การศึกษาทางพฤกษเคมีของผักหวานนก

นางสาว ทัตยา โลหกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชพฤกษศาสตร์ ภาควิชาเภสัชพฤกษศาสตร์
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2546

ISBN 974-17-4969-4

PHYTOCHEMICAL STUDY OF *SAUROPUS HIRSUTUS* BEILLE.

Miss Tattaya Lohakul

A Thesis Submitted in Partial Fulfillment of Requirements
for the Degree of Master of Science in Pharmacy in Pharmaceutical Botany

Department of Pharmaceutical Botany
Faculty of Pharmaceutical Sciences
Chulalongkorn University

Academic Year 2003
ISBN 974-17-4969-4

Thesis Title	PHYTOCHEMICAL STUDY OF *SAUROPUS HIRSUTUS* BEILLE.
By	Miss Tattaya Lohakul
Field of study	Pharmaceutical Botany
Thesis Advisor	Associate Professor Ekarin Saifah, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of Requirements for the Master's Degree

……..Dean of Faculty of Pharmaceutical Sciences

(Associate Professor Boonyong Tantisira, Ph.D.)

THESIS COMMITTEE

……..Chairman

(Associate Professor Rapepol Bavovada, Ph.D.)

……..Thesis Advisor

(Associate Professor Ekarin Saifah, Ph.D.)

……..Member

(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

……Member

(Assistant Professor Rutt Suttisri, Ph.D.)

ทัตยา โลหกุล : การศึกษาทางพฤกษเคมีของผักหวานนก
(PHYTOCHEMICAL STUDY OF *SAUROPUS HIRSUTUS* BEILLE.)
อ. ที่ปรึกษา : รศ. ดร. เอกรินทร์ สายฟ้า, 141 หน้า. ISBN 974-17-4969-4

จากส่วนที่อยู่เหนือดินของผักหวานนก (*Sauropus hirsutus* Beille.) สามารถสกัดแยกสารอัลคาลอยด์ ในกลุ่ม ไอโสควิโนลีน ชนิดใหม่ได้ 2 ชนิด คือ 4-methoxy-2-methyl-7, 8-methylenedioxy-1-isoquinolone และ 4, 6-dimethoxy-2-methyl-7, 8-methylenedioxy-1-isoquinolone สารในกลุ่มฟลาวาน 1 ชนิด คือ epicatechin รวมทั้งสารในกลุ่มสเตอรอลอีก 1 ชนิด คือ β-sitosterol การพิสูจน์เอกลักษณ์ของสารเหล่านี้ ทำโดยการวิเคราะห์ข้อมูลทางสเปกโทรสโกปี จาก UV, IR. MS, 1-D และ 2-D NMR ร่วมกับการเปรียบเทียบค่าที่ได้มีรายงานไว้แล้ว

ภาควิชาเภสัชพฤกษศาสตร์	ลายมือชื่อนิสิต.....
สาขาวิชาเภสัชพฤกษศาสตร์	ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา 2546	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4476574033 : MAJOR PHARMACEUTICAL BOTANY

KEYWORD : *SAUROPUS HIRSUTUS* / EUPHORBIACEAE / ISOQUINOLINE ALKALOID / FLAVAN / STEROID

TATTAYA LOHAKUL : PHYTOCHEMICAL STUDY OF *SAUROPUS HIRSUTUS* BEILLE. THESIS ADVISOR : ASSOCIATE PROFESSOR EKARIN SAIFAH, Ph.D.

141 pp. ISBN 974-17-4969-4

From the aerial part of *Sauropus hirsutus* Beille., two new isoquinoline alkaloids, 4-methoxy-2-methyl-7, 8- methylenedioxy-1-isoquinolone and 4, 6-dimethoxy-2-methyl-7, 8-methylenedioxy-1-isoquinolone, a flavan, epicatechin, together with a sterol, β-sitosterol, were isolated. Identification of these compounds was accomplished by analysis of their spectroscopy data: UV, IR, MS, 1-D and 2-D NMR, as well as comparison with reported values.

Department	Pharmaceutical Botany	Student's signature…….
Field of study	Pharmaceutical Botany	Advisor's signature…….
Academic year	2003	Co-advisor's signature……..

ACKNOWLEDGEMENTS

The author wished to express her sincere gratitude to her thesis advisor, Associate Professor Dr. Ekarin Saifah of the Department of Pharmaceutical Botany, Chulalongkorn University, for his guidance, suggestion, encouragement and kindness throughout the course of this study.

The author would also like to express her grateful thanks to Associate Professor Dr. Rapepol Bavovada of the Department of Pharmaceutical Botany and Associate Professor Dr. Nijsiri Ruangrungsi of the Department of Pharmacognosy for their concern, assistance and valuable suggestion.

The author is deeply indebted to Assistant Professor Dr. Rutt Suttisri of the Department of Pharmaceutical Botany for his extreme goodness and invaluable helps for structure elucidation.

Additional sincere thanks are expressed to all staff members of the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their kindness and help.

The author would like to thank the Graduate School of Chulalongkorn University for granting partial financial support to conduct this investigation.

Finally, The author wish to express infinite gratitude to her family for their love and encouragement.

CONTENTS

Page

CONTENTS (continued)

Page

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF SCHEMES

ABBREVIATIONS

br s	=	broad singlet (for NMR spectra)
^{0}C	=	degree celsius
CC	=	column chromatography
$CDCl_3$	=	Deuterated chloroform
CD_3OD	=	Deuterated methanol
$CHCl_3$	=	chloroform
cm	=	centimeter
^{13}C-NMR	=	Carbon-13 Nuclear Magnetic Resonance
COSY	=	Correlated Spectroscopy
δ	=	chemical shift
d	=	doublet (for NMR spectra)
dd	=	doublet of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Tranfer
DMSO-d_6	=	Deuterated dimethylsulfoxide
EIMS	=	Electron Impact Mass Spectroscopy
ESI TOFMS	=	Electrospray Ionization TOF Mass Spectroscopy
ε	=	molar absorptivity
g	=	gram
^{1}H-NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	^{1}H-detected Heteronuclear Multiple Bond Coherence
HMQC	=	^{1}H-detected Heteronuclear Multiple Quantum Coherence
Hz	=	Hertz
IR	=	Infrared
J	=	coupling constant
KBr	=	potassium bromide
λ_{max}	=	wavelength at maximum absorption (nm)
m	=	multiplet (for NMR spectra)
m	=	meter

M^+	=	molecular ion
mg	=	milligram
MHz	=	Megahertz
ml	=	milliliter
mm	=	millimeter
MS	=	Mass Spectrum
m/z	=	mass-to-charge ratio
nm	=	nanometer
NMR	=	Nuclear Magnetic Resonance
NOESY	=	Nuclear Overhauser Effect Spectroscopy
n.s.	=	not specified
ν_{max}	=	wavenumber at maximum absorption
ppm	=	part per million
q	=	quartet (for NMR spectra)
rel. int.	=	relative intensity
s	=	singlet (for NMR spectra)
sp.	=	species
t	=	triplet (for NMR spectra)
TLC	=	Thin Layer Chromatography
UV	=	Ultraviolet

CHAPTER I

INTRODUCTION

Sauropus hirsutus Beille. (Figure 1) is a plant belonging to the family Euphorbiaceae. In Thailand, this plant is called Phak wan nok or Tai bai (Kanchanaburi), Kom koi (Phetchabun), Kongkoi lot khon (Central) and Ra ngap manut (Chumphon) (ส่วนพฤกษศาสตร์ป่าไม้ สำนักวิชาการป่าไม้ กรมป่าไม้, 2544). *Sauropus hirsutus* is a prostrate woody herb to small shrub which can grow up to 40 cm–2 m high. The branches are round and hirsute, with 1.8-4 by 0.7-2 mm stipules. The petiole is hairy, 1.7-2.8 mm long. The leaf blade is ovate to elliptic to obovate, 1.4-7.7 by 1-3.8 cm (length/width ratio). The leaf base is rounded to cuneate. The leaf margin is sometimes revolute, the apex bluntly acute, usually mucronulate. The upper surface of the leaf is glabrous except for the margin (dark green) and the lower surface is slightly to completely hairy (light green). The number of nerves on each leaf are 5-9, often distinct.

The flowers are usually few (to many) together and their color is green to dark red. The diameter of staminate flowers are 1.5-4 mm. Its pedicel is subglabrous and 1.4-4.3 mm long. The size of the calyx lobes are 0.4-1.8 by 0.6-1.3 mm, slightly hair on the outside. The stamens of *Sauropus hirsutus* consist of androphore (0.2-0.3 mm long) and anther (0.2-0.3 by 0.2-0.4 mm). The pistillate flowers are 5-15 mm in diameter. Its pedicel is hirsute and 2.4-4 mm long. It has 3 smaller calyx lobes, 2.2-5.5 by 1.4-3 (to 5.8 by 3.8 in fruit) mm, 3 larger ones, 3.2-13 by 2.2-4.5 mm, hairy on the outside. The size of ovary is 1-1.8 by 1.3-2.7 mm. The stigmas are horizontal. The shape of its fruits is ovoid and 5-8 by 5-8 mm in size. Seeds of this plant are in triangular shape, in section, and 5 by 2.7 by 2.7 mm in size.

Sauropus hirsutus is distributed in Thailand, Laos and Cambodia. In Thailand, it could be found in the northern (Chiang Mai, Lampang, Phrae, Sukhothai and Nakhon Sawan), north-eastern (Phentchabun, Loei, Udon Thani, Sakhon Nakhon, Nakhon Phanom and Khon Kaen), eastern (Chaiyaphum, Nakhon Ratchasima, Buri Ram, Surin and Ubon Ratchatani), south-western (Uthai Thani, Kanchanaburi, Ratchaburi,

Phetchaburi and Prachuap Khiri Khan), central (Lop Buri and Nakhon Pathom) and south-eastern (Prachin Buri and Chon Buri).

The plant is distributed in deciduous forest, dry dipterocapus forest, bamboo forest, secondary growths, grassy (buffalo grazing) ground and waste land (along railways and roads) (Airy Shaw, 1972).

The only species of *Sauropus* that have been investigated is *Sauropus androgynus*. It is a vegetable cultivated in India, Malaysia, Indonesia, Southwest China and Vietnam (Chang *et al*.,1997). The leaves of this plant is widely comsumed after cooking in Malaysia (Luh *et al*., 1999). It contains large amounts of various nutrients (Padmavathi and Rao, 1990; Hulshof *et al*., 1997; Ching and Mohamed, 2001). It has been reported that excessive consumption of *Sauropus androgynus* leaves containing a considerable amount of the alkaloid papaverine may cause dizziness, drowsiness and constipation (Bander and Ismail, 1973; Yu and Cheah, 1979).

In late1994, *Sauropus androgynus* was imported into Taiwan from the Indo-Malaysia region. Its raw leaves or aqueous extract were used as weight reduction substance (Chag *et al*., 1997). In August 1995, an outbreak of *Sauropus androgynus* intoxication in Taiwan has been reported (Lai *et al*., 1996). The consumption of uncooked *Sauropus androgynus* for body weight reduction experienced constrictive a bronchiolitis obliterans (Chang *et al*., 1998; Hsiue *et al*,1998; Wang *et al*., 2000) and 3 cases reported arrhythmia (Chen *et al*., 1996).

In Thailand, the roots of *Sauropus androgynus* are used ethnomedically as a remedy for fever and externally for mumps. Its leaves can be used to treat wound and abscess, and the flowers are used as abortifacient (มาโนช วามานนท์ และ เพ็ญนภา ทรัพย์เจริญ, 2540; นันทวัน บุณยะ ประภัศร และ อรนุช โชคชัยเจริญพร, 2542).

To the present, phytochemical study of *Sauropus hirsutus* has never been reported. Preliminary examination of this plant revealed a positive test for alkaloids. Therefore, it is the purpose of this investigation to study the nature of the compounds in the aerial part of *Sauropus hirsutus*. The result of this investigation may serve as an additional information on the chemical nature of this plant family, which could be a valuable lead in the fields of chemotaxonomy and phytochemistry.

Figure 1. *Sauropus hirsutus* Beille.

CHAPTER II

HISTORICAL

1. Botanical description of euphorbiaceous plants.

The Euphorbiaceae family is a large and diverse family with about 300 genera and 8100 species. They grow extensively in the tropical and warmer regions of the world.

The plants in this family can be described as highly variable with plants growing as herbs, shrubs or trees with fleshy stems and milky or colored latex that can be irritating or toxic.

The leaves of these plants are usually alternate, but can also be opposite or even whorled. The stipules are present, large or small and gland-like. The leaves are usually simple but can also be palmately compound.

The flowers are regular and usually monoecious, but they can also be dioecious although this form is rare. The inflorescence are various in type, often compacted to form a special flower cluster called a cyathium. The perianth is usually 5-merous, distinct or connate. The androecium consists of one to many stamens that are free or united. The filaments are distinct or connate. Sometimes the nectary disk is present. The opening of anther is by longitudinal slits. The gynoecium of a flower consists of a compound pistil of 3 united carpels (but they can have 2 or 4), with as many locules. The ovary is superior and commonly 3-lobed. There are 1 or 2 ovules in each locule attached to apical-axile placentas. The styles can be distinct or connate into a single style.

The fruit is usually a dehiscent capsule, but can occasionally be an indehiscent utricle in 1-celled species. This schizocarp separates elastically into usually 3 segments that split ventrally.

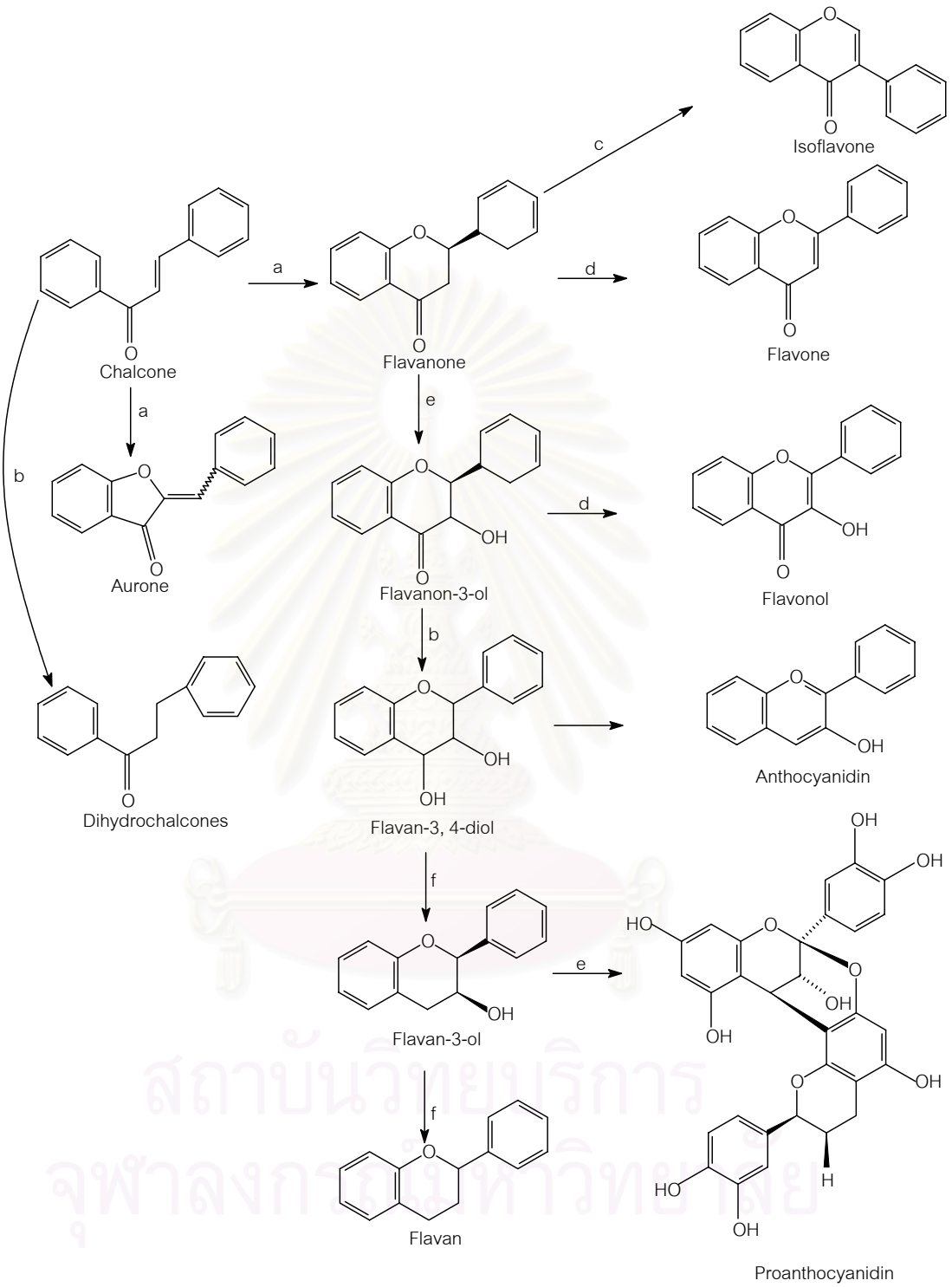
The seeds are abundant, having fleshy endosperm with the embryo straight or curved (Dennis, 2000).

2. Chemical constituents of the family Euphorbiaceae.

2.1 Flavonoids from euphorbiaceous plants.

Flavonoids are a group of natural products isolated from a wide variety of plants, and are responsible for much of the colouring found in vascular plants. A single plant may contain dozens of different flavonoids, and the distribution of flavonoids within a plant family can yield useful classifying information about that family. Flavonoids exhibit a wide range of biological activities (Harborne, 1994).

Flavonoids can be classified according to their biosynthetic origin. Some flavonoid types are both intermediates in biosynthesis as well as end-product, which can accumulate in plant tissues. These include chalcones (the first formed C_{15} structure derived from malonyl coenzyme A and *p*-coumaryl coenzyme A), flavanones, flavanon-3-ols and flavan-3,4-diols. Other classes are only known as end-products of biosynthesis e.g. anthocyanins, flavones and flavonols. Two further classes of flavonoids are those in which the 2-phenyl side chain of flavanone isomerizes to the 3-position (giving rise to isoflavones and related isoflavonoids) and then to the 4-position (give rise to the neoflavonoids) as shown in Scheme 1.



Scheme 1. Biosynthetic relationship of flavonoids

a=cyclization, b=bioreduction, c=aryl migration,

d=dehydrogenation, e=hydroxylation, f=dehydroxylation

Many flavonoids which occur naturally are associated with sugars in conjugated form and within any one class may be characterized as monoglycosidic, diglycosidic, etc. Glycosidic complexity is considerable. There are, for example, over 1,500 glycosides of the flavones and flavonols that have been isolated to date. Mono-, di- and tri-saccharides may be linked through a phenolic hydroxyl; and one or more such hydroxyl groups may carry a sugar substitution. Acylated O-glycosides are known, where aromatic or aliphatic acids are linked through the 6-hydroxyl of a glucose moiety. A special group of mainly flavone-based C-glycosides occurs in plant. Sulphate conjugates are common in the flavone and flavonol series, where the sulphation may be on a phenolic hydroxyl and/or on an aliphatic hydroxyl of glycoside moiety (Chapman and Hall, 1994).

Many types of flavonoid are found in family Euphorbiaceae and these are summarized in Table 1.

Table 1 Flavonoids from euphorbiaceous plants.

Compound	Source	Plant part	Reference
Flavanone			
Bonannione A **[1]**	*Macaranga pleiostemona*	leaves	Schutz *et al*., 1995
5,4′-Dihydroxy-[2′′-(1-hydroxyl) dihydrofurano]			
-(7,8:5′′,4′′) flavanone **[2]**	*Macaranga conifera*	leaves	Jang *et al*., 2002
5, 7-Dihydroxy-4′-methoxy-8			
-(3-methylbut-2-enyl) flavanone **[3]**	*Macaranga conifera*	leaves	Jang *et al*., 2002
5, 7-Dihydroxy-4′-methoxy-8			
-(2-hydroxy-3-methylbut-3-enyl) flavanone **[4]**	*Macaranga conifera*	leaves	Jang *et al*., 2002
6, 7-Dimethoxy-3′,4′-methylenedioxyflavanone **[5]**	*Macaranga indica*	leaves	Sultana and Ilyas, 1987
Euchrestaflavanone A **[6]**	*Macaranga pleiostemona*	leaves	Schutz *et al*., 1995
5-Hydroxy-4′-methoxy-2′′,2′′-dimethylpyrano			
-(7,8:6′′,5′′) flavanone **[7]**	*Macaranga conifera*	leaves	Jang *et al*., 2002
Lonchocarpol A **[8]**	*Macaranga conifera*	leaves	Jang *et al*., 2002
Macarangaflavanone A **[9]**	*Macaranga pleiostemona*	leaves	Schutz *et al*., 1995
Macarangaflavanone B **[10]**	*Macaranga pleiostemona*	leaves	Schutz *et al*., 1995
Sophoraflavanone B **[11]**	*Macaranga conifera*	leaves	Jang *et al*., 2002

Table 1. (continued)

Compound	Source	Plant part	Reference
Tomentosanol D **[12]**	*Macaranga conifera*	leaves	Jang *et al*., 2002
Flavanone glycoside			
Prunin **[13]**	*Euphorbia pekinensis*	aerial part	Ahn *et al*., 1996
Flavanonol			
Lupinifolinol **[14]**	*Macaranga conifera*	leaves	Jang *et al*., 2002
Isoflavone			
7-Methyltectorigenin **[15]**	*Macaranga indica*	leaves	Sultana and Ilyas, 1987
Flavone			
Apigenin **[16]**	*Euphorbia serpens microfilia*	n.s.	Del V. Galarza, Cabreya and Juliani, 1983
	Euphorbia minuta	n.s.	Del V. Galarza, *et al*., 1983

Table 1. (continued)

Compound	Source	Plant part	Reference
Chrysin **[17]**	*Acalypha indica*	whole	Hiremath, Rudresh and Badami, 1998
5-Hydroxy-7,4′-dimethoxyflavone **[18]**	*Croton betolaster*	n.s.	Barbosa *et al*., 2003
Luteolin **[19]**	*Euphorbia serpens microfilia*	n.s.	Del V. Galarza, *et al*., 1983
Pinnatin **[20]**	*Gelonium multiflorum*	roots	Das *et al*., 1994
Flavone glycosides			
Apigenin 7-O-glucoside **[21]**	*Euphorbia minuta*	n.s.	Del V. Galarza, *et al*., 1983
Isovitexin **[22]**	*Euphorbia serpens microfilia*	n.s.	Del V. Galarza, *et al*., 1983
	Euphorbia minuta	n.s.	Del V. Galarza, *et al*., 1983
	Jatropha curcas	leaves	Subramanian, Nagarajan and Sulochana, 1971
	Jatropha heynii	leaves	Subramanian *et al*., 1971

Table 1. (continued)

Compound	Source	Plant part	Reference
	Hevea brasiliensis	leaves	Subramanian *et al*., 1971
Luteolin-7-O-glucoside **[23]**	*Euphorbia minuta*	n.s.	Del V. Galarza *et al*., 1983
Swertisin **[24]**	*Aleurites moluccana*	leaves	Meyre-Silva *et al*., 1997
Vitexin **[25]**	*Macaranga triloba*	leaves	Vinh, Nguyen and Nguyen, 2002
	Euphorbia serpens microfilia	n.s.	Del V. Galarza *et al*., 1983
	Euphorbia minuta	n.s.	Del V. Galarza, *et al*., 1983
	Jatropha curcas	leaves	Subramanian *et al*., 1971
	Jatropha heynii	leaves	Subramanian *et al*., 1971
	Hevea brasiliensis	leaves	Subramanian *et al*., 1971
Favonol			
Ayanin **[26]**	*Croton schiedeanus*	n.s.	Puebla *et al*., 2003
Desmethoxykanugin **[27]**	*Gelonium multiflorum*	roots	Das *et al*., 1994

Table 1. (continued)

Compound	Source	Plant part	Reference
Ferrugin [28]	*Bridelia ferruginea*	stem bark	Cimanga *et al*., 2001
Galangin [29]	*Acalypha indica*	whole	Hiremath *et al*., 1998
Isolicoflavonol [30]	*Macaranga conifera*	leaves	Jang *et al*., 2002
Kaempferol [31]	*Euphorbia latifolia*	leaves, flowers and stems	Atalykova and Kukenov, 1981
	Euphorbia pachyrrhiza	leaves, flowers and stems	Atalykova and Kukenov, 1981
Kaempferol-3,6-dimethyl ether [32]	*Chamaesyce prostrata*	aerial part	Rojas *et al*., 1999
Kanugin [33]	*Gelonium multiflorum*	roots	Das *et al*., 1994
Myricetin [34]	*Bridelia ferruginea*	stem bark	Cimanga *et al*., 2001
3-O-Methylquercetin [35]	*Bridelia ferruginea*	stem bark	Cimanga *et al*., 2001
Quercetin [36]	*Sapium japonicum*	leaves	Ahn *et al*., 1996
	Euphorbia serpens microfilia	n.s.	Del V. Galarza *et al*., 1983
	Euphorbia minuta	n.s.	Del V. Galarza *et al*., 1983

Table 1. (continued)

Compound	Source	Plant part	Reference
	Euphorbia latifolia	leaves,flowers and stems	Atalykova and Kukenov, 1981
	Euphorbia pachyrrhiza	leaves,flowers and stems	Atalykova and Kukenov, 1981
	Euphorbia paralias	n.s.	Rizk *et al*., 1976
Quercetin 3,7-dimethyl ether **[37]**	*Croton schiedeanus*	n.s.	Guerrero *et al*., 2002 Puebla *et al*., 2003
Rutisin **[38]**	*Bridelia ferruginea*	stem bark	Cimanga *et al*., 2001
3,3′,4′,5′-Tetra-O-methylmyricetin **[39]**	*Bridelia ferruginea*	stem bark	Cimanga *et al*., 2001
Flavonol glycosides			
Afzelin **[40]**	*Sapium japonicum*	leaves	Ahn *et al*., 1996
	Euphorbia pekinensis	aerial part	Ahn *et al*., 1996
Astragalin **[41]**	*Sapium japonicum*	leaves	Ahn *et al*., 1996
	Euphorbia pekinensis	aerial part	Ahn *et al*., 1996
Euphorbianin **[42]**	*Euphorbia hirta*	leaves	Aqil and Khan, 1999
6-Hydroxykaempferol-7-rutinoside **[43]**	*Sapium euginiaefolium*	leaves	Ahmad *et al*., 1991

Table 1. (continued)

Compound	Source	Plant part	Reference
Isoquercitrin [44]	*Sapium japonicum*	leaves	Ahn *et al*., 1996
	Euphorbia pekinensis	aerial part	Ahn *et al*., 1996
	Phyllanthus sellowianus	n.s.	Hnatyszyn *et al*., 2002
	Euphorbia serpens microfilia	n.s.	Del V. Galarza *et al*., 1983
Isorhamnetin-3-rutinoside [45]	*Mercurialis annua*	n.s.	Dumkow *et al*., 1969
Isorhamnetin-3-rutinoside-4′-glucoside [46]	*Mercurialis annua*	whole plant	Harborne, 1994
Isorhamnetin-3-O-β-D-xyloside [47]	*Alchornea davidii*	leaves and twigs	Cui, Liu and Tan, 2003
Isorhamnetin-3-O-β-glucopyranoside -7-O-α-rhamnopyranoside [48]	*Chrozophora obliqua*	aerial part	Mohamed, 2001
Kaempferol-3-O-rutinoside [49]	*Euphorbia pekinensis*	aerial part	Ahn *et al*., 1996
	Manihot esculenta	leaves	Prawat *et al*., 1995
	Mercurialis perennis	n.s.	Dumkow, 1969
Kaempferol-3-sophoroside [50]	*Mercurialis perennis*	n.s.	Dumkow, 1969
Kaempferol-7-O-glucoside [51]	*Chamaesyce prostrata*	aerial part	Rojas *et al*., 1999
Kaempferol-3-O-β-D-glucosyl-7-O-α-L-rhamnosyl [52]	*Sauropus androgynus*	aerial part	Wang and Lee, 1997
Kaempferol-3-O-β-D-glucosyl-(1→6)-β-D-glucosyl [53]	*Sauropus androgynus*	aerial part	Wang and Lee, 1997

Table 1. (continued)

Compound	Source	Plant part	Reference
Kaempferol-3-O-β-D-glucosyl-(1→6)-β-D-glucosyl			
7-O-α-L-rhamnosyl **[54]**	*Sauropus androgynus*	aerial part	Wang and Lee, 1997
Quercetin-3-arabinoside **[55]**	*Euphorbia paralias*	n.s.	Rizk, Ahmed and Diab, 1979
Quercetin-3-galactoside **[56]**	*Euphorbia paralias*	n.s.	Rizk *et al*., 1979
	Euphorbia serpens microfilia	n.s.	Del V. Galarza *et al*., 1983
	Euphorbia minuta	n.s.	Del V. Galarza *et al*., 1983
Quercetin-3-glucosyl-(1→4)-rhamnoside **[57]**	*Euphorbia drancunculoides*	leaves	Harborne, 1994
	Euphorbia pekinensis	aerial part	Ahn *et al*., 1996
	Manihot esculenta	phloem sap	Calatayud, 1994
	Croton sparsiflorus	leaves	Subramanian *et al*., 1971
	Manihot utilissima	leaves	Subramanian *et al*., 1971
Quercetin-3-O-glucoside **[58]**	*Bridelia ferruginea*	stem bark	Cimanga *et al*., 2001
	Euphorbia minuta	n.s.	Del V. Galarza *et al*., 1983

Table 1. (continued)

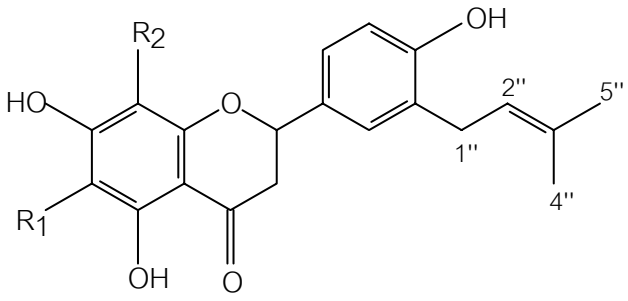
Compound	Source	Plant part	Reference
Quercetin-3-O-rutinoside **[59]**	*Croton sparsiflorus*	leaves	Subramanian *et al*., 1971
	Euphorbia pekinensis	aerial part	Ahn *et al*., 1996
	Manihot esculenta	leaves	Prawat *et al*., 1995
		phloem sap	Calatayud *et al*., 1994
	Manihot utilissima	leaves	Subramanian *et al.,* 1971
	Mercurialis annua	n.s.	Dumkow, 1969
	Phyllantus ussuriensis	n.s.	Ham *et al*., 2001
	Phyllanthus sellowianus	n.s.	Hnatyszyn *et al*., 2002
	Sapium japonicum	leaves	Ahn *et al*., 1996
Quercetin-3-O-rhamnoside **[60]**	*Euphorbia hirta*	leaves	Blanc and De Saqui-Sannes, 1972; Aqil and Khan, 1999;
	Euphorbia pekinensis	aerial part	Ahn *et al*., 1996
	Euphorbia minuta	n.s.	Del V. Galarza *et al*., 1983
Quercetin-3-O-(2″-O-galloyl)-β-D-glucoside **[61]**	*Euphorbia pekinensis*	aerial part	Ahn *et al*., 1996
Quercetin-3-O-(2″-O-galloyl)-α-L-rhamnoside **[62]**	*Euphorbia pekinensis*	aerial part	Ahn *et al*., 1996

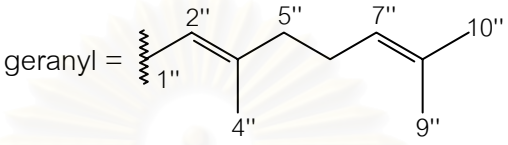
Table 1. (continued)

Compound	Source	Plant part	Reference
Quercetin-3-O-β-D-glucopyranosyl-[1→6]-O -α-L- rhamnoside **[63]**	*Homonia reparia*	leaves	Parveen,Singh and Khan, 1988
Quercetin-3′-xyloside **[64]**	*Euphorbia paralias*	n.s.	Rizk *et al*., 1976
Quercetin-3-O-β--glucopyranoside -7-O-α-rhamnopyranoside **[65]**	*Chrozophora obliqua*	aerial part	Mohamed, 2001
Trifolin **[66]**	*Sapium japonicum*	leaves	Ahn *et al*., 1996
Anthocyanidin			
Cyanidin-3-O-galactoside **[67]**	*Euphorbia minuta*	n.s.	Del V. Galarza *et al*., 1983
Cyanidin-3-O-glucoside **[68]**	*Euphorbia serpens microfilia*	n.s.	Del V. Galarza *et al*., 1983
Delphinidin-3-O-glucoside **[69]**	*Euphorbia serpens microfilia*	n.s.	Del V. Galarza *et al*., 1983

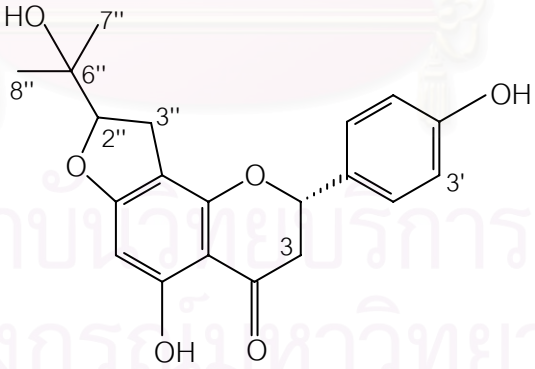
Table 1. (continued)

Compound	Source	Plant part	Reference
Flavanol			
Leucocyanidol **[70]**	*Euphorbia hirta*	n.s.	Blanc and De Saqui-Sannes, 1972
Flavans			
Gallocatechin-[4′-O-7]-epigallocatechin **[71]**	*Bridelia ferruginea*	stem bark	De Bruyne *et al*., 1997; Cimanga *et al*., 2001
Rotenoids			
Sumatrol **[72]**	*Macaranga indica*	leaves	Sultana and Ilyas, 1987

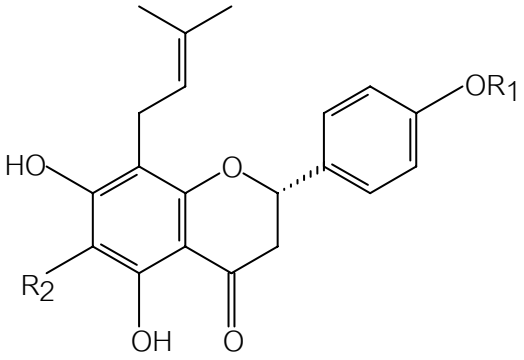




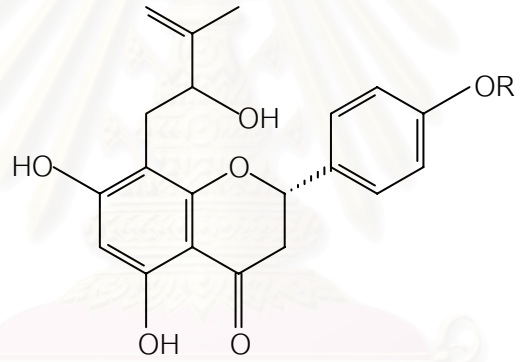
	R_1	R_2
Bonannione A **[1]**	H	geranyl
Macarangaflavanone A **[9]**	geranyl	H



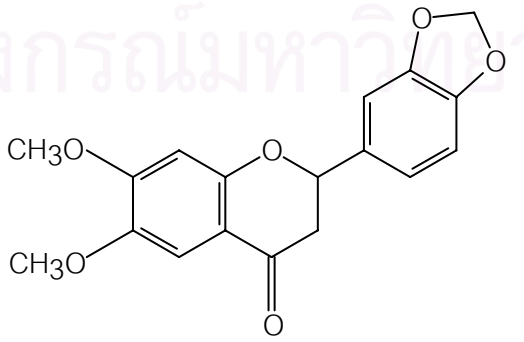
5, 4′-Dihydro-[2′′-(1-hydroxyl)dihydrofurano]-(7, 8:5′′, 4′′) flavanone **[2]**



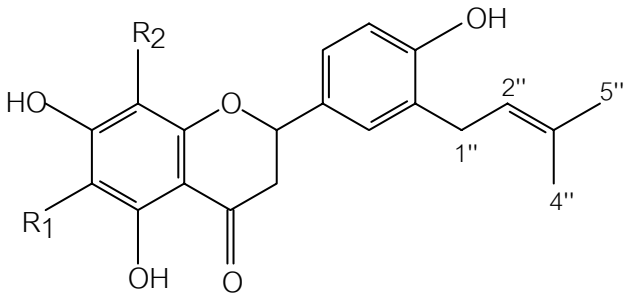
	R_1	R_2
5, 7-Dihydroxy-4′-methoxy-8-(3-methylbut-2-enyl) flavanone **[3]**	CH_3	H
Lonchocarpol A **[8]**	H	prenyl
Sophoraflavanone B **[11]**	H	H

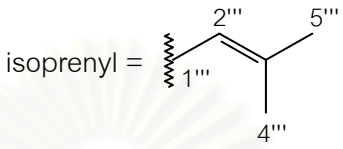


	R
5, 7-Dihydroxy-4′-methoxy-8-(2-hydroxy-3-methybut-3-enyl) flavanone **[4]**	CH_3
Tomentosanol D **[12]**	H

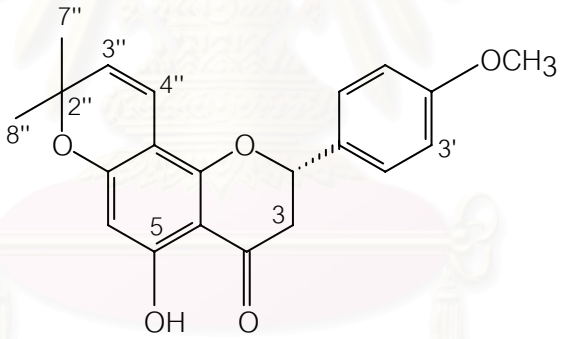


6, 7-Dimethoxy-3′, 4′-methylenedioxyflavanone **[5]**

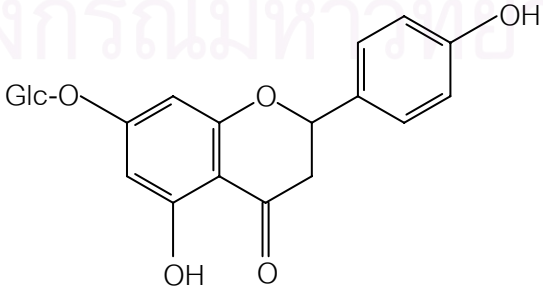




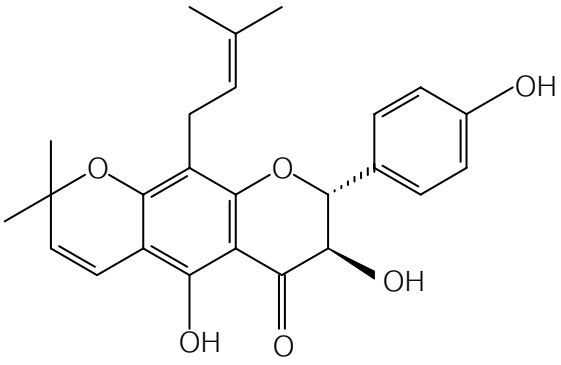
	R_1	R_2
Euchrestaflavanone A **[6]**	H	isoprenyl
Macarangaflavanone B **[10]**	isoprenyl	H



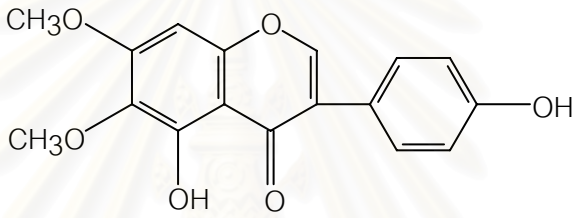
5-Hydroxy-4′-methoxy-2′′, 2′′-dimethylpyrano-(7, 8:6′′, 5′′) flavanone **[7]**



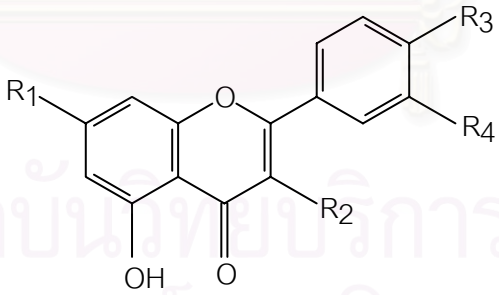
Prunin **[13]**



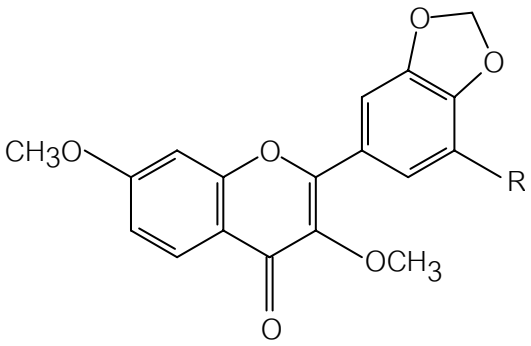
Lupinifolinol **[14]**



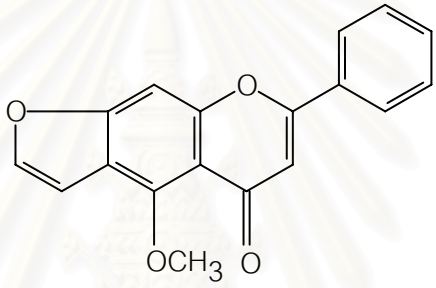
7-Methyltecotorigenin **[15]**



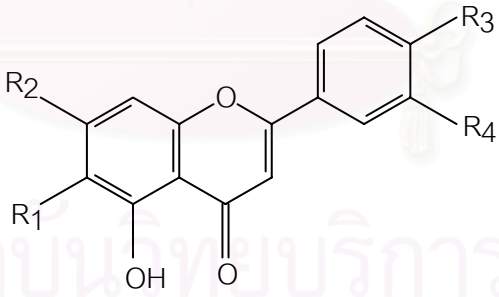
	R_1	R_2	R_3	R_4
Apigenin **[16]**	OH	H	OH	H
Chrysin **[17]**	OH	H	H	H
Galangin **[29]**	OH	OH	H	H
5-Hydroxy-7,4′-dimethylflavone **[18]**	OCH_3	H	OCH_3	H
Luteolin **[19]**	OH	H	OH	OH



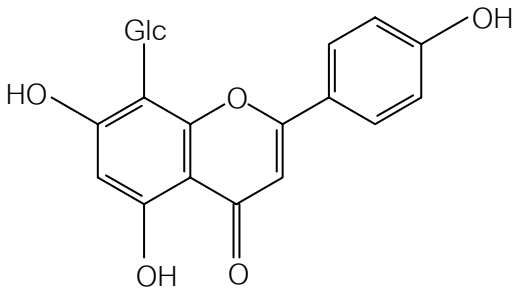
	R
Desmethoxykanugin **[27]**	H
Kanugin **[33]**	OCH_3



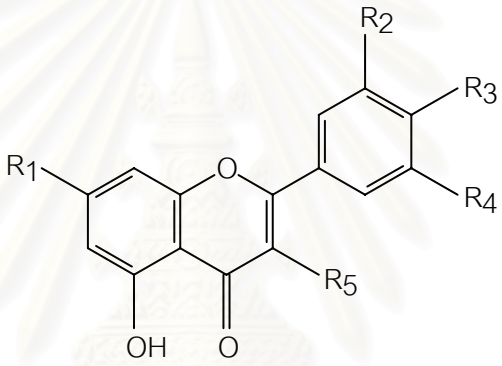
Pinnatin **[20]**



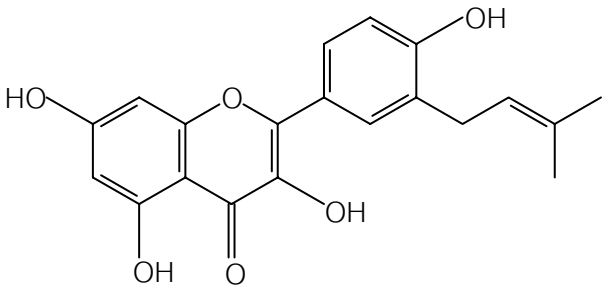
	R_1	R_2	R_3	R_4
Apigenin-7-O-glucoside **[21]**	H	OGlc	OH	H
Isovitexin **[22]**	Glc	OH	OH	H
Luteolin-7-O-glucoside **[23]**	H	OGlc	OH	OH
Swertisin **[24]**	Glc	OCH_3	OH	H



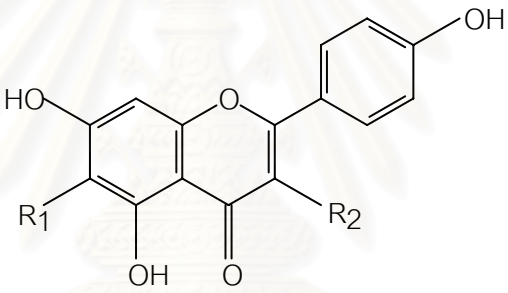
Vitexin **[25]**



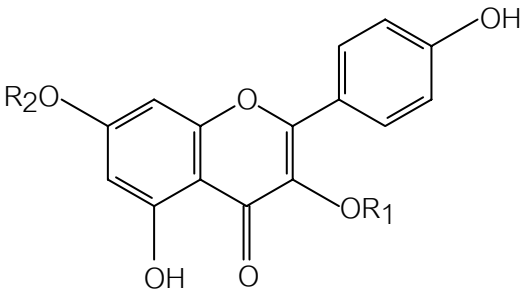
	R_1	R_2	R_3	R_4	R_5
Ayanin **[26]**	OCH_3	OH	OCH_3	H	OCH_3
Ferrugin **[28]**	OH	OCH_3	OCH_3	OCH_3	OH
Myricetin **[34]**	OH	OH	OH	OH	OH
3-O-Methylquercetin **[35]**	OH	OH	OH	H	OCH_3
Quercetin **[36]**	OH	OH	OH	H	OH
Quercetin 3, 7-dimethyl ether **[37]**	OCH_3	OH	OH	H	OCH_3
Rutisin **[38]**	OCH_3	OCH_3	OCH_3	H	OCH_3
3, 3′, 4′, 5′-Tetra-O-methylmyricetin **[39]**	OH	OCH_3	OCH_3	OCH_3	OCH_3



Isolicoflavonol **[30]**

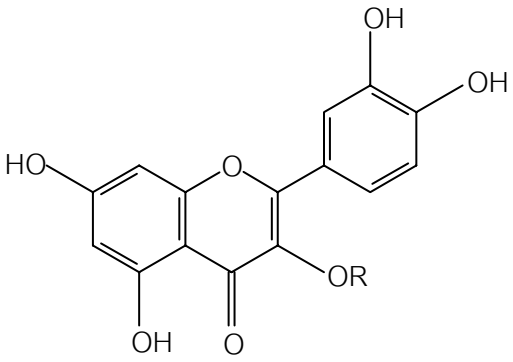


	R_1	R_2
Kaempferol **[31]**	H	OH
Kaempferol-3, 6-dimethyl ether **[32]**	OCH_3	OCH_3

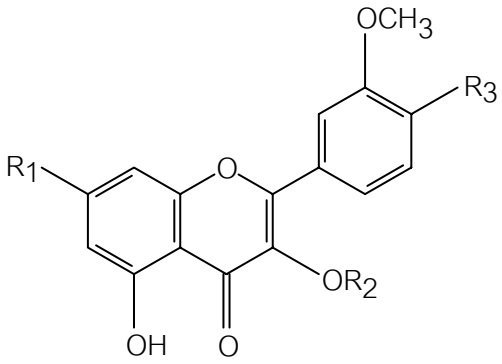


	R_1	R_2
Afzelin **[40]**	Rha	H
Astragalin **[41]**	Glc	H
Kaempferol-3-O-rutinoside **[49]**	Glc^6-Rha	H
Kaempferol-3-sophoroside **[50]**	Glc^2-Glc	H
Kaempferol-3-O-β-D-glucosyl-7-O-α-L-rhamnosyl **[52]**	β-Glc	α-Rha
Kaempferol-3-O-β-D-glucosyl-(1⟶6)-β-D-glucosyl **[53]**	β-Glc^6-β-Glc	H
Kaempferol-3-O-β-D-glucosyl-(1⟶6)-β-D-glucosyl 7-O-α-L-rhamnosyl **[54]**	β-Glc^6-β-Glc	α-Rha
Trifolin **[66]**	Gal	H

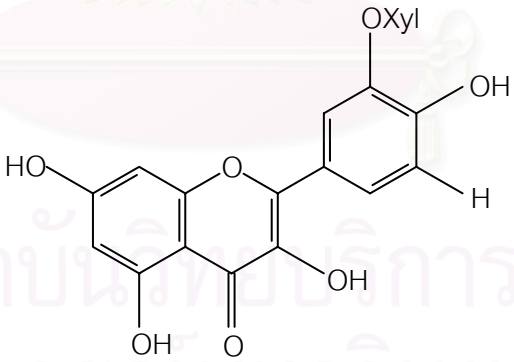
	R_1	R_2
6-Hydroxykaempferol-7-rutinoside **[43]**	OH	Glc^6-Rha
Kaempferol-7-O-glucoside **[51]**	H	Glc



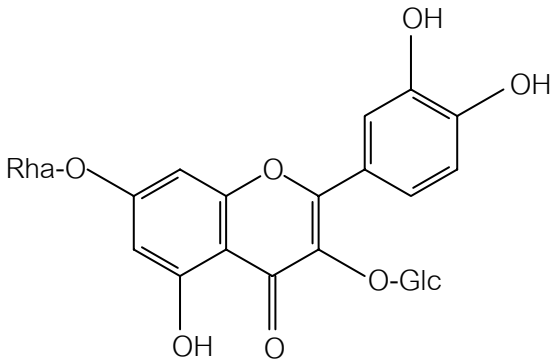
	R
Euphorbianin **[42]**	β-Gal^{3}-6‴-Ac-Glc
Isoquercitrin **[44]**	Glc
Quercetin-3-arabinoside **[55]**	Ara
Quercetin-3-galactoside **[56]**	Gal
Quercetin-3-glucosyl-(1→4)-rhamnoside **[57]**	Glc-Rha^{4}
Quercetin-3-O-glucoside **[58]**	Glc
Quercetin-3-O-rutinoside **[59]**	Glc^{6}-Rha
Quercetin-3-O-rhamnoside **[60]**	Rha
Quercetin-3-O-(2″-O-galloyl)-β-D-glucoside **[61]**	2″-O-galloyl-β-Glc
Quercetin-3-O-(2″-O-galloyl)-α-L-rhamnoside **[62]**	2″-O-galloyl-α-Rha
Quercetin-3-O-β-D-glucopyranosyl-(1→6)-O-α-L-rhamnoside **[63]**	β-Glc-α-Rha^{6}



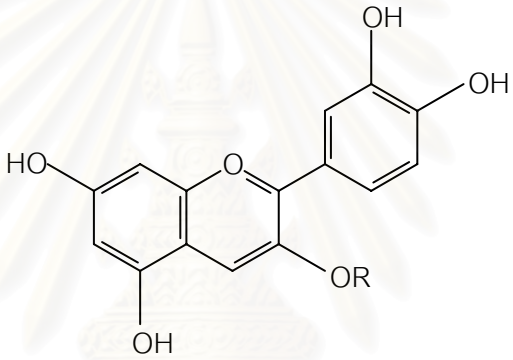
	R_1	R_2	R_3
Isorhamnetin-3-rutinoside **[45]**	OH	Glc^6-Rha	OH
Isorhamnetin-3-rutinoside-4′-glucoside **[46]**	OH	Glc^6-Rha	O-Glc
Isorhamnetin-3-O-β-D-xyloside **[47]**	OH	β-Xyl	OH
Isorhamnetin-3-O-β-glucopyranoside-7-O-α-rhamnopyranoside **[48]**	O-α-Rha	β-Glc	OH



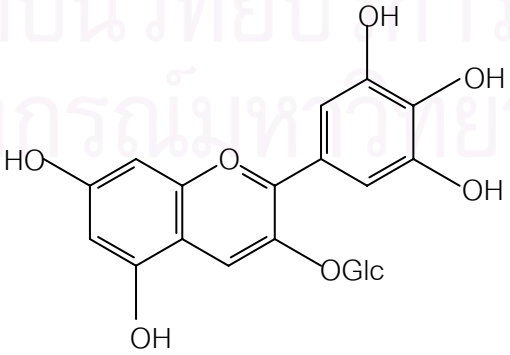
Quercetin-3′-xyloside **[64]**



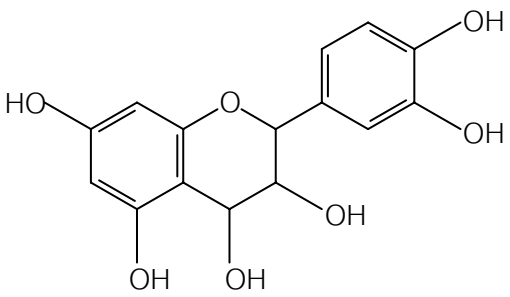
Quercetin-3-O-β-glucopyranoside-7-O-α-rhamnopyranoside **[65]**



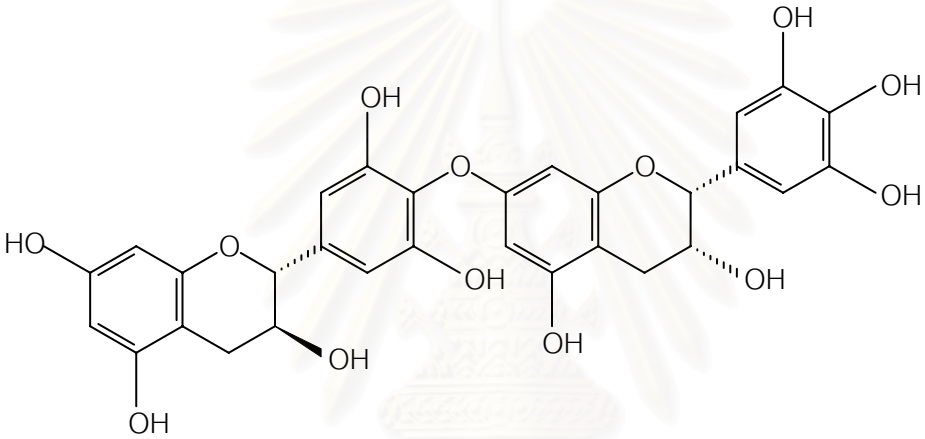
	R
Cyanidin-3-O-galactoside **[67]**	Gal
Cyanidin-3-O-glucoside **[68]**	Glc



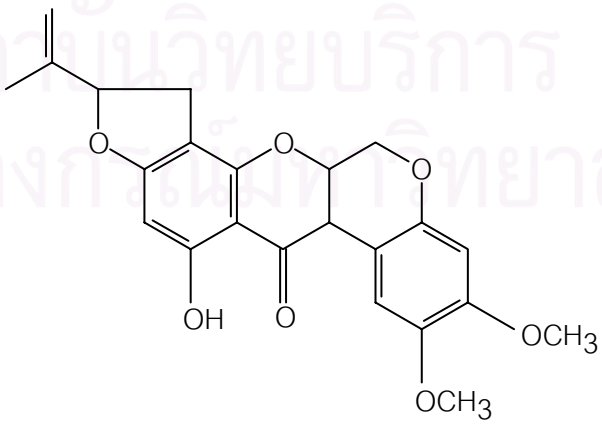
Delphinidin-3-O-glucoside **[69]**



Leucocyanidol **[70]**



Gallocatechin-[4′-O-7]-epigallocatechin **[71]**



Sumatrol **[72]**

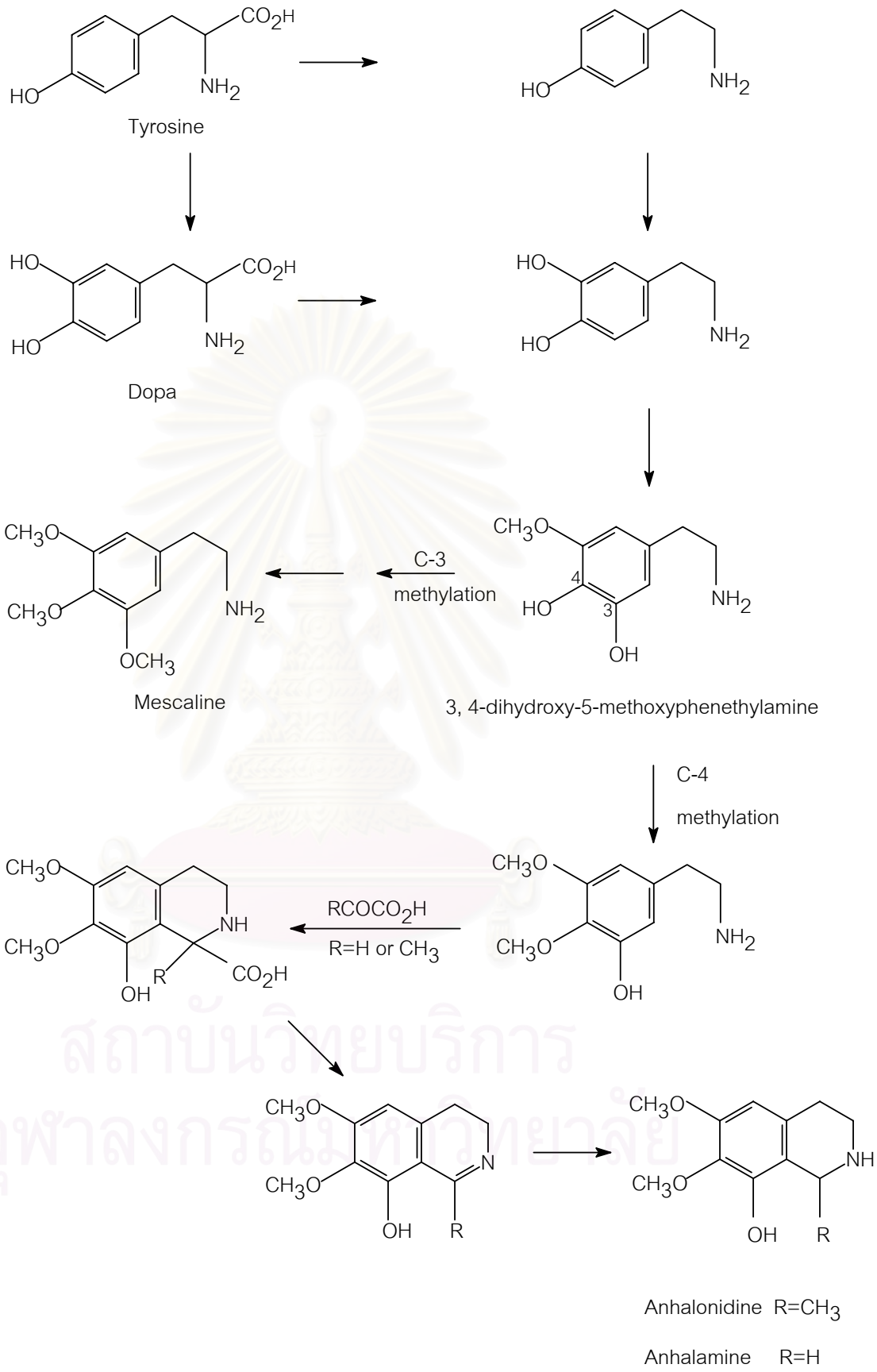
2.2 The isoquinoline alkaloids from euphorbiaceous plants.

The alkaloids are one of the largest groups of natural products, the most diverse group of secondary metabolites found in living organism. Then compounds have an array of structure types, biosynthesis pathways and pharmacological activities (Roberts and Wink, 1998).

Isoquinolines form one of the largest groups of plant alkaloids. Their structures comprise double carbon rings containing one nitrogen atom. This extremely large and enormously varied group can be divided into approximately twenty categories, which include a number of valuable clinical agents such as codeine, morphine, emetine and tubocurarine.

Simple isoquinoline are one category of the isoquinoline alkaloids which begin in primary metabolism with the ∝-amino-acid, tyrosin. This is the result of quite extensive experiments particularly with various phenethylamine precursors. A key intermediate is 3,4-dihydroxy-5-methoxyphenethylamine. Methylation at C-3 of 3,4-dihydroxy-5-methoxyphenethylamine leads to mescaline, whereas methylation at C-4 ultimately affords anhalonidine and anhalamine (Phillipson, Roberts and Zenk, 1985).

The biosynthesis scheme of isoquinoline alkaloids is shown in Scheme 2 and the distribution of isoquinoline alkaloids within the family Euphorbiaceae is summarized in Table 2.



Scheme 2. Biosynthesis of isoquinoline alkaloids.

Table 2 Isoquinoline alkaloids from euphorbiaceous plants.

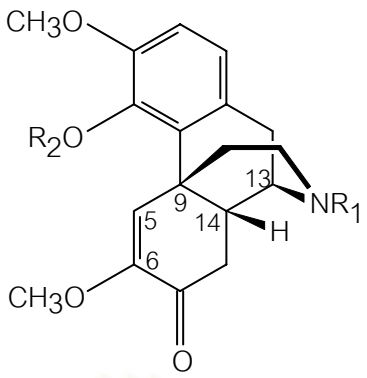
Compound	Source	Plant part	Reference
O-Acetyldihydrosalutaridine **[73]**	*Croton linearis*	n.s.	Barton *et al*., 1968; Haynes *et al*., 1968
Croton linearis Base E **[74]**	*Croton linearis*	n.s.	Haynes, Husbands and Stuart, 1966
Crotonosine **[75]**	*Croton linearis*	n.s.	Haynes *et al*., 1966
Crotsparine **[76]**	*Croton sparsiflorus*	n.s.	Bhakuni and Dhar, 1968;
			Bhakuni, Satish and Dhar, 1972
Crotsparinine **[77]**	*Croton sparsiflorus*	n.s.	Bhakuni and Dhar, 1969;
			Bhakuni, Satish and Dhar, 1972
8,14-Dihydronorsalutaridine **[78]**	*Croton linearis*		Barton *et al*., 1968; Haynes *et al*., 1968
8,14-Dihydrosalutaridine **[79]**	*Croton linearis*	n.s.	Barton *et al*., 1968; Haynes *et al*., 1968
	Croton discolor	n.s.	Barton *et al*., 1968; Haynes *et al*., 1968
N,O-Dimethylcrotsparine **[80]**	*Croton sparsiflorus*	n.s.	Bhakuni and Dhar, 1968
			Bhakuni *et al*., 1972
N,O-Dimethylhernovine **[81]**	*Croton wilsonii*	n.s.	Stuart and Chambers, 1967
Discolorine **[82]**	*Croton discolor*	n.s.	Cordell *et al*., 1989
Flavinantine **[83]**	*Croton flavens*	n.s.	Kotani and Tobinaga, 1973
Flavinine **[84]**	*Croton flavens*	n.s.	Kotani and Tobinaga, 1973
Hernovine **[85]**	*Croton wilsonii*	n.s.	Stuart and Chambers, 1967

Table 2. (continued)

Compound	Source	Plant part	Reference
Homolinearisine [86]	*Croton linearis*	n.s.	Haynes *et al*., 1966
Isocrotsparinine [87]	*Croton sparsiflorus*	aerial part	Casagrande *et al*., 1975
Jaculadine [88]	*Croton linearlis*	n.s.	Cordell, 1989
Jacularine [89]	*Croton linearlis*	n.s.	Stuart *et al*., 1968; Casagrande *et al*., 1975
Linearisine [90]	*Croton linearlis*	n.s.	Colombo, 1976; Haynes and Stuart, 1963; Haynes *et al*., 1966
3-Methoxy-4,6-dihydroxymorphinan -dien-7-one [91]	*Croton bonplandianum*		Cordell *et al*., 1989
N-Methylcrotsparine [92]	*Croton sparsiflorus*	n.s.	Bhakuni and Dhar, 1968; Bhakuni *et al*., 1972
N-Methylcrotsparinine [93]	*Croton sparsiflorus*	n.s.	Bhakuni and Dhar,1969; Bhakuni *et al*., 1972
N-Methylhernovine [94]	*Croton wilsonii*	n.s.	Stuart and Chambers, 1967
10-O- Methylhernovine [95]	*Croton wilsonii*	n.s.	Stuart and Chambers, 1967
N-Methylisocrotsparinine [96]	*Croton sparsiflorus*	n.s.	Casagrande *et al*., 1975

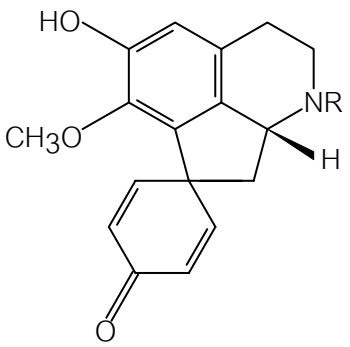
Table 2. (continued)

Compound	Source	Plant part	Reference
Norsinoacutine **[97]**	*Croton balsamifera*	n.s.	Kametani, 1969
	Croton flavens	n.s.	Stuart and Graham, 1973
Papaverine **[98]**	*Sauropus androgynus*	leaves	Ismail, 1973
Salutaridine **[99]**	*Croton salutaris*	leaves and twigs	Barnes and Soeiro, 1981
	Croton balsamifera	n.s.	Kametani, 1969
Salutarine **[100]**	*Croton salutaris*	leaves and twigs	Barnes and Soeiro, 1981
Sparsiflorine **[101]**	*Croton sparsiflorus*	leaves	Chatterjee *et al*., 1965
Tetrahydroglaziovine **[102]**	*Croton sparsiflorus*	n.s.	Casagrande *et al*., 1975
1,2,3,4,-Tetrahydro-6-hydroxy-1-methyl-3-isoquinoline carboxylic acid **[103]**	*Euphorbia myrsinites*	latex	Cordell *et al*., 1989
	Euphorbia tirucalli	latex	Cordell *et al*., 1989
Thaliporphine **[104]**	*Croton sp.*	n.s.	Tschesche *et al*., 1965
Wilsonirine **[105]**	*Croton wilsonii*	n.s.	Stuart and Chambers, 1967

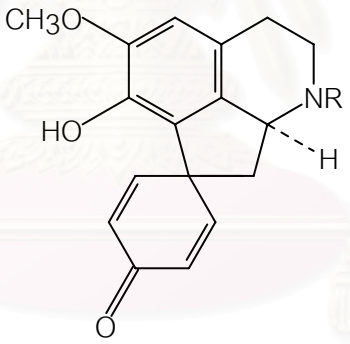


	R_1	R_2
O-Acetyldihydrosalutaridine **[73]**	CH_3	Acetyl
8, 14-Dihydronorsalutaridine **[78]**	H	H
8, 14-Dihydrosalutaridine **[79]**	CH_3	H

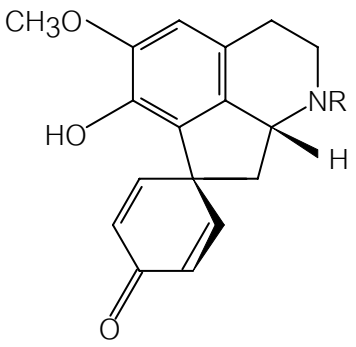
Croton linearis Base E **[74]**



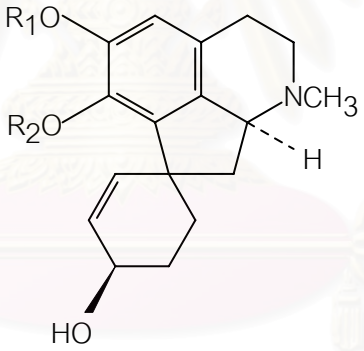
	R
Crotonosine **[75]**	H
Homolinearisine **[86]**	CH_3



	R
Crotsparine **[76]**	H
N-Methylcrotsparine **[92]**	CH_3
N,O-Dimethylcrotsparine **[80]**	O-Di-CH_3

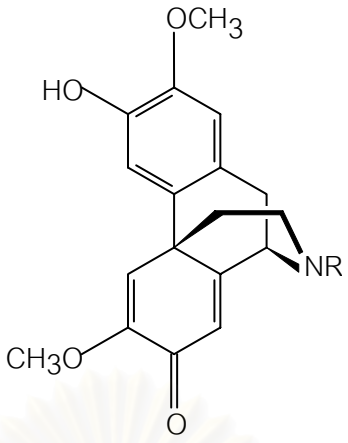


	R
Crotsparinine **[77]**	H
N-Methylcrotsparine **[93]**	CH_3

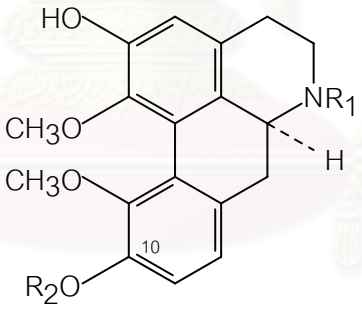


R_1=H, R_2=CH_3 or R_1= CH_3, R_2=H

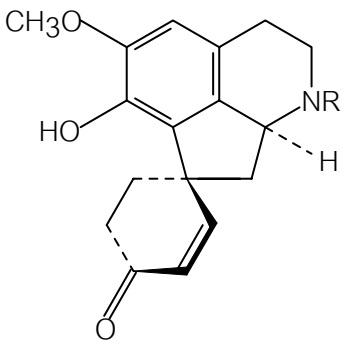
Discolorine **[82]**



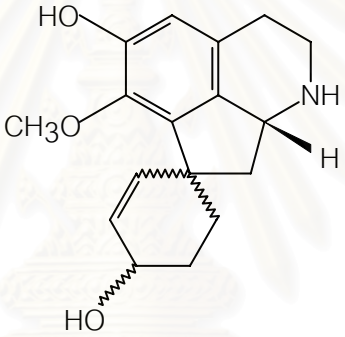
	R
Flavinantine **[83]**	CH_3
Flavinine **[84]**	H



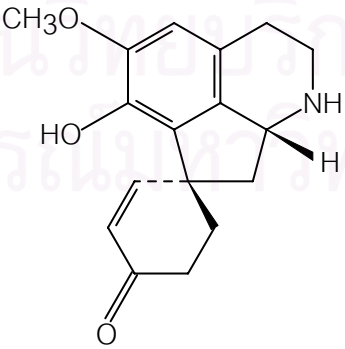
	R1	R2
N, O-Dimethylhernovine **[81]**	CH_3	CH_3
Hernovine **[85]**	H	H
N-Methylhernovine **[94]**	CH_3	H
10-O-Methylhernovine **[95]**	H	CH_3



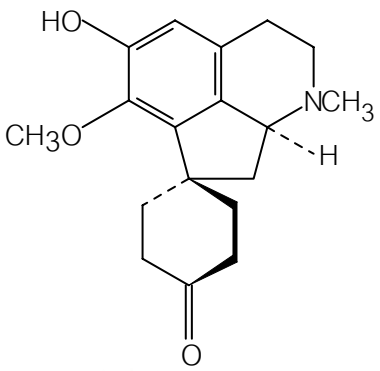
	R
Isocrotsparinine **[87]**	H
N-Methylisocrotsparinine **[96]**	CH_3



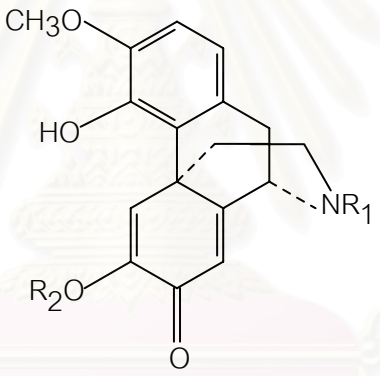
Jaculadine **[88]**



Jacularine **[89]**

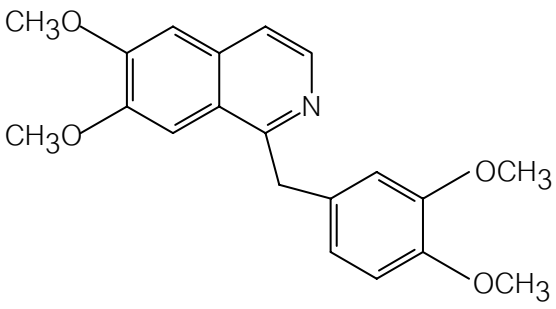


Linearisine **[90]**

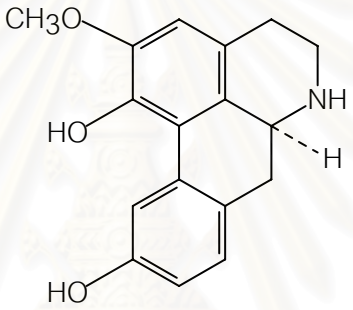


(-)-form absolute configuration

	R_1	R_2
3-Methoxy-4, 6-dihydroxymorphinandien-7-one **[91]**	H	H
Norsinoacutine **[97]**	H	CH_3
(+)-form Salutaridine **[99]**	CH_3	CH_3
(±)-form Salutarine **[100]**	CH_3	CH_3

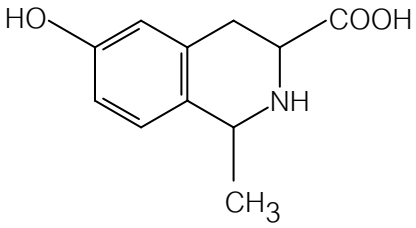


Papaverine **[98]**

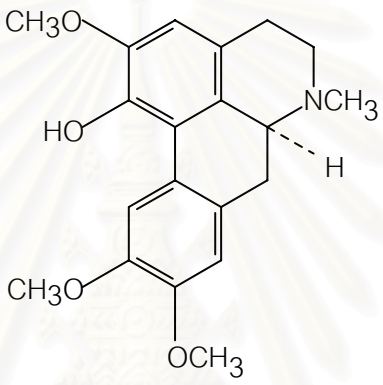


Sparsiflorine **[101]**

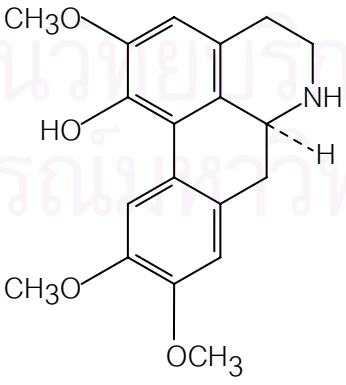
Tetrahydroglaziovine **[102]**



1,2,3,4-Tetrahydro-6-hydroxy-1-metyl-3-isoquinolinecarboxylic acid **[103]**



Thaliporphine **[104]**



Wilsonirine **[105]**

3. Chemical constituents of the genus *Sauropus*

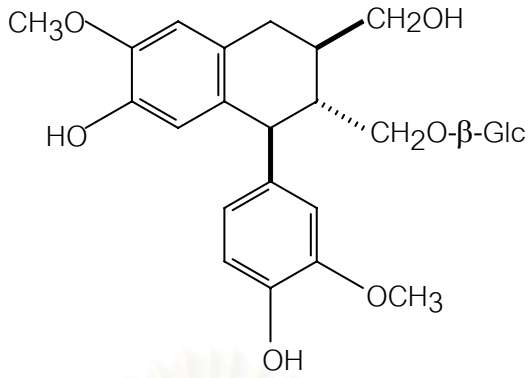
The genus *Sauropus* contains about 29 species (Airy Shaw, 1972). Chemical study of plants in this genus has been done on only two species, *Sauropus androgynus* and *S. quadrangularis*. The chemical consituents of *S.androgynus* and*S.quadrangularis* are summarized in Table 3.

Table 3. Chemical consituents of plants in the genus *Sauropus*.

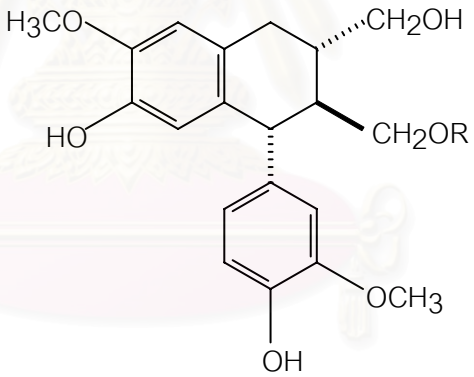
Plants	Chemical type	Name	Reference
Sauropus androgynus	Alkaloids	Papaverine [98]	Bender and Ismail, 1973
	Flavonoids	3-O-β-D-glucosyl-7-O-α-L-rhamnosyl-kaempferol [52]	Wang and Lee, 1997
		3-O-β-D-glucosyl-(1→6)-β-D-glucosyl-kaempferol [53]	Wang and Lee, 1997
		3-O-β-D-glucosyl-(1→6)-β-D-glucosyl-7-O-α-L-rhamnosyl-kaempferol [54]	Wang and Lee, 1997
		Quercetin [36]	Miean and Mohamed, 2001
	Lignan glycosides	(+)-isolariciresinol 3α-O-β-glucopyranoside [106]	Kanchanapoom *et al*., 2003
		(-)-isolariciresinol 3α-O-β-glucopyranoside [107]	Kanchanapoom *et al*., 2003
		(-)-isolariciresinol 3α-O-β-apiofuranosyl-(1→2)-O-β-glucopyranoside [108]	Kanchanapoom *et al*., 2003
		Liriodendrin [109]	Kanchanapoom *et al*., 2003
	Megastigmane glucoside	Corchoionoside C [110]	Kanchanapoom *et al*., 2003
		Sauroposide [111]	Kanchanapoom *et al*., 2003
	Nucleoside	Adenosine [112]	Wang and Lee, 1997
		5'-Deoxy-5'-methylsulphinyladenosine [113]	Wang and Lee, 1997
		Guanosine [114]	Kanchanapoom *et al*., 2003

Table 3. (continued)

Plants	Chemical type	Name	Reference
		Uridine **[115]**	Wang and Lee, 1997
Sauropus quadrangularis	Lignans	Benzoyl diphyllin **[116]**	Satyanarayna *et al*., 1995
		6-Bromo-3,4-dimethoxybenzoyl diphyllin **[117]**	Satyanarayna *et al*., 1995
		6-Bromo-3,4-methylenedioxybenzoyl diphyllin **[118]**	Satyanarayna *et al*., 1995
		Cinnamoyl diphyllin **[119]**	Satyanarayna *et al*., 1995
		Diphyllin **[120]**	Satyanarayna *et al*., 1995
		3,4-methylenedioxybenzoyl diphyllin **[121]**	Satyanarayna *et al*., 1995
		4-Nitro-benzoyl diphyllin **[122]**	Satyanarayna *et al*., 1995
		3,4,5-Trimethylbenzoyl diphyllin **[123]**	Satyanarayna *et al*., 1995
		trans-(3R,4S) bis (3',4'-methylenedioxybenzyl) tetrahydrofuran **[124]**	Satyanarayna *et al*., 1995



(+)-Isolariciresinol-3-α-O-β-glucopyranoside **[106]**

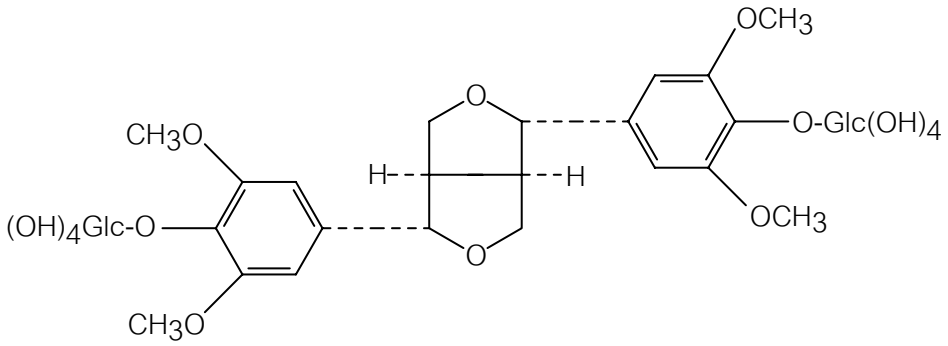


R=β-Glc

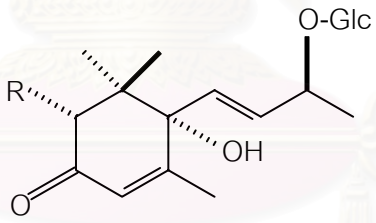
(-)-Isolariciresinol-3-α-O-β-glucopyranoside **[107]**

R=β-Api-O-β-Glc[2]

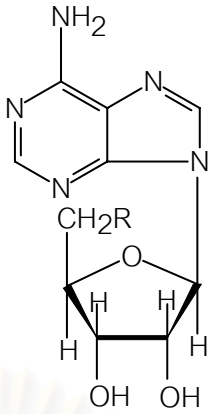
(-)-Isolariciresinol-3-α-O-β-apiofuranosyl-(1→2)-O-β-glucopyranoside **[108]**



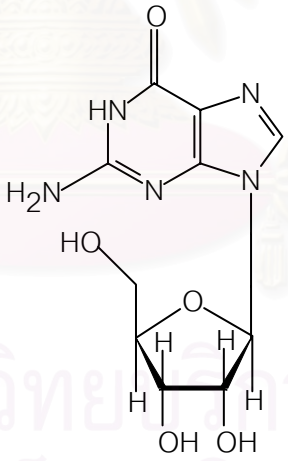
Liriodendrin **[109]**



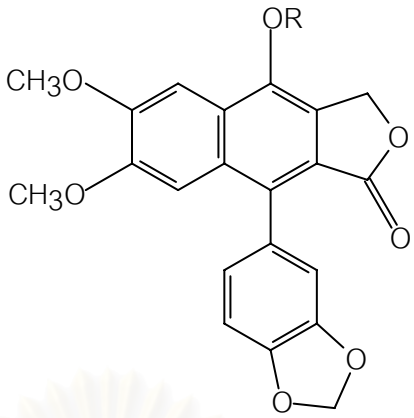
	R
Corchoionoside C **[110]**	H
Sauroposide **[111]**	OH



	R
Adenosine **[112]**	OH
5′-Deoxy-5′-methylsulphinyladenosine **[113]**	SCH_3

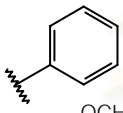


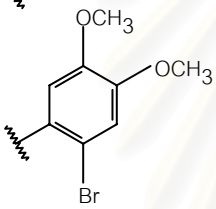
Guanosine **[114]**



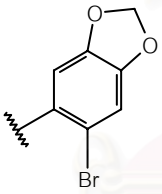
R

H Diphyllin **[120]**

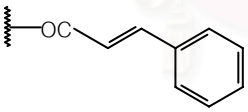

Benzoyl diphyllin **[116]**



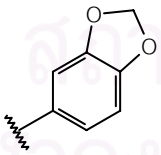
6-Bromo-3,4-dimethoxybenzoyl diphyllin **[117]**



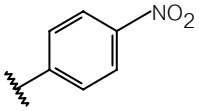
6-Bromo-3,4-methylenedioxybenzoyl diphyllin **[118]**



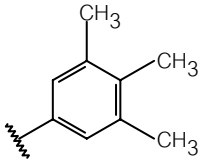
Cinnamoyl diphyllin **[119]**



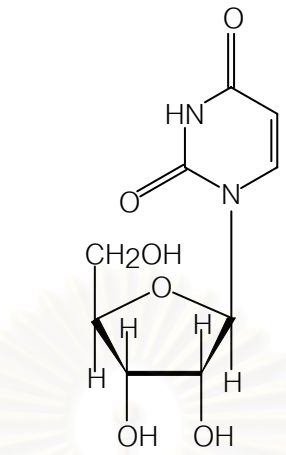
3,4-Methylenedioxybenzoyl diphyllin **[121]**



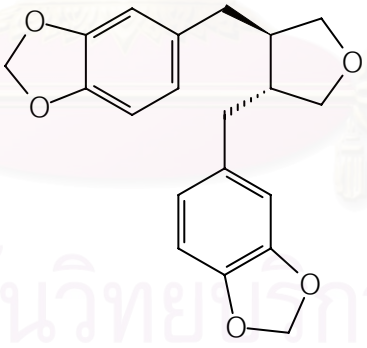
4-Nitro-benzoyl diphyllin **[122]**



3,4,5-Trimethylbenzoyl diphyllin **[123]**



Uridine **[115]**



trans-(3R, 4S) bis (3′,4′-Methylenedioxybenzyl) tetrahydrofuran **[124]**

CHAPTER III

EXPERIMENTAL

Source of plant material

The aerial part of *Sauropus hirsutus* was collected from Sakaerat Environmental Research Station, Wung Num Kiow District, Nakhon Ratchasima Province, Thailand, in October 2002. It was identified by comparision with herbarium specimen (S. Phengnaren No. 449) at the Royal Forest Department, Bangkok, Thailand.

Phytochemical Techniques

1. Chromatographic Techniques

1.1 Thin Layer Chromatography (TLC)

Technique	:	one way ascending
Stationary phase	:	TLC aluminium sheet silica gel 60F 254, Layer thickness 0.2 mm.
Distance	:	5 cm.
Temperature	:	28-35 ^{o}C (room temperature)
Detection	:	1) UV light at the wavelengths of 254 and 365 nm 2)10% sulfuric acid in ethanol and heating at 110^{o}C 3) Dragendorff Reagent
Solvent	:	Various solvent systems depending on materials

1.2 Column Chromatography (CC)

Column	:	Flat bottom glass column (various diameter)
Stationary phase	:	Silica gel 60 (No. 9385, E. Merck) particle size 0.040-0.063 mm (230-400 mesh ASTM)
Packing method	:	Dry and wet packing
Sample loading	:	1) Dry packing: The sample was dissolved in a small amount of suitable organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then loaded on top of the column.

2) Wet packing: The sample was dissolved in a small amount of the eluent, then loaded on top of the column.

Solvent system : Various solvents systems depending on materials.

Detection : Fractions were examined by TLC observing under UV light at the wavelengths of 254 and 365 nm, then the TLC plate was sprayed with 10% sulfuric acid in ethanol and heated at 110 °C or sprayed with dragendorff reagent. The fractions of similar TLC pattern were combined.

1.3 Gel Filtration Chromatography

Gel filter : Sephadex™ LH-20

Packing method : Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.

Sample loading : The sample was dissolved in a small volume of the eluent and applied on top of the column.

Solvent : Methanol 100%

Methanol-chloroform (1:1)

1.4 Preparative Thin Layer Chromatography (PTLC)

Stationary phase : Kieselgel 60 F 254, Layer thickness 1 mm

Distance : 15 cm

Temperature : 28-35 °C (room temperature)

Detection : UV light (254 and 365 nm)

Solvent : Hexane-acetone (3:2)

2. Spectroscopy

2.1 Utraviolet (UV) Absorption Spectra

UV spectra (in methanol) were obtained on a Milton Roy Spectronic 3000 Array Spectrometer (Pharmaceutical Research Equipment Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.2 Infrared (IR) Absorption Spectra

IR spectra (KBr disc and thin film) were obtained on a Perkin Elmer Infrared Spectrophotometer Model 1760X (Scientific and Technological Research Equipment Center, Chulalongkorn University)

2.3 Mass Spectra (MS)

Electron impact mass spectra (EIMS) were recorded on a Fison Micromass VG Platform II mass spectrometer (Faculty of Science, Mahidol University). The electrospray ionization time of flight mass spectroscopy (ESI TOFMS) were obtained using a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of accurate mass (The National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand Science Park)

2.4 Proton and Carbon-13 Nuclear Magnetic Resonance spectra

The ^{1}H and ^{13}C NMR spectra were obtained either on a JEOL JNM-A500 (Alpha series) 500 MHz NMR spectra (Science and Technological Research Equipment Center, Chulalongkorn University) or a Bruker Avance DPX-300 300 MHz NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

NMR solvents used in this study were deuterated dimethylsulfoxide (DMSO-d_6), deuterated chloroform ($CDCl_3$) and deuterated methanol (CD_3OD). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

3. Melting Point

Melting points were obtained on a Fisher-John Melting Point Apparatus

(Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

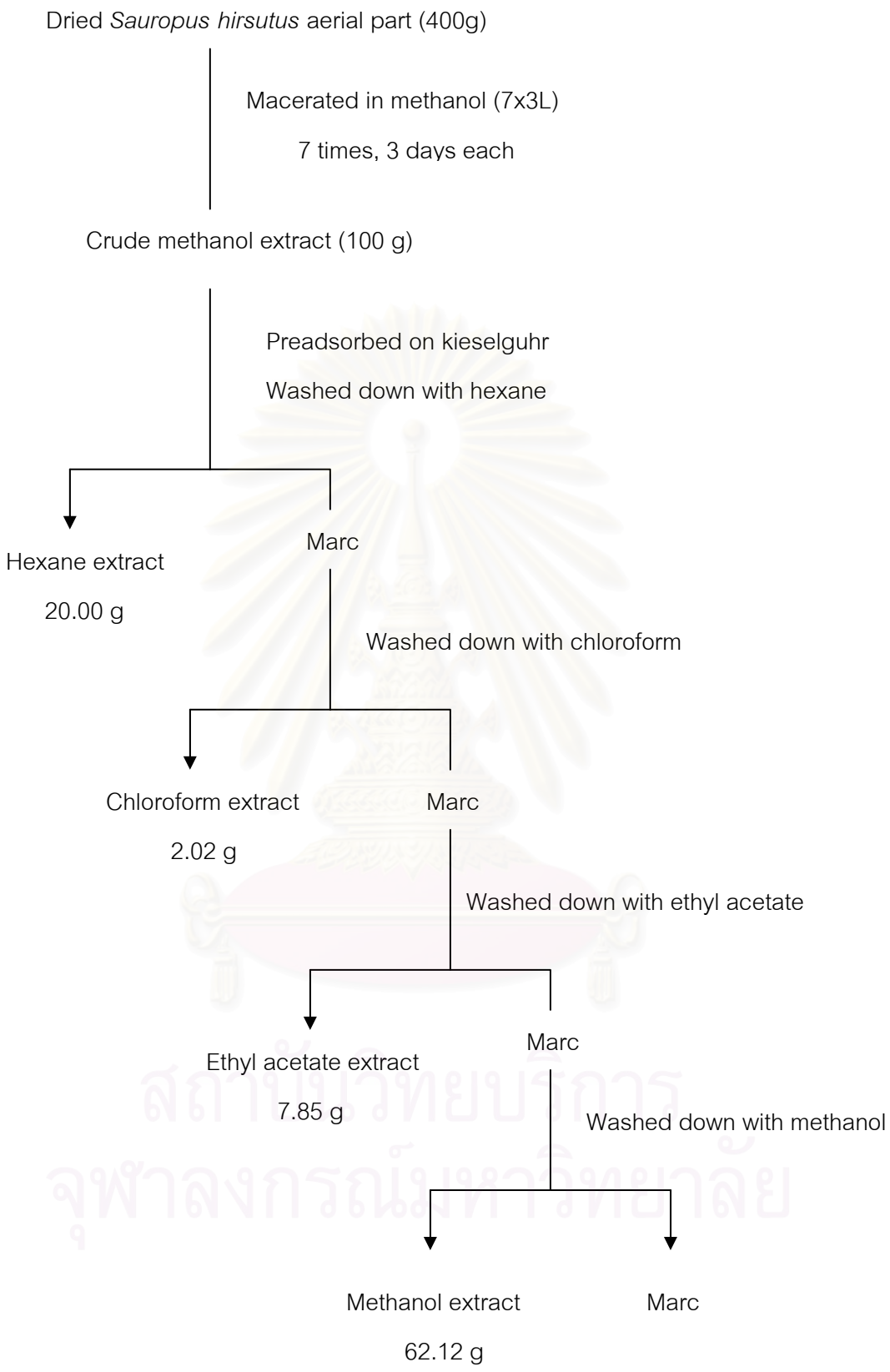
4. Solvent

Throughout this work, all organic solvents were of commercial grade and were redistilled prior to use.

Extraction

The dried aerial part of *Sauropus hirsutus* (400 g) were ground into small pieces. It was extracted by maceration with methanol (7 times 3 days each) and then filtered. The filtrate of each batch was combined and concentrated to remove methanol under reduce pressure to yield 100 g of crude methanol extract (25% of dried weight). The crude methanol extract was preadsorbed on kieselguhr and packed into a percolator, then washed down with hexane until the eluate stopped giving positive result to Liebermann-Burchard test. The eluate was evaporated to dryness to give 20.00 g of the hexane extract (5% of dried weight). The remaining kieselguhr residue was air dried and then eluted with chloroform until the eluate stopped giving positive result to Dragendorff reagent. The eluate was evaporated to dryness to give 2.02 g of the chloroform extract (0.51% of dried weight). The air dried kieselguhr residue was eluted with ethyl acetate to give, on evaporation, 7.85 g of the ethyl acetate extract (1.96% of dried weight). Finally, the dried kielselguhr residue was washed down with methanol to give 62.12 g of the methanol extract. These extracts were subjected to column chromatography for further purification.

Extraction of the aerial part of *Sauropus hirsutus* is summarized in Scheme 3



Scheme 3. Extraction of the aerial part of *Sauropus hirsutus*

Isolation

1. Fractionation of the hexane extract

The hexane extract (10 g) was subjected to silica gel column chromatography (300 g, 5x40 cm) using hexane-acetone (19:1) as the solvent system. One hundred and fifty-five 30-ml fractions were collected and combined according to their TLC pattern into nine major fractions (H01-H09) as shown in Table 4. Finally, the column was washed throughly with methanol to give H10.

Table 4 Combined fractions from the hexane extract

Fraction	Number of eluates	Weight (g)
H01	1-9	0.28
H02	10-19	0.40
H03	20-27	0.51
H04	28-29	0.25
H05	30-38	3.21
H06	39-68	1.18
H07	69-98	0.33
H08	99-143	0.38
H09	144-155	0.73
H10	methanol elute	2.46

1.1 Isolation of compound H1

Fraction H07, which displayed one major pink-violet spot on TLC upon detection with 10% sulfuric acid, was recrystallized in methanol to give 45.1 mg (0.02% yield) of compound H1 as colorless needles.

2. Fractionation of the chloroform extract

The chloroform extract (2.02 g) was subjected to silica gel column chromatography (100 g, 3x30 cm), using hexane-acetone (7:3) as the eluent. Forty one fractions (30 ml each) were collected and examined by TLC, those with similar pattern on TLC plates were combined and evaporated to dryness to give five major fractions (C01-C04). The last fraction was from elution with methanol (C05). The combined fractions from the chloroform extract are summarized in Table 5

Table 5. Combined fractions from the chloroform extract

Fraction	Number of eluates	Weight (mg)
C01	1-9	55.8
C02	10-13	18.3
C03	14-20	33.6
C04	21-41	23.8
C05	methanol eluate	1650

Fraction C03 (33.6 mg) was further chromatographed on silica gel column chromatography (18 g, 1x20 cm) using hexane-acetone (3:2) as the solvent system. Forty-two fractions (2 ml each) were collected and combined according to their TLC pattern into four major fractions (C06-C09), as shown in Table 6.

Table 6 Combined fractions from fraction C03

Fraction	Number of eluates	Weight (mg)
C06	1-18	3.3
C07	19-24	8.2
C08	25-35	5.0
C09	36-42	11.7

2.1 Isolation of compound C1

Fraction C07 displayed one major TLC spot similar to that of fraction C02. Therefore, they were combined to give fraction C10. It was then further separated by gel filtration chromatography using a Sephadex LH-20 column (1x40 cm). Elution was performed utilizing methanol-chloroform (1:1) as the solvent system. Thirty-two fractions (2 ml each) were collected. The fractions were monitored by silica gel TLC with hexane-acetone (3:2) as the solvent.. Those of similar pattern on TLC plates were combined into three major fractions (C11-C13) as shown in Table 7.

Table 7 Combined fractions from fraction C10

Fraction	Number of eluates	Weight (mg)
C11	1-20	7.9
C12	21-22	3.3
C13	23-33	12.5

Fraction C12 showed one orange-red spot on TLC plate upon detection with Dragendorff reagent. It was crystallized in chloroform to give compound C1 as pale yellow needle (3.3 mg, 0.000825% yield).

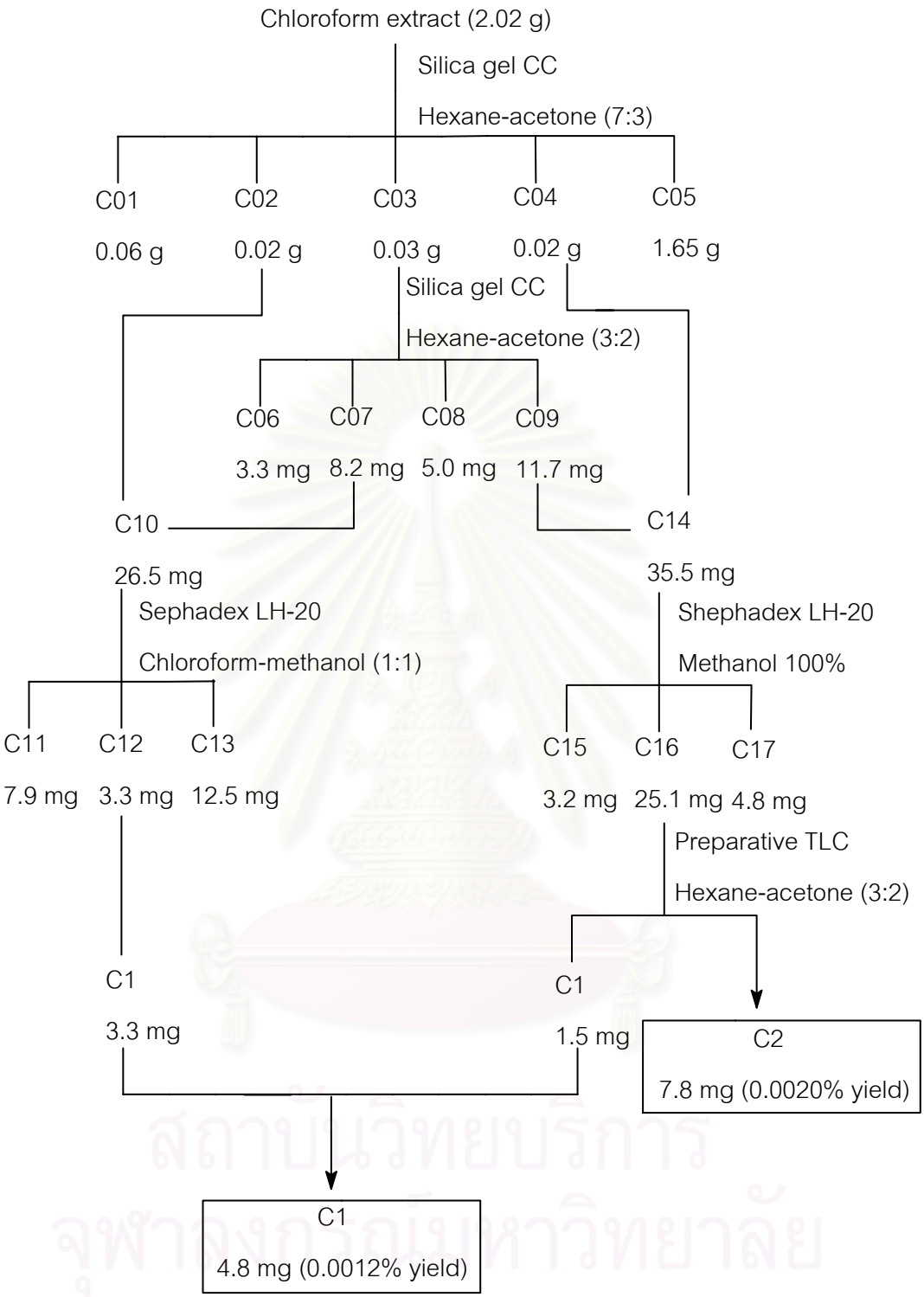
2.2 Isolation of compound C2

Fraction C08 showed interesting spots on TLC similar to those of fraction C04. They were combined to give fraction C14. It was then chromatographed on a Sephadex LH-20 column (1x40 cm), using methanol as the eluent. Twenty-five fractions (2 ml) were collected and combined according to their TLC pattern into three major fractions, as summarized in Table 8.

Table 8 Combined fractions from fraction C14

Fraction	Number of eluates	Weight
C15	1-11	3.2
C16	12-19	25.1
C17	20-25	4.8

Fraction C16 (25.1 mg), displaying two major spots (Rf=0.44 and 0.52) on TLC plate when detected under UV light. It was then further separated by preparative TLC, using hexane-acetone (3:2) as the solvent. The two major bands were separated, the adsorbent washed throughly with chloroform-methanol (3:7) and the eluate concentrated to afford compound C2 as pale yellow needles (4.8 mg) and an additional amount of compound C1 (1.5 mg) respectively. The fractionation of chloroform extract is summarized in Scheme 4.



Scheme 4. Fractionation of chloroform extract.

3. Fractionation of the ethyl acetate extract.

The ethyl acetate extract (7.85g) was subjected to silica gel column chromatography (240 g, 5x30 cm) using acetone-chloroform (3:2) as the eluent, to give seventy fractions (30 ml each). The column was then washed down with methanol. Combination of fractions according to their TLC pattern resulted eight major fractions (E01-E08), as shown in Table 9.

Table 9 Combined fractions from the ethyl acetate extract.

Fraction	Number of eluates	Weight (g)
E01	1-7	0.08
E02	8-12	0.12
E03	13-18	1.30
E04	19-34	0.74
E05	35-46	0.39
E06	47-61	0.40
E07	62-70	0.44
E08	methanol eluate	4.24

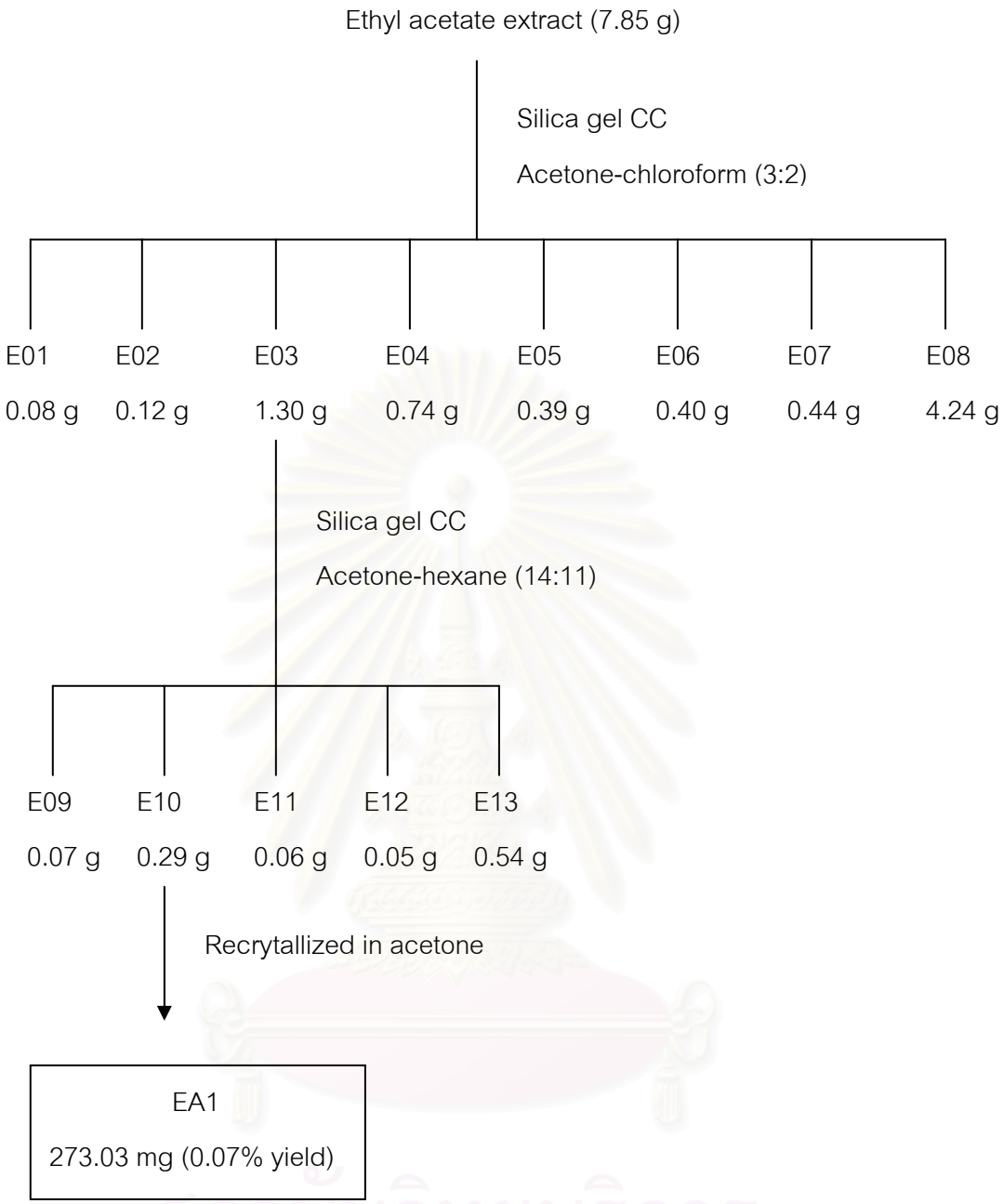
3.1 Isolation of compound EA1

Fraction E03, which displayed one permanent spot with trace of impurity on TLC plate, was selected for further investigation. It was rechromatographed on a silica gel column (70 g, 2.5x30 cm), using acetone-hexane (14:11) as the eluent. Thirty-one fractions (30 ml) were collected and then the column was washed down with methanol. Five major fractions (E09-E13) were obtained as shown in Table 10.

Table 10 Combined fractions from fraction E03.

Fraction	Number of eluates	Weight (g)
E09	1-8	0.07
E10	9-17	0.29
E11	18-21	0.06
E12	22-31	0.05
E13	methanol eluate	0.54

Fraction E10 showed yellow-brown spot on TLC plate. It was recrystallized in acetone to give 273.03 mg of compound EA1 as pale yellow crytals (0.07% yield). Fractionation of ethyl acetate extract is summarized in Scheme 5.



Scheme 5. Fractionation of the ethyl acetate extract.

Characterization of isolated compounds

1. **Compound H1**

Appearance : Colorless needles

Solubility : Soluble in chloroform

^{1}H-NMR (δ ppm, 300 MHz, $CDCl_3$) (Figure 2, page 94)

0.66 (3H), 0.78 (3H), 0.80 (3H), 0.83 (3H), 0.90 (3H), 0.99 (3H), 3.50 (1H), 5.35 (1H)

^{13}C-NMR (δ ppm, 75 MHz, $CDCl_3$) (Figures 3a-3b, page 95-96)

12.0 (*q*), 12.1 (*q*), 18.9 (*q*), 19.2 (*q*), 19.5 (*q*), 20.0 (*q*), 21.2 (*t*), 23.2 (*t*), 24.4 (*t*), 26.3 (*t*), 28.4 (*t*), 29.3 (*d*), 31.8 (*t*), 32.0 (*t*), 32.0 (*d*), 34.1 (*t*), 36.6 (*d*), 36.3 (*d*), 37.4 (*t*), 39.9 (*t*), 42.4 (*t*), 42.4 (*d*), 46.0 (*d*), 50.2 (*d*), 56.2 (*d*), 56.8 (*d*) 71.8 (*d*), 121.6 (*d*), 140.6 (*s*)

2. **Compound C1**

Appearance : Pale yellow needles

Solubility : Soluble in chloroform

Melting Point : 118-120 ^{0}C

EIMS *m/z* (% relative intensity) : 233(0.6), 219(2.7), 218(13.9), 217(100.0),216(15.2), 189(38.6), 188(29.8), 174(10.8), 161(12.4), 160(20.1), 149(13.3), 146(23.8), 118(10.9), 91(10.1) (Figure 5, page 98)

UV λ_{max} nm (log ε), in methanol : 217 (3.99), 245 (4.12), 295 (3.05), 335 (3.02) (Figure 7, page 100)

IR ν_{max} (thin flim) cm^{-1} : 2925, 2850, 1742, 1640, 1596, 1524, 1477, 1448, 1417, 1346, 1280, 1196, 1077, 1048, 946, 812 and 492 (Figure 6, page 99)

^{1}H-NMR (δ ppm, 500 MHz, $CDCl_3$) (Figures 11a-11b, page 104-105)

2.67 (3H, *s*), 3.99 (3H, *s*), 6.19 (2H, *s*), 6.47 (1H, *s*), 7.08 (1H, *d*, *J*=8.5 Hz) and 7.68 (1H, *d*, *J*=8.5 Hz)

^{13}C-NMR (δ ppm, 125 MHz, $CDCl_3$) (Figure 8, page 101)

26.0 (*q*), 55.5 (*q*), 99.1(*d*), 102.2(*t*), 108.6(*d*), 115.6 (*d*), 116.5 (*s*), 135.6 (*s*), 140.3 (*s*), 147.3 (*s*), 161.3 (*s*) and 162.5 (*s*)

3. Compound C2

Appearance : Pale yellow needles

Solubility : Soluble in methanol

Melting Point :164-166^{0}C

EIMS *m/z* (% relative intensity) : 263 (0.2), 249 (1.8), 247 (100.0), 246 (45.6), 232 (30.8), 218 (18.8), 204 (22.2), 189 (13.9), 176 (18.8), 160 (9.7), 149 (11.8), 133 (7.6), 118 (5.3), 91 (4.4) and 77 (6.7) (Figure 15, page 111)

UV λ_{max} nm (log ε), in methanol : 223 (4.14), 254 (4.29) and 307 (3.35) (Figure 17, page 113)

IR ν_{max} (KBr) cm^{-1} : 2968, 2938, 1721, 1592, 1534, 1465, 1416, 1345, 1297, 1163, 1148, 1064, 941 and 826 (Figure 16, page 112)

^{1}H-NMR (δ ppm, 500 MHz, $CDCl_3$/ CD_3OD) (Figure 21, page 119)

2.58 (3H,*s*), 3.94 (3H,*s*), 4.02 (3H,*s*), 6.19 (2H,*s*), 6.66 (1H,*s*) and 7.12 (1H,*s*)

^{13}C-NMR(δ ppm, 125 MHz, $CDCl_3$) (Figures 18a-18b, page 114-115)

24.8 (*q*), 56.3 (*q*), 56.6 (*q*), 96.90 (*d*), 100.9 (*d*), 103.9 (*t*), 117.4 (*s*), 132.2 (*s*), 139.1 (*s*), 142.2 (*s*), 145.2 (*s*), 159.8 (*s*) and 163.2 (*s*)

4. **compound EA1**

Appearance : Pale yellow crytals

Solubility : soluble in methanol

Melting Point : 240-242^{0}C

ESI TOFMS (*m/z*) : 290.0837 (Figure 24, page 126)

IR ν_{max} (KBr)cm^{-1} : 3457, 1625, 1521, 1260, 1144, 1095, 1016, 795, 627 and 461 (Figure 25, page 127)

^{1}H-NMR (δ ppm, 500 MHz, DMSO-d_6) (Figure 29, page 133)

2.48 (1H, *dd*, *J*= 16.3, 3.5 Hz), 2.67 (1H, *dd*, *J*= 16.3, 4.4 Hz), 4.00 (1H, *m*), 4.65 (1H, *d*, *J*=4.58 Hz), 4.73 (1H, *s*), 5.71 (1H, *d*, *J*=2.44 Hz), 5.88 (1H, *d*, *J*=2.44 Hz), 6.64 (1H, *dd*, *J*=8.1, 1.8 Hz), 6.66 (1H, *d*, *J*=8.1 Hz), 6.88 (1H, *d*, *J*=1.83 Hz), 8.72 (1H, *s*), 8.79 (1H, *s*), 8.96 (1H, *s*) and 9.11 (1H, *s*)

^{13}C-NMR (δ ppm, 125 MHz, DMSO d_6) (Figures 26a-26b, page 128-129)

28.3 (*t*), 64.9 (*d*), 78.1 (*d*), 94.1 (*d*), 95.1 (*d*), 98.5 (*s*), 114.8 (*d*), 114.9 (*d*), 118.0 (*d*), 130.7 (*s*), 144.4 (*s*), 144.5 (*s*), 155.8 (*s*), 156.3 (*s*) and 156.6 (*s*)

CHAPTER IV

RESULTS AND DISCUSSION

The investigation of chemical constituents of the methanol extract of the aerial part of *Sauropus hirsutus* Beille. by chromatographic techniques led to the isolation of four compounds. The identification and structure elucidation of these compounds were based on spectroscopic evidences (UV, IR, NMR and mass spectra) and also confirmed by comparison with those previously reported in the literature. The details can be discussed as follows.

1. Structure elucidation of compound H1

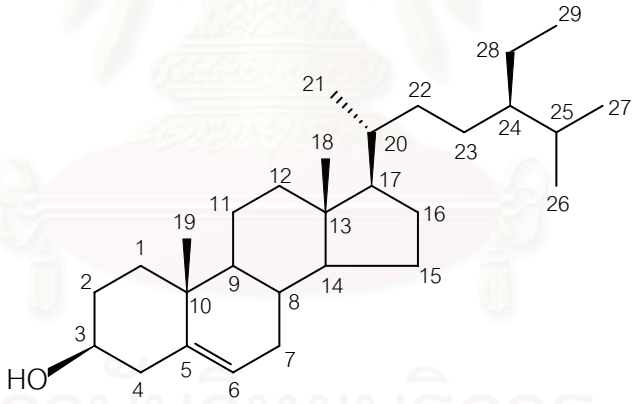
Compound H1 was obtained as colorless needles (0.02% yield). It gave purple color upon spraying with 10% H_2SO_4 in 95% ethanol and heated. Liebermann-Burchard test of this compound gave positive green color, suggesting the presence of a steroidal skeleton.

The ^{1}H-NMR spectrum (Figure 2) showed the signal at δ 5.35 ppm which could be assigned to the vinylic proton H-6, which another one-proton multiplet signal at δ 3.50 ppm was attributable to the proton geminal to the 3-OH group. The signals between δ 0.66-0.99 ppm are the signals of methyl protons, which at δ 0.66 ppm (H-18), 0.78 ppm (H-27), 0.80 ppm (H-26), 0.83 ppm (H-29), 0.90 ppm (H-21) and 0.99 ppm (H-19). The signal at δ 1.1-2.3 ppm were those of methylene and methine protons.

The ^{13}C-NMR spectrum (Figures 3a-3b) showed the signals of 29 carbon atoms, supporting the assignment of this compound as a steroid derivative. The DEPT experiments (Figure 4) were performed to differentiate these 29 signals in to those of three quaternary carbons at δ 36.6 (C-10), 42.4 (C-13) and 140.6 (C-5) ppm, nine methine carbons at δ 29.3 (C-25), 32.0 (C-8), 36.3 (C-20), 46.0 (C-24), 50.2 (C-9), 56.2 (C-17), 56.8 (C-14), 71.8 (C-3) and 121.6 (C-6) ppm, eleven methylene carbons at δ 21.2 (C-11), 23.2 (C-28), 24.4 (C-15), 26.3 (C-23), 28.4 (C-16), 31.8 (C-2), 32.0 (C-7),

34.1 (C-22), 37.4 (C-1), 39.9 (C-12) and 42.4 (C-4) ppm, and six methyl carbons at δ 12.0 (C-18), 12.1 (C-29), 18.9 (C-21), 19.2 (C-27), 19.5 (C-19) and 20.0 (C-26) ppm. The two most downfield signals at δ 140.6 and 121.6 could be assigned to the olefenic C-5 and C-6 of double bond, respectively.The carbon signal at δ 71.8 ppm represented the hydroxyl substituted position C-3.

Comparison of the ^{13}C-NMR data of compound H1 with those values previously reported for β-sitosterol (De-Eknamkul and Potduang, 2003) revealed them to be fully in agreement, as summarized in Table 11. Therefore, compound H1 was identified as β-sitosterol.



β-sitosterol

Table 11. Comparison of the ^{13}C-NMR spectral data of β-sitosterol and Compound H1 (in $CDCl_3$)

Carbon	Chemical shift (ppm)	
	β-sitosterol	H1
1	37.2	37.4
2	31.6	31.8
3	71.8	71.8
4	42.2	42.4
5	140.7	140.6
6	121.7	121.6
7	31.9	32.0
8	31.9	32.0
9	50.1	50.2
10	36.5	36.6
11	21.1	21.2
12	39.7	39.9
13	42.3	42.4
14	56.7	56.8
15	24.3	24.4
16	28.2	28.4
17	56.0	56.2
18	11.8	12.0
19	19.4	19.5
20	36.1	36.3
21	18.8	18.9
22	33.9	34.1
23	26.0	26.3
24	45.8	46.0
25	29.1	29.3
26	19.8	20.0
27	19.0	19.2
28	23.0	23.2
29	12.0	12.1

2. Structure elucidation of compound C1

Compound C1 was recrystallized as pale yellow needles from $CHCl_3$ (4.8 mg, 0.0012% yield). The EIMS spectrum of this compound (Figure 5) showed a molecular ion peak at m/z 233, suggesting its molecular formula as containing odd number of nitrogen atom, which corresponded to the molecular formula $C_{12}H_{11}O_4N$, supported by the number of carbon signals and proton integration in ^{13}C-NMR (Figure 8) and ^{1}H-NMR (Figures 11a-11b) spectrum respectively.

The IR spectrum (Figure 6) revealed absorption bands at 1596 and 946 cm^{-1}, suggesting the presence of tertiary amide and methylene dioxy functionalities (Weinstein and Hylton, 1964)

The ^{13}C-NMR spectrum (Figure 8) of C1 exhibited the signals of 12 carbon atoms. The DEPT (Figure 9) and ^{1}H-^{13}C HMQC (Figure 10) experiment were performed to differentiate these 12 signals into those of one methoxyl carbon at δ 55.5 ppm (4-OCH_3), six quaternary carbons at δ 116.5 (C-4a), 135.6 (C-8a), 140.3 (C-8), 147.3 (C-7), 161.3 (C-1) and 162.5 ppm (C-4), three methine carbons at δ 99.1 (C-3), 108.6 (C-6) and 115.6 ppm (C-5), one methylene carbon at δ 102.2 ppm (C-9) and another one N-methyl carbon at δ 26.0 (NCH_3)

The ^{1}H-NMR spectrum (Figures 11a-11b) showed six signals at δ 2.67 (3H, *s*, N-CH_3), 3.99 (3H, *s*, 4-OCH_3), 6.19 (2H,*s*, H-9), 6.47 (1H, *s*, H-3), 7.08 (1H, *d*, *J*=8.5 Hz, H-6) and 7.68 ppm (1H, *d*, *J*=8.5 Hz, H-5). The last two signals were ortho-coupled as could also be observed in the ^{1}H-^{1}H COSY experiment (Figure 12).

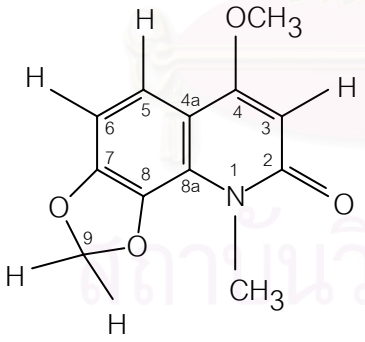
In addition, the elucidation of C1 structure was chiefly done by comparison of the ^{13}C and ^{1}H-NMR of this compound with those corresponding signals of the quinolone alkaloid casimiroin (4-methoxy-1-methyl-7,8-methylenedioxy-2-quinolone), previously reported from the seed and bark of *Casimiroa edulis* (family Rutaceae) (Ito *et al*., 1998). Comparison of their ^{1}H- and ^{13}C-NMR data is presented in Table 12.

Table 12. Comparison of ^{13}C NMR and ^{1}H NMR data of compound C1, compound C2 and Casimiroin (Ito et al., 1998) (in $CDCl_3$)

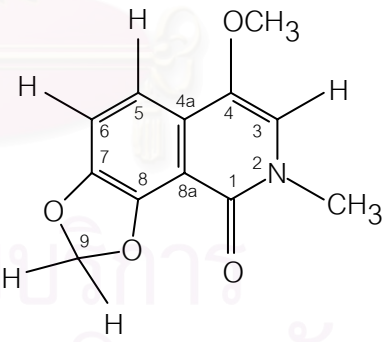
Position	Chemical shift (δ) ppm					
	Casimiroin		C1		C2*	
	C	H	C	H	C	H
C=O	164.1	-	161.3	-	159.8	-
N-CH_3	29.1	3.84, *s*	26.0	2.67, *s*	24.8	2.58
3	94.6	5.89, *s*	99.1	6.47, *s*	100.9	6.66
4	162.7	-	162.5	-	163.2	-
4a	113.0	-	116.5	-	117.4	-
5	118.0	7.53, *d*, *J*=8.6 Hz	115.6	7.68, *d*, *J*=8.5 Hz	96.9	7.12
6	104.3	6.78, *d*, *J*=8.6 Hz	108.6	7.08, *d*, *J*=8.5 Hz	145.2	-
7	149.9	-	147.3	-	139.1	-
8	133.5	-	140.3	-	142.2	-
8a	126.5	-	135.6	-	132.2	-
9	101.0	6.04, *s*	102.2	6.19, *s*	103.9	6.19
4-OCH_3	55.8	3.91, *s*	55.5	3.99, *s*	56.3	-
6-OCH_3	-		-		56.6	3.94

* in $CDCl_3/CD_3OD$

The ^{1}H-^{13}C HMBC experiment (Figures 13a-13c) was useful to confirm the structure of compound C1. The proton at δ 7.08 displayed three-bond correlations with C-4a (116.5 ppm) and C-8 (140.3 ppm) and two-bond correlation with C-7 (147.3 ppm), confirming its position at C-6, while the signal of H-5 at δ=7.68 showed three-bond correlations with C-4 (162.5 ppm), C-7 (147.3 ppm) and C-8a (135.6 ppm). The methoxy proton at (δ 3.99, *s*) displayed long-range coupling with C-4 (162.5), confirming its attachment at C-4. The H-3 methine proton at δ 6.47(*s*) showed three-bond correlations with C-4a (116.5ppm) and N-methyl carbon (26.0 ppm) and two-bond correlation with C-4 (162.5 ppm), confirming its position at C-3 (99.1 ppm). The singlet signal of methylene dioxy proton at δ 6.19 ppm exhibited three-bond correlation with both C-7 (147.3 ppm) and C-8 (140.3 ppm), indicating its attachment between these two positions. The signal of N-methyl proton (2.67, *s*) showed long-range cross peaks with both C-3 (99.1 ppm) and C-1 (161.3 ppm) placing this group between the amide carbonyl and C-3, making its position is different from casimiroin.

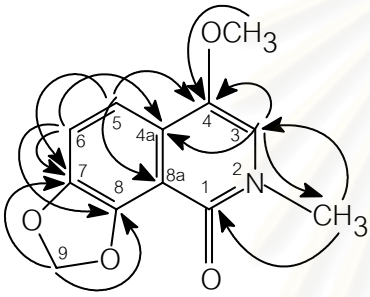


Casimiroin

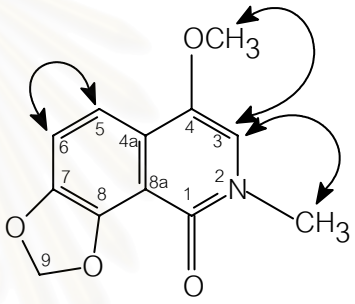


Compound C1

The structure of C1 was also confirmed by NOESY experiment (Figure 14). The proton signal at δ 6.47 ppm (H-3) showed cross peaks with both the methoxy proton (δ 3.99, 4-OCH_3) and N-methyl proton (δ 2.67). The complete carbon and proton assignments of C1, together with HMBC and NOESY results are shown in Table 13 Major HMBC and NOESY correlations are shown below.



Major HMBC correlation of Compound C1



Major NOESY correlation of Compound C1

From the above data, the structure of C1 was elucidated as a new isoquinoline alkaloid, 4-methoxy-2-methyl-7, 8-methylenedioxy-1-isoquinolone.

Table 13.The ^{1}H- NMR, ^{13}C-NMR, HMBC and NOESY data of compound C1

Position	δC	δH	HMBC correlations	NOESY correlations
1	161.3	-	-	-
2-NCH_3	26.03	2.67, *s*	C-1, C-3	H-3
3	99.1	6.47, *s*	C-4, C-4a, 2-NCH_3	2-NCH_3, 4-OCH_3
4	162.5	-	-	-
4-OCH_3	55.5	3.99, *s*	C-4	H-3
4a	116.5	-	-	-
5	115.6	7.68, *d*, *J*=8.5	C-4, C-8a, C-7	H-6
6	108.6	7.08, *d*,*J*=8.5	C-4a, C-7, C-8	H-5
7	147.3	-	-	-
8	140.3	-	-	-
9	102.2	6.19, *s*	C-7, C-8	-

3. Structure elucidation of compound C2

Compound C2 was crystallized as pale yellow needles from $CHCl_3$-MeOH (3:7) (7.8 mg, 0.0020% yield). The EIMS spectrum (Figure 15) showed a molecular ion peak at *m/z* 263, suggesting its molecular formula as containing odd number of nitrogen atom, corresponding to the molecular formula of $C_{13}H_{13}O_5N$, supported by number of carbon signals and proton integration in ^{13}C-NMR (Figures18a 18b) and ^{1}H-NMR (Figure 21) spectrum.

The IR spectrum (Figure 16) revealed absorption bands at 1592 and 941 cm^{-1}, suggesting the presence of tertiary amide and methylene dioxy groups (Weinstein and Hylton, 1964).

The ^{13}C-NMR spectrum (Figures 18a-18b) showed the signals of 13 carbon atoms. DEPT (Figure 19) and HMQC (Figures 20a-20b) experiments indicated these as the signals of two methoxyl carbons at δ 56.3 (4-OCH_3) and δ 56.6 ppm (6-OCH_3), seven quaternary carbons at δ 117.4 (C-4a), 132.2 (C-8a), 139.1 (C-7), 142.2 (C-8), 145.2 (C-6), 159.8 (C-1) and 163.2 ppm (C-4), two methine carbons at δ 96.9 (C-5) and 100.9 ppm (C-3), one methylene carbon at δ 103.9 ppm (C-9) and one methyl carbon at δ 24.8 ppm (N-CH_3)

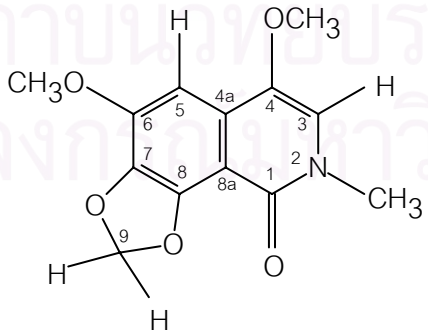
The ^{1}H-NMR spectrum (Figure 21) exhibited 6 singlet signals at δ 2.58 (3H, *s*, N-CH_3), 3.94 (3H, *s*, 6-OCH_3), 4.02 (3H, *s*, 4-OCH_3), 6.19 (2H, *s*, H-9), 6.66 (1H, *s*, H-3) and 7.12 (1H, *s*, H-5). The spectrum was similar to that of compound C1. The compound also gave similar orange-red spot on TLC plate upon detected with Dragendorff reagent. Therefore, the elucidation of the structure of C2 was mainly accomplished by comparison of the ^{13}C-NMR and ^{1}H-NMR chemical shift data with casimiroin and C1 (Table 12) supported by HMBC experiment which helped in assigning all carbon and proton positions within its structure.

The ^{1}H-^{13}C HMBC experiment (Figures 22a-22b) exhibited long-rang correlation between, the methoxy proton at δ 3.94 ppm and C-6 (δ 145.2 ppm), whereas another methoxy proton at δ 4.02 ppm showed long-range correlation with C-4 (δ 163.2 ppm),

indicated their positions as at C-6 and C-4, respectively. The signal of singlet methine proton at δ 7.12 ppm (H-5) showed three-bond correlations with C-4 (δ 163.2 ppm), C-7 (δ 139.1ppm) and C-8a (δ 132.2 ppm) and two bond correlation with C-6 (δ 145.2 ppm). Another one methine proton at δ 6.66 ppm (H-3) displayed three-bond coupling with C-4a (δ 117.4 ppm) and 2N-CH_3 (δ 24.8 ppm) and two-bond coupling with C-4 (δ 163.2 ppm), while the N-methyl proton exhibited three-bond correlations with both C-1 (δ 159.8 ppm) and C-3 (δ 100.9 ppm), indicating the proximity of then positions. The methylene dioxy protons at δ 6.19 ppm showed long-range coupling with both C-7 (δ 139.1 ppm) and C-8 (δ 142.2 ppm), that confirming the position of this group as between the protons 7 and 8.

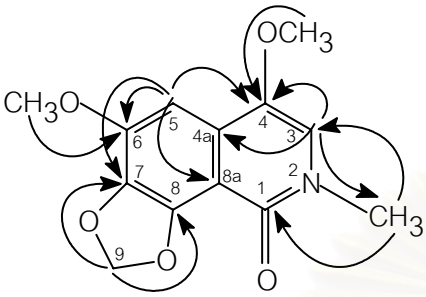
The ^{1}H-^{1}H NOESY (Figure 23) showed cross peaks between H-3 proton and both N-CH_3 protons and 4-OCH_3 protons, while H-5 proton exhibited cross peaks with both 4-OCH_3 and 6-OCH_3 signals, confirming the position of both methane protons.

Consequently, It could be concluded that the structure of compound C2 contains an additional methoxy group at position C-6, which is different from compound C1 and its chemical name is therefore 4,6-Dimethoxy-2-methyl-7,8-methylenedioxy-1-isoquinolone.The structure of compounds C2 is shown below

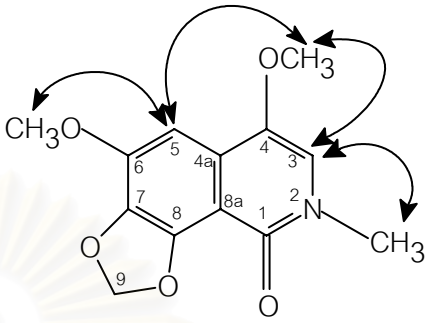


Compound C2

The complete carbon and proton assignment of C2 together with HMBC and NOESY experiment is shown in Table 14 and the HMBC and NOESY correlation of compound C2 are shown below



Major HMBC correlation of Compound C2



Major NOESY correlation of Compound C2

From the above data, the structure of C2 was elucidated as a new isoquinoline alkaloid, 4, 6-dimethoxy-2-methyl-7, 8-methylenedioxy-1-isoquinolone

Table 14. The ^{1}H- NMR, ^{13}C-NMR , HMBC and NOESY data of compound C2

Position	δC	δH	HMBC correlations	NOESY correlations
1	159.8	-	-	-
2-NCH_3	24.8	2.58, *s*	C-1, C-3	H-3
3	100.9	6.66, *s*	C-4, C-4a, 2-NCH_3	2-NCH_3, 4-OCH_3
4	163.2	-	-	-
4-OCH_3	56.3	4.02, *s*	C-4	H-3, H-5
4a	117.4	-	-	-
5	96.9	7.12, *s*	C-4, C-6, C-7, C-8a	4-OCH_3, 6-OCH_3
6	145.2	-	-	-
6- OCH_3	56.6	3.94, *s*	C-6	H-5
7	139.1	-	-	-
8	142.2	-	-	-
9	103.9	6.19, *s*	C-7, C-8	-

4. Structure elucidation of compound EA1

Compound EA1 was recrystallized as pale yellow crystals (0.07% yield) from acetone. The EIS TOF mass spectrum of this compound (Figure 24) showed a molecular ion peak at m/z 290.0938, which corresponded to the molecular formula of $C_{15}H_{14}O_6$ (calculated for $C_{15}H_{14}O_6$ = 290.0790) The presence of the alcohol functionality in the molecule was confirmed by IR absorption peak at 3457 cm^{-1} (Figure 25).

The ^{13}C-NMR spectrum of EA1 (Figures 26a-26b) showed 15 carbon signals. The DEPT (Figure 27) and HMQC (Figures 28a-28b) experiments were employed to classify these signals into those of seven quaternary carbons at δ 156.6 (C-7), 156.3 (C-5), 155.8 (C-9), 144.5 (C-4′), 144.4 (C-3′), 130.7 (C-1′), and 98.5 (C-10) ppm, seven methine carbons at δ 118.0 (C-6′), 114.9 (C-2′), 114.8 (C-5′), 95.1 (C-6), 94.1 (C-8), 78.1 (C-2) and 64.9 (C-3) ppm, and a methylene carbon at δ 28.3 (C-4) ppm.

The ^{1}H-NMR spectrum of EA1 (Figure 29) showed the substitution pattern of the aromatic proton. The meta-substitution pattern of two hydroxyl groups on ring A (5-OH and 7-OH) was substantiated by the meta-coupling of the two aromatic proton signals at δ 5.88 ppm (*d*, *J*=2.4Hz) and δ 5.71 ppm (*d*, *J*=2.4 Hz), which could be assigned to H-6 and H-8, respectively. Both signals appeared as sharp doublets that showed cross peak in the ^{1}H-^{1}H COSY spectrum (Figure 30).

Substitution pattern on ring B was deduced from the meta-coupling of aromatic proton signals at δ 6.88 ppm (1H, *d*, *J*=1.8 Hz, H-2′) and δ 6.64 ppm (1H, *dd*, *J*=8.1, 1.8 Hz, H-6′), whereas the latter signal also ortho-coupled to a doublet at δ 6.66 ppm (1H, *d*, *J*=8.1 Hz, H-5′). Therefore, two hydroxy groups could be assigned to the 3′ and 4′ positions of this ring.

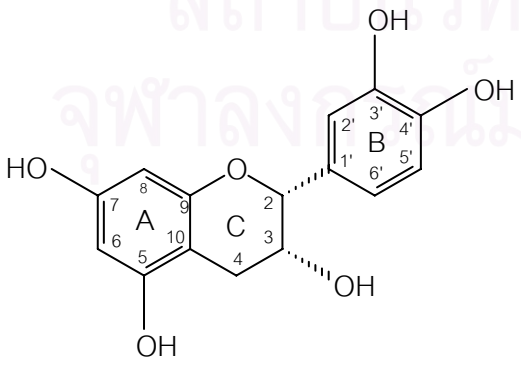
On the ring C, a methylene carbon at δ 28.3 ppm (C-4) showed cross peak with two proton signals at δ 2.48 (1H, *dd*, *J*=16.3, 3.5 Hz) and 2.67 ppm (*dd*, *J*=16.3, 4.4 Hz) in the HMQC spectrum, indicating both signals as those of the methylene H-4, which showed COSY cross peak with another signal at δ 4.00 ppm (m, H-3). A hydroxy proton signal at δ 4.65 (*d*, *J*=4.6 Hz) gave cross peak with H-3, confirming its position at C-3.

Another methine proton signal at δ 4.73 (H-2) appeared as a broad singlet because its dihedral angle with H-3 was 90^0. This is different from catechin, of which the signal of H-2 appears as a doublet (Meulenbeld et al, 1999).

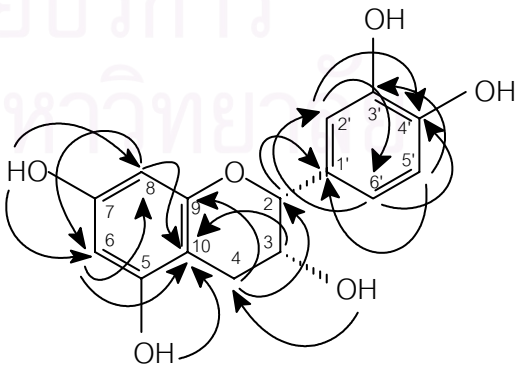
Compound EA1 was therefore identified as the flavan-3-ol, epicatechin Comparison of its carbon chemical shift with those previously reported for epicatechin (Lin and Lin, 1999) is shown in Table 15.

The NMR assignments of EA1 were also confirmed by the ^{1}H-^{13}C HMBC experiment (Figures 31a-33f). Correlations of aromatic proton at position 6 (δ 5.88 ppm) and C-8 (δ 94.1 ppm) and C-10 (δ 98.5 ppm) could be observed, as well as between H-8 (δ 5.71ppm) and C-6 (δ 95.1 ppm) and C-10. H-4 Methylene proton showed three-bond coupling with C-2 (δ 78.1 ppm), C-5 (δ 156.3 ppm) and C-9 (δ 155.8 ppm), while H-2 methine proton signal at δ 4.72 ppm showed long-range coupling with both C-1′ and C-2′

The signal of aromatic proton in ring B at δ 6.64 ppm (H-6′) showed long-rang coupling with C-2′ and C-4′ and the doublet signal at δ 6.66 ppm (H-5′) showed three bond coupling with C-1′ and C-3′, while another olefinic proton at δ 6.89 ppm (H-2′) display HMBC cross peak with C-4′and C-6′. The ^{1}H-^{13}C HMBC correlation in structure EA1 were summarized in Table 16



Epicatechin



Major HMBC correlation of Compound EA1

Table 15. Comparison of ^{13}C NMR data of epicatechin (Lin and Lin, 1999) and compound EA1 (in DMSO-d_6).

Carbon	Chemical shift (δ) ppm	
	Epicatechin	EA1
2	78.1	78.1
3	65.0	65.0
4	28.3	28.3
5	156.3	156.3
6	95.1	95.1
7	156.6	156.6
8	94.1	94.1
9	155.8	155.8
10	98.6	98.5
1′	130.7	130.7
2′	114.8	114.9
3′	144.5	144.5
4′	144.5	144.5
5′	114.9	114.8
6′	118.0	118.0

Table 16. The ^{1}H-NMR, ^{13}C-NMR and HMBC data of compound EA1.

Position	δC	δH	HMBC correlation
2	78.1	4.73, *br s*	C-2′, C-1′, C-6′
3	65.0	4.00, *m*	C-10
3-OH	-	4.65, *d*, *J*=4.6	C-4
4	28.3	2.67, *dd*, *J*=16.3, 4.4 2.48, *dd*, *J*=16.3, 3.5	C-2, C-3, C-5, C-9, C-10
5	156.3	-	-
5-OH	-	9.11, *s*	-
6	95.1	5.88, *d*, *J*=2.4	C-5, C-7, C-8, C-10
7	156.6	-	-
7-OH	-	8.90, *s*	C-6, C-8
8	94.1	5.71, *d*, *J*=2.4	C-6, C-9, C-10
9	155.8	-	-
10	98.5	-	-
1′	130.7	-	-
2′	114.9	6.88, *d*, *J*=1.8	C-4′, C-6′
3′	144.5	-	-
4′	144.5	-	-
5′	114.8	6.66, *d*, *J*=8.1 Hz	C-1′, C-3′
6′	118.0	6.64, *dd*, *J*=8.1, 1.8 Hz	C-2′, C-4′

CHAPTER V

CONCLUSION

Four compounds were isolated from the aerial part of *Sauropus hirsutus* by chromatographic techniques. Their chemical structures were elucidated using spectroscopic techniques. Two of them isolated from the chloroform extract, were elucidated as the new isoquinolone alkaloids named 4-methoxy-2-methyl-7,8-methylenedioxy-1-isoquinolone and 4,6-dimethoxy-2-methyl-7,8-methylenedioxy-1-isoquinolone. From the ethyl acetate extract, a flavonoid, epicatechin, was isolated, whereas β-sitosterol was found in the hexane extract.

This is the first chemical investigation of the constituents of this *Sauropus* species and the data obtained would be valuable in the chemotaxonomic and phytochemical studies of this plant genus.

REFERENCES

Thai

นันทวัน บุณยะประภัศร และ อรนุช โชคชัยเจริญพร. 2542. สมุนไพรไม้พื้นบ้าน (3). กรุงเทพมหานคร:บริษัท ประชาชน จำกัด. หน้า 134-135.

มาโนช วามานนท์ และ เพ็ญนภา ทรัพย์เจริญ. 2540. ผักพื้นบ้าน ความหมาย และภูมิปัญญาของสามัญชนไทย:โรงพิมพ์ องค์การสงเคราะห์ทหารผ่านศึก. หน้า 18.

ส่วนพฤกษศาสตร์ป่าไม้ สำนักวิชาการป่าไม้ กรมป่าไม้. 2544. ชื่อพรรณไม้แห่งประเทศไทย เต็ม สมิตินันทน์. กรุงเทพมหานคร:บริษัท ประชาชน จำกัด. หน้า 466-467.

English

Airy Shaw, H. K., 1972. Kew Bulletin. London: Her Majesty ' s Stationery office.

Ahmad, M., Jain, N., Kamil, M. and Ilyas, M. 1991. 6-Hydroxykaempferol 7-rutinoside from leaves of *Sapium euginiaefolium*. Phytochemistry 30 : 2815-2816.

Ahn, B. T., Zhang, B., Lee, S. C., Kim, J. G., Ro, J. S. and Lee, K. S. 1996. Phenolic compounds of aerial parts of *Euphorbia pekensis*. Yakhak Hoechi 40 : 170-176.

Ahn, Y. J., Lee, S. H., Kang, S. T., Hwang, B. Y., Park, W. Y., Ahn, B. T., Ro, J. S. and Lee, K. S. 1996. The phenolic components of *Sapium japonicum*. Yakhak Hoechi. 40 : 183-192.

Aqil, M. and Kham, I. Z. 1999. Euphorbianin a new flavonol glycoside from *Euphorbia hirta* Linn. Global J. Pure Appl Sci. 5 : 371-373.

Atalykova, F. M. and Mukenov, M. K. 1981. Flavonoid containing plant of the Tarbagatai natural flora. Ser Biologicheskaya 4 : 4-8.

Barbosa, P. R., Fascio, M., Martins, D., Guedus, M. L. S. and Roque, N. F. 2003. Triterpenes of *Croton betulaster* (Euphorbiaceae). Biochem Syst Ecol. 31 : 307-308.

Barnes, R. A. and Soeiro, O. M. The alkaloids of *Croton salutaris*. 1981. Phytochemistry 20 : 543-544.

Barton, D. H. R., Kirby, A. J. and Kirby, G. W. 1968. Phenol oxidation and biosynthesis. Part XVII. Investigation on the biosynthesis of sinomenine. J. Chem. Soc. (C). : 929-936.

Bender, A. E. and Ismail, K. S. 1973. Nutritive value and toxicity of *Sauropus androgynus*. Proc. Nutr. Soc. 32 : 79A-80A.

Bhakuni, D. S. and Dhar, M. M. 1968. Crotsparine, a new proaporphine alkaloid from *Croton sparsiflorus* Morong. Experientia 24 : 10-11.

Bhakuni, D. S. and Dhar, M. M. 1969. Crotsparinine, a dihydroproaporphine alkaloid from *Croton sparsiflorus*. Experientia 25 : 354.

Bhakuni, D. S., Satish, S. and Dhar, M. M. 1972. Absolute configuration of crotsparine, crotsparinine and sparsiflorine. Tetrahedron 28 : 4579-4582.

Blance, P. and De Saqui-Sannes, G. 1972. Flavonoids of *Euphorbia hirta* (Euphorbiaceae). Plant Med Phytother. 6 : 106-109.

Calatayud, P. A., Rahbe, Y., Delobel, B., Khuong-Huu, F., Tertuliano, M. and Ru, B. L. 1994. Influence of secondary compounds in the phloem sap of cassava on expression of antibiosis towards the mealybug *Phenacoccus manihoti*. Entomol. Exp. Appl. 72 : 47-57.

Casagrande, C., Canonica, L. and Severini-Rica, S. 1975. Studies on proaporphine and aporphine alkaloids. Part VII. Stereochemistry of reduced proaporphines of *Croton sparsiflorus* and *C. linearis*. J. Chem. Soc. Perkin Trans I. 1659-1663.

Chapman and Hall. 1994. Dictionary of Natural Products London : Great Britain.

Chang, H., Wang, J. S., Tseng, H. H., Lai, R. S. and Su, J. M. 1997. Histopathological study of *Sauropus androgynus* associated constrictive bronchiolitis obliterans. Am. J. Surg. Pathol. 21 : 35-42.

Chang, Y. L., Yao, Y. T., Wang, N. S. and Lee, Y. C. 1998. Segmental necrosis of small bronchi after prolonged in take of *Sauropus androgynus* in Taiwan. Am. J. Respir. Crit. Care Med. 157 : 594-598.

Chatterjee, A., Majumder, P.L., Mukherjee, R. Saha, S. K. and Talapatra, S.K. 1965. Structure of sparsiflorine, an alkaloid of *Croton sparsiflorus* Morung. Tetrahadron Lett. 21 :1539-1544.

Chen, I. C., Chang, K. C., Hsieh, Y. K. and Wu, D. 1996. Torsade de pointes due to consumption of *Sauropus androgynus* as a weight-reducing vegetable. Am. J. Cardiol. 78 :1186-1187.

Ching, L. S. and Mohamed, S. 2001. Alpha-tocopherol content in 62 edible tropical plants. J. Agric. Food Chem. 49 : 3101-3105.

Cimanga, K., Ying, L., De Bruyne, T., Aper, S., Cos, P., Hermans, N., Bakana, P., Tona, L., Kambu, K., Kalenda, D. T., Pieters, L., Vanden Berghe, D. and Vlietinck, A. J. 2001. Radical scavenging and xanthine oxidas inhibitory activity of phenolic compounds from *Bridelia ferruginea* stem bark. J. Pharm. Pharmacol. 53 :757-761.

Colombo, A. 1976. Crystal structure and relative configuration of (±) – 11, 12 dihydroglaziovine. Stereochemistry of reduced proaporphines. J. Chem. Soc. Perkin Trans II. 1218-1221.

Cordell, G. A., Saxton, J. E., Shamma, M. and Smith, G. F. 1989. Dictionary of alkaloids.London New York :Chapman and Hall Ltd.

Cui, G. Y., Liu, J. Y. and Tan, R. X. 2003. A new antimicrobial flavonol glycoside from *Alchornea clavidii*. Chin Chem Lett. 14 : 179-180.

Das, B., Chakravarty, A. K., Masuda, K., Suzuki, H. and Ageta, H. 1994. A diterpenoid from roots of *Gelonium multiflorum*. Phytochemistry 37 : 1363-1366.

De Bruyne, T., Cimanga, K., Pieters, L., Claeys, M., Dommisse, R. and Vlietinck, A. 1997. Gallocatechin- (4′→O→7)-epigallocatechin, a new biflavonoid isolated from *Bridelia ferruginea*. Nat. Prod. Lett. 11 : 47-52.

De- Ehnamkul, W. and Potduang, B. 2003. Biosynthesis of β-sitosterol and stigmasterol in *Croton sublyratus* proceeds via a mixed origin of isoprene units. Phytochemistry 62 : 389-398.

Del V. Galarza, S., Cabreya, J. L. and Juliani, H. R. 1983. Constitutents of Euphorbiaceae from Argentina. Part I. An. Asoc. Quim. Argent. 71 : 505-508.

Dennis, W. 2000. Contemporary Plant Systematics USA : Andrews University Press.

Dumkow, K. 1969. Flavonoids of native Euphorbiaceae. IV. Flavonol glycosides from *Mercurialis annua*. Z Naturforsch. 24 : 1203.

Gurrero, M. F., Puebla, P., Carron, R., Martin, M. L. and Roman, L. S. 2002. Quercetin 3, 7-dimethyl ether : a vasorelaxant flavonoid isolated from *Croton schiedeanus* Schlecht. J. Pharm. Pharmacol. 54 : 1373-1378.

Ham, I., Wang, T., Cho, E. S., Cho, H. K. and Wang, W. K. 2001. Phenolic compounds from *Phyllanthus ussuriensis*. Yakhak Hoechi. 45 : 237-244.

Harborne, J. B. 1994. The flavonoids : advance in research since 1986. London : Chapman and Hall.

Haynes, L. J. and Stuart, K. L. 1963. Alkaloids from *Croton* species. Part I. The isolation of alkaloids from *C. linearis* Jacq. and the detection of alkaloids in *C. glabellus* L., *C. humilis* L. and *C. flavans* L. J. Chem. Soc. 1784-1788.

Haynes, L. J., Husbands, G. E. M. and Stuart, K. L. 1966. Alkaloids from *Croton* species. Part IV. The isolation and structure of a New proaporphine derivative from *Croton linearis* Jacq. J. Chem. Soc. (C). 1680-1681.

Haynes, L. J., Husbands, G. E. M. and Stuart, K. L. 1968. Alkaloids from *Croton* species. Part VII. Morphinandienone derivatives from *Croton linearis* Jacq. J. Chem. Soc. (C). 951-957.

Haynes, L. J., Stuart, K. L., Barton, D. H. R. and Kirby, G. W. 1966. Alkaloids from *Croton* species. Part III. The constitution of the proaporphines crotonosine, “Homolinearisine”, Base A, and the dihydroproaporphine linearisine. J. Chem. Soc (C). 1676-1679.

Hiremath, S. P., Rudresh, K. and Badami, S. 1998. Flavonoids of *Acalypha indica* Linn. Indian J. Heterocycl. Chem. 8 : 163-164.

Hnatyszyn, O., Mino, J., Ferraro, G., Acevedo, C. 2002. The hypoglycemic effect of *Phyllanthus sellowianus* fractions in streptozotocin – induced diabetic mice. Phytomedicine 9 : 556-559.

Hsiue, T. R., Guo, YL. L., Chen, K. W., Chen, C. W., Lee, C.H. and Chang, H. Y. 1998. Dose response relationship and irreversible obstructive ventilatory defect in patients with consumption of *Sauropus androgynus*. Chest 113 :71-76.

Ito, A. Shamon, L. A., Yu, B., Meta-Greenwood, E., Lee, S. K., Breemen, R. B., Mehta, R. G., Farnsworth, N. R., Fong, H. H. S., Pezzuto, J. M. and Kinghorn, A. D. 1998. Antimutagenic constituents of *Casimiroa edulis* with potential cancer chemopreventive activity. J. Agric. Food Chem. 46 : 3509-3516.

Hulshof, P. J. M., Xu, G., Bovenkamp, P., Muhilal and West, C. E. 1997. Application of a validated method for the determination of provitamin A carotenoids in Indonesian foods of different maturity and origin. J. Agric. Food Chem. 45 :1174-1179.

Jang, D. S., Cuendet, M., Hawthorne, M. E., Kardono, L. B. S., Kawanishi, K., Fong, H. H. S., Mehta, R. G., Pezzuto, J. H. and Kinghorn, A. D. 2002. Prenylated flavonoids of the leaves of *Macaranga conifera* with inhibitory activity against cyclooxygenase-2. Phytochemistry 61 : 867-876.

Kanchanapoom, T., Chumsri, P., Kasai, R., Otsuka, H. and Yamasaki, K. 2003. Lignan and megastigmane glycosides form *Sauropus androgynus*. Phytochemistry 63 :985-988.

Kametani, T., Ihara, M., Fukumoto, K. and Yagi, H. 1969. Studies on syntheses of heterocyclic compounds. Part CCC. Syntheses of salutaridine, sinoacutine and thebaine. Formal total syntheses of morphine and sinomenine. J. Chem. Soc (C). 2030-2033.

Kotani, E. and Tobinaga, S. 1973. Total synthesis of the morphinandienone alkaloids, (±) – amurine, (±) – flavinantine and (±) – pallidine by anodic oxidation. Tetrahedron Lett. 48 : 4759-4762.

Lai, R. S., Chiang, A. A., Wu, M. T., Wang, J. S., Lai, N. S., Lu, J. Y., Ger, L. P., and Roggli, V. 1996. Outbreak of bronchiolitis obliterans associated with consumption of *Sauropus androgynus* in Taiwan. Lencet 348 : 83-85.

Lin, J. H. and Lin, Y.T. 1999. Flavonoids from leaves of *Loranthus Kaoi* (Chao) Kiu. J. Food Drug Anal. 7 : 185-190.

Luh, S. P., Lee, Y. C., Wu, H. D., Kuo, S. H. and Chu, S. H. 1999. Lung transplantation for patients with end-stage *Sauropus androgynus* induced bronchiolitis obliterans (SABO) syndrome. Clin. Transplantation 13 : 496-503.

Meulenbeld, G. H., Zuilhof, H., Veldhuizen, A., Heuvel, R. H. H. and Hartmans, S. 1999. Enhanced (+)-Catechin transglucosylating activity of *Steptococcus mutans* GS-5 glucosyltranferase-D due tofructose removal. Appl. Environ. Microbiol. 65 : 4141-4147.

Meyre-Silva, C., Mora, T. C., Santos, A. R. S., Dal margo, J., Yunes, R. A., Delle Monache, F. and Cechinel-Filho, V. 1997. A triterpene and a flavonoid C-glycoside from *Aleurites moluccana* L. Wild. (Euphorbiaceae). Acta. Farm. Bonaerense 16 : 169-172.

Miean, K. H. and Mohamed, S. 2001. Flavonoid (myricetin, quercetin, kaempferol,luteolin and apigenin) content of edible tropical plants. J. Agri. Food Chem. 49 : 3106-3112.

Mohamed, K. M. 2001. Phenylpropanoid glucosides from *Chrozophora obliqua*. Phytochemistry 58 :615-618.

Padmavathi, P. and Rao, M. P. 1990. Nutritive value of *Sauropus androgynus* leaves. Plant Foods Hum Nutr. 40 : 107-113.

Parveen, N., Singh, M.P. and Khan, N. U. 1988. Chemical examination of the leaves of *Homonoia reparia* Lour (Euphorbiaceae). J. Indian Chem. Soc. 65 : 815-816.

Phillipson, J. D., Roberts, M. F. and Zenk, M. H. 1985. The Chemistry and Biology of Isoquinoline alkaloids. Geramany : Springer-Verlag Berlin Heidelberg.

Prawat, H., Mahidol, C., Ruchirawat, S., Prawat, U., Tuntiwachawuttikul, P., Tooptakong, U., Taylor, W. C., Pakawatchai, C., Skelton, B. W. and White, A. H. 1995. Cyanogenic and non-cyanogenic glycosides from *Manihot esculenta*. Phytochemistry 40 : 1167-1173.

Puebla, P., Lopez, J. L., Guerrero, M., Carron, R., Martin, M. L., Roman, L. S. and Feliciano, A. S. 2003. Neo-clerodane diterpenoids from *Croton schiedeanus*. Phytochemistry 62 :551-555.

Rizk, A. M., Ahmed, S. S. and Diab, M. A. 1979. Constituents of Egyptian Euphorbiaceae. VII. Further investigation of the flavonoids of *Euphorbia paralias* L. Planta Med. 36 : 189-190.

Rizk, A. M., Youssef, A. M., Diab, M. A. and Salem, H. M. 1976. Constituents of Egyptain Euphorbiaceae. Part 2 : Flavonoids of *Euphorbia paralias*. Pharmazie. 31 : 405.

Roberts, M. F. and Wink, M. 1998. Alkaloids Biochemistry, Ecology and Medicinal Appications New York : Plenum Press.

Rojas, S., Macias, M., Castaneda, P., Bye, R., Linares, E. and Mata, R. 1999. A New Lanostan-type triterpenoid from *Chamaesyce prostrata*. Planta Med. 65 : 478-479.

Satyanarayana, P. and Ramu, K.S. 1995. Characterisation of a new series of diphyllin esters from *Sauropus quadrangularis* Muell-Arg. Indian J. Chem. Sec.B. 34B : 571-572.

Schutz, B. A., Wright, A. D., Rali, T. and Sticher, O. 1995. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochemistry 40 : 1273-1277.

Stuart, K. L. and Chamber, C. 1967. New aporphine alkaloids from *Croton wilsonii* Griseb. Tetrahedron Lett. 42 : 4135-4138.

Stuart, K. L. and Graham, L. 1973. Alkaloid biosynthesis in *Croton flavens*. Phytochemistry 12 :1967-1972.

Stuart, K. L., Haynes,L.J., Barrett, M. and Husbands, G. E. M. 1968. Jacularine, a new reduced proaporphine from *Croton linearis* Jacq. Tetrahedron Lett. 42 :4473-4474.

Subramanian, S. S., Nagarajan, S. and Sulochana, N. 1971. Flavonoids of some Euphorbiaceous plants. Phytochemistry 10 : 2548-2549.

Sultana, S. and Ilyas, M. 1987. Chemical investigation of *Macaranga indica* Wight. Indian J. Chem. 26B :801-802.

Tschesche, R., Welzel, P. and Legler, G. 1965. Zur struktur einiger tetrasubstituierter aporphinalkaloide. Tetrahedron Lett. 8 :445-450.

Vinh, D. Nguyen, M.D. and Nguyen, V. T. 2002. A flavonoid compound from leaves of *Macaranga tribola* Muell-Arg. Euphorbiaceae. Tap. Chi. Duoc. Hoc. 8 : 15-17.

Wang, J. S., Tseng, H. H., Lai, R. S., Hsu, H. K. and Ger, L. P. 2000. *Sauropus androgynus* constrictive obliterative bronchitis/bronchiolitis-histopathological study of pneumonectomy and biopsy specimens with emphasis on the inflammatory process and disease progression. Histopathology 37 : 402-410.

Wang, P. H. and Lee, S. S. 1997. Active chemical consituents from *Sauropus androgynus*. J. Chin. Chem. Soc.-Taip. 44 : 145-149. Chem abstract 127 (1997) : Abstract No. 92667q.

Weinstein, B. and Hylton, T. A. 1964. Heterocyclic compounds V. The synthesis of casimiroin. Tetrahedron 20 : 1725-1728.

Yu, S. Y. and Cheah, C. H. 1979. Nutritive value and toxicity of *Sauropus albicans*, a Malaysian leafy vegetable. Food and Nutr. 5 : 38.

APPENDIX

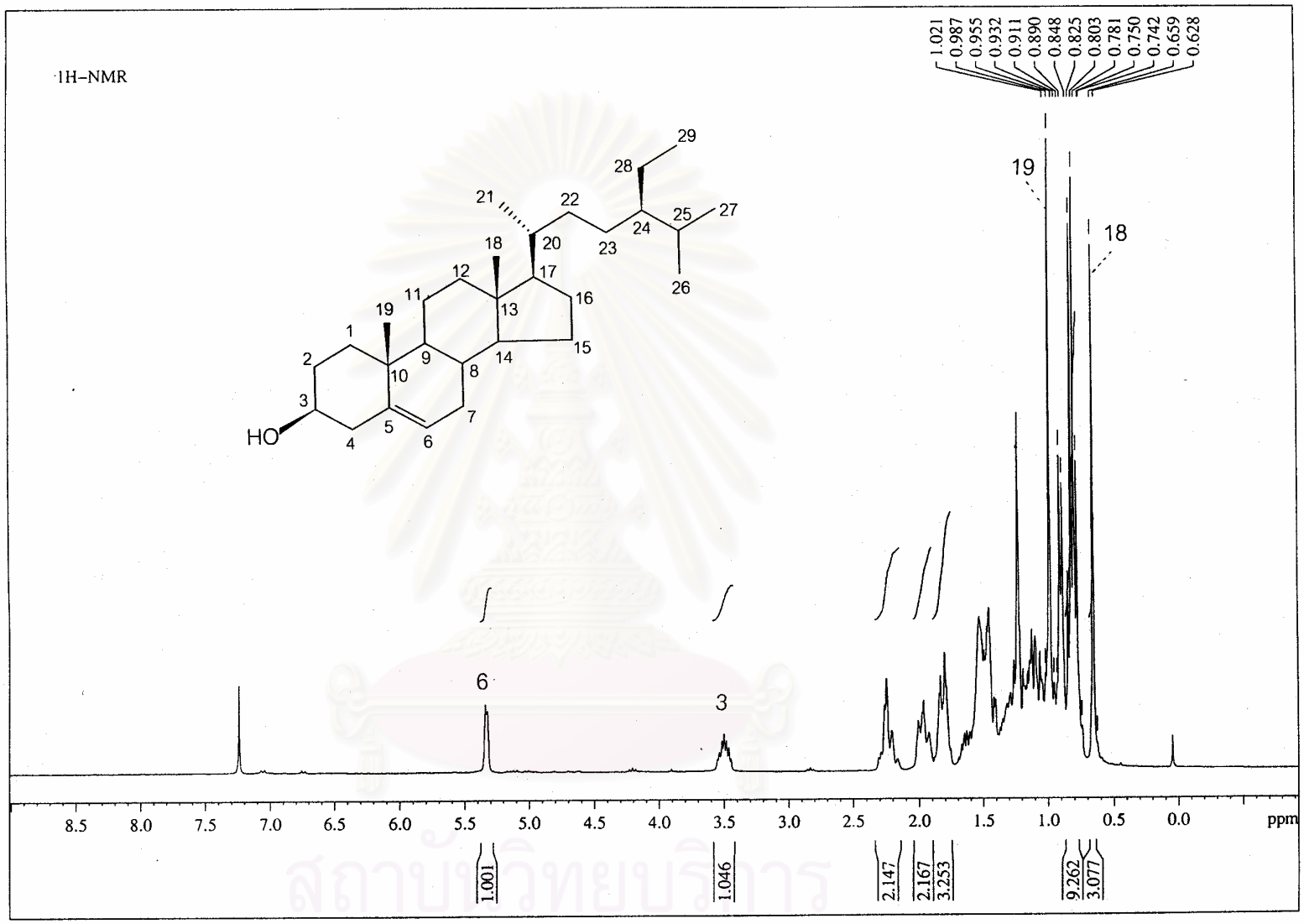


Figure 2. The 300MHz ^{1}H-NMR spectrum of compound H1. (in $CDCl_3$)

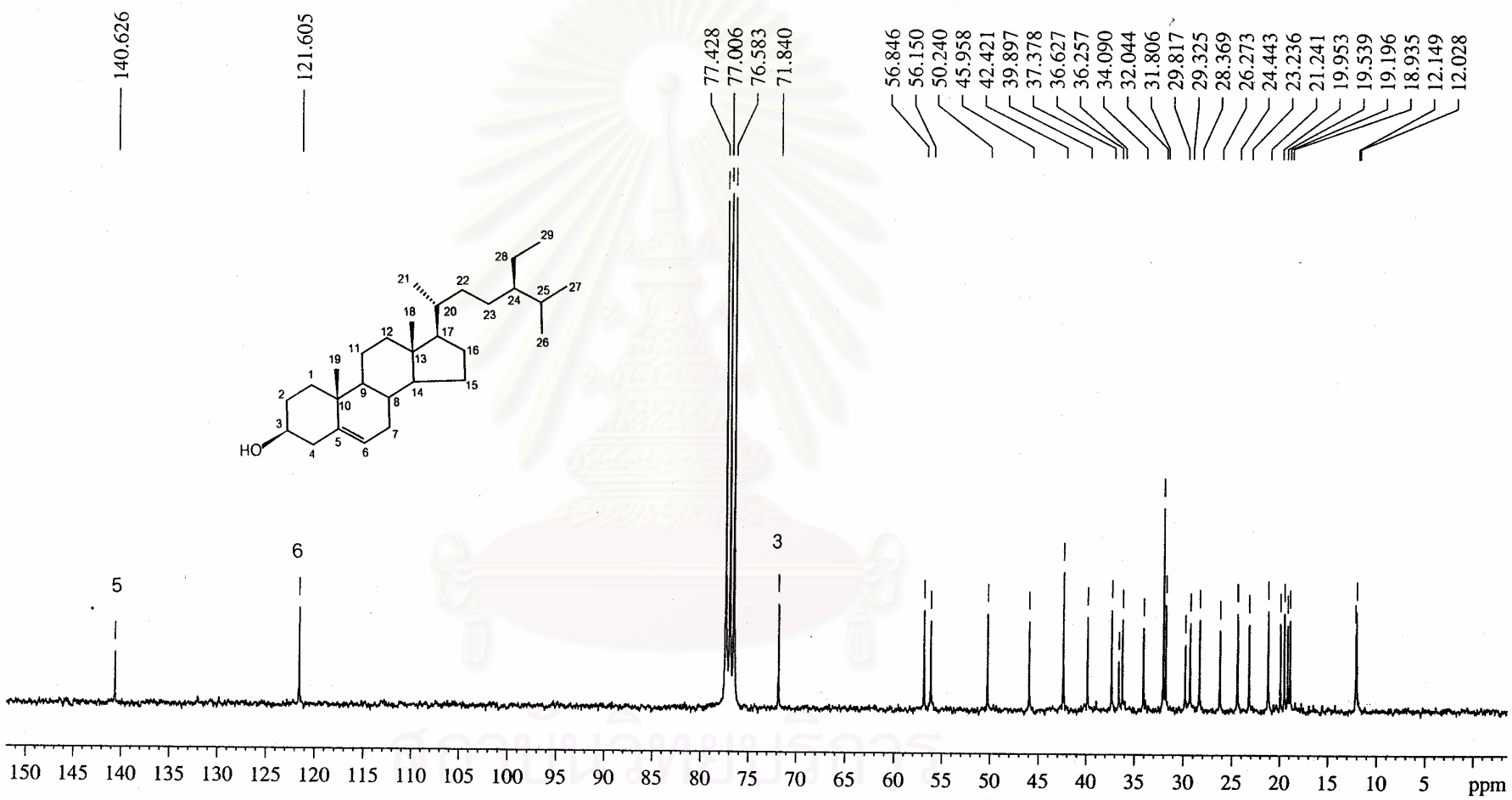


Figure 3a. The 75 MHz ^{13}C-NMR spectrum of compound H1. (in $CDCl_3$)

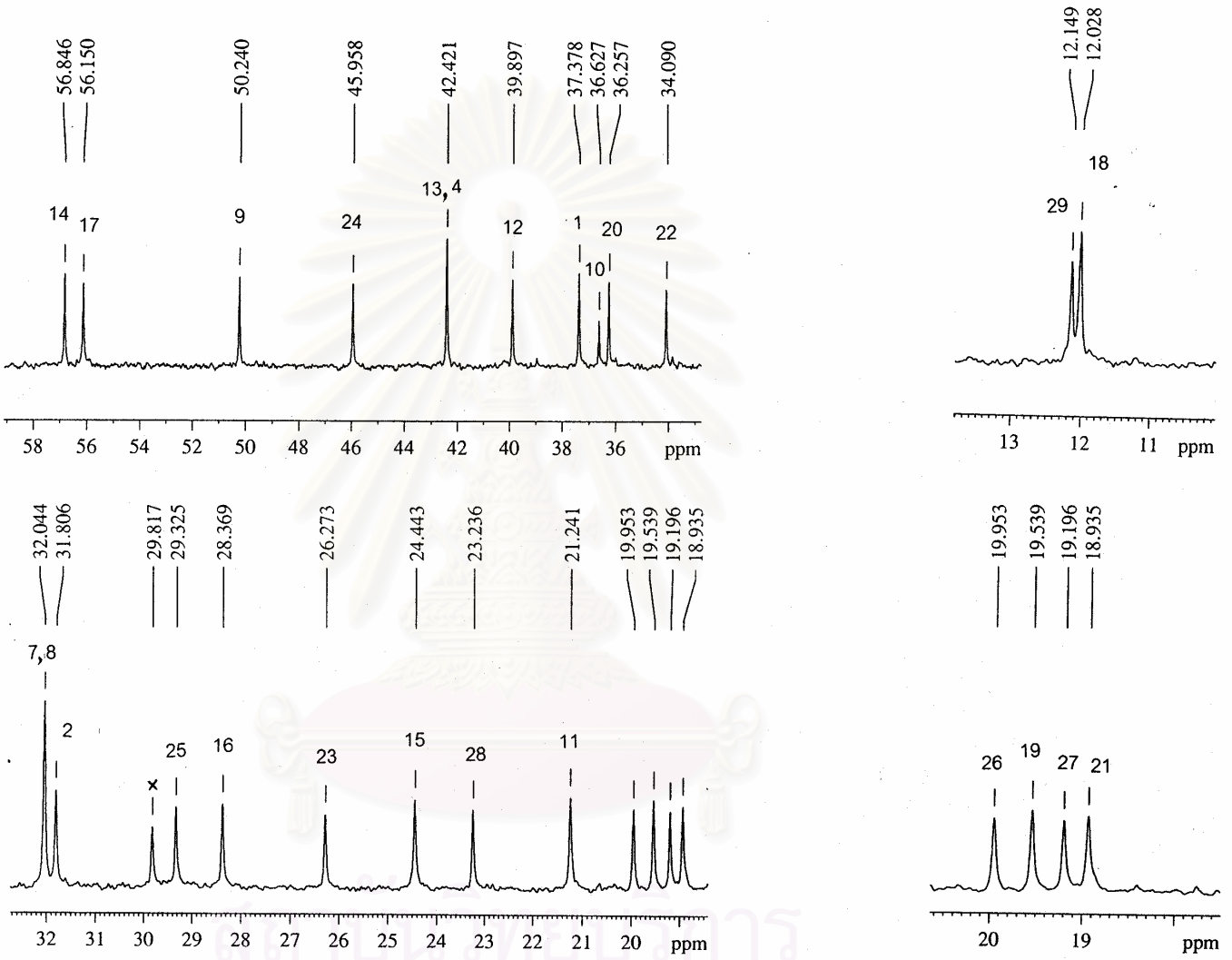
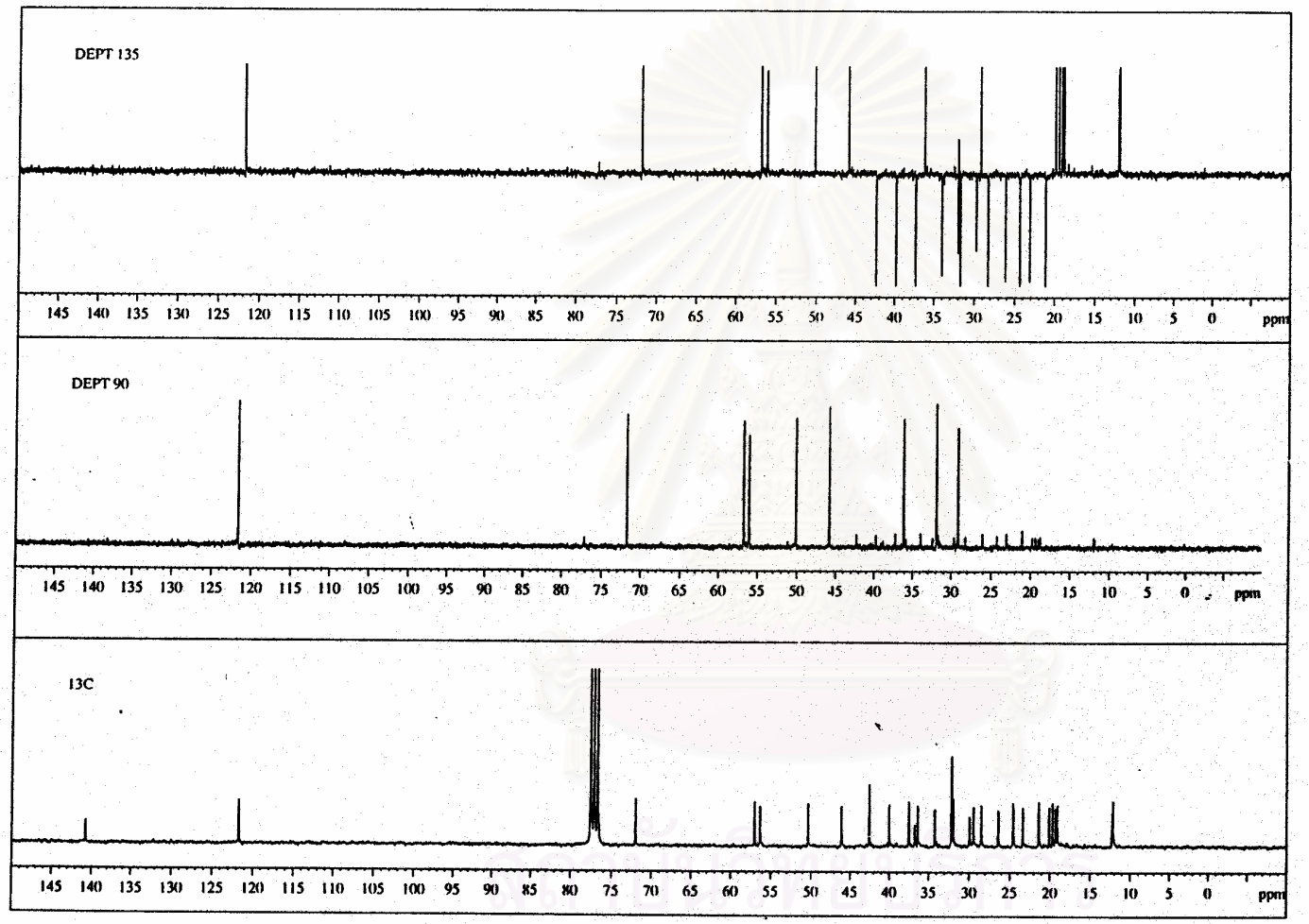


Figure 3b. The 75 MHz ^{13}C-NMR spectrum of compound H1. (expanded)



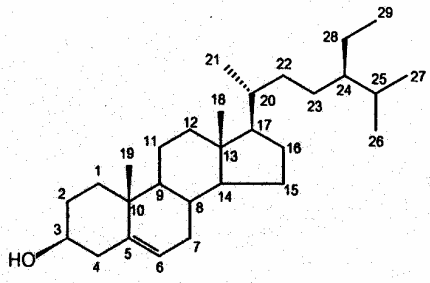


Figure 4. The 75 MHz ^{13}C- DEPT NMR sprectrum of compound H1.

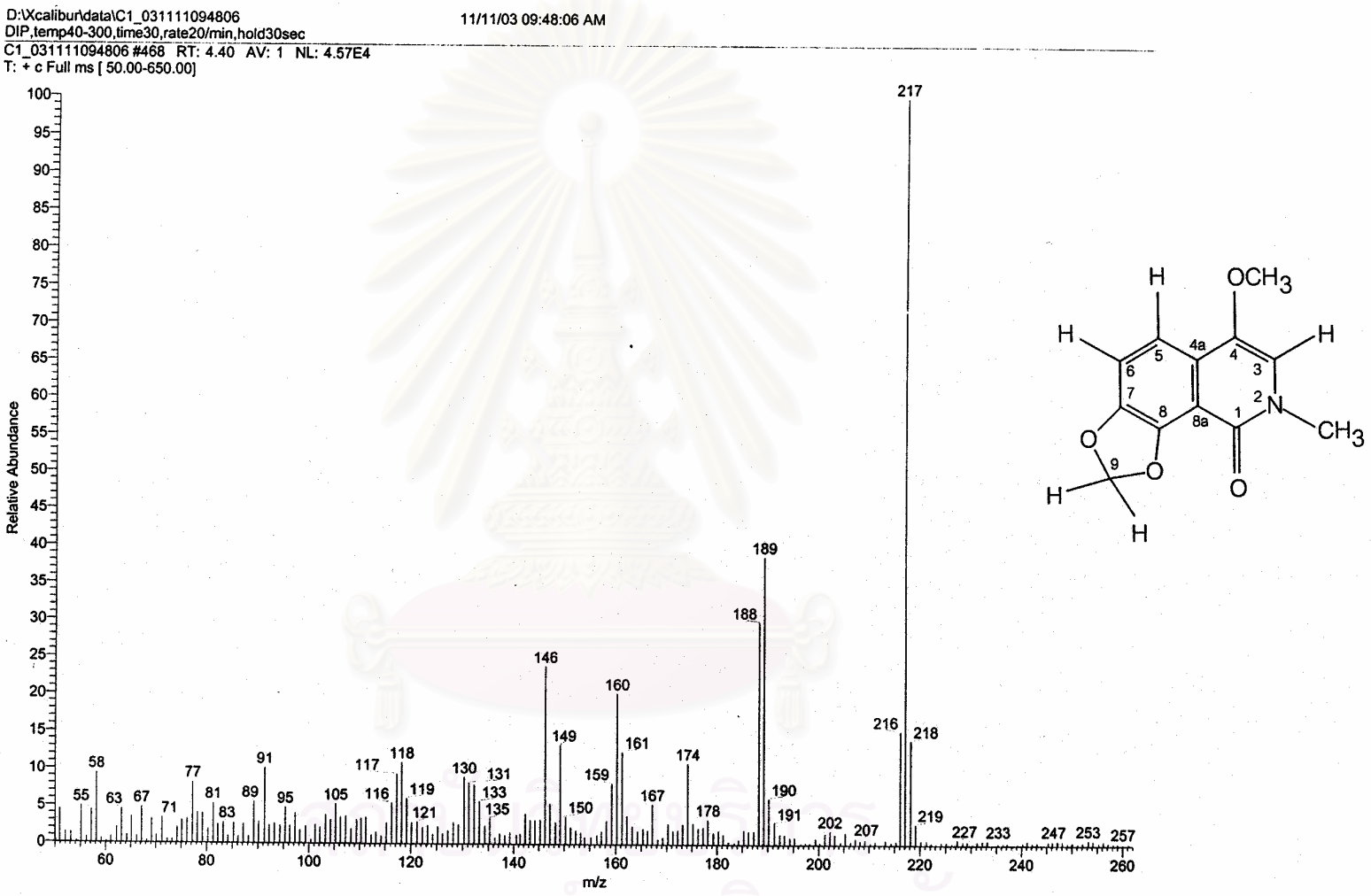
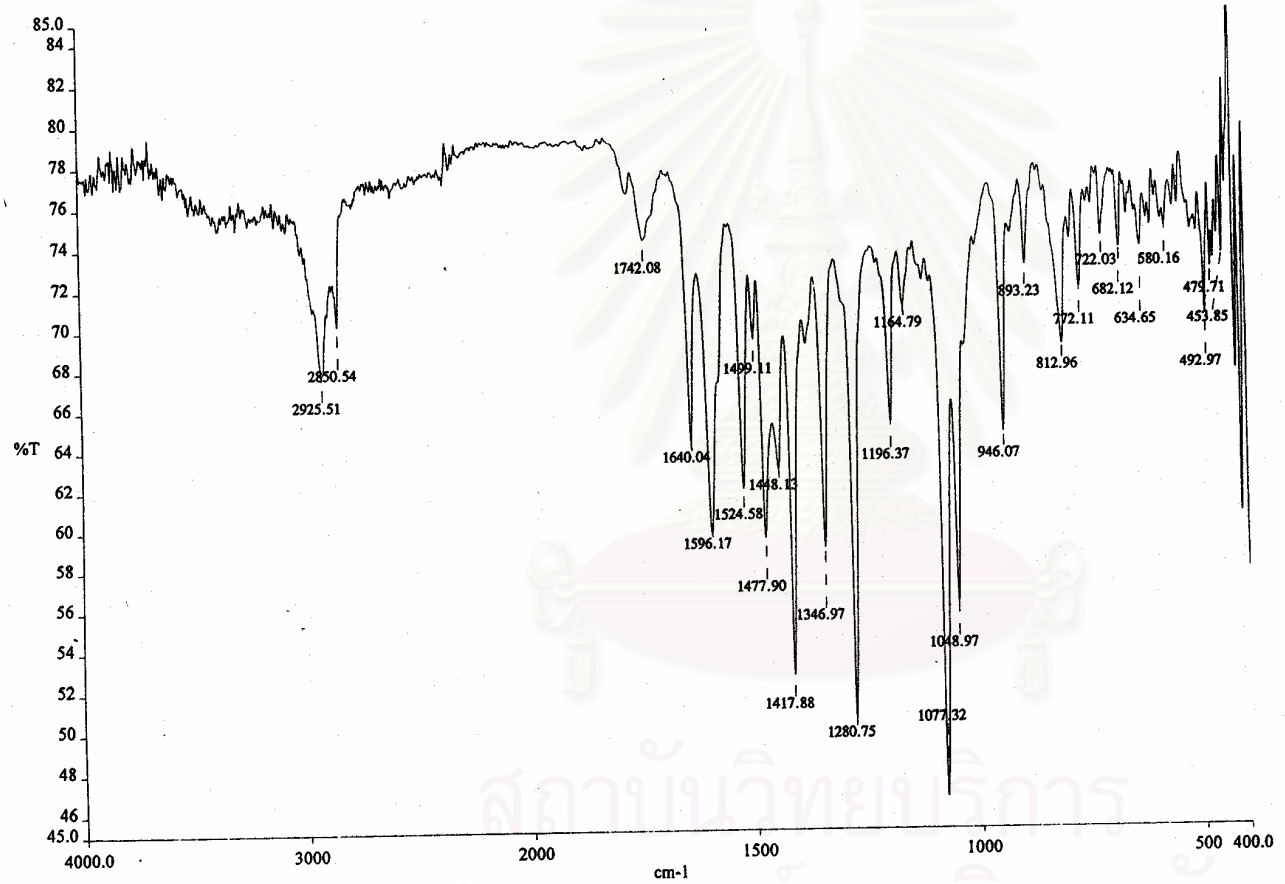


Figure 5. The EIMS spectrum of compound C1.



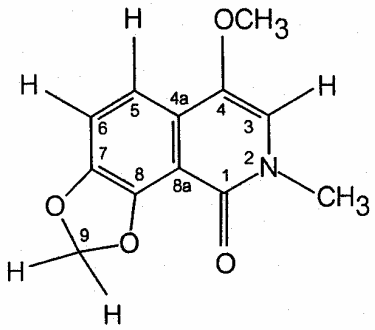


Figure 6. IR spectrum of compound C1.

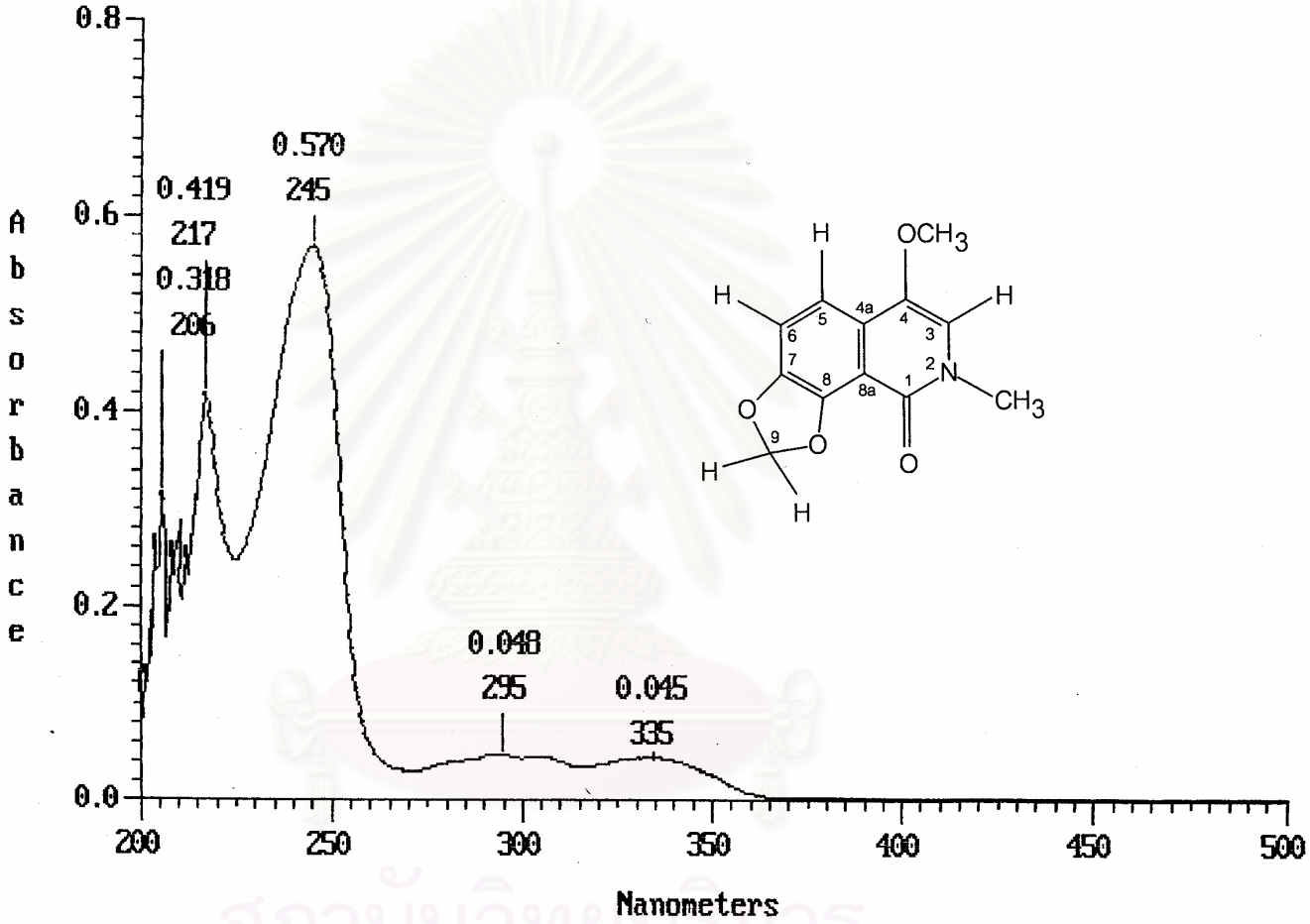
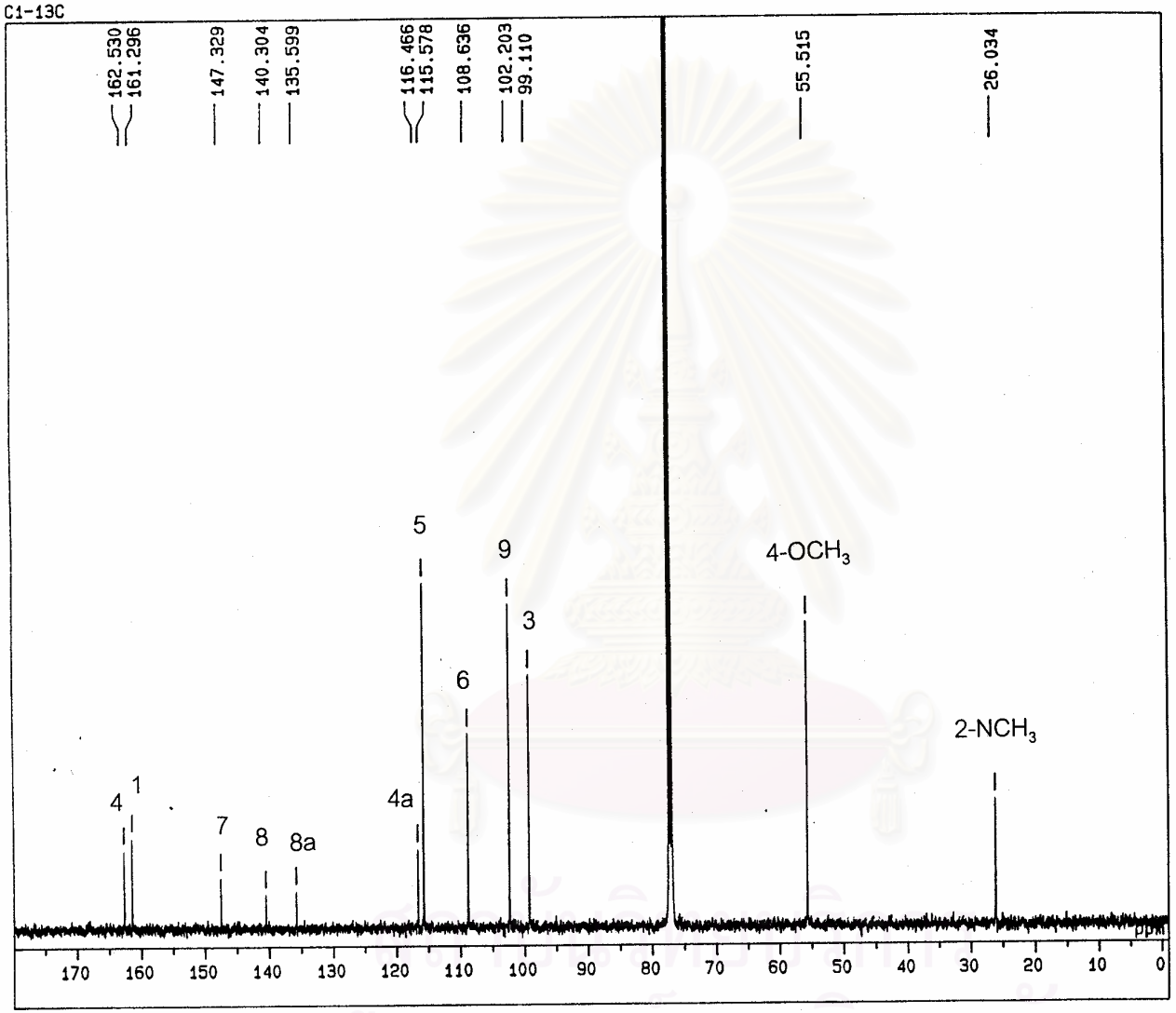


Figure 7. UV spectrum of compound C1.



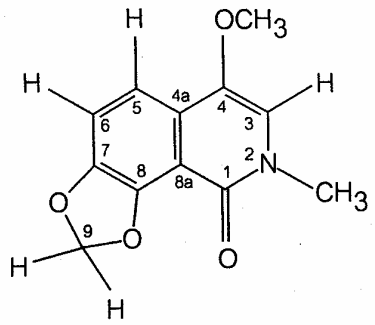
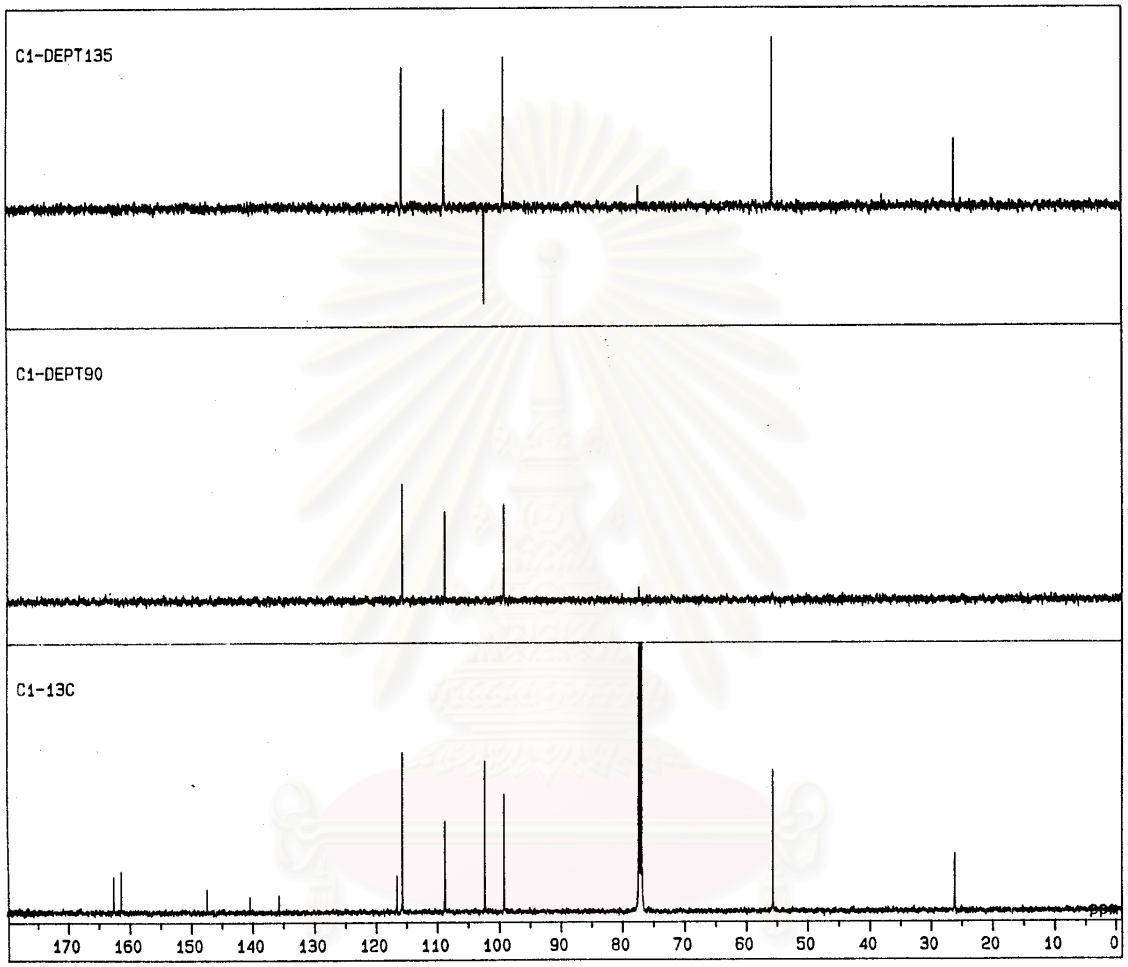


Figure 8. The 125 MHz ^{13}C-NMR spectrum of compound C1. (in $CDCl_3$)



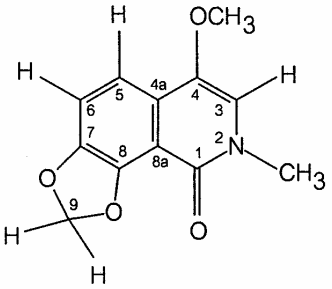


Figure 9. The 125 MHz ^{13}C-DEPT NMR spectrum of compound C1. (in $CDCl_3$)

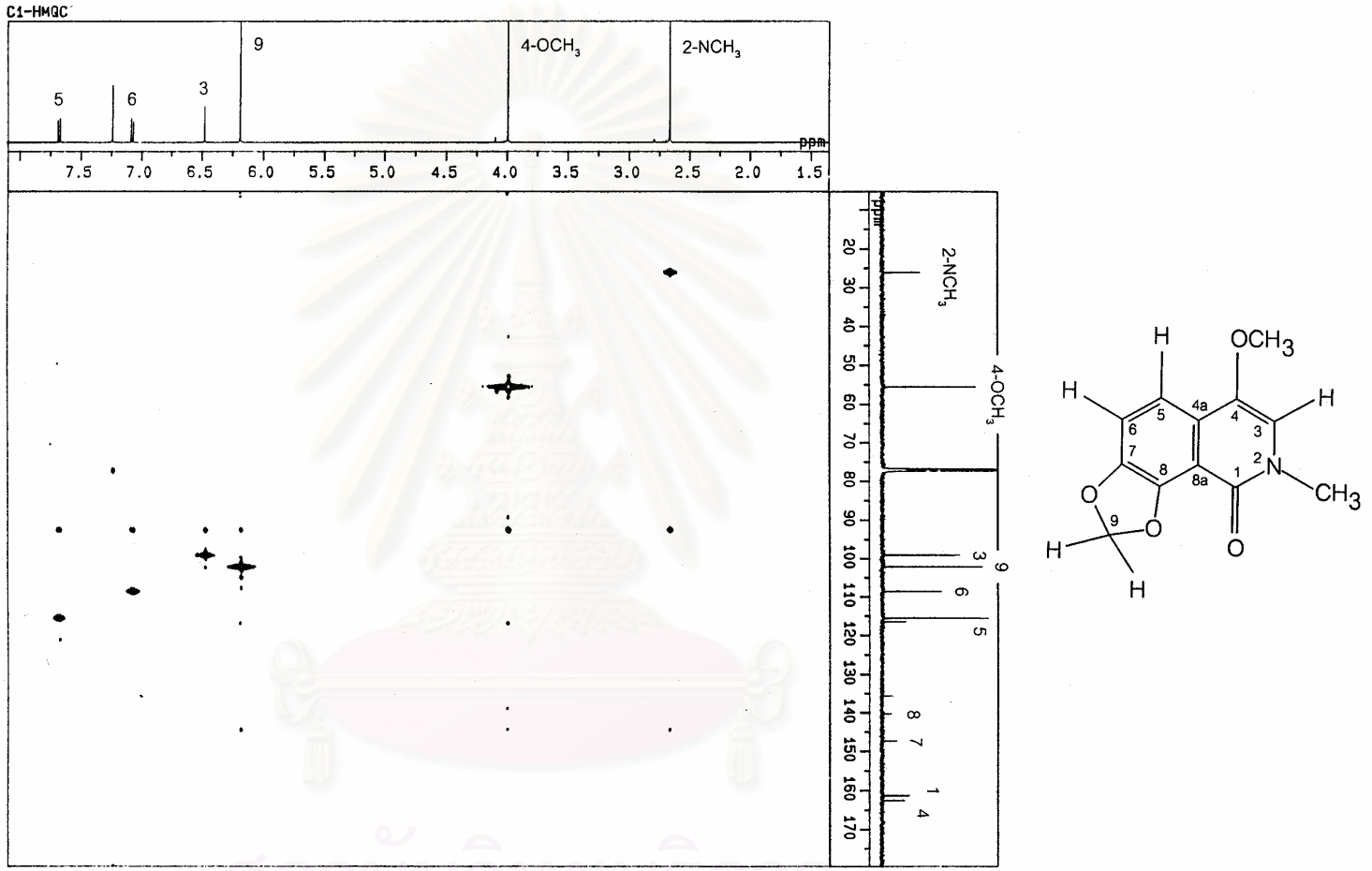


Figure 10. The 500 MHz ^{1}H-^{13}C HMQC spectrum of compound C1. (in $CDCl_3$)

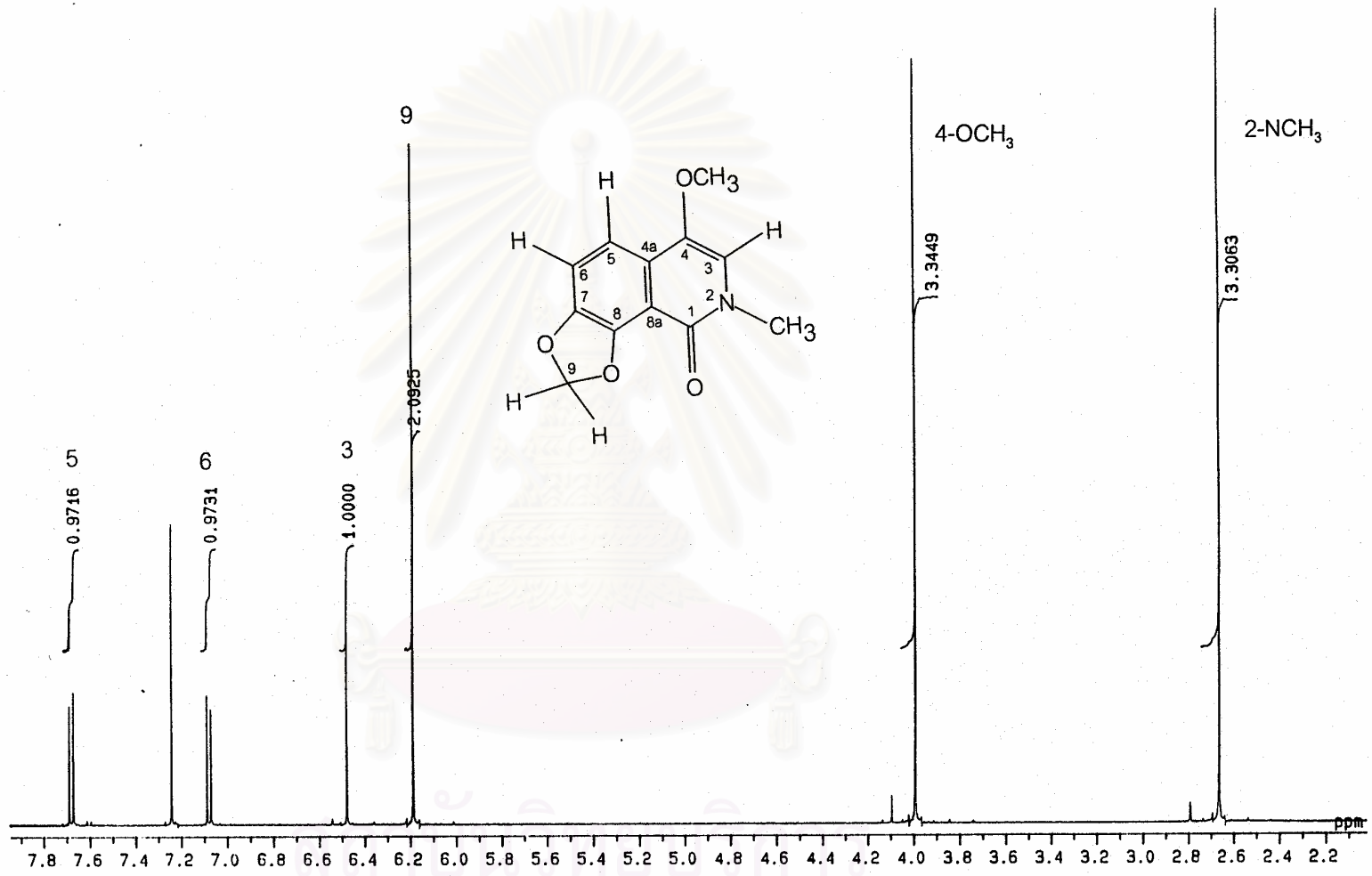


Figure 11a. The 500 MHz ^{1}H-NMR spectrum of compound C1. (in $CDCl_3$)

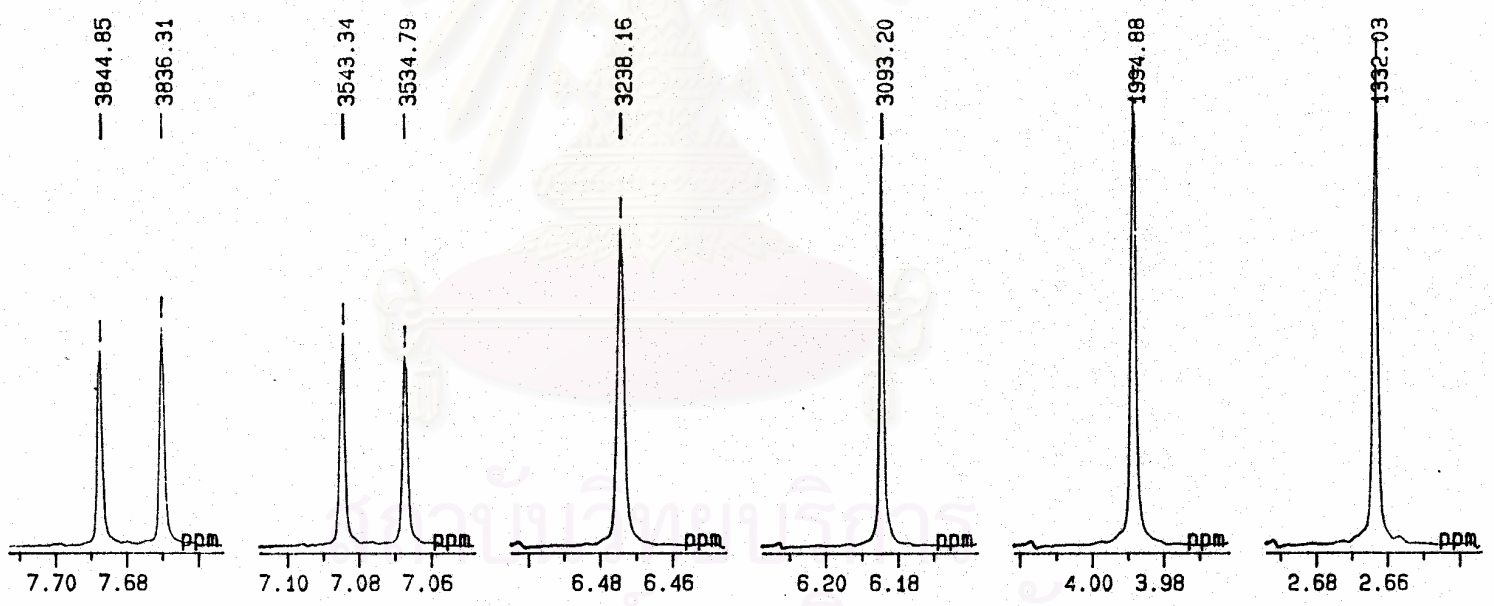
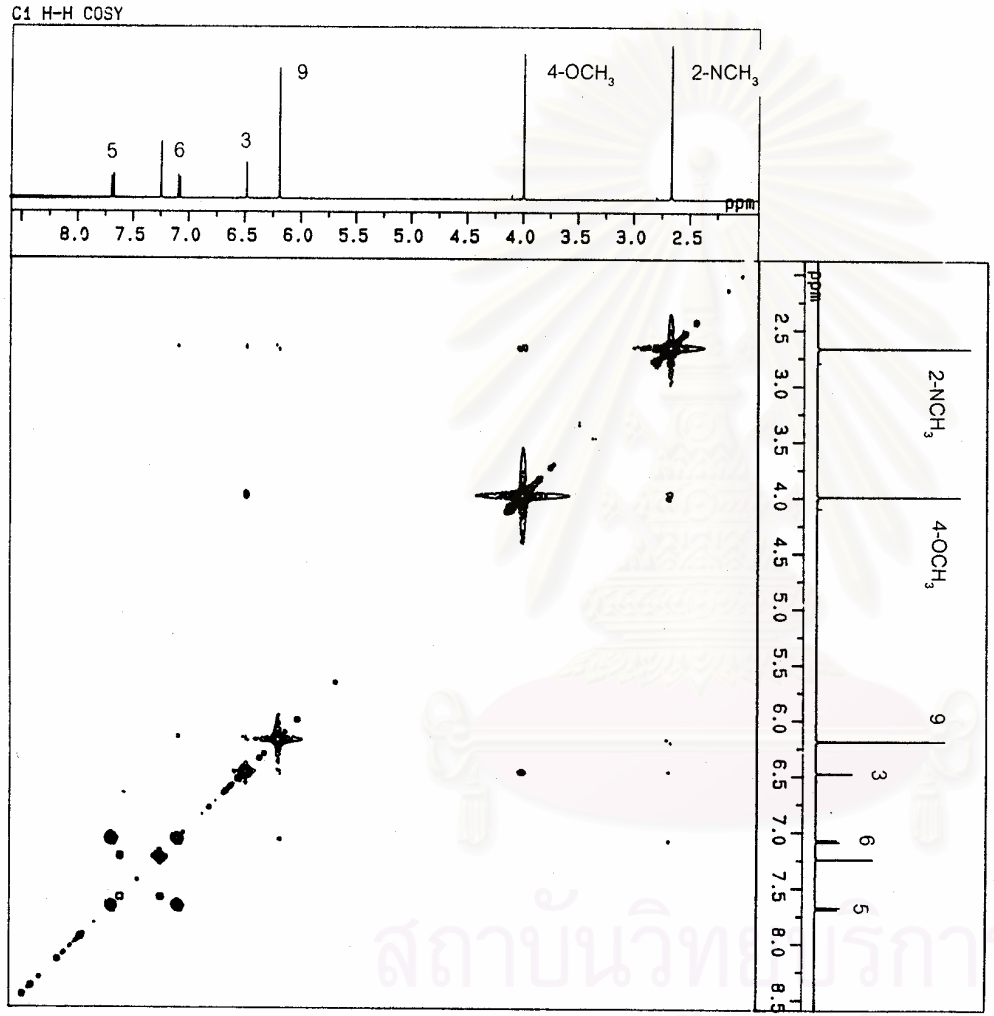


Figure 11b. The 500 MHz ^{1}H-NMR spectrum of compound C1. (in $CDCl_3$) (expanded).



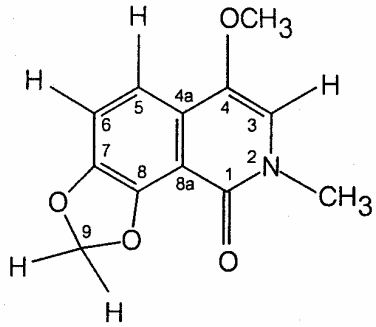
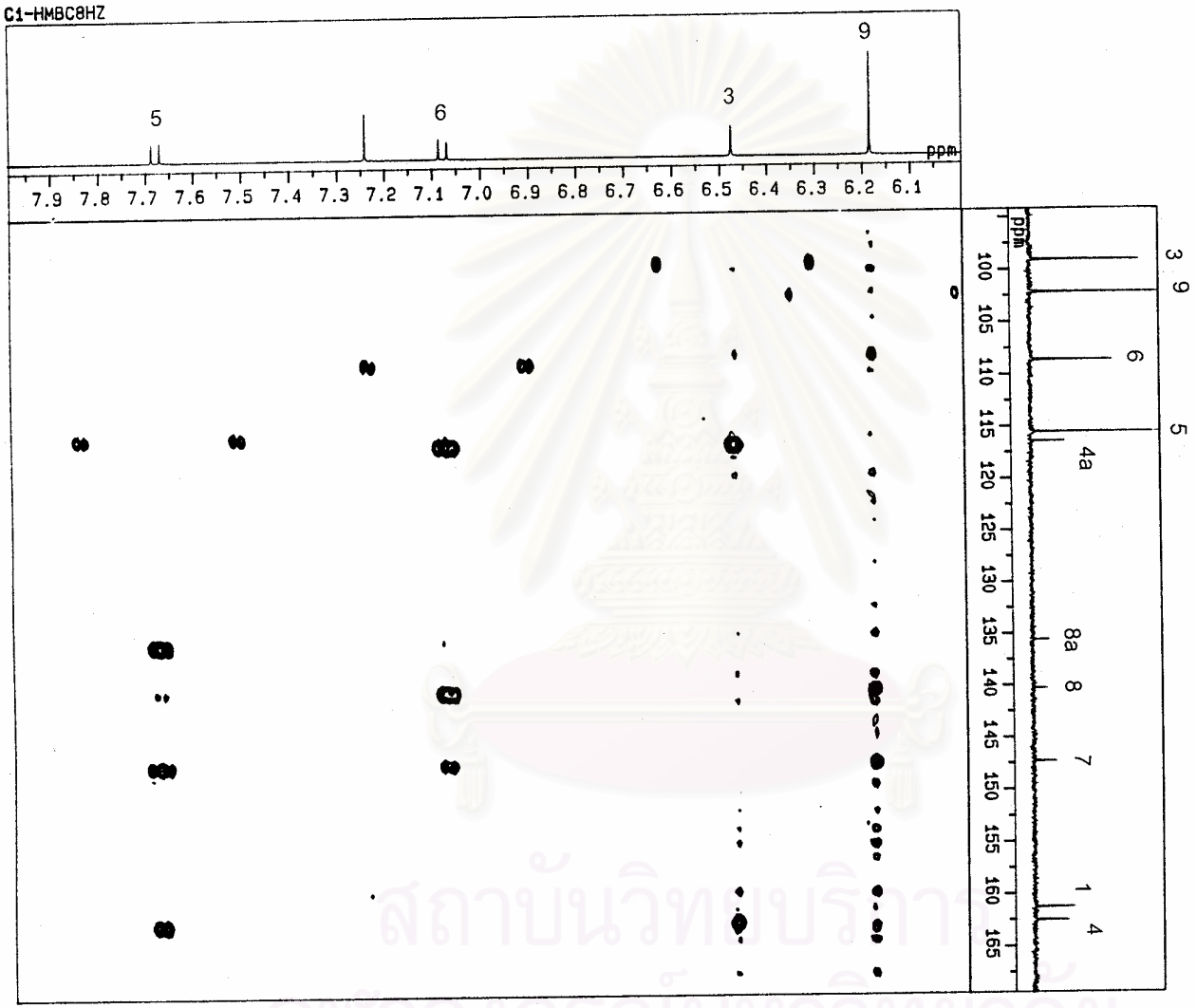


Figure 12. The 500 MHz ^{1}H-^{1}H COSY spectrum of compound C1. (in $CDCl_3$)



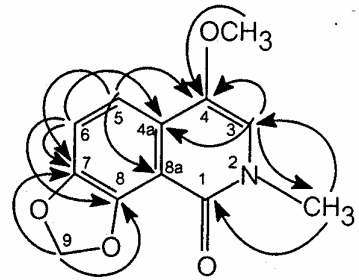
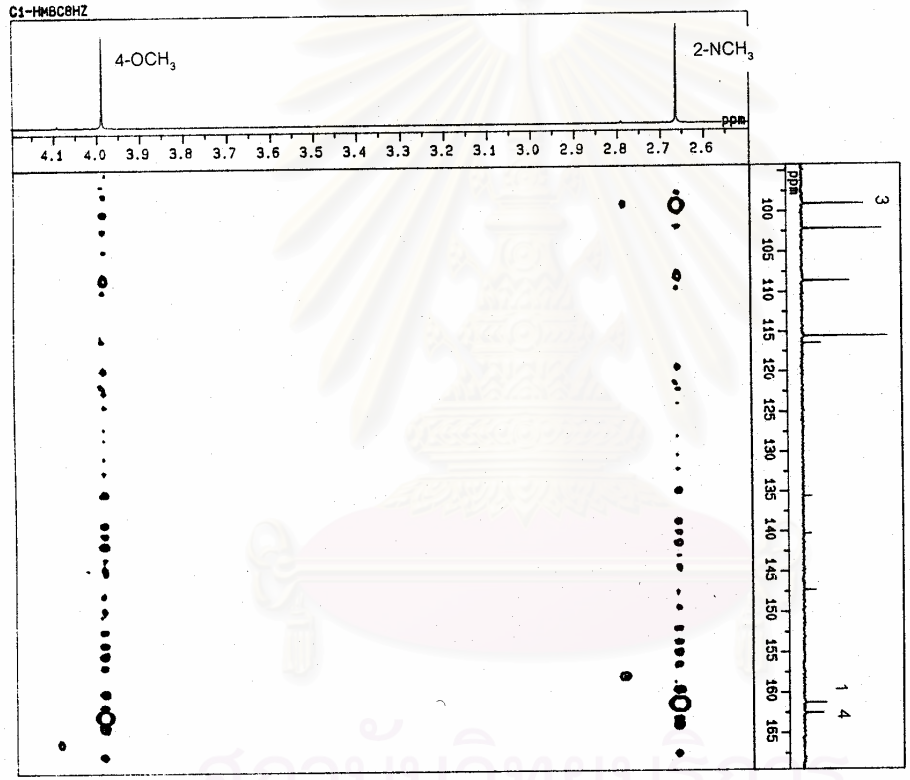


Figure 13a. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound C1. (in $CDCl_3$)



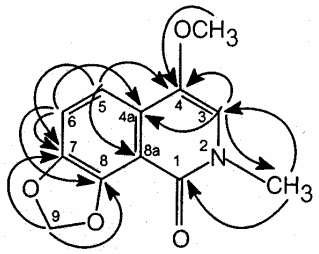
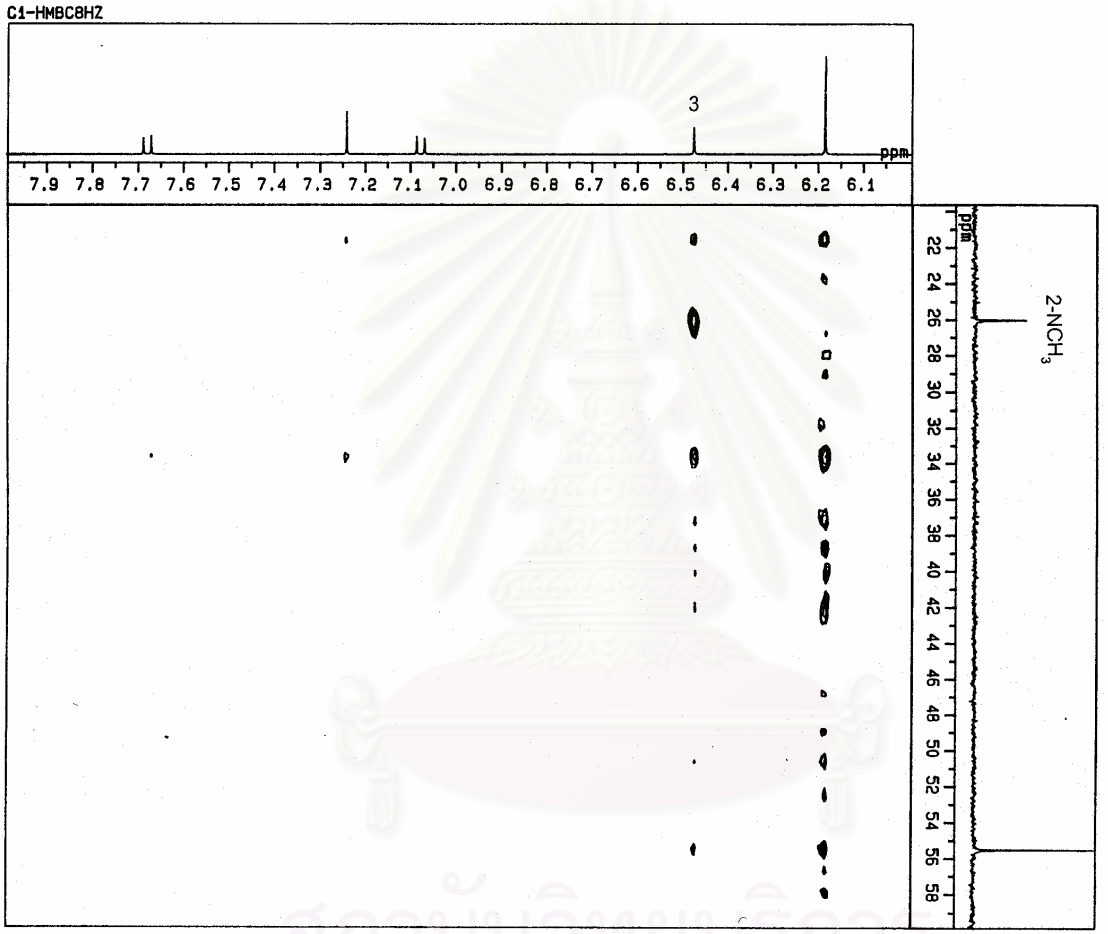


Figure 13b. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound C1. (in $CDCl_3$)



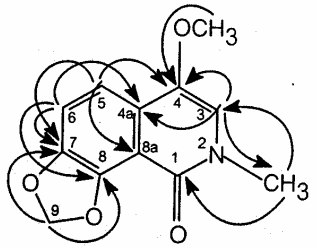
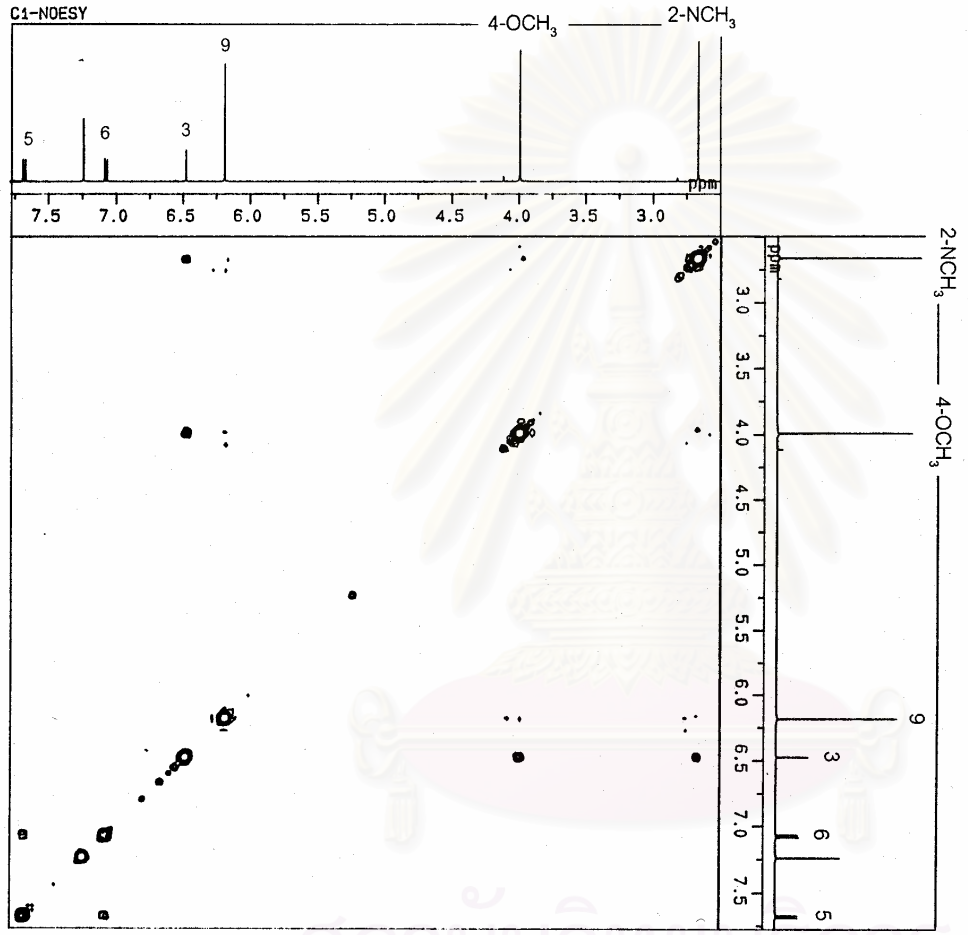


Figure 13c. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound C1. (in $CDCl_3$)



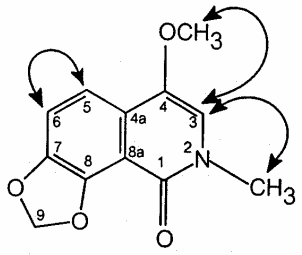
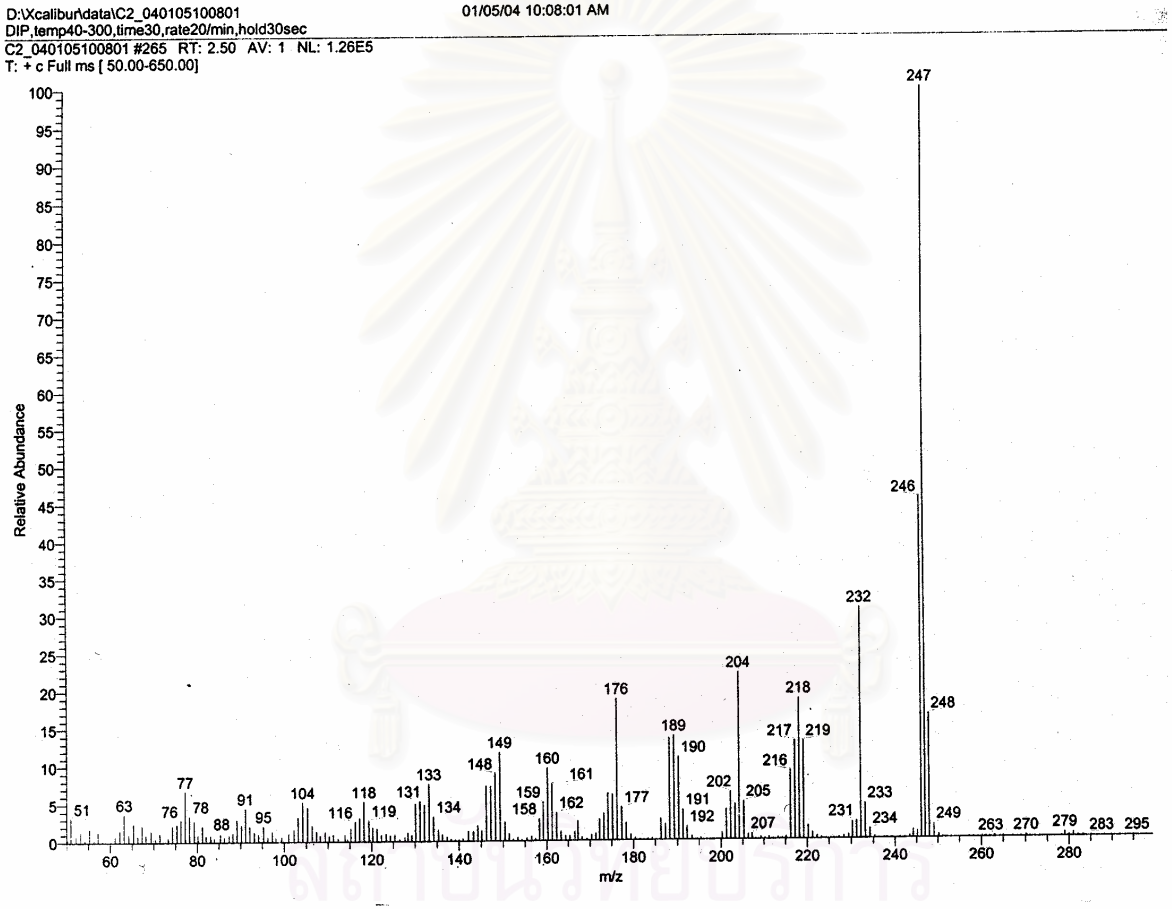


Figure 14. The 500 MHz ^{1}H-^{1}H NOESY spectrum of compound C1. (in $CDCl_3$)



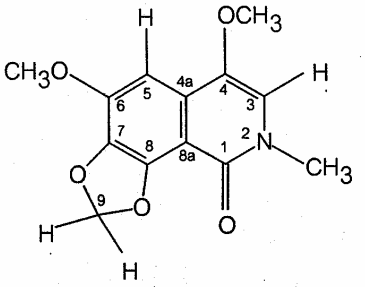
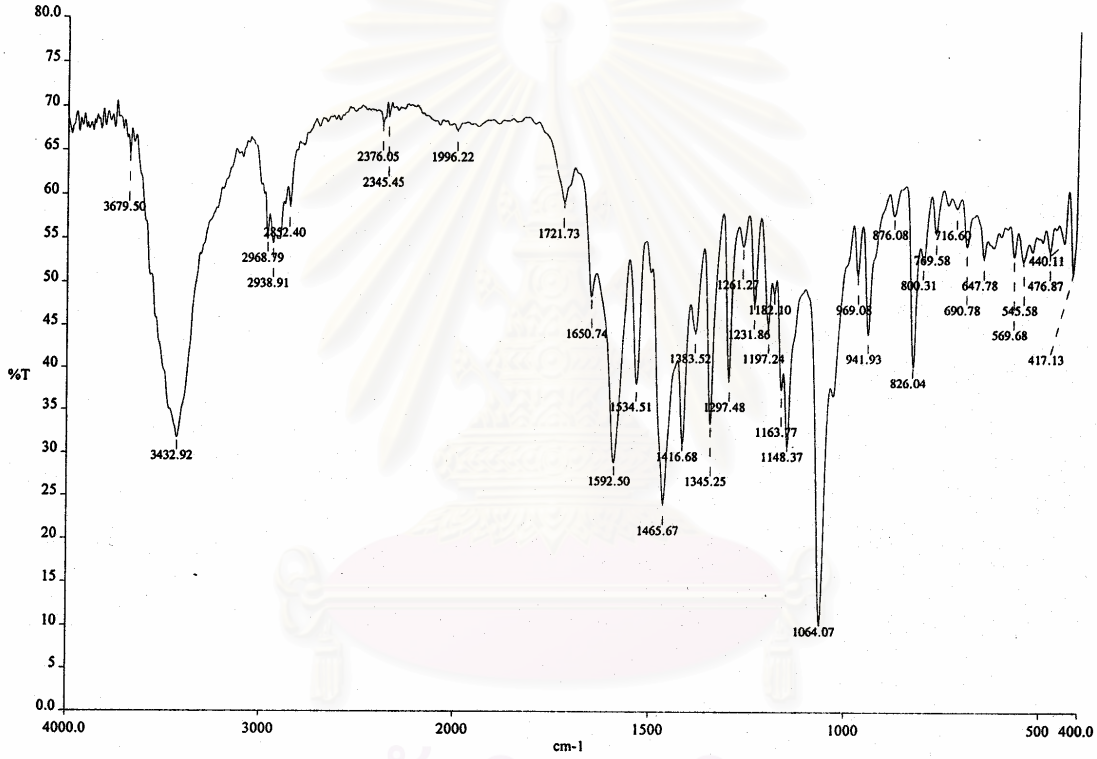


Figure 15. EIMS spectrum of compound C2.



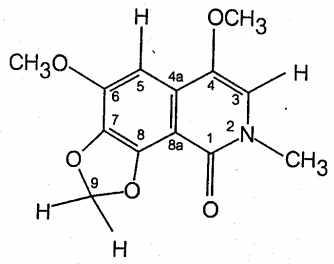


Figure 16. IR spectrum of compound C2.

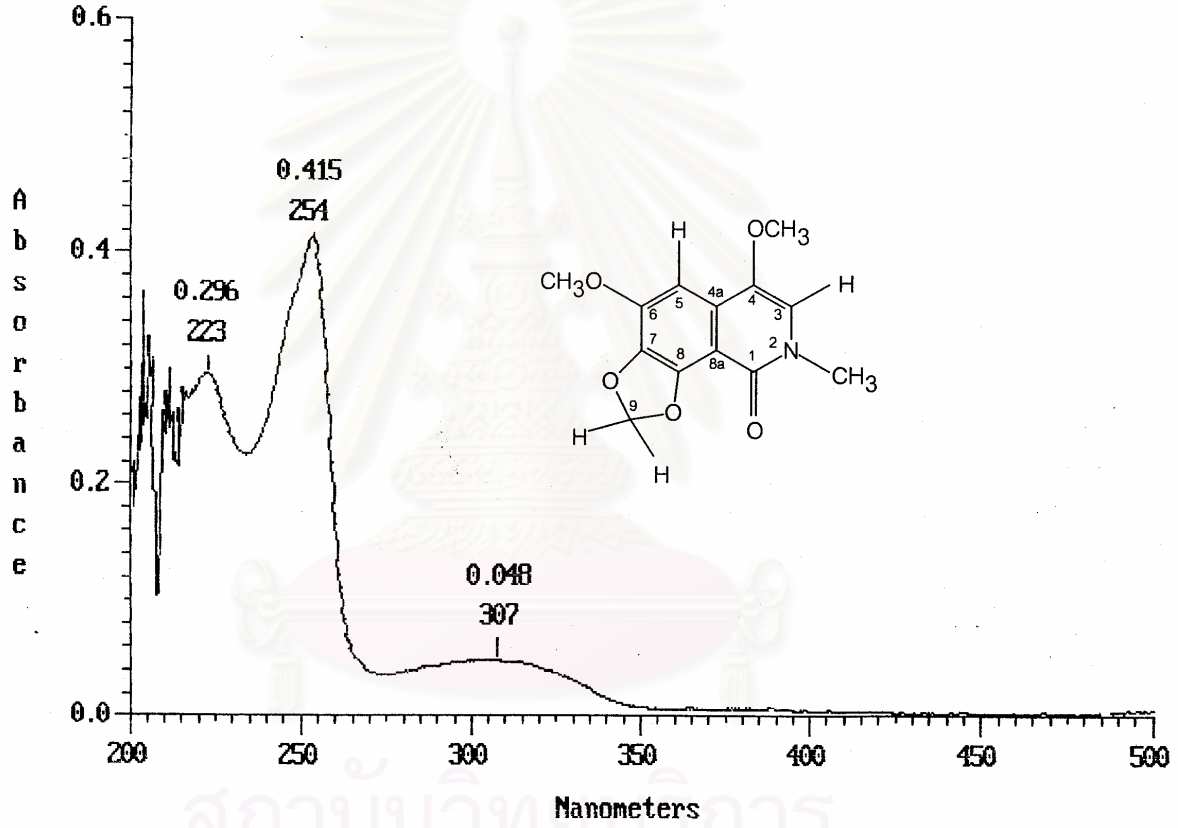
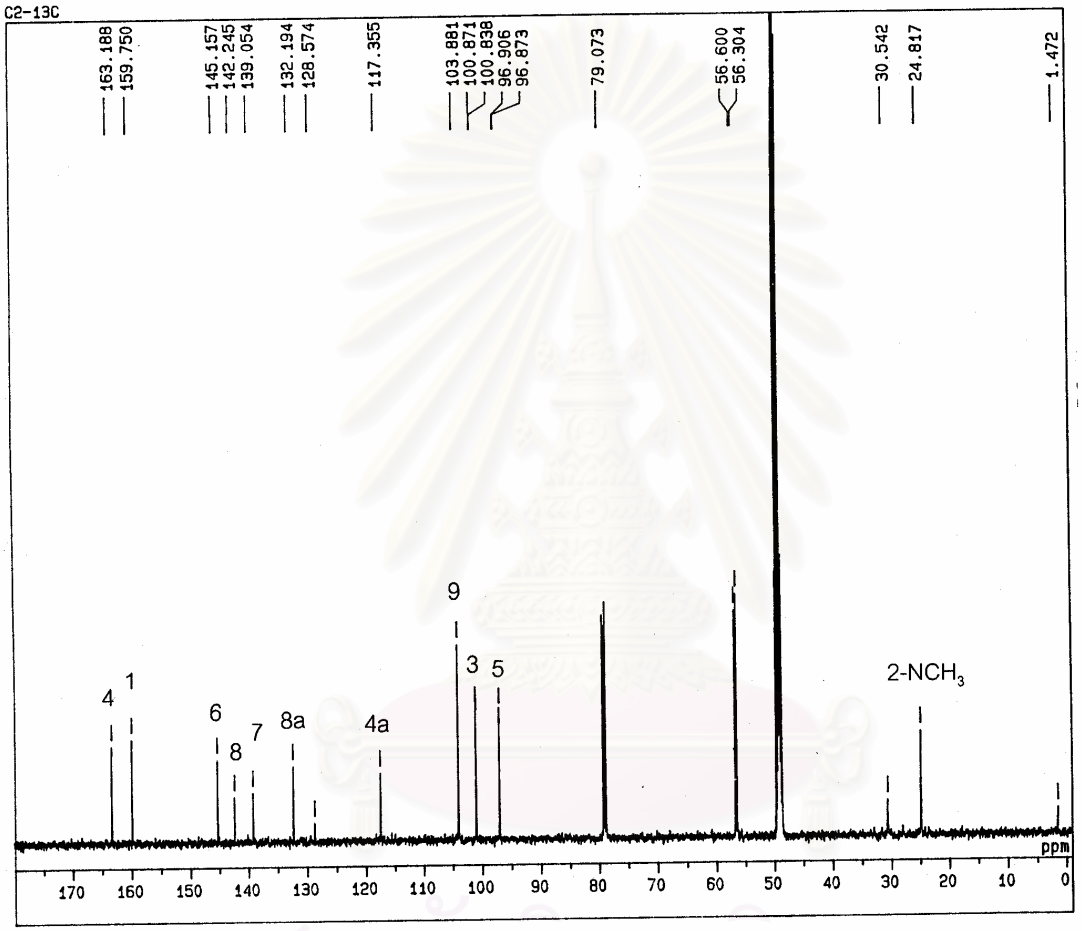


Figure 17. UV spectrum of compound C2.



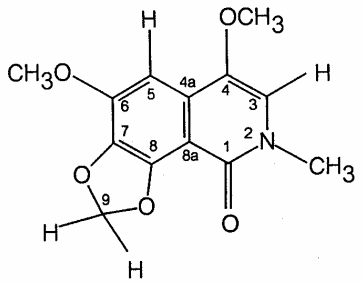


Figure 18a. The 125 MHz ^{13}C-NMR spectrum of compound C2. (in $CDCl_3/CD_3OD$)

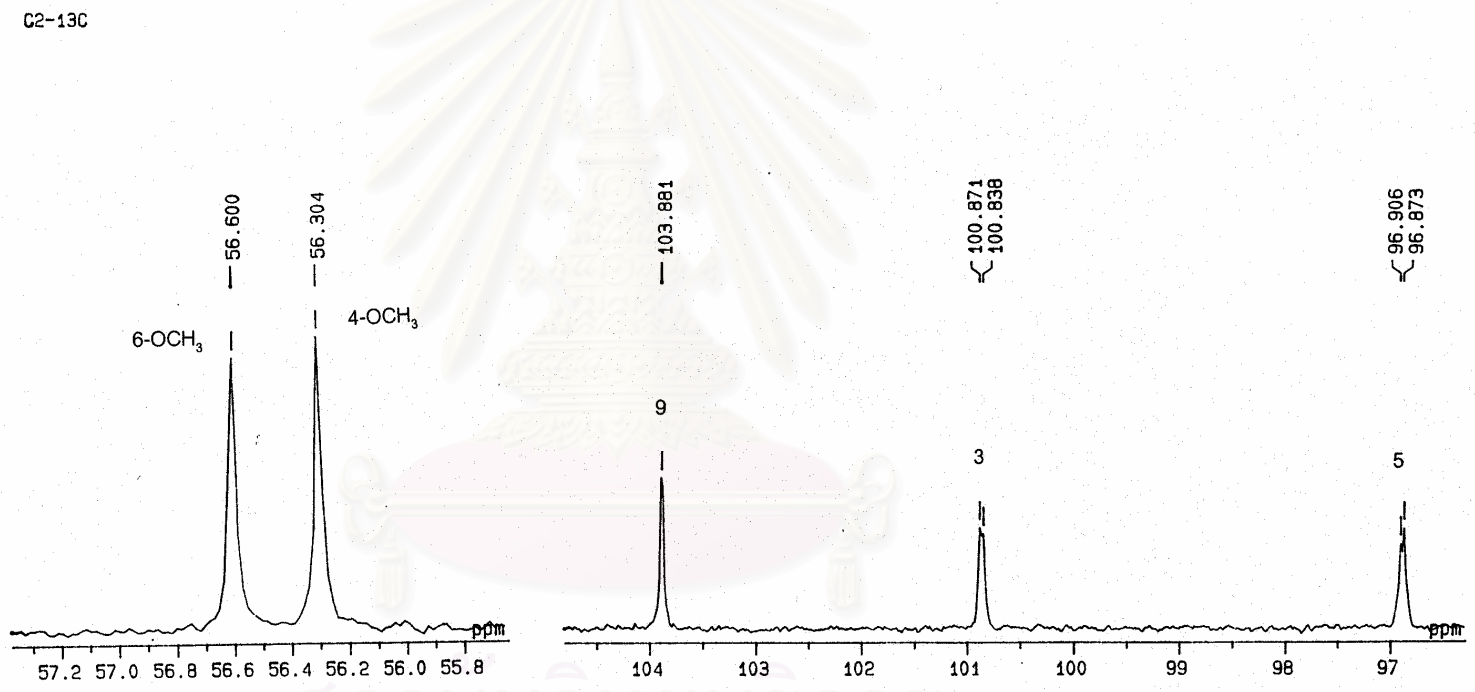
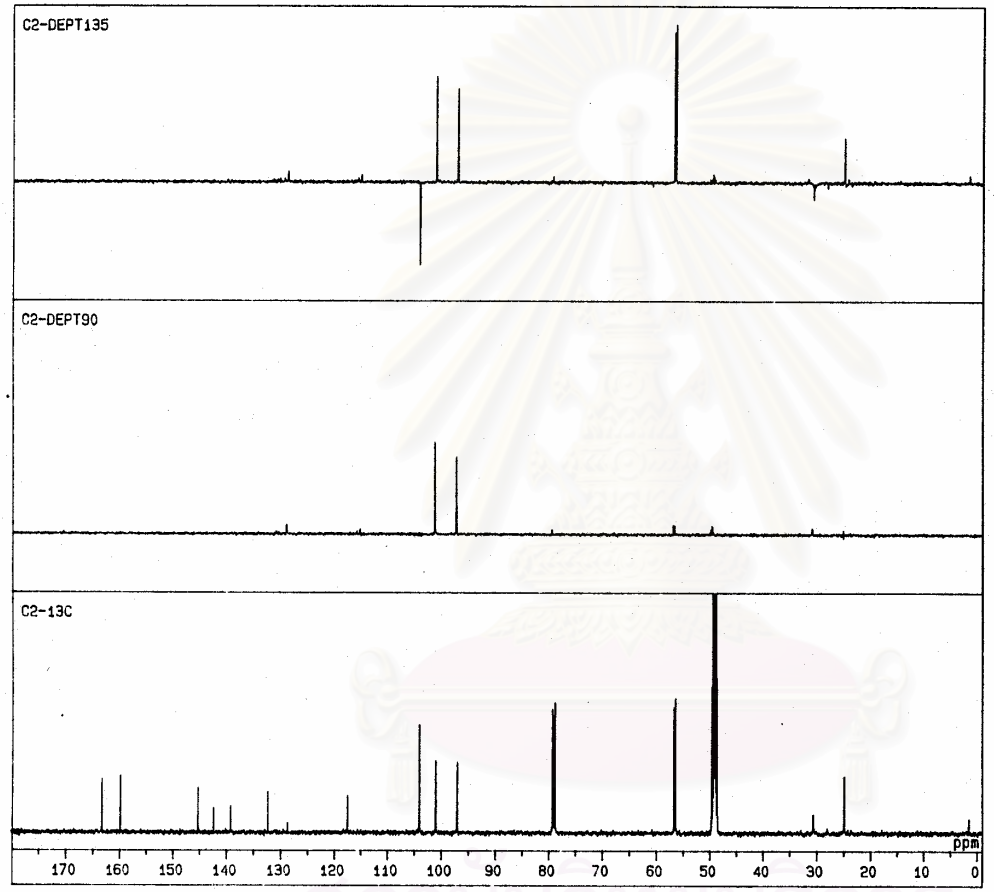


Figure 18b. The 125 MHz ^{13}C-NMR spectrum of compound C2. (in $CDCl_3/CD_3OD$) (expanded)



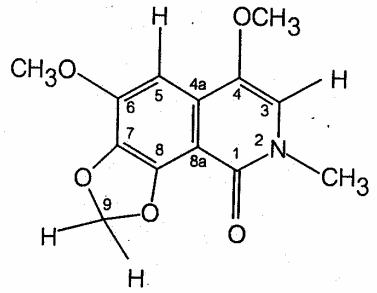
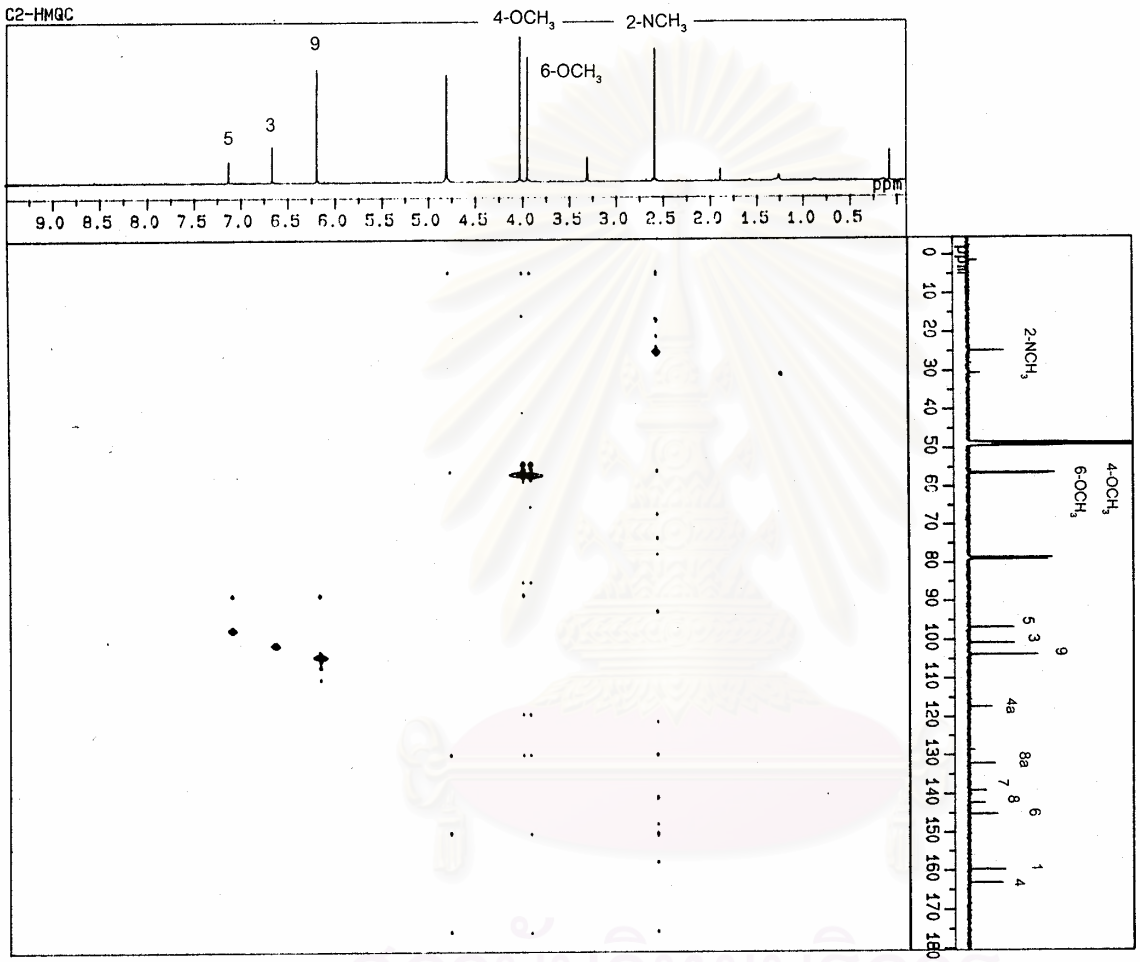


Figure 19. The 125 MHz ^{13}C-DEPT NMR spectrum of compound C2. (in $CDCl_3/CD_3OD$)



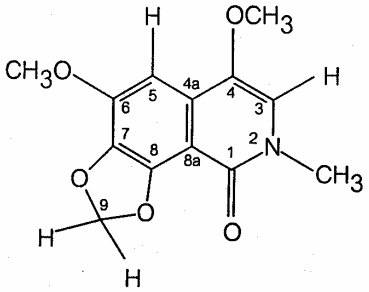
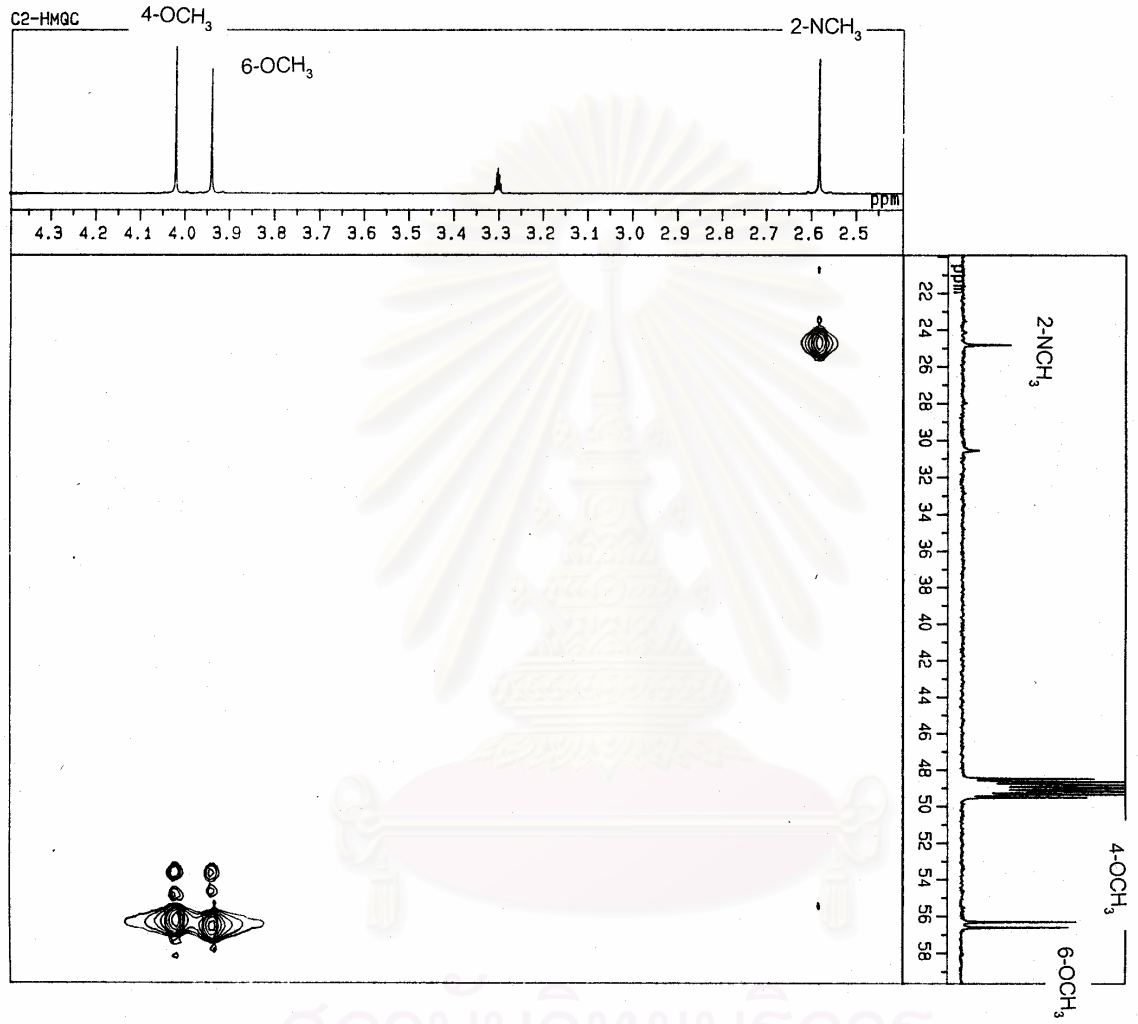


Figure 20a. The 500 MHz ^{1}H-^{13}C HMQC spectrum of compound C2. (in $CDCl_3/CD_3OD$)



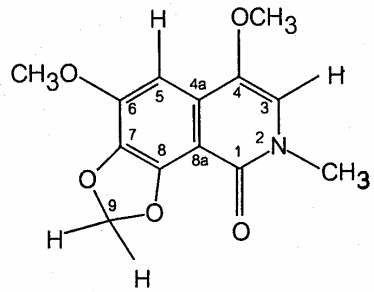


Figure 20b. The 500 MHz ^{1}H-^{13}C HMQC spectrum of compound C2. (in $CDCl_3/CD_3OD$)

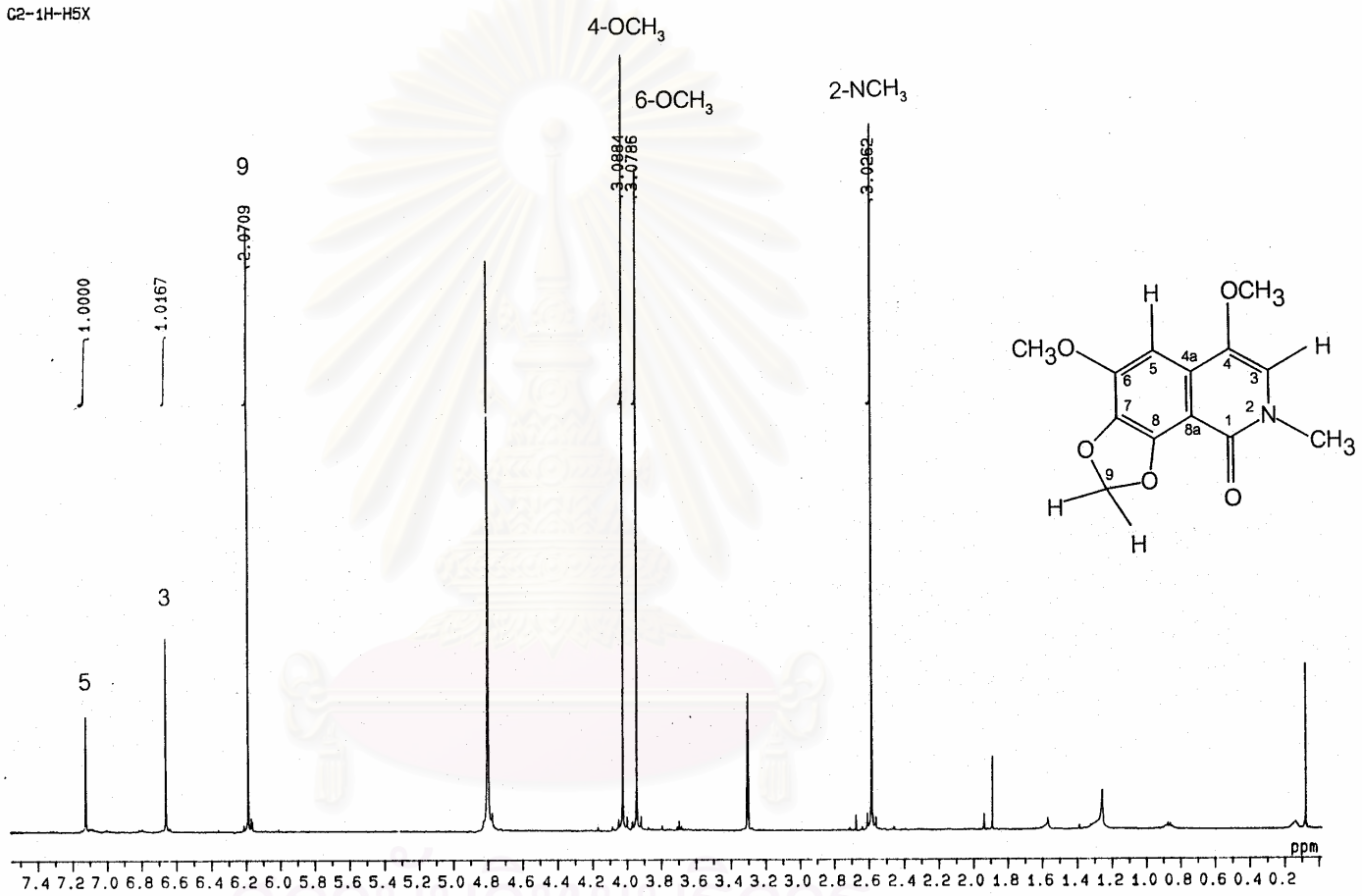
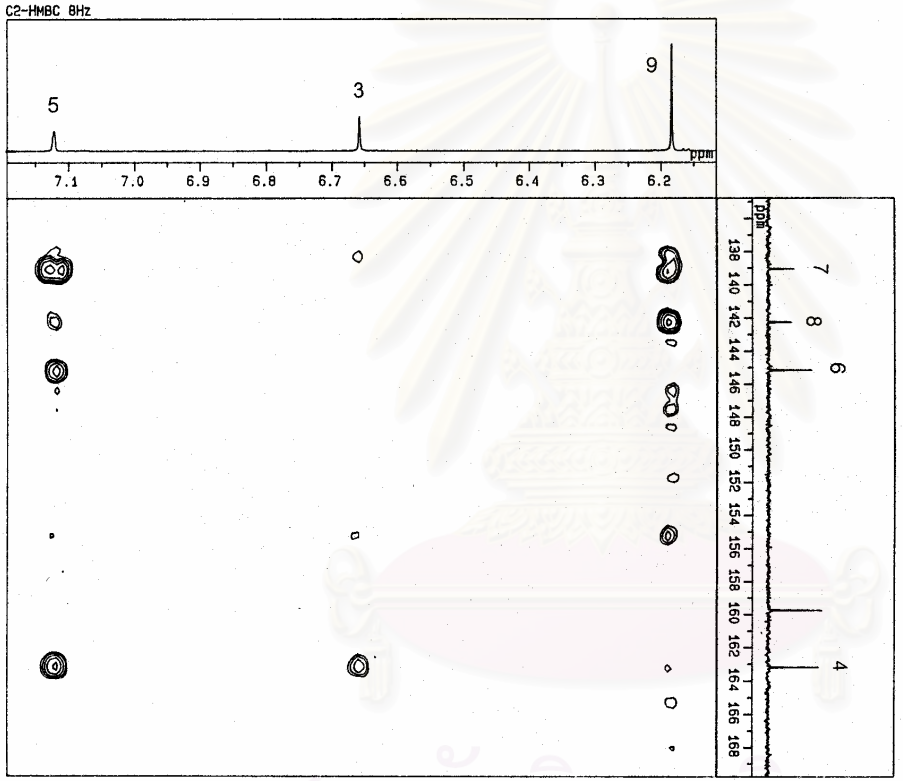


Figure 21. The 500 MHz ^{1}H-NMR spectrum of compound C2. (in $CDCl_3/CD_3OD$)



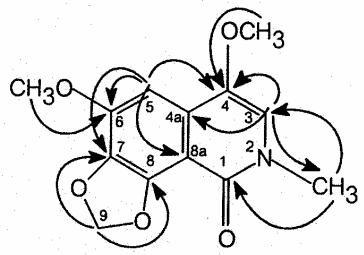


Figure 22a. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound C2. (in $CDCl_3/CD_3OD$)

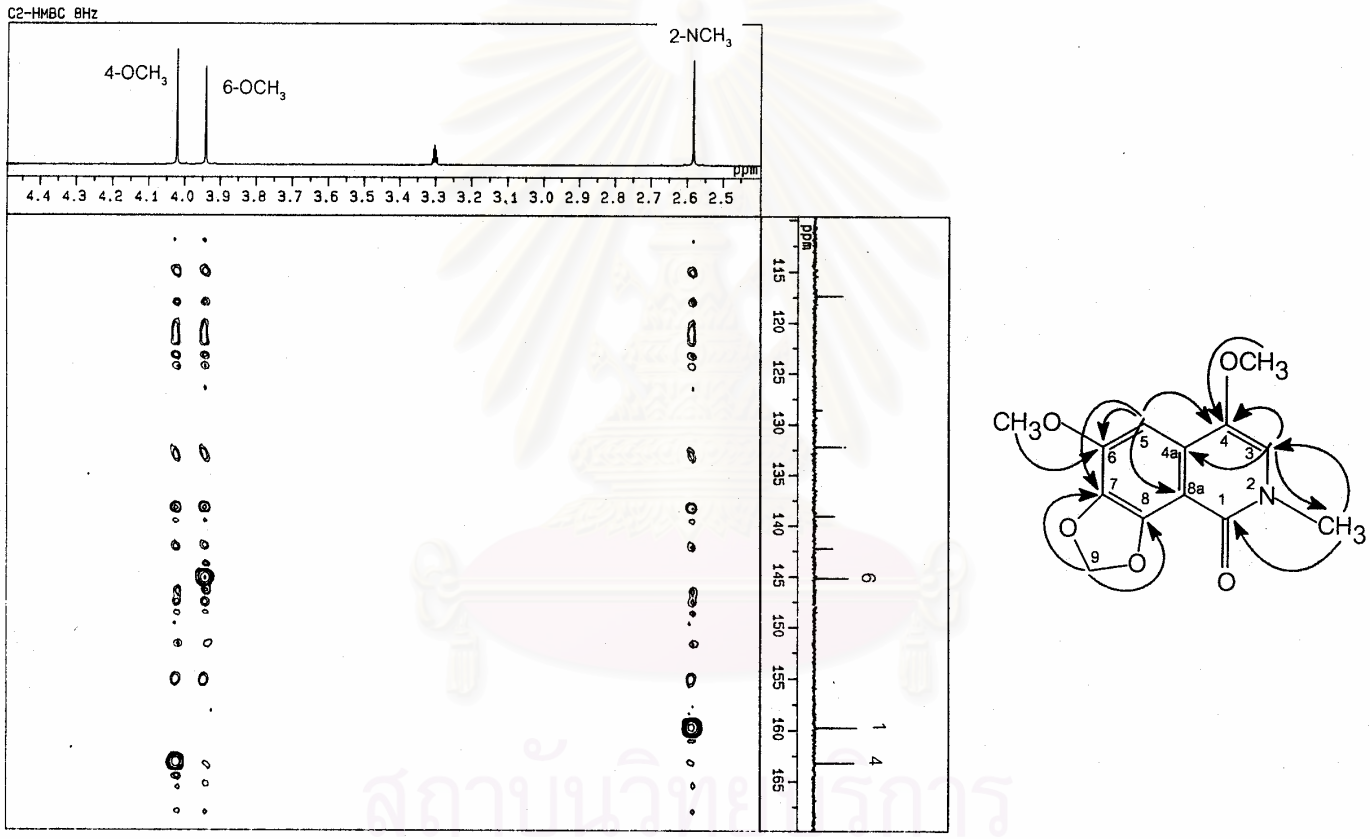
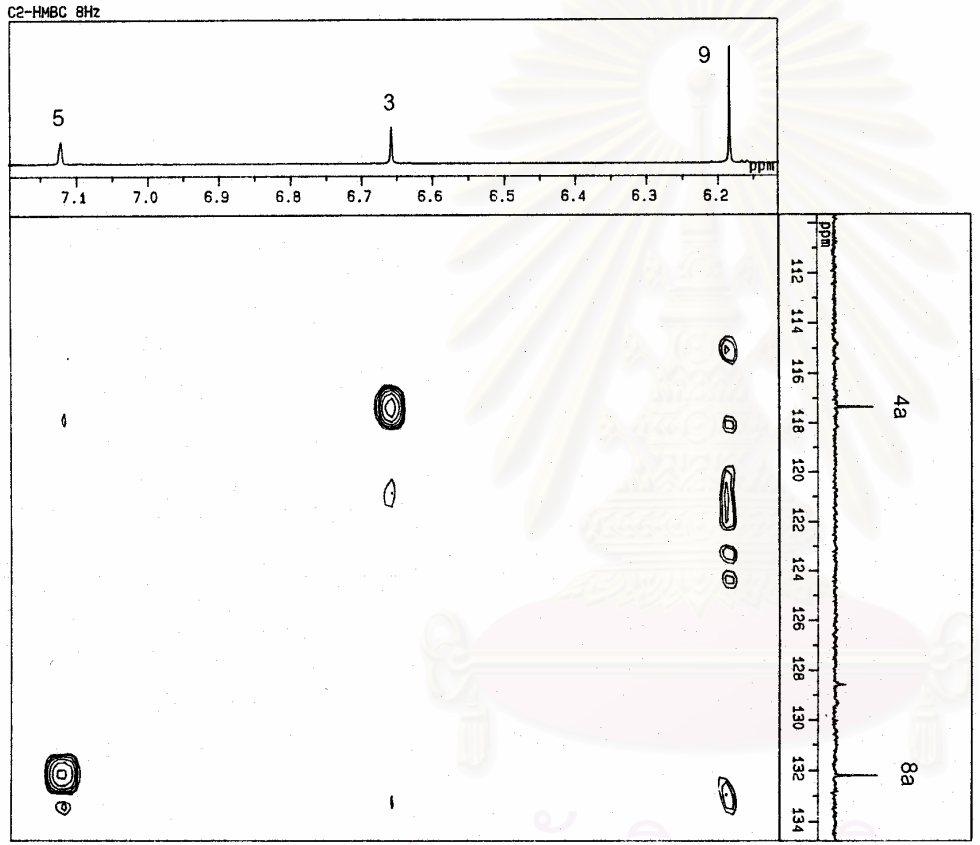


Figure 22b. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound C2. (in $CDCl_3/CD_3OD$)



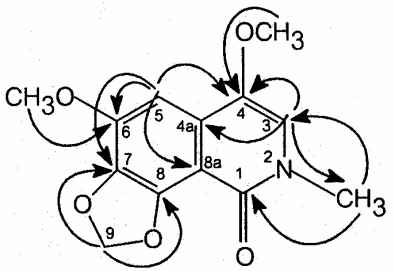
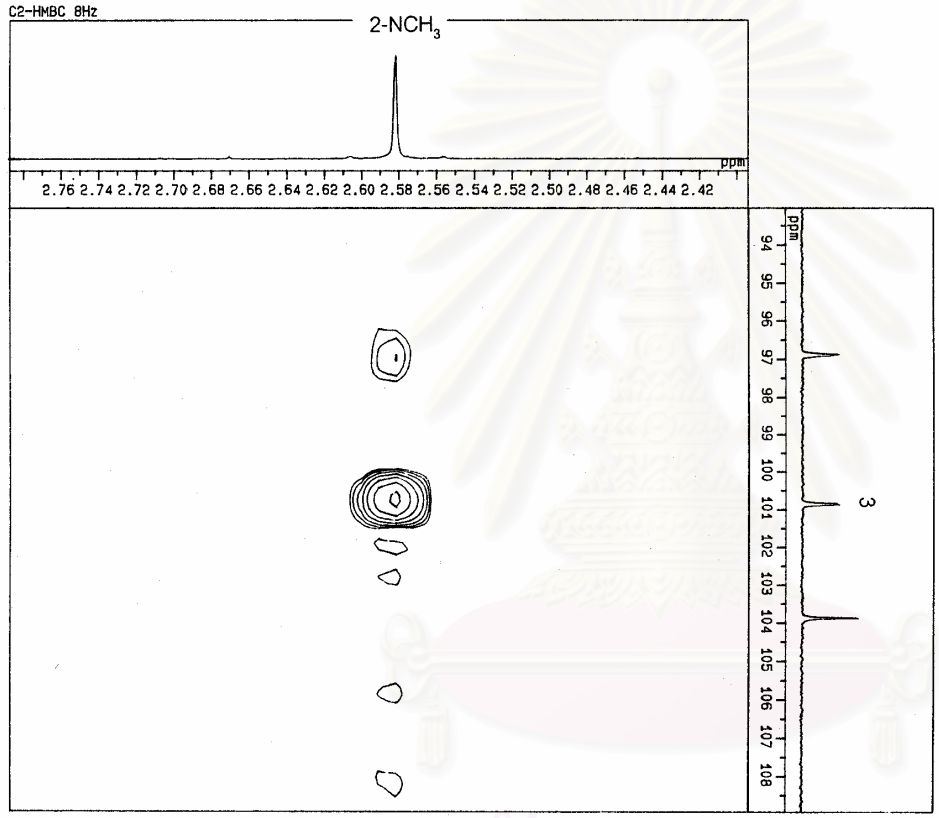


Figure 22c. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound C2. (in $CDCl_3/CD_3OD$)



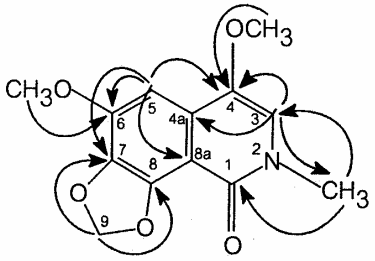
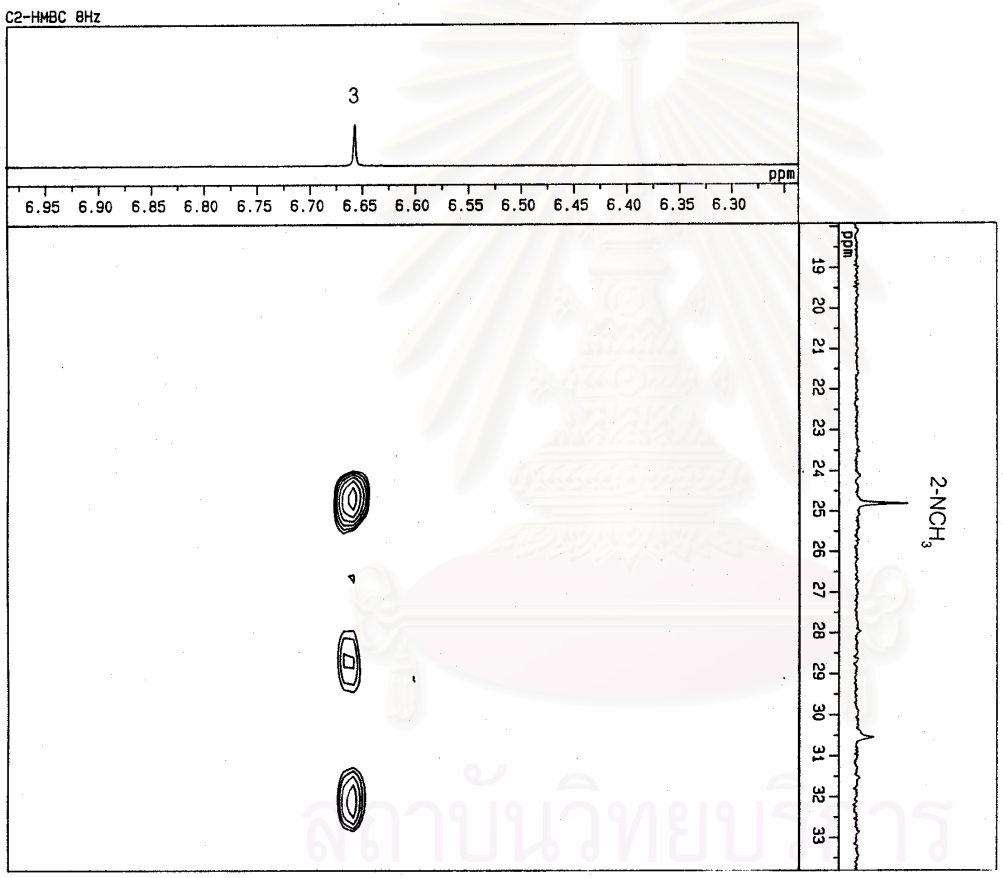


Figure 22d. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound C2. (in $CDCl_3/CD_3OD$)



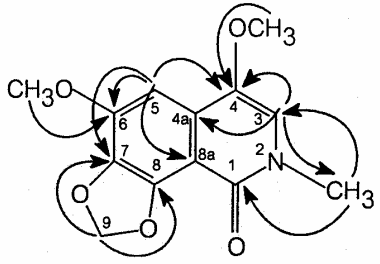


Figure 22e. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound C2. (in $CDCl_3/CD_3OD$)

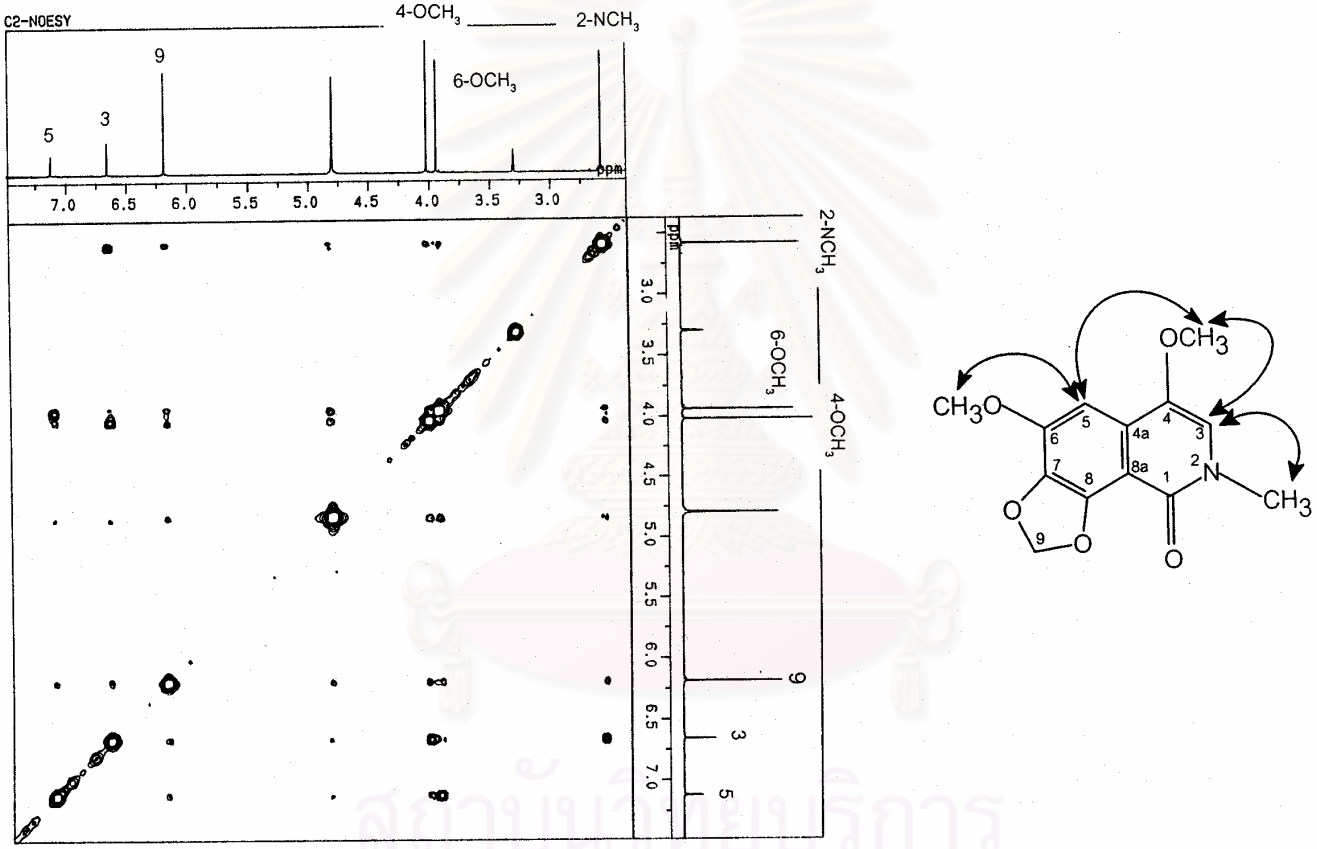


Figure 23. The 500 MHz ^{1}H-^{1}H NOESY spectrum of compound C2. (in $CDCl_3/CD_3OD$)

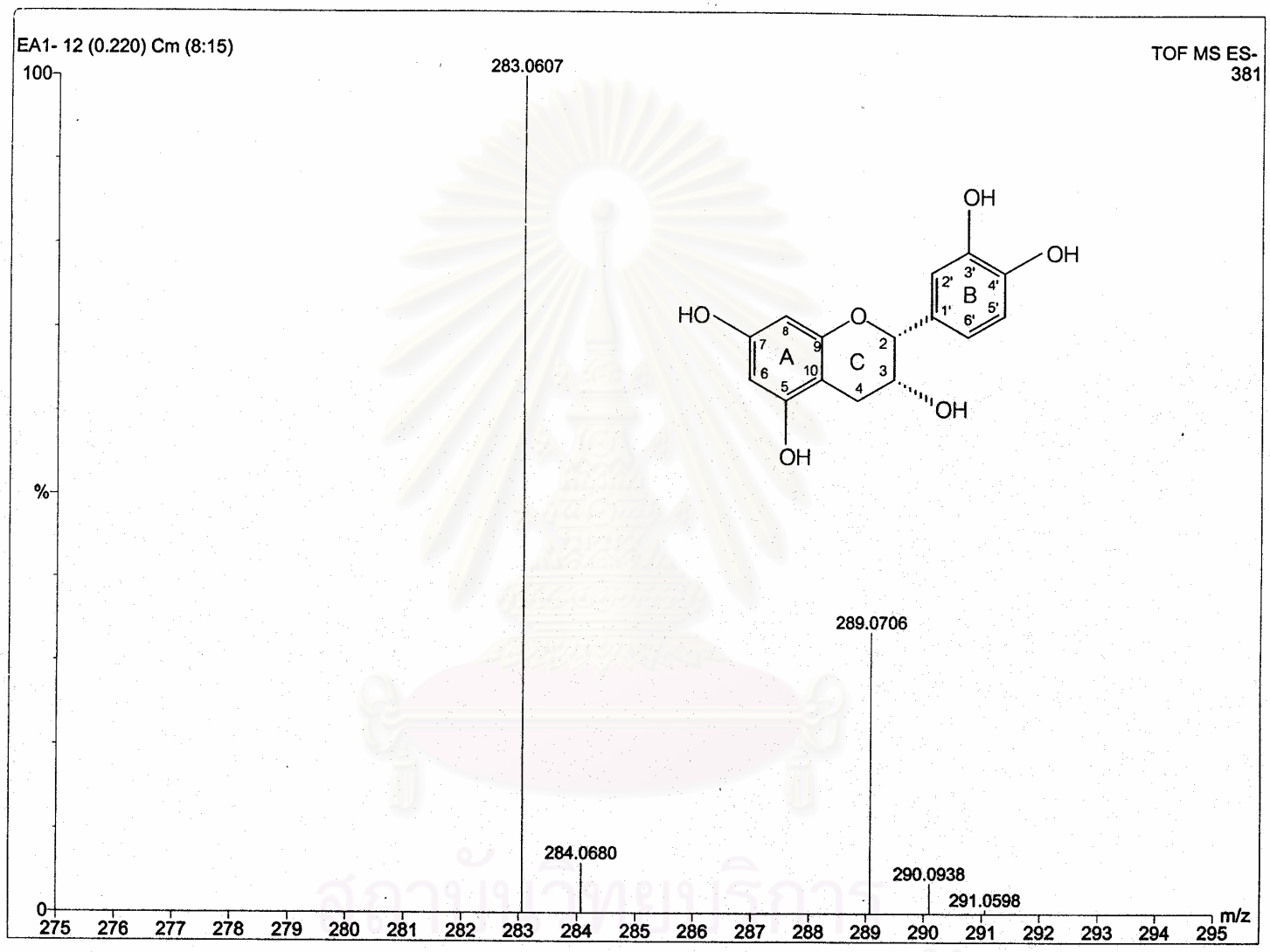
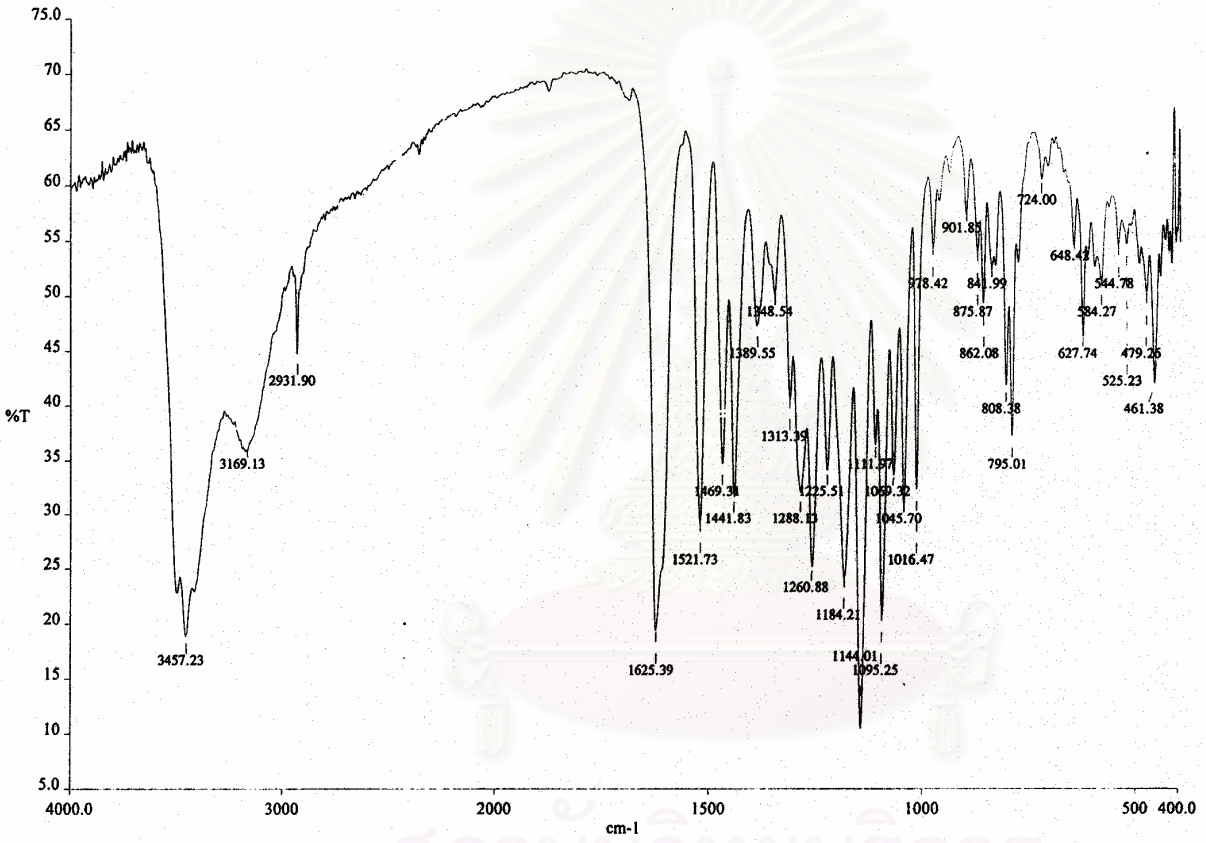


Figure 24. ESI TOFMS spectrum of compound EA1.



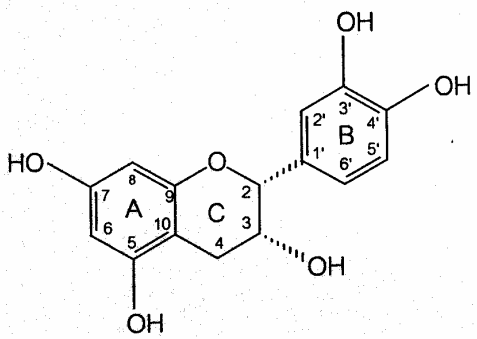
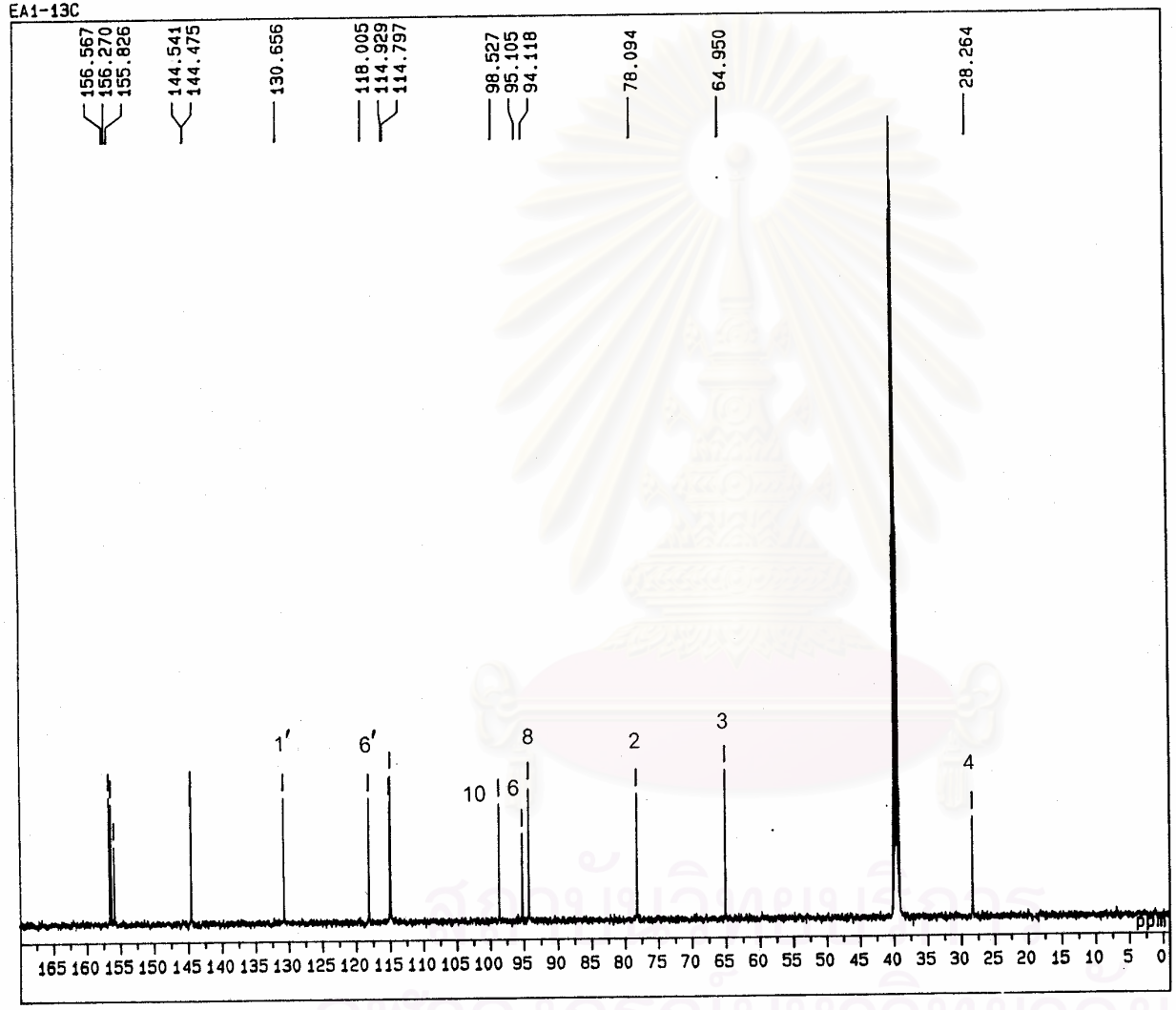


Figure25. IR spectrum of compound EA1.



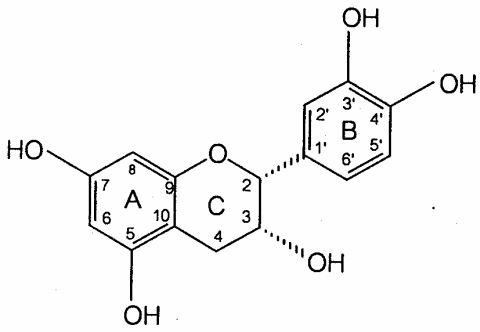


Figure26a. The 125 MHz ^{13}C-NMR spectrum of compound EA1. (in DMSO-d_6)

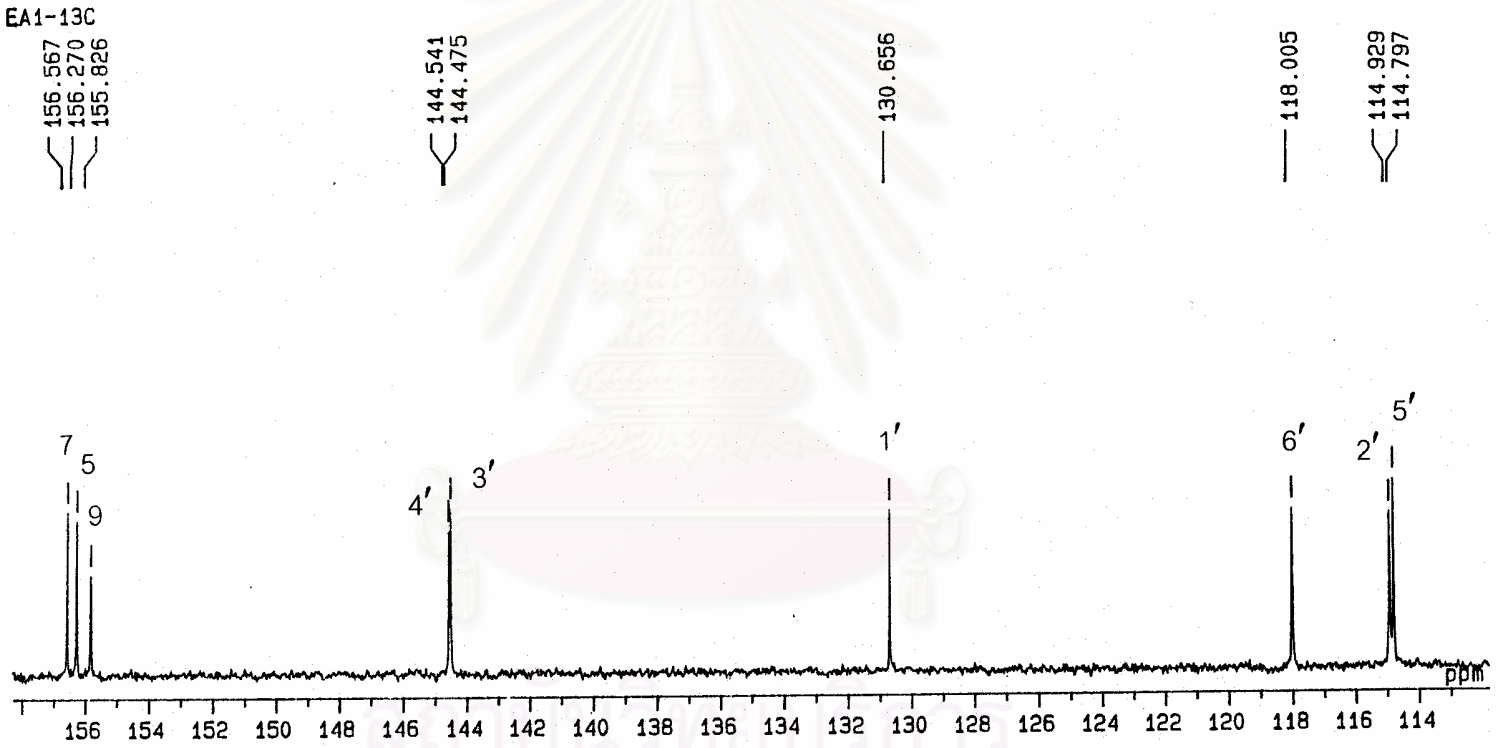
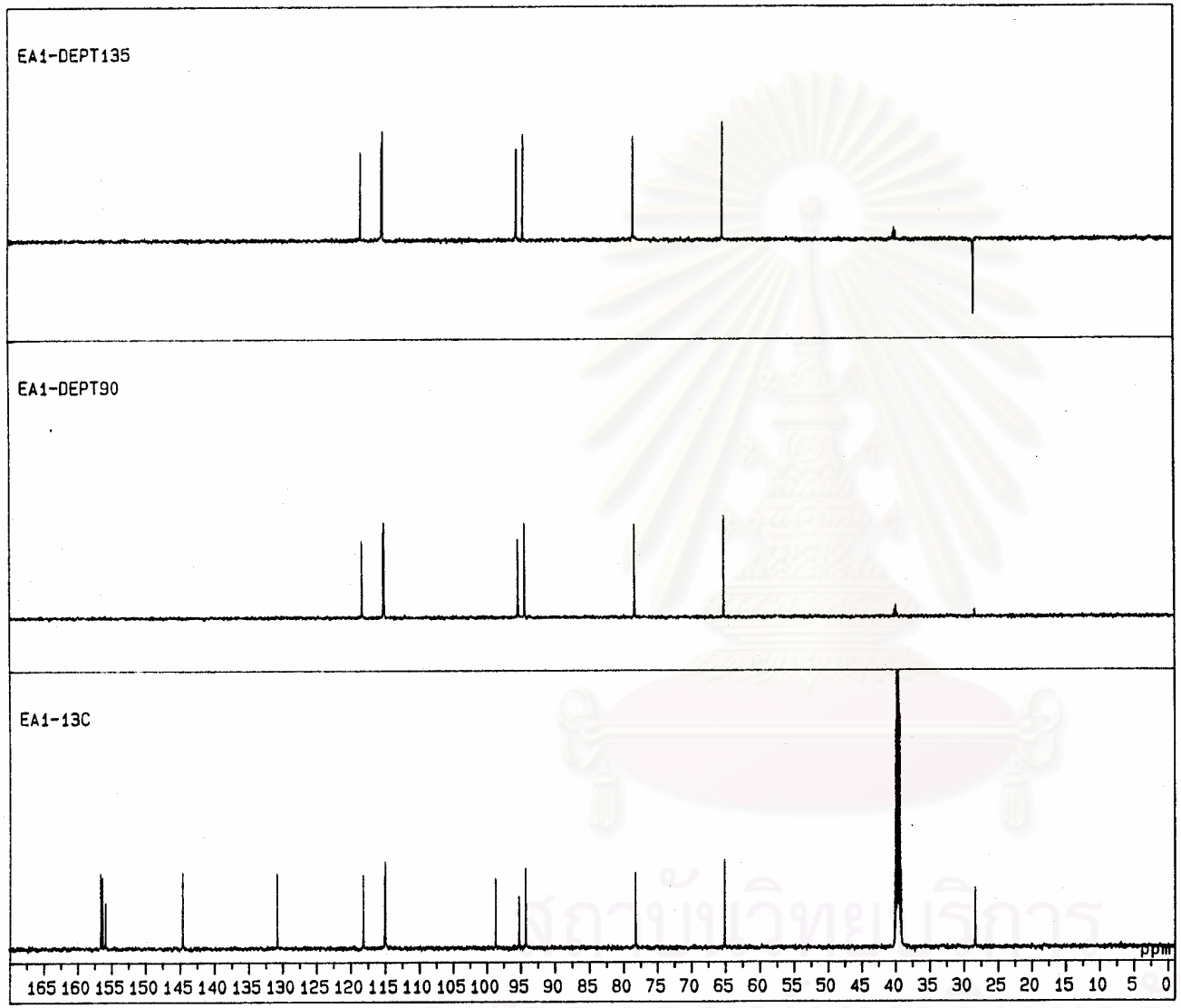


Figure 26b. The 125 MHz ^{13}C-NMR spectrum of compound EA1. (in DMSO-d_6) (expanded)



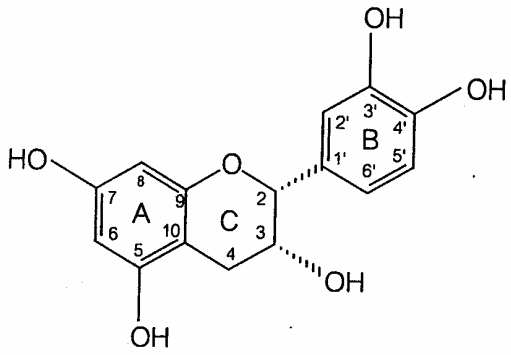
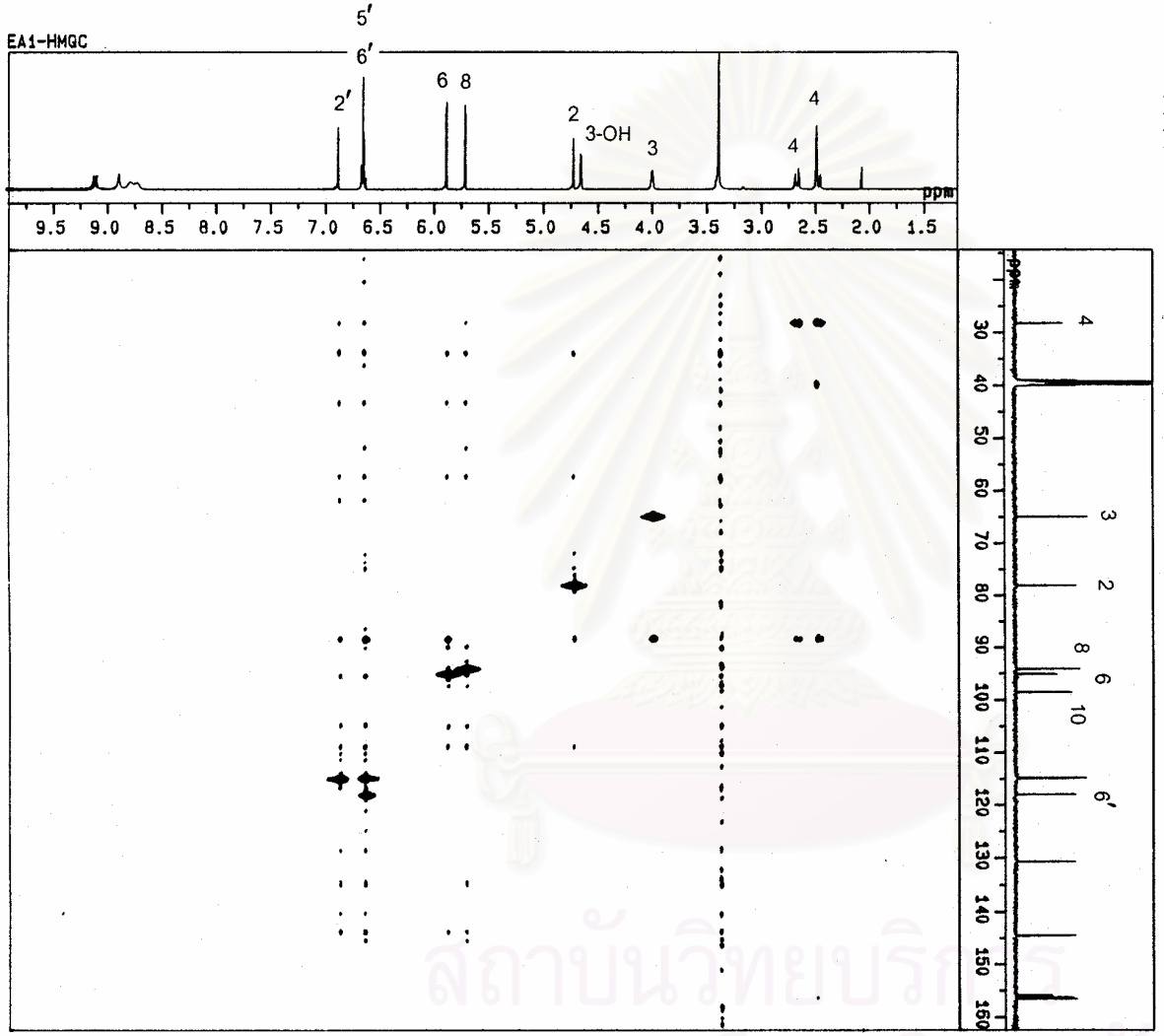


Figure 27. The 125 MHz ^{13}C-DEPT NMR spectrum of compound EA1. (in DMSO-d_6)



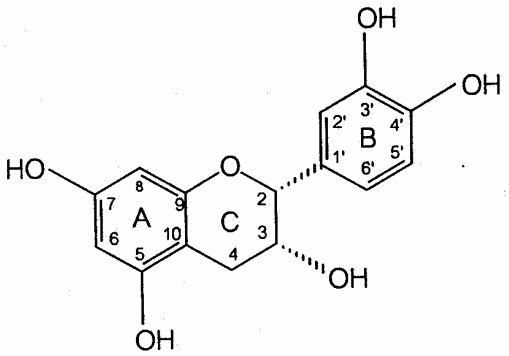
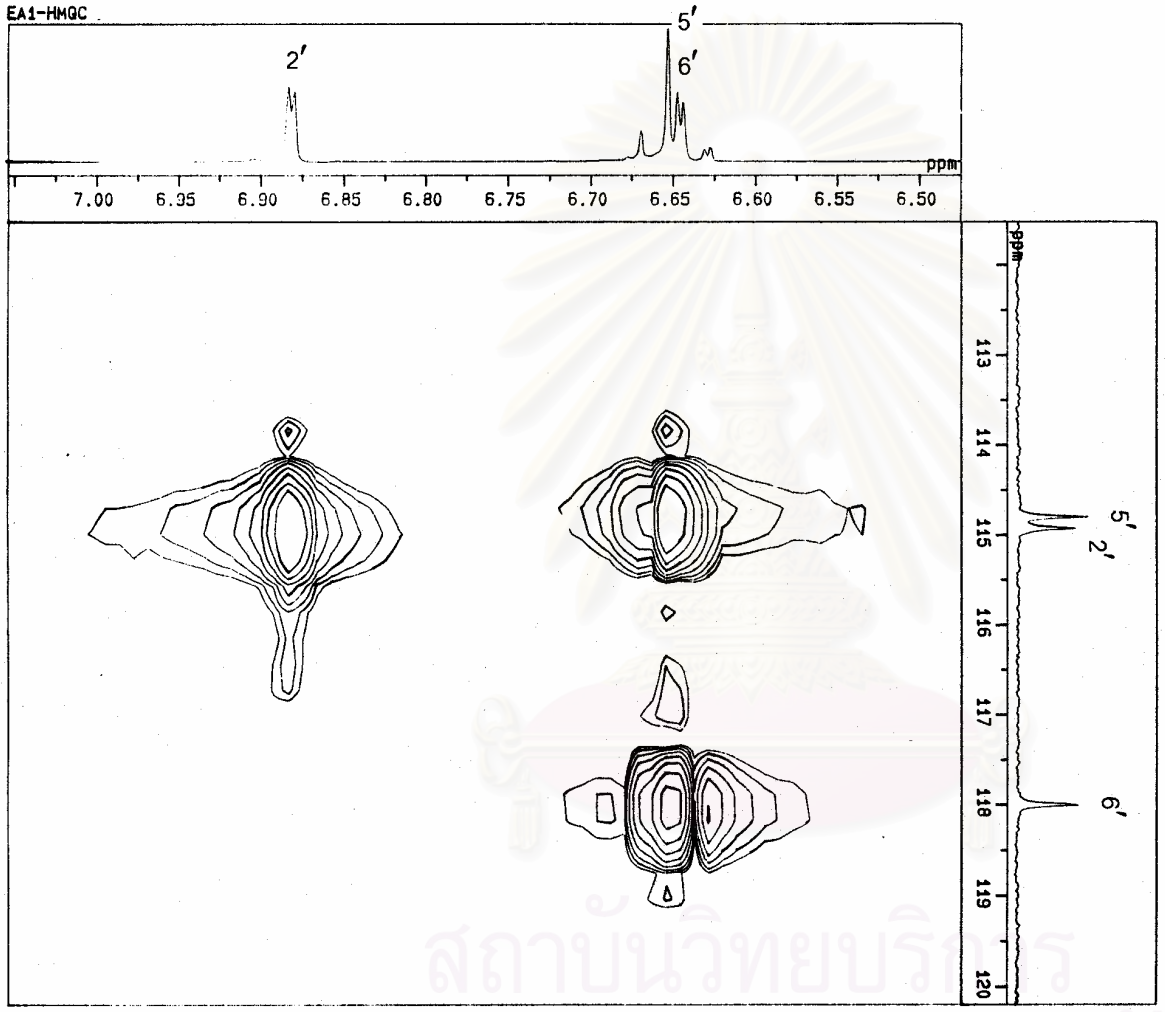


Figure 28a. The 500 MHz ^{1}H-^{13}C HMQC spectrum of compound EA1. (in DMSO-d_6)



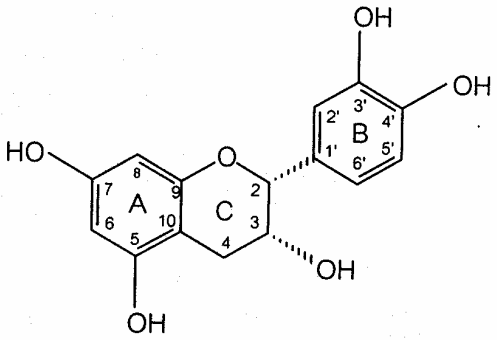
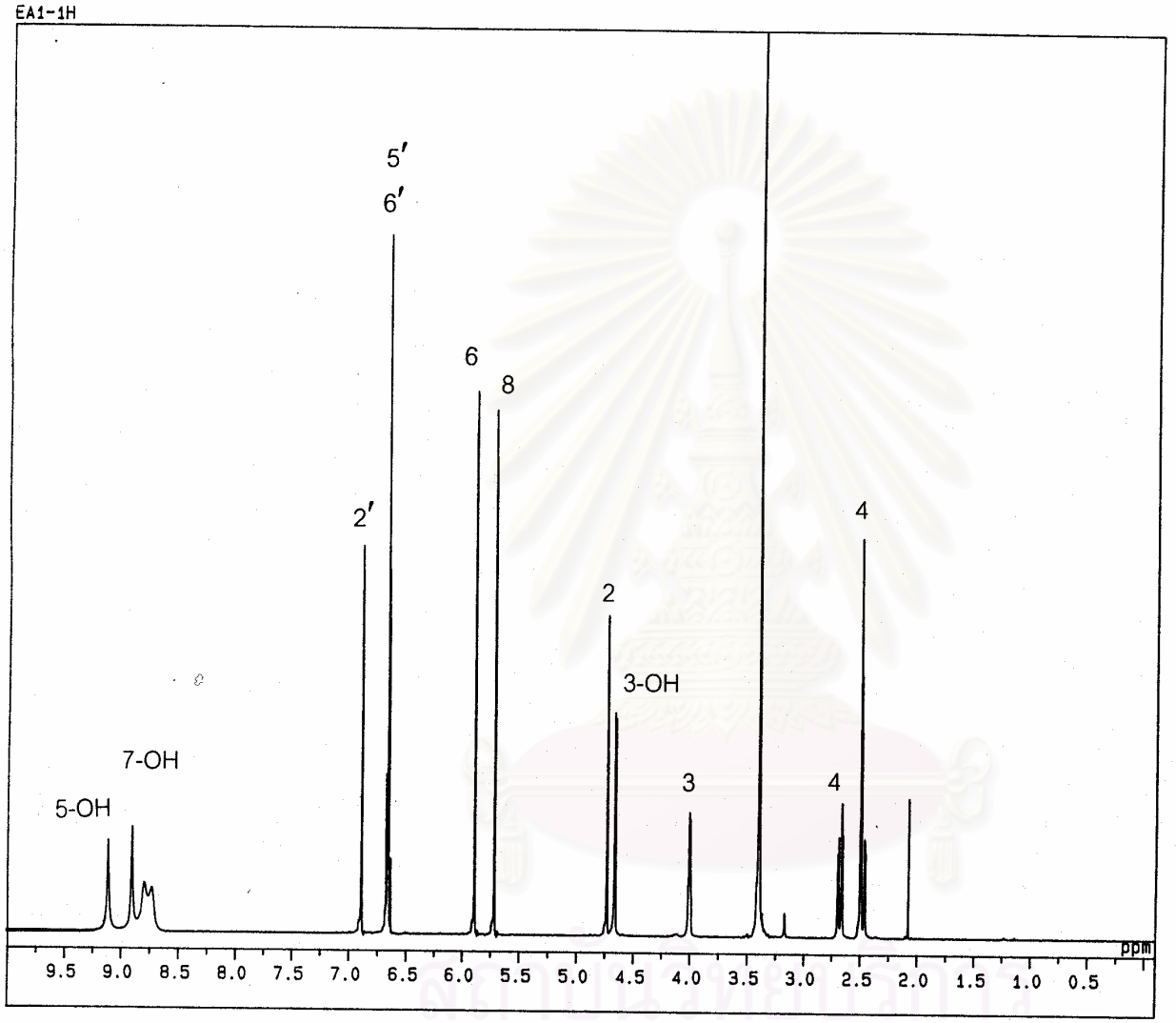


Figure 28b. The 500 MHz ^{1}H-^{13}C HMQC spectrum of compound EA1. (in DMSO-d_6)



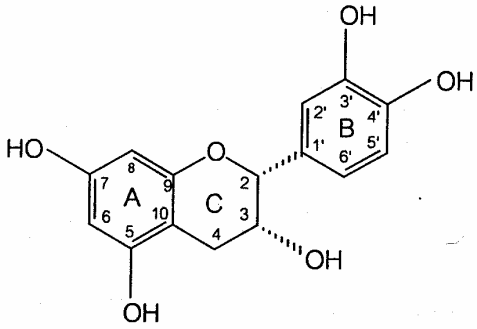
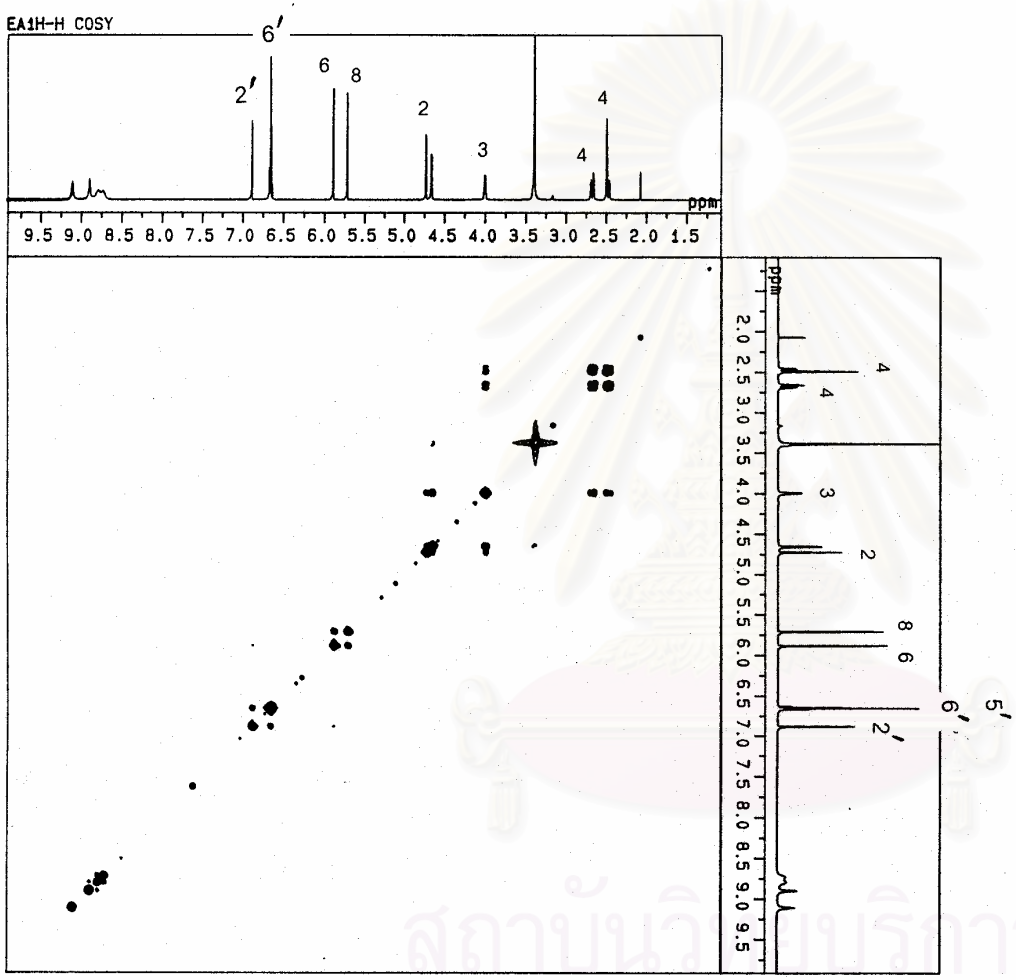


Figure 29. The 500 MHz ^{1}H-NMR spectrum of compound EA1. (in DMSO-d_6)



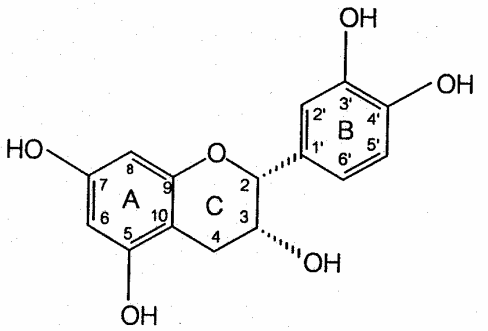
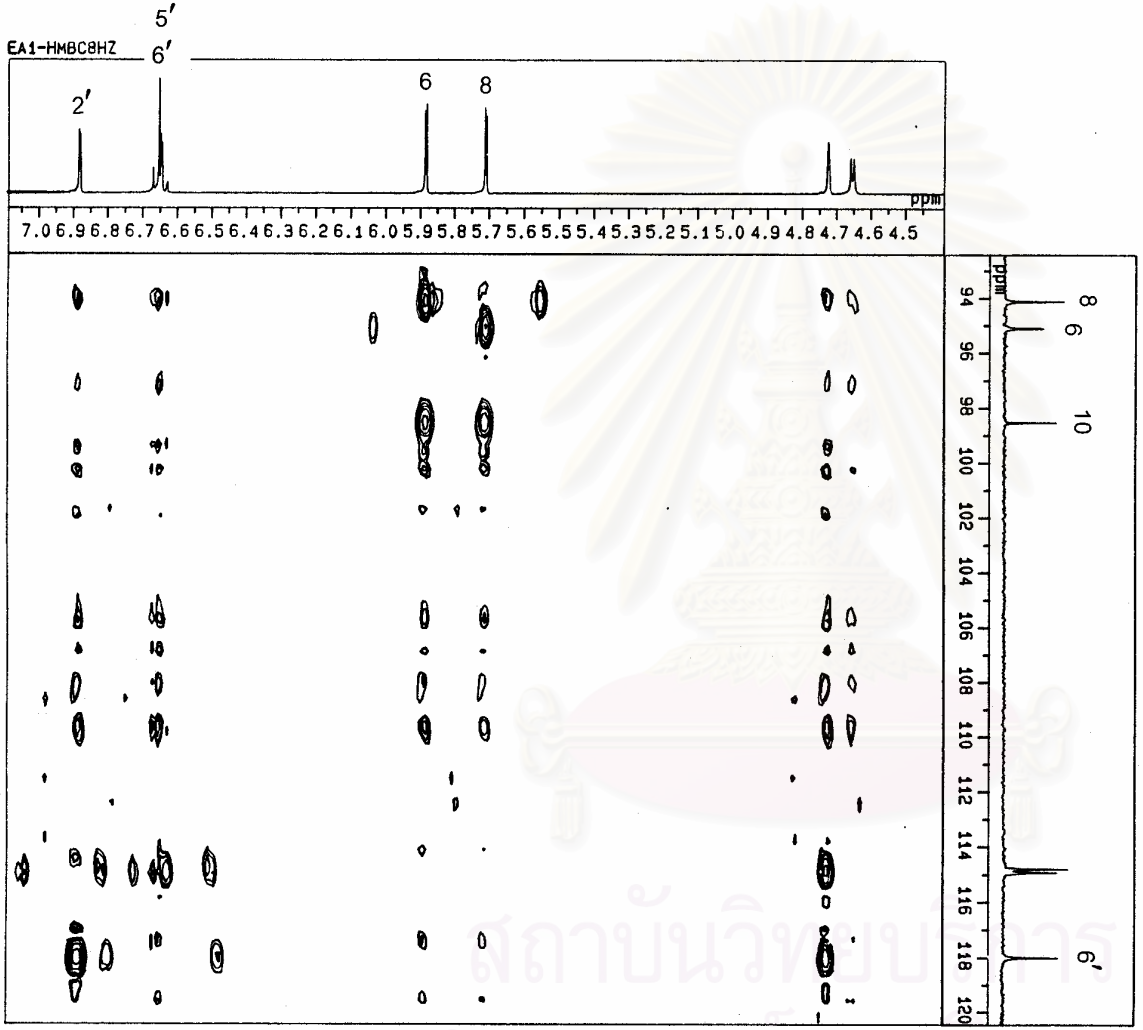


Figure 30. The 500 MHz ^{1}H-^{1}H COSY spectrum of compound EA1. (in DMSO-d_6)



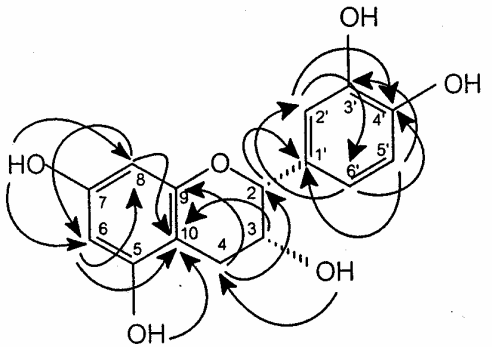
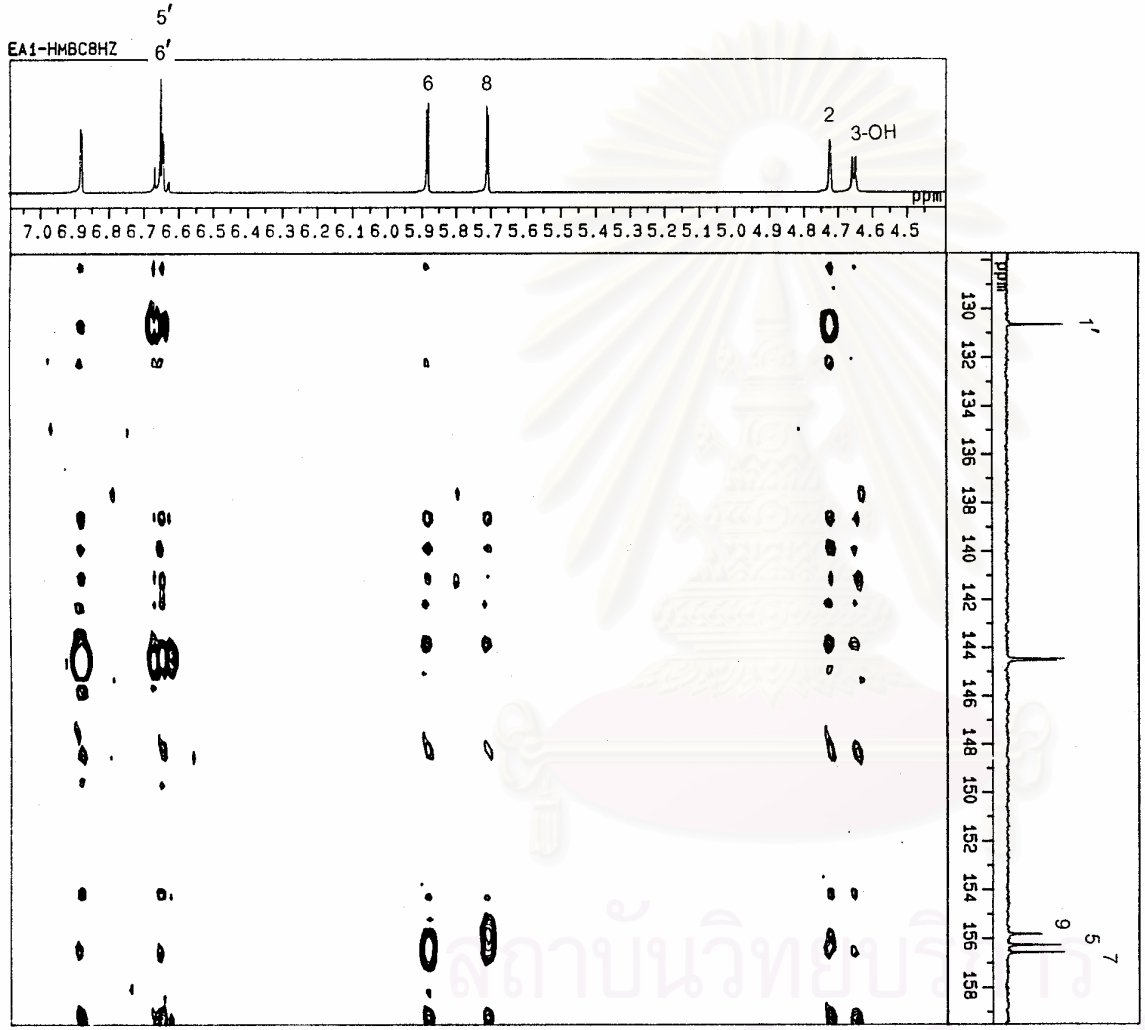


Figure 31a. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound EA1. (in DMSO-d_6)



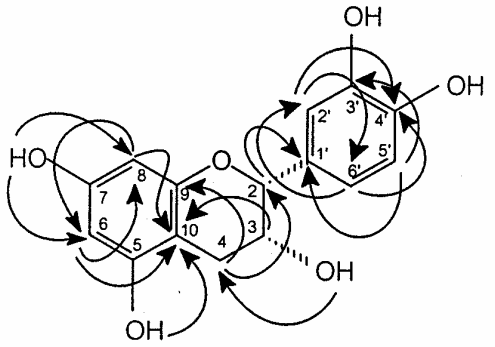


Figure 31b. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound EA1. (in DMSO-d_6)

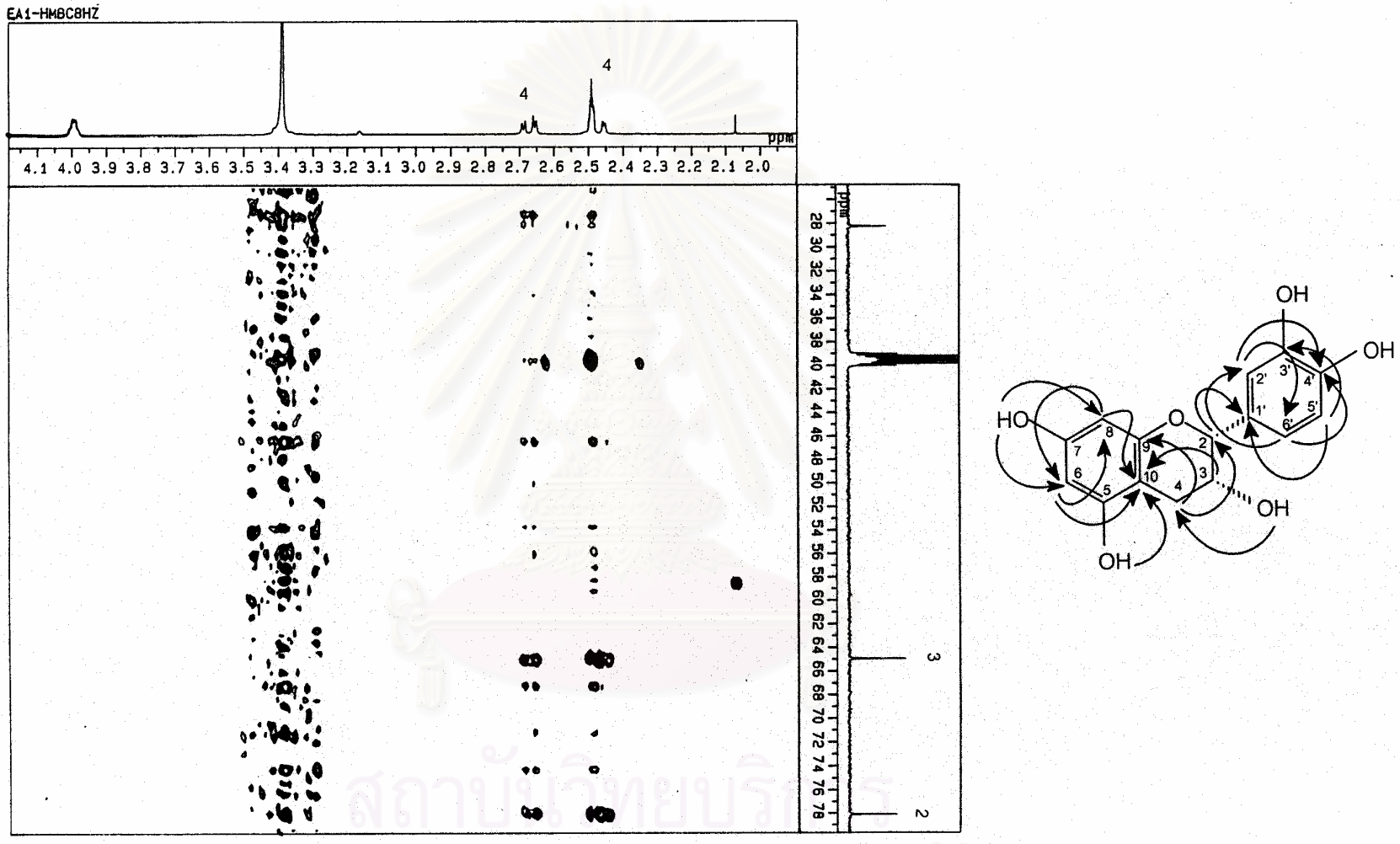
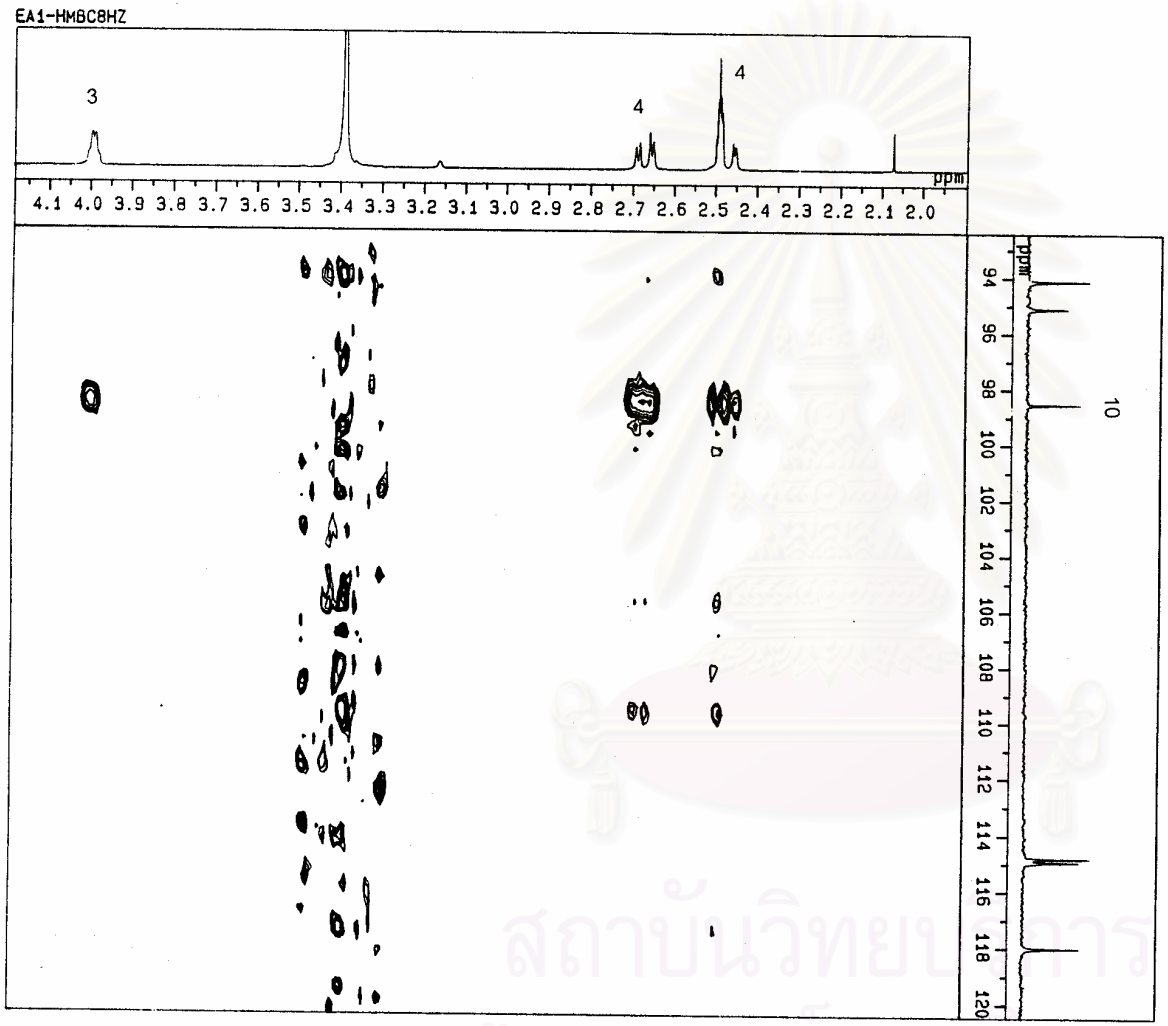


Figure 31c. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound EA1. (in DMSO-d_6)



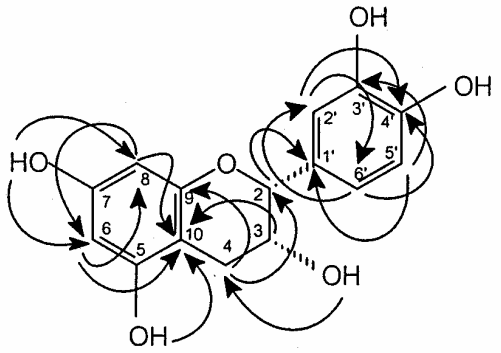
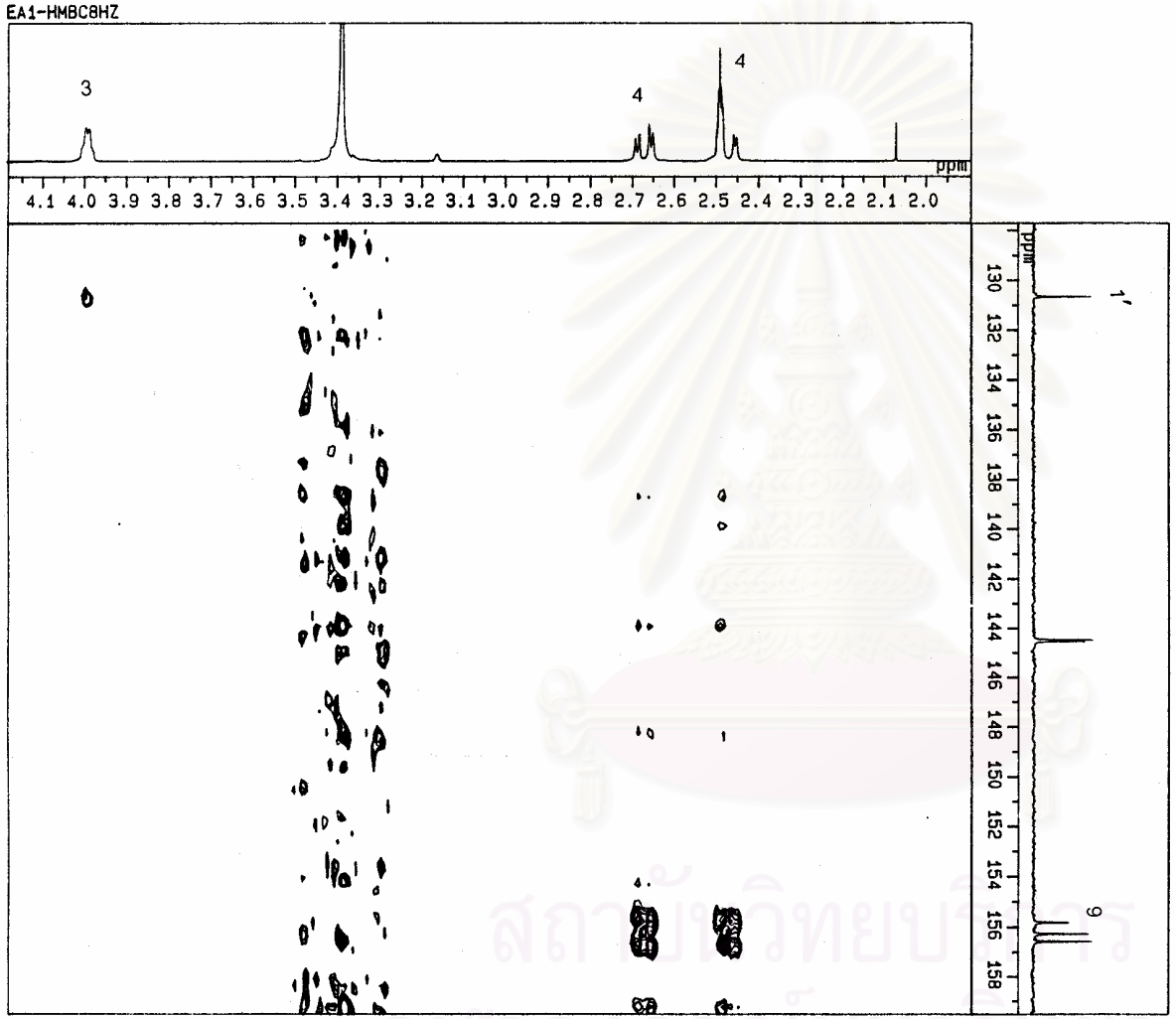


Figure 31d. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound EA1. (in DMSO-d_6)



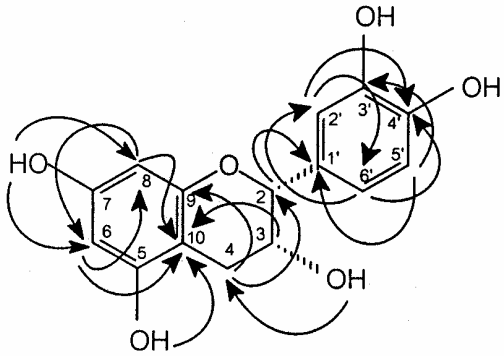
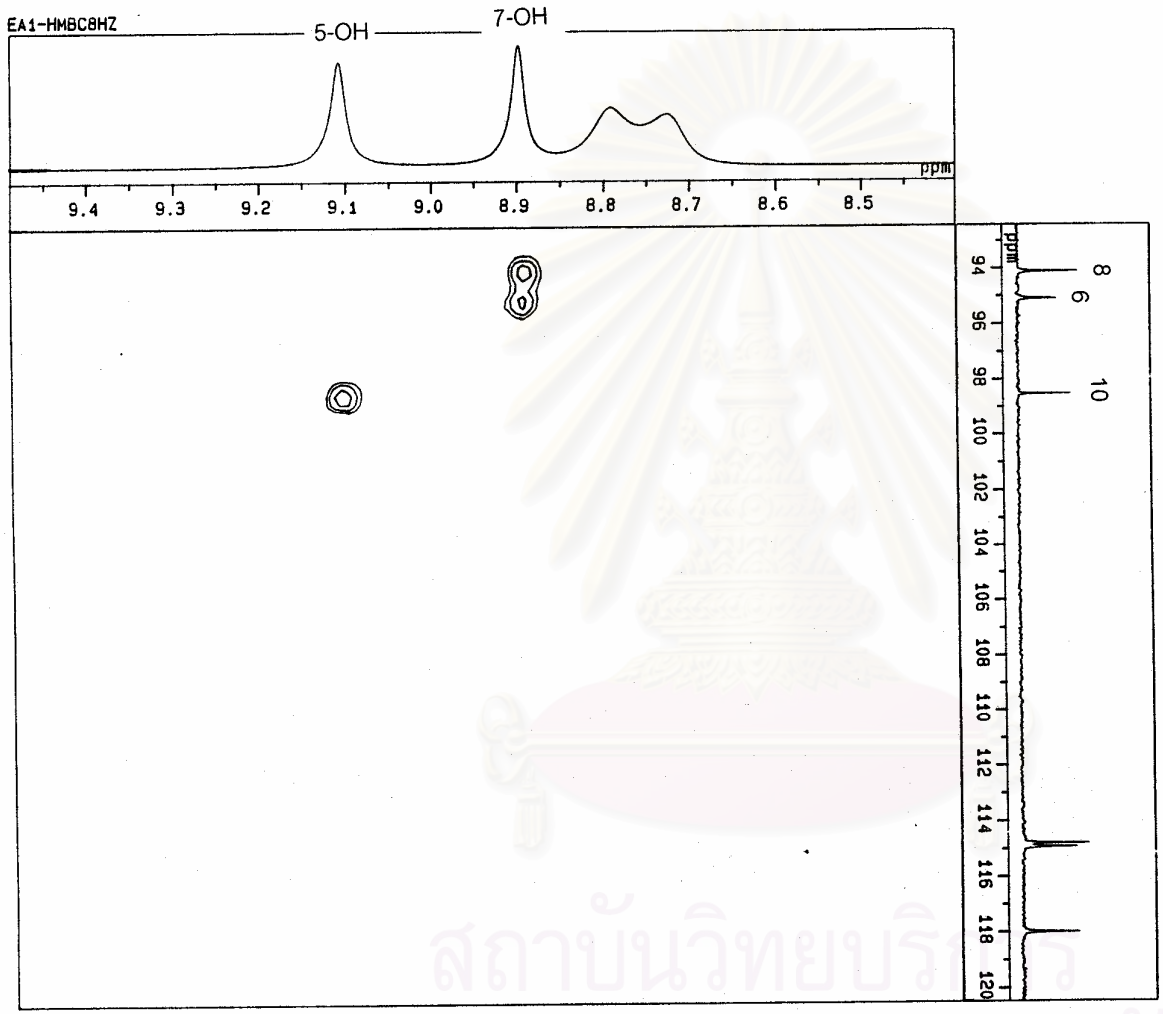


Figure 31e. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound EA1. (in DMSO-d_6)



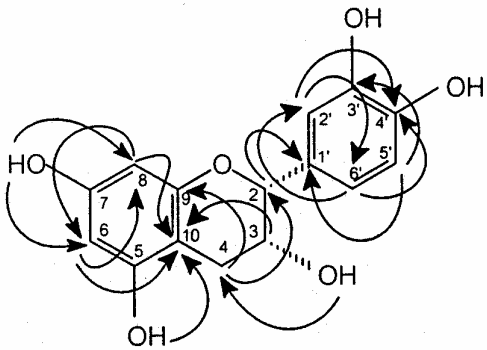


Figure 31f. The 500 MHz ^{1}H-^{13}C HMBC spectrum of compound EA1. (in DMSO-d_6)

VITA

Miss Tattaya Lohakul was born on March 22, 1976 in Phang-nga, Thailand. She has received her Bachelor of Pharmaceutical Sciences in 1998 from the Faculty of Pharmaceutical Sciences , Prince of Songkla university, Songkhla, Thailand. She is now working as a hospital pharmacist at Bantakhun hospital, Surattani, Thailand.