78, 56.88 and 71.80).

The spectra were compared with previously reports of β-sitosterol and stigmasterol (Elgendy and Al-Ghamdy, 2007; Subhadhirasakul and Pechpongs, 2005; Sritularak, 1998a)



β-sitosterol



stigmasterol

Table 4.11 ^{13}C NMR spectrum data of mixture H-3 (in $CDCl_3$-*d*; ^{1}H and ^{13}C NMR (300 and 75 MHz, respectively)), β-sitosterol and stigmasterol (in $CDCl_3$-*d*; ^{1}H and ^{13}C NMR (300 and 75 MHz, respectively))

Position	β-sitosterol		stigmasterol		Mixture H-3	
	δ_C (ppm)	δ_H (ppm) (multiplicity)	δ_C (ppm)	δ_H (ppm) (multiplicity)	δ_C (ppm)	δ_H (ppm) (multiplicity)
1	36.2	-	39.0	-	36.14, 37.27	-
2	39.8	-	39.4	-	39.80, 39.70	-
3	71.8	3.50 (m)	70.5	3.52 (m)	71.80	3.54 (m)
4	42.3	-	50.6	-	42.32, 51.23	-
5	140.8	5.35 (s)	140.4	5.34 (s)	140.77	5.36 (d)
6	121.7	-	120.6	-	121.68	-
7	31.5	-	31.9	-	31.92	-
8	29.2	-	31.2	-	29.21, 31.86	-
9	45.8	-	49.5	-	45.88, 50.17	-
10	34.0	-	40.0	-	33.98, 40.43	-
11	21.1	-	20.6	-	21.09	-
12	31.9	-	36.7	-	31.92, 37.27	-
13	42.2	-	41.5	-	42.32	-
14	56.1	-	55.2	-	56.10, 56.00	-
15	24.3	-	24.8	-	24.29, 24.36	-
16	23.1	-	23.7	-	23.10	-
17	56.8	-	56.2	-	56.88, 56.78	-

Table 4.11 ^{13}C NMR spectrum data of mixture H-3 (in $CDCl_3$-*d*; ^{1}H and ^{13}C NMR (300 and 75 MHz, respectively)), β-sitosterol and stigmasterol (in $CDCl_3$-*d*; ^{1}H and ^{13}C NMR (300 and 75 MHz, respectively)) (continued)

Position	β-sitosterol		stigmasterol		Mixture H-3	
	δ_C (ppm)	δ_H (ppm) (multiplicity)	δ_C (ppm)	δ_H (ppm) (multiplicity)	δ_C (ppm)	δ_H (ppm) (multiplicity)
18	19.1	0.69 s	20.4	0.69 s	19.79, 21.09	0.70, 0.71 s
19	19.4	1.01 s	20.5	1.01 s	21.04, 21.20	1.02 s
20	28.3	-	31.0	-	28.88, 31.68	-
21	12.0	0.87 d	11.7	1.02 d	12.21, 11.97	0.93, 1.09 d
22	37.3	-	128.5	5.00 dd	37.27, 129.31	5.02 dd
23	36.5	-	137.7	5.14 dd	36.51, 138.04	5.17 dd
24	50.1	-	41.7	-	50.17, 42.32	-
25	31.7	-	35.9	-	31.86, 36.14	-
26	18.3	0.93 d	18.4	0.79 d	18.78, 18.98	0.94, 0.79 d
27	18.7	0.93 d	18.8	0.84 d	19.04, 19.38	0.94, 0.81d
28	26.1	-	28.3	-	26.16, 28.23	-
29	11.9	0.84 dd	11.2	0.80 t	12.04, 11.85	0.83 dd, 0.80 t

4.3 Anti-tyrosinase activity of pure compounds

Tyrosinase inhibitory activity of pure compounds were showed in table 4.12. The Artocarpanone (M-3) was the most potent anyi-tyrosinase activity with IC_{50} 44.56 μg/mL.

Artocarpanone is a compound of flavonoid group and related 4-substituted resorcinols, suggested that compounds with the 4-substituted resorcinol skeleton (see the figure 4.2) have potent tyrosinase inhibitory activity (Kim and Uyama, 2005). However, the additional group (such as hydroxyl and prenyl) at the 3 position (see the figure 4.2) also somewhat affected the activity (Kim and Uyama, 2005; Shimizu ***et al***, 2000). Thus, Artocarpin (M-1) and Cudraflavone C (M-2) did not showed tyrosinse inhibition.

Table 4.12 IC_{50} of pure compounds on tyrosinase inhibitory activity

Compound	IC_{50} (μg/mL)
Artocarpin (M-1)	> 200
Cudraflavone C (M-2)	> 200
Artocarpanone (M-3)	44.56
Long chain alkene (H-1)	> 200
Lanosterol (H-2)	> 200
Mixture of β-sitosterol and stigmasterol (H-5)	> 200
Kojic acid	31.43
Artocarpus lakoocha[W]	6.41
Artocarpus integer[E]	10.81

W = water wood extract; E = ethanol root extract

In the other hand, H-1 (long chain alkene), H-2 (mixture of β-sitosterol and stigmasterol) and H-3 (lanosterol), may be not showed functional group or side chain for interaction with active site of tyrosinase. Thus, these compounds did not show tyrosinase inhibition.

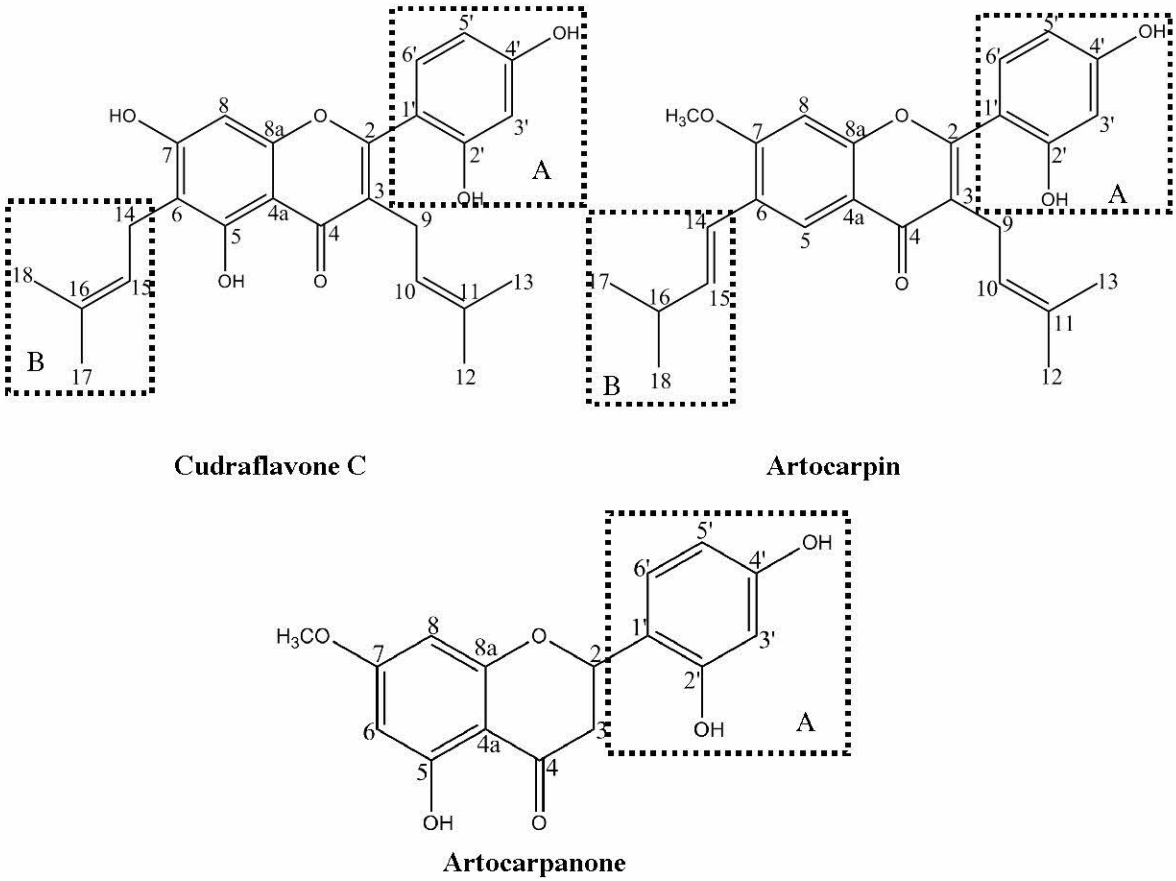


Figure 4.2 The chemical structures of Cudraflavone C, Artocarpin and Artocarpanone

The A box part: 4-substituented resorcinol skeleton

The B box part: long chain aliphatic group

4.4 Anti-microbial activity of pure compounds

Artocarpin (M-1) and Cudraflavone C (M-2) showed strong inhibitory effect against *Propionibacterium acnes, Staphylococcus aureus* and *S. epidermidis*. Artocarpin and Cudraflavone C are flavonoid compounds. The substituents of flavonoid as long chain aliphatic group at 6 or 8 positions and hydroxyl group at 2' and 4' positions (see figure 4.2) are important for anti-bacterial activity (Cushnie and Lamb, 2005). The MIC and MBC values of compounds showed in Table 4.13

Table 4.13 MIC and MBC of pure compounds against *P. acnes*, *S. aureus* and *S. epidermidis*

Compound	*P. acnes*		*S. aureus*		*S. epidermidis*	
	MIC	MBC	MIC	MBC	MIC	MBC
	µg/mL		µg/mL		µg/mL	
M-1	2	32	2	32	4	32
M-2	2	32	2	32	4	32
M-3	32	64	64	256	64	128
H-1	128	128	128	>256	128	256
H-2	128	128	128	256	128	256
H-3	128	128	128	256	128	256
Oxacillin	0.062	-	0.5	-	0.5	-

Even though, Artocarpin did not show tyrosinase inhibitory activity. In previously report, it showed cytotoxic activity and inhibitory activity on melanin biosynthesis on B16 melanoma cells (Arung ***et al***, 2008). Cudraflavone C (M-2) has the similar skeleton to Artocarpin, so it might be interested for studies of anti-melanogenesis and cytotoxic. By the way, Artocarpanone has been reported that it has inhibitions of both tyrosinase activity and melanin production in B16 melanoma cells (Arung ***et al***, 2006). Therefore, Artocarpin, Cudraflavone C and Artocarpanone are interesting to further study for whitening agent and anti-acne.

CHAPTER 5

CONCLUSION

In this investigation, the crude extracts from family Moraceae showed the potential inhibitory effects of anti-tyrosinase and anti-mocrobial activities, such as ***Artocarpus integer***, ***Ficus racemosa*** and ***Cudrania javanesis***. The root of ***A. integer*** was selected for phytochemical investigation because it showed the highest potential of anti-tyrosinase and anti-microbial activities. Five pure compounds and one mixture compound were isolated from the root of ***A. integer***.

Three pure compounds as Artocarpin (M-1), Cudraflavone C (M-2) and Artocarpanone (M-3) were isolated from methanol extract. The other two pure compounds and one mixture compound as Long chain alkene (H-1), Lanosterol (H-2) and mixed of β-Sitosterol with Stigmasterol (H-3), they were isolated from hexane extract.

Artocarpanone showed the potential of anti-tyrosinase activity. While Artocarpin and Cudraflavone C showed anti-microbial activity. Whereas, the other compounds as Long chain alkene, Lanosterol and the mixture of β-Sitosterol and Stigmasterol showed low activities on anti-tyrosinase and anti-mocrobial.

Thus, Artocarpin, Cudraflavone C and Artocarpanone are interesting for further study in order to provide possibilities for the development of new whitening and anti-acne agents from ***A. integer***.

BIBLIOGRAPHY

Achmad, S.A. Hakim, E.H., Juliawaty, L.D., Makmur, L., Suyatno, A.N. and Ghisalberti, E.L. 1996. A new prenylated flavone from ***Artocarpus champeden***. **Journal of Natural Products**. 59, 878-879.

Aida, M., Shinomiya, K., Hano, Y. and Nomura, T. 1993. Artonins J, K and L, new isoprenylated flavones from the root bark of ***Artocarpus heterophyllus*** Lamk. **Heterocycles**. 36, 575-583.

Aida, M., Shinomiya, K., Matsuzawa, K., Hano, Y. and Nomra, T. 1994. Artonins Q, R, S, T and U, five new prenylated phenols from the bark of ***Artocarpus heterophyllus*** Lamk. **Heterocycles**. 39, 847-858.

Aida, M., Yamaguchi, N., Hano, Y. and Nomura, T. 1997. Artonols A, B, C, D and E, five new isoprenylated phenols from the bark of ***Artocarpus communis*** Forst. **Heterocycles**. 45, 163-175.

Altman, L.J. and Zito, S.W. 1976. Sterol and triterpenes from the fruit of ***Artocarpus altilis***. **Phytochemistry**. 15, 829-830.

An, B.J., Choi, J.Y., Kwon, I.B., Nishioka, I. and Choi, C. 1992. Structure and isolation of glucosyltransferase inhibitor from jackfruit. Korean Biochemical Journal. 25 (4), 347-353.

Anonymous, 2010. Chromatographic techniques [online] Available from URL: http://en.wikipedia.org/wiki/Chromatography [Accessed October 14, 2010]

Anonymous, 2010. Column chromatography [online] Available from URL: http://en.wikipedia.org/wiki/Column_chromatography [Accessed October 14, 2010]

Anonymous, 2010. Thin layer chromatography [online] Available from URL: http://en.wikipedia.org/wiki/Thin_layer_chromatography [Accessed October 14, 2010]

Anonymous, 2010. Spectroscopic techniques [online] Available from URL: www.oc-praktikum.de/en/articles/pdf/Spectroscopy_en.pdf [Accessed October 14, 2010]

Arora, J.S., Sanhu, R.S., Kamboj, S.S. and Chopra, S.K. 1987. Occurrence and characterization of lympho-agglutinins in India plants. **Vox Sanguinis**. 52, 134-137.

Arung, E.T., Muladi, S., Sukaton, E., Shimizu, K. and Kondo, R. 2008. Artocarpin, a promising compound as whitening agent and anti-skin cancer. **Journal of Tropical Wood Science and Technology**. 6 (1), 33-36.

Arung, E.T., Shimizu, K. and Kondo, R. 2006. Inhibitory effect of Artocarpanone from ***Artocarpus heterophyllus*** on melanin biosynthesis. **Biological and Pharmaceutical Bulletin.** 29 (9), 1966-1969.

Athikomkulchai, S., Watthanachaiyingcharoen, R., Tunvichien, S., Vayumhasuwan, P., Karnsomkiet, P., Sae-Jong, P. and Ruangrungsi, N. 2008. The development of anti-acne products from ***Eucalyptus globulus*** and ***Psidium guajava*** oil. **Journal of Health Research**. 22 (3), 109-113.

Barik, B.R., Bhaumik, T., Dey, A.K. and Kundu, A.B. 1994. Triterpenoids of ***Artocarpus heterophyllus***. **Phytochemistry**. 35, 1001-1004.

Barton, D.H.R. 1951. Cycloartenone, a triterpenoid ketone. **Journal of the Indian Chemical Society**. 74, 1444-1451.

Cao, S., Butler, M.S. and Buss, A.D. 2003. Flavonoids from *Artocarpus lanceifolius*. **Natural Product Research**. 17, 79-81.

Chaisawadi, S., Thongbute, D., Methawiriyasilp, W., Chaisawadi, A., Pitakworrarat, N, Jaturronrasamee, K., and Suthipinitthum, A. 2008. Preliminary study of antimicrobial activities on medicinal herbs of Thai food's ingredients. Available: **http://www.scisoc.or.th/stt/28/web/content/J_10/J11.htm** (Accessed:2008, July 2)

Chakraborty, D.P. and Mandal, A.K. 1981. Aurantinamide acetate from *Artocarpus intregrifolia* Linn. **Journal of the Indian Chemical Society**. 58, 103.

Chan, S.C., Ko, H.H. and Lin, C.N. 2003. New prenylflavonoids from *Artocarpus communis*. **Journal of Natural Products**. 66, 427-430.

Chauhan, J.S. and Kumari, G. 1979. A new glycoflavanol from the root bark of *Artocarpus lakoocha*. **Planta Medica**. 37, 85-99.

Chauhan, J.S. and Kumari, G., Kumar, S. and Chaturvedi, R. 1982. Chemical examination of the root bark of *Artocarpus lakoocha*. **Proceedings of the National Academy of Sciences, India. Section A**. 52, 217-218.

Chen, C.C., Huang, Y.L., Ou, J.C., Lin, C.F. and Pan, T.M. 1993. Three new prenylflavones from *Artocarpus altilis*. **Journal of Natural Products**. 56, 1594-1597.

Chomnawang, M.T., Surassmo, S., Nukoolkarn, V.S. and Gritsanapan, W. 2005. Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. **Journal of Ethnopharmacology.** xxx, xxx-xxx.

Chung, M.I., Lu, C.M., Huang, P.L. and Lin, C.N. 1995. Prenylflavonoids of *Artocarpus heterophyllus*. **Phytochemistry**. 40, 1279-1281.

Chung, M.I., Ko, H.H., Yen, M.H., Lin, C.N., Yang, S.Z., Tsao, L.T. and Wang, J.P. 2000. Artocarpol A, a novel constituent with potent anti-inflammatory effect, isolated from *Artocarpus rigida*. **Helvetica Chimica Acta**. 83, 1200-1204.

Cidade, H.M., Nacimento, M.S., Pinto, M.M.M., Kijjoa, A., Silva, A.M. and Herz, W. 2001 Artelastocarpin and carpelastofuran, two new flavones and cytotoxicities of prenylflavonoids from *Artocarpus elasticus* against three cancer cell lines. **Planta Medica**. 67, 867-870.

Clausa, H. and Decker, H. 2006. Bacterial tyrosinases. **Systematic and Applied Microbiology**. 29, 3–14.

Cunha, C. and Socorro, M.P. 1994. Two flavonoids from *Clarisia racemosa*. **Journal of the Brazilian Chemical Society**. 5 (2), 101-105.

Cushnie, T.P.T. and Lamb, A. 2005. Antimicrobial activity of favonoids. **International Journal of Antimicrobial Agents**. 26, 343–356.

Daulatabad, C.D. and Mirajkar, A.M. 1989. Ricinoleic acid in *Artocarpus integrifolia* seed oil. **Journal of the American Oil Chemists' Society**. 66, 1631.

Dayal, R. and Seshadri, T.R. 1974. Colourless components of the roots of *Artocarpus heterophyllus*: isolation of a new compound, artoflavanone. **Indian Journal of Chemistry**. 12, 895-896.

Department of Medical Science (DMSC), Ministry of Public Health, Thailand. 2001. สารทำให้ผิวขาว. **ความรู้ทั่วไปเกี่ยวกับสิ่งเป็นพิษ**. 15, 9 – 14.

Durand, E., Ellington, E.V., Feng, P.C., Haynes, L.J., Magnus, K.E. and Philip, N. 1962. Simple hypotensive and hypertensive principle from some West Indian medicinal plants. **Journal of Pharmacy and Pharmacology**. 14,562-566.

Elgendy, E.M. and Al-Ghamdy, H. 2007. Thermal and photooxidation reactions of the steroids: β-Sitosterol, Stigmasterol and Diosgenin. **Taiwan Pharmaceutical Journal**, 59,113-132.

Emmons, G.T., Wilson, W.K. and Schroepfer, G.J. 1989. ^{1}H and ^{13}C NMR assignments of lanostan-3β-ol derivatives: Revised assignments for lanosterol. **Magnetic Resonance in Chemistry**. 27, 1012-1024.

Ersam, T., Achmad, S.A., Ghisalberti. E.L., Hakim, E.H., Makmur, L. and Syah, Y.M. 2002. A new isoprenylated chalcone, artoindonesianin J, from the root and tree bark of *Artocarpus bracteata*. **Journal of Chemical Research Synopsis**. 4, 186-187.

Ettre, L.S. 1993. Nomenclature for chromatography. **Pure and Applied Chemistry.** 65 (4), 819-872.

Euis, H., Hakim, L.D., Juliawaty, Y.M.. Syah, A.A. and Sjamsul, A.A. 2005. Molecular diversity of *Artocarpus champeden* (Moraceae): A species endemic to Indonesia. **Molecular Diversity**. 9, 149-158.

Ferreira de Miranda-Santos, I.K., Delgado, M., Bonini, P.V., Bunm, M.M.M. and Campos, N.A. 1992. A crude extract of *Artocarpus integrifolia* contains two lectins with distinct biological activities. **Immunology Letters**. 31, 65-71.

Field, F.H. 1968. Physical and inorganic chemistry: Chemical ionization mass spectrometry. VII. alkenes and alkynes. **Journal of The American Chemical Society**. 90 (21), 5649-5656.

Fujimoto, Y., Zhang, X.X., Kirisawa, M., Uzawa, J. and Sumatra, M. 1990. New flavones from *Artocarpus communis* Forst. **Chemical and Pharmaceutical Bulletin**. 38, 1787-1789.

Gąsowska, B., Frąckowiak, B., and Wojtasek, H. 2006. Indirect oxidation of amino acid phenylhydrazides by mushroom tyrosinase. **Biochemica et Biophysica Acta**, 1373–1379.

García-Molina, F., Fenoll, L.G., Morote, J.C., García-Ruiz, P.A., Rodríguez-López, J.N., García-Cánovas, F. and Tudela, J. 2005. Opposite effects of peroxidase in the initial stages of tyrosinase-catalysed melanin biosynthesis. **The International Journal of Biochemistry & Cell Biology.** 37, 1179–1196.

Giwanon, R., Rungsri, S., Rerk-am, U., Tangstirapekdee, S., Srisom, T. and Suntomtanasart, T. 2006. Antimicrobial efficacy of *Curcuma aromatics* L. extract and *Morus alba* L. extract against *Propionibacterium acnes*, *Staphylococcus epidermedis* and *Staphylococcus aureus*. **47th Annual meeting on Folk Botanical Wisdom: Towards Global Markets**. June 5-9, 2006. Chiang Mai, Thailand.

Hakim, E.H., Fahriyati, A., Kau, M.S., Achmad, S.A., Makmur, L., Ghisalberti, E.L. and Nomura, T. 1999. Artoindonesianins A and B, two new prenylated flavones from the root of *Artocarpus champeden*. **Journal of Natural Products**. 62, 613-615.

Hakim, E.H., Asnizar, Y., Aimi, N., Kitajima, M. and Takayama, H. 2002a. Artoindonesianin P, a new prenylated flavone with cytotoxicity from *Artocarpus lanceifolius*. **Fitoterapia**. 73, 668-673.

Hakim, E.H., Ulinnuha, U.Z., Syah, Y.M. and Ghisalberti. E.L. 2002b. Artoindonesianins N and O, new prenylated stilbene and prenylated arylbenzofuran derivatives from *Artocarpus gomezianus*. **Fitoterapia**. 73, 597-603.

Hakim, E.H., Achmad, S.A., Juliawaty, L.D., Makmur, L., Syah, Y.M., Aimi, N., Kitajima, M., Takayama, H. and Ghisalberti, E.L. 2006. Prenylated flavonoids and related compounds of the Indonesia *Artocarpus* (Moraceae). **Journal of Natural Medicines**. 60, 161-184.

Han, A.R., Kang, Y.J., Windono, T., Lee, S.K. and Seo, E.K. 2006. Prenylated flavonoids from the heartwood of *Artocarpus communis* with inhibitory activity on lipopolysaccharide-induced nitric oxide production. **Journal of Natural Products**. 69, 719-721.

Hano, Y., Aida, M., Shiina, M., Nomura, T., Kawai, T., Ohe, H. and Kagei, K. 1989. Artonins A and B, two new prenylflavones from the root bark of *Artocarpus heterophyllus* Lamk. **Heterocycles**. 29, 1447-1453.

Hano, Y., Aida, M. and Nomura, T. 1990a. Two new natural Diels-Alder-type adducts from the root bark of *Artocarpus heterophyllus*. **Journal of Natural Products**. 53, 391-395.

Hano, Y., Inami, R. and Nomura, T. 1990b. Components of the bark of *Artocarpus rigida* Bl. I. Structures of to new isoprenylated flavones, artonins G and H. **Heterocycles**. 31, 2137-2179.

Hano, Y., Yamagami, Y., Kobayashi, M., Isohata, R. and Nomura, T. 1990c. Artonins E and F, two new prenylflavones from the bark of ***Artocarpus communis*** Forst. **Heterocycles**. 31, 877-882.

Hano, Y., Inami, R. and Nomura, T. 1993. Component of the bark of ***Artocarpus rigida*** Bl. II. Structures of four new isoprenylated flavone derivatives, artonins M, N, O and P. Heterocycles. 35, 1341-1350.

Hano, Y., Inami, R. and Nomura, T. 1994. Constituents of Moraceae plant. XX. A novel flavone, artonin V, from the root bark of ***Artocarpus altilis***. **Journal of Chemical Research Synopsis**. 349.

Hoogduijn, M.J., Cemeli, E., Ross, K., Anderson, D., Thody, A.J. and Wooda, J.M. 2004. Melanin protects melanocytes and keratinocytes against H_2O_2-induced DNA strand breaks through its ability to bind Ca^{2+}. **Experimental Cell Research**. 294, 60– 67.

Jablonski, G.N. and Chaplin, G. 2002. Throughout the world; human skin color has evolved to be dark enough to prevent sunlight from destroying the nutrient folate but light enough to foster the production of vitamin D. **Journal Scientific American**. 287; 4, 50-55.

Jayasinghe, L., Balasooriya, B.A.I.S., Padmini, W.C., Hara, N. and Fujimoto, Y. 2004. Geranyl chalcone derivative with antifungal and radical scavenging properties from the leaves of ***Artocarpus nobilis***. **Phytochemistry**. 65, 1287-1290.

Jayasinghe, L., Rupasinghe, G.K., Hara, N. and Fujimoto, Y. 2006. Geranylated phenolic constituents from fruits of ***Artocarpus nobilis***. **Phytochemistry**. 67, 1353-1358.

Kapil, R.S. and Joshi, S.S. 1960. Chemical constituents of *Artocarpus lakoocha*. **Journal of Science India Research (India)**. 19B, 498.

Karioti, A., Protopappa, A., Megoulasb, N. and Skaltsaa, H. 2007. Identification of tyrosinase inhibitors from *Marrubium velutinum* and *Marrubium cylleneum*. **Bioorganic & Medicinal Chemistry**. 15, 2708–2714.

Kielland, I.S. and Malterud, K.E. 1994. Triterpenoids from *Artocarpus integrifolia* fruits. **Planta Medica**. 60, 196.

Kijjoa, A., Cidade, H.M., Pinto, M.M.M., Gonzales, M.J.T.G., Anantachoke, C., Gedris, T.E. and Herz, W. 1996. Prenylflavonoids from *Artocarpus elasticus*. **Phytochemistry**. 43, 691-694.

Kijjoa. A., Cidade, H.M., Gonzalez, M.T.G., Afonzo, C.M., Silva, A.M.S. and Herz, W. 1998. Further prenylflavonoids from *Artocarpus elasticus*. **Phytochemistry**. 47, 875-878.

Kiken, D.A. and Cohen, D.E., 2002. Contact dermatitis to botanical extracts. **American Journal of Contact Dermatitis**. 13, 148–152.

Kim, Y.J. and Uyama H. 2005. Tyrosinase inhibitors from natural and synthetic sources : structure, inhibition mechanism and perspective. **Cellular and Molecular Life Sciences**. 65, 1707 – 1723.

Kingroungpet, B. 1994. Phytochemical study of *Artocarpus gomezinus* Wall. ex Tréc. Leaves. **Master's thesis**, Department of pahrmacognosy, Graduate School, Chulalongkorn University.

Ko, H.H., Lin, C.N. and Yang, S.Z. 2000. New constituents of *Artocarpus rigida*. **Helvetica Chimica Acta**. 83, 3000-3005.

Ko, H.H., Lin, Y.H., Yang, S.Z., Won, S.J. and Lin, C.N. 2005. Cytotoxic prenylflavonoids from *Artocarpus elasticus*. **Journal of Natural Products**. 68, 1692-1695.

Kongcharoensuntorn, W., Chomosot, N., Jindamol, J., Arrayasillapathon, J., Charadram, P., Ounarom, S., and Pittayaphasertgul, A. 2005. Screening of some Thai medicinal plants for antimicrobial activity and antioxidant activity against microorganisms, **31st Congress on Science and Technology of Thailand at Suranaree University of Technology**, 18-20 October 2005.

Kumar, G.S., Jayaveera, K.N., Kumar, C.K.A., Sanjay, U.P., Swamy. B.M.V. and Kumar, D.V.K. 2007. Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. **Journal of Pharmaceutical Research**. 6 (2), 717-723.

Kumar, N.S., Pavanasasivam, G., Sultanbawa, M.U.S. and Mageswaran, R. 1977. Chemical investigation of Ceylonese plants. Part 24. New chromenoflavonoids from the bark of *Artocarpus nobilis* Thw. (Moraceae). **Journal of the Chemical Society, Perkin Transactions 1**, 1243-1251.

Kummee, S. and Intaraksa, N. 2008. Antimicrobial activity of *Desmos chinensis* leaf and *Maclura cochinchinensis* wood extract. **Songklanarin Journal of Science and Technology**. 30 (5), 635-639.

Lee, S.H., Choi, S.Y., Kim, H., Hwang, J.S., Lee, B.G., Gao, J.J. and Kim, S.Y. 2002. Mulberroside F isolated from the leaves of *Morus alba* inhibits melanin biosynthesis. **Biological and Pharmaceutical Bulletin**. 25, 1045 -1048.

Likhitwitayawuid, K., Sritularak, B. and De-Eknamkul, W. 2000. Tyrosinase inhibitors from *Artocarpus gomezianus*. **Planta Medica**. 66, 275-277.

Likhitwitayawuid, K. and Sritularak, B. 2001. A new dimeric stilbene with tyrosinase inhibitory activity from *Artocarpus gomezianus*. **Journal of Natural Products**. 64, 1457-1459.

Likhitwitayawuid, K., Chaiwiriya, S., Sritularak, B. and Lippipun, V. 2006. Antiherpetic flavones from the heartwood of *Artocarpus gomezianus*. **Chemistry and Biodiversity**. 3, 1138-1143.

Lin, C.N. and Shieh, W.L. 1991. Prenylflavonoids and a pyranodihydrobenzoxanthone from *Artocarpus communis*. **Phytochemistry**. 30, 1669-1671.

Lin, C.N., Shieh, W.L. and Jong, T.T. 1992. A pyranodihydrobenzoxanthone epoxide from *Artocarpus communis*. **Phytochemistry**. 31, 2563-2564.

Lin, C.N., Lu, C.M. and Huang, P.L. 1995. Flavonoids from *Artocarpus heterophyllus*. **Phytochemistry**. 39, 1447-1451.

Lorian, V. 2005. **Antibiotics in laboratory medicine**. 5th ed., Lippincott Williams & Wilkins, USA, pp.1-265.

López-Serrano, D., Solano, F. and Sanchez-Amat, A. 2004. Identification of an operon involved in tyrosinase activity and melanin synthesis in *Marinomonas mediterranea*. **Gene**. 342, 179 - 187.

Lu, C.M. and Lin, C.N. 1993. Two 2', 4', 6'-trioxygenated flavanones from *Artocarpus heterophyllus*. **Phytochemistry.** 33, 909-911.

Lu, C.M. and Lin, C.N. 1994. Flavonoids and 9-hydroxytridecyl docosanoate from *Artocarpus heterophyllus*. **Phytochemistry**. 35, 781-783.

Lu, Y.H., Lin, C.N., Ko, H.H., Yang, S.Z., Tsao, L.T. and Wang, J.P. 2002. Two novel and anti-inflammatory constituents of *Artocarpus rigida*. **Helvetica Chimica Acta**. 85, 1626-1632.

Lu, Y.H., Lin, C.N., Ko, H.H., Yang, S.Z., Tsao, L.T. and Wang, J.P. 2003. Novel anti-inflammatory constituents of *Artocarpus rigida*. **Helvetica Chimica Acta**. 86, 2566-2572.

Mahato, S.B., Banerjee, S.K. and Chakravatri, R.N. 1971. Triterpenes of the stem bark of *Artocarpus chaplasha*. **Phytochemistry**. 10, 1351-1354.

Marienthal, M. And Franklin, P.J. 1995. Preparation of lanosterol from bromalanosterol. **The Journal of Organic Chemistry**. 20 (12), 1627-1630.

Makmur, L., Tukiran, S., Achmad, S.A., Aimi, N., Hakim, E.H., Kitajima, M. and Takayama, H. 2000. Artoindonesianin C, a new xanthone derivative from *Artocarpus teymanii*. **Journal of Natural Products**. 63, 243-244.

Mongolsuk, S., Robertson, A. and Towers, R. 1957. 2, 4, 3'. 5'-Tetrahydroxystilbene from *Artocarpus lakoocha*. **Journal of Chemical Society.** 2231-2233.

National Science Museum, Ministry of Science and Technology, Thailand. 2007. เมลานิน Available: **http://www.nsm.or.th/modules.php?name=News&file=article&sid=164** (Accessed: 2007, June 6)

Niyomkam, P. 2007. Screening of antibacterial activity of Thai medicinal plants against *Propionobacterium acnes* and preliminary formulation study. **A Thesis in Partial Fulfillment of the Requirements for the Degree of Mater of Pharmacy in Pharmaceutical Sciences**. Prince of Songkla University. Songkhla, pp. 47-51.

No, J.K., Soung, D.Y., Kim, Y.J., Shim, K.H., Jun, Y.S., Rhee, S.H., Yokozawa, T. and Chung, H.Y. 1999. Inhibition of tyrosinase by green tea components. **Pharmacology Letters**. 65 (21). 241-246.

Nogueira, P.L. and Correia, A.A. 1958. Chemical study of a tropical wood. **Garcia de Orta**. 6, 679-688.

Okunji, C., Komarnytsky, S., Fear, G., Poulev, A., Ribnicky, D.M., Awachie, P.I., Ito, Y., Raskin, I. 2007. Preparative isolation and identification of tyrosinase inhibitors from the seeds of *Garcinia kola* by high-speed counter-current chromatography. **Journal of Chromatography A**. 1151, 45–50.

Pant, T. and Chaturvedi, K. 1989. 4-Hydroxyundecyl docosanoate and cycloartenone in *Artocarpus integra* latex. **Phytochemistry**. 28, 2197-2199.

Parthasarathy, P.C., Radhakrishnan, P.V., Rathi, S.S. and Venkataraman, K. 1969. Coloring mattres of the wood of *Artocarpus heterophyllus*. Part V. Cycloartocarpesin and oxydihydroartocarpesin, two new flavones. **Indian Journal of Chemistry**. 7, 101-102.

Pavanasasivam, G. and Sultanbawa, M.U.S. 1973. Cycloartenyl acetate, cycloartenol and cycloartenone in the bark of *Artocarpus* species. **Phytochemistry**. 12, 2725-2726.

Pendse, A.D., Pendse, R., Rao, A.V.R. and Venkataraman, K. 1976. Integrin, cyclointegrin and oxyisocyclointegrin, three new flavones from the heartwood of *Artocarpus integer*. **Indian Journal of Chemistry**. 14B, 69-72.

Peñalver, M.J., Fenoll, L.G., Rodríguez-López, J.N., García-Ruiz, P.A., García-Molina, F., Varón, R., García-Cánovas, F. and Tudela, J. 2005. Reaction mechanism to explain the high kinetic autoactivation of tyrosinase. **Journal of Molecular Catalysis B: Enzymatic.** 33, 35–42.

Pereira, J.R., Medina, H. and Bustos, R.E. 1962. On the presence of acetylcholine in seeds of *Artocarpus integrifolia* (Moraceae) and of *Annona squamosa* (Annonaceae). **Annals of Faculty of Medical of University Parana**. 5, 45-47.

Petit, L. and Piérard, G.E. 2003. Skin-lightening products revisited. **International Journal of Cosmetic science**. 25, 169-181.

Phongpaichit, S., Kummee, S., Nilrat, L. and Itarat, A. 2006. Antimicrobial activity of *Cinnamomum porrectum* oil. **Songklanakarin Journal of Science and Technology**. 29 (1), 11-16.

Puntumchai, A., Kittakoop, P., Rajviroongit, Vimuttipong, S., Likhitwitayawuid, K. and Thebtaranonth, Y. 2004. Lakoochins A and B, new antimycobacterial stilbene derivatives from *Artocarpus lakoocha*. **Journal of Natural Products**. 67, 485-486.

Radhakrishnan, P.V., Rao, A.V.R. and Venkataraman, K. 1965. Two new flavones from *Artocarpus heterophyllus*. **Tetrahedron Letters**. 663-667.

Rao, A.V.R., Rathi, S.S. and Venkataraman, K. 1972. Chaplashin, a flavone contaning an oxepine ring from the heartwood of *Artocarpus chaplasha* Roxb. **Indian Journal of Chemistry**. 10, 909-907.

Sasaki, K. and Yoshizaki, F. 2002. Nobiletin as a tyrosinase inhibitor from the peel of *Citrus* fruit. **Biological and Pharmaceutical Bulletin**. 25, 806-808.

Sawai, S., Akashi, T., Sakurai, N., Suzuki, H., Shibata, D., Ayabe, A.-I. and Aoki, T. 2006. Plant lanosterol synthase: Divergence of the sterol and triterpene biosynthetic pathways in eukaryotes. **Plant and Cell Physiology**. 47; 5, 673-677.

Seo, E.K., Lee, D., Shin, Y.G., Chai, H.B., Navarro, H.A., Kardono, L.B.S., Rahman, I., Cordell, G.A., Farnsworth, N.R., Pezzuto, J.M., Kinghorn, A.D., Wani, M.C. and Wall, M.E. 2003. Bioactive prenylated flavonoids from the stem bark of *Artocarpus kemando*. **Archives of Pharmacal Research**. 26, 124-127.

Shieh, W.L. and Lin, C.N. 1992. A quinonoid pyranobenzoxanthone and pyranodihydrobenzoxanthone from *Artocarpus communis*. **Phytochemistry**. 31, 364-367.

Shimizu, K., Kondo, R. and Sakai, K. 1997. A stilbene derivative from *Artocarpus incisus*. **Phytochemistry**. 45, 1297-1298.

Shimizu, K., Kondo, R., Sakai, K., Lee, S.H. and Sato, H. 1998. The inhibitory components from *Artocarpus incisus* on melanin biosynthesis. **Planta Medica**. 64, 408-412.

Shimizu, K., Kondo, R., Sakai, K., Buabarn, S. and Dilokkunanant, U. 2000. A geranylated chalcone with 5α-reductase inhibitory properties from *Artocarpus incisus*. **Phytochemistry**. 54, 737-739.

Shinomiya, K., Aida, M., Hano, Y. and Nomura, T. 1995. A Diels-Alder-type adduct from *Artocarpus heterophyllus*. **Phytochemistry**. 40, 1317-1319.

Soekamto, N.H., Achmad, S.A., Ghisalberti, E.L., Hakim, E.H. and Syah, Y.M. 2003. Artoindonesianins X and Y, two isoprenylated 2-arylbenzofurans, from *Artocarpus fretessi* (Moraceae). **Phytochemistry**. 64, 831-834.

Sritularak, B. 1998a. Chemical constituents of ***Artocarpus lakoocha*** and ***A. gomezianus***. **Master's thesis**, Department of Pharmacognosy, Gradute School, Chulalongkorn University.

Sritularak, B., De-Eknamkul, W. and Likhitwitayawuid, K. 1998b. Tyrosinase inhibitors from *Artocarpus lakoocha*. **Thai Journal Pharmaceutical Science**. 22, 149–155.

Stoddard, J.M., Nguyen, L., Mata-Chavezand, H. and Nguyen, K. 2007. TLC plates as a convenient plat form for solvent-free reactions. **Chemical Communication**. 1240-1241.

Su, B.N., Cuendet, M., Hawthorne, M.E., Kardono, L.B.S., Riswan, S., Fong, H.H.S., Mehta, R.G., Pezzuto, J.M. and Kinghorn, A.D. 2002. Constituents of the bark and twigs of *Artocarpus dadah* with cyclooxygenase inhibitory activity. **Journal of Natural Products**. 65, 163-169.

Subhadhirasakul, S. and Pechpongs, P. 2005. A terpenoid and two steroids from the flowers of *Mammea siamensis*. **Songklanakarin Journal Science and Technology**. 27 (2), 555-561

Suhartati, T., Achmad, S.A., Aimi, N., Hakim, E.H., Kitajima, M., Takayama, H. and Takeya, K. 2001. Artoindonesianin L, a new prenylated flavone with cytotoxic activity from *Artocarpus rotunda*. **Fitoterapia**. 72, 912-918.

Sultanbawa, M.U.S. and Surendrakumar, S. 1989. Two pyranodihydrobenzoxanthones from *Artocarpus nobilis*. **Phytochemistry**. 28, 599-605.

Suresh, G.K., Appukuttan, P.S. and Basu, D.K. 1982. α-D-Galactose specific lectin from jackfruit (*Artocarpus integra*) seed. **Journal of Biological Sciences**. 4, 257-261.

Syah, Y.M., Achmad, S.A., Ghisalberti, E.L., Hakim, E.H., Makmur, L. and Mujahidin, D. 2001. Artoindonesianins G-I, three new isoprenylated flavones from *Artocarpus lanceifolius*. **Fitoterapia**. 72 765-773.

Syah, Y.M., Achmad, S.A., Ghisalberti, E.L., Hakim, E.H., Makmur, L. and Mujahidin, D. 2002b. Artoindonesianins Q-T, four isoprenylated flavones from *Artocarpus chempeden* Spreng. (Moraceae). **Phytochemistry**. 61, 949-953.

Syah, Y.M., Achmad, S.A., Ghisalberti, E.L., Hakim, E.H. and Mujahidin, D. 2004. Two new cytotoxic isoprenylated flavones, artoindonesianins U and V, from the heartwood of *Artocarpus champeden*. **Fitoterapia**. 75, 134-140.

Syah, Y.M., Achmad, S.A., Aimi, N., Hakim, E.H., Juliawawty, L.D. and Takayama, H. 2006a. Two prenylated flavones from the tree bark of *Artocarpus lanceifolius*. **Zeitschrift für Naturforsch**. 61, 1134-1137.

Syah, Y.M., Juliawawty, L.D., Achmad, S.A., Hakim, E.H. and Ghisalberti, E.L. 2006b. Cytotoxic prenylated flavones from *Artocarpus champeden*. **Journal of Natural Medicines**. 60, 308-312.

Tengamnuay, P., Pengrungruangwong, K., Pheansri, I. and Likhitwitayawuid, K. 2006. *Artocarpus lakoocha* heartwood extract as novel cosmetic ingredient: evaluation of the ***in vitro*** anti-tyrosinase and ***in vivo*** skin whitening activities. **International Journal of Cosmetic Science**. 28, 269-276.

Ultee, A.J. 1949. Components of samples of latex (from a number of tropical trees). **Pharmaceutisch weekblad**. 84, 65-70.

Venkataraman, K. 1972. Wood phenolics in the chemotaxonomy of the Moraceae. **Phytochemistry**. 11, 1571-1586.

Wang, K.H., Lin. R.D., Hsud. F.L., Huange. Y.H., Chang. H.C., Huang. C.Y., Lee. M.H. 2006. Cosmetic applications of selected traditional Chinese herbal medicines. **Journal of Ethnopharmacology**. 106, 353–359.

Wang, N. and Hebert, N.D. 2006. Tyrosinase maturation through the mammalian secretory pathway: bringing color to life. **Pingment Cell Research**. 19, 3-18.

Wang, Y.H., Hou, A.J., Chen, L., Chen, D.F., Sun, H.D., Zhao, Q.S., Bastow, K.F., Nakanish, Y., Wang, X.H. and Lee, K.H. 2004. New isoprenylated flavones, artochamins A-E and cytotoxic principles from *Artocarpus chama*. **Journal of Natural Products**. 67, 757-761.

Wang, Y.H., Hou, A.J. and Chen, D.F. 2007a. Two new isoprenylated stilbene from *Artocarpus chama*. **Journal of Integrative Plant Biology.** 49, 605-608.

Wang, Y.H., Xu, K., Lin, L., Pan, Y. And Zheng, X. 2007b. Geranyl flavonoids from the leaves of *Artocarpus altilis*. **Phytochemistry**. 68, 1300-1306.

Wei, B.L., Weng, G.R., Chiu, P.H., Hung, C.F., Wang, J.P. AND Lin, C.N. 2005. Antiinflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. **Journal of Agricultural and Food Chemistry**. 53, 3867-3871.

Weng, J.R., Chan, S.C., Lu, Y.H., Lin, H.C., Ko, H.H. and Lin, C.N. 2006. Antiplatelet prenylflavonoids from *Artocarpus communis*. **Phytochemistry**. 67, 824-829.

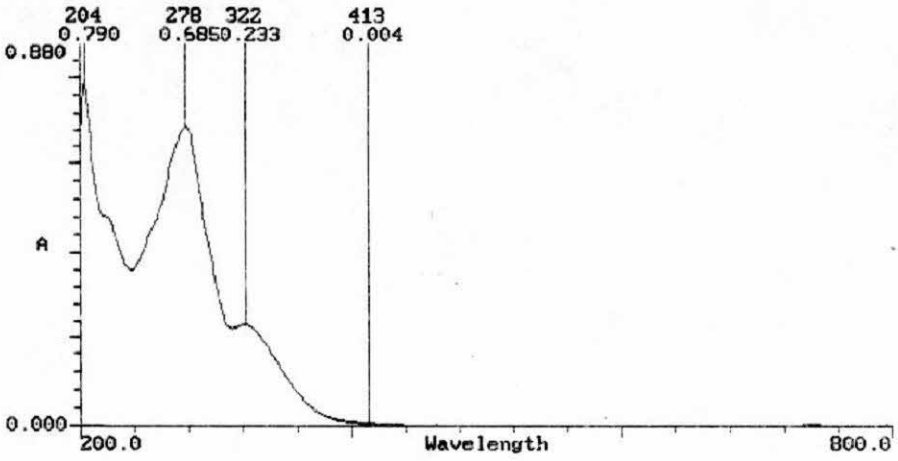
Wetwitayaklung, P. 1994. Chemical constituents from the heartwood of ***Artocarpus lakoocha*** Roxb. **Master's thesis**, Department of Pharmacognosy, Graduate School, Chulalongkorn University.

Widyawaruyanti, A., Subehan, Kalauni, S.K., Awale, S., Nindatu, M., Zajni, N.C., Syzfruddin, D., Asih, P.B.S., Tezuka, Y. and Kadota, S. 2007. New prenylated flavones from ***Artocarpus champeden*** and their antimalarial activity ***in vitro***. **Journal of Natural Medicines**. 61, 410-413.

APPENDIX

TEST SETUP
GENESYS 6 v2.001 2M9M008001

Scanning	23:11 4May10
Test Name	C-3-3
Measurement Mode	Absorbance
Start Wavelength	200.0nm
Stop Wavelength	800.0nm
Sample Positioner	Auto 6
Scan Speed	Fast
Interval	1.0nm
Cell Correction	Off
ID# (0=OFF)	1
Auto Print	On
Auto Save Data	Off



ID#: 1
Smoothing [On]

Wavelength	Abs	
204.0	0.790	Peak
278.0	0.685	Peak
322.0	0.233	Peak
413.0	0.004	Peak

Figure A1 UV-Visible spectrum of M-1 in Methanol

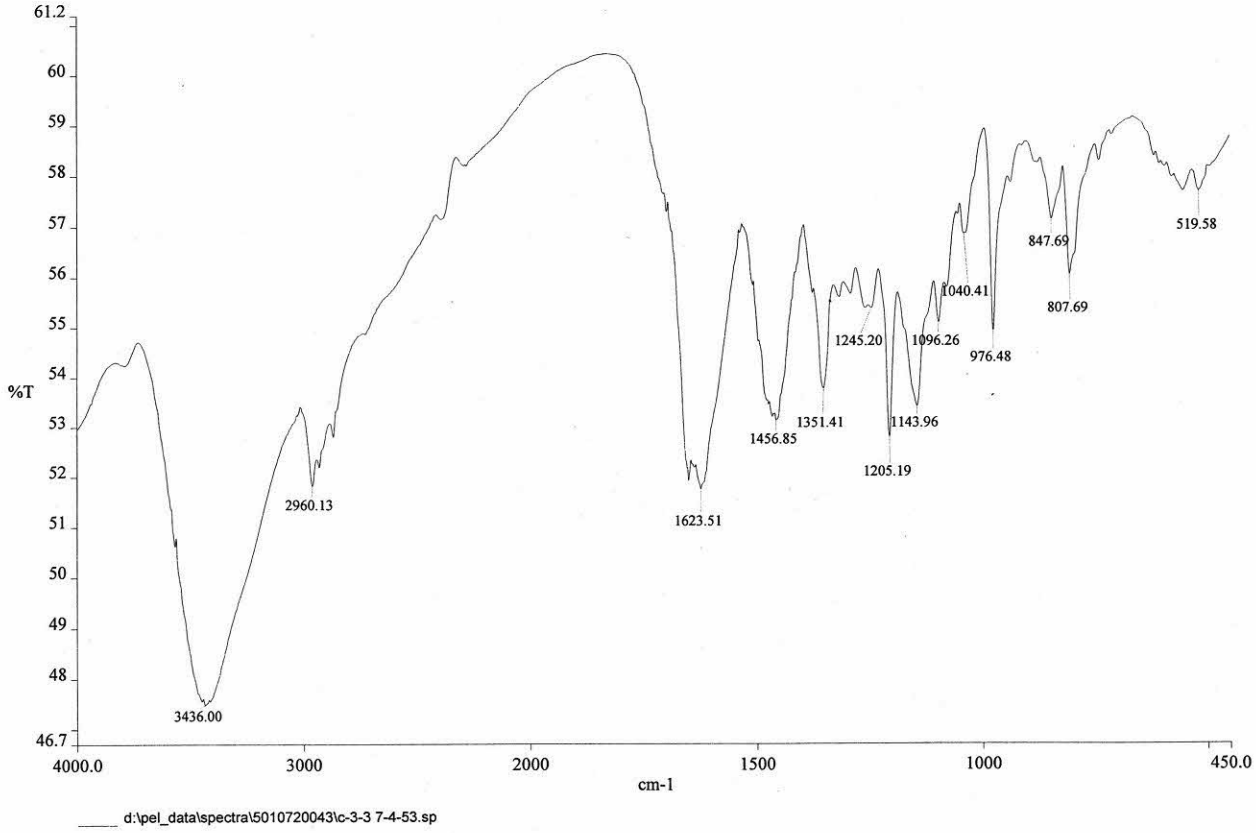


Figure A2 IR spectrum of compound M-1 (KBr disc)

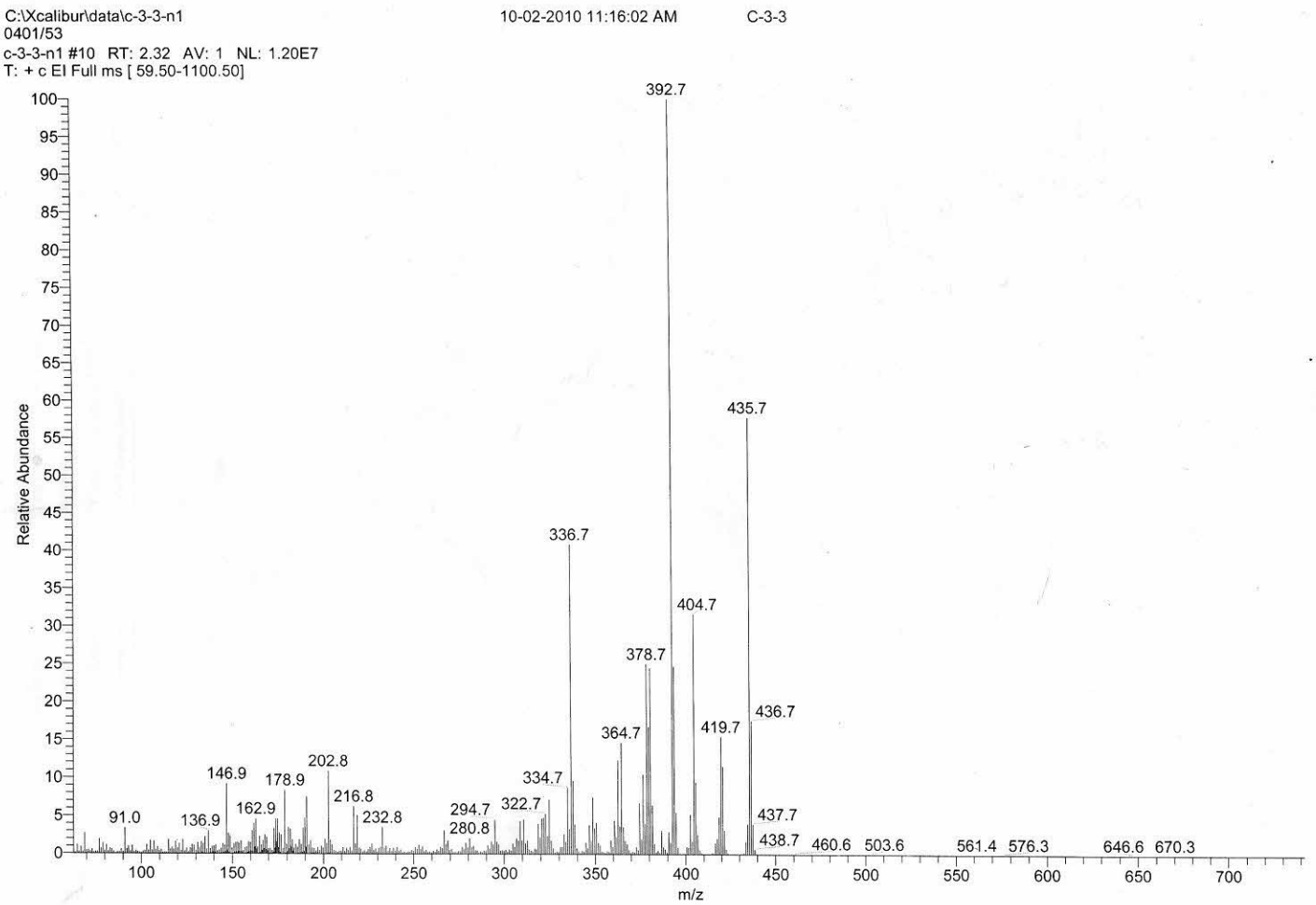


Figure A3 EI mass spectrum of compound M-1

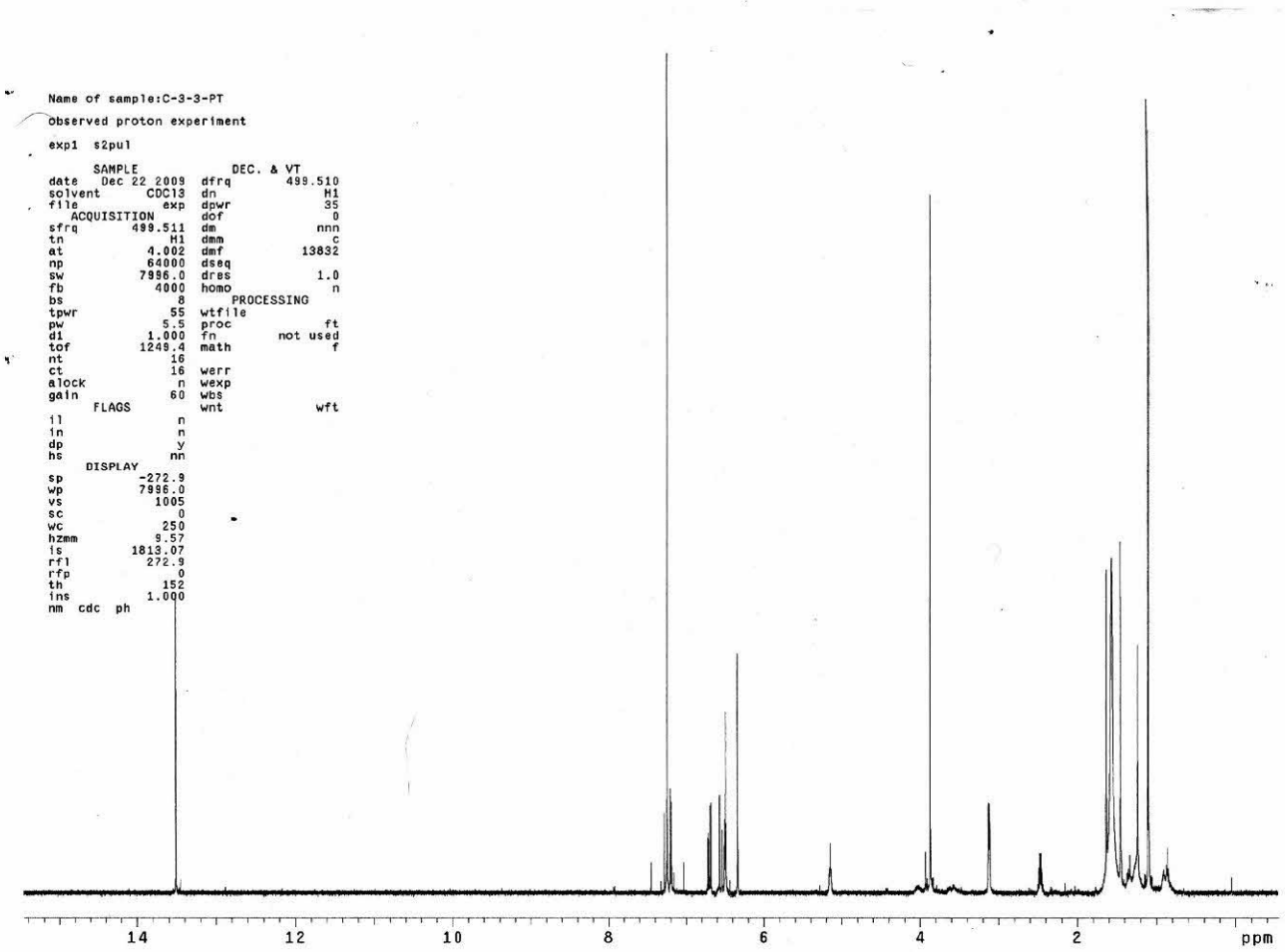


Figure A4 500 MHz ^{1}H spectrum of compound M-1 (in chloroform –*d*)

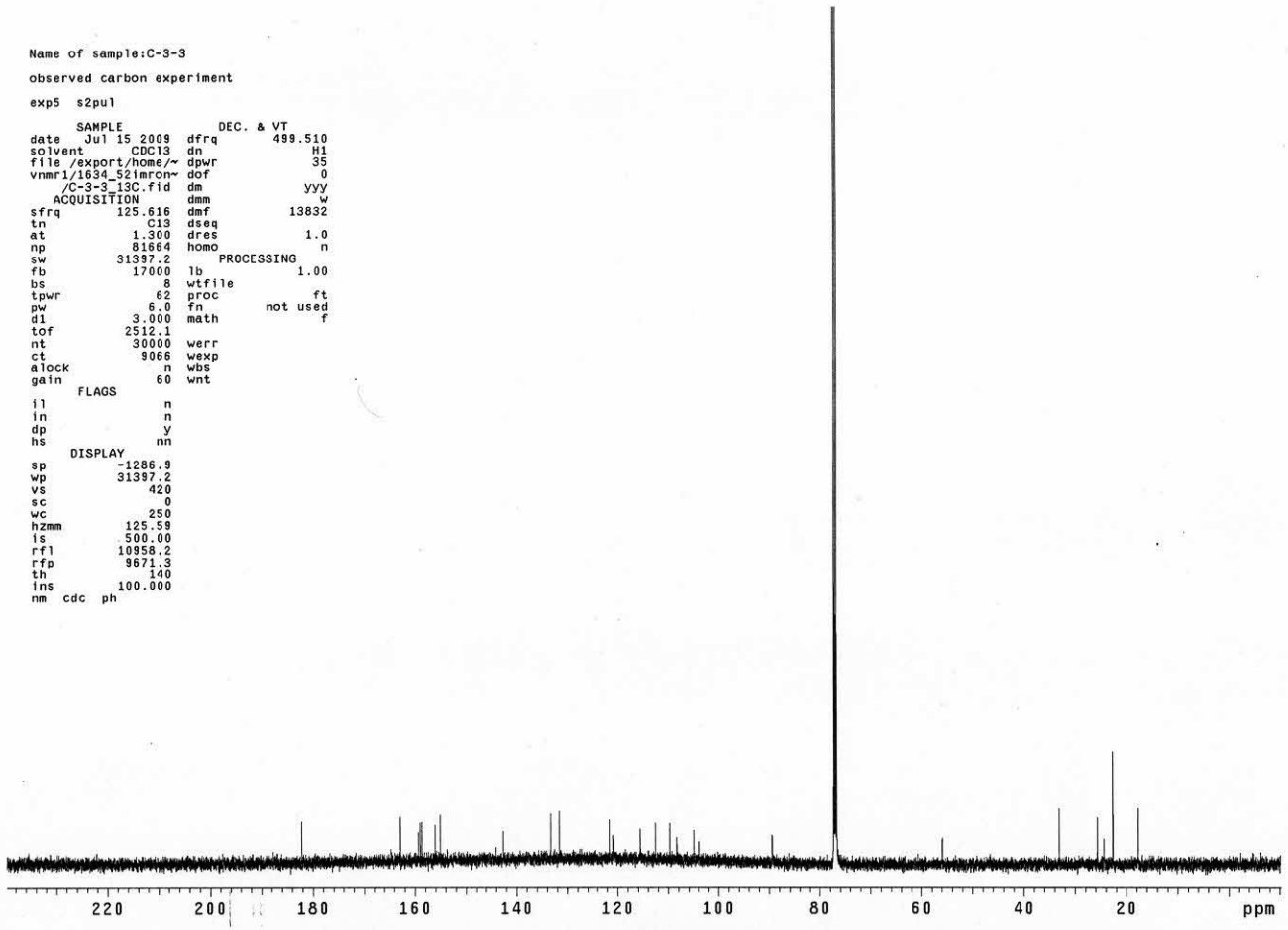


Figure A5 125 MHz ^{13}C spectrum of compound M-1 ((in chloroform –*d*)

Name of sample:C-3-3

DEPT90 experiment

CH only

exp2 dept

SAMPLE		DEC. & VT	
date	Jul 21 2009	dfrq	499.510
solvent	cdcl3	dn	H1
file	exp	dpwr	38
ACQUISITION		dof	0
sfrq	125.611	dm	nny
tn	C13	dmm	ccw
at	1.301	dmf	11768
np	58816	dseq	
sw	22605.3	dres	1.0
fb	12000	homo	n
bs	8	PROCESSING	
ss	8	lb	1.00
tpwr	54	wtfile	
pw	9.5	proc	ft
d1	2.000	fn	not used
tof	-1739.0	math	f
nt	2000		
ct	1531	werr	
alock	n	wexp	procplot
gain	60	wbs	
pp	10.5	wnt	
pplvl	61		
j	140.0		
mult	1.0		
satdly	0		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-1142.7		
wp	22605.3		
vs	3820		
sc	0		
wc	250		
hzmm	90.42		
is	500.00		
rfl	19055.8		
rfp	17913.1		
th	6		
ins	100.000		

ai cdc ph

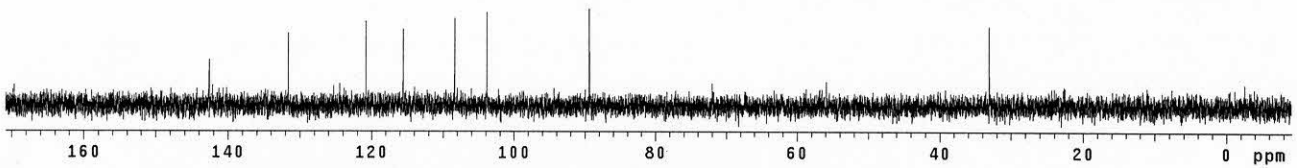


Figure A6.1 DEPT 90 spectrum of compound M-1 (in chloroform –*d*)

Name of sample:C-3-3

DEPT135 experiment

CH,CH up & CH2 down

exp1 dept

SAMPLE		DEC. & VT	
date	Jul 21 2009	dfrq	499.510
solvent	cdcl3	dn	H1
file	exp	dpwr	38
ACQUISITION		dof	0
sfrq	125.611	dm	nny
tn	C13	dmm	ccw
at	1.301	dmf	11768
np	58816	dseq	
sw	22605.3	dres	1.0
fb	12000	homo	n
bs	8	PROCESSING	
ss	8	lb	1.00
tpwr	54	wtfile	
pw	9.5	proc	ft
d1	2.000	fn	not used
tof	-1739.0	math	f
nt	5000		
ct	2970	werr	
alock	n	wexp	procplot
gain	60	wbs	
pp	10.5	wnt	
pplvl	61		
j	140.0		
multf	1.5		
satdly	0		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-1142.0		
wp	22605.3		
vs	4421		
sc	0		
wc	250		
hzmm	90.42		
is	500.00		
rfl	19055.1		
rfp	17913.1		
th	6		
ins	100.000		

al cdc ph

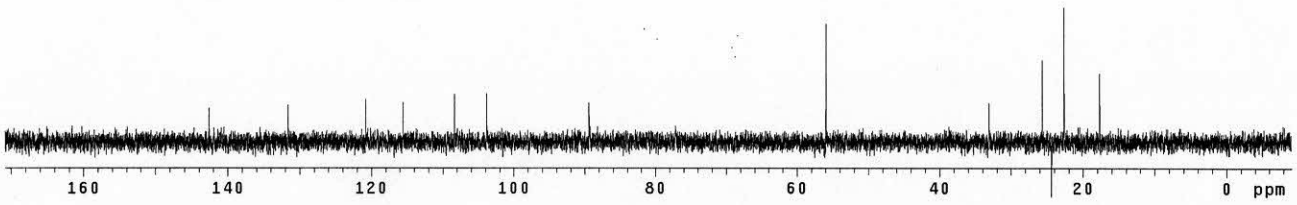


Figure A6.2 DEPT 135 spectrum of compound M-1 (in chloroform –*d*) (continued)

Name sample:C-3-3

gCOSY experiment

expl gcosy

SAMPLE		DEC. & VT	
date	Jul 16 2009	dn	H1
solvent	CDCl3	dof	0
file	/export/home/~	dm	nnn
vnmr1/1634_52imron~		dmm	c
/C-3-3_gcosy.fid		dmf	13832
ACQUISITION		dpwr	35
sfrq	499.511	homo	n
tn	H1	temp	not used
at	0.128		
np	2048	lp	-0.4
sw	7996.0	rp	-89.5
fb	4000	rp1	0
bs	8	lp1	0
ss	2	PROCESSING	
tpwr	55	sb	0.037
pw	7.6	sbs	0.001
p1	7.6	proc	ft
d1	1.000	fn	2048
tof	1249.0	math	f
nt	4		
ct	4	werr	
alock	n	wexp	
gain	40	wbs	
FLAGS		wnt	wft
il	n	2D PROCESSING	
in	n	lb1	0.32
dp	y	proc1	ft
hs	nn	fn1	2048
2D ACQUISITION		DISPLAY	
sw1	7996.0	sp	-272.5
ni	512	wp	7996.0
d2	0	vs	2693
GRADIENTS		sc	0
gzlvl1	600	wc	150
gt1	0.001900	hzmm	53.31
grise	0.000100	is	654.63
gstab	0	rf1	3889.0
qlvl	1.0	rfp	3616.4
2D DISPLAY		th	2
sp1	-269.0	ins	1.000
wp1	7996.0	ai cdc av	
sc2	0		
wc2	155		
rf11	3885.4		
rfp1	3616.4		

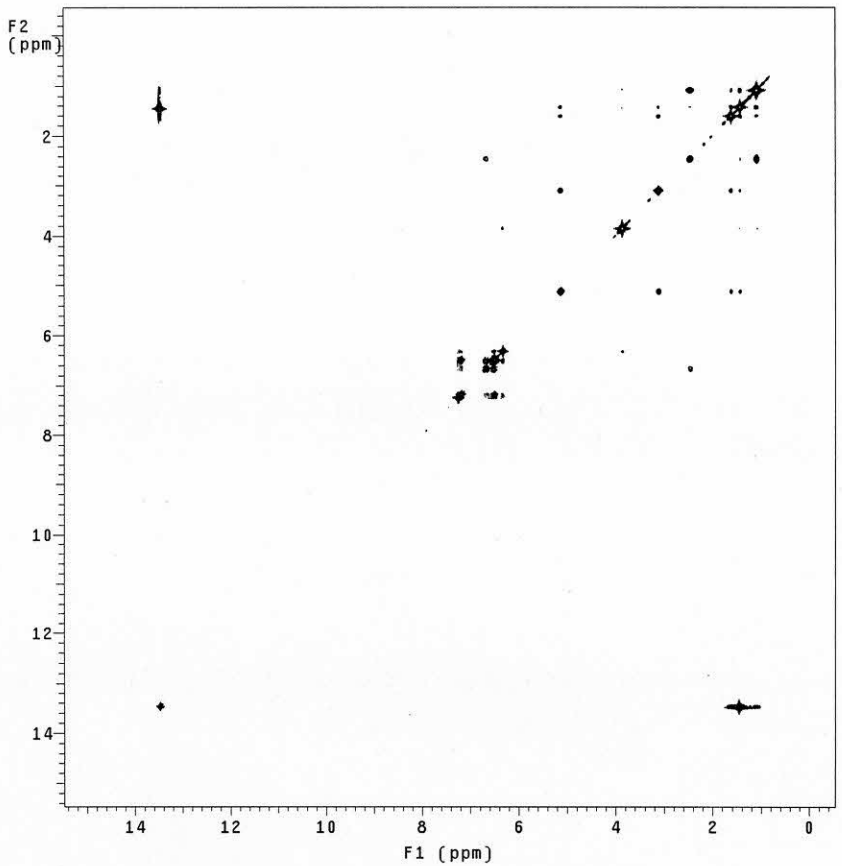


Figure A7 COSY spectrum of compound M-1 (in chloroform –*d*)

Name sample:C-3-3

gHMQC experiment

exp3 ghmqc_da

SAMPLE		DEC. & VT	
date	Jul 16 2009	dfrq	125.615
solvent	CDCl3	dn	C13
file	/export/home/~	dpwr	50
vnmr1/1634_52imron~		dof	2112.0
/C-3-3_ghmqc.fid		dm	nny
ACQUISITION		dmm	ccg
sfrq	499.511	dmf	27173
tn	H1	dseq	garp1
at	0.137	dres	1.0
np	2048	homo	n
sw	7492.7	PROCESSING	
fb	4000	sb	-0.037
bs	8	sbs	-0.015
ss	16	gf	0.054
tpwr	58	gfs	0.053
pw	7.5	awc	0.044
d1	2.000	proc	ft
tof	999.0	fn	2048
nt	128	math	f
ct	128	2D PROCESSING	
gain	60	sb1	0.007
FLAGS		sbs1	not used
il	n	gf1	0.005
dp	y	gfs1	0.001
hs	nn	proc1	ft
2D ACQUISITION		fn1	2048
sw1	30007.0	DISPLAY	
ni	512	vs	141799
phase	1	th	0
SEQUENCE		rp	179.8
pwx	12.0	lp	0
pwxlvl	60	rfl	3881.9
j	140	rfp	3616.4
mbond	n	sc	0
taumb	0.0625	wc	150
GRADIENTS		hzmm	49.95
gzlvl1	2000	ai	av
gt1	0.002000	2D DISPLAY	
gzlvl2	2000	rp1	0
gt2	0.002000	lp1	0
gzlvl3	-1010	rfl1	10677.8
gt3	0.002000	rfp1	9672.1
gstab	0.000050	sc2	0
		wc2	150

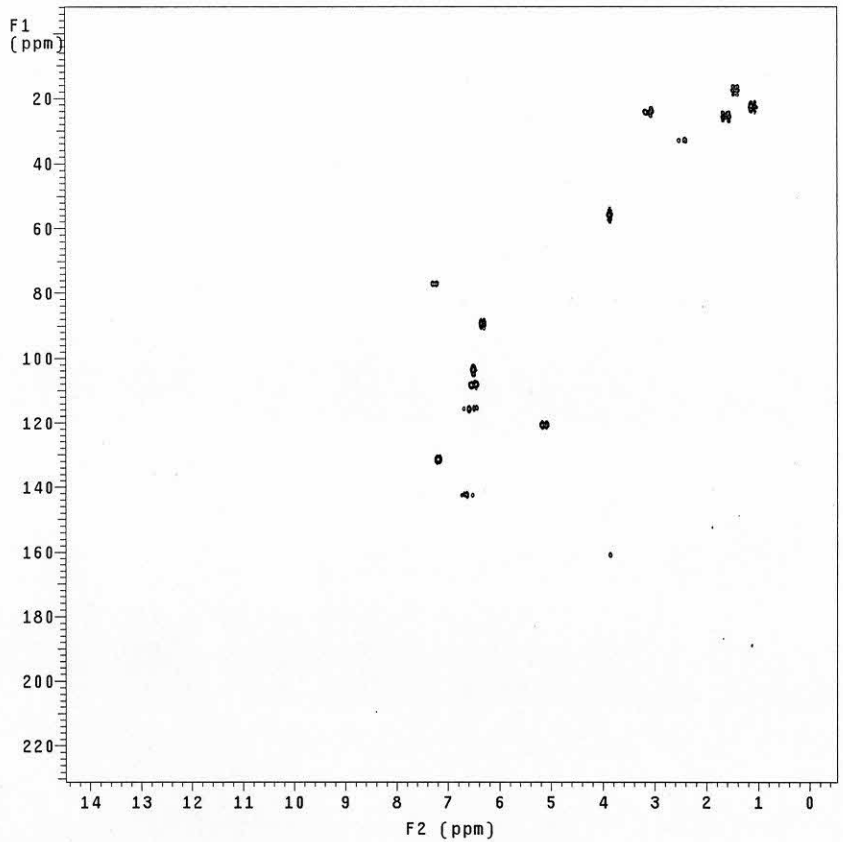


Figure A8 HMQC spectrum of compound M-1 (in chloroform –*d*)

Name sample:C-3-3

gHMBC experiment

using ghmqc pulse sequence

exp2 ghmqc_da

	SAMPLE		DEC. & VT
date	Jul 17 2009	dfrq	125.615
solvent	CDCl3	dn	C13
file	exp	dpwr	50
	ACQUISITION	dof	2112.0
sfrq	499.511	dm	nnn
tn	H1	dmm	ccg
at	0.137	dmf	27173
np	2048	dseq	garp1
sw	7492.7	dres	1.0
fb	4000	homo	n
bs	8		PROCESSING
ss	16	sb	-0.097
tpwr	58	sbs	-0.051
pw	7.5	gf	0.036
d1	2.000	gfs	0.068
tof	999.0	awc	0.044
nt	256	proc	ft
ct	240	fn	2048
gain	60	math	f
	FLAGS		2D PROCESSING
il	n	sb1	0.007
dp	y	sbs1	not used
hs	nn	gf1	0.005
	2D ACQUISITION	gfs1	0.001
sw1	30007.0	proc1	ft
ni	512	fn1	2048
phase	1		DISPLAY
	SEQUENCE	vs	141799
pwx	12.0	th	0
pwxlvl	60	rp	179.8
j	140	lp	0
mbond	y	rfl	3882.6
taumb	0.0625	rfp	3616.4
	GRADIENTS	sc	0
gzlvl1	2000	wc	150
gt1	0.002000	hzmm	49.95
gzlvl2	2000	ai	av
gt2	0.002000		2D DISPLAY
gzlvl3	-1010	rp1	0
gt3	0.002000	lp1	0
gstab	0.000050	rfl1	10687.4
		rfp1	9672.1
		sc2	0
		wc2	150

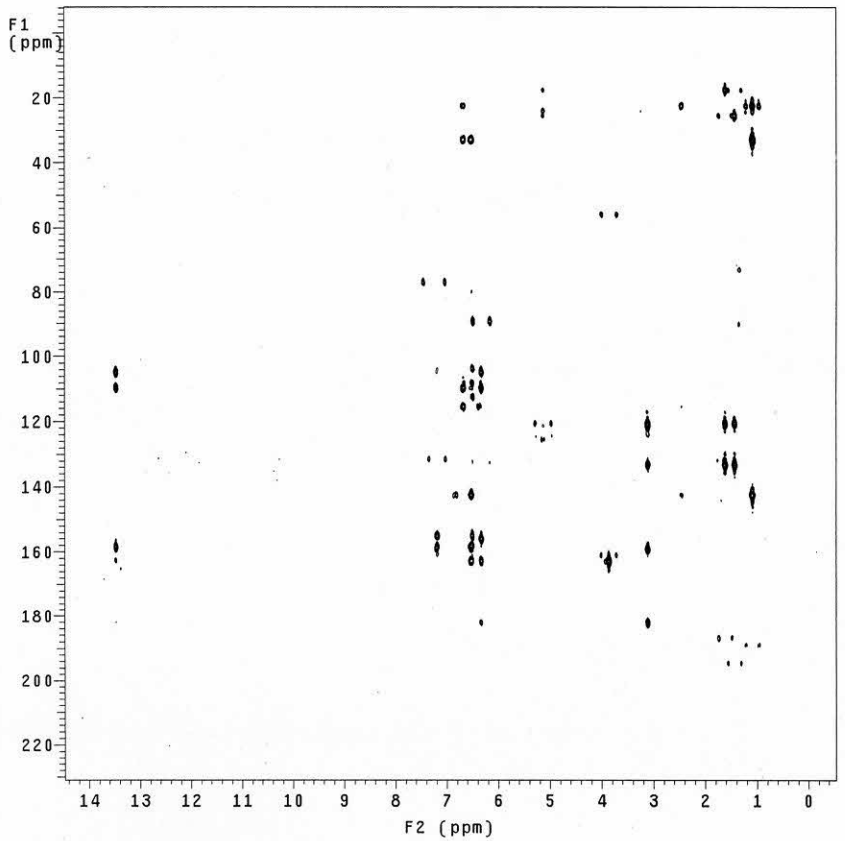
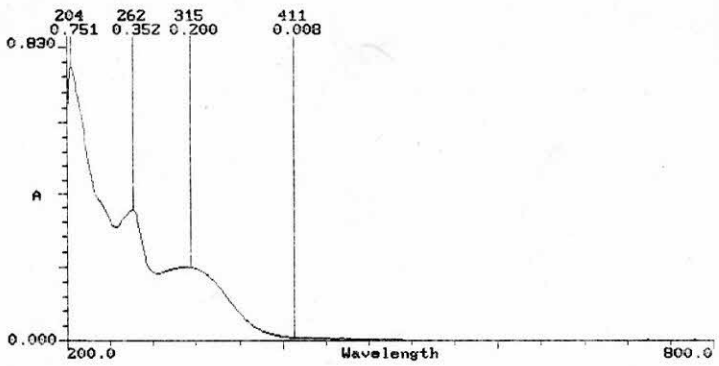


Figure A9 HMBC spectrum of compound M-1 (in chloroform –*d*)

TEST SETUP
GENESYS 6 v2.001 2M9M008001

Scanning	23:18 4May10
Test Name	D-3-1a
Measurement Mode	Absorbance
Start Wavelength	200.0nm
Stop Wavelength	800.0nm
Sample Positioner	Auto 6
Scan Speed	Fast
Interval	1.0nm
Cell Correction	Off
ID# (0=OFF)	1
Auto Print	On
Auto Save Data	Off



ID#: 1
Smoothing [On]

Wavelength	Abs	
204.0	0.751	Peak
262.0	0.352	Peak
315.0	0.200	Peak
411.0	0.008	Peak

Figure B1 UV-Visible spectrum of M-2 in Methanol

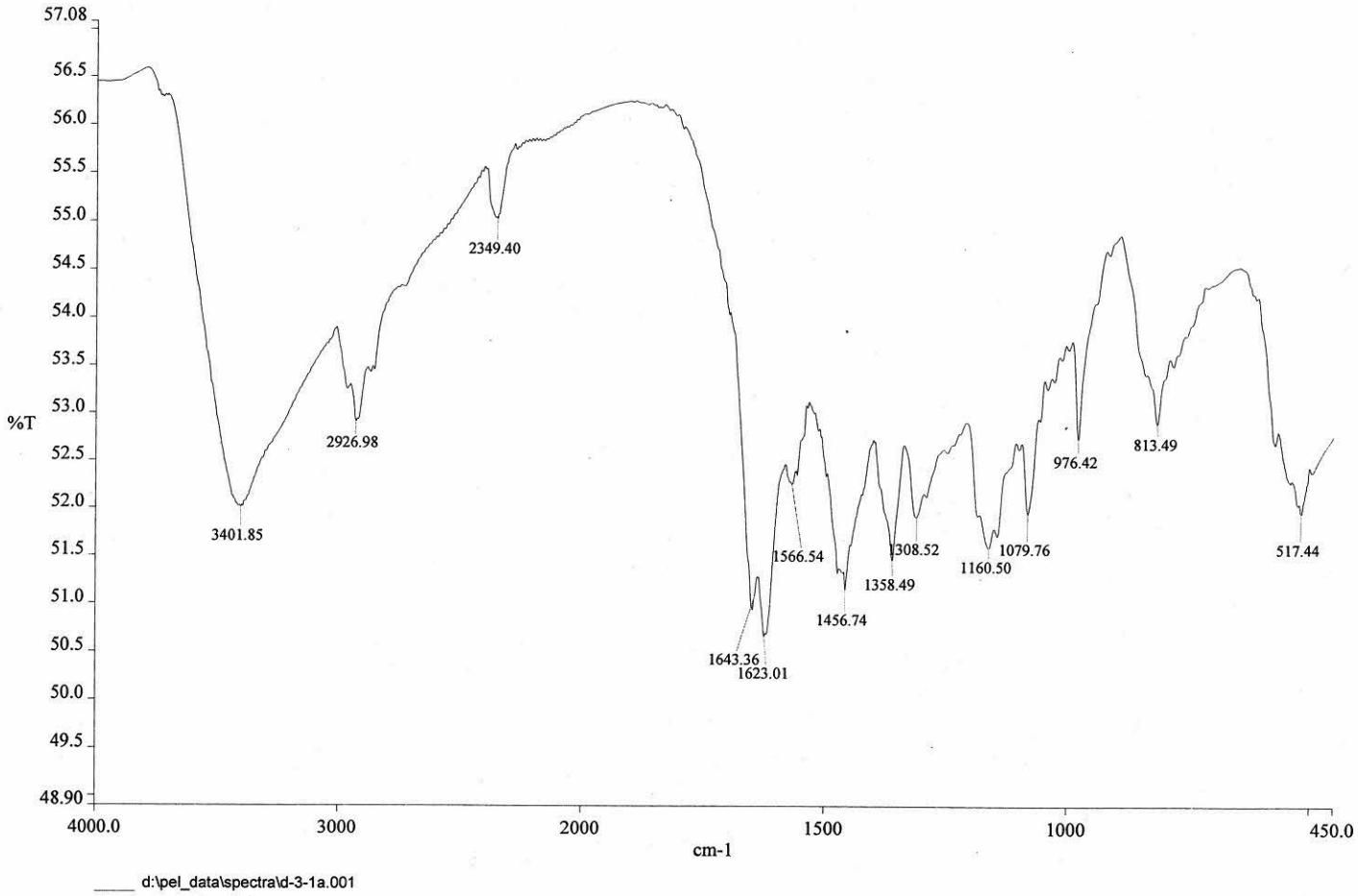


Figure B2 IR spectrum of compound M-2 (KBr disc)

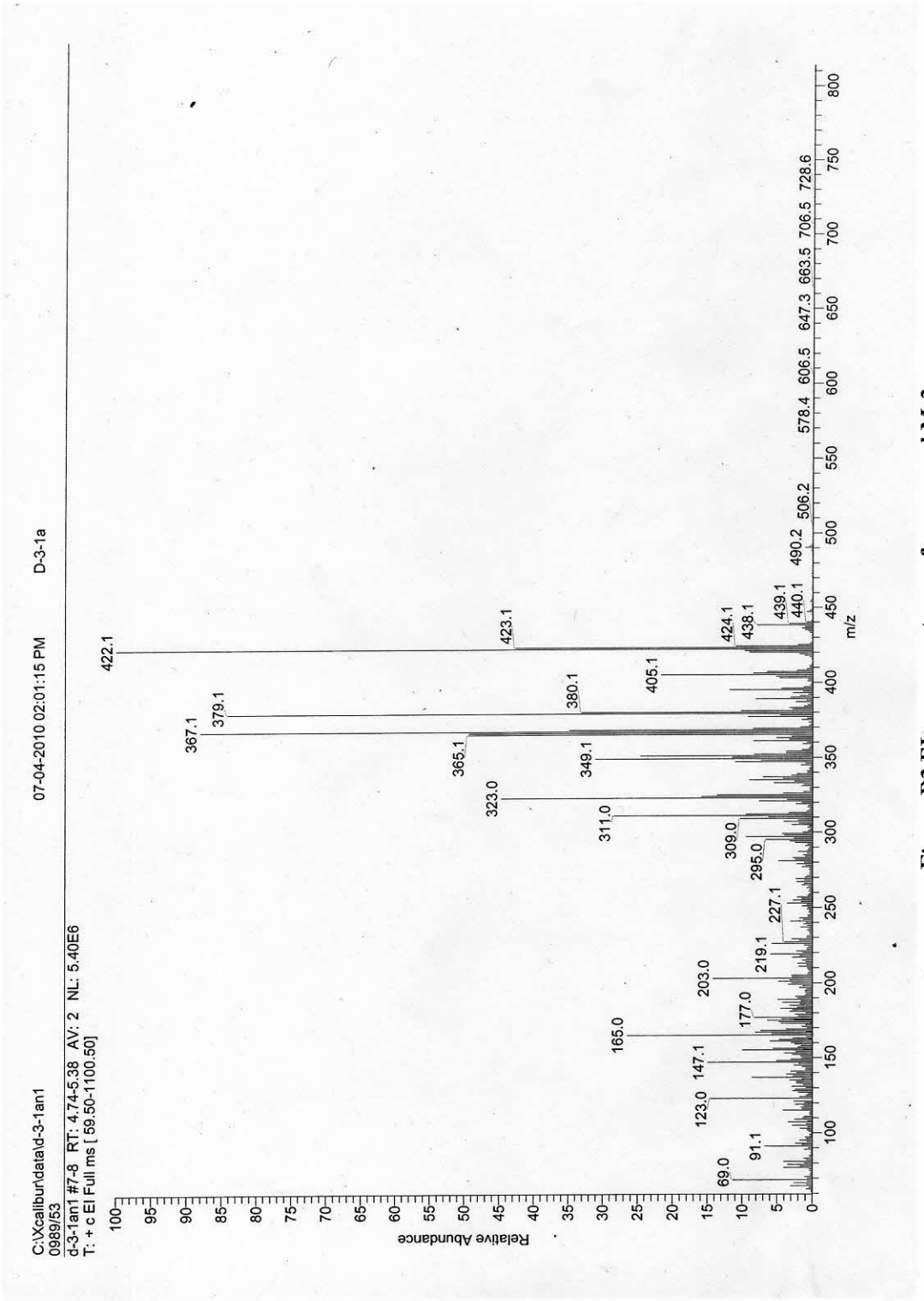


Figure B3 EI mass spectrum of compound M-2

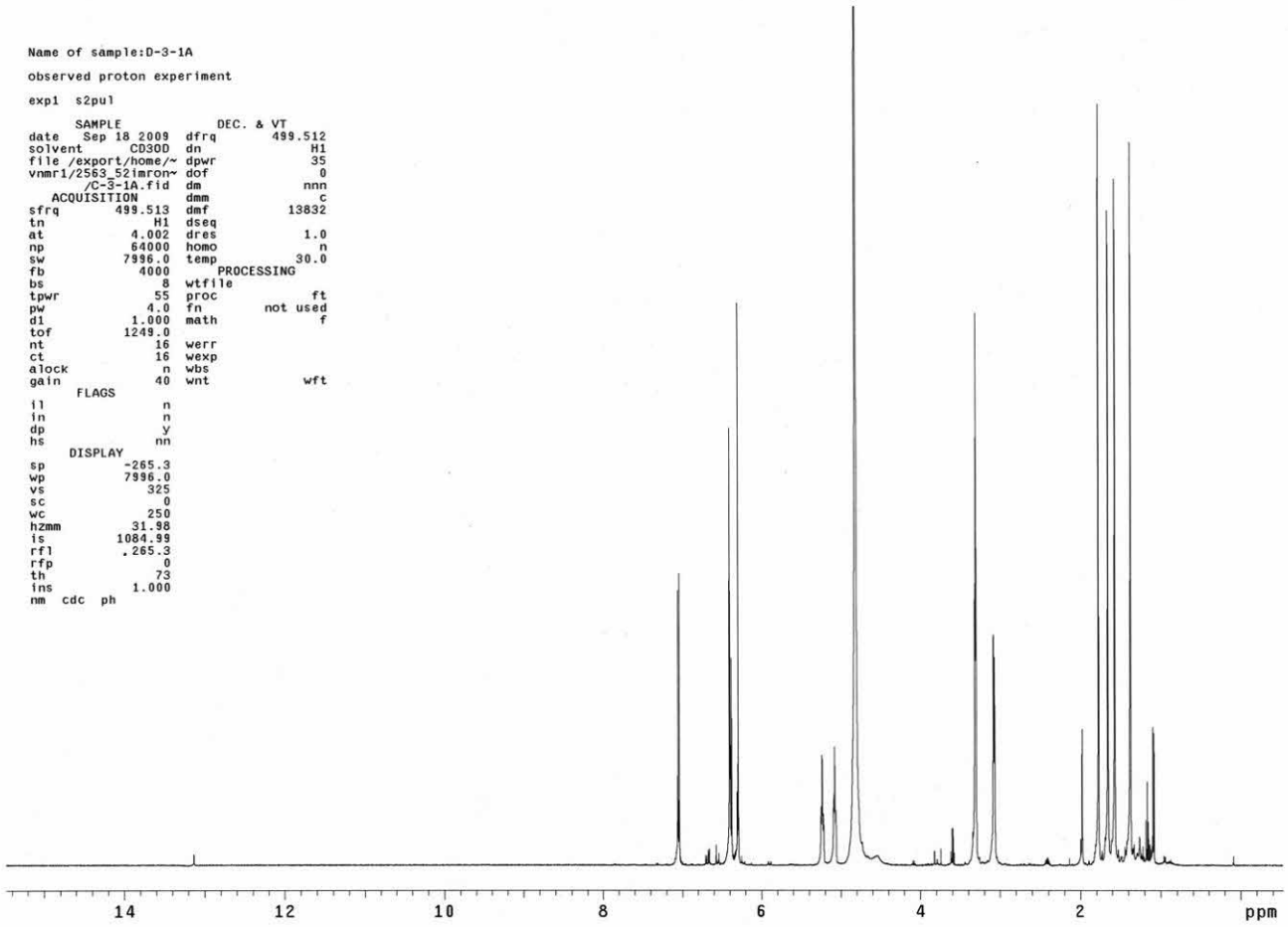


Figure B4 500 MHz ^{1}H spectrum of compound M-2 (in methanol $-d_4$)

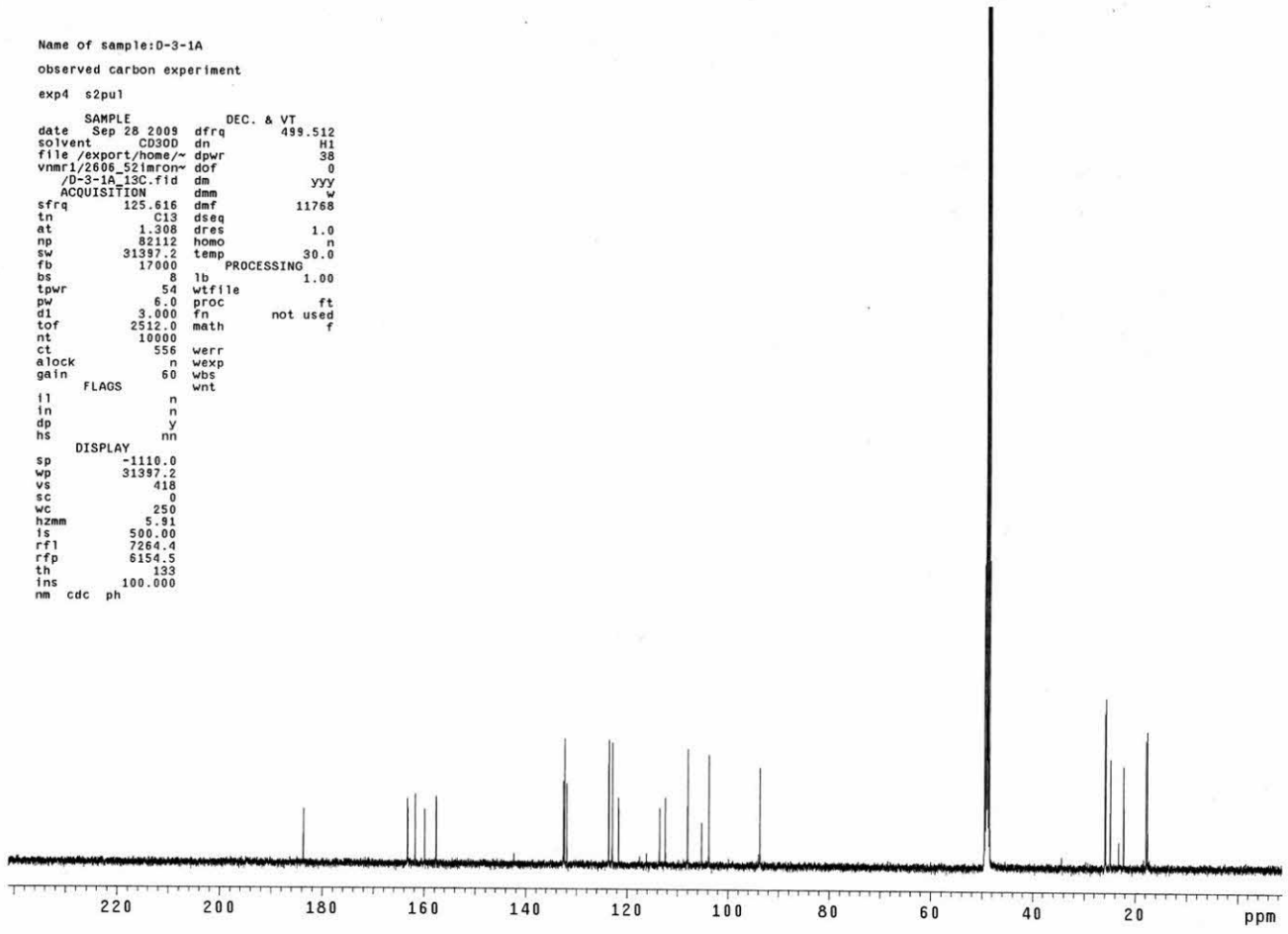


Figure B5 125 MHz ^{13}C spectrum of compound M-2 (in methanol $-d_4$)

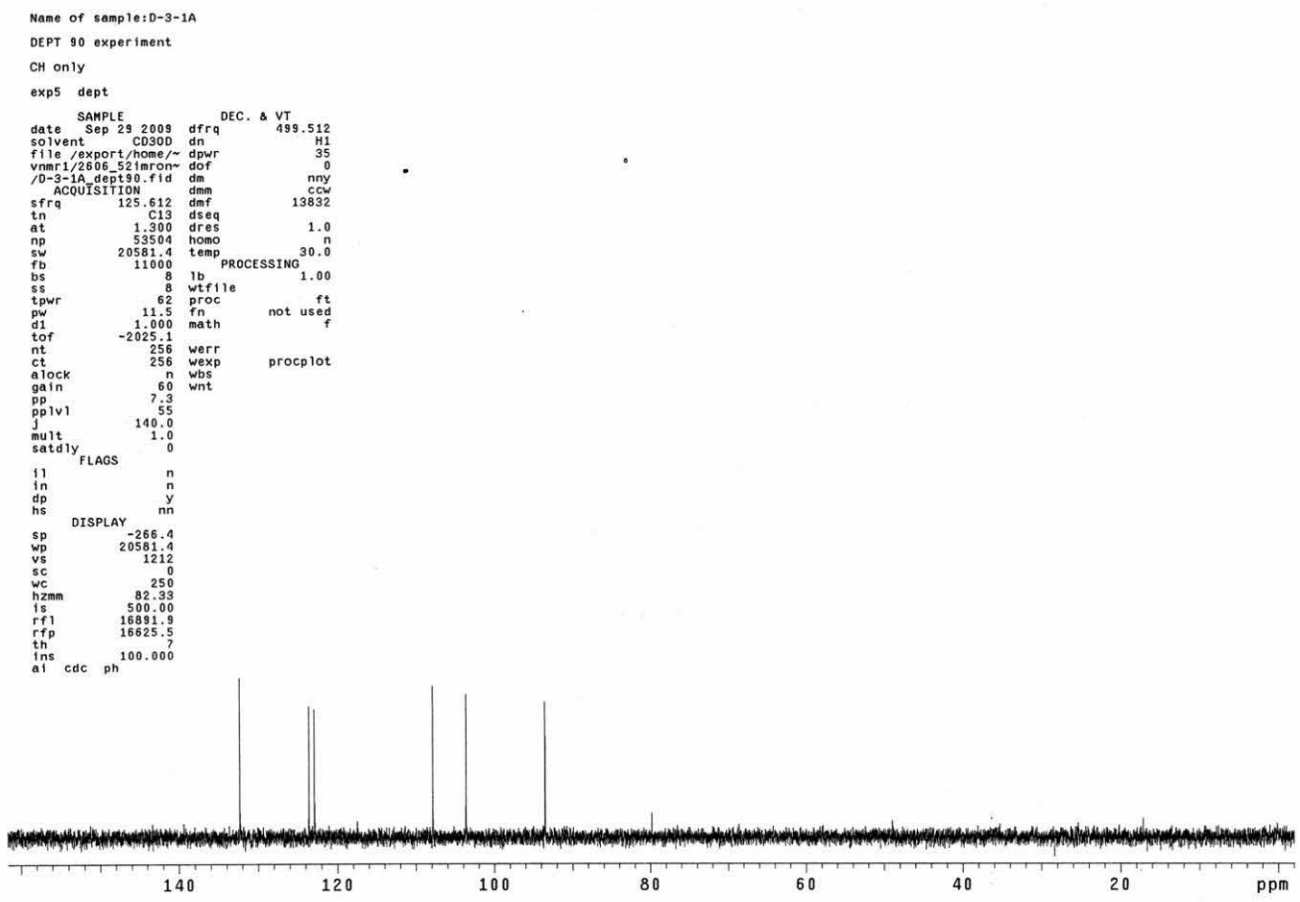


Figure B6.1 DEPT 90 spectrum of compound M-2 (in methanol $-d_4$)

Name of sample:D-3-1A

DEPT 135 experiment

CH,CH3 up & CH2 down

exp5 dept

SAMPLE		DEC. & VT	
date	Sep 29 2009	dfrq	499.512
solvent	CD3OD	dn	H1
file	/export/home/~	dpwr	35
vnmr1/2606_521mron~		dof	0
/D-3-1A_dept135.fi~		dm	nny
	d	dmm	ccw
ACQUISITION		dmf	13832
sfrq	125.612	dseq	
tn	C13	dres	1.0
at	1.300	homo	n
np	53504	temp	30.0
sw	20581.4	PROCESSING	
fb	11000	lb	1.00
bs	8	wtfile	
ss	8	proc	ft
tpwr	62	fn	not used
pw	11.5	math	f
d1	1.000		
tof	-2025.1	werr	
nt	512	wexp	procplot
ct	512	wbs	
alock	n	wnt	
gain	60		
pp	7.3		
pplvl	55		
j	140.0		
mult	1.5		
satdly	0		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-266.4		
wp	20581.4		
vs	1174		
sc	0		
wc	250		
hzmm	82.33		
is	500.00		
rfl	16891.9		
rfp	16625.5		
th	7		
ins	100.000		

ai cdc ph

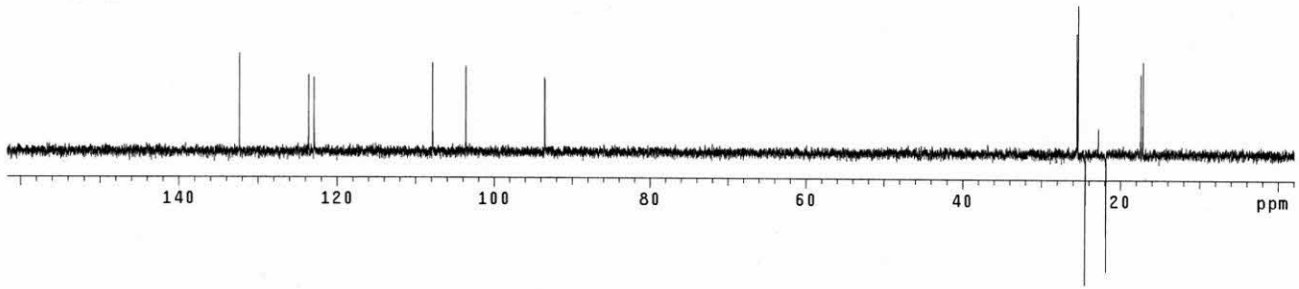


Figure B6.2 DEPT 125 spectrum of compound M-2 (in methanol $-d_4$)

Name of sample:D-3-1A

gCOSY experiment

exp4 gcosy

SAMPLE		DEC. & VT	
date	Sep 29 2009	dn	H1
solvent	CD3OD	dof	0
file	/export/home/~	dm	nnn
vnmr1/2607_52imron~		dmm	c
/D-3-1A_gcosy.fid		dmf	13832
ACQUISITION		dpwr	35
sfrq	499.511	homo	n
tn	H1	temp	30.0
at	0.241		
np	2048	lp	1.3
sw	4245.4	rp	89.0
fb	2000	rp1	0
bs	8	lp1	0
ss	2	PROCESSING	
tpwr	55	sb	0.045
pw	7.6	sbs	not used
p1	7.6	proc	ft
d1	1.000	fn	2048
tof	-618.9	math	f
nt	4		
ct	4	werr	
alock	n	wexp	
gain	40	wbs	
FLAGS		wnt	wft
il	n	2D PROCESSING	
in	n	lb1	0.32
dp	y	proc1	ft
hs	nn	fn1	2048
2D ACQUISITION		DISPLAY	
sw1	4245.4	sp	-258.1
ni	512	wp	4245.4
d2	0	vs	317
GRADIENTS		sc	0
gzlvl1	600	wc	150
gt1	0.001900	hzmm	28.30
grise	0.000100	is	681.53
gstab	0	rfl	258.1
glvl	1.0	rfp	0
2D DISPLAY		th	2
sp1	-258.2	ins	1.000
wp1	4245.4	ai cdc av	
sc2	0		
wc2	155		
rfl1	258.2		
rfp1	0		

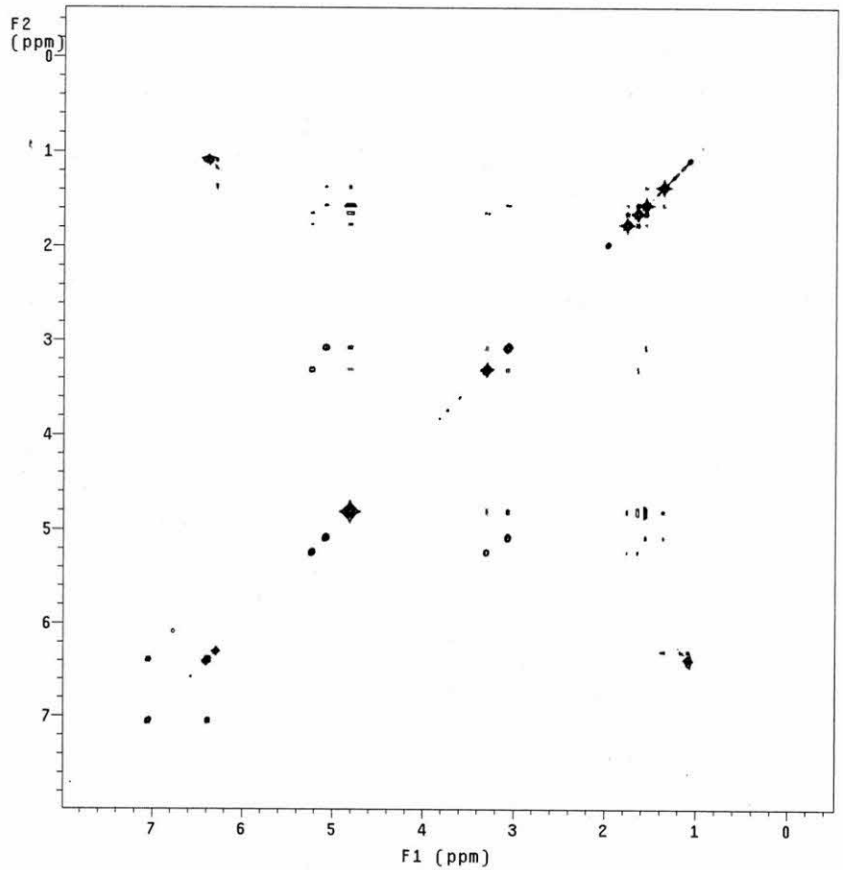


Figure B7 COSY spectrum of compound M-2 (in methanol $-d_4$)

Name of sample:D-3-1A

gHMQC experiment

exp3 ghmqc_da

SAMPLE		DEC. & VT	
date	Nov 9 2009	dfrq	125.616
solvent	CD3OD	dn	C13
file	exp	dpwr	50
ACQUISITION		dof	2112.0
sfrq	499.511	dm	nny
tn	H1	dmm	ccg
at	0.237	dmf	27173
np	2048	dseq	garp1
sw	4311.7	dres	1.0
fb	2000	homo	n
bs	8	temp	30.0
ss	16	PROCESSING	
tpwr	58	sb	-0.066
pw	7.5	sbs	not used
d1	2.000	gf	0.010
tof	-593.1	gfs	0.014
nt	64	awc	0.044
ct	64	proc	ft
gain	60	fn	2048
FLAGS		math	f
il	n	2D PROCESSING	
dp	y	sb1	0.007
hs	nn	sbs1	not used
2D ACQUISITION		gf1	0.005
sw1	30007.0	gfs1	0.001
ni	512	proc1	ft
phase	1	fn1	2048
SEQUENCE		DISPLAY	
pwx	12.0	vs	232550
pwxlvl	60	th	0
j	140	rp	179.8
mbond	n	lp	0
taumb	0.0625	rfl	1918.2
GRADIENTS		rfp	1648.4
gzlvl1	2000	sc	0
gt1	0.002000	wc	150
gzlvl2	2000	hzmm	28.74
gt2	0.002000	ai av	
gzlvl3	-1010	2D DISPLAY	
gt3	0.002000	rp1	0
gstab	0.000050	lp1	0
		rfl1	6997.8
		rfp1	6155.0
		sc2	0
		wc2	150

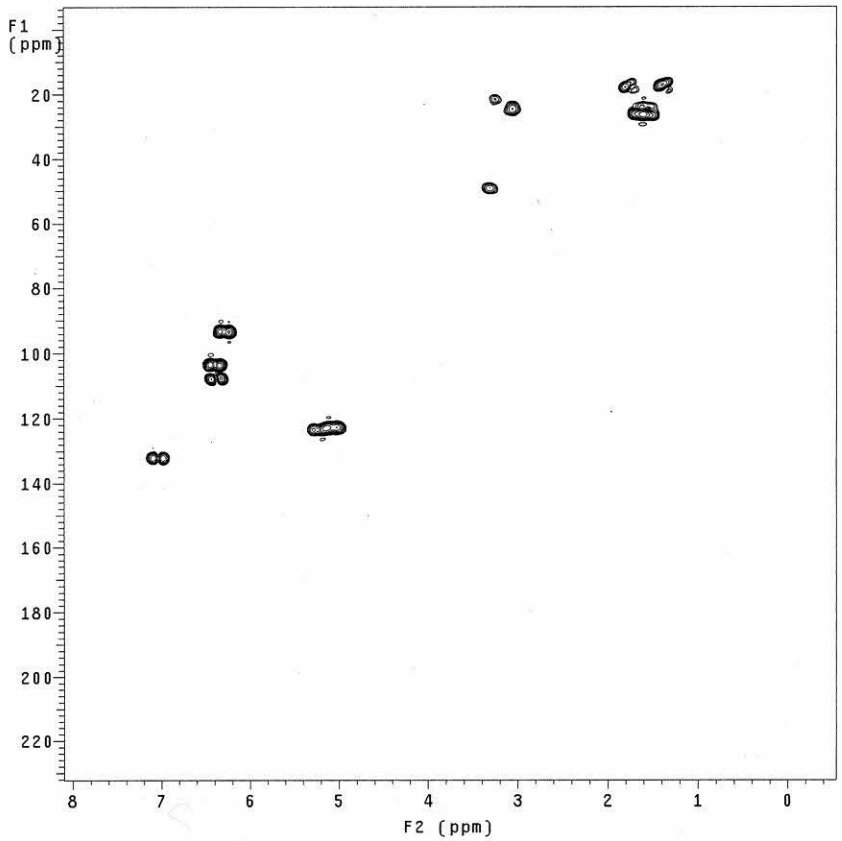


Figure B8 HMQC spectrum of compound M-2 (in methanol $-d_4$)

Name of sample:D-3-1A

gHMBC experiment

using ghmqc pulse sequence

exp2 ghmqc_da

SAMPLE		DEC. & VT	
date	Nov 9 2009	dfrq	125.616
solvent	CD3OD	dn	C13
file	exp	dpwr	50
ACQUISITION		dof	2112.0
sfrq	499.511	dm	nnn
tn	H1	dmm	ccg
at	0.237	dmf	27173
np	2048	dseq	garp1
sw	4311.7	dres	1.0
fb	2000	homo	n
bs	8	temp	30.0
ss	16	PROCESSING	
tpwr	58	sb	-0.162
pw	7.5	sbs	-0.160
d1	2.000	gf	0.042
tof	-593.1	gfs	0.075
nt	64	awc	0.044
ct	32	proc	ft
gain	60	fn	2048
FLAGS		math	f
il	n	2D PROCESSING	
dp	y	sb1	0.007
hs	nn	sbs1	not_used
2D ACQUISITION		gf1	0.005
sw1	30007.0	gfs1	0.001
ni	512	proc1	ft
phase	1	fn1	2048
SEQUENCE		DISPLAY	
pwx	12.0	vs	141799
pwxlvl	60	th	0
j	140	rp	179.8
mbond	y	lp	0
taumb	0.0625	rfl	1908.5
GRADIENTS		rfp	1648.4
gzlvl1	2000	sc	0
gt1	0.002000	wc	150
gzlvl2	2000	hzmm	3.26
gt2	0.002000	ai av	
gzlvl3	-1010	2D DISPLAY	
gt3	0.002000	rp1	0
gstab	0.000050	lp1	0
		rfl1	6987.4
		rfp1	6155.0
		sc2	0
		wc2	150

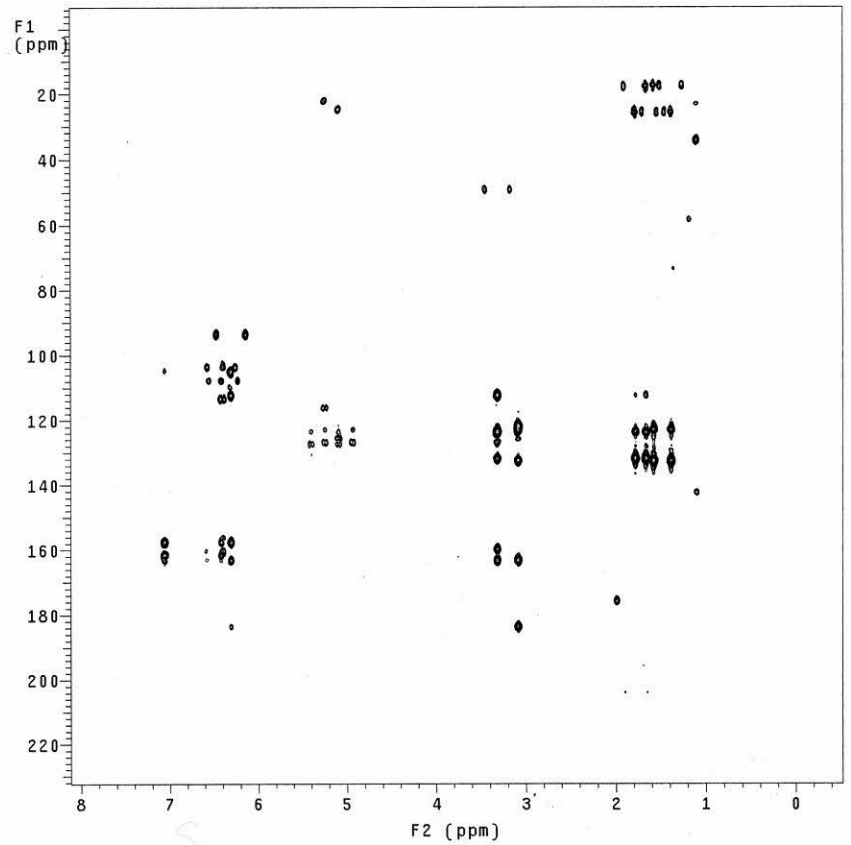
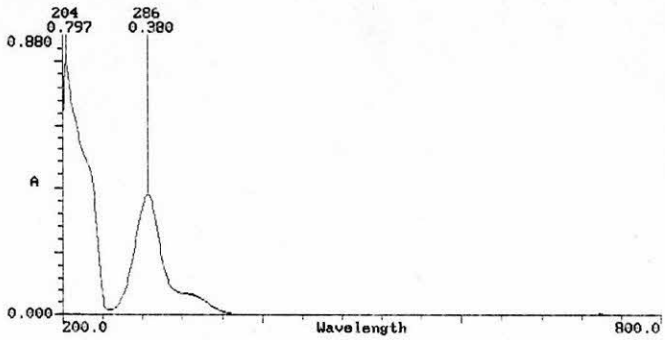


Figure B9 HMBC spectrum of compound M-2 (in methanol $-d_4$)

TEST SETUP
GENESYS 6 v2.001 2M9M008001

Scanning	23:04 4May10
Test Name	D-5
Measurement Mode	Absorbance
Start Wavelength	200.0nm
Stop Wavelength	800.0nm
Sample Positioner	Auto 6
Scan Speed	Fast
Interval	1.0nm
Cell Correction	Off
ID# (0=OFF)	1
Auto Print	On
Auto Save Data	Off



ID#: 1
Smoothing [On]

Wavelength	Abs	
204.0	0.797	Peak
286.0	0.380	Peak

Figure C1 UV-Visible spectrum of M-3 in Methanol

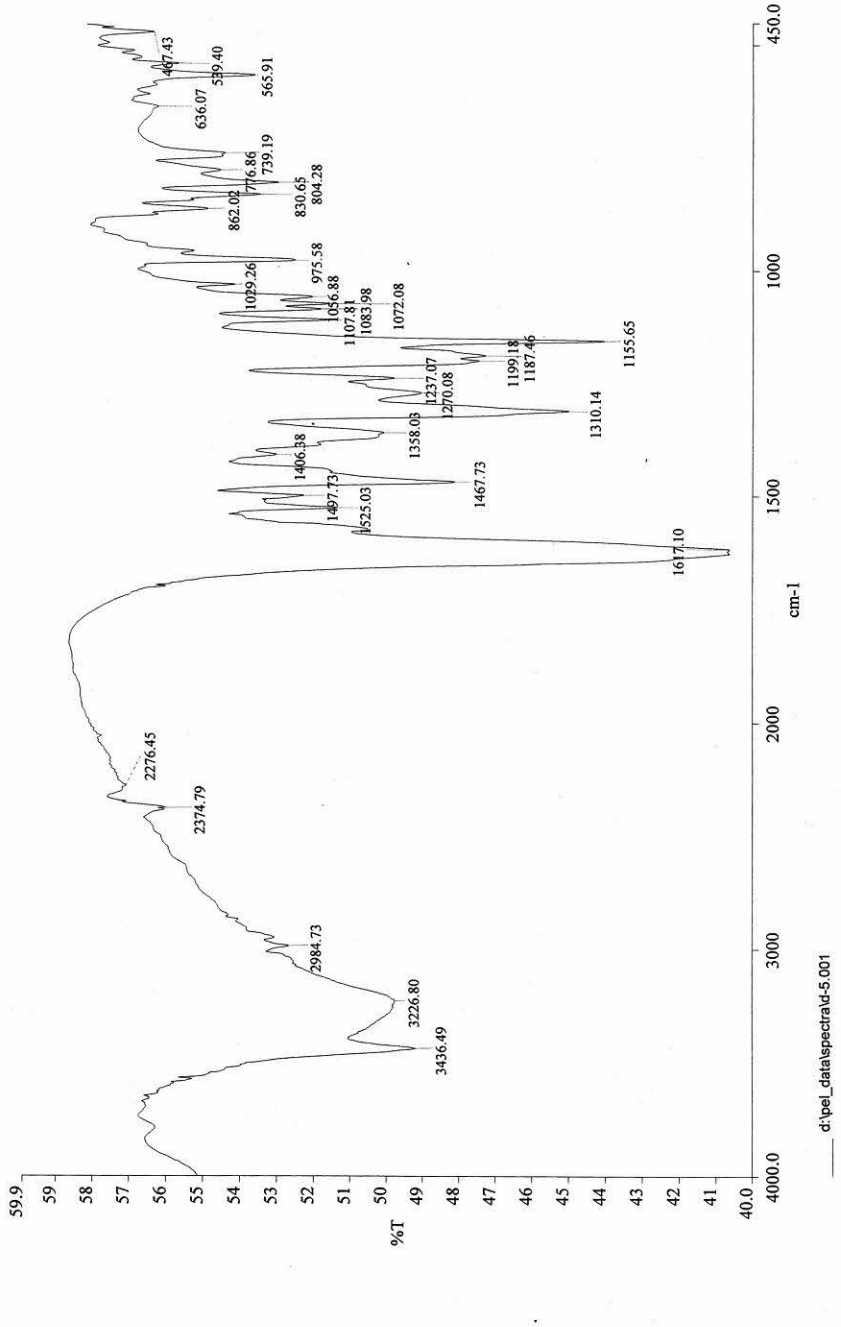


Figure C2 IR spectrum of compound M-3 (KBr disc)

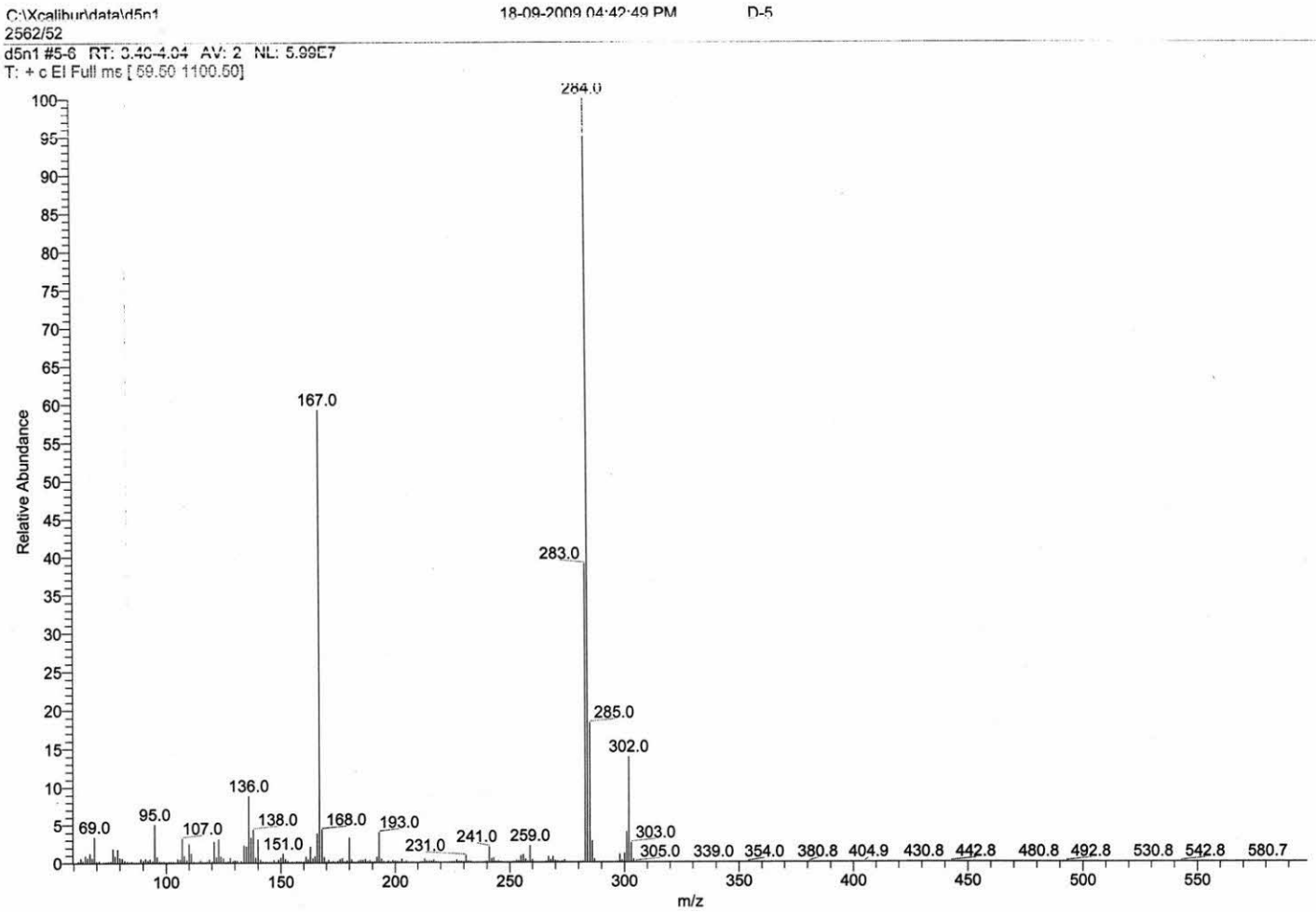


Figure C3 EI mass spectrum of compound M-3

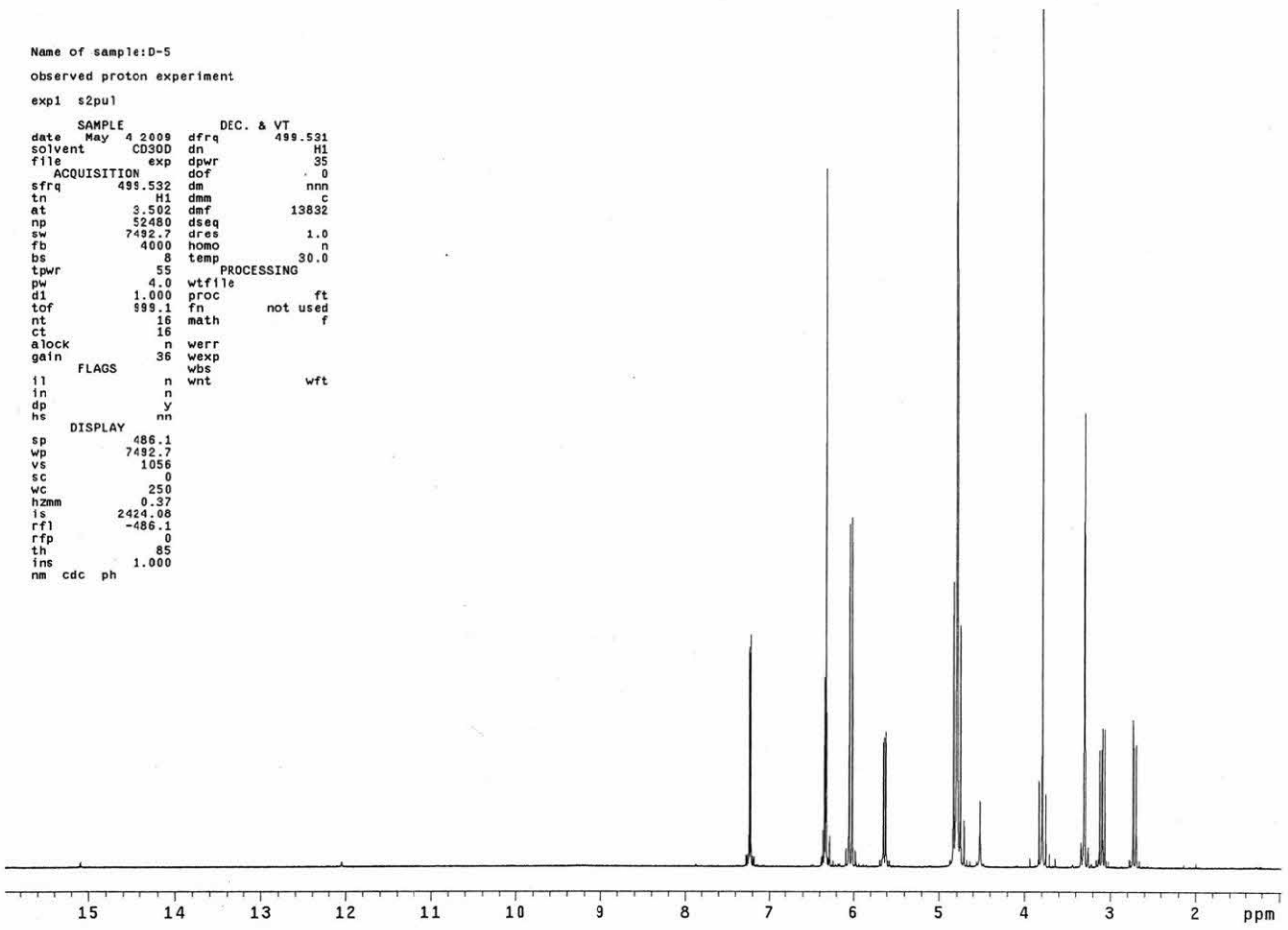


Figure C4 500 MHz ^{1}H spectrum of compound M-3 (in methanol-d_4)

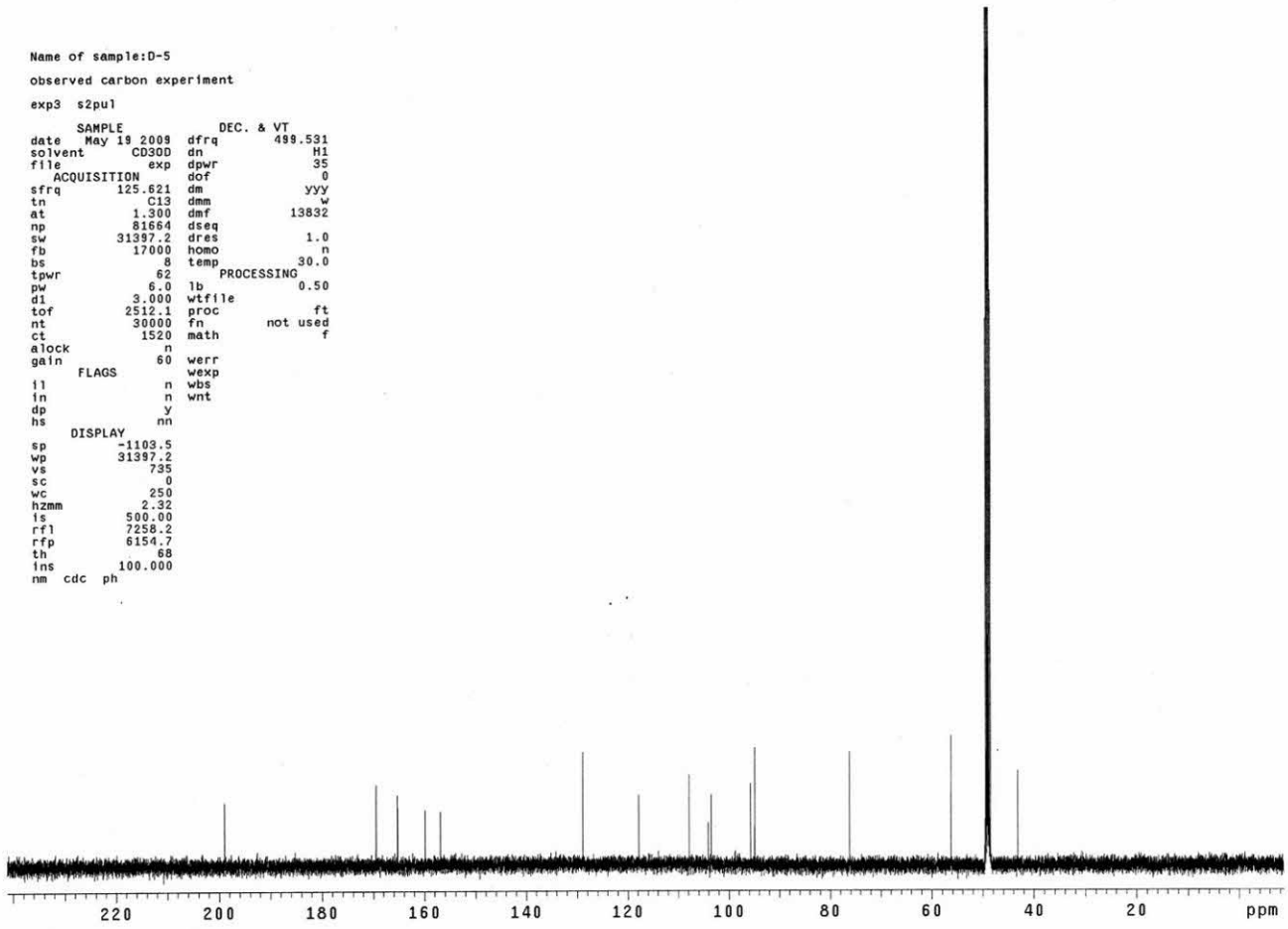


Figure C5 125 MHz ^{13}C spectrum of compound M-3 (in methanol-d_4)

Name of sample:D-5

DEPT 90 experiment

CH only

exp3 dept

SAMPLE		DEC. & VT	
date	May 28 2009	dfrq	499.531
solvent	CD3OD	dn	H1
file	/export/home/~	dpwr	38
vnmr1/1382_52imron~		dof	0
/D_5_dept90.fid		dm	nny
ACQUISITION		dmm	ccw
sfrq	125.618	dmf	11768
tn	C13	dseq	
at	1.301	dres	1.0
np	62208	homo	n
sw	23916.3	temp	30.0
fb	13000	PROCESSING	
bs	8	wtfile	
ss	8	proc	ft
tpwr	54	fn	not used
pw	9.5	math	f
d1	2.000		
tof	-387.0	werr	
nt	1024	wexp	procplot
ct	1024	wbs	
alock	n	wnt	
gain	60		
pp	10.5		
pplvl	61		
j	140.0		
mult	1.0		
satdly	0		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-262.8		
wp	23916.3		
vs	441		
sc	0		
wc	250		
hzmm	5.47		
is	500.00		
rfl	16453.9		
rfp	16191.2		
th	6		
ins	100.000		
ai cdc ph			

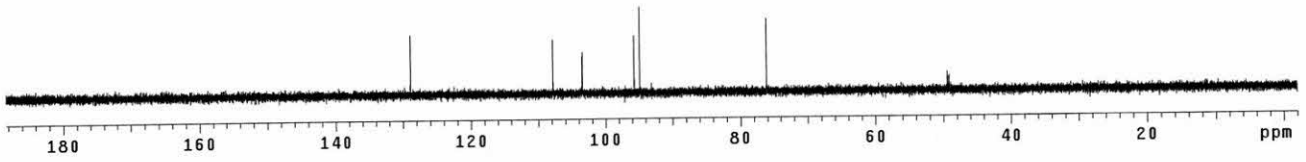


Figure C6.1 DEPT 90 spectrum of compound M-3 (in methanol-d_4)

Name of sample:D-5

DEPT 135 experiment

CH,CH3 up & CH2 down

exp3 dept

SAMPLE		DEC. & VT	
date	May 28 2009	dfrq	499.531
solvent	CD3OD	dn	H1
file	/export/home/~	dpwr	38
vnmr1/1382_521mron~		dof	0
/D-5_DEPT135.fid		dm	nny
ACQUISITION		dmm	ccw
sfrq	125.618	dmf	11768
tn	C13	dseq	
at	1.301	dres	1.0
np	62208	homo	n
sw	23916.3	temp	30.0
fb	13000	PROCESSING	
bs	8	lb	1.00
ss	8	wtfile	
tpwr	54	proc	ft
pw	9.5	fn	not used
d1	2.000	math	f
tof	-387.0		
nt	15000	werr	
ct	2092	wexp	procplot
alock	n	wbs	
gain	60	wnt	
pp	10.5		
pplvl	61		
j	140.0		
mult	1.5		
satdly	0		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-262.8		
wp	23916.3		
vs	3155		
sc	0		
wc	250		
hzmm	95.67		
is	500.00		
rfl	16453.9		
rfp	16191.2		
th	11		
ins	100.000		

ai cdc ph

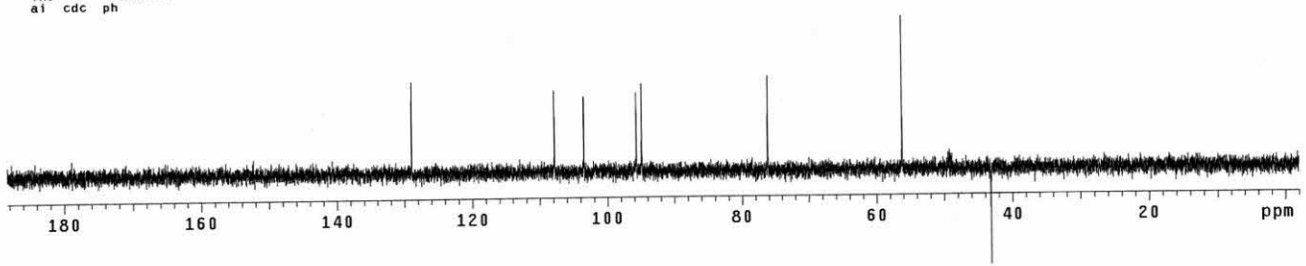


Figure C6.2 DEPT 135 spectrum of compound M-3 (in methanol-d_4)

Name of sample:D-5

gCOSY experiment

exp3 gcosy

SAMPLE		DEC. & VT	
date	May 29 2009	dn	H1
solvent	CD3OD	dof	0
file	/export/home/~	dm	nnn
vnmrl	/1382_521mron~	dmm	c
	/D-5_gcosy.fid	dmf	13832
ACQUISITION		dpwr	35
sfrq	499.530	homo	n
tn	H1	temp	30.0
at	0.215		
np	2048	lp	17.1
sw	4766.7	rp	-0.2
fb	3000	rp1	0
bs	8	lp1	0
ss	2	PROCESSING	
tpwr	55	sb	0.051
pw	7.6	sbs	0.001
p1	7.6	proc	ft
d1	1.000	fn	2048
tof	-933.3	math	f
nt	4		
ct	4	werr	
alock	n	wexp	
gain	36	wbs	
FLAGS		wnt	wft
il	n	2D PROCESSING	
in	n	lb1	0.32
dp	y	proc1	ft
hs	nn	fn1	2048
2D ACQUISITION		DISPLAY	
sw1	4766.7	sp	-401.8
ni	512	wp	4268.2
d2	0	vs	1897
GRADIENTS		sc	0
gzlv11	600	wc	150
gt1	0.001900	hzmm	10.44
grise	0.000100	is	2424.08
gstab	0	rfl	2469.6
glv1	1.0	rfp	1648.4
2D DISPLAY		th	2
sp1	-174.0	ins	1.000
wp1	3956.0	ai cdc av	
sc2	0		
wc2	155		
rfl1	3875.6		
rfp1	3158.5		

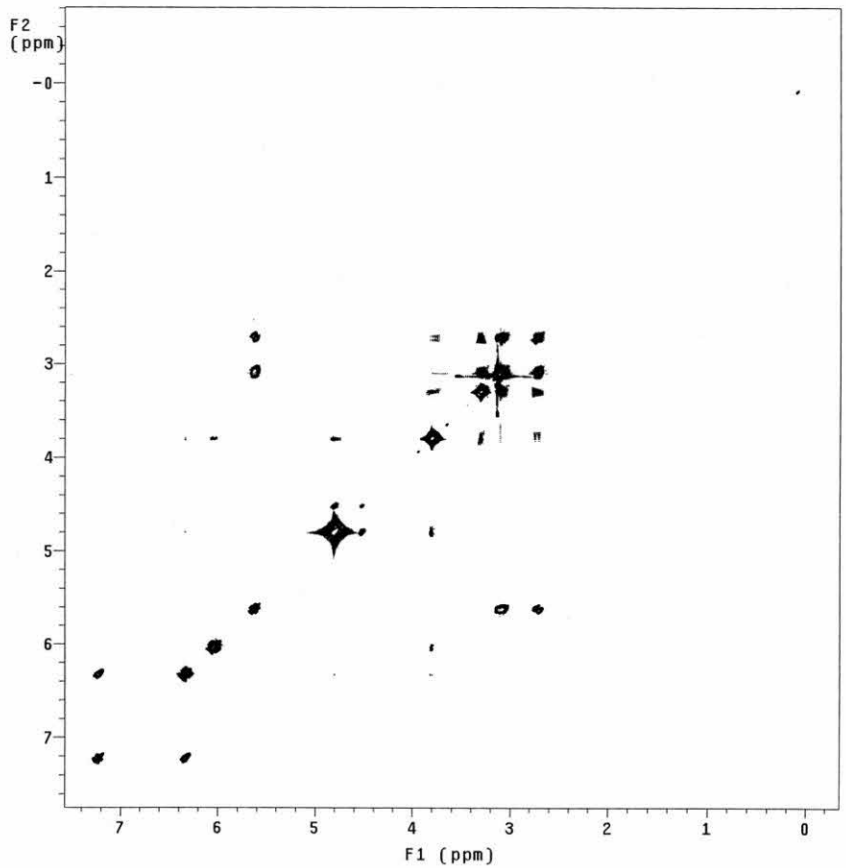


Figure C7 COSY spectrum of compound M-3 (in methanol-d_4)

Name of sample:D-5

gHMQC experiment

exp3 ghmqc_da

SAMPLE		DEC. & VT	
date	May 29 2009	dfrq	125.620
solvent	CD3OD	dn	C13
file	/export/home/~	dpwr	50
vnmr1/1382_52imron~		dof	2112.0
/D-5_ghmqc.fid		dm	nny
ACQUISITION		dmm	ccg
sfrq	499.530	dmf	27173
tn	H1	dseq	garp1
at	0.215	dres	1.0
np	2048	homo	n
sw	4767.0	temp	30.0
fb	3000	PROCESSING	
bs	8	sb	-0.059
ss	16	sbs	-0.054
tpwr	58	gf	0.020
pw	7.5	gfs	0.026
d1	2.000	awc	0.044
tof	-933.0	proc	ft
nt	64	fn	2048
ct	64	math	f
gain	60	2D PROCESSING	
FLAGS		sb1	0.007
il	n	sbs1	not used
dp	y	gf1	0.005
hs	nn	gfs1	0.001
2D ACQUISITION		proc1	ft
sw1	30007.0	fn1	2048
n1	512	DISPLAY	
phase	1	vs	386517
SEQUENCE		th	0
pwx	12.0	rp	179.8
pwxlvl	60	lp	0
j	140	rfl	4425.9
mbond	n	rfp	3606.6
taumb	0.0625	sc	0
GRADIENTS		wc	150
gzlvl1	2000	hzmm	31.78
gt1	0.002000	ai av	
gzlvl2	2000	2D DISPLAY	
gt2	0.002000	rp1	0
gzlvl3	-1010	lp1	0
gt3	0.002000	rfl1	17019.1
gstab	0.000050	rfp1	16192.5
		sc2	0
		wc2	150

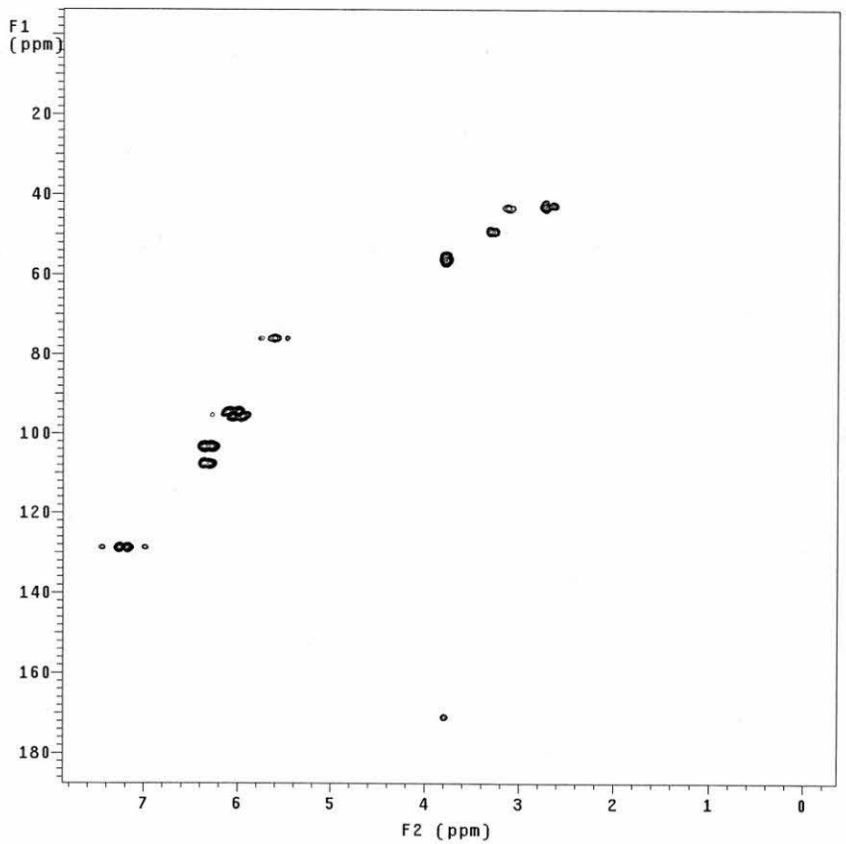


Figure C8 HMQC spectrum of compound M-3 (in methanol-d_4)

Name of sample:D-5

gHMBC experiment

using ghmqc pulse sequence

exp3 ghmqc_da

SAMPLE		DEC. & VT	
date	May 29 2009	dfrq	125.620
solvent	CD3OD	dn	C13
file	/export/home/~	dpwr	50
vnmr1/1382_52imron~		dof	2112.0
/D-5_ghmbc.fid		dm	nnn
ACQUISITION		dmm	ccg
sfrq	499.530	dmf	27173
tn	H1	dseq	garp1
at	0.215	dres	1.0
np	2048	homo	n
sw	4767.0	temp	30.0
fb	3000	PROCESSING	
bs	8	sb	-0.064
ss	16	sbs	-0.013
tpwr	58	gf	0.029
pw	7.5	gfs	0.062
d1	2.000	awc	0.044
tof	-933.0	proc	ft
nt	128	fn	2048
ct	128	math	f
gain	60	2D PROCESSING	
FLAGS		sb1	0.007
il	n	sbs1	not used
dp	y	gf1	0.005
hs	nn	gfs1	0.001
2D ACQUISITION		proc1	ft
sw1	30007.0	fn1	2048
ni	512	DISPLAY	
phase	1	vs	102968
SEQUENCE		th	0
pwx	12.0	rp	179.8
pwxlvl	60	lp	0
j	140	rfl	4428.3
mbond	y	rfp	3606.6
taumb	0.0625	sc	0
GRADIENTS		wc	150
gzlvl1	2000	hzmm	8.20
gt1	0.002000	ai	av
gzlvl2	2000	2D DISPLAY	
gt2	0.002000	rp1	0
gzlvl3	-1010	lp1	0
gt3	0.002000	rfl1	20904.0
gstab	0.000050	rfp1	16192.6
		sc2	0
		wc2	150

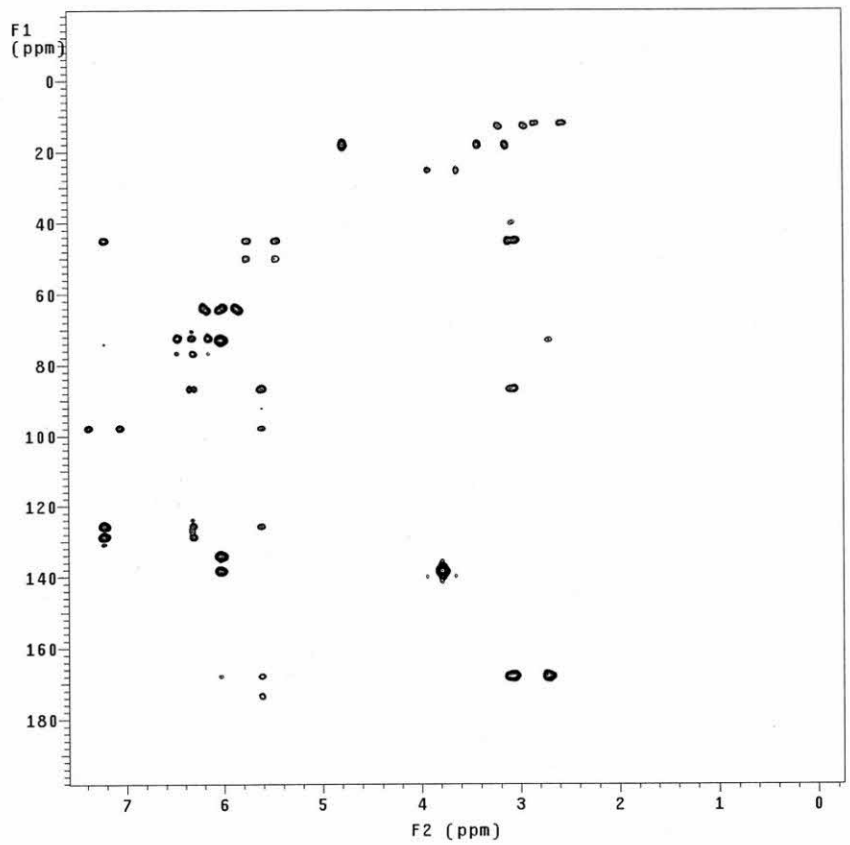
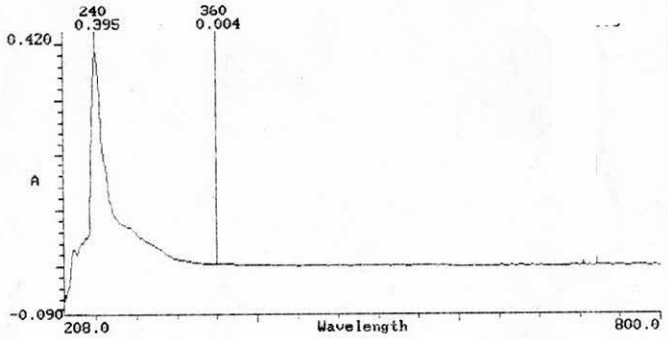


Figure C9 HMBC spectrum of compound M-3 (in methanol-d_4)

TEST SETUP
GENESYS 6 v2.001 2M9M008001

Scanning	21:54 8Apr10
Test Name	CH-1
Measurement Mode	Absorbance
Start Wavelength	200.0nm
Stop Wavelength	800.0nm
Sample Positioner	Auto 6
Scan Speed	Fast
Interval	2.0nm
Cell Correction	Off
ID# (0=OFF)	1
Auto Print	On
Auto Save Data	Off



ID#: 1
Smoothing [On]

Wavelength	Abs	
240.0	0.395	Peak
360.0	0.004	Peak

Figure D1 UV-Visible spectrum of H-1 in Chloroform

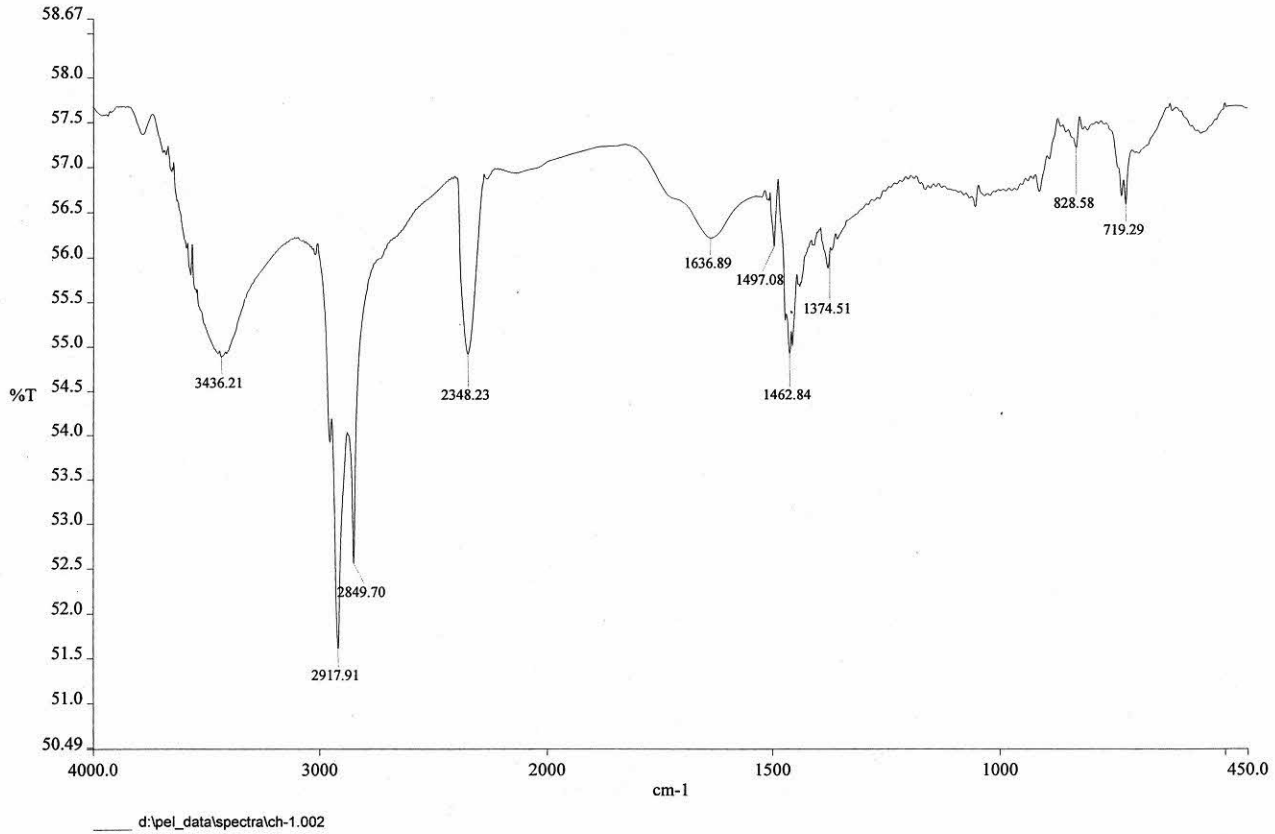


Figure D2 IR spectrum of compound H-1 (KBr disc)

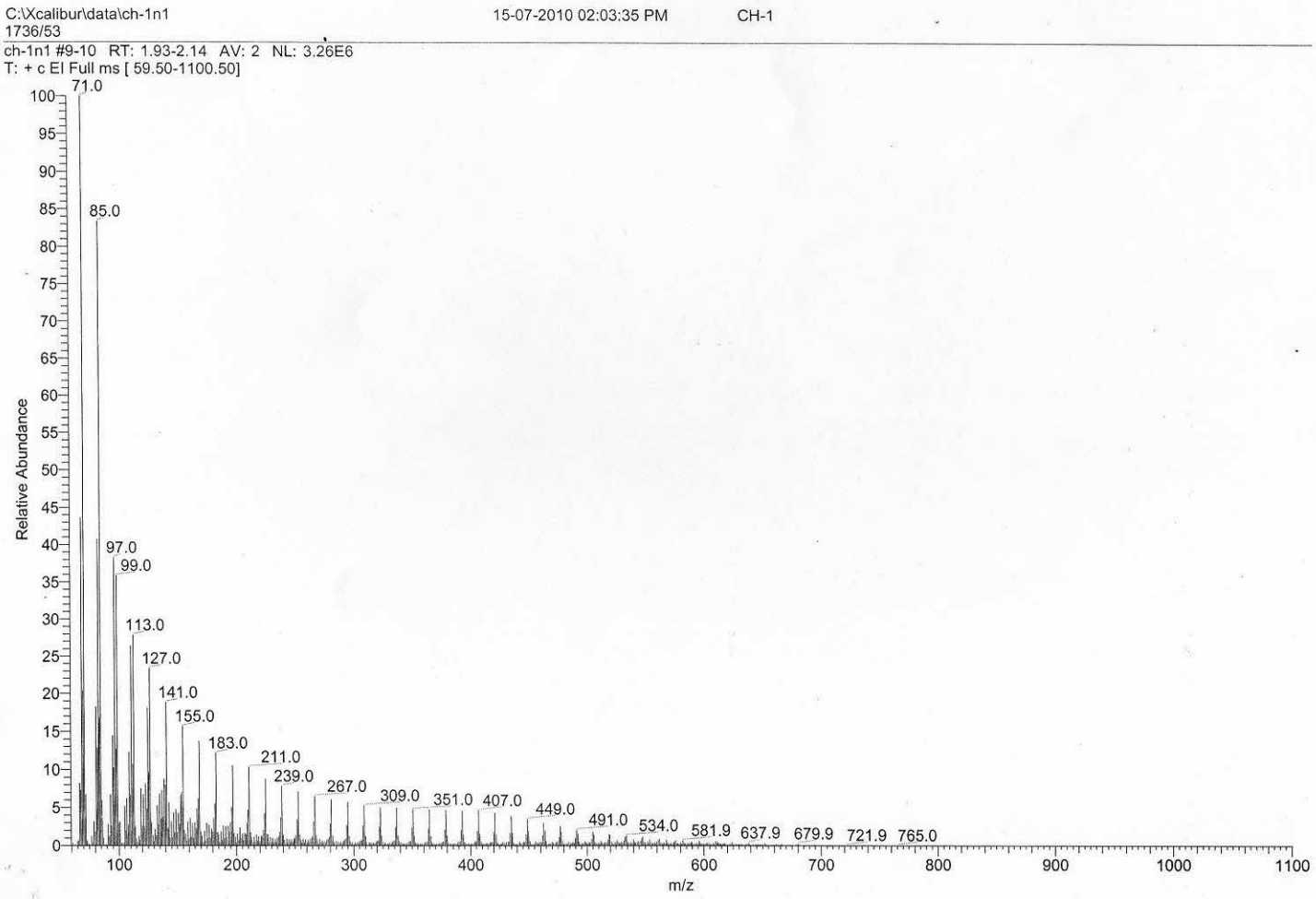


Figure D3 EI mass spectrum of compound H-1

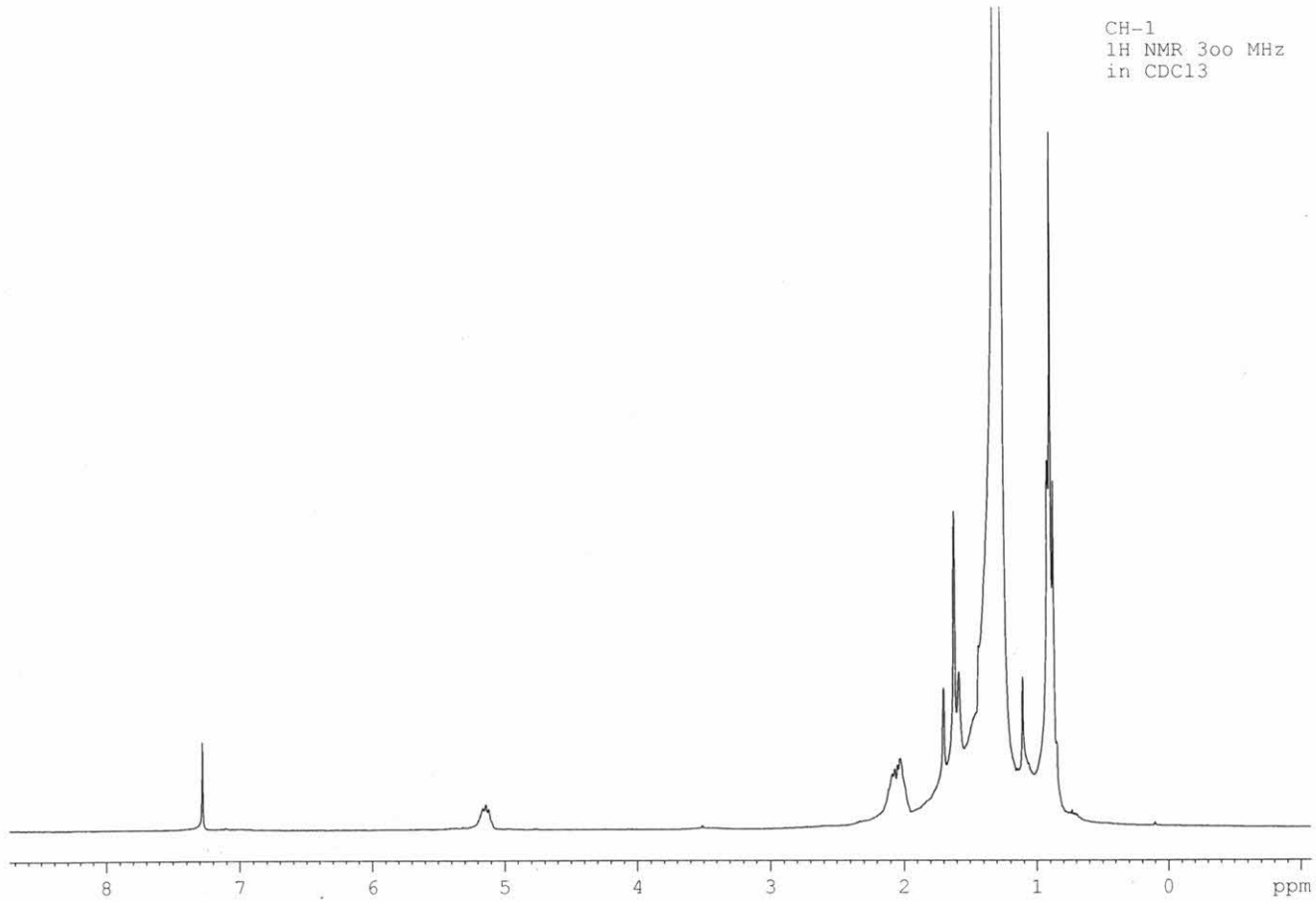


Figure D4 300 MHz ^{1}H spectrum of compound H-1 (in chloroform –*d*)

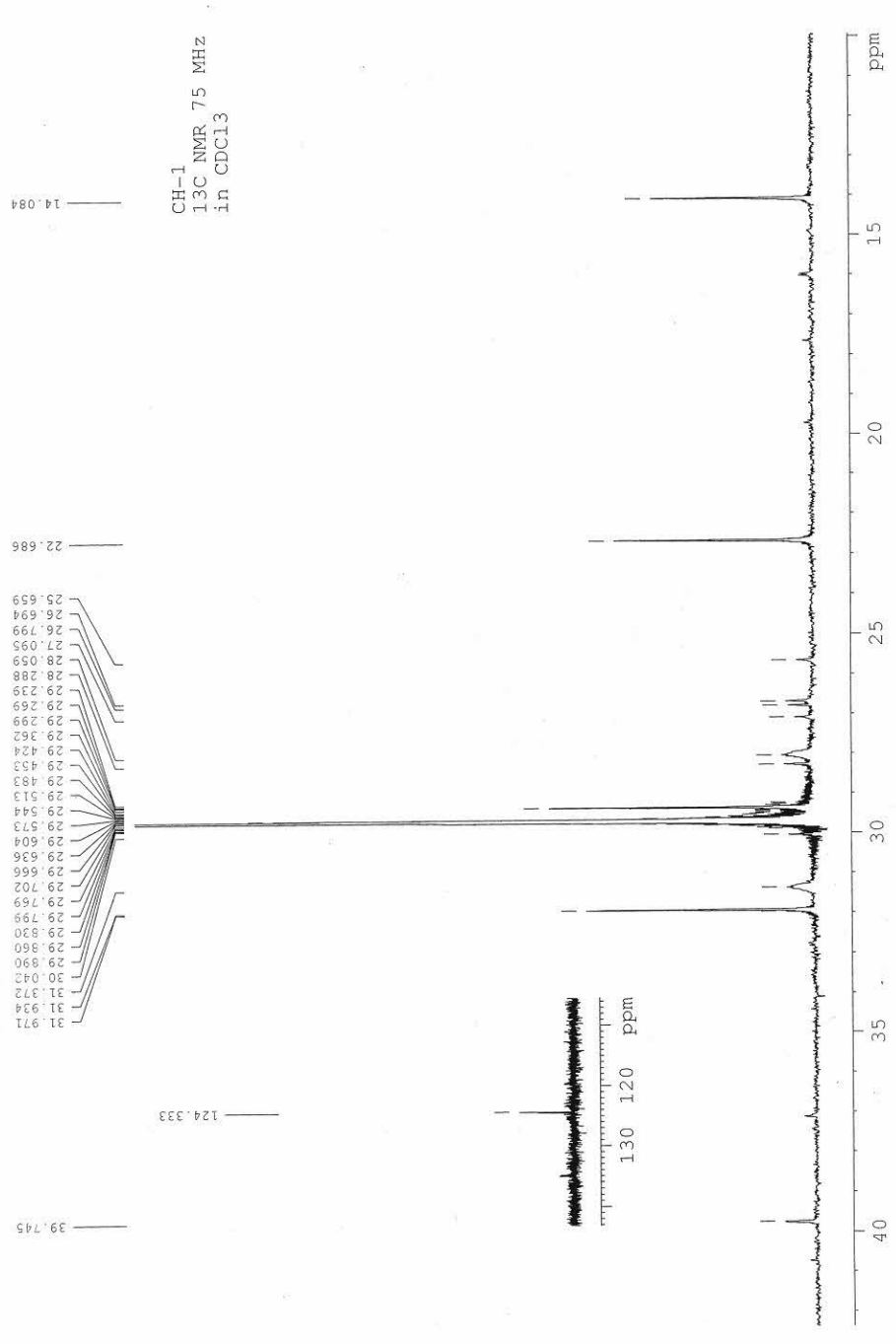
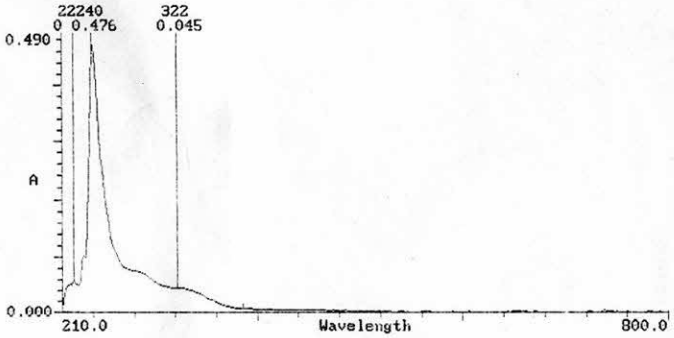


Figure D5 75 MHz ^{13}C spectrum of compound H-1 (in chloroform –*d*)

TEST SETUP
GENESYS 6 v2.001 2M9M008001

Scanning	21:40 8Apr10
Test Name	CH-2
Measurement Mode	Absorbance
Start Wavelength	210.0nm
Stop Wavelength	800.0nm
Sample Positioner	Auto 6
Scan Speed	Fast
Interval	2.0nm
Cell Correction	Off
ID# (0=OFF)	1
Auto Print	On
Auto Save Data	Off



ID#: 1
Smoothing [On]

Wavelength	Abs	
240.0	0.476	Peak
222.0	0.057	Peak
322.0	0.045	Peak

Figure E1 UV-Visible spectrum of H-2 in Chloroform

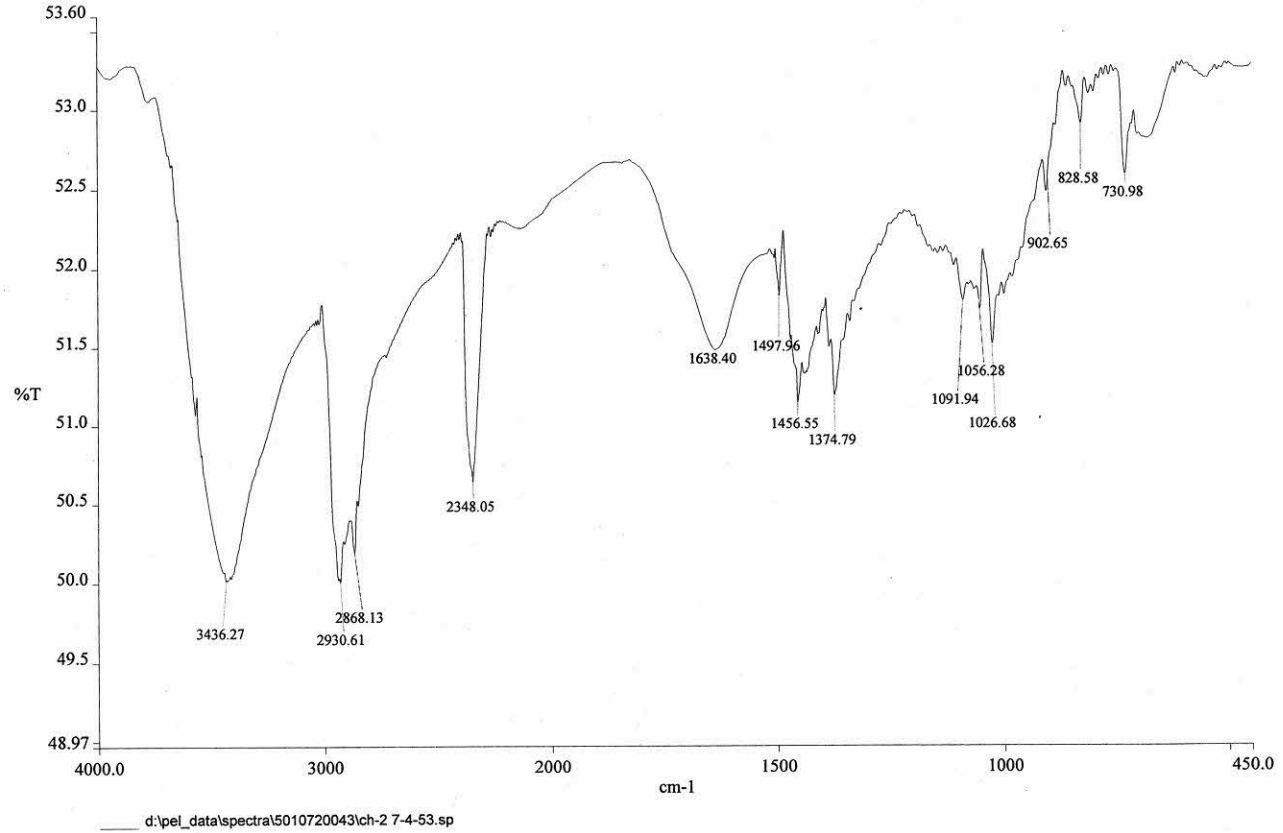


Figure E2 IR spectrum of compound H-2 (KBr disc)

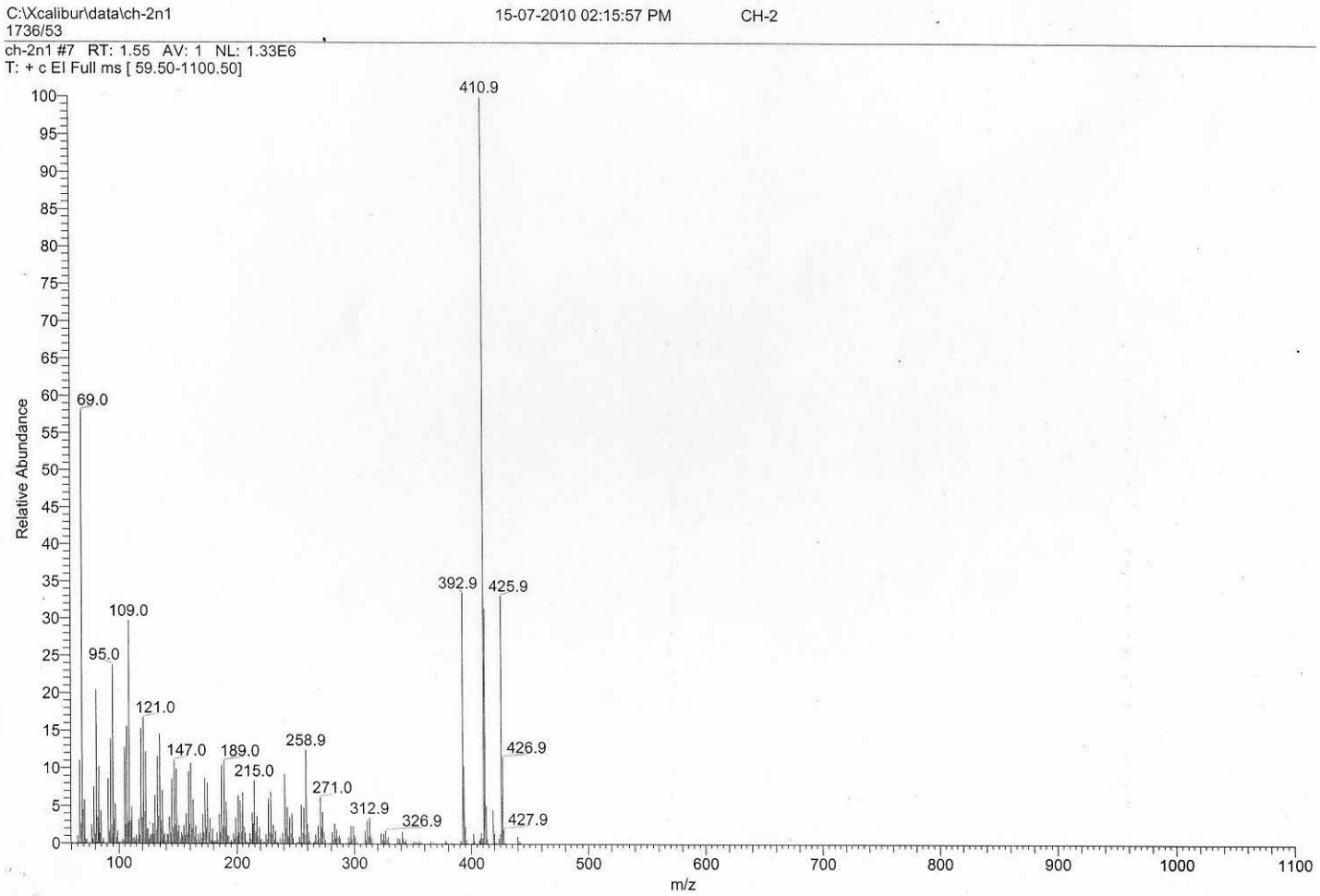


Figure E3 EI mass spectrum of compound H-2

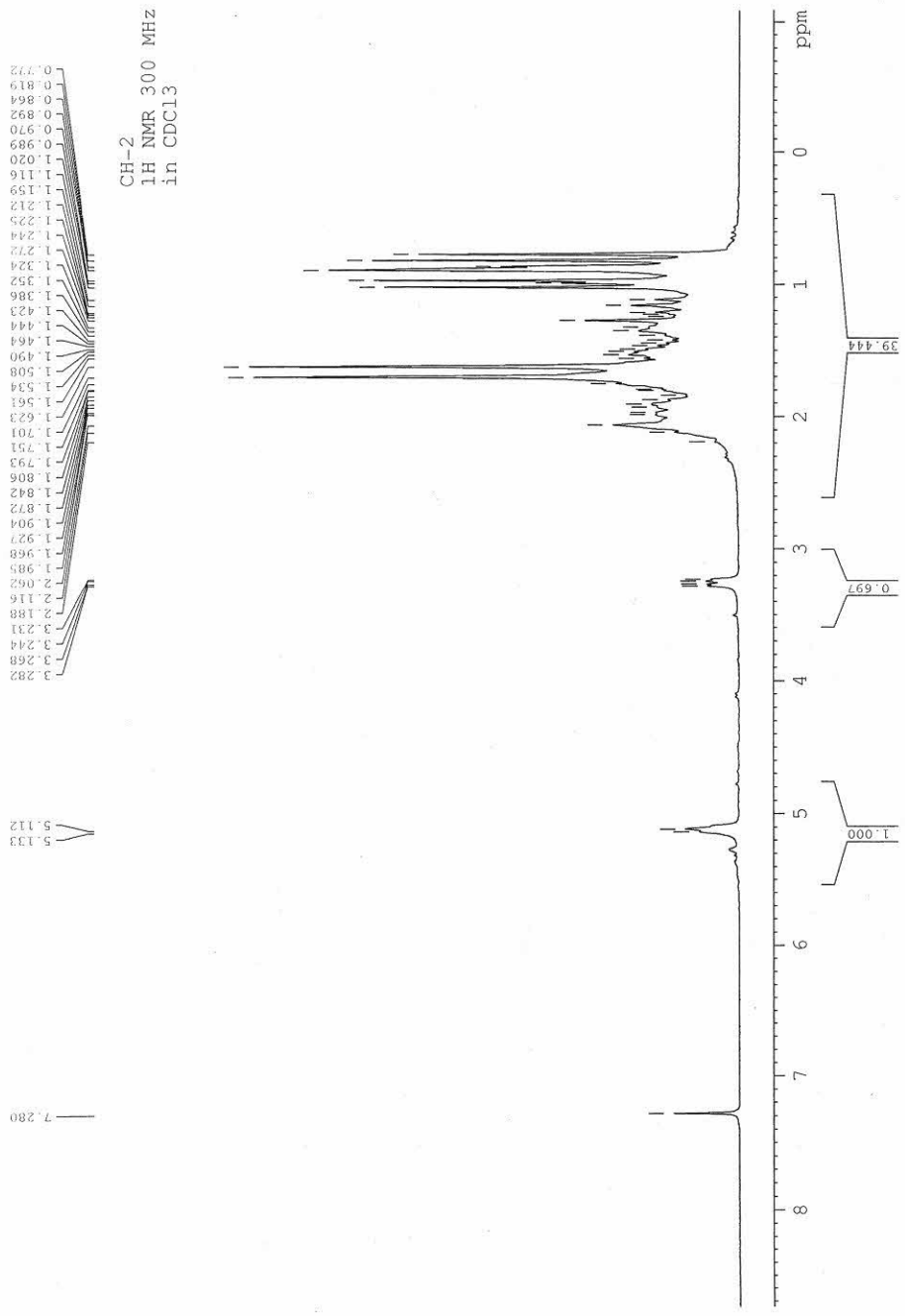


Figure E4 300 MHz ^{1}H spectrum of compound H-2 (in chloroform –*d*)

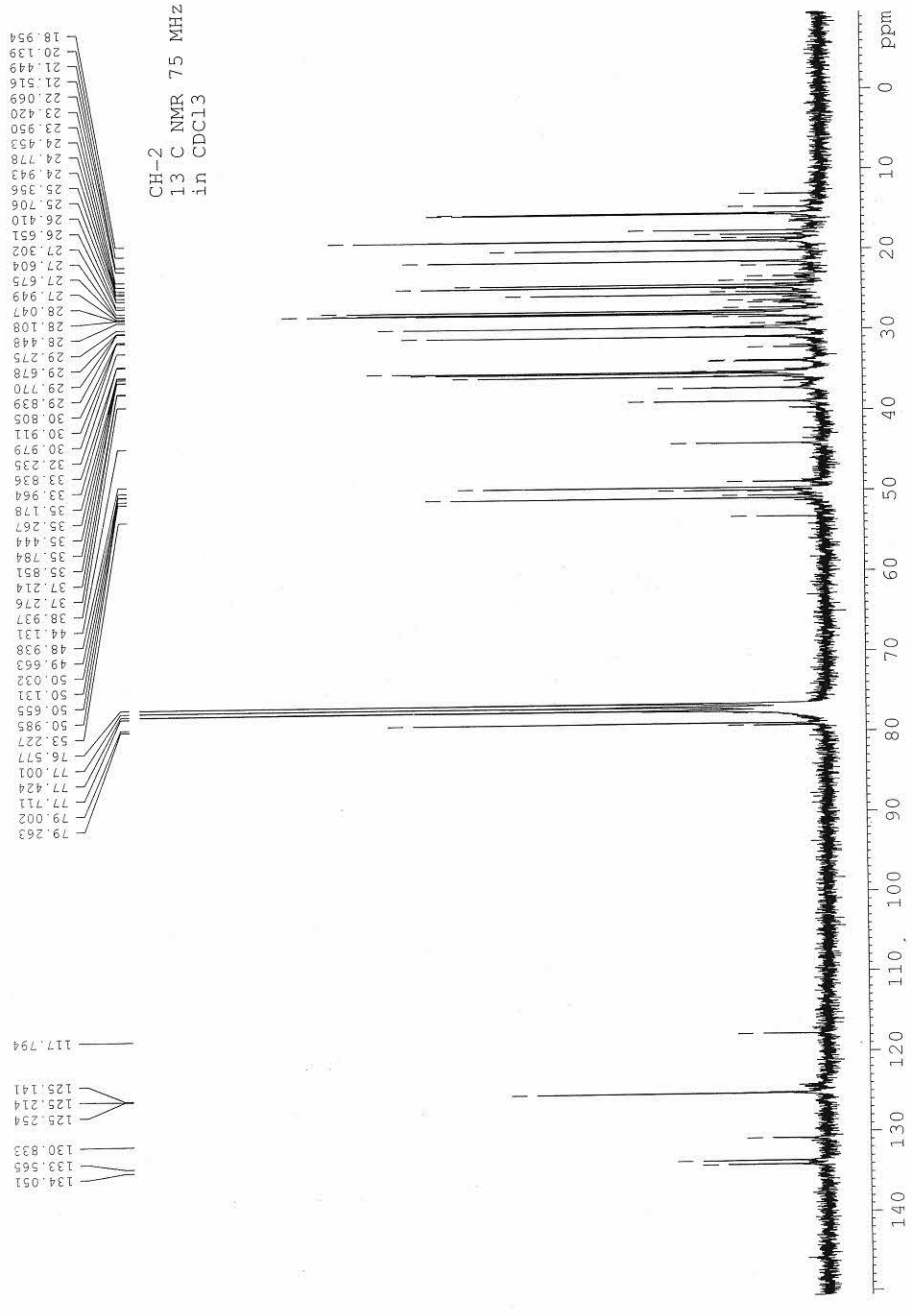
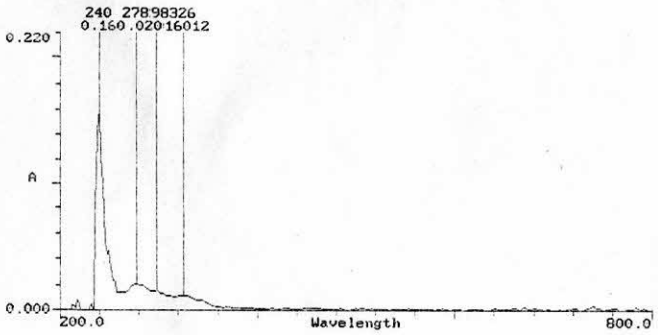


Figure E5 75 MHz ^{13}C spectrum of compound H-2 (in chloroform –*d*) (continued)

TEST SETUP
GENESYS 6 v2.001 2M9M008001

Scanning	21:12 8Apr10
Test Name	CH-5
Measurement Mode	Absorbance
Start Wavelength	200.0nm
Stop Wavelength	800.0nm
Sample Positioner	Auto 6
Scan Speed	Fast
Interval	2.0nm
Cell Correction	Off
ID# (0=OFF)	1
Auto Print	On
Auto Save Data	Off



ID#: 1
Smoothing [On]

Wavelength	Abs	
240.0	0.160	Peak
278.0	0.020	Peak
298.0	0.016	Peak
326.0	0.012	Peak

Figure F1 UV-Visible spectrum of H-3 in Chloroform

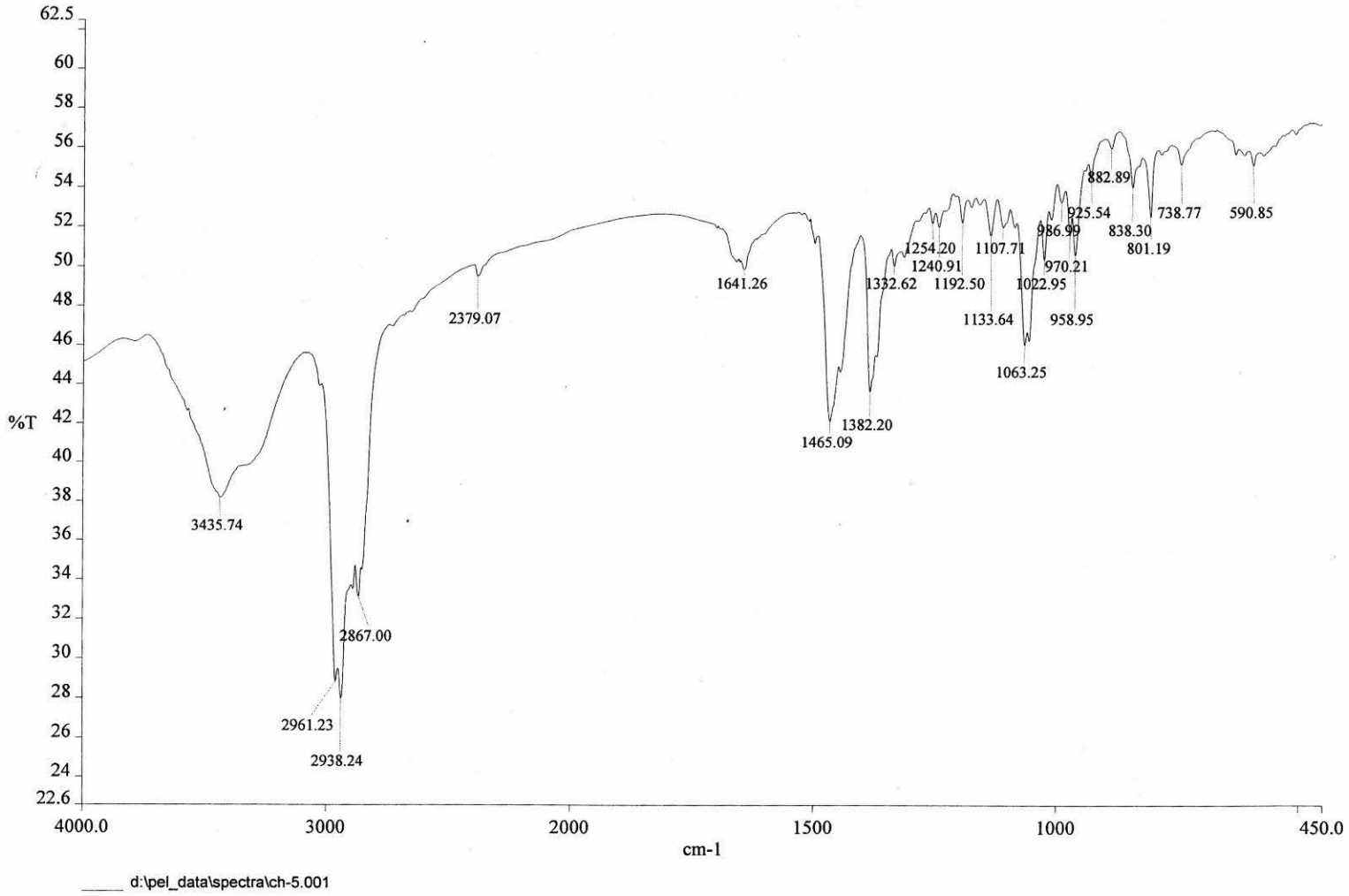


Figure F2 IR spectrum of compound H-3 (KBr disc)

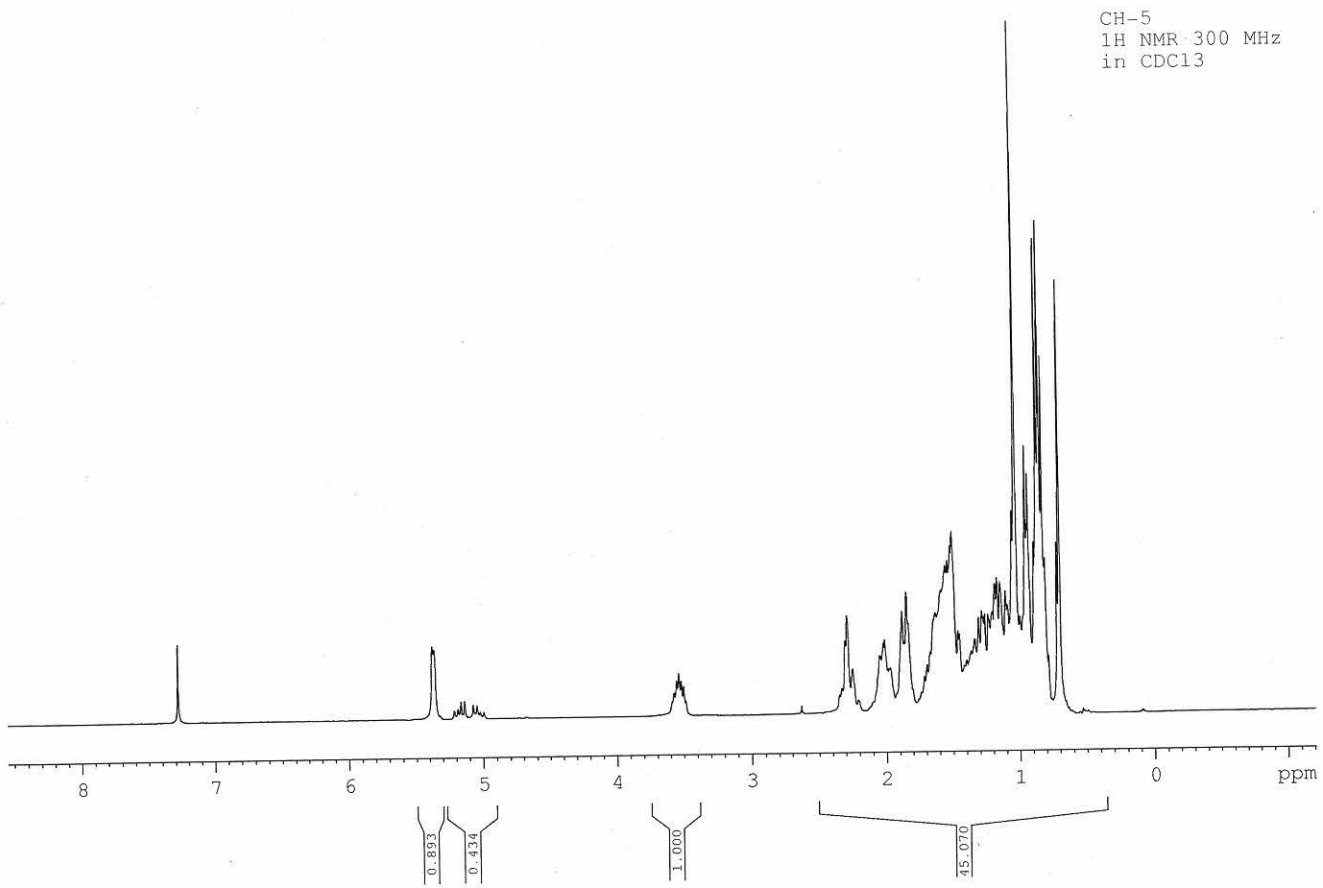


Figure F3 300 MHz ^{1}H spectrum of compound H-3 (in chloroform –*d*)

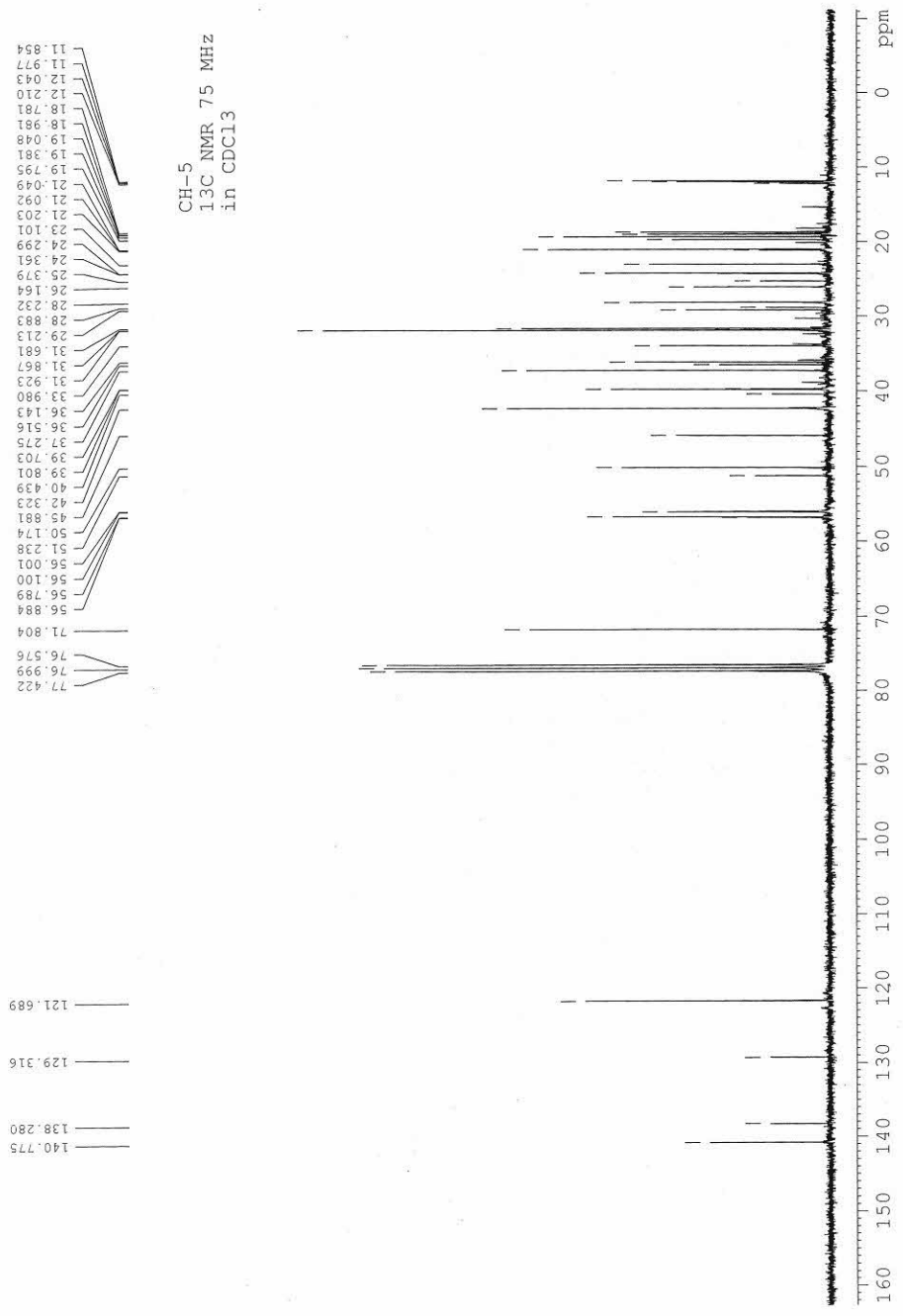


Figure F4 75 MHz ^{13}C spectrum of compound H-3 (in chloroform –*d*)

VITAE

Name Mr. Imron Meechai

Student 5010720043

Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Chemistry)	Yala Rajabhat University	2006

List of Publication and Proceedings

Meechai, I., Puripattanavong, J. and Dej-adisai, S. 2010. Anti-tyrosinase and anti-microbial activities from the root of ***Artocarpus integer***. Thailand Research Symposium 2010 Proceedings, 26-31 August, 2010 at Bangkok Convention Centre, Central World, Bangkok, Thailand (poster presentation).

Meechai, I., Puripattanavong, J. and Dej-adisai, S. 2010. Screening of anti-tyroisnase activity from Thai medicinal planta. NRCT-JSPS Core University Program on Natural Medicine in Pharmaceutical Sciences ***The 9th Joint Seminar*** Natural Medicine Research for the Next Decade: New Challenges and Future Collabroraotion Proceedings, 8-9 December, 2010. Facluty of Pharmaceutical Sciences, Chulalonhkorn University, Bangkok, Thailand (poster presentation).