องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของโงงังและปารี

นายประเสริฐ พัฒนาประทีป

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเภสัชเคมีและผลิตภัณฑ์ธรรมชาติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547

ISBN : 974-17-7005-7

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *CRATOXYLUM ARBORESCENS* AND *MILLETTIA DECIPIENS*

Mr. Prasert Pattanaprateeb

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Pharmaceutical Chemistry and Natural Products
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2004

ISBN : 974-17-7005-7

Thesis Title	CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *CRATOXYLUM ARBORESCENS* AND *MILLETTIA DECIPIENS*
By	Mr. Prasert Pattanaprateeb
Field of Study	Pharmaceutical Chemistry and Natural Products
Thesis Advisor	Associate Professor Nijsiri Ruangrungsi, Ph.D.

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

……Dean of the Faculty of Pharmaceutical Sciences
(Associate Professor Boonyong Tantisira, Ph.D.)

THESIS COMMITTEE

……. Chairman
(Associate Professor Sumphan Wongseripipatana, Ph.D.)

……..…… Thesis Advisor
(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

…….. Member
(Associate Professor Ekarin Saifah, Ph.D.)

……..... Member
(Associate Professor Mayuree Chuankamnerdkarn, Ph.D.)

……..Member
(Associate Professor Sunit Suksamrarn, Ph.D.)

ประเสริฐ พัฒนาประทีป: องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของโงงังและปารี (CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *CRATOXYLUM ARBORESCENS* AND *MILLETTIA DECIPIENS*) อาจารย์ที่ปรึกษา: รศ. ดร. นิจศิริ เรืองรังษี, 204 หน้า. ISBN: 974-17-7005-7

การศึกษาทางพฤกษเคมีของเปลือกต้นโงงัง สามารถแยก xanthone ที่พบครั้งแรกในธรรมชาติ 1 ชนิด คือ 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone รวมทั้ง xanthone และ anthraquinone ที่เคยมีรายงานแล้ว คือ fuscaxanthone C, 1,8-dihydroxy-3-geranyloxy-6-methylanthraquinone และ 2-geranylemodin ในส่วนรากของต้นโงงังสามารถแยกสารได้เหมือนในส่วนเปลือกต้น และ 1,7-dihydroxyxanthone การศึกษาทางพฤกษเคมีของเปลือกต้นปารีสามารถแยกสารใหม่ได้ 2 ชนิดคือ (+)-*S*-3,4-methylenedioxy-2′,4′,6′,*β*-tetramethoxy-dihydrochalcone และ 2,4-dimethoxy-3′,4′-methylenedioxydihydrochalcone รวมทั้งสารที่เคยมีรายงานแล้ว 9 ชนิด คือ 3′,4′-methylenedioxy-2,4,6,*β*-tetramethoxychalcone, lanceolatin B, pongaflavone, karanjin, milletenone, desmethoxykanugin, dihydro-isomilletenone methyl ether, dihydromilletenone methyl ether และ pongaglabrone ในส่วนรากของต้นปารีสามารถแยกสารได้เหมือนกับในส่วนของเปลือกต้น และ ovalitenin B การพิสูจน์โครงสารที่แยกได้อาศัยการวิเคราะห์ทางสเปคโตรสโคปี ร่วมกับการเปรียบเทียบข้อมูลของสารที่ทราบโครงสร้างแล้ว สารที่แยกได้ทั้งหมดจากต้นโงงัง นำไปทดสอบฤทธิ์การต้านเชื้อมาลาเรีย และฤทธิ์ความเป็นพิษต่อเซลล์ ของ NCI-H187 cells, KB cells และ Vero cells พบว่า 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone และ 2-geranylemodin มีฤทธิ์ปานกลางต่อ NCI-H187 cells สารทั้งหมดที่แยกได้จากต้นปารี นำไปทดสอบฤทธิ์การต้านเชื้อมาลาเรีย ต้านวัณโรค และฤทธิ์จับอนุมูลอิสระ พบว่าสารส่วนมากมีฤทธิ์อ่อนในการต้านวัณโรค ยกเว้น pongaflavone ที่มีฤทธิ์ปานกลาง สารทั้งหมดไม่มีฤทธิ์จับอนุมูลอิสระ และสารทั้งหมดจากต้นโงงัง และปารี ไม่มีฤทธิ์ต้านเชื้อมาลาเรีย

ภาควิชา เภสัชเวท ลายมือชื่อนิสิต……...
สาขาวิชา เภสัชเคมีและผลิตภัณฑ์ธรรมชาติ ลายมือชื่ออาจารย์ที่ปรึกษา…….....
ปีการศึกษา 2547

#4376957633: MAJOR: PHARMACEUTICAL CHEMISTRY AND NATURAL PRODUCTS

KEY WORD: *CRATOXYLUM ARBORESCENS* / *MILLETTIA DECIPIENS* / XANTHONES ANTHRAQUINONES / CHALCONES / ANTIMALARIAL ACTIVITY / ANTIMYCO-BACTERIAL ACTIVITY / CYTOTOXIC ACTIVITY / FREE RADICAL SCAVENGING ACTIVITY

PRASERT PATTANAPRATEEB: CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *CRATOXYLUM ARBORESCENS* AND *MILLETTIA DECIPIENS* THESIS ADVISOR: ASSOCIATE PROFESSOR NIJSIRI RUANGRUNGSI, Ph.D. 204 pp. ISBN 974-17-7005-7

Phytochemical study of the stem bark of *Cratoxylum arborescens* (Vahl) Blume (Cratoxylaceae) has shown the presence of a new natural xanthone, 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone, together with a known xanthone and anthraquinones, fuscaxanthone C, 1,8-dihydroxy-3-geranyloxy-6-methylanthraquinone, and 2-geranyl-emodin. The roots of *C. arborescens* led to the isolation a known xanthone, 1,7-dihydroxyxanthone, together with the same compounds as from the stem bark. Phytochemical investigation of the stem bark of *Millettia decipiens* Prain (Fabaceae) led to the isolation of two new compounds, (+)-*S*-3,4-methylenedioxy-2′,4′,6′,*β*-tetramethoxydihydrochalcone and 2,4-dimethoxy-3′,4′-methylenedioxydihydrochalcone, along with nine known compounds. The nine known compounds were 3′,4′-methylenedioxy-2,4,6,*β*-tetramethoxychalcone, lanceolatin B, pongaflavone, karanjin, milletenone, desmethoxykanugin, dihydroisomilletenone methyl ether, dihydro-milletenone methyl ether, and pongaglabrone. The roots of *M. decipiens* led to the isolation of a known *β*-methoxydihydrochalcone, ovalitenin B, and the same compounds as from the stem bark. The structure determination of all of the isolates was accomplished by spectroscopic analyses and compared with the literature data of the known compounds. All of the isolates from *C. arborescens* were subjected for biological activity evaluation, including antimalarial and cytotoxic activity against NCI-H187 cells, KB cells, BC cells, and Vero cells. The new natural xanthone, 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone and 2-geranyl- emodin showed moderate activity against NCI-H187. All of the isolates from *M. decipiens* were subjected for biological activity evaluation, including antimalarial, antimyco- bacterial, and free radical scavenging activity. Most of them showed mild activity and no antimycobacterial activity , except for pongaflavone which showed moderate activity. All of the compounds from *M. decipiens* exhibited inactive free radical scavenging activity. The isolates from *C. arborescens* and *M. decipiens* showed no antimalarial activity.

Field of study Pharmaceutical Chemistry and Natural Products Student's signature……..
Advisor's signature…….

Academic year 2004

ACKNOWLEDGEMENTS

The author is sincerely grateful to the following persons and institutions who encouraged and supported his research fulfillment:

Associate Professor Dr. Nijsiri Ruangrungsi, his thesis advisor, for his deeply and constantly generosity, valuable guidance and encouragement throughout this study.

The Thailand Research Fund for a 2001 Royal Golden Jubilee Scholarship for granting financial support.

Professor Dr. Geoffrey A. Cordell, Department of Medicinal Chemistry and Pharmacognosy College of Pharmacy, University of Illinois at Chicago, his thesis co-advisor, for providing research opportunities and invaluable suggestions during his stay in US and for his helpful advice on structure elucidation.

Ms. Somporn Tiyasri, Department of Chemistry, Faculty of Science, Srinakharinwirot University for NMR spectral measurements.

Dr. Mayuso Kuno, Department of Chemistry, Faculty of Science, Srinakharinwirot University for molecular modeling calculations.

The Department of Chemistry, Faculty of Science, Srinakharinwirot University and Ramkhamhaeng University, Thailand, for providing the NMR measurements.

The Bioassay Research Facility of BIOTEC, NSTDA, Science Park, Thailand for conducting the biological activity tests.

The staff of Department of Medicinal Chemistry and Pharmacognosy College of Pharmacy, University of Illinois at Chicago, for their sincere friendships and memorable supports.

The thesis committee for their constructive suggestions and critical review of his thesis.

The author is also grateful to his friends at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their friendship, kind support and encouragement through out the period.

His parents and family for their invaluably, understanding, grate loves, supports and encouragements.

CONTENTS

Page

Page

Page

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

Figure Page

Figure Page

Figure Page

Figure Page

Figure Page

LIST OF SCHEMES

LIST OF ABBREVIATIONS AND SYMBOLS

α	=	Alpha
$[\alpha]^{28}_{D}$	=	Specific rotation at 28° and sodium D line (589 nm)
ax	=	Axial
β	=	Beta
br d	=	Broad doublet (for NMR spectra)
br t	=	Broad triplet (for NMR spectra)
br s	=	Broad singlet (for NMR spectra)
CD	=	Circular Dichroism
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chloroform
C_6D_6	=	Deuterated benzene
^{o}C	=	Degree Celsius
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
calcd	=	Calculated
cm	=	Centimeter
cm^{-1}	=	Reciprocal centimeter (unit of wave number)
δ	=	Delta
d	=	Doublet (for NMR spectra)
dd	=	Doublet of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMSO-d_6	=	Deuterated dimethyl sulfoxide
δ	=	Chemical shift

EC_{50}	=	50% Effective Concentration
EIMS	=	Electrospray Impact Mass Spectrometry
EtOAc	=	Ethyl acetate
EtOH	=	Ethanol
eq	=	Equatorial
g	=	Gram
γ	=	Gramma
h	=	Hour
^{1}H-^{1}H COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
^{1}H NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	^{1}H-Detected Heteronuclear Multiple Bond Coherence
HMQC	=	^{1}H-Detected Heteronuclear Multiple Quantum Coherence
HRESI	=	High Resolution Electrospray Ionization Mass Spectrometry
HRFABMS	=	High Resolution Fast Atom Bombardment Mass Spectrum
Hz	=	Hertz
IC_{50}	=	Median Inhibitory Concentration
IR	=	Infrared Spectrum
in	=	Inch
J	=	Coupling constant
KBr	=	Potassium bromide
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
MeOH	=	Methanol

MHz	=	Megahertz
MIC	=	Minimum Inhibitory Concentration
M^+	=	Molecular ion
$[M+H]^+$	=	Protonated molecular ion
MW	=	Molecular weight
MS	=	Mass Spectrometry
m	=	Multiplet (for NMR spectra)
mg	=	Milligram
ml	=	Milliliter
mm	=	Millimeter
m/z	=	Mass to charge ratio
μl	=	Microliter
$[\theta]$	=	Molar ellipticity
NMR	=	Nuclear Magnetic Resonance Spectroscopy
NOESY	=	Nuclear Overhauser Effect Spectroscopy
nm	=	Nanometer
o	=	Ortho
Pet. ether	=	Petroleum ether
ppm	=	Part per million
p	=	Para
ν_{max}	=	Wave number at maximal absorption
s	=	Singlet (for NMR spectra)
spp.	=	Species
TLC	=	Thin Layer Chromatography
t	=	Triplet (for NMR spectra)

UV = Ultraviolet

CHAPTER I

INTRODUCTION

The genus *Cratoxylum* belongs to the family Cratoxylaceae, with at least six species of this genus distributed in several Southeast Asian countries (Bennett and Lee, 1989).

According to Smitinand (2001), the species of genus *Cratoxylum* found in Thailand are as follows.

Cratoxylum arborescens (Vahl) Blume	ลอแง Lo-ngae (Malay-Pennisular, กะลอแง Ka-lo-ngae (Malay- Narathiwat), โงงัง Ngong- ngang (Narathiwat).
C. cochinchinense (Lour.) Blume	กุ่ยฉ่องบ้าง Kui-chong-bang (Karen-Lam-pang), ขี้ติ้ว Khi tio, ติ้วเกลี้ยง Tio kliang, ติ้วใบเลื่อม Tio bai luean (Northern).
C. formosum (Jack) Dyer	ติ้วขาว Tiokhao (Bangkok), ติ้วส้ม Tio som (Nakhon Ratchasima), แต้วหอม Taeo hom (Philsanulok), มูโต้ะ Mu-to (Malay-Narathiwat).
C. formosum (Jack) Dyer subsp. *pruniflorum* (Kurz) Gogel	กวยโชง Kuai-chong (Karen-Kanchanaburi), กุยฉ่องเซ้า Kui-chong-sao (Karen-Lampang), ตาว Tao (Satun), ติ้วขน Tio khon (Nakhon Ratchasima), ติ้วแดง Tio daeng, ติ้วยาง Tio yang, ติ้วเลือด Tio lueat (Northern), ติ้วเหลือง Tio lueang (Central), แต้วหิน Taeo hin (Lampang), เน็กเครแย่ Nek-khre-yae (Lawa-chiang Mai), ราเง้ง Ra-ngeng (Khmer-surin)
C. harmandii Pierre	= *C. maingayi* Dyer

C. maingayi Dyer	แต้ว Taeo (Trang, Nakhon Si Thammarat, แต้วคา Taeo kha (yala), ต้าว Tao (Narathiwat).
C. neriifolium Kurz	= *C. sumatranum* (Jack) Blume subsp. *neriifolium* Gogel
C. polyanthum Korth	= *C. cochichinense* (Lour.) Blume
C. polyanthum Korth var. *wightii* Dyer	= *C. cochichinense* (Lour.) Blume
C. pruniflorum Kurz	= *C. formosum* (Jack) Dyer subsp. *pruni-florum* (Kurz) Gogel
C. sumatranum (Jack) Blume subsp. *neriifolium* Gogel	ขี้ติ้ว Khi tio (Chiang Mai), ติ้วดำ Tio dam, ติ้วเสลา Tio salao (Northern), สลิว Salio (Central)

Cratoxylum arborescense (Vahl) Blume is a medium to large sized tree, 25-40 m high; scattered in peat swamp forest; crown pyramidal to cylindric; bark red-brown, very thick, laminated, sometimes up to 10 cm in thickness; inner bark thin, leathery, 2-3 mm in thickness, light brown to yellow-pink, with opaque yellowish exudation. Leaves simple, opposite, decussate; blade elliptic-oblong to obovate lanceolate, 8-14 by 4-6 cm, glabrous; upper surface dark green; lower surface pale; apex acute to acuminate; base cuneate; secondary nerves many, very faint; petiole, 0.3-1.2 cm long. Flower dark red, *ca*. 8 mm across, in terminal and axillary panicles, (5-)10-20 cm long; sepals 5, free, imbricate, persistent in fruit; petals 5, imbricate; stamens numerous. Fruit an ovoid-lanceolate capsule, 7-9 by 5-6 mm, loculicidally dehiscent into 3 valves; many seeded. Seeds very small, oblong, winged (Ekavibhata, Phengklai and Niyomdham., 1991).

Plants in the genus *Millettia* belong to the family Fabaceae, subfamily Papilionoideae. These plants are trees or climbing shrubs, leaves odd-pinnate. Flower showy, in auxiliary racemes, often fascicled, simple or paniculate and terminal. Calyx campanulate; teeth short. Petals white or pink; standard ovate or orbicular; wing oblong. Stamens monodelphous or diadelphous, filaments filiform; anthers uniform.

Ovary sessile, linear, few-ovuled; style filiform, incurved, glabrous, stigma capitate. Pod linear or oblong, coriaceous or woody, flattened or thick. Seeds lenticular or rainform (Chopra, Badhwar and Ghosh., 1965).

According to Smitinand (2001), the species of the genus *Millettia* found in Thailand are as follows (Smitinand, 2001).

Millettia atropurpurea Wall.	= *Collerya atropurpurea* (Wall.) Schott
M. brandisiana Kurz	กระพี้จั่น Kra phi chan, จั่น Chan, พี้จั่น Phi Chan (General); ปี๊จั่น Pi Chan (Northern).
M. caerulea Baker.	ปัวเปาะเด๊าะ Pua-po-do (Karen Mae Hong Son); ผักเยี่ยววัว Phak yiao wua (Nakhonsawan, Northern); หางไหลแดง Hang Lai daeng (Kanchanaburi).
M. decipiens Prain	ปารี Pa ri (Malay-Narathiwat).
M. extensa Benth.	ก๋าวเครือ Kao khruea, กวาวเครือ Kwao khruea (Chiang Mai); ตานครบ Tan krop (Lampang).
M. glaucescens Kurz	ยะดา Ya-daa (Malay-Narathiwat); หยีน้ำ Yi nam (Peninsular).
M. kangensis Craib	กระเจาะ Kra cho, ขะเจาะ Kha cho, ขะเจาะน้ำ Kha cho nam (Chiang Mai).
M. kityana Craib	เครือข้าวเย็น Khruea khao yen, ลางเย็น Lang yen, ฮางเย็น Hang yen (Northern).
M. latifolia Dunn	ขะเจาะ Kha cho (General).

M. leucantha Kurz var. *leucantha*	กะเซาะ Kaso (Central); กระเจาะ Kra ขะเจาะ Kha cho (Northern); กระพี้เขาควาย Kra phi khao khwai (Prachuap Khiri Khan); ขะแมบ Kha maep, คำแมบ Kham maep (Chiang Mai).
M. leucantha Kurz var. *buteoides* (Gagnep.) P.K. Loc [(*M. buteoides* Gagnep. var. *siamensis* Craib, *M. pendula* (Benth.)]	กระเจ๊าะ Kra cho, ขะเจ๊าะ Kha cho (Lampang); กระท้อน Kra thon, (Phetchabun Phitsanulok); ไม้กระทงน้ำผัก Mai kra tong nam phak (Loei); สะท้อน Sa thon (Saraburi); สาธร Sa thon (Ubon Ratchathani).
M. macrostachya Collett & Hemsl. var. *macrostachya*	ขะเจาะน้ำ Kha cho nam (Chiang Mai).
M. macrostachya Collett & Hemsl. var. *tecta* Craib	ขะเจาะหลวง Kha cho luang, ขะเจาะใหญ่ Kha cho yai (Narathiwat).
M. pachycarpa Benth.	เกถะ Ke-tha (Karen-Chiang Mai); เครือไหล Khruea lai (Chiang Mai).
M. peguensis Ali (*M. ovalifolia* Kurz)	ตอหิ To-hi (Karen-Kanchanaburi).
M. pulcha Benth. Kurz	จันพอ Chan pho (Northern).
M. racemosa (Roxb.) Benth.	= *Endosamara racemosa* (Roxb.) R Geesink
M. sericea (Vent.) Benth.	จะไนโค๊ะ Cha-nai-kho, ปาตู Paa-tu (Malay-Narathiwat); นอเราะ No-ro

(Malay-Yala, Pattani); ยิมแมเก๊าะ Yim-mae-ko (Malay-Yala); อ้อยสามสวน Oi sam suan (Nong Khai).

M. thorelii Gagnep. = *Derris thorelii* Craib

M. utilis Dunn สะท้อนน้ำผัก Sathon nam phak (Loei).

M. xylocarpa Miq. กะเจ๊าะ Ka cho, ขะเจาะ Kha cho (General); คะแมด Kha maet (Chiang Mai); จักจั่น Chakkachan (Loei); พี้พง Phi phong (Phrae); ยะดา Ya-da (Malay-Yala); ไย่ยี Yai-yi (Karen-Mae Hong Son); สาธร Sa thon, หยีน้ำ Yi nam (Pattani-Yala).

Millettia decipiens Prain known in Thai as Pa ri. This plant is a big tree, 40 to 60 ft. tall. Stem 2 to 3 ft. through, glabrous. Leaves 8 to 9 in. long, leaflets 4 to 5 pairs, membranous, elliptic acuminate (lowest ovate acuminate) beneath glaucous 2 to 3.5 in. long, 1.25 to 1.5 in. wide; petiolules ½ in long. Racemes axillary 6 in. long, puberulous to glabrous; pedicels 2 in long. Calyx wide cup-shaped, obscurely toothed 0.15 in long. Corolla ¼ in. long, pink or white tinged pink; standard orbicular, silky outside (Ridley, 1922).

Millettia decipiens is a plant growing in the south of Thailand with no previous records of chemical or biological examination.

The main objectives in this investigation are as follows

1. to isolate and purify components from the stem bark of *Cratoxylum arborescense* and *Millettia decipiens*.
2. to determine the chemical structure of each isolated compound.
3. to evaluate the biological activities of the isolated compounds.

Figure 1. *Cratoxylum arborescens* (Vahl) Blume

Figure 2. *Millettia decipiens* Prain

CHAPTER II

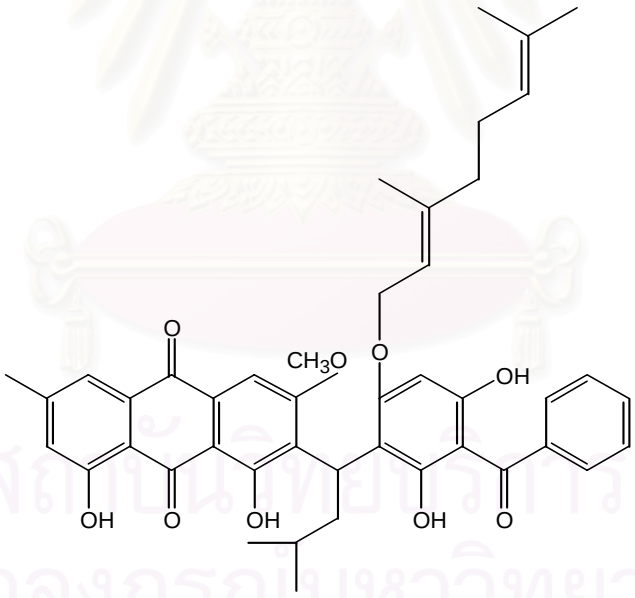
HISTORICAL

1. Chemical Constituents of *Cratoxylum* species

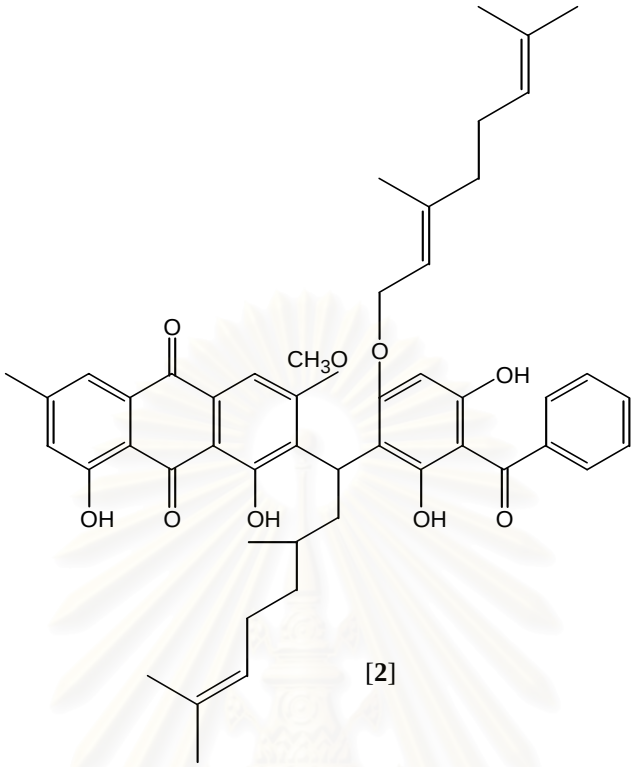
Cratoxylum species contain a number of xanthones. In addition, *Cratoxylum* species also produce anthraquinonebenzophenones, flavanones, prenylated anthranoid, triterpenes, and triterpene quinones.

1.1 Anthraquinonebenzophenones.

In 2002, Seo *et al*. isolated two novel anthraquinonebenzophenone, cratoxyarborequinone A [**1**] and cratoxyarborequinone B [**2**] from the stem bark of *Cratoxylum sumatranum* (Seo *et al*., 2002). Cytotoxicity evaluation against the KB (human oral epidermoid) cancer cell line of the two compounds indicated no activity.



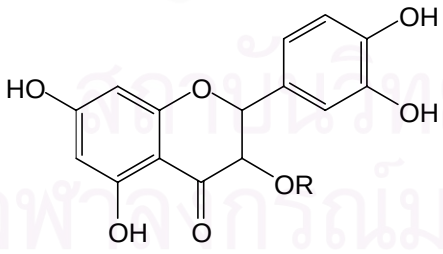
[**1**]



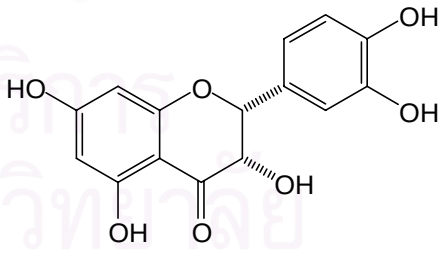
[**2**]

1.2 Flavanones

In 1996, Iinuma *et al*. isolated two flavanones, astilbin [**3**] and (-) epi-catechin [**4**] from the root of *C. formosanum* (Iinuma *et al*., 1996)



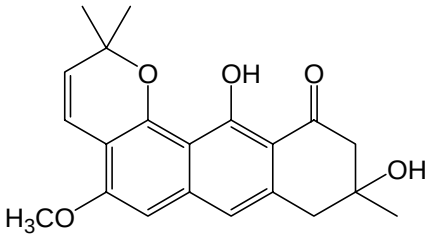
[**3**] R= rhamnosyl



[**4**]

1.3 Prenylated anthranoid

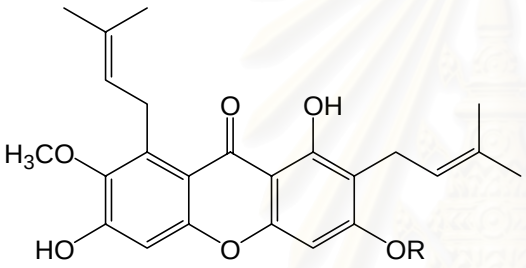
Vismone B [**5**] was isolated from the stem bark of *C. sumatranum* and exhibited moderated cytotoxic activity, EC_{50} = 1.3±0.1 µg/ml, against the KB (human oral epidermoid) cancer cell line (Seo *et al*., 2002).



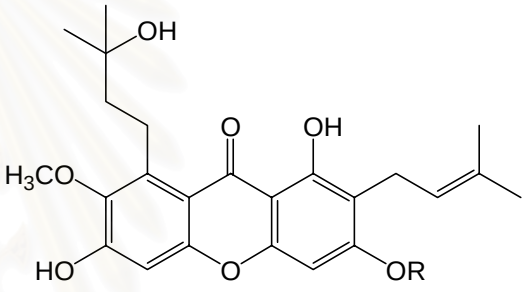
[**5**]

1.4 Xanthones

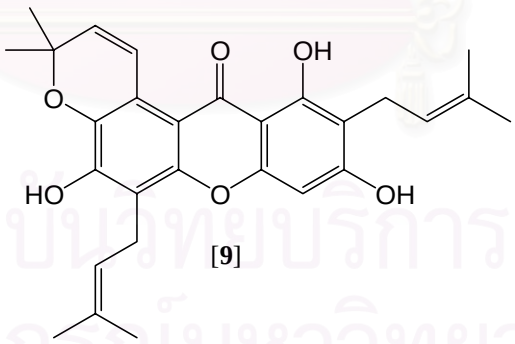
Cratoxylum species are a rich source of xanthones. In 1993, Bennett *et al*. isolated mangostin [**6**], *β*-mangostin [**7**], garcinone D [**8**], and tovophyllin A [**9**] from the stem bark of *C. cochinchinense* (Bennett *et al*., 1993).



[**6**] R = H
[**7**] R = CH_3

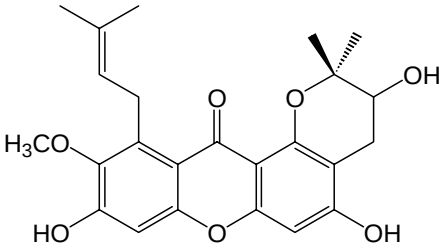


[**8**]

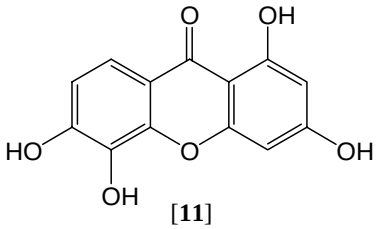


[**9**]

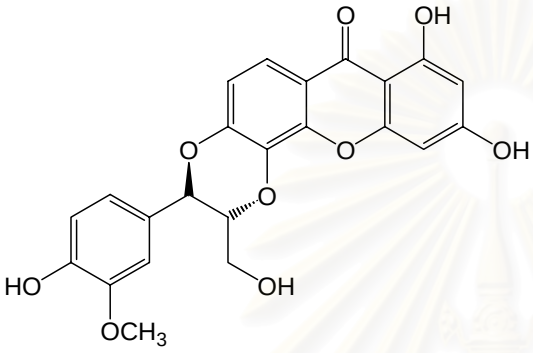
In 1995, Sia *et al*. isolated 11-hydroxy-1-isomangostin [**10**], 1,3,5,6-tetrahydroxyxanthone [**11**], 5′-demethoxycadensin [**12**], and cratoxyxanthone [**13**] from the stem bark of *C. cochinchinense* (Sia *et al*., 1995)



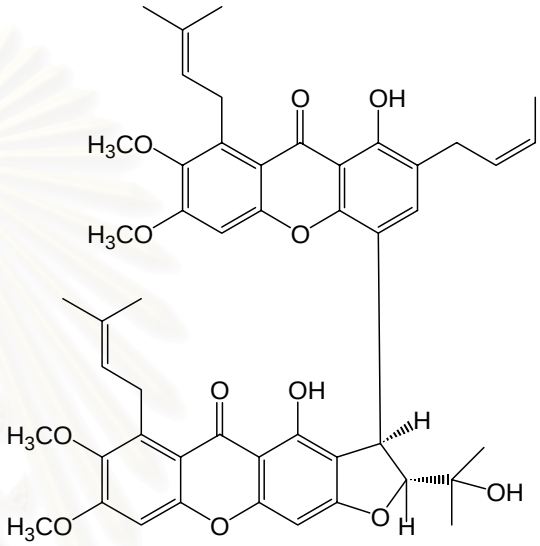
[**10**]



[**11**]

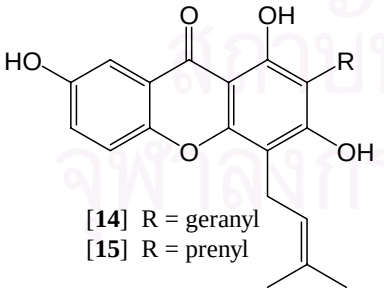


[**12**]

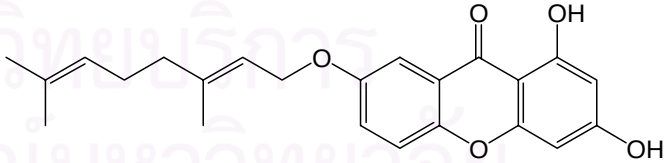


[**13**]

In 1998, Nguyen and Harrison isolated 1,3,7-trihydroxy-2,4-di(3-methyl-but-2-enyl)-xanthone [**14**], 2-prenyl-1,3,7-trihydroxy-4-(3-methyl-but-2-enyl)-xanthone [**15**], and 1,3-dihydroxy-7-geranyloxyxanthone [**16**] from the stem bark of *C. cochinchinense* (Nguyen and Harrison, 1998).



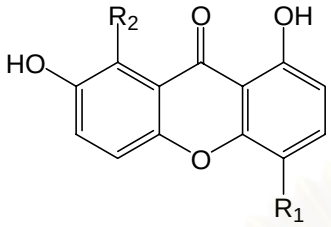
[**14**] R = geranyl
[**15**] R = prenyl



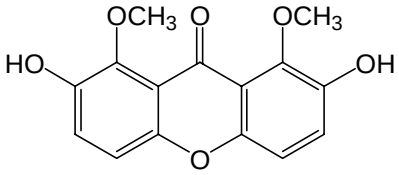
[**16**]

Chemical investigation of the roots of *C. formosanum* led to the isolation of 1,7-dihydroxyxanthone [**17**], 1,7-dihydroxy-4-methoxyxanthone [**18**], 1,7-dihydroxy-8-methoxyxanthone [**19**], 2,7-dihydroxy-1,8-dimethoxyxanthone [**20**], 8-methoxy-1,4,7-trihydroxyxanthone [**21**], 1,4,7-trihydroxyxanthone [**22**],

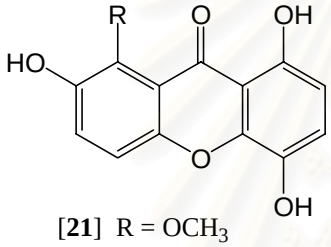
1,2,3,4,8-pentamethoxyxanthone [**23**], and macluraxanthone [**24**] (Iinuma *et al*., 1996).



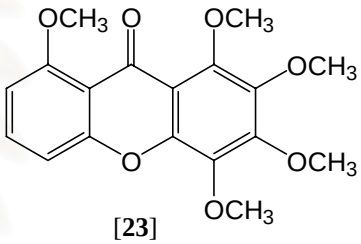
[**17**] $R_1 = R_2 = H$
[**18**] $R_1 = OCH_3$, $R_2 = H$
[**19**] $R_1 = H$, $R_2 = OCH_3$



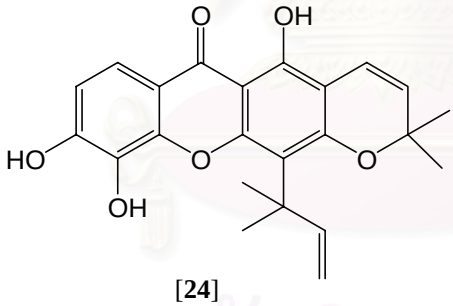
[**20**]



[**21**] $R = OCH_3$
[**22**] R = H

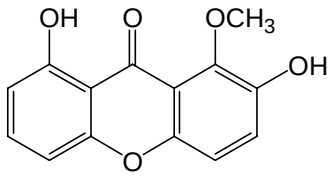


[**23**]

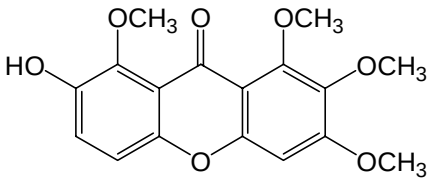


[**24**]

The wood of *C. maingayi* yielded 2,8-dihydroxy-1-methoxyxanthone [**25**] and 7-hydroxy-1,2,3,8-tetramethoxyxanthone [**26**] (Kijjoa *et al*., 1998).

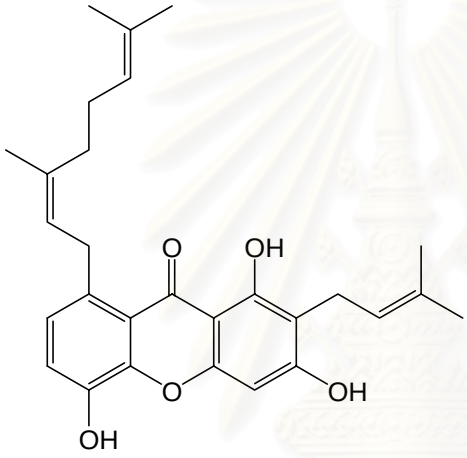


[**25**]

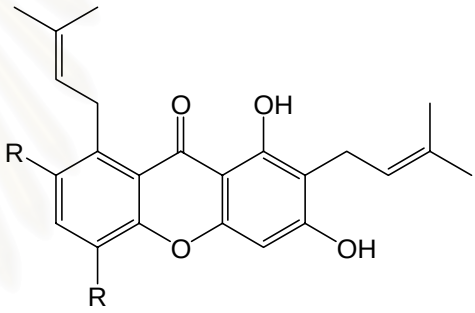


[**26**]

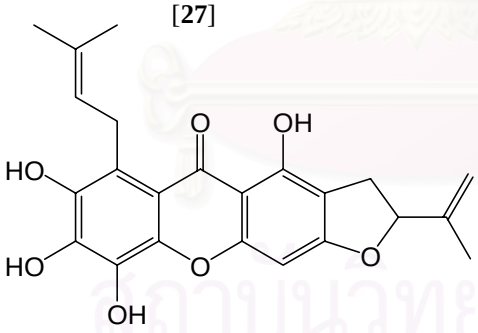
In 2002, cratoxyarborenones A–F [**27**-**32**] were isolated from the leaves and twigs of *C. sumatranum*, which exhibited moderate cytotoxic activity against the KB (human oral epidermoid) cancer cell line. Values in the range of EC_{50} 1.0-4.30 µg/ml were obtained (Seo *et al*., 2002).



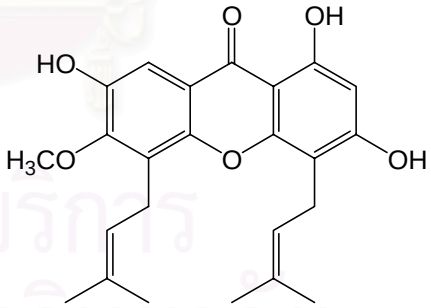
[**27**]



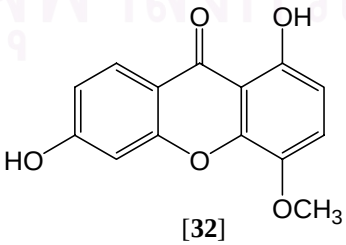
[**28**] R = OH
[**29**] R = OCH_3



[**30**]



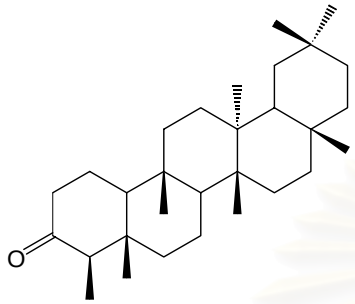
[**31**]



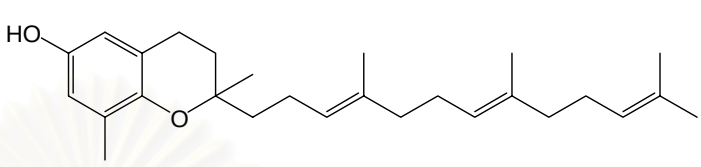
[**32**]

1.5 Triterpenes

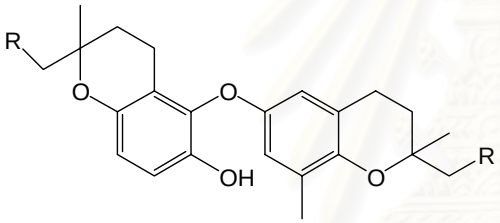
In 1993, Bennett *et al*. isolated friedelin [**33**], δ-tocotrienol [**34**], δ-tocotrienol dimer [**35**] and 5-(γ-tocotrienyl- γ-tocotrienol [**36**] from the stem bark of *C. cochinchinense* (Bennett *et al*., 1993).



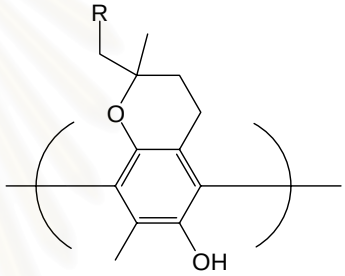
[**33**]



[**34**]



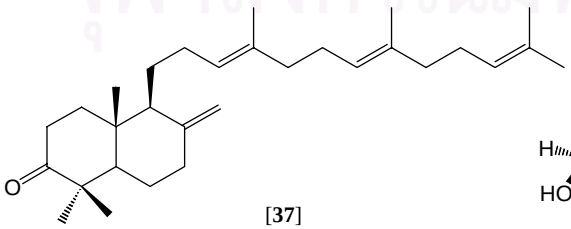
[**35**] R = farnesyl



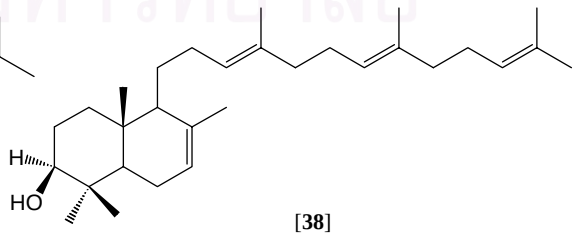
[**36**] R = farnesyl

1.6 Triterpene quinones

Triterpene quinones, polypoda-8(26)-13,17,21-tetraen-3-one [**37**] and (13*E*,17*E*)-polypoda-7,13,17,21-tetraen-3*β*-ol [**38**] were found in the stem bark of *C. cochinchinense* (Bennett *et al*., 1993; Nguyen and Harrison, 1998).



[**37**]



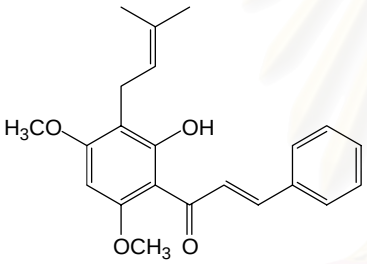
[**38**]

2. Chemical constituents of *Millettia spp.*

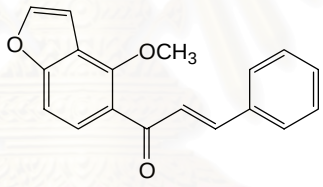
Chemical investigation of *Millettia* species have shown them to be a good source of chalcones, flavans, isoflavans, flavanones, isoflavanones, flavones and isoflavones. In addition, other classes natural products such as alkaloids, coumarins, quinones and rotenoids were also found.

2.1 Chalcones

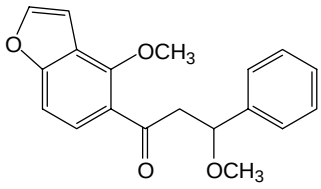
Chalcone derivatives, 4′,6′-dimethoxy-2′-hydroxy-3-*C*-prenylchalcone [**39**], ovalitenins A-C [**40**-**42**], and ovalitenone [**43**] and its enol form [**44**] were isolated from the seeds of *Millettia ovalifolia* (Gupta and Krishnamurti, 1976; 1977; Islam *et al*., 1980). In 1986, Saxena *et al*. isolated 1-(4-hydroxy-5-benzofuranyl)-3-phenyl-2-propen-1-one [**45**] from the roots of *M. ovalifolia* (Saxena *et al*., 1987).



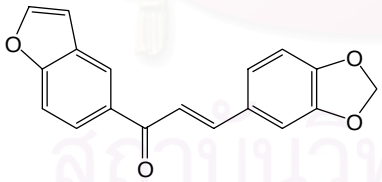
[**39**]



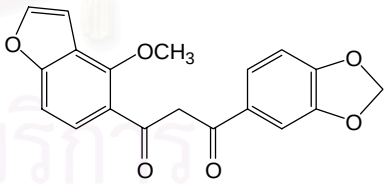
[**40**]



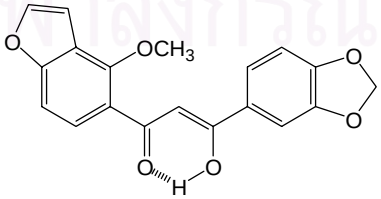
[**41**]



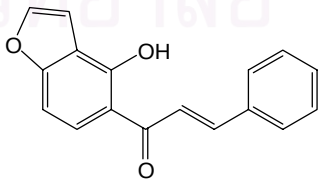
[**42**]



[**43**]



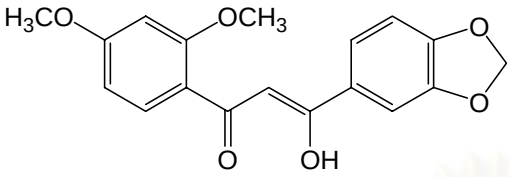
[**44**]



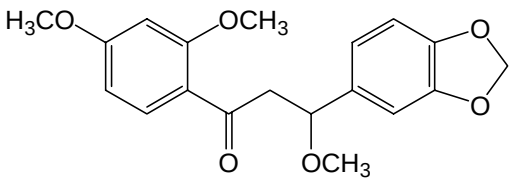
[**45**]

Three chalcones, milletenone [**46**], dihydromilletenone methyl ether [**47**], and dihydroisomilletenone methyl ether [**48**] were isolated from the stem

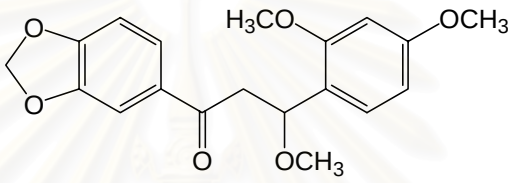
bark of *M. hemsleyama* (Mahmoud and Waterman, 1985). Compound **47** was also found in *M. leucantha* (Phrutivorapongkul *et al*., 2003).



[**46**]

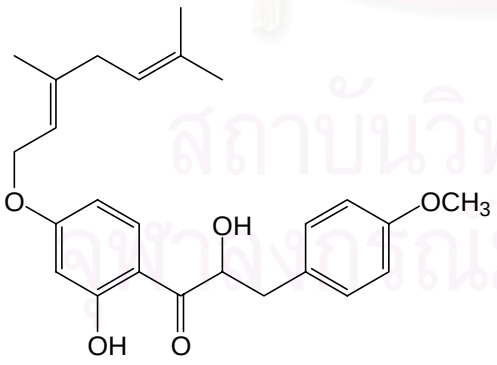


[**47**]

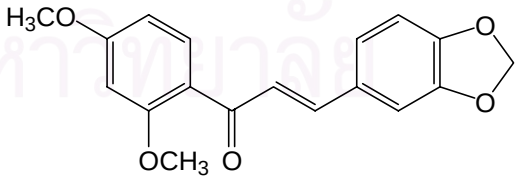


[**48**]

A new *α*-hydroxydihydrochalcone [**49**] was isolated from the stem bark of *M. usaramensis* subsp. *usaramensis* (Yenesew, Midiwo and Waterman, 1998). In 2002, Sritularak *et al*. isolated a new chalcone [**50**] from *M. erythrocalyx* (Sritularak *et al*., 2002a).

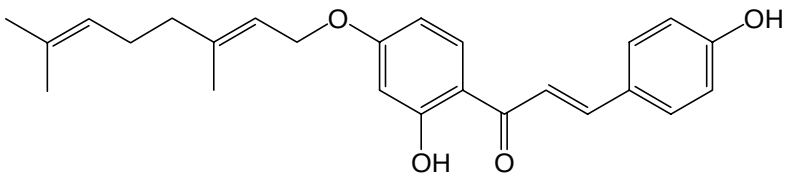


[**49**]



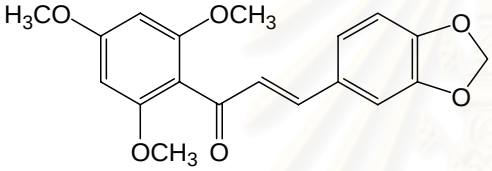
[**50**]

A novel 4′-*O*-geranylisoliquiritigenin [**51**] was isolated from the bark and seed pods of *M. ferruginea* (Dagne, Bekele and Waterman, 1989) and the root bark of *M. griffoniana* (Yankep, Fomun and Dagne, 1997).

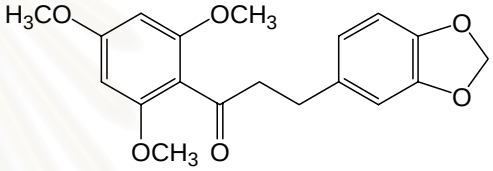


[**51**]

In 2003, Phrutivorapongkul *et al*. isolated the anti-Herpes Simplex Virus (HSV) compounds **50** and **52** and the cytotoxic compounds ([**47**] and [**53**]) from the stem bark of *M. leucantha* (Phrutivorapongkul *et al*., 2003).

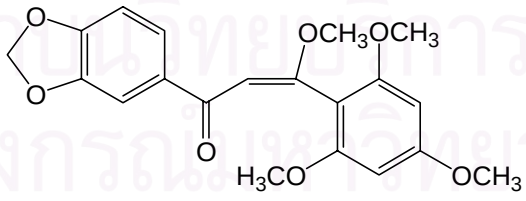


[**52**]



[**53**]

A new chalcone, 3′,4′-methylenedioxy-2,4,6,*β*-tetramethoxychalcone [**54**] was also found in *M. leucantha* (Phrutivorapongkul *et al*., 2003).

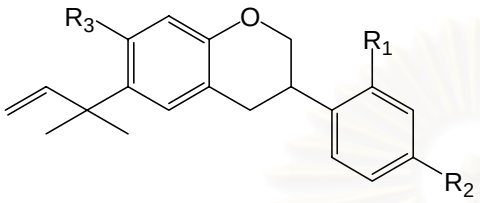


[**54**]

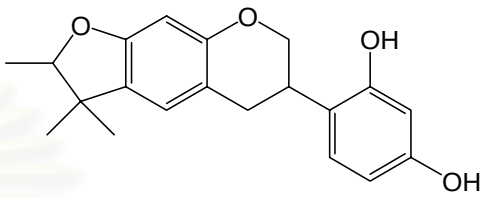
2.2 Flavans and Isoflavans

Isoflavans, 3*R*(+)-millinol [**55**], 3*R*(+)-millinol-B [**56**] and 3*R*(+)-cyclomillinol [**57**] were isolated from the stem bark of *M. racemosa* (Kumar, Krupadanam and Srimannarayana, 1989). In 1994, Rao and Krupadanam isolated

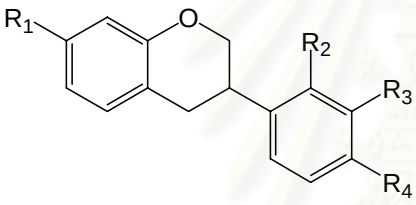
compounds **55**, **56**, **57**, 3*R*(+)-isomillinol-B [**58**], 3*R*(-)-vestitol [**59**], and 3*R*(-)-laxifloran [**60**] from *M. race- mosa* (Rao and Krupadanam, 1994). Compounds **57** and **59** showed significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Two prenylated isoflavans, neomillinol [**61**] and millinol [**62**] were isolated from the stem bark of *M. racemosa* (Rao, Prashat and Krupadanam, 1996).



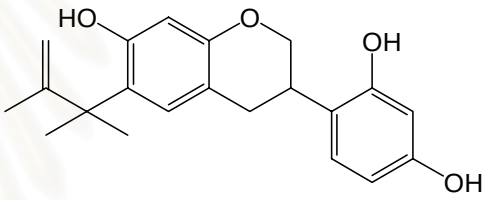
[**55**] $R_1 = R_2 = R_3 = OH$
[**56**] $R_1 = OCH_3$, $R_2 = R_3 = OH$
[**58**] $R_1 = R_3 = OH$, $R_2 = OCH_3$



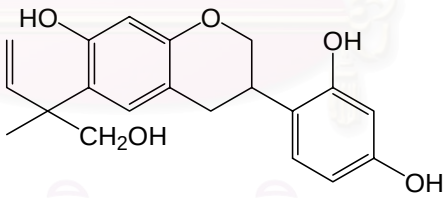
[**57**]



[**59**] $R_1 = R_4 = OH$, $R_2 = OCH_3$, $R_3 = OCH_3$
[**60**] $R_1 = R_2 = R_3 = OH$, $R_4 = OCH_3$

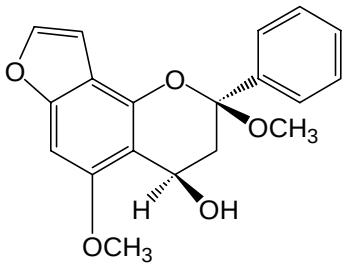


[**61**]



[**62**]

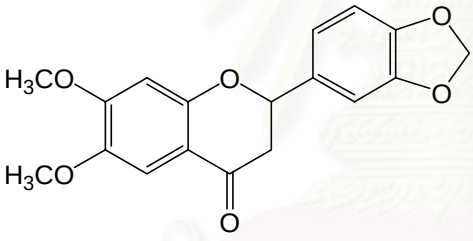
A new furanoflavan, 2,5-dimethoxy-4-hydroxy-[2´´,3´´:7,8] furano-flavan [**63**] was isolated from the roots of *M. erythrocalyx* (Sritularak *et al*., 2002a).



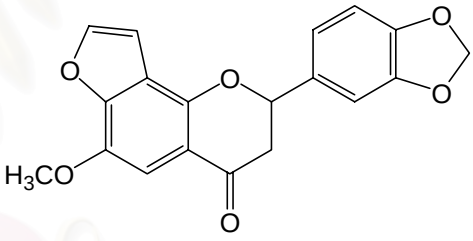
[**63**]

2.3 Flavanones and Isoflavanones

M. ovalifolia is the source of flavanones and isoflavanones. The flavanones, milletenin A [**64**] and milletenin B [**65**] were isolated from the leaves of *M. ovalifolia* (Khan and Zaman, 1974).

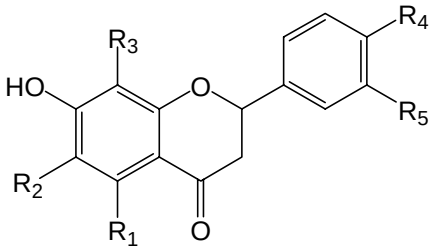


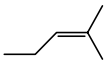
[**64**]

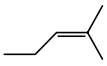


[**65**]

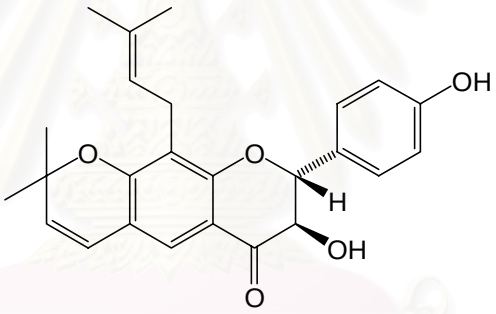
In 1980, Islam *et al*. isolated 6,8-di-*C*-prenyl-7-hydroxyflavone [**66**] and 8-di-*C*-prenyl-7-hydroxyflavone [**67**] from the seeds of *M. ovalifolia* (Islam, Gupta and Krishnamurti, 1980).



[**66**] $R_1 = R_4 = R_5 = H$, $R_2 = R_3 =$ 

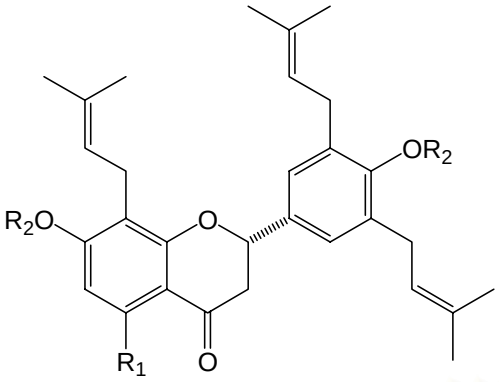
[**67**] $R_1 = R_2 = R_4 = R_5 = H$, $R_3 =$ 

In 1980, *M. pachycarpa* was found to contain a prenylated dihydroflavanol [**68**] (Singhal *et al*., 1980).

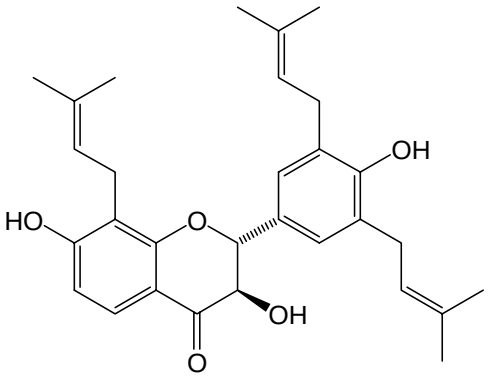


[**68**]

In 1984, Baruah *et al*. isolated a dihydroflavanol, (2*S*)-3,7,4′-trihydroxy-8,3′,5′-triprenylflavanone [**69**] and two flavanones, (-)-sophoranone [**70**] and its 5-hydroxy derivative [**71**] from *M. pulchra* (Baruah *et al*., 1984).

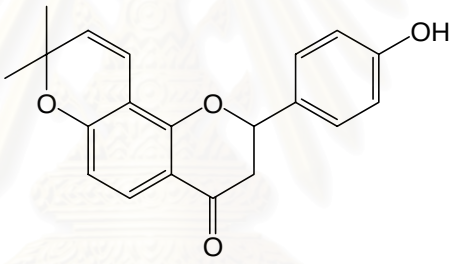


[**69**] $R_1 = R_2 = H$
[**70**] R_1= OH, R_2 = H



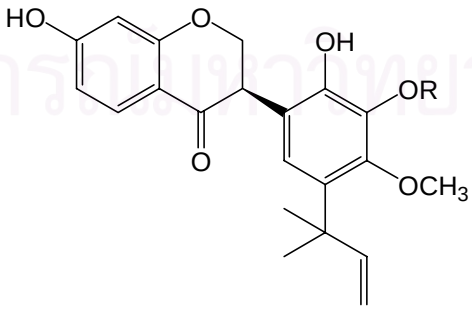
[**71**]

The pyranoflavanone, 4′-hydroxyisolonchocarpin [**72**], was isolated from the bark of *M. ferruginea* subsp. *ferruginea* (Dagne, Bekele and Waterman, 1989).



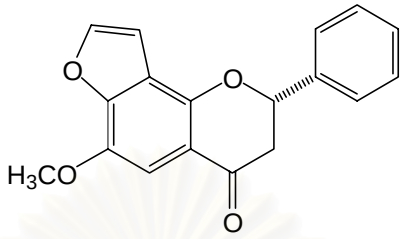
[**72**]

Pervillenone [**73**] and its 3′-*O*-demethyl derivative [**74**] were isolated from the root bark of *M. pervilleana* and showed the cytotoxic activity against KB cell (Galeffi *et al*., 1997).



[**73**] R = CH_3
[**74**] R = H

A new furanoflavanone, 6-methoxy-[2′′,3′′:7,8]-furanoflavanone [**75**] was isolated from the roots of *M. erythrocalyx* (Sritularak *et a*l., 2002a).

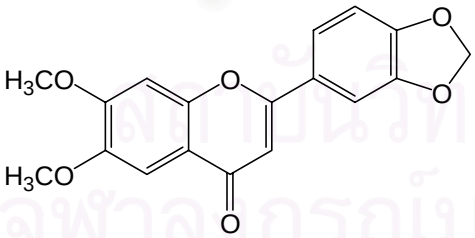


[**75**]

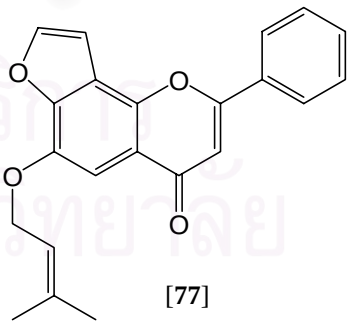
2.4 Flavones and Isoflavones

Flavones and isoflavones were found in *Millettia* species, for example *M. ovalifolia*, *M. hemsleyana*, *M. auriculata*, *M. pachycarpa*, *M. zechiana*, *M. sanagana*, *M. ferruginea*, *M. ferruginea* subsp. *ferruginea*, *M. pulchra*, *M. griffoniana*, *M. conraui*, *M. ichthyochtona*, *M. thonningii*, *M. usaramensis* subsp. *usaramensis*, and *M. erythrocalyx*.

The flavones milletenin C [**76**] and ovalifolin A [**77**] were isolated from leaves of *M. ovalifolia* (Khan and Zaman, 1974).

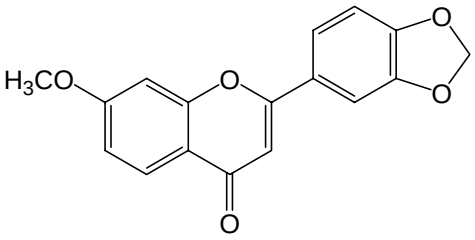


[**76**]



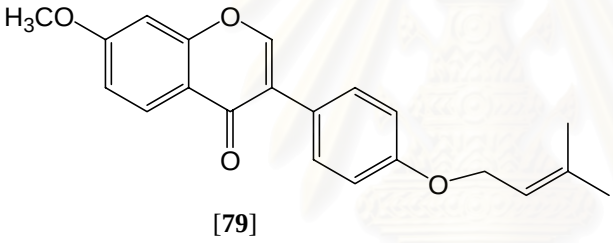
[**77**]

In 1985, Mahmoud and Waterman isolated 7-methoxy-3′,4′-methylenedioxyflavone [**78**] from the stem bark of *M. hemsleyana* (Mahmoud and Waterman, 1985).

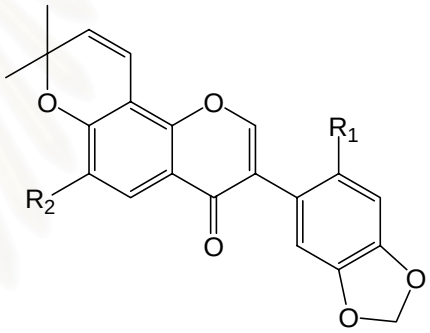


[**78**]

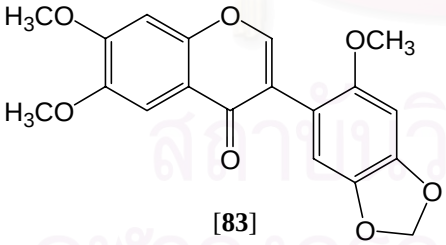
M. dura Dunn is widely distributed in East Africa and has yielded isoflavones. In 1967, Ollis *et al*. isolated durlettone [**79**], durmillone [**80**], jamaicin [**81**], ichthynone [**82**], and milldurone [**83**] from the seeds of *M. dura* (Ollis, Rhodes and Sutherland, 1967).



[**79**]

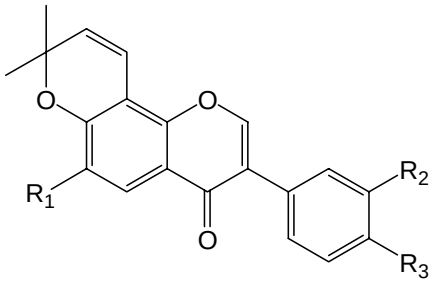


[**80**] R_1 = H, R_2 = OCH_3
[**81**] R_1 = OCH_3, R_2 = H
[**82**] R_1 = R_2 = OCH_3

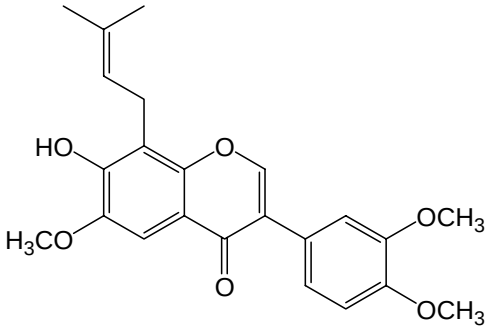


[**83**]

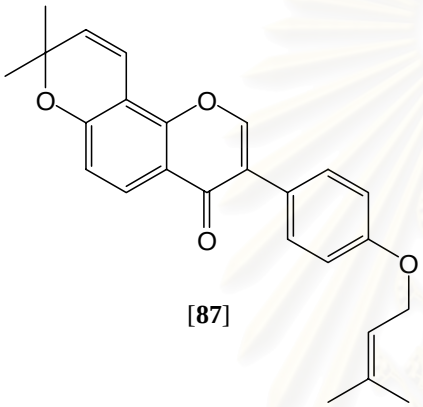
In the seed pods of *M. dura* durallone [**84**], 6-demethyldurallone [**85**], predurallone [**86**], and isoerythrinin-A [**87**] were found (Yenesew, Midio and Waterman, 1996). From the stem bark of *M. dura*, durmillone [**80**], calopogonium isoflavone-A [**88**], maximaisoflavone-D [**89**], and maximaisoflavone-H [**90**] were isolated (Yenesew, Midiwo and Waterman, 1996).



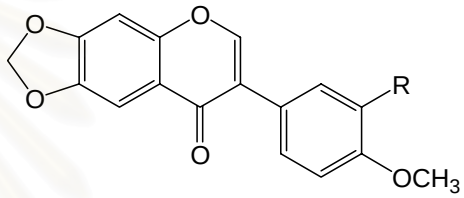
[**84**] $R_1 = R_2 = R_3 = OCH_3$
[**85**] $R_1 = OH, R_2 = R_3 = OCH_3$
[**88**] $R_1 = R_2 = H, R_3 = OCH_3$



[**86**]

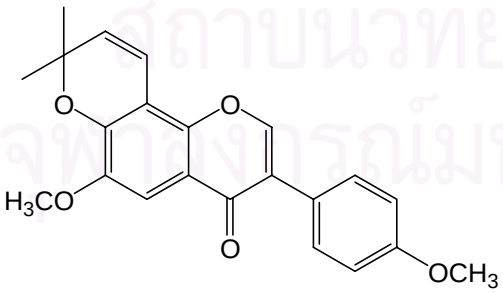


[**87**]

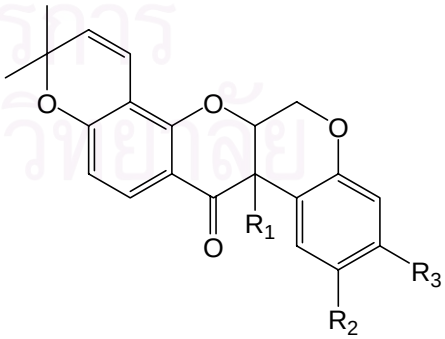


[**89**] $R = OCH_3$
[**90**] R = H

In 1997, Yenesew *et al*. isolated 6-methoxycalopogonium isoflavone A [**91**], millettone [**92**], and tephrosin [**93**] from the seed pods of *M. dura* (Yenesew, Midiwo and Waterman, 1997).

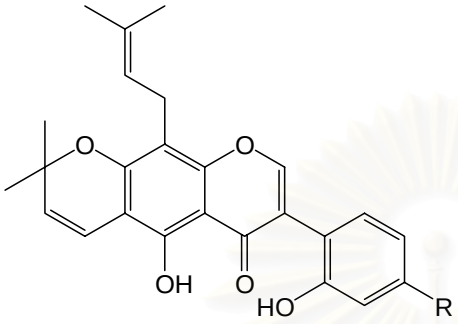


[**91**]

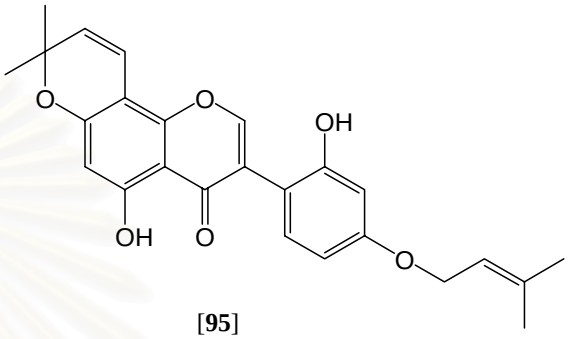


[**92**] $R_1 = H, R_2,R_3 = OCH_2O$
[**93**] $R_1 = OH, R_2 = R_3 = OCH_3$

M. auriculata has been studied in the period 1968-1992. In 1968 and 1970 auriculatin [**94**], isoauriculatin [**95**], and auriculin [**96**] were isolated from the roots by Shabbir and Zaman (Shabbir and Zaman, 1968 and 1970).

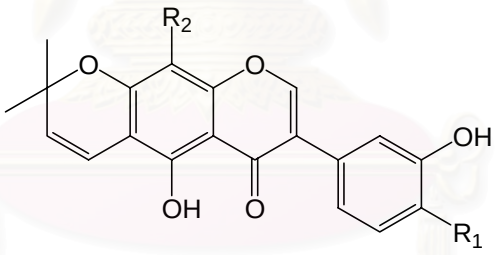


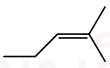
[**94**] R = OH
[**96**] R = OCH_3

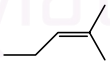


[**95**]

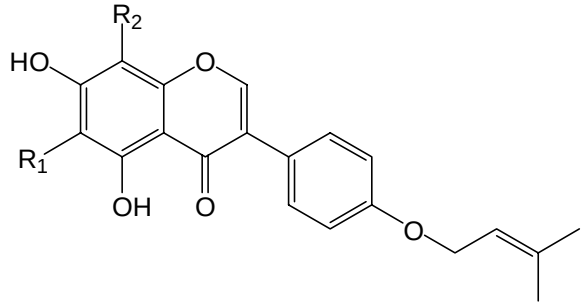
Auriculasin [**97**] and isoauriculasin [**98**] were isolated from the leaves of *M. auriculata* (Minhaj *et al*., 1976).



[**97**] R_1 = OH, R_2 = 

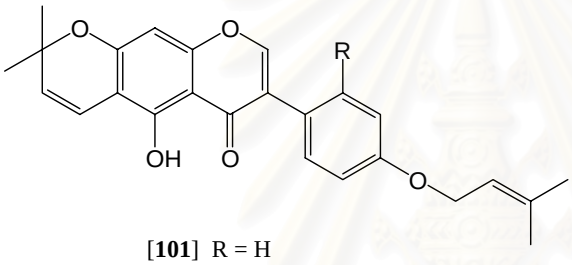
[**98**] $R_1 = R_2$ = 

A new isoflavone, aurmillone [**99**] was isolated from the seeds of *M. auriculata* (Raju and Srimannarayana, 1978), while Gupta's group isolated isoaurimillone [**100**] from the seed and pods (Gupta *et al*., 1983).

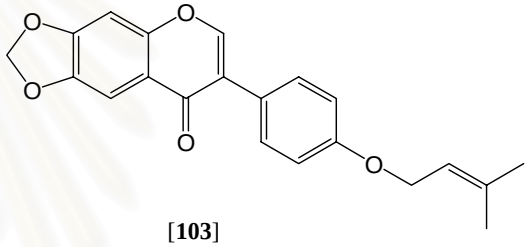


[**99**] R_1 = H, R_2 = OCH_3
[**100**] R_1 = OCH_3, R_2 = H

Three new prenylated flavones, 2′-deoxyisoauriculatin [**101**], 2′-*O*-methylisoauriculatin [**102**], and auricularin [**103**] were isolated from the roots of *M. auriculata* (Rao, Prasad and Ganapaty, 1992).

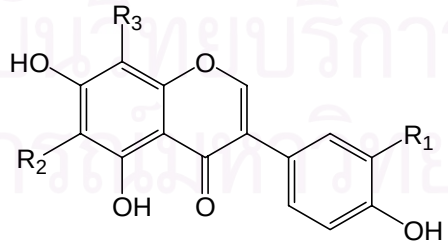


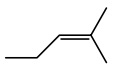
[**101**] R = H
[**102**] R = OCH_3

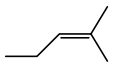


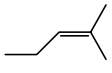
[**103**]

In 1980, Singhal *et al*. isolated the prenylated isoflavones, 6,8-diprenyl-5,7,4′-trihydroxyisoflavone [**104**], 6,3′-diprenyl-5,7,4′-trihydroxyisoflavone [**105**], and 6,8-diprenyl-5,7,3′,4′-tetrahydroxyisoflavone [**106**] from the aerial parts of *M. pachycarpa* (Singhal *et al*., 1980).

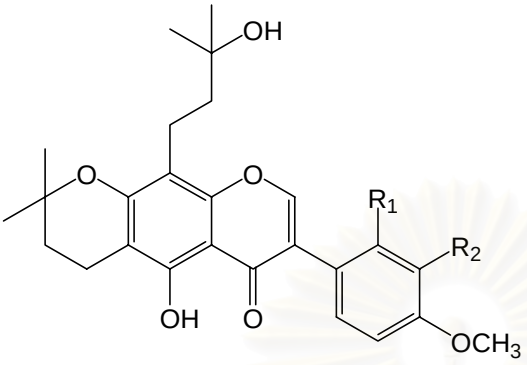


[**104**] R_1 = H, R_2 = R_3 = 

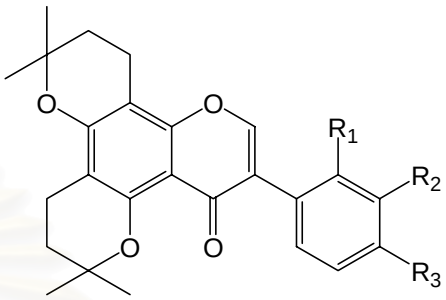
[**105**] R_3 = H, R_1 = R_2 = 

[**106**] R_1 = OH, R_2 = R_3 = 

In 1981, *M. pachycarpa* yielded four new prenylated 5-hydroxy-isoflavones with 3,3-dimethyl-3-hydroxypropyl group [**107**] and their isomers [**108**], [**109**] and [**110**] by Singhal's group (Singhal *et al*., 1981).

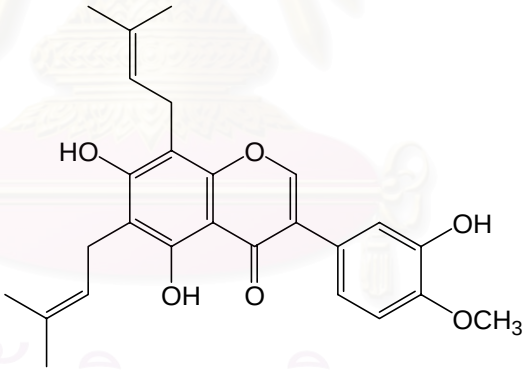


[**107**] R_1 = H, R_2 = OCH_3
[**108**] R_1 = OCH_3, R_2 = H



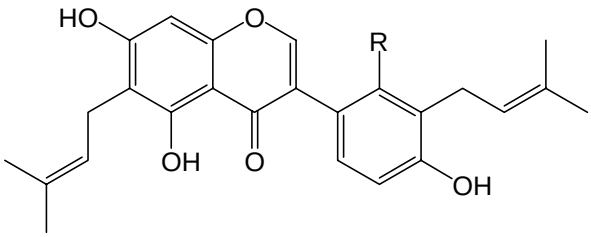
[**109**] R_1 = R_3 = H, R_2 = OCH_3
[**110**] R_1 = OH, R_2 = H, R_3 = OCH_3

In 1983, a new prenylated isoflavone, 6,8-diprenyl-5,7,3′-trihydroxy-4′-methoxyisoflavone [**111**] was isolated from the seeds of *M. pachycarpa* (Singhal *et al*., 1983).



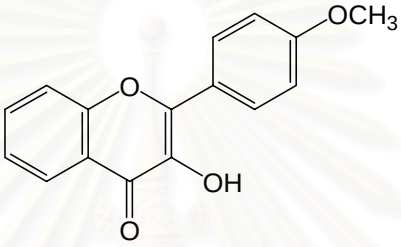
[**111**]

In 1984, two new prenylated isoflavones 6,3′-diprenyl-5,7,2′,4′-tetrahydroxyisoflavone [**112**], together with its 2′-*O*-methyl derivative [**113**] were isolated from *M. pluchra* (Baruah *et al*., 1984).



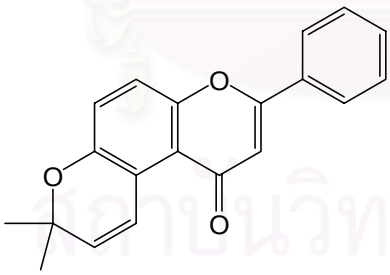
[**112**] R = OH
[**113**] R = OCH_3

In 1990, Parvez and Ogbeide isolated 3-hydroxy-4′-methoxyflavone [**114**] from the flowers of *M. zechiana* (Parvez and Ogbeide, 1990).

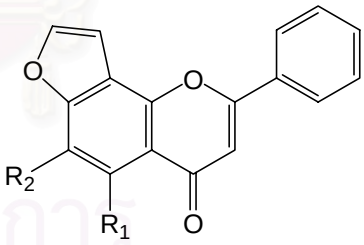


[**114**]

Four furanoflavones, sanaganone [**115**], lanceolatin B [**116**], kanjone [**117**], and 5-methoxyfurano [7,8:4′′,5′′] flavone [**118**] were isolated from the root bark of *M. sanagana* (Mbafor *et al*., 1995).

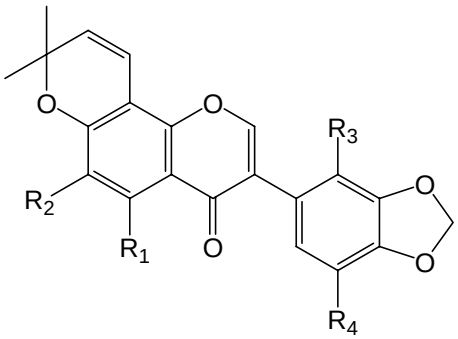


[**115**]



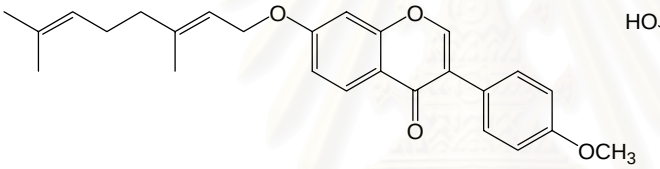
[**116**] $R_1 = R_2 = H$
[**117**] $R_1 = H$, $R_2 = OCH_3$
[**118**] $R_1 = OCH_3$, $R_2 = H$

In 1989, Dagne, Bekele, and Waterman isolated the isoflavones, 5-methoxydurimillone [**119**] and ferrugone [**120**] from the bark of *M. ferruginea* subsp. *ferruginea* (Dagne, Bekele and Waterman, 1989).

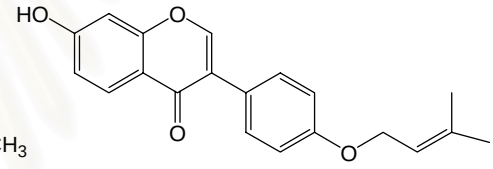


[**119**] $R_1 = R_2 = OCH_3$, $R_3 = R_4 = H$
[**120**] $R_1 = R_2 = H$, $R_3 = R_4 = OCH_3$

In 1990, 7-*O*-geranylformononetin [**121**] and nordurlettone [**122**] were isolated from the root bark of *M. ferruginea* subsp. *darassana* (Dagne *et al*., 1990).

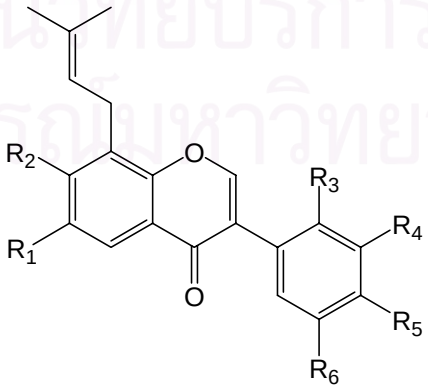


[**121**]



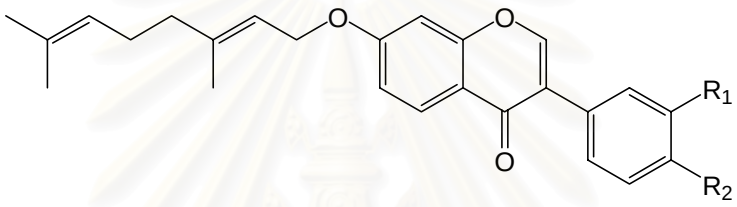
[**122**]

In 1990, Dagne and Bekele isolated the *C*-prenylated isoflavones, preferrugone [**123**], predurmillone [**124**], and prebarbigerone [**125**] from the root bark of *M. ferruginea* subsp. *darassana* (Dagne and Bekele, 1990).



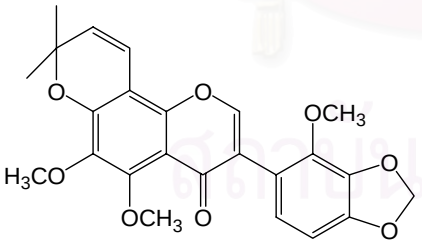
[**123**] $R_1 = H$, $R_2 = OH$, $R_3 = R_6 = OCH_3$, R_4, $R_5 = OCH_2O$
[**124**] $R_3 = R_6 = H$, $R_1 = OCH_3$, $R_3 = OH$, R_4, $R_5 = OCH_2O$
[**125**] $R_1 = R_4 = H$, $R_2 = R_3 = R_5 = R_6 = OCH_3$

An *O*-geranylated isoflavone was also isolated from *M. griffoniana*. In 1997, a new *O*-geranylated isoflavone, 7-*O*-[(E)-3,7-dimethyl-2,6-octadienyl] 3′,4′-methylenedioxyisoflavone [**126**] was isolated from the root bark of *M. griffoniana* (Yankep *et al*., 1997). In 1998, two new *O*-geranylated isoflavones, 4′-methoxy-7-*O*-[(E)-3-methyl-7-hydroxymethyl-2,6-octadienyl]isoflavone [**127**] and 3′,4′-dihydroxy-7-*O*-[(E)-3,7-dimethyl-2,6-octa- dienyl]isoflavone [**128**] were isolated from the root bark of *M. griffoniana* (Yankep *et al*., 1998).

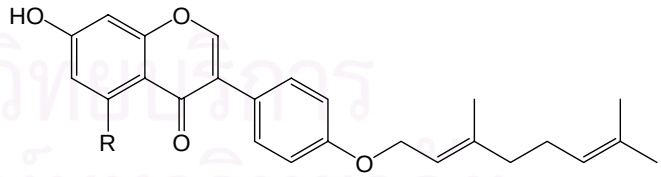


[**126**] R_1,R_2 = OCH_2O, R_3 = CH_3
[**127**] R_1 = H, R_2 = OCH_3, R_3 = CH_2OH
[**128**] R_1 = R_2 = OH, R_3 = CH_3
[**130**] R_1,R_2 = OCH_2O, R_3 = OH

Conrauinones A,B,C, and D [**129**-**132**] were isolated from the stem bark of *M. conraui* (Fuendjiep *et al*., 1998a; b).

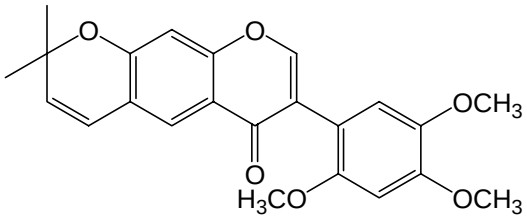


[**129**]

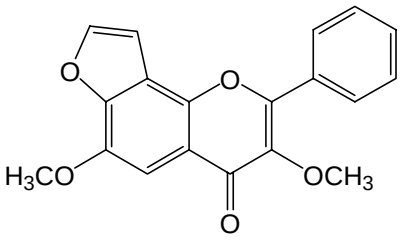


[**131**] R = OCH_3
[**132**] R = H

In 1998, Kamperdick *et al*. isolated 2′,4′,5′-trimethoxy-2′′,2′′-dimethylpyrano[5′′,6′′:6,7]isoflavone [**133**] and 3,6-dimethoxyfurano[4′′,5′′: 8,7] flavone [**134**] from the leaves and branches of *M. ichthyochtona* (Kamperdick *et al*., 1998).

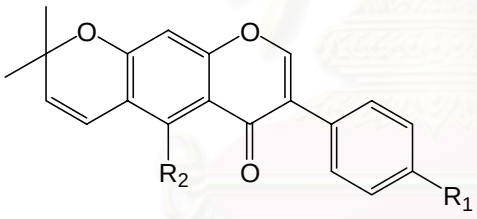


[**133**]

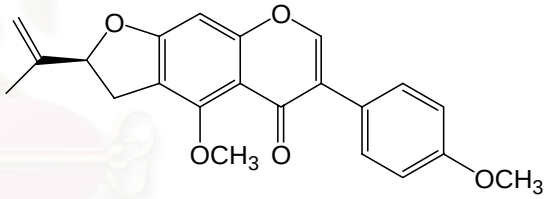


[**134**]

Chemical studies on *M. thonningii* have shown the presence of isoflavones. Olivares *et al.* isolated alpinum isoflavone [**135**] from seeds of *M. thonningii* (Olivares *et al*., 1982). Dimethylalpinumisoflavone [**136**] and 4′-methylalpinumisoflavone [**137**] were isolated from the seeds of *M. thonningii* (Khalid and Waterman, 1983). In 1995, Asomaning *et al*. isolated thonningin-isoflavone [**138**] from the roots of *M. thonningii* (Asomaning *et al*., 1995).

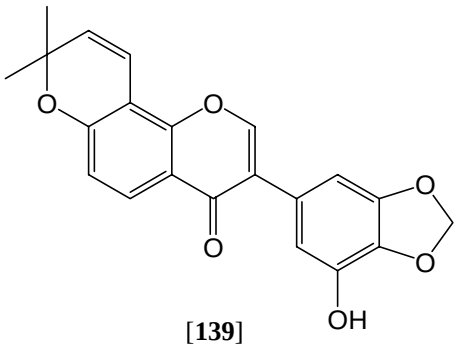


[**135**] $R_1 = R_2 = OH$
[**136**] $R_1 = R_2 = OCH_3$
[**137**] $R_1 = OH$, $R_2 = OCH_3$

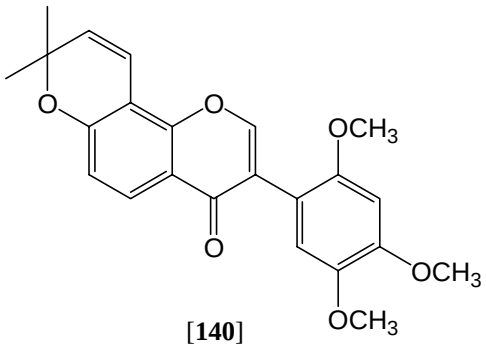


[**138**]

In 1998, norisojamicin [**139**] and barbigerone [**140**] were isolated from the stem bark of *M. usaramensis* subsp. *usaramensis* (Yenesew, Midiwo and Waterman, 1998).

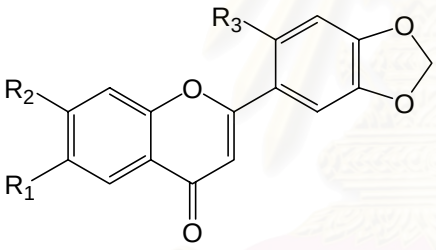


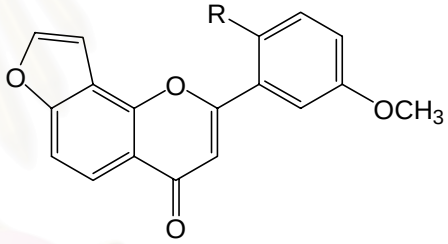
[**139**]



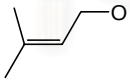
[**140**]

Three new flavones, millettocalyxins A-C [**141**-**143**] and the new natural product pongol methyl ether [**144**] were isolated from the stem bark of *M. erythrocalyx* (Sritularak *et al*., 2002b).





[**141**] R_1 = H, R_2 = R_3 = OCH_3

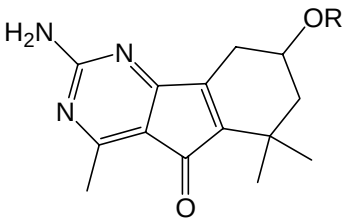
[**142**] R_1 =  , R_2 = OCH_3, R_3 = H

[**143**] R = OCH_3

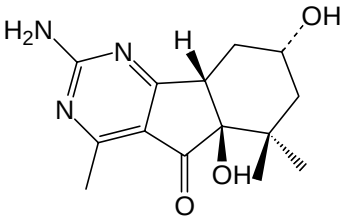
[**144**] R = H

2.5 Alkaloids

Millaurine [**145**], acetylmillaurine [**146**], and 5a,9a-dihydro-5a-hydroxymillaurine [**147**] were isolated from the seeds of *M. laurentii* (Ngamga, Free and Fomun, 1993 and 1994).

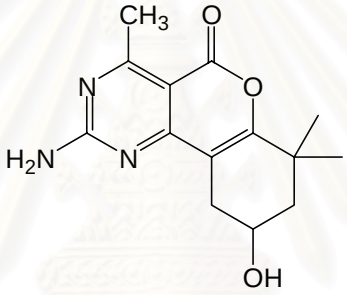


[**145**] R = H
[**146**] R = Ac



[**147**]

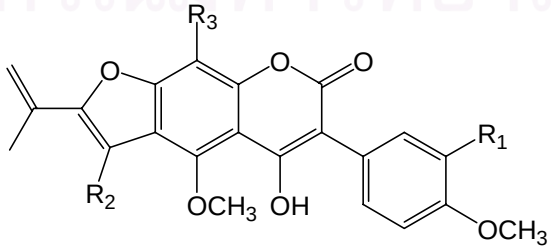
In 1994, Kamnaing *et al*. isolated millettonine [**148**] from the stem bark of *M. laurentii* (Kamnaing *et al*., 1994).



[**148**]

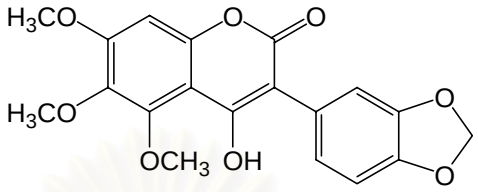
2.6 Coumarins

The novel 3-phenylcoumarins, thonningine A [**149**], thonningine B [**150**] and thonningine C [**151**] were isolated from seeds and roots of *M. thonningii* (Khalid and Waterman 1983; Asomaning *et al*., 1999).



[**149**] $R_1 = R_3 = OCH_3$, $R_2 = H$
[**150**] $R_3 = OCH_3$, $R_1 = R_2 = H$
[**151**] $R_1 = R_2 = OCH_3$, $R_3 = H$

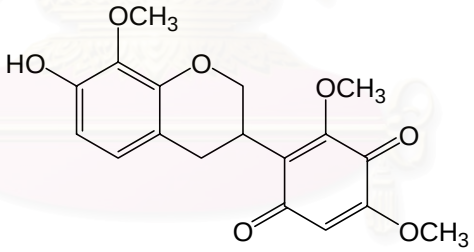
A new 3-phenylcoumarin, 4-hydroxy-5,6,7-trimethoxy-3-(3′,4′-methylenedioxy)phenylcoumarin [**152**] was isolated from the root bark of *M. griffoniana* (Yankep *et al*., 1998).



[**152**]

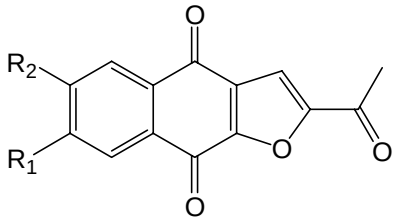
2.7 Quinones

In 1999, Kamnaing *et al*. isolated a new isoflavan-quinone, laurenti-quinone [**153**] from the wood of *M. laurentii* (Kamnaing *et al*, 1999).



[**153**]

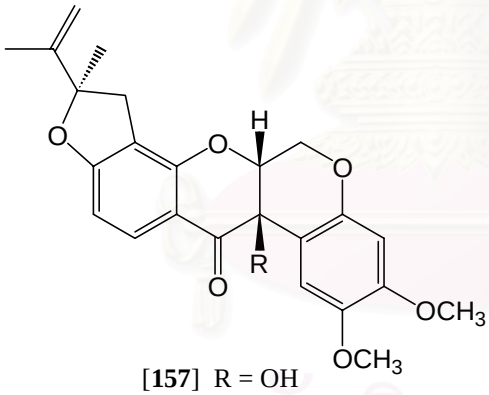
An anti-inflammatory furoquinone [**154**], along with two known furoquinone **155** and **156**, were isolated from the stem bark of *M. versicolor* (Fotsing *et al*., 2003).



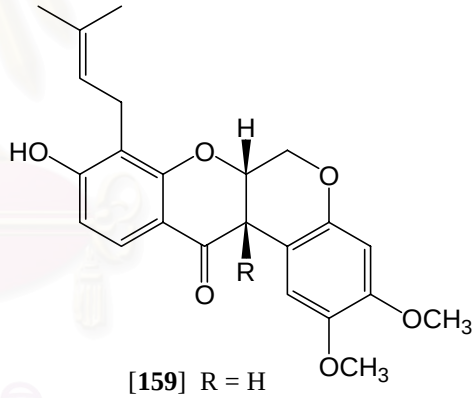
[**154**] R_1 = H, R_2 = OCH_3
[**155**] R_1 = R_2 = H
[**156**] R_1 = OCH_3, R_2 = H

2.8 Rotenoids

In 1982, Singhal *et al*. isolated a new rotenone, *cis*-12a-hydroxyrot-2-enoic acid [**157**] and three known compounds [**158-160**] from *M. pachycarpa* (Singhal *et al*., 1982).

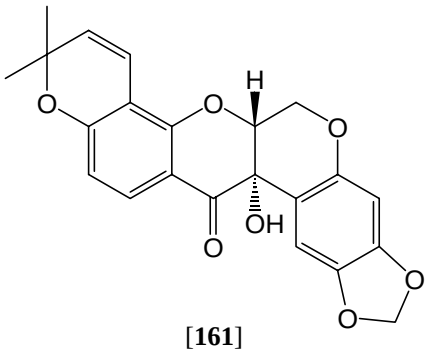


[**157**] R = OH
[**158**] R = H

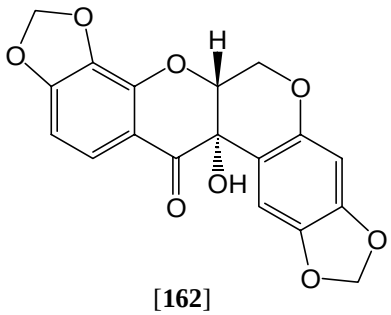


[**159**] R = H
[**160**] R =OH

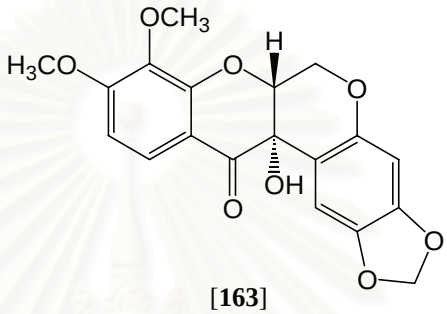
In 1998, (+)12a-epimillettosin [**161**], (+)-usararotenoid-A [**162**], and (+)-usararotenoid-B [**163**] were isolated from the stem bark of *M. usaramensis* (Yenesew, Midiwo and Waterman, 1998).



[**161**]

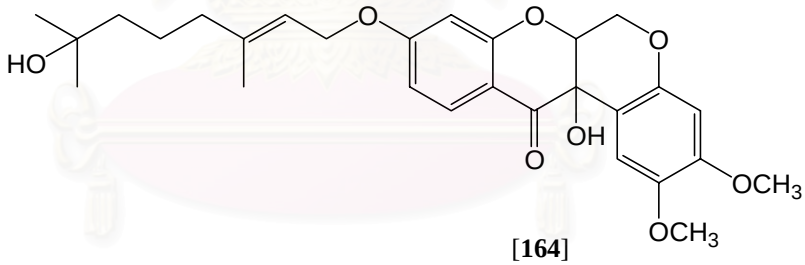


[**162**]



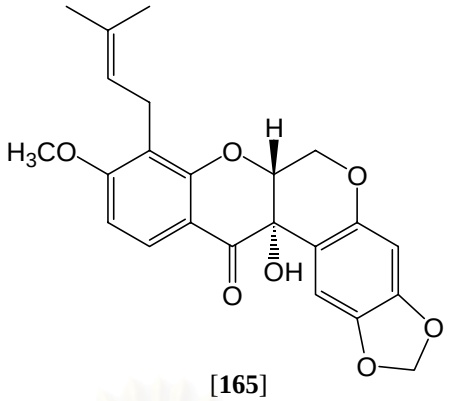
[**163**]

In 2001, a new rotenone, griffonianone [**164**] was isolated from the root bark of *M. griffoniana* (Yankep *et al*., 2001).



[**164**]

In 2003, Yenesew *et al*. isolated an anti-plasmodial rotenoid, usara-rotenoid C [**165**] from *M. usaramensis* subsp. *usaramensis* (Yenesew *et al*., 2003).



[**165**]

CHAPTER III

EXPERIMENTAL

1. Sources of Materials

The stem bark and roots of *Cratoxylum arborescens* (Vahl) Blume. and *Millettia decipiens* Prain. were collected from Cha-Nae District, Narathiwat Province, Southern of Thailand, in July 2002. Authentications were achieved by comparison with the herbarium specimen (BKF No. 084524) and (BKF No. 106940) at the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand. The voucher specimens have been deposited at herbarium of the Faculty of Pharmacutical Science, Chulalongkorn University, Bangkok, Thailand.

2. General Techniques

2.1 Thin-Layer Chromatography (TLC)

Technique	:	One dimension, ascending.
Adsorbent	:	Silica gel 60 F_{254} precoated on aluminium plate (E. Merck).
Layer thickness	:	0.2 mm
Plate size	:	2 x 5.0 and 5 x 5 cm
Detection	:	1. Under ultraviolet light at wavelengths of 254 and 365 nm.
		2. Dyeing reagents.
		2.1 Anisaldehyde-H_2SO_4 reagent. (0.5% ethanolic solution of anisaldehyde with 5% sulphuric acid). Stained TLC plates give specific color spots with this reagent after heating at 80-100˚ C for 2-3 minutes.

2.2 Column Chromatography

2.2.1 Vacuum Liquid Column Chromatography

Adsorbent	:	a. Silica gel 60 (No. 7734) particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck). b. Silica gel 60 (No. 9385) particle size 0.040-0.063 nm (70-230 mesh ASTM) (E. Merck).
Packing method	:	Dry packing method.
Sample loading	:	A sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried, and placed on the top of column.

2.2.2 Flash Column Chromatography

Adsorbent	:	a. Silica gel 60 (No. 7734) particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck). b. Silica gel 60 (No. 9385) particle size 0.040-0.063 nm (70-230 mesh ASTM) (E. Merck).
Packing method	:	Slurry method.
Sample loading	:	A portion of sample was dissolved in a small amount of organic solvent and added to a small quantity of silica gel 60 with particle size 0.063-0.200 nm, air dried and added onto the top of this column, for further elution.

2.3 Spectroscopy

2.3.1 Infrared (IR) Absorption Spectra

IR spectra (KBr disc and neat film) were obtained on a Perkin Elmer BX spectrophotometer (Department of Chemistry, Faculty of Science, Srinakharinwirot University).

2.3.2 Ultraviolet (UV) Absorption Spectra

UV (in methanol) spectra were recorded on a Shimadzu UV-2401 PC spectrophotometer (Department of Chemistry, Faculty of Science, Srinakharinwirot University).

2.3.3 Mass Spectra

Electrospray ionization mass spectra (ESIMS), HRESIMS and HRFABMS were measured on a Bruker BiO TOF II (Department of Chemistry University of Minnesota, USA).

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

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a BRUKER AV300 spectrometer (Department of Chemistry, Faculty of Science, Srinakharinwirot University).

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a BRUKER AV400 spectrometer (Department of Chemistry, Faculty of Science, Ramkhamhaeng University).

2.4 Physical Properties

2.4.1 Optical rotations

Optical rotations were recorded in chloroform on a Perkin-Elmer 341 polarimeter. (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.4.2 Circular Dichroism (CD) Spectra

CD spectra were recored on a JASCO J-715 spectropolarimeter (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5 Solvents

Column chromatography	:	All solvents are of commercial grade and are redistilled prior to use.
NMR	:	All deuterated solvents are NMR grade.

3. Extraction and Isolation

3.1 Extraction and Isolation of Compounds from the stem bark of *Cratoxylum arborescens*

3.1.1 Extraction

The dried, powdered stem bark (5 kg) was successively macerated with chloroform ($CHCl_3$, 3 x 25 L) and then 95% ethanol (EtOH, 3 x 25 L). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40 ^{o}C to afford the corresponding chloroform (250 g) and ethanol (120 g) extracts, respectively.

3.1.2 Isolation of Compounds from the chloroform extract

The chloroform extract (75 g) was fractionated by vacuum liquid column chromatography using a sintered glass column of silica gel (No. 7734, 350 g). Elution was performed in a polarity gradient manner with mixture of hexane and EtOAc (90:10 to 0:100). The eluates were collected (500 ml per fraction) and examined by TLC (silica gel, EtOAc-hexane 1:3). Fraction (45 fractions) with similar chromatographic pattern were combined to yield seven fractions, CA-1 to CA-7.

3.1.3 Isolation of Compound CT-01

Fraction CA-1 was purified by column chromatography (silica gel 60 No. 9385, 200 g); 5% EtOAc in hexane. Thirty fractions (50 ml per fraction) were collected and combined according to their TLC behavior (silica gel, EtOAc-hexane 1:8) to afford compound CT-01 as an orange solid (60 mg). This compound was subsequently identified as 1,8-dihydroxy-3-geranyloxy-6-methylanthraquinone [**166**].

3.1.4 Isolation of Compound CT-02

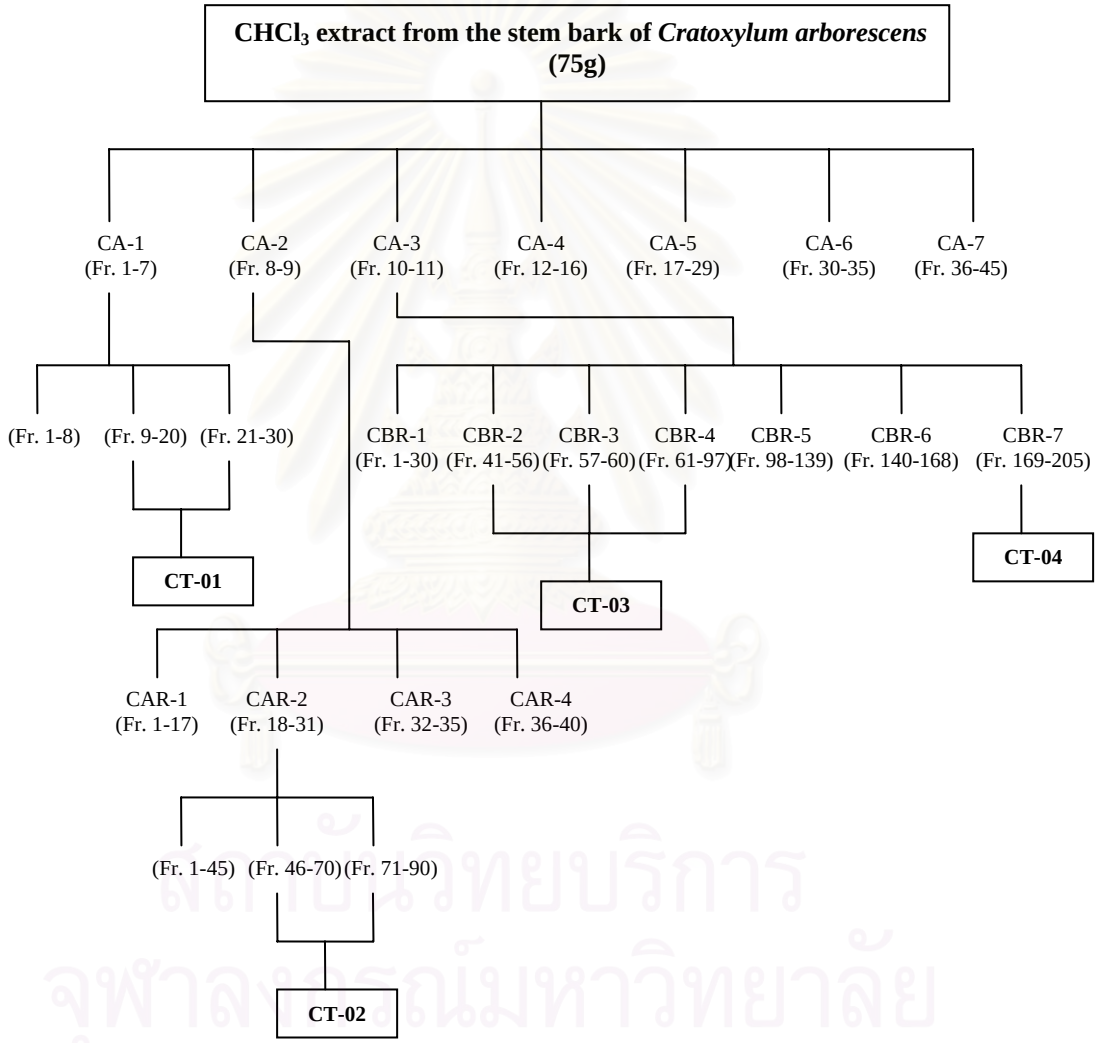
Fraction CA-2 was purified by column chromatography (silica gel 60 No. 9385, 300 g); 10% EtOAc in hexane. Forty fractions (50 ml per fraction) were collected and combined according to their TLC behavior (silica gel, EtOAc-hexane 1:5) to give four fractions (CAR-1 to CAR-4). Fraction CAR-2 was re-chromatographed on a silica gel 60 (No. 9385, 100 g), 10% EtOAc in hexane) to afford compound CT-02 as a yellow powder (80 mg). Compound CT-02 was identified as fuscaxanthone C [**167**].

3.1.5 Isolation of Compounds CT-03 and CT-04

Fraction CA-3 was subjected to flash column chromatography (silica gel 60 No. 7734, 300 g) gradient 10% - 50% EtOAc in hexane. Two hundred and five fractions were obtained and combined according to their TLC behavior (silica gel, EtOAc-hexane 1:5) to give seven fractions (CBR-1 to CBR-7). Fractions CBR-2 to CBR-4 were combined and re-purified on silica gel 60 (No. 9385, 100g) column. Gradient elution (10% EtOAc in hexane to 15%

EtOAc in hexane) to afford of compound CT-03 (120 mg) as a brown solid. This compound was identified as 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone [**168**].

Fractions CBR-7 were combined and re-chromatographed on silica gel 60 (No. 9385, 100 g) column. Gradient elution (15% EtOAc in hexane to 20% EtOAc in hexane) afforded compound CT-04 as orange needles (10 mg). Compound CT-04 was identified as a 2-geranylemodin [**169**].



Scheme 1. Separation of the chloroform extract from the stem bark of *Cratoxylum arborescens*

3.2 Extraction and Isolation of Compounds from the roots of *Cratoxylum arborescens*

3.2.1 Extraction

The dried roots of *Cratoxylum arborescens* (5 kg) were chopped, ground, and macerated with chloroform (3 x 25 L). The filtrates were pooled and evaporated *in vacuo* until dryness to yield a syrupy mass (120 g).

3.2.2 Isolation of Compounds from chloroform extract

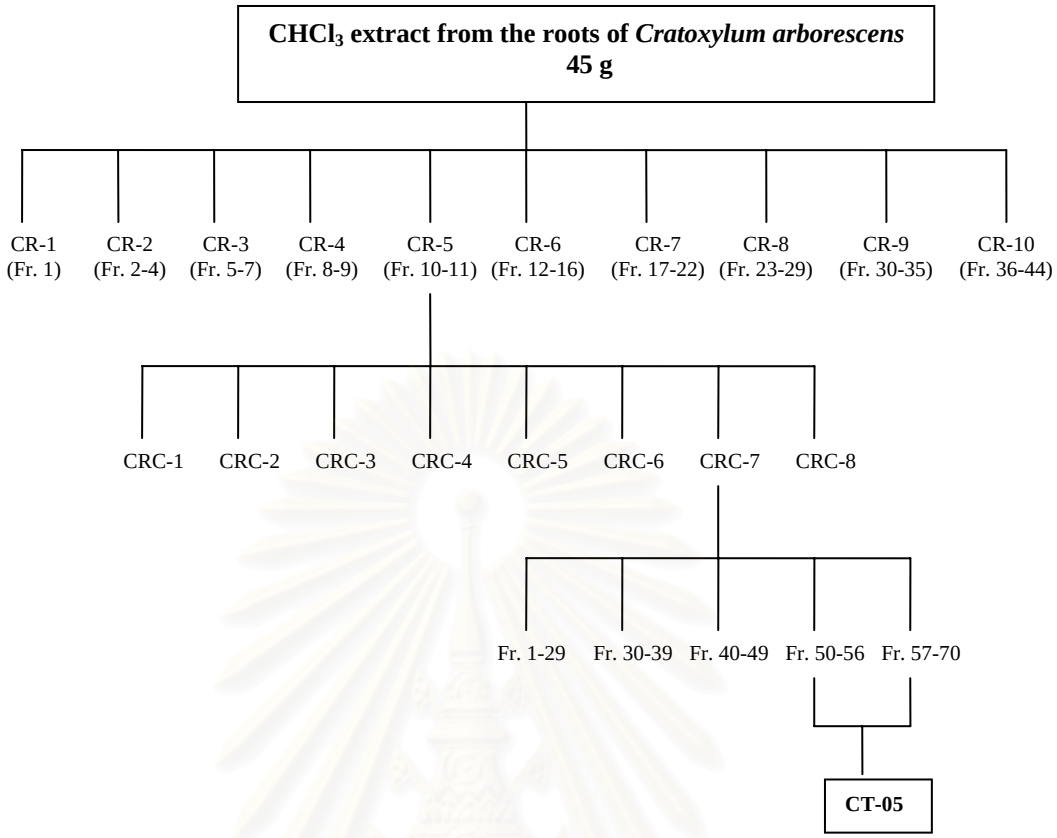
The chloroform extract (45 g) was fractionated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (No. 7734, 100 g). Successive gradient elution with mixture of EtOAc-hexane. The eluates were collected (500 ml per fraction) and examined by TLC (silica gel, EtOAc-hexane 1:3). Fractions (44 fractions) with similar chromatographic pattern were combined to yield ten fractions: fractions CR-1 to CR-10.

Fractions CR-1 to CR-4 and CR-6 were purified by column chromatography to afford compounds CT-01, CT-02, CT-03, and CT-04.

3.2.3 Isolation of Compound CT-05

Fraction CR-05 was purified by column chromatography (silica gel 60 No. 9385, 100 g); 5% EtOAc in hexane. Two hundred and sixty three fractions (10 ml per fraction) were collected and combined according to their TLC behavior (silica gel, EtOAc-hexane 1:5) to give eight fractions (CRC-1 to CRC-8).

Fraction CRC-7 was re-chromatographed on a silica gel 60 No. 9385, 50 g) column. Gradient elution (5% EtOAc in hexane to 10% EtOAc in hexane) to afford compound CT-05 as a white solid, 10 mg. This compound was identified as 1,7-dihydroxyxanthone [**17**].



Scheme 2. Separation of the chloroform extract from the roots of *Cratoxylum arborescens*

3.3 Extraction and Isolation of Compounds from the stem bark of *Millettia decipiens*

3.3.1 Extraction

The dried, powdered stem bark of *Millettia decipiens* (6 kg) was extracted with chloroform (3 x 25 L), filtered and evaporated, yielding dark brown gum (250g).

3.3.2 Isolation of Compounds from chloroform extract

The chloroform extract (150g) was fractionated by vacuum liquid column chromatography using a sintered glass filter column of silica gel (No. 7734, 500 g). Elution was performed in a polarity gradient manner with a mixture of pet. ether and EtOAc (60:40 to 0:100). The eluates were collected

(1000 ml per fraction) and examined by TLC (silica gel, EtOAc-pet. ether 2:5). Fractions (17 fractions) with similar chromatographic pattern were combined to yield five fractions : fractions ME-1 to ME-5.

3.3.3 Isolation of Compound MD-01

Compound MD-01 was crystallized from the mother liquor of fractions ME-02 to ME-03. Recrystallization with EtOAc and pet. ether to afford compound MD-01 (28 g). This compound was identified as 3′,4′-methylenedioxy -2,4,6,β-tetramethoxychalcone [**54**]

3.3.4 Isolation of Compound MD-02

Fraction ME-02 was fractionated by column chromatography using silica gel (No. 7734, 300 g). Elution was performed in a polarity gradient manner with mixture of pet. ether and EtOAc (95:5 to 75:25). The eluates were collected 500 ml per fraction and examined by TLC (silica gel, EtOAc-pet. ether 2:5). Fraction (24 fractions) with similar chromatographic pattern were combined to yield eight fractions: fractions MEA-1 to MEA-8.

Fraction MEA-04 was re-chromatographed on a silica gel (No. 7734, 200 g) column. Gradient (5% EtOAc in pet. ether to 20% EtOAc in pet. ether). The eluates were collected (20 ml per fraction) and examined by TLC (silica gel, EtOAc-pet. ether 2:5). Fractions (670 fractions) with similar chromatographic pattern were combined to yield six fractions MEAB-1 to MEAB-6.

Fraction MEAB-1 was re-chromatographed on a silica gel 60 (No. 9385, 100 g) column. Gradient (3% EtOAc in pet. ether to 7% EtOAc in pet. ether) to afford compound MD-02 as a white solid (80 mg). This compound was identified as lanceolatin B [**116**].

3.3.5 Isolation of Compound MD-03

Fraction MEAB-6 was re-chromatographed on a silica gel 60 (No. 9385, 80 g) column. Gradient elution (10% EtOAc in pet. ether to 15%

EtOAc in pet. ether) afforded compound MD-03 as a white solid (50 mg). This compound was identified as pongaflavone [**170**].

3.3.6 Isolation of Compounds MD-04 and MD-07

Fraction MEAB-3 was re-chromatographed on silica gel 60 (No. 9385, 150 g) column. Gradient (5% EtOAc in pet. ether to 10% EtOAc in pet. ether) to afford compound MD-04 as a white solid (23 mg) from fractions 1-16 and 17-50. This compound was identified as karanjin [**171**]. Compound MD-07 was purified from fractions 51-97 and 98-135 as a white solid (832 mg). This compound was identified as dihydroisomilletenone methyl ether [**48**].

3.3.7 Isolation of Compounds MD-05 and MD-09

Fractions MEAB-4 and MEAB-5 were combined and re-chromatographed on silica gel 60 (No. 9385, 150 g) column. Gradient elution (7% EtOAc in pet. ether to 15% EtOAc in pet. ether) afforded compound MD-05 as yellow crystals (40 mg) and compound MD-09 as a brown amorphous solid (40 mg). Compound MD-05 was identified as milletenone [**46**] and compound MD-09 was identified as dihydromilletenone methyl ether [**47**].

3.3.8 Isolation of Compound MD-10

Fraction MEAB-2 was re-chromatographed on silica gel 60 (No. 9385, 80 g) column. Gradient elution (3% EtOAc in pet. ether to 7% EtOAc in pet. ether) afforded compound MD-10 as a white solid (60 mg). This compound was identified as 2,4-dimethoxy-3′,4′-methylenedioxydihydrochalcone [**174**]. This is the first isolation of compound MD-10 [**174**] from a natural source.

3.3.9 Isolation of Compound MD-06

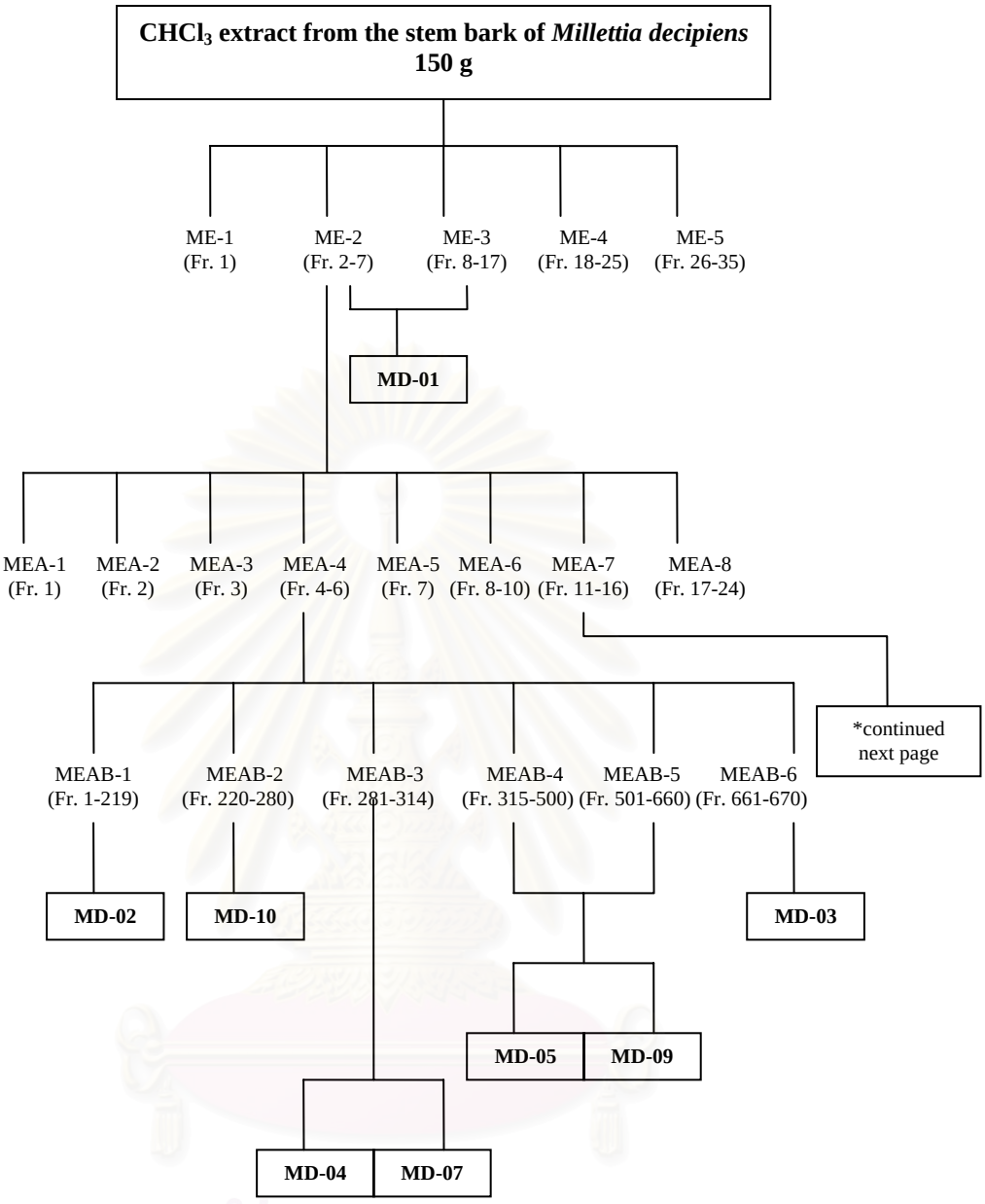
Fraction MEA-7 was re-chromatographed on silica gel (No. 7734, 150 g) column. The eluate from the gradient elution (10% EtOAc in pet. ether to 25% EtOAc in pet. ether) were collected (20 ml per fraction) and examined by TLC (silica gel, EtOAc-pet. ether 2:5). Fractions (112 fractions) with a similar chromatographic pattern were combined to yield nine fractions MEAC-1 to MEAC-9.

Fraction MEAC-8 was re-chromatographed on a silica gel 60 (No. 9385, 85 g) column. Gradient elution (15% EtOAc in pet. ether to 25% EtOAc in pet. ether) afforded compound MD-06 as a white solid (50 mg). This compound was identified as desmethoxykanugin [**172**].

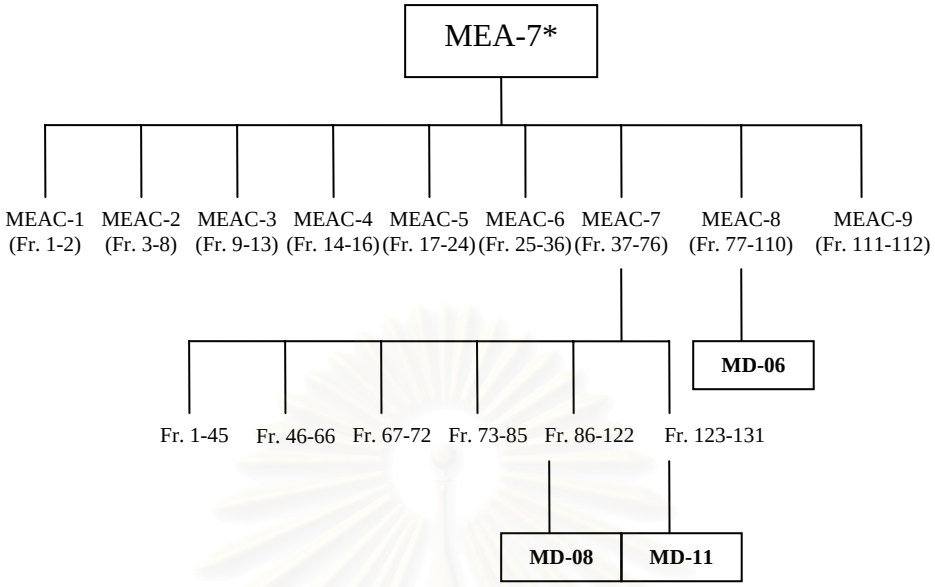
3.3.10 Isolation of Compounds MD-08 and MD-11

Fraction MEAC-7 was re-chromatographed on a silica gel 60 (No. 9385, 90 g) column. Gradient elution (15% EtOAc in pet. ether to 25% EtOAc in pet. ether) afforded compound MD-08 as a yellow powder (30 mg). This compound was identified as 3,4-methylenedioxy-2′,4′,6′,*β*-tetramethoxydihydro- chalcone [**173**]. This is the first isolation of compound MD-08 [**173**] from a natural source.

Compound MD-11 was isolated from fr. 123-131 as a white solid (5 mg). This compound was identified as 3′,4′-methylenedioxy-(2′′,3′′:7,8) furano flavone or pongaglabrone[**175**].



Scheme 3. Separation of the chloroform extract from the stem bark of *Millettia decipiens*



Scheme 3. Separation of the chloroform extract from the stem bark of *Millettia decipiens* (continued).

3.4 Extraction and Isolation of Compounds from the roots of *Millettia decipiens*

3.4.1 Extraction

The dried roots of *Millettia decipiens* (5 kg) were chopped, ground, and macerated with chloroform (3 x 30L). The filtrates were pooled and evaporated *in vacuo* to yield a brown syrupy mass (150 g).

3.4.2 Isolation of Compounds from chloroform extract

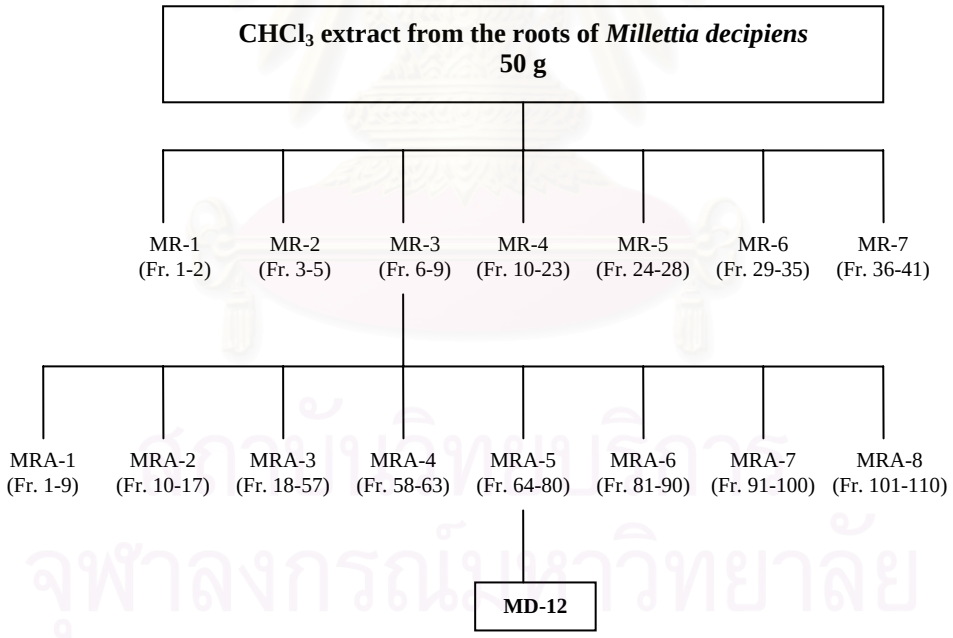
The chloroform extract (50 g) was fractionated by vacuum liquid column chromatogarphy using a sintered glass filter column of silica gel (No. 7734, 500 g). Successive gradient elution with mixtures of EtOAc-pet. ether. The eluates were collected (500 ml per fraction) and examined by TLC (silica gel, EtOAc-pet. ether 2:5). Fractions (41 fractions) with similar chromatographic patterns were combined to yield 7 fractions : fractions MR-1

to MR-7. Fractions MR-3 to MR-6 were purified by column chromatography to afford compounds MD-01 to MD-11.

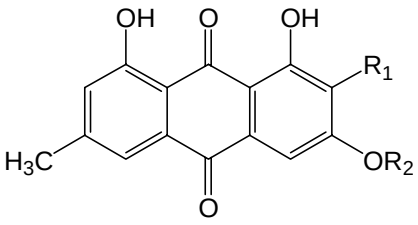
3.4.3 Isolation of Compound MD-12

Fraction MR-3 was re-chromatographed on silica gel (No. 7734, 150 g) column. The eluates from the gradient elution (5% EtOAc in pet. ether to 20% EtOAc in pet. ether) were collected (20 ml per fraction) and examined by TLC (silica gel, EtOAc-pet. ether, 2:5). Fractions (110 fractions) with similar chromatographic patterns were combined to yield eight fractions: fractions MRA-1 to MRA-8.

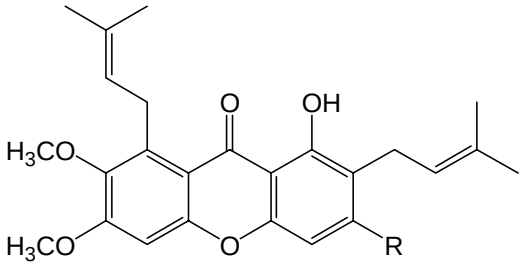
Fraction MRA-5 was rechromatographed on a silica gel 60 (No. 9385, 80 g) column. Gradient elution (5% EtOAc in pet. ether to 10% EtOAc in pet. ether) afforded compound MD-12 as a brown amorphous solid (60 mg). This compound was identified as ovalitenin B [**41**].



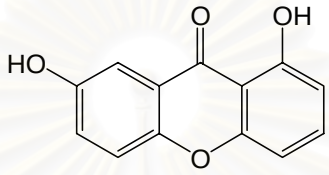
Scheme 4. Separation of the chloroform extract from the roots of *Millettia decipiens*



[**166**] R_1 = H, R_2 = geranyl
[**169**] R_1 = geranyl, R_2 = H

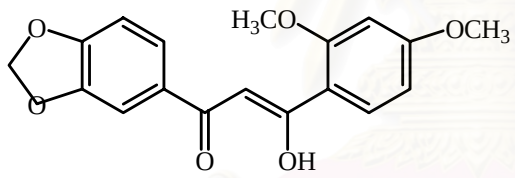


[**167**] R = OCH_3
[**168**] R = OH

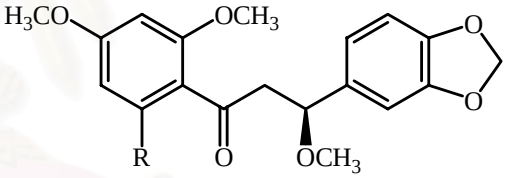


[**17**]

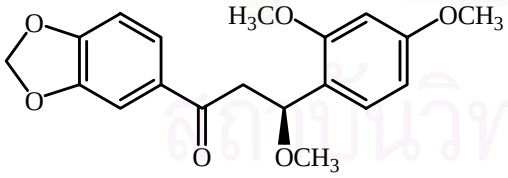
Figure 3. Structure of the Compounds isolated from *Cratoxylum arborescens*



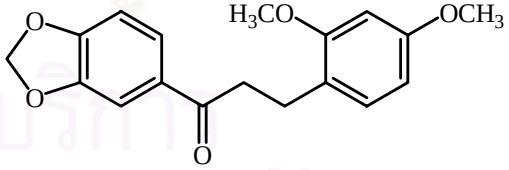
[**46**]



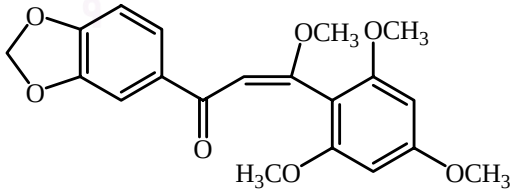
[**47**] R = H
[**173**] R = OCH_3



[**48**]



[**174**]



[**54**]

Figure 4. Structure of the Compounds isolated from *Millettia decipiens*

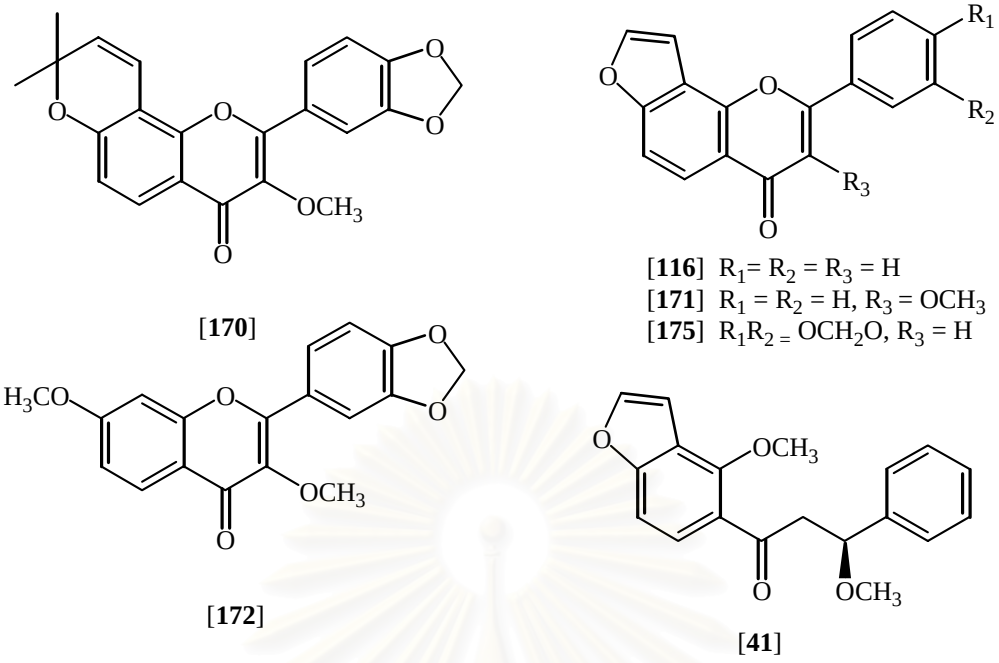


Figure 4. Structures of the Compounds isolated from *Millettia decipiens* (continued)

4 Physical and Spectral Data of the Isolated Compounds

4.1 Compound CT-01

Compound CT-01 was obtained as an orange solid, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 287 (4.36), 436 (4.20); Figure 5

IR : ν_{max} cm^{-1}, KBr; 2915, 1677, 1631, 1562, 1480; Figure 6

EIMS : *m/z* (% relative intensity); 406 (M^+, 19), 270 (89), 242 (8), 241 (9), 93 (32), 69 (100); Figure 13

HRESI : 429.16822 $[M+Na]^+$, (calcd. for $C_{25}H_{26}O_5Na$, 429.16724)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 1, Figure 7

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 1, Figure 8

4.2 Compound CT-02

Compound CT-02 was obtained as a yellow solid, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 269 (4.12), 312 (4.39), 347 (3.83) Figure 14

IR : ν_{max} cm^{-1}, KBr; 2923, 1648, 1598, 1458; Figure 15

EIMS : *m/z* (% relative intensity); 438 (M^+, 90), 395 (70), 383 (56), 382 (46), 367 (100), 339 (28), 313 (17); Figure 22

HRESI :461.19276 $[M+Na]^+$, (calcd. for $C_{26}H_{30}O_6Na$, 461.19345)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 2, Figure 16

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 2, Figure 17

4.3 Compound CT-03

Compound CT-03 was obtained as a brown solid, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 270 (2.7), 314 (5.0), 350sh (4.4); Figure 23

IR : ν_{max} cm^{-1}, KBr; 3419, 2974, 2953, 1651, 1608, 1455, 1275; Figure 24

EIMS : *m/z* (% relative intensity); 424 (M^+, 100), 381 (91), 353 (86), 337 (33); Figure 33

HRESI : 447.17786 $[M+Na]^+$, (calcd. for $C_{25}H_{28}O_6Na$, 447.17780)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 3, Figure 25

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 3, Figure 26

4.4 Compound CT-04

Compound CT-04 was obtained as orange crystals, soluble in DMSO

UV : λ_{max} nm (log ε), in CH_3OH; 283 (4.11), 438 (3.79); Figure 34

IR : ν_{max} cm^{-1}, KBr; 3374, 2924, 1667, 1616, 1587, 1476, 1420; Figure 35

EIMS : *m/z* (% relative intensity); 406 (M^+, 20), 363 (16), 337 (100), 295 (29), 284 (83), 283 (80), 123 (62), 69 (50); Figure 44

HRESI : 429.16866 $[M+Na]^+$, (calcd. for $C_{25}H_{46}O_5Na$, 429.16724)

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Table 4, Figure 36

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Table 4, Figure 37

4.5 Compound CT-05

Compound CT-05 was obtained as a white solid, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 202 (4.3), 234 (4.42), 259 (4.47), 287 (4.51); Figure 45

IR : ν_{max} cm^{-1}, KBr; 3311, 2921, 1638, 1607, 1578, 1479; Figure 46

EIMS : *m/z* (% relative intensity); 228 (M^+, 100), 200 (20), 172 (7), 144 (10), 115 (21); Figure 53

^{1}H NMR : δ ppm, 300 MHz, in acetone-d_6; Table 5, Figure 47

^{13}C NMR : δ ppm, 75 MHz, in acetone-d_6; Table 5, Figure 48

4.6 Compound MD-01

Compound MD-01 was obtained as pale yellow needles, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 271 (4.08), 320 (4.15); Figure 54

IR : ν_{max} cm^{-1}, KBr; 2942, 1660, 1581, 1504; Figure 55

EIMS : *m/z* (% relative intensity); 372 (M^+, 1), 341 (100), 311 (3), 195 (3), 149 (12), 121 (4); Figure 63

HRESI : 395.11106 $[M+Na]^+$, (calcd. for $C_{20}H_{20}O_7Na$, 395.11012)

^1H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 6, Figure 56

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 6, Figure 57

4.7 Compound MD-02

Compound MD-02 was obtained as white solids, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 273 (3.64), 327 (3.42); Figure 64

IR : ν_{max} cm^{-1}, KBr; 2936, 1628, 1591, 1440, 1397; Figure 65

EIMS : *m/z* (% relative intensity); 262 (M^+, 94), 234 (53), 160 (93), 132 (66), 76 (100); Figure 72

^1H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 7, Figure 66

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 7, Figure 67

4.8 Compound MD-03

Compound MD-03 was obtained as a white solid, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 282 (4.36), 326 (4.20); Figure 73

IR : ν_{max} cm^{-1}, KBr; 2974, 2930, 1628, 1483, 1440; Figure 74

EIMS : *m/z* (% relative intensity); 334 (M^+, 48), 333 (51), 219 (9), 187 (21), 159 (10), 105 (7), 77 (14); Figure 81

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 8, Figure 75

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 8, Figure 76

4.9 Compound MD-04

Compound MD-04 was obtained as a white solid, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 280 (4.12), 305 (4.36); Figure 82

IR : ν_{max} cm^{-1}, KBr; 1631, 1570, 1459, 1406, 1164; Figure 83

EIMS : *m/z* (% relative intensity); 293 (M^+, 6), 292 (35), 291 (100); Figure 90

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 9, Figure 84

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; see Table 9, Figure 85

4.10 Compound MD-05

Compound MD-05 was obtained as yellow crystals, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 275 (3.89), 314 (4.09), 370(4.47); Figure 91

IR : ν_{max} cm^{-1}, KBr; 1616, 1507, 1454, 1242; Figure 92

EIMS : *m/z* (% relative intensity); 328 (M^+, 34), 297 (55), 165 (100), 149 (34), 122 (12); Figure 102

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 10, Figure 93

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 10, Figure 95

4.11 Compound MD-06

Compound MD-06 was obtained as a white solid, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 314 (4.40), 339 (4.31); Figure 108

IR : ν_{max} cm^{-1}, KBr; 1619, 1500, 1448, 1387, 1251; Figure 109

EIMS : *m/z* (% relative intensity); 326 (M^+, 63), 325 (100), 253 (8); Figure 115

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 11, Figure 110

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 11, Figure 111

4.12 Compound MD-07

Compound MD-07 was obtained as a white solid, soluble in $CHCl_3$

$[\alpha]_D^{28}$: +46.3 ^{o} (*c* 0.3; $CHCl_3$)

UV : λ_{max} nm (log ε), in CH_3OH; 277 (4.08), 308 (4.08); Figure 116

CD : (*c* 0.3; $CHCl_3$): $[\theta]_{262.5}$ +345, $[\theta]_{281}$ +2744, $[\theta]_{305.5}$ -668 Figure 125

IR : ν_{max} cm^{-1}, KBr; 1667, 1602, 1500, 1449, 1358, 1269; Figure 117

EIMS : *m/z* (% relative intensity); 344 (M^+, 2), 281 (3), 181 (100), 149 (17), 121 (8); Figure 124

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 12, Figure 118

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 12, Figure 119

4.13 Compound MD-08

Compound MD-08 was obtained as a yellow solid, soluble in $CHCl_3$

$[\alpha]_D^{28}$: +37.3 ^{o} (*c* 0.3; $CHCl_3$)

UV : λ_{max} nm (log ε), in CH_3OH; 285 (4.13); Figure 126

CD : (*c* 0.3; $CHCl_3$): $[\theta]_{269.5}$ -1828, $[\theta]_{282}$ -895, $[\theta]_{298}$ -2671; Figure 139

IR : ν_{max} cm^{-1}, KBr; 1681, 1607, 1488, 1416, 1276, 1125; Figure 127

EIMS : *m/z* (% relative intensity); 374 (M^+, no observed), 359 (10), 314 (13), 195 (100), 165 (15), 149 (6); Figure 138

HRFAB : 375.14439 $[M+H]^+$, (calcd. for $C_{20}H_{23}O_7$, 375.14433)

^{1}H NMR : δ ppm, 400 MHz, in $CDCl_3$; Table 13, Figure 128

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$; Table 13, Figure 129

4.14 Compound MD-09

Compound MD-09 was obtained as a brown amorphous solid, soluble in $CHCl_3$

$[\alpha]_D^{28}$: $+46.0^o$ (*c* 0.3; $CHCl_3$)

UV : λ_{max} nm (log ε), in CH_3OH; 292 (4.38), 305 (4.30), 370(3.60); Figure 140

CD : (*c* 0.3: $CHCl_3$): $[\theta]_{232.5}$ +4151, $[\theta]_{262.5}$ -2324, $[\theta]_{280}$ -1517, $[\theta]_{297.5}$ -3000 Figure 149

IR : ν_{max} cm^{-1}, KBr; 2935, 1659, 1601, 1441, 1214, 1159; Figure 141

EIMS : *m/z* (% relative intensity); 344 (M^+, no observed), 329 (29), 165 (100), 149 (23); Figure 148

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 14, Figure 142

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 14, Figure 143

4.15 Compound MD-10

Compound MD-10 was obtained as a white solid, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 276 (3.80), 308 (3.90); Figure 150

IR : ν_{max} cm^{-1}, KBr; 1677, 1614, 1587, 1502, 1432, 1370, 1211; Figure 151

EIMS : *m/z* (% relative intensity); 314 (M^+, 14), 296 (5), 165 (2), 151 (100), 149 (24), 121 (54); Figure 162

HRFAB : 315.12323 $[M+H]^+$, (calcd. for $C_{18}H_{19}O_5$, 315.12326)

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 15, Figure 152

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 15, Figure 153

4.16 Compound MD-11

Compound MD-11 was obtained as a white solid, soluble in $CHCl_3$

UV : λ_{max} nm (log ε), in CH_3OH; 224 (4.58), 240 (4.52), 328 (4.47); Figure 163

IR : ν_{max} cm^{-1}, KBr; 2921, 1640, 1592, 1502, 1449, 1347, 1256; Figure 164

EIMS : *m/z* (% relative intensity); 306 (M^+, 100), 160 (11), 146 (15), 145 (36); Figure 170

^{1}H NMR : δ ppm, 300 MHz, in $CDCl_3$; Table 16, Figure 165

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Table 16, Figure 166

4.17 Compound MD-12

Compound MD-12 was obtained as a brown amorphous solid, soluble in $CHCl_3$

$[\alpha]_D^{28}$: +42.3 ^{o} (*c* 0.3; $CHCl_3$)

UV : λ_{max} nm (log ε), in CH_3OH; 205 (4.07), 235(4.33), 305 (3.16); Figure 171

CD : (*c* 0.3; $CHCl_3$): $[\theta]_{278}$ ±0, $[\theta]_{305}$ -931, $[\theta]_{331.5}$ ±0; Figure 180

IR : ν_{max} cm^{-1}, KBr; 1671, 1559, 1578, 1474, 1419, 1357, 1102; Figure 172

EIMS : *m/z* (% relative intensity); 310 (M^+, no observed), 295 (27), 175 (100), 160 (29); Figure 179

^{1}H NMR : δ ppm, 400 MHz, in $CDCl_3$; Table 17, Figure 173

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$; Table 17, Figure 174

5. Biological Activities

5.1 Antimycobacterial Activity

The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA). The standard drugs, isoniazid and kanamycin sulfate, used as reference compounds for the antimycobacterial assay, showed MIC values of 0.040-0.090 and 2.0-5.0 μg/ml, respectively, in the test systems (Collins and Franzblau, 1997).

5.2 Antimalarial Activity

The antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), which was cultured continuously according to the method of Trager and Jensen (Trager and Jansen, 1976). Quantitative assessment of antimalarial activity *in vitro* was determined by the microculture radioisotope technique based upon the method described by Desjardins, *et al.* The inhibitory concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An IC_{50} value of 1 ng/ml was observed for the standard compound, artemisinin, in the same test system (Desjardins *et al.*, 1979).

5.3 Cytotoxic Activity

The purified compounds from *C. arborescens* were tested for cytotoxic activity against the small cell lung cancer cell line NCI-H187, KB cells, BC cells, and Vero cells using the colorimetric; 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method (Skehamp *et al*., 1990) previously described in detail by Jane A. Plumb *et al* (Plumb, Milroy and Kaye, 1989) briefly, cells were diluted to 10^5 cells/ml (20000 cell/well). Test compounds were diluted in distilled water and added to microtiter plates in total volume of 200 µl. Plates were incubated at 37^{o}C, 5% CO_2 for 5 days, 50 µl of 2 mg/ml MTT solution (3-[4,5-dimethylthiazol2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) was added to each well of the plate. Plates were wrapped with aluminium foil and incubated for 4 h. After incubation period, the micro plates were spined down at 200 x g for 5 min. MTT was then removed from the wells and the formazan crystals were dissolved in 200 µl of 100% DMSO and 25 µl of Sorensen' glycine buffer. OD was read in microtiter plate reader at wavelength of 510 nm. The compounds were tested in triplicate. The IC_{50} values of the tested compounds were represented in µg/ml. Ellipticine was used as a positive control.

6. Determination of Free Radical Scavenging Activity

6.1 TLC Screening Assay (Takao *et al*., 1994)

Reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl or 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazyl) radical. TLC screening assay : after developing and drying, TLC plates (with amounts of sample ranging from 0.1 to 100 µg) were sprayed with 0.2 % (2 mg/ml) of DPPH solution in methanol. The plates were examined half an hour after spraying. Active compounds appeared as yellow spots against a purple background (Takao *et al*., 1994).

CHAPTER IV

RESULTS AND DISCUSSION

The chloroform extract of the dried stem bark of *Cratoxylum arborescens* (Vahl) Blume (5 kg) was investigated by means of column chromatographic methods to yield five compounds classified as three xanthones [**17**, **167**, and **168**], and two anthraquinones [**166** and **169**]. The structure determinations of these compounds were achieved by interpretation of their UV, IR, MS, and NMR data, and confirmed by comparison with the literature values. The antimalarial and cytotoxic activities of these compounds were evaluated.

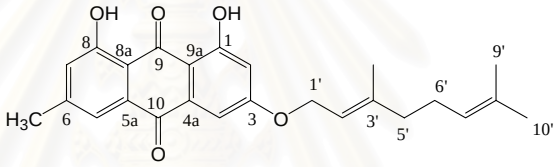
The chloroform extract of the dried stem bark of *Millettia decipiens* Prain (6 kg) was investigated by means of column chromatographic methods to yield twelve compounds classified as seven chalcones [**41**, **46**, **47**, **48**, **54**, **173**, and **174**], and five flavones [**116**, **170**, **171**, **172**, and **175**]. Their complete structures were determined based on their UV, IR MS, and NMR data, and comparison with the literature values. The antimycobacterial, antimalarial, cytotoxic, and free radical scavenging activities of these compounds were evaluated.

1. Structure Determination of Compounds Isolated from *Cratoxylum arborescens*

1.1 Structure Determination of Compound CT-01 (1,8-Dihydroxy-3-geranyloxy-6-methylanthraquinone)

Compound CT-01 was obtained as an orange solid with an observed molecular formula of $C_{25}H_{26}O_5$. The UV spectrum (Figure 5) showed λ_{max} at 287 and 436 nm. The IR absorption spectrum (Figure 6) displayed ν_{max} at 2915 (CH stretching), 1677 and 1631 (C=O stretching), 1562 and 1480 (aromatic ring) cm^{-1}. The EI mass spectrum (Figure 13) exhibited a molecular ion peak at *m/z* 406. HRESI showed *m/z* 429.16822 $[M+Na]^+$, (calcd. for $C_{25}H_{26}O_5Na$: 429.16724). The ^1H NMR spectrum (Table 1, Figure 7) obtained in $CDCl_3$ showed two chelated phenolic hydroxyl groups (δ 12.25, OH-1, 1H, *s*) and (δ 12.09, OH-8, 1H, *s*). Ring A showed the long-range coupling of two aromatic protons at δ 6.64 (H-2, 1H, *d*, 2.5) and at δ 7.32 (H-4, 1H, *d*, 2.5). Ring B

showed the long-range coupling of two aromatic protons at δ 7.58 (H-5, 1H, *d*, 1.4) and at δ 7.04 (H-7, 1H, *d*, 1.4) a methyl singlet (δ 2.43, CH_3-6, 3H, *s*). The signals at δ 4.66 (H-1′, 2H, *d*, 6.5), 5.47 (H-2′, 1H, *t*, 6.5), 1.78 (CH_3-4′, 3H, *s*), 2.08 [H-5′and H-6′, 2H (each 1H), *m*], 5.07 (H-7′,1H, *m*), 1.67 (CH_3-9′, 3H, *s*), and 1.59 (CH_3-10′, 3H, *s*) indicated the presence of an *O*-geranyl group. The *O*-geranyl group was placed at C-3 by the HMBC experiment. The H_2-1′ protons resonating at δ 4.66 (2H, *d*, 6.5) showed long-range hetero-nuclear connectivities with C-3 (δ 165.9) (Figure 12). Thus, this compound was assigned as 1,8-dihydroxy-3-geranyloxy-6-methylanthraquinone [**166**] from the spectroscopic data and comparison of the ^{1}H NMR data with the literature values (Botta *et al*., 1983).



[166]

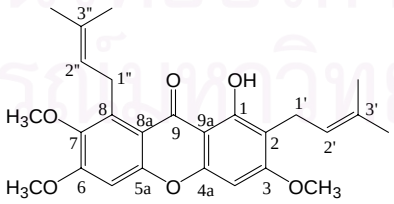
Table 1 : The ^{1}H and ^{13}C NMR Spectral Data of Compound CT-01 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm) of Compound CT-01 (in $CDCl_3$)
	1,8-Dihydroxy-3-geranyloxy-6-methyl-anthraquinone* (in $CDCl_3$)	Compound CT-01 (in $CDCl_3$)		
			1	165.0
2	6.60 (1H, *d*, 2.5)	6.64 (1H, *d*, 2.5)	2	107.4
			3	165.9
4	7.27 (1H, *d*, 2.5)	7.32 (1H, *d*, 2.5)	4	108.7
			4a	135.1
5	7.50 (1H, *br d*, 1.8)	7.58 (1H, *d*, 1.4)	5	121.9
			5a	133.1
			6	148.3
7	7.00 (1H, *br d*, 1.8)	7.04 (1H, *d*, 1.4)	7	124.4
			8	162.4
			8a	113.6
			9	190.6
			9a	110.0
			10	181.9
1′	4.60 (2H, *d*, 7.0)	4.66 (2H, *d*, 6.5)	1′	65.8
2′	5.43 (1H, *t*, 7.0)	5.47 (1H, *t*, 6.5)	2′	118.0
			3′	142.8
4′	1.77 (3H, *s*)	1.78 (3H, *s*)	4′	17.7
5′	2.10 (2H, *m*)	2.08 (2H, *m*)	5′	39.5
6′	2.10 (2H, *m*)	2.08 (2H, *m*)	6′	26.5
7′	5.05 (1H, *br*)	5.07 (1H, *m*)	7′	123.5
			8′	132.0
9′	1.67 (3H, *s*)	1.67 (3H, *s*)	9′	25.6
10′	1.60 (3H, *s*)	1.59 (3H, *s*)	10′	16.8
CH_3-6	2.40 (3H, *s*)	2.43 (3H, *s*)	CH_3-6	22.1
OH-1	12.23 (1H, *s*)	12.25 (1H, *s*)	-	-
OH-8	12.08 (1H, *s*)	12.09 (1H, *s*)	-	-

*Botta *et al*.. 1983

1.2 Structure Determination of Compound CT-02 (Fuscaxanthone C)

Compound CT-02 was obtained as a yellow powder and an observed molecular formula of $C_{26}H_{30}O_6$. The UV spectrum (Figure 14) showed λ_{max} at 269, 312, and 347 nm. The IR absorption spectrum (Figure 15) displayed ν_{max} at 2923 (CH stretching), 1648 (C=O stretching), 1598 and 1458 (aromatic ring) cm^{-1}. The EI mass (Figure 22) exhibited a molecular ion peak at *m/z* 438. HRESI showed *m/z* 461.19276 $[M+Na]^+$, (calcd. for $C_{26}H_{30}O_6Na$: 461.19345). The ^1H NMR spectrum (Table 2, Figure 16) showed one chelated phenolic hydroxyl group (δ 13.50, OH-1, 1H, *s*), and signals due to three methoxy groups, two prenyl groups and two lone aromatic protons. The locations of the substituents on ring A were established by HMBC correlations (Figure 21) from C-2 (δ 111.6) to a hydrogen bonded OH (δ 13.50, 1H, *s*) and the lone H-4 (δ 7.32, 1H, *d*, 2.5), and from C-1 (δ 159.9) to H-1′ (δ 4.13, 2H, *br d*, 6.6) of a prenyl group, and to C-3 (δ 163.5) having a OCH_3. The locations of the substituents on ring C were established by the HMBC correlation (Figure 21) from C-7 (δ 144.1) to H-1′′ (δ 3.37, 2H, *br d*, 7.0) of a prenyl group and from C-7 (δ 144.1) to H-5 (δ 6.76, 1H, *s*). A NOESY correlation (Figure 19) was observed between OCH_3-3 (δ 3.92, 3H, *s*) and H-4 (δ 6.34, 1H, *s*) and there was also a NOESY correlation between OCH_3-6 (δ 3.98, 3H, *s*) and H-5 (δ 6.76, 1H, *s*). Thus, this compound was assigned to fuscaxanthone C [**167**] by spectroscopic data and comparison of the ^1H NMR data with the literature (Ito *et al*., 2003).



[**167**]

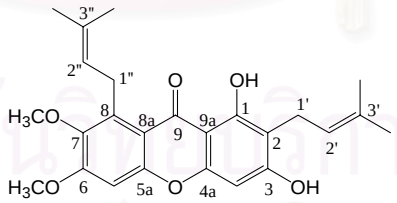
Table 2 : The ^{1}H and ^{13}C NMR Spectral Data of Compound CT-02 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm) of	
	Fuscaxanthone C* (in $CDCl_3$)	Compound CT-02 (in $CDCl_3$)		Fuscaxanthone C * (in $CDCl_3$)	Compound CT-02 (in $CDCl_3$)
			1	159.8	159.9
			2	111.5	111.6
			3	163.4	163.5
4	6.33 (1H, *s*)	6.34 (1H, *s*)	4	88.6	88.7
			4a	155.2	155.3
5	6.75 (1H, *s*)	6.76 (1H, *s*)	5	98.2	98.3
			5a	155.4	155.5
			6	158.0	158.1
			7	144.0	144.1
			8	137.0	137.3
			8a	112.1	112.2
			9	182.0	182.1
			9a	104.0	104.1
1′	3.36 (2H, *d*, 7.0)	3.37 (2H, *br d*, 7.0)	1′	26.2	21.5
2′	5.24 (1H, *m*)	5.23 (1H, *m*)	2′	123.2	123.4
			3′	131.8	131.9
4′	1.85 (3H, *s*)	1.87 (3H, *s*)	4′	18.2	18.3
5′	1.68 (3H, *s*)	1.70 (3H, s)	5′	25.8	26.0
1″	4.13 (2H, *d*, 6.6)	4.14 (2H, *br d*, 6.5)	1″	21.4	26.3
2″	5.25 (1H, *m*)	5.23 (1H, *m*)	2″	122.3	122.5
			3″	131.7	131.8
4″	1.80 (3H, *s*)	1.82 (3H, *s*)	4″	17.8	17.9
5″	1.68 (3H, *s*)	1.70 (3H, *s*)	5″	25.9	26.0
OH-1	13.48 (1H, *s*)	13.50 (1H, *s*)			
OCH_3-3	3.91 (3H, *s*)	3.92 (3H, *s*)	OCH_3-3	-	55.9
OCH_3-6	3.96 (3H, *s*)	3.98 (3H, *s*)	OCH_3-6	56.6	56.1
OCH_3-7	3.80 (3H, *s*)	3.81 (3H, *s*)	OCH_3-7	60.9	61.0

*Ito *et al*., 2003

1.3 Structure Determination of Compound CT-03 (1,3-Dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone)

Compound CT-03 was obtained as a yellow powder with a HRESI *m/z* 447.17786 (calcd. for $C_{25}H_{28}O_6Na$: 447.17780). The UV spectrum (Figure 23) showed λ_{max} at 270, 314, and 350sh nm. The IR spectrum showed (Figure 24) ν_{max} at 3419 (OH stretching), 2974 (CH stretching), 1651 (C=O stretching), and 1608 (aromatic ring) cm^{-1}. The ^1H NMR spectrum (Table 3, Figure 25) showed signals due to two methoxy groups, two prenyl groups, and two isolated aromatic protons. The locations of the substituents on ring A of the xanthone were established by HMBC correlations (Figure 31-32). C-2 was correlated (δ 108.0) to the hydrogen bonded OH (δ 13.50, 1H, *s*) and a lone H-4 (δ 6.28, 1H, *s*), and from C-1 (δ 160.6) to H-1′ (δ 3.43, 2H, *d*, 7.0) of a prenyl group, which was also correlated to C-3 (δ 161.5) having a hydroxyl group. Two methoxy groups and a prenyl group were therefore attached to the C ring, and this was confirmed by the HMBC correlation of C-7 (δ 144.0) to H-1″ (δ 4.12, 2H, *d*, 6.0) and H-5 (δ 6.74, 1H, *s*), together with the NOESY interactions (Figure 29) of OCH_3-6 (δ 3.90, 3H, *s*) with H-5 (δ 6.74, 1H, *s*). Thus, **1** was identified as 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone [**168**]. Compound **168** was synthesised by Lu *et al*. in 1998 (Lu *et al*., 1998).



[**168**]

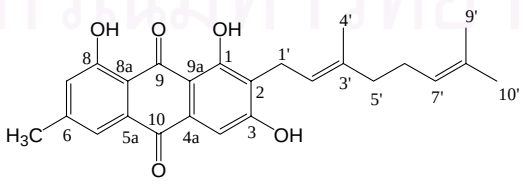
Table 3 : The ^{1}H and ^{13}C NMR Spectral Data of Compound CT-03 in $CDCl_3$

Position	δ_H (ppm), *J*(Hz)	δ_C (ppm)
1	-	160.6
2	-	108.0
3	-	161.5
4	6.28 (1H, *s*)	93.1
5	6.74 (1H, *s*)	98.3
6	-	158.1
7	-	143.9
8	-	137.3
9	-	182.0
4a	-	155.0
5a	-	155.4
8a	-	112.0
9a	-	103.7
1′	3.43 (2H, *d*, 7.0)	26.1
2′	5.25 (1H, *m*)	122.3
3′	-	135.8
4′	1.85 (3H, *s*)	17.9
5′	1.77 (3H, s)	25.8
1″	4.12 (2H, *d*, 6.0)	21.4
2″	5.24 (1H, *m*)	121.4
3″	-	131.8
4″	1.85[a] (3H, *s*)	18.2 [b]
5″	1.68[a] (3H, *s*)	25.9[b]
OH-1	13.50 (1H, *s*)	-
OH-3	6.16 (1H, *br s*)	-
OCH_3-6	3.90 (3H, *s*)	56.0
OCH_3-7	3.79 (3H, *s*)	60.9

[a,b]Assignments may be interchanged

1.4 Structure Determinatin of Compound CT-04 (2-Geranylemodin)

Compound CT-04 was obtained as orange crystals. The UV spectrum (Figure 34) showed λ_{max} at 283 and 438 nm. The IR absorption spectrum (Figure 35) displayed ν_{max} at 3374 (OH stretching), 2924 (CH stretching), 1667 (C=O stretching), 1616 and 1587 (aromatic ring) cm^{-1}. The EI mass spectrum (Figure 44) exhibited a molecular ion peak at *m/z* 406. The HRESI showed *m/z* 429.16866 $[M+Na]^+$, (calcd. for $C_{25}H_{46}O_5Na$: 429.16724). The ^1H NMR spectrum (Table 4, Figure 36) showed two chelated phenolic hydroxyl groups [(δ 11.96, OH-8, 1H, *s*) and (δ 12.45, OH-1, 1H, *s*)]. The signals at δ 3.27 (H-1′, 2H, *d*, 7.0), 5.14 (H-2′, 1H, *m*), 1.72 (CH_3-4′, 3H, *s*), 1.89 (H-5′, 2H, *m*), 1.92 (H-6′, 2H, *m*), 5.00 (H-7′, 1H, *m*), 1.55 (CH_3-9′, 3H, *s*), and δ 1.49 (CH_3-10′, 3H, *s*) indicated the presence of a geranyl group. The long-range heteronuclear correlation spectrum (Figure 41-43) showed the connectivities of δ 3.27 (H-1′, 2H, *d*, 7.0) with C-1 (δ 162.2) and C-3 (δ 163.0). Thus, the geranyl group was place at C-2 by HMBC. On ring A showed the long-range heteronuclear connectivities of OH-1 (δ 12.45, *s*) with C-2 (δ 121.4) and C-9a (δ 109.0), OH-3 (δ 11.36, 1H, *s*) with C-2 (δ 121.4) and C-4 (δ 108.3), and H-4 (δ 7.20, 1H, *s*) with C-10 (δ 180.0) and C-2 (δ 121.4). Ring B showed the long-range heteronuclear connectivities of OH-8 (δ 11.96, 1H, *s*) with C-8a (δ 113.7) and C-7 (δ 124.3), of H-5 (δ 7.44, 1H, *dd*, 1.6, 1.1) with C-10 (δ 180.0) and C-7 (δ 124.3), and of H-7 (δ 7.12, 1H, *dd*, 1.5, 1.5) with C-8a (δ 113.7) and C-5 (δ 120.9). Thus compound CT-04 was assigned as 2-geranylemodin [**169**] by spectroscopic data and comparison of the ^1H NMR data with literature values (Botta *et al*., 1985).



[**169**]

Table 4: The ^{1}H and ^{13}C NMR Spectral Data of Compound CT-04 in DMSO-d_6

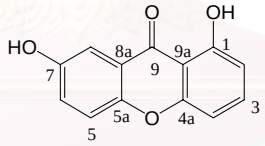
H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm) of Compound CT-04 (in DMSO-d_6)
	2-Geranylemodin* (in DMSO-d_6)	Compound CT-04 (in DMSO-d_6)		
			1	**162.2**
			2	**121.4**
			3	**163.0**
4	7.20 (1H, *s*)	7.20 (1H, s)	4	**108.3**
			4a	**132.4**
5	7.40 (1H, *d*, 2.0)	7.44 (1H, *dd*, 1.1, 1.6)	5	**120.9**
			5a	**133.1**
			6	**148.5**
7	7.06 (1H, *d*, 2.0)	**7.12 (1H, *dd*, 1.5, 1.5)**	7	**124.3**
			8	**161.6**
			8a	**113.7**
			9	**190.3**
			9a	**109.0**
			10	**180.0**
1′	3.40 (2H, *br d*, 7.0)	**3.27 (2H, *d*, 7.0)**	1′	**40.0**
2′	5.30-4.90 (1H, *m*)	**5.14 (1H, *m*)**	2′	**120.6**
			3′	**135.2**
4′	1.76 (3H, *s*)	1.72 (3H, *s*)	4′	**16.3**
5′	2.10 – 1.95 (2H, *m*)	**1.89 (2H, *m*)**	5′	**19.9**
6′	2.10 – 1.95 (2H, *m*)	**1.92 (2H, *m*)**	6′	**25.7**
7′	5.30 – 4.90 (1H, *m*)	**5.00 (1H, *m*)**	7′	**124.3**
			8′	**131.0**
9′	1.57 (3H, *s*)	1.55 (3H, *s*)	9′	**26.4**
10′	1.50 (3H, *s*)	1.49 (3H, *s*)	10′	**17.7**
CH_3-6	2.35 (3H, *s*)	2.38 (3H, *s*)	CH_3-6	**21.8**
OH-1	12.30 (1H, *s*)	12.45 (1H, *s*)	-	-
OH-3		**11.36 (1H, *s*)**	-	-
OH-8	11.90 (1H, *s*)	11.96 (1H, *s*)	-	-

*Botta *et al*., 1985

1.5 Structure Determination of Compound CT-05

(1,7-Dihydroxyxanthone)

Compound CT-05 was obtained as a white solid. The UV spectrum (Figure 45) showed λ_{max} at 202, 234, 259 and 287 nm. The IR absorption spectrum (Figure 46) displayed ν_{max} at 2942 (CH stretching), 1660 (C=O stretching), 1581 and 1504 (aromatic ring) cm^{-1}. The EI mass spectrum (Figure 53) exhibited a molecular ion peak at *m/z* 372, consistent with $C_{13}H_8O_4$ as the molecular formula. The ^1H NMR spectrum (Table 5, Figure 47) showed two phenolic hydroxyl groups at δ 12.71 (OH-1, 1H, *s*) and δ 9.04 (OH-7, 1H, *br s*), the former hydroxyl group being chelated. Ring A showed H-2 (δ 6.73, *d*, 8.2) coupling with H-3 (δ 7.68, *t*, 8.3) and H-4 (δ 6.97, *d*, 8.4) coupling with H-3 (δ 7.68, *t*, 8.3). Ring B showed the ABX splitting pattern of H-5 (δ 7.49, *d*, 9.0), H-6 (δ 7.42, *dd*, 9.0, 2.8) and H-8 (δ 7.58, *d*, 2.8). Thus, the structure of this compound was assigned as 1,7-dihydroxyxanthone by spectroscopic data and by comparison the ^1H NMR data with the literature values (Cardona and Seoane, 1982).



[**17**]

Table 5: The ^{1}H and ^{13}C NMR Spectral Data of Compound CT-05 in Acetone-d_6

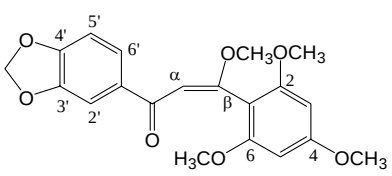
H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm) of Compound CT-05 (in Acetone-d_6)
	1,7-Dihydroxyxanthone* (in DMSO-d_6)	Compound CT-05 (in Acetone-d_6)		
			1	161.8
2	6.53 (1H, *d*, 10.0)	6.73 (1H, *d*, 8.2)	2	109.6
3	7.66 (1H, *t*, 10.0)	7.68 (1H, *t*, 8.3)	3	136.9
4		6.97 (1H, *d*, 8.4)	4	106.8
			4a	156.3
5	7.42 (1H, *m*)	7.49 (1H, *d*, 9.0)	5	119.3
			5a	150.0
6	7.42 (1H, *m*)	7.42 (1H, *dd*, 9.0, 2.8,)	6	125.2
			7	120.9
8	7.56 (1H, *s*)	7.58 (1H, *d*, 2.8)	8	108.2
			8a	154.1
			9	182.8
			9a	103.9
OH-1	11.10 (1H, *s*)	12.71 (1H, *s*)		
OH-7	10.04 (1H, *s*)	9.04 (1H, *s*)		

*Cardona and Seoane, 1982

2. Structure Determination of Compounds Isolated from *Millettia decipiens*

2.1 Structure Determination of Compound MD-01 (3′,4′-Methylenedioxy-2,4,6,β-tetramethoxychalcone)

Compound MD-01 was obtained as pale yellow needles from ethyl acetate-hexane. The UV spectrum (Figure 54) displayed λ_{max} at 271 and 320 nm. The IR spectrum (Figure 55) showed absorption bands at 2942 (CH stretching), 1660 (C=O stretching), 1581 and 1504 (aromatic ring) cm^{-1}. The EI mass spectrum (Figure 63) showed the molecular ion peak at *m/z* 372. HRESI showed *m/z* 395.11106 $[M+Na]^+$ (calcd. for; $C_{20}H_{20}O_7Na$: 395.11012). The ^1H NMR spectral data (Table 6, Figure 56) exhibited an olefinic proton as singlet at δ 6.32 assigned as H-α, a singlet (3H) at δ 3.81 belonging to the β-methoxy group was confirmed by HMBC correlation (Figure 62) of methoxy proton with C-β (δ 165.7). The other three methoxy groups were substituted on the B ring at δ 3.71 [OCH_3-2 and OCH_3-6, 6H (each 3H), *s*], and δ 3.78 (OCH_3-4, 3H, *s*). Their locations were confirmed by the NOESY correlations (Figure 60) of H-3 (δ 6.09, 1H, *s*) with OCH_3-2 and OCH_3-4, and the correlation of H-5 (δ 6.09, 1H, *s*) with OCH_3-4, and OCH_3-6. In the DEPT 135 experiment (Figure 58), the presence of a methylene carbon signal at δ 101.4 also supported that compound MD-01 contains only one methylenedioxy group (δ 5.95, 2H, s), whereas the methylene carbon signal at δ 90.8 belonged to both C-3 and C-5. The configuration of the olefinic function was determined by NOESY experiment (Figure 60). The NOESY spectrum showed a correlation between H-α (δ 6.32, 1H, *s*) and OCH_3-β (δ 3.81, 3H, *s*). Thus double bond is an *E*-isomer. This compound was assigned to 3′,4′-methylenedioxy-2,4,6,β-tetramethoxychalcone [**54**] by spectroscopic data and comparison with the literature (Phrutivorapongkul *et al*., 2003)



[**54**]

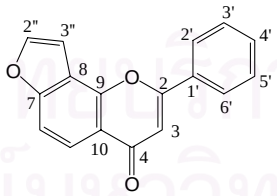
Table 6: The ^{1}H NMR and ^{13}C NMR Spectral Data of Compound MD-01 in $CDCl_3$

H	δ_H (ppm), J(Hz)		C	δ_C (ppm)	
	3′,4′-Methylenedioxy - 2,4,6,β-tetramethoxy chalcone* (in $CDCl_3$)	Compound MD-01 (in $CDCl_3$)		3′,4′-Methylenedioxy - 2,4,6,β-tetramethoxy chalcone* (in $CDCl_3$)	Compound MD-01 (in $CDCl_3$)
			1	107.0	107.0
			2	158.5	158.5
3	6.10 (1H, *s*)	6.09 (1H, *s*)	3	90.8	90.8
			4	162.1	162.1
5	6.10 (1H, *s*)	6.09 (1H, *s*)	5	90.8	90.8
			6	158.5	158.5
			1′	134.7	134.7
2′	7.32 (1H, *d*, 2.0)	7.31 (1H, *br s*)	2′	108.2	108.2
			3′	147.5	147.5
			4′	150.4	150.4
5′	6.76 (1H, *d*, 8.4)	6.73 (1H, *d*, 8.0)	5′	107.4	107.4
6′	7.46 (1H, *dd*, 8.4, 2.0)	7.43 (1H, *d*, 8.0)	6′	123.4	123.4
α	6.33 (1H, *s*)	6.32 (1H, *s*)	α	101.2	101.2
			β	165.7	165.7
OCH_2O	5.98 (2H, *s*)	5.92 (2H, *s*)	OCH_2O	101.4	101.4
			C=O	188.4	188.3
OCH_3-β	3.83 (3H, *s*)	3.81 (3H, *s*)	OCH_3-β	55.9	55.9
OCH_3-2	3.73 (3H, *s*)	3.71 (3H, *s*)	OCH_3-2	55.2	55.2
OCH_3-4	3.80 (3H, *s*)	3.78 (3H, *s*)	OCH_3-4	55.2	55.2
OCH_3-6	3.73 (3H, *s*)	3.71 (3H, *s*)	OCH_3-6	55.9	55.9

*Phrutivorapongkul *et al*., 2003

2.2 Structure Determination of Compoud MD-02 (Lanceolatin B)

Compound MD-02 was obtained as white solids. The UV spectrum (Figure 64) showed λ_{max} at 273 and 327 nm. The IR spectrum (Figure 65) showed ν_{max} at 2936 (CH stretching) and 1628 (C=O stretching). The EI mass spectrum (Figure 72) indicated a molecular ion peak at *m/z* 262, consistent with $C_{17}H_{10}O_3$ as the molecular formula. The ^{1}H NMR spectrum (Table 7, Figure 66) demonstrated that compound MD-02 was a furanoflavonone showing the furan protons at (δ 7.73, H-2″, 1H, *d*, 2.0) and (δ 7.16, H-3″, 1H, *d*, 2.0). The *ortho*-related aromatic protons on ring A were observed at δ 8.13 (H-5, 1H, *d*, 8.7) and δ 7.52 (H-6, 1H, *m*). This spectrum also exhibited two multiplet signals at δ 7.52 [3H (each 1H, *m*)] assigned to H-3′, H-4′ and H-5′, and at δ 7.92 [2H (each 1H, *m*)] belonging to H-2′ and H-6′. Additionally, a singlet proton at δ 6.84 (H-3, 1H, *s*) was observed. This compound was identified as lanceolatin B [**116**], which was previously isolated from many plants, including *Pongamia glabra* (Talapatra, Mallik and Talapatra, 1982), *P. pinnata* (Tanaka *et al*., 1992), *Dahlstedtia pinnata* (Garcez *et al*., 1988), and *Millettia erythrocalyx* (Sritularak *et al*., 2002a). Comparison of the ^{1}H NMR and ^{13}C NMR spectra between compound MD-02 from this work and the literature values for lanceolatin B [**116**] (Garcez *et al*., 1988).



[**116**]

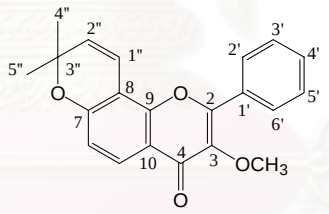
Table 7: The ^{1}H NMR and ^{13}C NMR Spectral Data of Compound MD-02 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm)	
	Lanceolatin B* (in $CDCl_3$)	Compound MD-02 (in $CDCl_3$)		Lanceolatin B* (in $CDCl_3$)	Compound MD-02 (in $CDCl_3$)
			2	162.7	163.0
3	6.90 (1H, *s*)	6.84 (1H, *s*)	3	108.1	108.3
			4	178.2	178.5
5	8.18 (1H, *d*, 9.0)	8.13 (1H, *d*, 8.7)	5	121.8	121.7
6	7.58 (1H, *m*)	7.52 (1H, *m*)	6	110.2	110.5
			7	158.4	158.7
			8	117.2	117.5
			9	150.9	151.1
			10	119.4	119.7
			1′	131.8	131.6
2′	7.97 (1H, *m*)	7.92 (1H, *m*)	2′	126.2	126.5
3′	7.58 (1H, *m*)	7.52 (1H, *m*)	3′	129.1	129.1
4′	7.58 (1H, *m*)	7.52 (1H, *m*)	4′	131.5	131.9
5′	7.58 (1H, *m*)	7.52 (1H, *m*)	5′	129.1	129.1
6′	7.97 (1H, *m*)	7.92 (1H, *m*)	6′	126.2	126.5
2″	7.79 (1H, *d*, 2.0)	7.73 (1H, *d*, 2.0)	2″	145.8	146.2
3″	7.26 (1H, *d*, 2.0)	7.16 (1H, *d*, 2.0)	3″	104.2	104.5

*Garcez *et al*., 1988

2.3 Structure Determination of Compound MD-03 (Pongaflavone)

Compound MD-03 was obtained as a white solid. The UV spectrum (Figure 73) showed λ_{max} at 282 and 326 nm. The IR spectrum (Figure 74) showed ν_{max} at 2974 (CH stretching) and 1628 (C=O stretching). The EI mass spectrum (Figure 81) showed a molecular ion peak at *m/z* 334, consistent with $C_{21}H_{18}O_4$ as the molecular formula. The ^{1}H NMR spectrum (Table 8, Figure 75) for the pyrano ring showed singlet resonance at δ 1.47 [CH_3-4″and CH_3-5″, 6H (each 3H), *s*] assigned to the methyl protons, and olefinic protons at δ 5.66 (H-2″, 1H, *d*, 9.9) and δ 6.85 (H-1″, 1H, *d*, 9.9). The singlet signal at δ 3.88 (3H, *s*) was assigned to OCH_3-3. Additionally, there were seven aromatic protons. Two *ortho* protons on the A ring H-6 (δ 6.82, 1H, *d*, 8.7), and H-5 (δ 7.9, 1H, *d*, 8.7) were coupled. Five other protons on ring B δ 7.51 [H-3′ and H-5′, 2H (each 1H), *m*], δ 7.53 (H-4′, 1H, *m*), and δ 8.08 [H-2′ and H-6′, 2H (each 1H), *m*] were displayed in the spectrum. This compound was eventually identified as pongaflavone [**170**] (Tanaka *et al*., 1992).



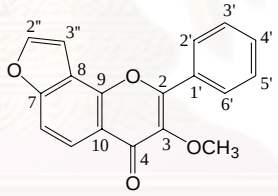
[**170**]

Table 8 : The ^{1}H and ^{13}C NMR Spectral Data of Compound MD-03 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)	C	δ_C (ppm)
		2	154.5
		3	141.2
		4	174.5
5	7.90 (1H, *d*, 8.7)	5	125.6
6	6.82 (1H, *d*, 8.7)	6	115.0
		7	157.2
		8	110.7
		9	151.3
		10	118.0
		1′	131.6
2′	8.08 (1H, *m*)	2′	128.5
3′	7.51 (1H, *m*)	3′	128.2
4′	7.53 (1H, *m*)	4′	130.2
5′	7.51 (1H, *m*)	5′	128.2
6′	8.08 (1H, *m*)	6′	128.5
1″	6.85 (1H, *d*, 9.9)	1″	114.9
2″	5.66 (1H, *d*, 9.9)	2″	130.4
		3″	77.7
4″	1.47 (3H, *s*)	4″	28.0
5″	1.47 (3H, *s*)	5″	28.0
OCH_3-3	3.88 (3H, *s*)	OCH_3-3	60.1

2.4 Structure Determination of Compound MD-04 (Karanjin)

Compound MD-04 was obtained as a white solid. The UV spectrum (Figure 82) showed λ_{max} at 280 and 305 nm. The IR spectrum (Figure 83) displayed absorption bands at 1631 (C=O stretching), 1570 and 1459 (aromatic ring) cm^{-1}. The EI mass spectrum (Figure 90) showed the molecular ion peak at *m/z* 292, consistent with $C_{18}H_{12}O_4$. The ^1H NMR (Table 9, Figure 84) showed the *ortho*-coupled proton H-5 at δ 8.21 (1H, *d*, 8.8) and H-6 (δ 7.56, 1H, *m*) overlapped with three other aromatic protons, including H-3′, H-4′ and H-5′, due to this position showed multiplet signal. Another multiplet signal at δ 8.16 belonged to H-2′ and H-6′. The ^1H NMR spectrum also provided the coupled signals of an angular furan ring at δ 7.76 (H-2″, *d*, 2.2) and δ 7.19 (H-3″, 1H, *d*, 2.2). The singlet signal at δ 3.93 (3H, *s*) was assigned to OCH_3-3. Thus, this compound was identified as karanjin [**171**] (Tanaka *et al*., 1992).



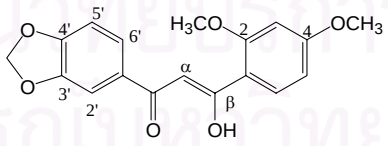
[**171**]

Table 9 : The ^{1}H and ^{13}C NMR Spectral Data of Compound MD-04 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)	C	δ_C (ppm)
		2	154.8
		3	141.8
		4	175.0
5	8.21 (1H, *d*, 8.8)	5	121.9
6	7.56 (1H, *m*)	6	109.9
		7	158.1
		8	117.0
		9	149.9
		10	119.7
		1′	131.0
2′	8.16 (1H, *m*)	2′	128.3
3′	7.56 (1H, *m*)	3′	128.6
4′	7.56 (1H, *m*)	4′	130.6
5′	7.56 (1H, *m*)	5′	128.6
6′	8.16 (1H, *m*)	6′	128.3
2″	7.76 (1H, *d*, 2.2)	2″	145.7
3″	7.19 (1H, *d*, 2.2)	3″	104.2
OCH_3-3	3.93 (3H, *s*)	OCH_3-3	60.2

2.5 Structure Determination of Compound MD-05 (Milletenone)

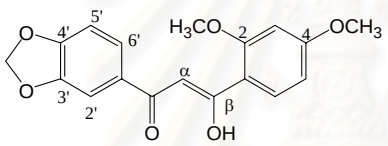
Compound MD-05 was obtained as yellow crystals from ethyl acetate-hexane. The UV spectrum (Figure 91) showed λ_{max} at 275, 314 and 370 nm. The IR spectrum (Figure 92) showed the conjugated carbonyl at 1616 cm^{-1}. The EI mass spectrum (Figure 102) showed the molecular ion peak at *m/z* 328, corresponding to $C_{18}H_{16}O_6$. Compound MD-05 should be a chelated hydroxychalcone as indicated by the ^{1}H NMR. The ^{1}H NMR spectrum (Table 10, Figure 93) showed the chelated hydroxyl at δ 17.11 (OH-β, 1H, *s*) and an olefinic proton at δ 7.07 (H-α, 1H, *s*) affected by keto-enol tautomerism also appeared at δ 185.5 and 181.8 (C=O and C-β). This suggested that compound MD-05 was a hydroxychalcone. Two methoxy groups were located at C-2 and C-4 on ring A. The methylenedioxy group was located at C-3′ and C-4′ on ring B. For ring B, the ABX splitting system consisted of two doublets at δ 6.86 (H-5′, 1H, *d*, 8.2) and δ 7.43 (H-2′, 1H, *d*, 1.6) and a doublet of doublets at δ 7.55 (H-6′, 1H, *dd*, 8.2, 1.6), together with the HMBC correlation (Figure 99-101) of H-2′ and H-6′ with C=O. For ring A, the ABX splitting system consisted of two doublets at δ 6.50 (H-3, 1H, *d*, 2.2), δ 8.00 (H-6, 1H, *d*, 8.7) and a double doublet δ 6.58 (H-5, 1H, *dd*, 8.7, 2.2), together with the HMBC correlation (Figure 99-101) of H-6 with C-β. Thus, compound MD-05 was identified as milletenone [**46**] (Khan and Zaman, 1974).



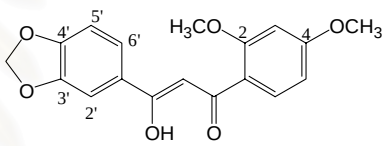
[**46**]

When milletenone [**46**] was kept in chloroform-*d* solution, the structure of milletenone appeared in the keto-enol form that was supported by the ^{1}H NMR spectrum (Figure 94). Three keto-enol forms, structure, **46a**, **46b**, and **46c** were studied using molecular modeling by the density function theory (DFT)

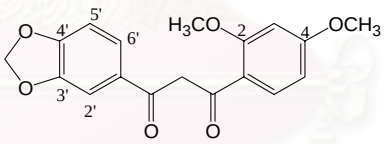
at B3LYP/6-31G(d)(Becke, 1988; Lee, Yang and Parr, 1988). All calculations were carried out using the Gaussion 03 package (Frisch *et al*., 2004). The structure **46a** (Figure 103), **46b** (Figure 105), **46d** (Figure 107), and the transition state structures (Figure 104) were fully optimized at *Cs* symmetry, but **46c** (Figure 106) without symmetry constrained. The total energy of the structures are shown in Table 20, and the total energy levels are in the sequence **46a**< **46b**< **46c**. The relative energies of these structures are shown in Table 21. The relative energy data showed that structure **46a** can change to **46b**. From the total energy levels, structure **46a** showed the lowest total energy, and thus the structure of milletenone [**46**] is proposed as **46a** in agreement with the ^{1}H NMR spectral data (Table 10, Figure 93).



[**46a**]



[**46b**]



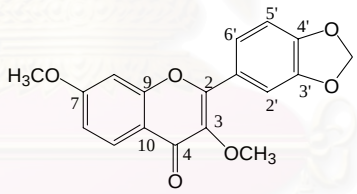
[**46c**]

Table 10 : The ^{1}H and ^{13}C NMR Spectral Data of Compound MD-05 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)	C	δ_C (ppm)
		1′	130.8
2′	7.43 (1H, *d*, 1.6)	2′	107.3
		3′	148.0
		4′	150.9
5′	6.86 (1H, *d*, 8.2)	5′	132.0
6′	7.55 (1H, *dd*, 8.2, 1.6)	6′	122.7
		1	117.4
		2	160.3
3	6.50 (1H, *d*, 2.2)	3	98.8
		4	163.9
5	6.58 (1H, *dd*, 8.7, 2.2)	5	105.2
6	8.00 (1H, *d*, 8.7)	6	108.1
α	7.07 (1H, *s*)	α	96.9
OH-β	17.11 (1H, *s*)	β	181.8
		C=O	185.5
OCH_2O	6.04 (2H, *s*)	OCH_2O	101.7
OCH_3-2	3.94 (3H, *s*)	OCH_3-2	55.8
OCH_3-4	3.87 (3H, *s*)	OCH_3-4	55.5

2.6 Structure Determination of Compound MD-06 (Desmethoxykanugin)

Compound MD-06 was obtained as a white solid. The UV spectrum (Figure 108) showed the λ_{max} at 314 and 339 nm. The IR spectrum (Figure 109) displayed ν_{max} at 1619 and 1500 (aromatic ring) cm^{-1}. The EI mass spectrum (Figure 115) showed the molecular ion peak at *m/z* 326, corresponding to the molecular formula $C_{18}H_{14}O_6$. The ^1H NMR (Table 11, Figure 110) showed the signal of two methoxy groups at δ 3.88 (OCH_3-3, 3H, *s*) and δ 3.91 (OCH_3-7, 3H, *s*). One methoxy group was located on the A ring together with an ABX splitting pattern, δ 6.88 (H-8, 1H, *d*, 2.3), δ 6.96 (H-6, 1H, *dd*, 8.8, 2.3) and δ 8.13 (H-5, 1H, *d*, 8.8). The methylenedioxy group (δ 6.06, 2H, *s*) was located on the B ring together with a separate ABX splitting pattern, δ 6.94 (H-5′, 1H, *d*, 8.3), δ 7.61 (H-2′, 1H, *d*, 1.7), and δ 7.70 (H-6′, 1H, *dd*, 8.3, 1.7). These data were identical with those in the literature (Das *et al*., 1994), and thus this compound was identified as desmethoxykanugin [**172**].



[**172**]

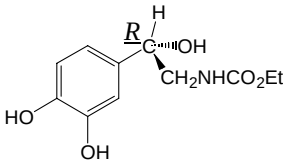
Table 11 : The ^{1}H and ^{13}C NMR Spectral Data of Compound MD-06 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm)	
	Desmethoxykanugin* (in $CDCl_3$)	Compound MD-06 (in $CDCl_3$)		Desmethoxykanugin* (in $CDCl_3$)	Compound MD-06 (in $CDCl_3$)
			2	154.7	154.6
			3	140.8	140.7
			4	174.4	174.3
5	8.14 (1H, *d*, 8.8)	8.13 (1H, *d*, 8.8)	5	127.1	127.0
6	6.96 (1H, *dd*, 8.8, 2.4)	6.96 (1H, *dd*, 8.8, 2.3)	6	114.3	114.2
			7	156.8	**163.9**
8	6.89 (1H, *d*, 2.4)	6.88 (1H, *d*, 2.3)	8	99.9	99.8
			9	164.0	**156.8**
			10	118.0	118.0
			1′	124.8	124.7
2′	7.61 (1H, *d*, 1.8)	7.61 (1H, *d*, 1.7)	2′	108.6	108.3
			3′	147.9	147.8
			4′	149.5	149.4
5′	6.94 (1H, *d*, 8.2)	6.94 (1H, *d*, 8.3)	5′	108.4	108.5
6′	7.69 (1H, *dd*, 8.8, 1.8)	7.70 (1H, *dd*, 8.3, 1.7)	6′	123.4	123.3
OCH_2O	6.06 (2H, s)	6.06 (2H, s)	OCH_2O	101.6	101.6
OCH_3-3	3.88 (3H, s)	3.88 (3H, s)	OCH_3-3	60.0	59.9
OCH_3-7	3.91 (3H, s)	3.91 (3H, s)	OCH_3-7	55.8	55.8

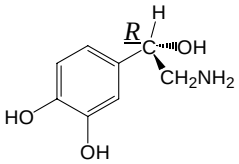
*Das *et al.*, 1994

2.7 Structure Determination of Compound MD-07 (Dihydroisomilletenone methyl ether)

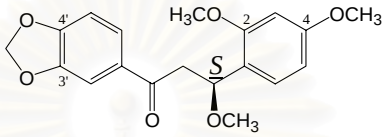
Compound MD-07 was obtained as a white solid, $[\alpha]_D^{28}$ +46.3^o (*c* 0.30; $CHCl_3$). The UV spectrum (Figure 116) showed λ_{max} at 277 and 308 nm. The IR spectrum (Figure 117) showed ν_{max} at 1667 (C=O stretching), 1602 and 1500 (aromatic) cm^{-1}. The EI mass spectrum (Figure 125) showed a molecular ion peak at *m/z* 344. Compound MD-07 was a *β*-methoxy-dihydrochalcone as indicated by the ^{1}H NMR. The ^{1}H NMR (Table 12, Figure 118) showed a signal for OCH_3-*β* at *δ* 3.24 (3H, *s*), *α*-Heq at *δ* 3.08 (1H, *dd*, 15.7, 3.2), *α*-Hax at *δ* 3.29 (1H, *dd*, 15.7, 9.2) and *β*-Hax at *δ* 5.14 (1H, *dd*, 9.2, 3.2), together with the HMBC correlation (Figure 123) of *β*-Hax with C=O at *δ* 196.3 ppm. The methylenedioxy group was located on ring A. This was confirmed by the EI mass fragment ion at *m/z* 149 (3′,4′-OCH_2O-$C_6H_3CO^+$) and the ABX splitting system consisting of two doublets at *δ* 6.83 (H-5′, 1H, *d*, 8.1) and *δ* 7.48 (H-2′, 1H, *d*, 1.6) and a doublet of doublets at *δ* 7.59 (H-6′, 1H, *dd*, 8.1, 1.6), together with the HMBC correlation (Figure 123) of H-2′ and H-6′ with the C=O at *δ* 196.3 ppm. Two methoxy groups were located on the B ring. The ^{1}H NMR (Table 12, Figure 118) exhibited two singlet resonances at *δ* 3.81 (OCH_3-2, 3H, *s*) and *δ* 3.82 (OCH_3-4, 3H, *s*). This was confirmed by the EI mass spectrum which showed the base peak at *m/z* 181 ($C_{10}H_{13}O_3^+$), together with HMBC correlations (Figure 123) of H-6, *δ* 7.33 (1H, *d*, 8.3) with C-2 (*δ* 157.8), C-4 (*δ* 160.2), and C-*β* (*δ* 74.3) ppm. Thus, compound MD-07 was identified as dihydroisomilletenone methyl ether [**48**] (Mahmoud and Waterman, 1985). The absolute configuration of compound MD-07 was established by comparison of its optical rotation with (-)*N*-ethoxycarbonylnorepinephrine [**176]**. Compound **176** was prepared from (-)-norepinephrine [**177**] and showed $[\alpha]_D^{25}$ = -34.8 ^{o} (*c* 1.08 w/v in 50% EtOH). It possesses the *R*-configuration (Pratesi *et.al*., 1959). Compound MD-07 showed $[\alpha]_D^{28}$ = +46.3 ^{o} (*c* 0.30 in $CHCl_3$), thus the absolute configuration of compound MD-07 at the *β*-position is *S*.



[**176**]



[**177**]



[**48**]

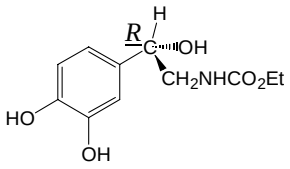
Table 12 : The ^{1}H and ^{13}C NMR Spectral Data of Compound MD-07 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm) of Compound MD-07 (in $CDCl_3$)
	Dihydroisomilletenone methyl ether* (in $CDCl_3$)	Compound MD-07 (in $CDCl_3$)		
			1	121.8
			2	157.8
3	6.47 (1H, *d*, 2.0)	6.47 (1H, *d*, 2.0)	3	98.4
			4	160.2
5	6.53 (1H, *dd*, 8.0, 2.0)	6.53 (1H, *dd*, 8.3, 2.0)	5	104.3
6	7.33 (1H, *d*, 8.0)	7.33 (1H, *d*, 8.3)	6	127.2
			1′	132.2
2′	7.49 (1H, *d*, 2.0)	7.48 (1H, *d*, 1.6)	2′	108.2
			3′	148.0
			4′	151.5
5′	6.84 (1H, *d*, 8.0)	6.83 (1H, *d*, 8.1)	5′	107.7
6′	7.59 (1H, *dd*, 8.0, 2.0)	7.59 (1H, *dd*, 8.1, 1.6)	6′	124.6
α-eq	3.10 (1H, *dd*, 15.9, 3.2)	3.08 (1H, *dd*, 15.7, 3.2)	α	45.8
α-ax	3.29 (1H, *dd*, 15.9, 9.2)	3.29 (1H, *dd*, 15.7, 9.2)		
β-ax	5.14 (1H, *dd*, 9.2, 3.2)	5.14 (1H, *dd*, 9.2, 3.2)	β	74.3
			C=O	196.3
OCH_2O	6.04 (2H, *s*)	6.02 (2H, *s*)	OCH_2O	101.7
OCH_3-2	3.81 (3H, *s*)	3.81 (3H, *s*)	OCH_3-2	57.1
OCH_3-4	3.82 (3H, *s*)	3.82 (3H, *s*)	OCH_3-4	57.1
OCH_3-β	3.24 (3H, *s*)	3.24 (3H, *s*)	OCH_3-β	55.3

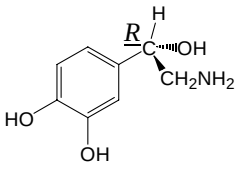
*****Mahmoud and Waterman, 1985

2.8 Structure Determination of Compound MD-08 (+)-*S*-3,4-methylene-dioxy- 2′,4′,6′,*β*-tetramethoxydihydrochalcone

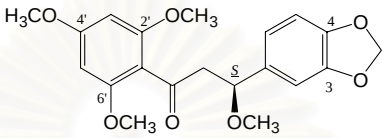
Compound MD-08 was obtained as a yellow powder, $[\alpha]_D^{28}$ +37.3 ^{o} (*c* 0.30; $CHCl_3$), which exhibited a molecular formula $C_{20}H_{22}O_7$, with a HRFAB *m/z* $[M^+ + H]$ 375.14439 (calcd. for $C_{20}H_{23}O_7$; 375.14433). The IR bands (Figure 127) were observed at 1681 (C=O stretching) and 1607 (aromatic ring) cm^{-1}, and the UV absorption at 280 nm suggested a chalcone skeleton (Markham, 1982) (Figure 126). The ^{1}H NMR spectrum (Table 13, Figure 128) showed a methylenedioxy group at δ 5.42 (2H, *dd*, 2.6, 1.3) and an ABX splitting system with a signals for three double doublet protons at δ 3.36 (α-H*eq*, 1H, *dd*, 17.1, 5.0), δ 3.68 (α-H*ax*, 1H, *dd*, 17.1, 8.7), and δ 5.05 (β-H*ax*, 1H, *dd*, 7.6, 4.1) due to the partial structure $COCH_2CH(O)$. The β-methoxy group was observed at δ 3.26 (3H, s). These spectral data indicated that compound MD-08 is a β-methoxydihydrochalcone. The EI mass spectrum (Figure 138) showed that a peak at *m/z* 195 [2′,4′,6′-$(MeO)_3C_6H_2CO^+$, 100 %] arose from the ketonic A ring fragment substituted by three methoxy groups. This was confirmed by the NOESY interactions (Figure 132) of OCH_3-2′ (δ 3.29, 3H, *s*) with H-3′ (δ 6.05, 1H, *s*), of OCH_3-4′ (δ 3.40, 3H, *s*) with H-3′ (δ 6.05, 1H, *s*) and H-5′ (δ 6.05, 1H, *s*) for OCH_3-6′ (δ 3.29, 3H, *s*) with H-5′. On the B ring, the ^{1}H NMR ABX aromatic system at δ 7.09 (H-2, 1H, *d*, 1.5,), δ 6.74 (H-5, 1H, *d*, 8.0), and δ 6.88 (H-6, 1H, *dd*, 8.0, 1.5), together with the HMBC correlations (Figure 134-137) of C-β (δ 79.6) with H-2 and H-6, indicated the placement of the methylenedioxy group at C-3 and C-4. Thus, compound MD-08 was identified as 3,4-methylenedioxy-2′,4′,6′,β-tetramethoxydihydrochalcone [**173**]. The absolute configuration of compound MD-08 was established by comparison of its optical rotation with (-)*N*-ethoxy-carbonylnorepinephrine [**176]**. Compound **176** was prepared from (-)-nor-epinephrine [**177**] and showed $[\alpha]_D^{25}$ = -34.8 ^{o} (*c* 1.08 w/v in 50% EtOH). It possesses the *R*-configuration (Pratesi *et al*., 1959). Compound MD-08 showed $[\alpha]_D^{28}$ = +37.3 ^{o} (*c* 0.30 in $CHCl_3$), and thus the absolute configuration of compound MD-08 at the β-position is *S*.



[**176**]



[**177**]



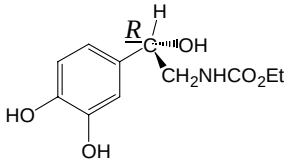
[**173**]

Table 13 : ^{1}H and ^{13}C NMR Spectral Data of Compound MD-08 in C_6D_6

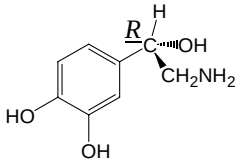
H	δ_H (ppm), *J* (Hz)	C	δ_C (ppm)
		1	136.8
2	7.09 (1H, *d*, 1.5)	2	107.6
		3	148.3
		4	147.3
5	6.74 (1H, *d*, 8.0)	5	120.8
6	6.88 (1H, *dd*, 8.0, 1.5)	6	120.8
		1′	114.8
		2′	158.8
3′	6.05 (*s*)	3′	91.2
		4′	162.5
5′	6.05 (*s*)	5′	91.2
		6′	158.8
α-eq	3.36 (1H, *dd*, 17.1, 5.0)	α	54.0
α-ax	3.68 (1H, *dd*, 17.1, 8.7)		
β-ax	5.05 (1H, *dd*, 8.7, 4.1)	β	79.6
		C=O	198.9
OCH_2O	5.42 (2H, *d*, 3.0)	OCH_2O	100.8
OCH_3-2′	3.29 (3H, *s*)	OCH_3-2′	55.3
OCH_3-4′	3.40 (3H, *s*)	OCH_3-4′	54.8
OCH_3-6′	3.29 (3H, *s*)	OCH_3-6′	55.3
OCH_3-β	3.26 (3H, *s*)	OCH_3-β	56.4

2.9 Structure Determination of Compound MD-09 (Dihydromilletenone methyl ether)

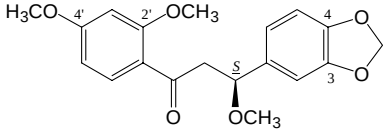
Compound MD-09 was obtained as a brown amorphous solid, $[\alpha]_D^{28}$ +46.0 ^{o} (*c* 0.30; $CHCl_3$). The UV spectrum (Figure 140) showed λ_{max} at 292, 305 and 370 nm. The IR spectrum (Figure 141) showed ν_{max} at 2935 (CH stretching), 1659 (C=O stretching) cm^{-1}. The EI mass spectrum (Figure 148) did not displayed the molecular ion peak. Compound MD-09 was a *β*-methoxydihydrochalcone as indicated by the ^{1}H NMR spectrum. The ^{1}H NMR (Table 14, Figure 147) showed the signal of a OCH_3-*β* at *δ* 3.18 (3H, *s*), and *α*-Heq at *δ* 3.22 (1H, *dd*, 16.7, 4.9), *α*-Hax at *δ* 3.46 (1H, *dd*, 16.7, 8.0) and *β*-Hax at 4.73 (1H, *dd*, 8.0, 4.9). Two methoxy groups were located on the A ring. This was confirmed by the EI mass spectrum which showed a base peak at *m/z* 165 [2′,4′-$(OCH_3)_2C_6H_3CO^+$, 100%], together with the HMBC correlations (Figure 142) of H-6′ [*δ* 7.77 (1H, *d*, 8.7)] with C-2′ (*δ* 161.0), C-4′ (*δ* 164.8) and C=O (*δ* 197.2). The methylenedioxy group was located at C-3 and C-4 on the B ring as supported by the ABX splitting pattern at *δ* 6.75 (H-5, 1H, *dd*, 8.0) and *δ* 6.87 (H-2, 1H, *d*, 1.1) and the doublet of doublets at *δ* 6.81 (H-6, 1H, *dd*, 8.0, 1.0), and was confirmed by the HMBC correlations (Figure 147) of H-2 and H-6 with C-*β* (*δ* 79.9). Thus compound MD-09 was identified as dihydromilletenone methyl ether [**47**] (Mahmoud and Waterman, 1985). The absolute configuration of compound MD-09 was established by comparison of its optical rotation with (-)*N*-ethoxycarbonyl-norepinephrine [**176]**. Compound **176** was prepared from (-)-norepinephrine [**177**], and showed $[\alpha]_D^{25}$ = -34.8 ^{o} (*c* 1.08 w/v in 50% EtOH). It possesses the *R*-configuration (Pratesi *et al*., 1959). Compound MD-09 showed $[\alpha]_D^{28}$ = +46.0^{o} (*c* 0.30 in MeOH) and thus the absolute configuration of compound MD-09 at the *β*-position is *S*.



[**176**]



[**177**]



[**47**]

Table 14 : The ^{1}H and ^{13}C NMR Spectra Data of Compoud MD-09 in $CDCl_3$

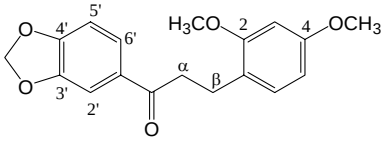
H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm) of Compound MD-09 (in $CDCl_3$)
	Dihydromilletenone methyl ether* (in $CDCl_3$)	Compound MD-09 (in $CDCl_3$)		
			1	135.9
2	6.88 (1H, *d*, 2.0)	6.87 (1H, *d*, 1.1)	2	107.9
			3	146.9
			4	147.8
5	6.76 (1H, *d*, 8.0)	6.75 (1H, *d*, 8.0)	5	106.9
6	6.82 (1H, *dd*, 8.0, 2.0)	6.81 (1H, *dd*, 8.0, 1.1)	6	120.4
			1′	121.5
			2′	160.6
3′	6.43 (1H, *d*, 2.0)	6.43 (1H, *d*, 2.0)	3′	98.2
			4′	164.4
5′	6.51 (1H, *dd*, 8.0, 2.0)	6.50 (1H, *dd*, 8.7, 2.0)	5′	105.2
6′	7.78 (1H, *d*, 8.0)	7.77 (1H, *d*, 8.7)	6′	132.7
α-eq	3.22 (1H, *dd*, 16.9, 4.9)	3.22 (1H, *dd*, 16.7, 4.9)	α	52.0
α-ax	3.47 (1H, *dd*, 16.9, 8.0)	3.46 (1H, *dd*, 16.7, 8.0)		
β-ax	4.73 (1H, *dd*, 8.0, 4.9)	4.73 (1H, *dd*, 8.0, 4.9)	β	79.5
			C=O	197.2
OCH_2O	5.95 (2H, *s*)	5.92 (2H, *s*)	OCH_2O	100.9
OCH_3-2′	3.85 (3H, *s*)	3.84 (3H, *s*)	OCH_3-2′	55.7
OCH_3-4′	3.84 (3H, *s*)	3.82 (3H, *s*)	OCH_3-4′	55.7
OCH_3-β	3.18 (3H, *s*)	3.18 (3H, *s*)	OCH_3-β	56.5

* Mahmound and Waterman, 1985

2.10 Structure Determination of Compound MD-10

(2,4-Dimethoxy -3′,4′-methylenedioxydihydrochalcone)

Compound MD-10 was obtained as pale yellow needles which exhibited a molecular formula $C_{18}H_{18}O_5$, with a HRFAB *m/z* [M^++H] 315.12323 (calcd. for $C_{18}H_{19}O_5$: 315.12326). The IR bands (Figure 151) were observed at 1677 (C=O stretching) and 1614 (aromatic ring) cm^{-1}, and the UV absorptions appearing at 308 and 276 nm indicated a chalcone skeleton (Markham, 1982) (Figure 150). The ^{13}C NMR spectral data (Table 13, Figure 153) showed 18 signals, corresponding to two methoxys, three methylenes, six methines, and seven quaternary carbons. Two methoxy groups were therefore attached to the B ring, and this was confirmed by the NOESY interaction (Figure 156) of OCH_3-2 (δ 3.79, 3H, s) with H-3 (δ 6.44, 1H, *d*, 2.3), and of OCH_3-4 (δ 3.81, 3H, *s*) with H-3 (δ 6.44, 1H, *d*, 2.3) and H-5 (δ 6.42, 1H, *dd*, 8.0, 2.3) respectively. The two sets of methylene protons (Table 14, Figure 152) [δ 3.11 (H-α, 2H, *m*), δ 2.92 (H-β, 2H, *m*,)], together with the HMBC correlation (Figure 158-161) of C-β (δ 25.5) with H-6 (δ 7.09, 1H, *d*, 8.0), and C-2 (δ 130.7) with H-β (δ 2.92) substantiated these relationships. On ring A, an ABX splitting system consisting of two doublets at δ 7.47 (H-2′, 1H, *d*, 1.7,) and δ 6.80 (H-5′, 1H, *d*, 8.0,), and a doublet of doublet at δ 7.59 (H-6′, 1H, *dd*, 8.0, 1.7), together with HMBC correlation of C=O (δ 198.6) with H-2′ and H-6′, indicated the placement of the methylenedioxy at C-3′ and C-4′. This was confirmed by the EI mass (Figure 162) which showed the fragment ions at *m/z* 149 (3,4-OCH_2O-C_6H_3-CO^+, 24%) and 121 (3,4-OCH_2O-$C_6H_3^+$, 53%). Thus, compound MD-10 was determined to be 2,4-dimethoxy-3′,4′-methylenedioxydihydrochalcone [**174**].



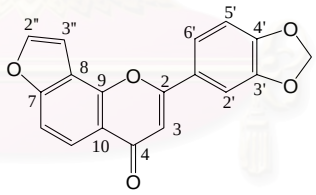
[**174**]

Table 15: ^{1}H and ^{13}C NMR Spectral Data of Compound MD-10 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)	C	δ_C (ppm)
		1	122.3
		2	130.7
3	6.44 (1H, *dd*, 2.3)	3	104.2
		4	158.7
5	6.42 (1H, *dd*, 8.0, 2.3)	5	98.9
6	7.09 (1H, *d*, 8.0)	6	159.8
		1′	131.9
2′	7.47 (1H, *d*, 1.7)	2′	108.2
		3′	148.4
		4′	151.9
5′	6.80 (1H, *d*, 8.0)	5′	108.4
6′	7.59 (1H, *dd*, 8.0, 1.7)	6′	124.6
α	3.10 (2H, *m*)	α	39.4
β	2.92 (2H, *m*)	β	25.8
		C=O	198.6
OCH_2O	6.03 (2H, *s*)	OCH_2O	102.1
OCH_3-2	3.79 (3H, *s*)	OCH_3-2	55.6
OCH_3-4	3.81 (3H, *s*)	OCH_3-4	55.7

2.11 Structure Determination of Compound MD-11 (Pongaglabrone)

Compound MD-11 was obtained as a white solid. The UV spectrum (Figure 163) showed the λ_{max} at 224, 240, and 328 nm. The IR spectrum (Figure 164) showed absorptions at ν_{max} 2921 (CH stretching), 1640 (C=O stretching), 1592 and 1502 (aromatic ring) cm^{-1}. The EI mass spectrum (Figure 170) showed the molecular ion peak at *m/z* 306, corresponding to the molecular formula $C_{18}H_{10}O_5$. The ^1H NMR spectrum (Table 16, Figure 165) showed the signals of a furan ring at δ 7.20 (H-3″, 1H, *dd*, 2.1, 1.0) and δ 7.78 (H-2″, 1H, *d*, 2.1).The furan ring was fused in the angular position at C-7 and C-8 of ring A as supported by the *ortho*-coupling of the protons H-5 (δ 8.18, 1H, *d*, 8.0) and H-6 (δ 7.55, 1H, *d*, 8.0). The methylenedioxy group was placed at C-3′ and C-4′ as supported by the ABX splitting pattern at δ 7.41 (H-2′, 1H, *d*, 1.7), δ 6.99 (H-5′, 1H, *d*, 8.1) and δ 7.55 (H-6′, 1H, *dd*, 8.1, 1.7). Thus, compound MD-11 was assigned to pongaglabrone [**175**] by spectroscopic data and comparison of the ^1H NMR data with the literature (Garcez *et al*., 1988).



[**175**]

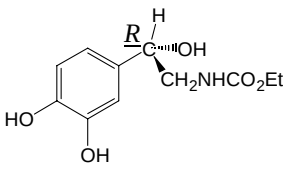
Table 16 : ^{1}H and ^{13}C NMR Spectral Data of Compound MD-11 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm) of Compound MD-11 (in $CDCl_3$)
	Pongaglabrone* (in $CDCl_3$)	Compound MD-11 (in $CDCl_3$)		
			2	162.6
3	6.80 (1H, *s*)	6.76 (1H, *s*)	3	107.3
			4	178.4
5	8.22 (1H, *d*, 8.0)	8.18 (1H, *d*, 8.0)	5	122.0
6	7.59 (1H, *d*, 8.0)	7.55 (1H, *d*, 8.0)	6	110.4
			7	158.6
			8	117.3
			9	150.9
			10	119.5
			1′	126.0
2′	7.44 (1H, *d*, 2.0)	7.41 (1H, *d*, 1.7)	2′	106.5
			3′	148.8
			4′	150.9
5′	7.00 (1H, *d*, 8.0)	6.99 (1H, *d*, 8.1)	5′	109.1
6′	7.59 (1H, *dd*, 8.0, 2.0)	7.55 (1H, *dd*, 8.1, 1.7)	6′	121.6
2″	7.82 (1H, *d*, 2.0)	7.78 (1H, *d*, 2.1)	2″	146.1
3″	7.20 (1H, *d*, 2.0)	7.20 (1H, *dd*, 2.1, 1.0)	3″	104.4
OCH_2O	6.14 (2H, *s*)	6.10 (2H, *s*)	OCH_2O	102.2

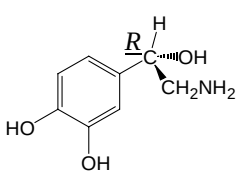
* Garcez *et al*., 1988

2.12 Structure Determination of Compound MD-12 (Ovalitenin B)

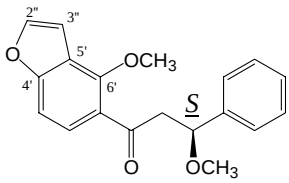
Compound MD-12 was obtained as a brown amorphous solid, $[\alpha]_D^{28}$ +42.3 ^{o} (*c* 0.30; $CHCl_3$). The UV spectrum (Figure 171) showed λ_{max} at 205, 235, and 305 nm. The IR spectrum (Figure 172) showed ν_{max} at 1671 (C=O stretching) cm^{-1}. The EI mass spectrum (Figure 179) did not showed the molecular ion peak at *m/z* 310. The ^{1}H NMR spectrum (Table 17, Figure 173) showed a *β*-methoxy group at *δ* 3.18 (3H, *s*). Three doublets of doublets at *δ* 3.24 (*α*-Heq, 1H, *dd*, 16.5, 4.5), *δ* 3.56 (*α*-Hax, 1H, *dd*, 8.5, 4.5), and *δ* 4.48 (*β*-Hax, 1H, *dd*, 8.5, 4.5) were assigned as H_2-*α* and H-*β*, respectively. These data suggested that compound MD-12 was a *β*-methoxydihydrochalcone. On the A ring two doublet proton signals for a furan ring at *δ* 6.96 (H-3″, 1H, *d*, 2.0) and *δ* 7.58 (H-2″, 1H, *d*, 2.0) were observed. The appearance of two, one-proton doublet signals with *ortho*-aromatic coupling at *δ* 7.19 (H-3′, 1H, *d*, 8.6) and *δ* 7.62 (H-2′, 1H, *d*, 8.6) and singlet signal of methoxy group at *δ* 4.09 (OCH_3-6′, 3H, *s*), together with the HMBC correlation (Figure 178) between C=O (*δ* 199.4) and H-2′ clearly indicated that the furan ring was fused in an angular form on ring A at C-4′ and C-5′. The signals for an unsubstituted B ring were observed at *δ* 7.25 (H-3, H-4, and H-5, each 1H, *m*) and *δ* 7.30 (H-2 and H-6, each 1H, *m*). Based on the spectral data and comparison with the literature (Gupta and Krishnamurti, 1977), compound MD-12 was identified as ovalitenin B [**41**]. The absolute configuration of compound MD-12 was established by comparison of its optical rotation with (-)*N*-ethoxycarbonyl-norepinephrine [**176]**. Compound **176** was prepared from (-)-norepinephrine [**177**] and showed $[\alpha]_D^{25}$ = -34.8 ^{o} (*c* 1.08 w/v in 50% EtOH). It possesses the *R*-configuration (Pratesi *et al*., 1959). Compound MD-12 showed $[\alpha]_D^{28}$ = +42.3 ^{o} (*c* 0.30 in $CHCl_3$) and thus the absolute configuration of compound MD-12 at the *β*-position is *S*.



[**176**]



[**177**]



[**41**]

Table 17 : ^{1}H and ^{13}C NMR Spectral Data of Compound MD-12 in $CDCl_3$

H	δ_H (ppm), *J* (Hz)		C	δ_C (ppm) of Compound MD-12 (in $CDCl_3$)
	Ovalitenin B* (in $CDCl_3$)	Compound MD-12 (in $CDCl_3$)		
			1	141.8
2	7.40 (5H, *s*)	7.30 (1H, *m*)	2	126.7
3		7.25 (1H, *m*)	3	128.4
4		7.25 (1H, *m*)	4	127.6
5		7.25 (1H, *m*)	5	128.4
6		7.30 (1H, *m*)	6	126.7
			1′	125.3
2′	7.70 (1H, *d*, 9.0)	7.62 (1H, *d*, 8.6)	2′	126.7
3′	7.07 (1H, *d*, 9.0)	7.19 (1H, *d*, 8.6)	3′	106.5
			4′	159.0
			5′	118.8
			6′	153.8
2″	7.60 (1H, *d*, 2.0)	7.58 (1H, *d*, 2.0)	2″	144.6
3″	6.98 (1H, *d*, 2.0)	6.96 (1H, *d*, 2.0)	3″	105.5
α-eq	3.40 (2H, *m*)	3.24 (1H, *dd*, 16.5, 4.5)	α	52.0
α-ax	3.40 (2H, *m*)	3.56 (1H, *dd*, 8.5, 4.5)		
β-ax	4.85 (1H, *m*)	4.48 (1H, *dd*, 8.5, 4.5)	*β*	80.0
			C=O	199.4
OCH_3-6′	4.10 (3H, *s*)	4.09 (3H, *s*)	OCH_3-6′	60.6
OCH_3-*β*	3.21 (3H, *s*)	3.18 (3H, *s*)	OCH_3-*β*	56.7

***** Gupta and Krishnamurti, 1977

3. Biological Activities of isolated Compounds from *Cratoxylum arborescens*

3.1 Cytotoxic activity

All of the isolates from *C. arborescens* were tested for their cytotoxic activity using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against the small cell lung cancer cell line NCI-H187, human epidermoid carcinoma cell line of nasopharynx (KB cell), and human breast cancer cell line (BC cell), and Vero cell as shown in Table 18. For the cell line NCI-H187, compounds CT-03 and CT-04 were found to be moderately cytotoxic with IC_{50} values of 3.69±1.27 and 3.08±0.73 μg/ml against NCI-H187, respectively. Compounds CT-01, CT-02, and CT-05 were inactive. For KB cells, BC cells, and Vero cells all of the compounds were inactive.

3.2 Antimalarial activity

All of the isolates from *C. arborescens* were tested for antimalarial against *Plasmodium falciparum*, K1 strain using microculture radioisotope technique. All of the compounds were inactive (data not shown).

4. Biological Activities of isolated Compounds from *Millettia decipiens*

4.1 Antimalarial activity

All of the isolates from *M. decipiens* were tested for antimalarial against *Plasmodium falciparum*, K1 strain using microculture radioisotope technique. All of the compounds were inactive (data not shown).

4.2 Antimycobacterial activity

All of the isolates were tested for their antimycobacterial activity using the microplate alamar blue assay against *Mycobacterium tuberculosis* H37Ra. As shown in Table 20, compound MD-03 showed moderate activity with a MIC value of 6.25 μg/ml, compounds MD-06-MD-09, and MD-12 showed mild activity with MICs of 25, 50, 50, 25, and 25 μg/ml, respectively.

Compounds MD-01, MD-02, MD-04, MD-05, MD-10, and MD-11 were inactive.

4.3 Free radical scavenging activity

All of the isolates were tested for their free radical scavenging activity by the TLC screening assay. All of the compounds were inactive. (data not shown).

Table 18: Cytotoxic activity of isolates from *C. arborescens*

Compound	Cytotoxic IC_{50} (μg/ml)*			
	NCI-H187[a]	KB cell[b]	BC cell[c]	Vero cell[d]
CT-01	N.D.	N.D.	N.D.	> 50
CT-02	N.D.	N.D.	N.D.	> 50
CT-03	3.69±1.27	N.D.	N.D.	> 50
CT-04	3.08±0.73	N.D.	N.D.	> 50
CT-05	N.D.	N.D.	N.D.	> 50

[a]NCI-H187, Human small cell lung cancer cell line

[b]KB cell, Human epidermoid carcinoma cell line of nasopharynx

[c]BC cell, Human breast cancer cell line

[d]Vero cell

N.D. ; Not determined

*IC_{50} (μg/ml) > 10; inactive

5-10; weakly active

<5; moderately active

<1 ; strongly active

Ellipticine is used as a positive control ; IC_{50} = 0.35±0.15 μg/ml

Table 19 : Antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra of isolates from *M. decipiens*

Compound	MIC (μg/ml)
MD-01	Inactive
MD-02	Inactive
MD-03	6.25
MD-04	Inactive
MD-05	Inactive
MD-06	25
MD-07	50
MD-08	50
MD-09	25
MD-10	Inactive
MD-11	Inactive
MD-12	25
Rifampicin	0.0047
Kanamycin	2.5
Isoniazide	0.05

CHAPTER V

CONCLUSION

Phytochemical investigation of the stem bark of *Cratoxylum arborescens* (Vahl) Blume (Cratoxylaceae), afforded a new natural xanthone, 1,3-dihydroxy-6,7-dimethoxy-2,8-diprenylxanthone [**168**] which was isolated together with three known compounds. These compounds are fuscaxanthone C [**167**], 1,8-dihydroxy-3-geranyloxy-6-methylanthraquinone [**166**], and 2-geranylemodin [**169**]. The roots of *C. arborescens* led to the isolation of a known xanthone, 1,7-dihydroxyxanthone [**17**] and the same compounds as from the stem bark. All of the isolates were tested for cytotoxic activity against NCI-H187 cells, KB cells, BC cells, and Vero cells. The isolates from *C. arborescens* were inactive against all cell lines, but compounds **168** and **169** showed moderate cytotoxic activity against the human small cell lung cancer cell line NCI-H187, IC_{50} 3.69±1.27 and 3.08±0.73 μg/ml, respectively. All of the isolates from *C. arborescens* showed no antimalarial activity against *Plasmodium falciparum*, K1 strain. From the stem bark of *Millettia decipiens* Prain. (Fabaceae), two new compounds, (+)-*S*-3,4-methylenedioxy-2,′4′,6′,*β*-tetramethoxydihydrochal-cone [**173**] and 2,4-dimethoxy-3′,4′-methylenedioxydihydrochalcone [**174**] were isolated, along with nine known compounds. The nine known compounds were 3′,4′-methylenedioxy-2,4,6,*β*-tetramethoxychalcone [**54**], lanceolatin B [**116**], ponga-flavone [**170**], karanjin [**171**], milletenone [**46**], desmethoxykanugin [**172**], dihydro-isomilletenone methyl ether [**48**], dihydromilletenone methyl ether [**47**], and ponga-glabrone [**175**]. The roots of *M. decipiens* led to the isolation of ovalitenin B [**41**], and the same compounds as from the stem bark. All of the isolates showed no antimalarial activity or free radical scavenging activity. All of the isolates were tested for their antimycobacterial activity, compound **170** showed moderate activity with a MIC value of 6.25 μg/ml, compounds **172**, **48**, **173**, **47**, and **41** showed weak activity with MICs of 25, 50, 50 25, and 25 μg/ml, respectively. Compounds **46**, **54**, **116**, **171**, **174**, and **175** were inactive.

REFFERENCES

Asomaning, W. A., Amoako, C., Oppong, I. V., Phillips, W. R., Addac Mensah, I., Twum, E. Y. O., Waibel, R., and Achenbach, H. 1995. Pyrano- and dihydrofurano-isoflavones from *Millettia thonningii*. Phytochemistry 39(5): 1215-1218.

Asomaning, W. A., Otoo, E., Akoto, O., Oppong, I. V., Addae-Mensah, I., Waibel, R., and Achenbach, H. 1999. Isoflavones and coumarins from *Millettia thonningii*. Phytochemistry 51: 937-941.

Baruah, P., Baruah, N. C., Sharma, R. P., Baruah, J. N., Kulanthaivel, P., and Herz, W. 1984. Flavonoids from *Millettia pulchra*. Phytochemistry 23(2): 443-447.

Becke, A. D. 1988. Density-functional exchange-energy approximation with correct asymptotic behavior. 1988. Physical Review A 38: 3098.

Bennett, G. J., and Lee, H. H. 1989. Xanthones from Guttiferae. Phytochemistry 28: 967-998.

Bennett, G. J., Harrison, L. J., Sia, G-L., and Sim, K-Y. 1993. Triterpenoids, tocotrienols and xanthones from the bark of *Cratoxylum cochinchinense*. Phytochemistry 32(5): 1245-1251.

Botta, B., Monache, F. D., Monache, G. D., Marini Bettolo, G. B., and Oguakwa, J. U. 1983. 3-Geranyloxy-6-methyl-1,8-dihydroxyanthraquinone and vismones C, D and E from *Psorospermum febrifugum*. Phytochemistry 22(2): 539-542.

Botta, B., Monache, F. D., Monache, G. D., Marini Bettolo, G. B., and Msonthi, J. D. 1985. Prenylated bianthrones and vismone F from *Psorospermum febrifugum*. Phytochemistry 24(4): 827-830.

Cardona, M. L., and Seoane, E. 1982. Xanthone constituents of *Hypericum ericoides*. J. Nat Prod. 45(2): 134-136.

Chopra, R. N., Badhwar, R. L., and Ghosh, S. 1965. Poisonous plants of India vol.I. 289-356. New Dehli: The National Printing Works.

Collins, L., and Franzblau, S.G. 1997. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Antimicrob. Agents Chemother 41(5): 1004-1009.

Dagne, E., and Bekele, A. 1990. C-prenylated isoflavones from *Millettia ferruginea*. Phytochemistry 29(8): 2679-2682.

Dagne, E., Bekele, A., Noguchi, H., Shibuya, M., and Sankawa, U. 1990. *O*-Geranylated and *O*-prenylated flavonoids from *Milletia ferruginea*. Phytochemistry 29(8): 2671-2673.

Dagne, E., Bekele, A., and Waterman, P. E. 1989. The flavonoids of *Millettia ferruginea* subsp. *ferruginea* and subsp. *darassana* in Ethiopia. Phytochemistry 28(7): 1897-1900.

Das, B., Chakravarty, A. K., Masuda, K., Suzuki, H., and Ageta, H. 1994. A diterpenoid from roots of *Gelonium multiflorum*. Phytochemistry 37(5): 1363-1366.

Desjardins, R. E., Canfield, C. J., Haynes, J. D., and Chulay, J. D. 1979. Quantitative assessment of antimalarial activity *in vivo* by semiautomated microdilution technique. Antimicrob. Agents Chemother 16: 710-718.

Ekavibhata, K., Phengklai, C and Niyomdham, C., 1991. Flora in peat swamp areas of Naratiwat; Phikul thong study centre. 197. Bangkok. S. Sombun Press.

Fotsing, M. T., Yankep, E., Njamen, D., Fomun, Z. T., Nyasse, B., Bodo, B., Recio, M. C., Giner, R. M., and Rios, J. L. 2003. Identification of an anti-inflammatory principle from the stem bark of *Millettia versicolor*. Planta Med. 69: 767-770.

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Montgomery, Jr., J. A., Vreven, T., Kudin, K. N., Burant, J. C., Millam, J. M., Iyengar, S. S., Tomasi, J., Barone, V., Mennucci, B., Cossi, M., Scalmani, G., Rega, N., Petersson, G. A., Nakatsuji, H., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, E., Yazyev, O., Austin, A. J., Cammi, R., Pomelli, C., Ochterski, J. W., Ayala, P. Rabuck, A. D., Raghavachari, K., Foresman, J. B., Ortiz, J. V., Cui, Q., Baboul, A. G., Clifford, S., Cioslowski, J., Stefanov, B. B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Martin, R. L., Fox, D. J., Keith, T., Al-Laham, M. A., Peng, C. Y., Nanayakkara, A., Challacombe, M., Gill, P. M. W., Johnson, B., Chen, W., Wong, M. W., Gonzalez, C., and Pople, J. A. 2004. Gaussian 03, Revision C.02., Wallingford CT: Gaussian.

Fuendjiep, V., Nkengfack, A. E., Fomun, Z. T., Sondengam, B. L., and Bodo, B. 1998a. Conrauinones A and B, two new isoflavones from stem bark of *Millettia conraui*. J. Nat. Prod. 61: 380-383.

Fuendjiep, V., Nkengfack, A. E., Fomun, Z. T., Sondengam, B. L., and Bodo, B. 1998b. Conrauinones C and D, two isoflavonoids from stem bark of *Millettia conraui*. Phytochemistry 47(1): 113-115.

Galeffi, C., Rasoanaivo, P., Federici, E., Palazzino, G., Nicoletti, M., and Rasolondratovo, B. 1997. Two prenylated isoflavanones fron *Millettia pervilleana*. Phytochemistry 45: 189-192.

Garcez, F. R., Scramin, S., Nascimento, M. C., and Mors, W. B. 1988. Prenylated flavonoids as evolutionary indicators in the genus *Dahlstedtia*. Phytochemistry 27(4): 1079-1083.

Gupta, R. K., Bhattacharyya, A. B., Mitra, S. R., and Adityachaudhury, N. 1983. Isoaurmillone, and isoflavone from the pods of *Millettia auriculata*. Phytochemistry 22(5): 1306-1307.

Gupta, R. K., and Krishnamurti, M. 1976. Prenylated flavanones from *Millettia ovalifolia* seeds. Phytochemistry 15: 832-833.

Gupta, R. K., and Krishnamurti, M. 1977. New dibenzoylmethane and chalcone derivatives from *Millettia ovalifolius*. Phytochemistry 16: 1104-1105.

Iinuma, M., Tosa, H., Ito, T., Tanaka, T., and Madulid, D. A. 1996. Two xanthones from roots of *Cratoxylum formosanum*. Phytochemistry 42(4): 1195-1198.

Islam A., Gupta, R. K., and Krishnamurti. 1980. Furano chalcone and prenylated flavanone from *Millettia ovalifolia* seeds. Phytochemistry 19: 1558-1559.

Ito, C., Itoigawa, M., Takakura, T., Ruangrungsi, N., Enjo, F., Tokuda, H., Nishino, H., and Furukawa. 2003. Chemical constituents of *Garcinia fusca*: Structure elucidation of eight new xanthones and their cancer chemopreventive activity. J. Nat. Prod. 66: 200-205.

Kalra, J., Krishnamurti, M., and Nath, M. 1977. Chemical investigation of Indian yam beans (*Pachyrrhizus erosus*): Isolation & structures of two new rotenoids & a new isoflavanone, erosenone. Indian Journal of Chemistry 15B: 1084-1086.

Kamnaing, P., Free, S. N. Y. F., Nkengfack, A. E., Folefoc, G., and Fomun, Z.T. 1999. An isoflavan-quinone and a flavonol from *Millettia laurentii*. Phytochemistry 51: 829-832.

Kamnaing, P, Free, S. N., Fomum, Z. T., and Martin M-T. 1994. Millettonine, a guanidine alkaloid from *Millettia laurentii*. Phytochemistry 36(6): 1561-1562.

Kamperdick, C., Phuong, N. M., Sung, T. V., and Adam, G. 1998. Flavones and isoflavones from *Millettia ichthyochtona*. Phytochemistry 48(3): 577-579.

Khalid, S. A., and Waterman, P. E. 1983. Thonningine-A and Thonningine-B: two 3-phenylcoumarins from the seeds of *Millettia thonningii*. Phytochemistry 22(4): 1001-1003.

Khan, H., and Zaman, A. 1974. Extractives of *Millettia ovalifolia*. Tetrahedron 30: 2811-2815.

Kijjoa, A., Jose, M., Gonzalez, T. G., Pinto, M. M. M., Damas, A. M., Mondranondra, I., Silva A. M., and Herz, W. 1998. Xanthones from *Cratoxylum maingayi*. Phytochemistry 49(7): 2159-2162.

Kumar, D. T., Krupadanam, G. L. D., and Srimannarayana, G. 1989. Isoflavans from *Millettia racemosa*. Phytochemistry, 28(3): 913-916.

Lee, C., Yang, W., and Parr, R. G. 1988. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Physical Review B 37, 785-789.

Lu, Z. X., Hasmeda, M., Mahabusarakam, W., Ternai, B., Ternai Chamsuksai, P., and Polya, G. M. 1998. Inhibition of eukaryote protein kinases and of a cyclic nucleotide-binding phosphatase by prenylated xanthones. Chemico-Biological Interaction 114: 121-140.

Markham, K. R. 1982. Techniques of flavonoid identification. London: Academic Press, pp. 38-39.

Mahmoud, E. N., and Waterman, P. E. 1985. Flavonoids from the stem bark of *Millettia hemsleyana*. Phytochemistry 24(2): 369-371.

Mbafor, J. T., Atchade, A. T., Nkengfack, A. E., Fomum, Z. T., and Sterner, O. 1995. Furanoflavones from root bark of *Millettia sanagana*. Phytochemistry 40(3): 949-952.

Minhaj, N., Khan, H., Kapoor, S. K., and Zaman, A. 1976. Extractives of *Millettia auriculata*. Tetrahedron 32: 749-751.

Ngamga, D., Free, S. N. Y. F., and Fomum Z. T. 1993. Millaurine and acetylmillaurine:Alkaloid from *Millettia laurentii*. J. Nat. Prod. 56(12): 2126-2132.

Ngamga, D., Free, S. N. Y. F., and Fomum Z. T. 1994. A new guanidine alkaloid from *Millettia laurentii*. J. Nat. Prod, 57(7): 1022-1024.

Nguyen, L. H. D., and Harrison, L. L. 1998. Triterpenoid and xanthone constituents of *Cratoxylum cocnincninense*. Phytochemistry 50: 471-476.

Olivares, E. M., Lwande, W., Monache, F. D., and Bettolo, G. B. M. 1982. A pyrano-isoflavone from seeds of *Millettia thonningii*. Phytochemistry 21(7): 1763-1765.

Ollis, W. D., Rhodes, C. A., and Sutherland, I. O. 1967. The extractives of *Millettia dura* (Dunn). The constitutions of durlettone, durmillone, milldurone, millettone and millettosin. Tetrahedron 23: 4741-4760.

Parvez, M., and Ogbeide, O. N. 1990. 3-Hydroxy-4'-methoxyflavone from *Millettia zechiana*. Phytochemistry 29(6): 2043-2044.

Pratesi, P., La Manna, A., Campiglio A., and Ghislandi V. 1959. The configuration of noradrenaline. J. Chem. Soc, 4062-4065.

Phrutivorapongkul, A., Lipipun, V., Ruangrungsi, N., Kirtikara, K., Nishikawa, K., Maruyama, S., Watanabe, T., and Ishikawa, T. 2003. Studies on the chemical constituents of stem bark of *Millettia leucantha*: Isolation of new chalcones with cytotoxicity, anti-herpes simplex virus and anti-inflammatory avtivities. Chem. Pharm. Bull. 51(2): 187-190.

Plum, J. A., Milroy, R., and Kaye, S. B. 1989. Effect of the pH dependence of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. Cancer Research 49: 4435-4440.

Raju, K. V. S., and Srimannarayana, G. 1978. Aurmillone, a new isoflavone from the seeds of *Millettia auriculata*. Phytochemistry 17: 1065-1066.

Rao, C. P., and Krupadanam, G. L. D. 1994. An isoflavan from *Millettia racemosa*. Phytochemistry 35(6): 1597-1599.

Rao, C. P., Prasad, Y. R., and Ganapaty, S., 1992. Three prenylated isoflavones from *Millettia auriculata*. Phytochemistry 31(3): 1015-1017.

Rao, C. P., Prashant, A., and Krupadanam, G. L. D. 1996. Two prenylated isoflavans from *Millettia racemosa*. Phytochemistry 41(4): 1223-1224.

Ridley H. N. 1922. The flora of the Malay Peninsula vol. 1 Polypetale. 585. London L. Reeve and Co., LTD.

Saxena, D. B., Tomar, S. S., Singh, R. P., and Mukerjee, S. K. 1987. A new chalcone from *Milletia ovalifolia*. Indian Journal of Chemistry 26B: 704.

Seo, E. K., Kim, N-C., Wani, M. C., Wall, M. E., Navarro, H. A., Burgess, J. P., Kawanishi, K., Kardono, L. B. S., Riswan, S., Rose, W. C., Fairchild, C. R., Farnsworth, N. R., and Kinghorn, A. D. 2002. Cytotoxic prenylated xanthones and the unusual compounds anthraquinonbenzophenones from *Cratoxylum sumatranum*. J. Nat. Prod. 65(8): 300-305.

Shabbir, M., and Zaman, A. 1968. Structure of auriculatin, extractives of *Millettia auriculata*. J. Chem. Soc. (C). 1899-1901.

Shabbir, M., and Zaman, A. 1970. Structure of isoauriculatin and auriculin, extractives of *Millettia auriculata*-II. Tetrahedron 26: 5041-5044.

Sia, G-L., Bennett, G. J., Harrison, L. J., and Sim, K-Y. 1995. Minor xanthones from the bark of *Cratoxylum cochinchinense*. Phytochemistry 38(6): 1521-1528.

Silveira, E. R., and McChesney, J. D. 1994. 6,7-Oxygenated neo-clerodane furan diterpenes from I. Phytochemistry 36(6): 1457-1463.

Singhal, A. K., Baruan, N. C., Sharma, R. P., and Baruan, J. N. 1983. A chalcone and an isoflavone from *Millettia pachycarpa* seeds. Phytochemistry 22(4): 1005-1006.

Singhal, A. K., Sharma, R. P., Thyagarajan, G., Herz, W., and Govindan, S. V. 1980. New prenylated isoflavones and a prenylated dihydroflavonol from *Millettia pachycarpa*. Phytochemistry 19: 929-934.

Singhal, A. K., Sharma, R. P., Madhusudanan, K. P., Thyagarajan, G., Herz, W., and Govindan, S. V. 1981. New prenylated isoflavones from *Millettia pachycarpa*. Phytochemistry 20(4): 803-804.

Singhal, A. K., Sharma, R. P., Baruan, J. N., Govindan, S. V., and Herz, W. 1982. Rotenoids from roots of *Millettia pachycarpa*. Phytochemistry 21(4): 946-951.

Skehamp, P., Storeng, R., Scudiero, D., Monks, A., McMahom, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer. Inst., 82(13): 1107-1112.

Smitinand, T., 2001. Thai Plant names: (botanical names-verancular names) revised edition. The Forest Herbarium, Royal Forest Deparment, Bangkok.

Sritularak, B., Likhitwitayawuid, K., Conrad, J., and Kraus, W., 2002a. Flavonoids from the roots of *Millettia erythrocalyx*. Phytochemistry 61: 943-947.

Sritularak, B., Likhitwitayawuid, K., Conrad, J., Vogler, B., Reeb, S., Klaiber, I., and Kraus, W. 2002b. New flavones from *Millettia erythrocalyx*. J. Nat. Prod. 65: 589-591.

Talapatra, S. K., Mallik, A. K., and Talapatra, B. 1982. Isopongaglabol and 6-methoxyisopongaglabol two new hydroxyfuranoflavones from Pongamia glabra. Phytochemistry 21(3): 761-766.

Takao, T., Kitatani, F., Watanabe, N., Yaki, A., and Sakata, K. 1994. A simple screening method for antioxidants and isolation of serveral antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotech. Biochem. 58: 1780-1783.

Tanaka, T., Iinuma, M., Yuki, K., Fujii, Y., and Mizuno, M. 1992. Flavonoids in the root bark of *Pongamia pinnata*. Phytochemistry, 31: 993-998.

Trager, W., and Jansen, J. B. 1976. Human malaria parasites in continuous culture. Science 193(4254): 673-674.

Yankep, E., Fomun, Z. T., Bisrat, D., Dagne, E., Hellwig, V., and Steglich, W. 1998. *O*-Geranylated isoflavones and a 3-phenylcourmarin from *Millettia griffoniana*. Phytochemistry 49(8): 2521-2523.

Yankep, E., Fomun, Z. T. and Dagne, E., 1997. An *O*-geranylated isoflavone from *Millettia griffoniana*. Phytochemistry 46(3): 591-593.

Yankep, E., Mbafor, J. T., Fomun, Z. T., Steinbeck, C., Messanga, B. B., Ntasse, B., Budzikiewicz, H., Lenz, C., and Schmickler, H. 2001. Further isoflavoid metabolites from *Millettia griffoniana* (Bail). Phytochemistry 56: 363-368.

Yankep, E., Njamen, D., Fotsing, M. T., Fomum, Z. T., Mbanya, J. C., Giner, R. M., Recio, M. C., Máñez, S., and Ríos, J. L. 2003. Griffonianone D, an isoflavone with anti-inflammatory activity from the root bark of *Millettia griffoniana*. J. Nat. Prod. 66(9): 1288-1290

Yenesew, A., Midiwo, J. O., and Waterman, P. E. 1996. Four isoflavones from seed pods of *Millettia dura*. Phytochemistry 41(3): 951-955.

Yenesew, A., Midiwo, J. O. and Waterman P. E. 1997. 6-Methoxycalpognium isoflavone A: A new isoflavone from the seed pods of *Millettia dura*. J. Nat. Prod. 60: 806-807.

Yenesew, A., Midiwo, J. O., and Waterman, P. E. 1998. Rotenoids, isoflavones and chalcones from the stem bark of *Millettia usaramensis* subspecies *usaramensis*. Phytochemistry 47(2): 295-300.

Yenesew, A., Derese, S., Midiwo, J.O., Oketch-Rabah, H. A., Lisgarten, J., Palmer, R., Heydenreich, M., Peter, M. G., Akala, H., Wangui, J., Liyala, P., and Waters, N. C. 2003. Anti-plasmodial activities and x-ray crystal structures of rotenoids from *Millettia usaramensis* subspecies *usaramensis*. Phytochemistry 64: 773-779.

APPENDICES

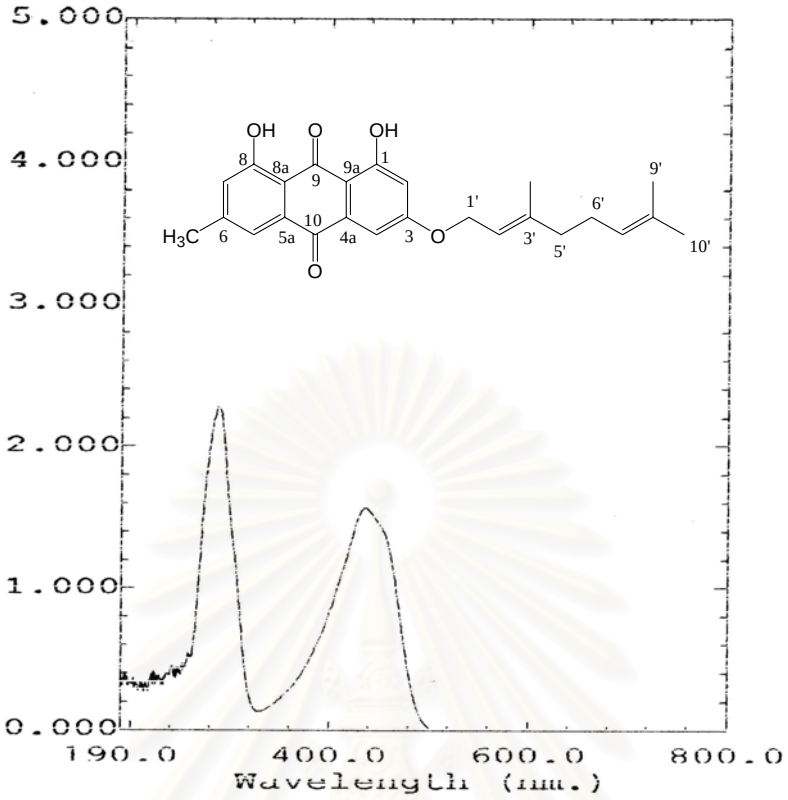


Figure 5. UV Spectrum of Compound CT-01 (methanol)

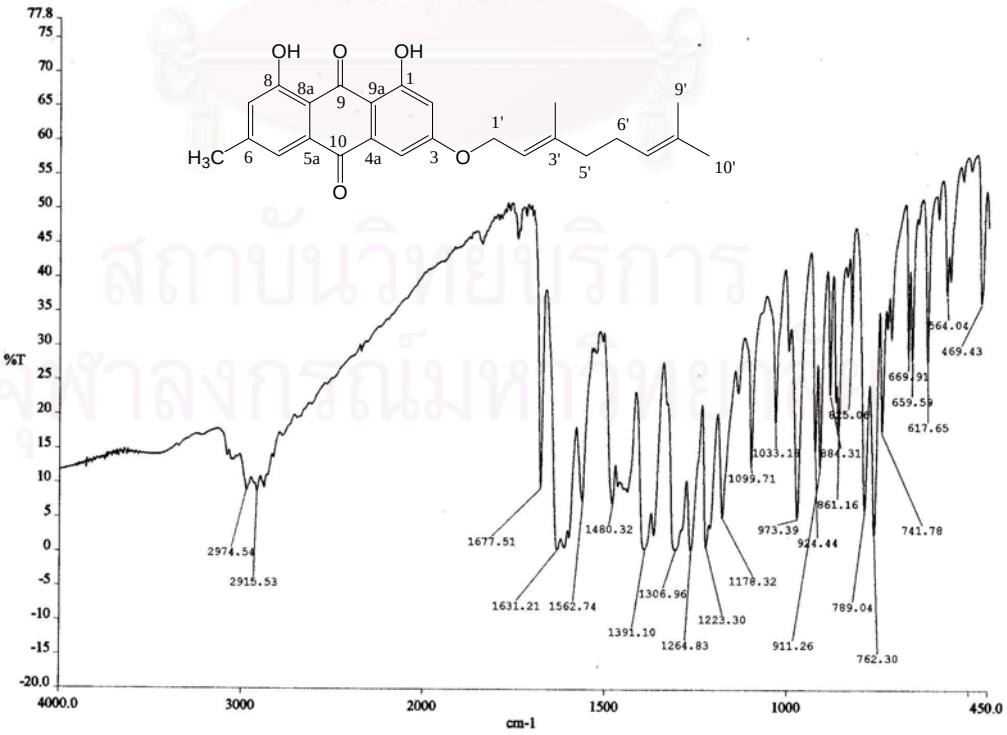


Figure 6. IR Spectrum of Compound CT-01 (KBr disc)

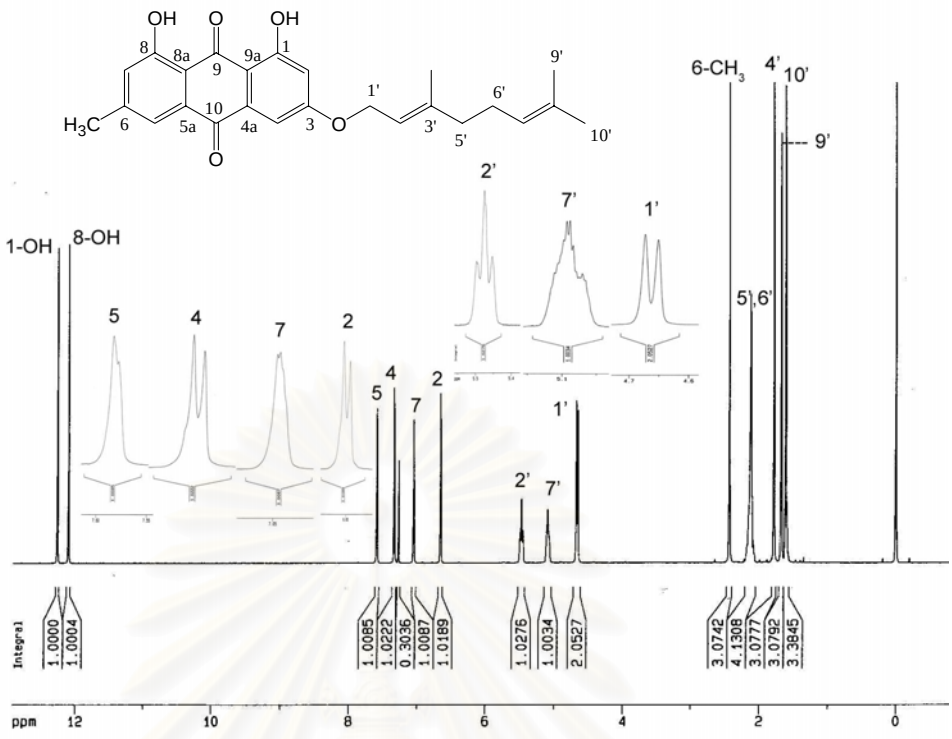


Figure 7. ^{1}H NMR (300 MHz) Spectrum of Compound CT 01 ($CDCl_3$)

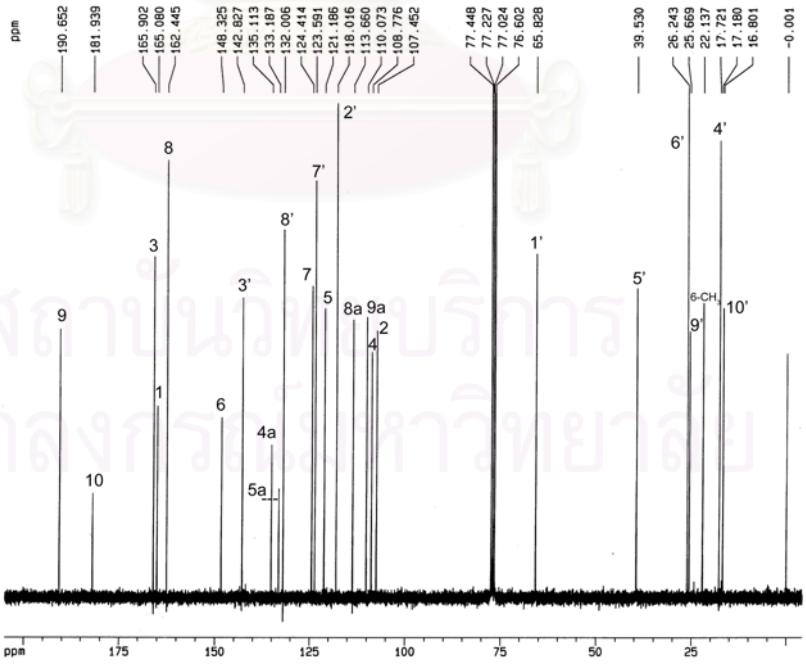


Figure 8. ^{13}C NMR (75 MHz) Spectrum of Compound CT-01 ($CDCl_3$)

5´

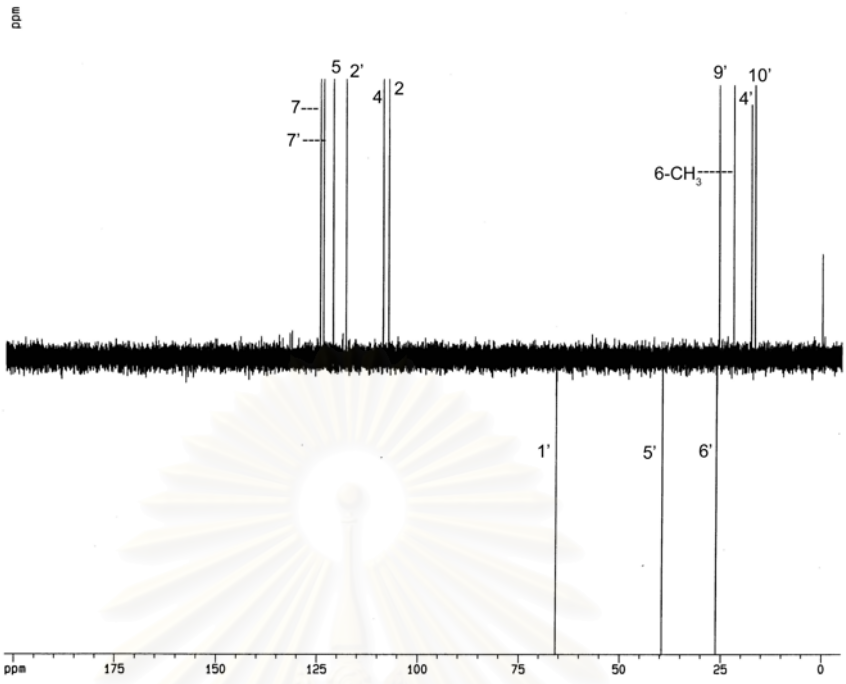


Figure 9. Dept 135 Spectrum of Compound CT-01 ($CDCl_3$)

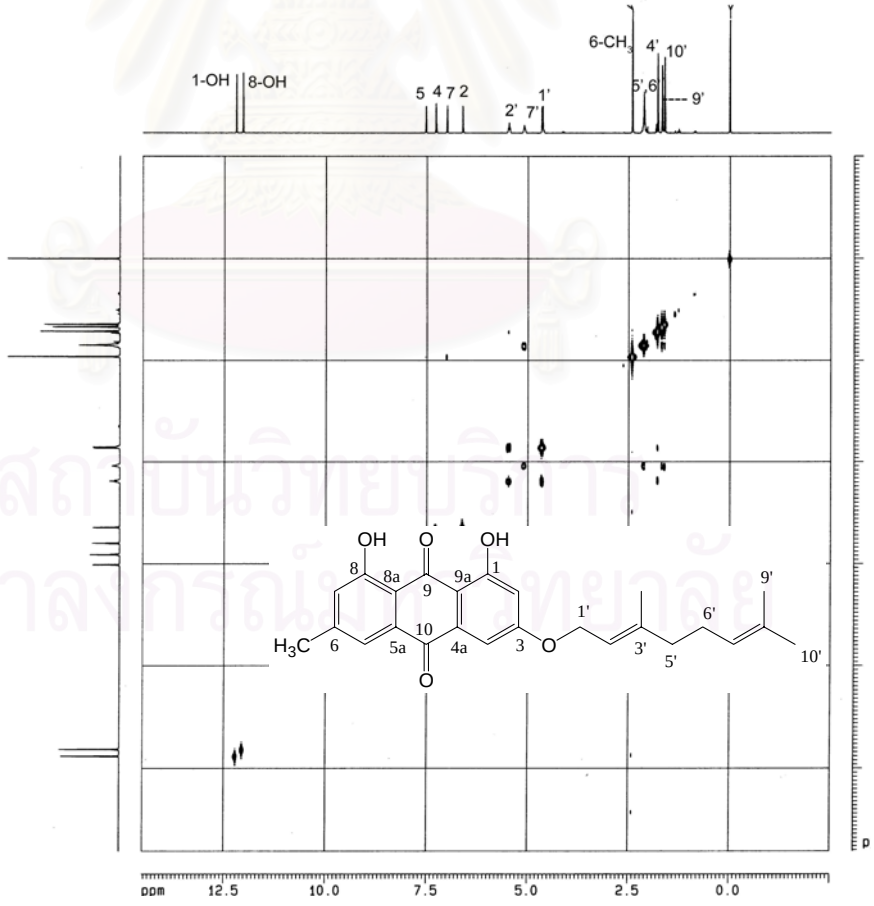


Figure 10. ^{1}H-^{1}H COSY Spectrum of Compound CT-01 ($CDCl_3$)

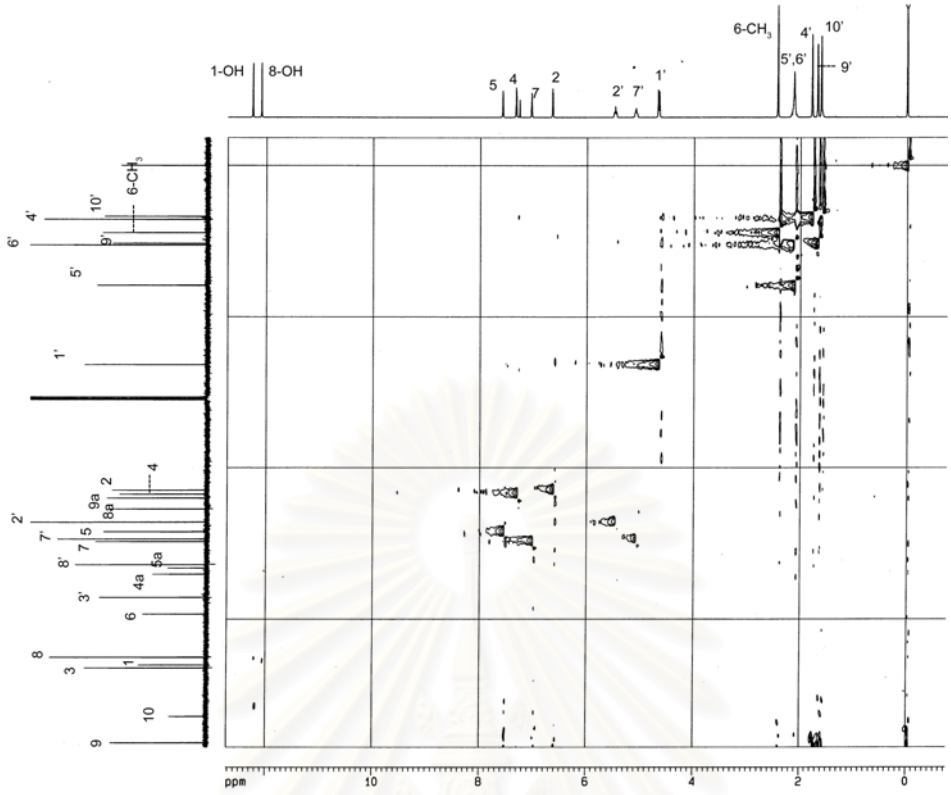


Figure 11. HMQC Spectrum of Compound CT-01 ($CDCl_3$)

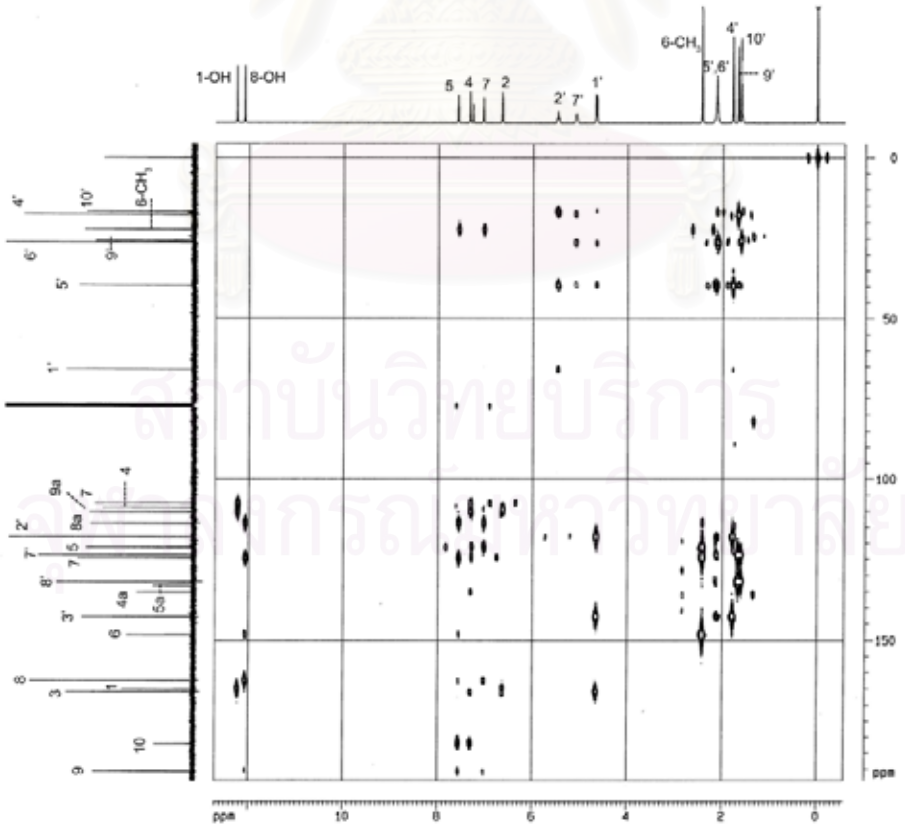


Figure 12. HMBC Spectrum of Compound CT-01 ($CDCl_3$)

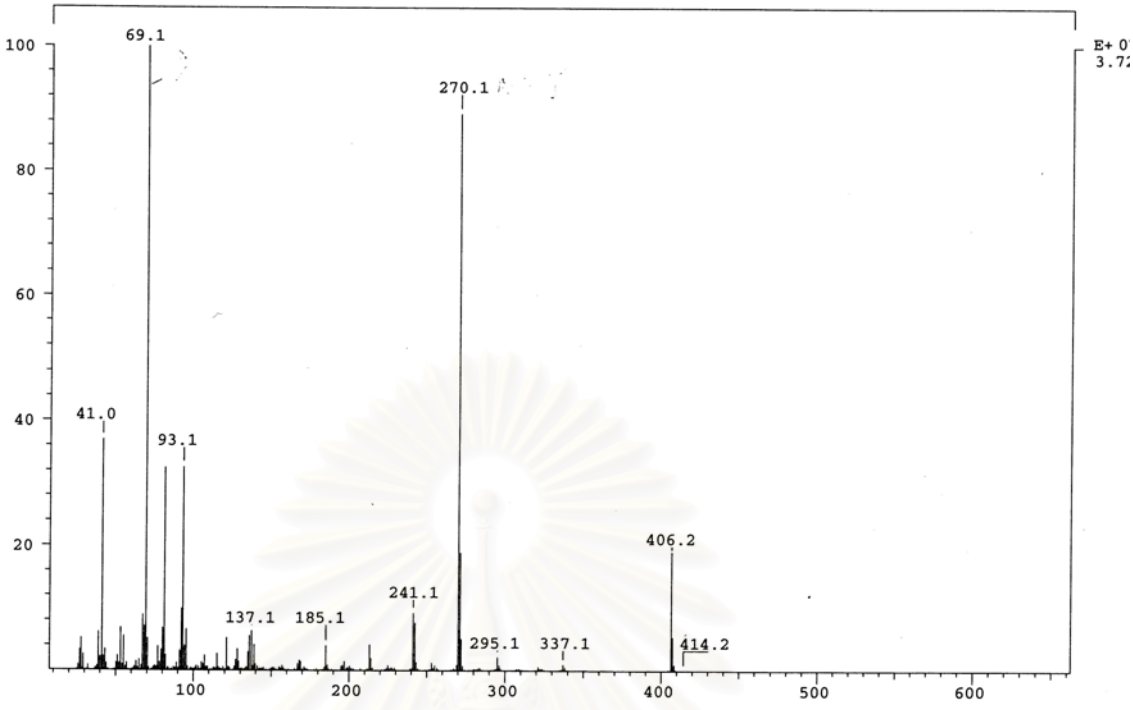


Figure 13. EI Mass Spectrum of Compound CT-01 ($CDCl_3$)

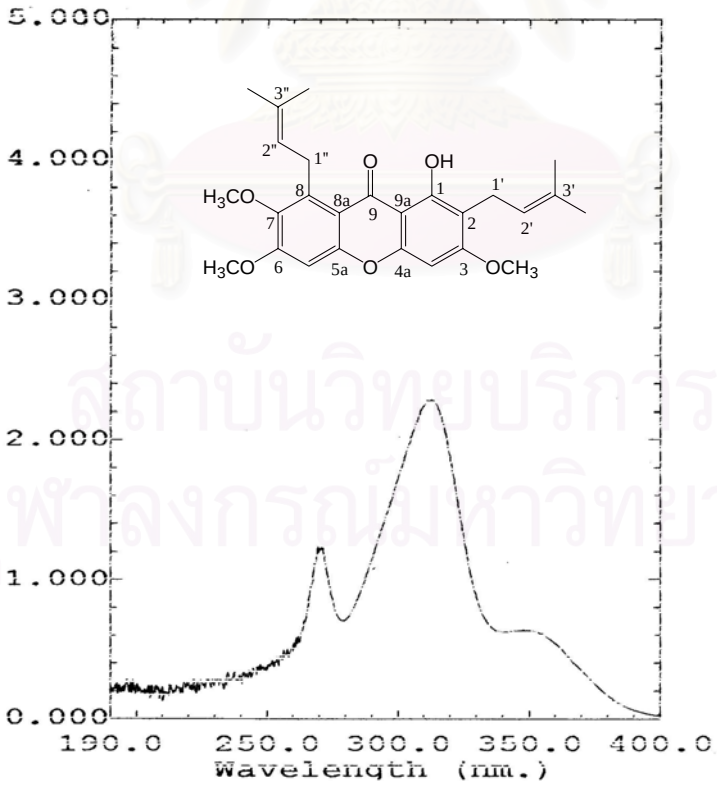


Figure 14. UV Spectrum of Compound CT-02 (methanol)

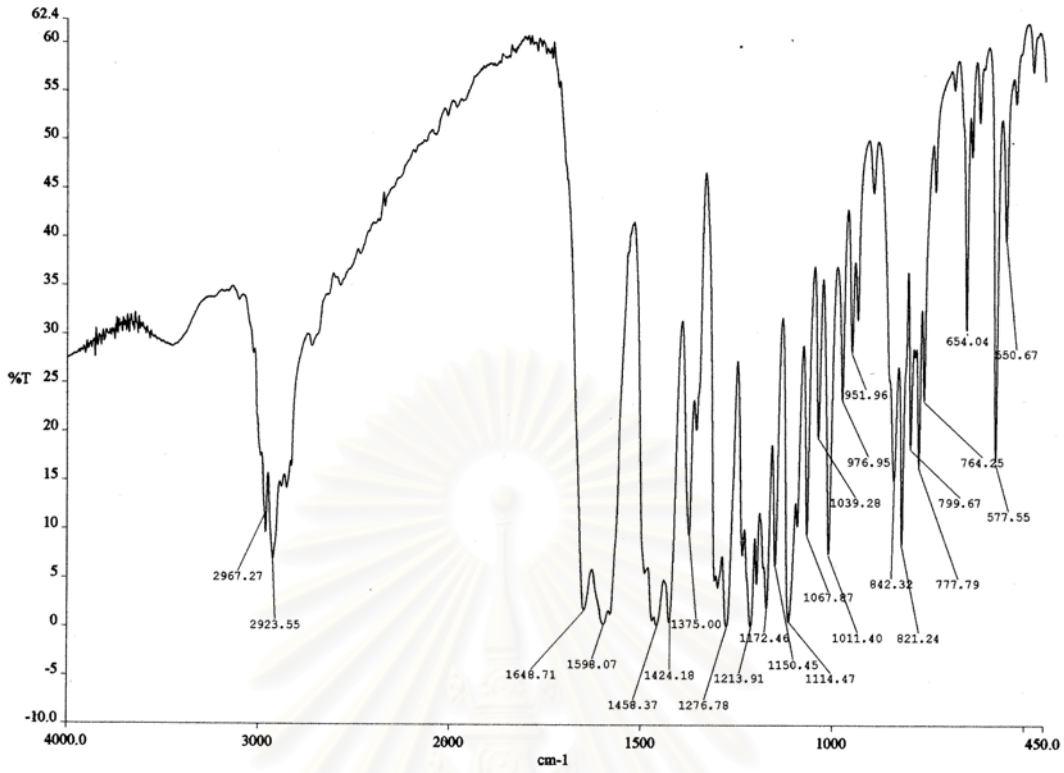


Figure 15. IR Spectrum of Compound CT-02 (KBr disc)

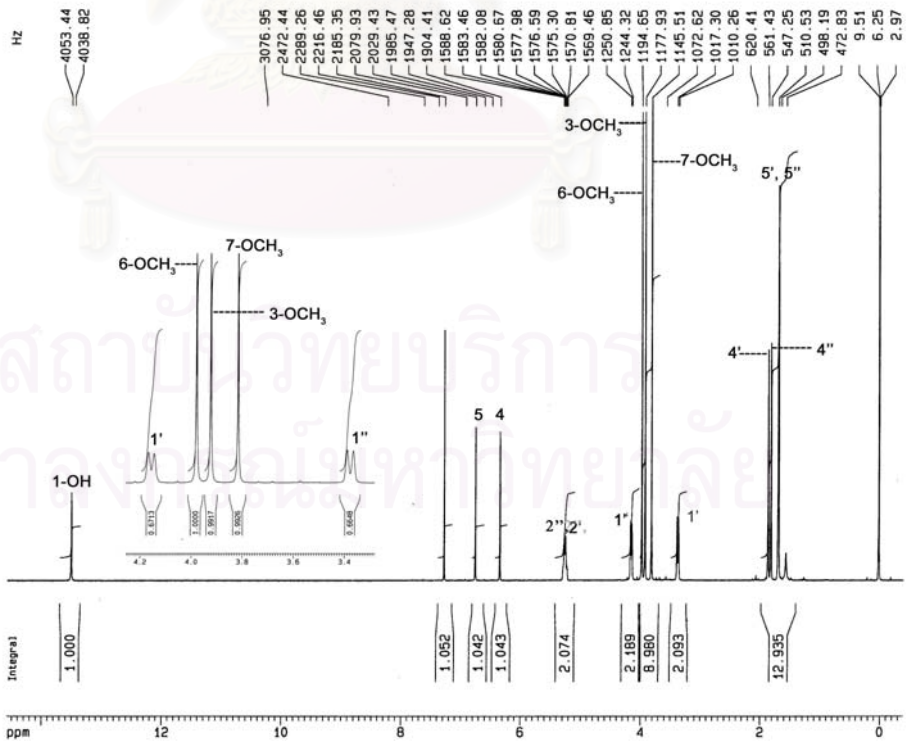


Figure 16. ^{1}H NMR (300 MHz) Spectrum of Compound CT-02 ($CDCl_3$)

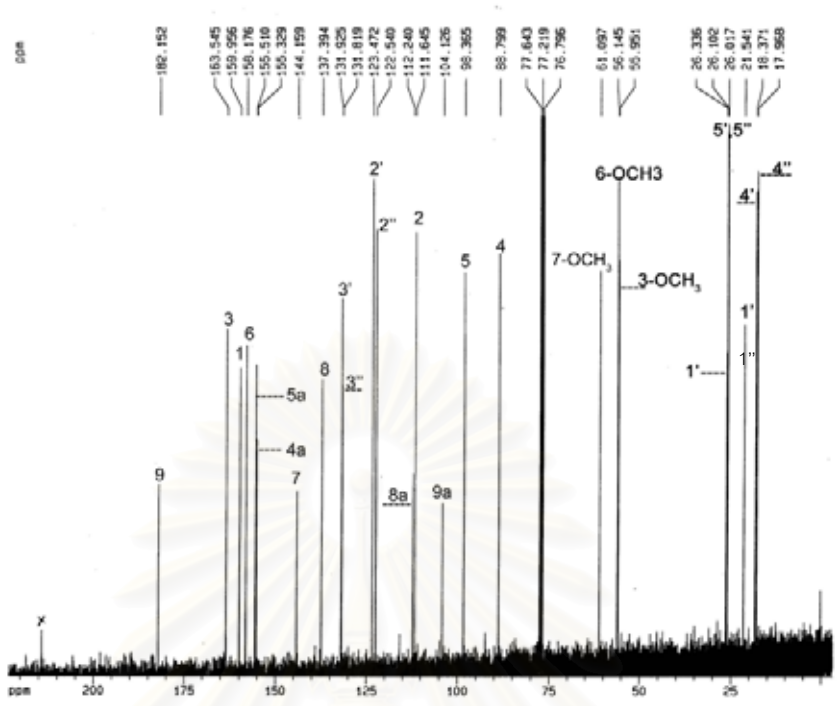


Figure 17. ^{13}C NMR (75 MHz) Spectrum of Compound CT-02 ($CDCl_3$)

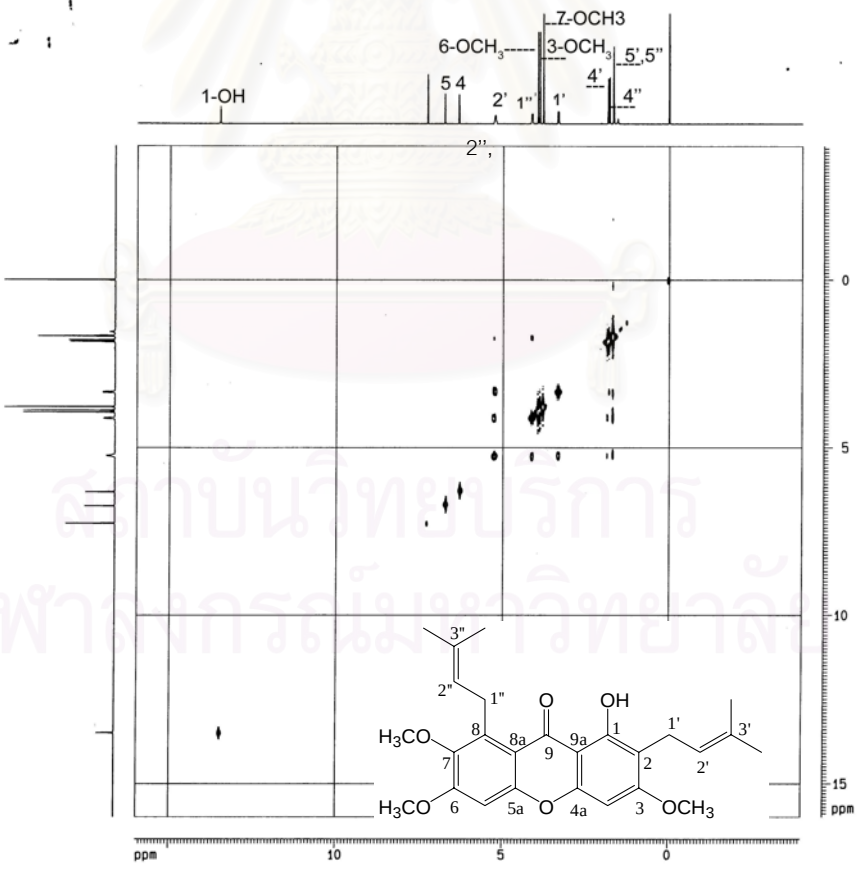


Figure 18. ^{1}H-^{1}H COSY Spectrum of Compound CT-02 ($CDCl_3$)

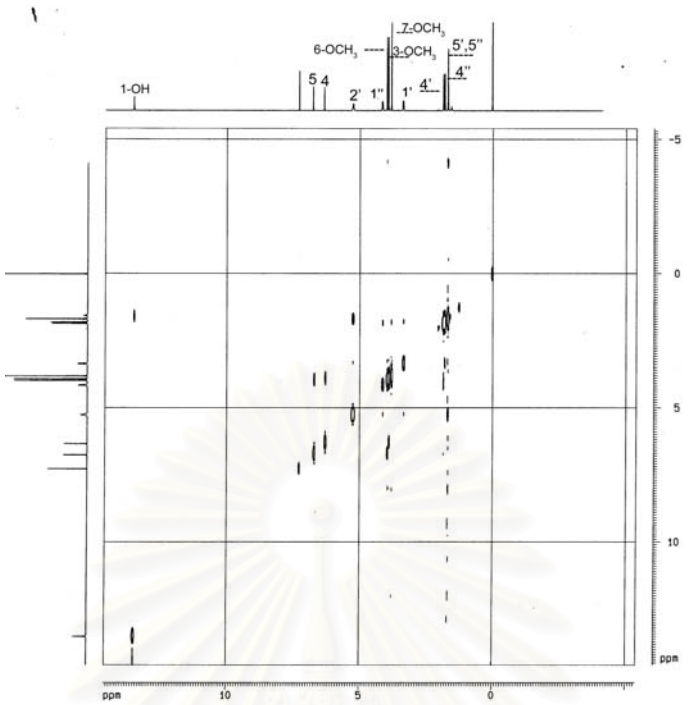


Figure 19. NOESY Spectrum of Compound CT-02 ($CDCl_3$)

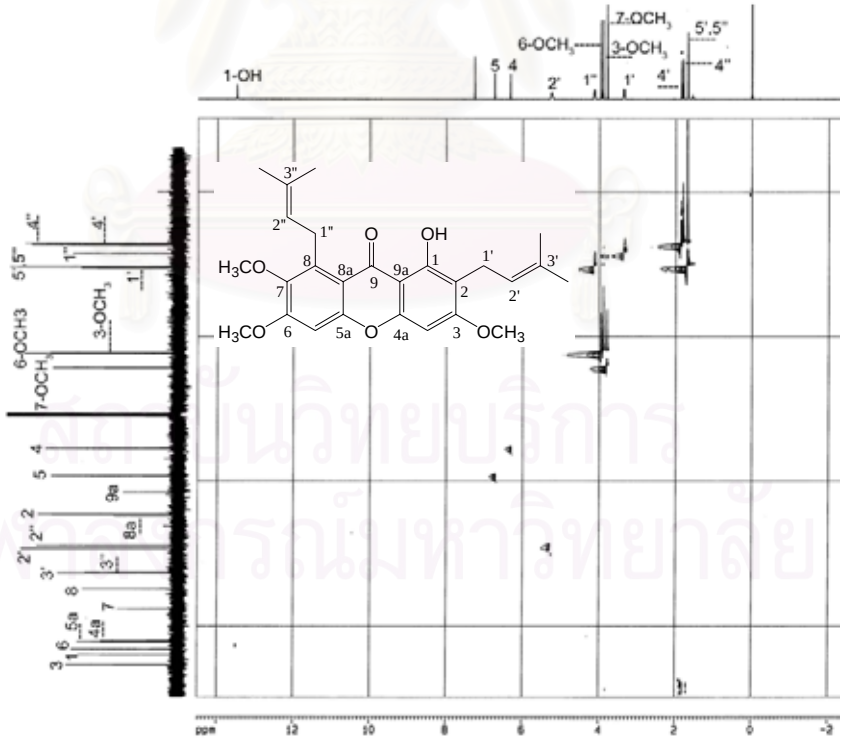


Figure 20. HMQC Spectrum of Compound CT-02 ($CDCl_3$)

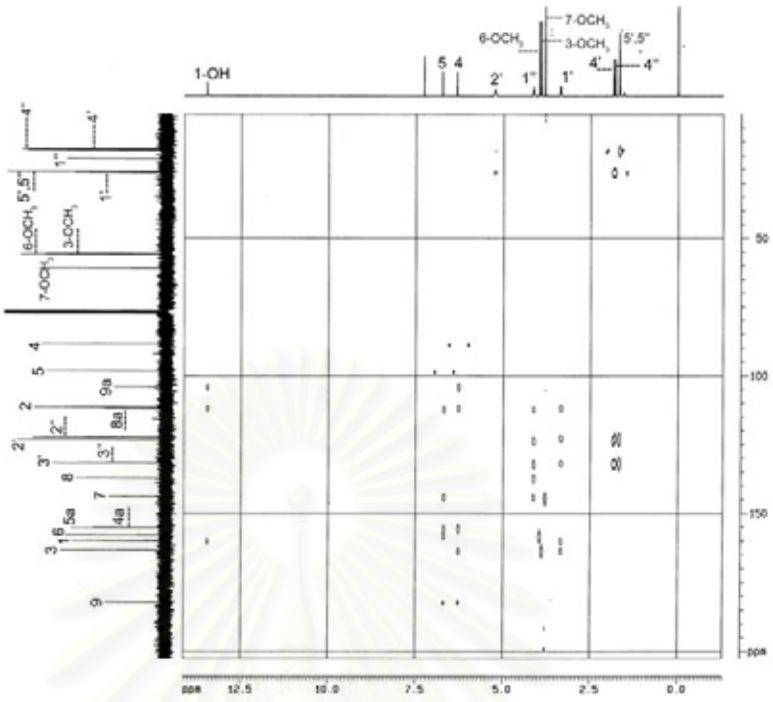


Figure 21. HMBC Spectrum of Compound CT-02 ($CDCl_3$)

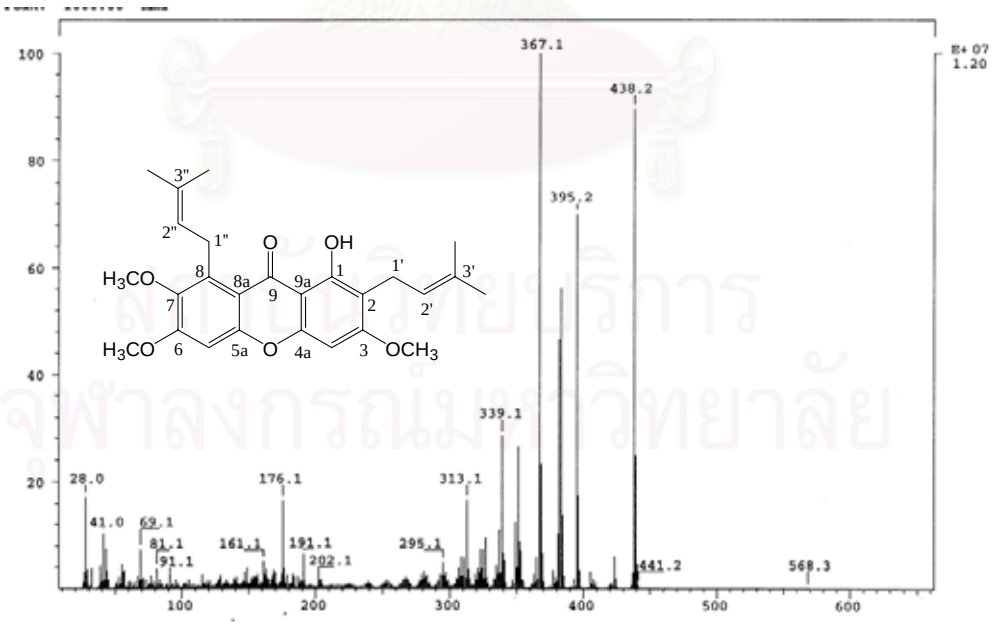


Figure 22. EI Mass Spectrum of Compound CT-02 ($CDCl_3$)

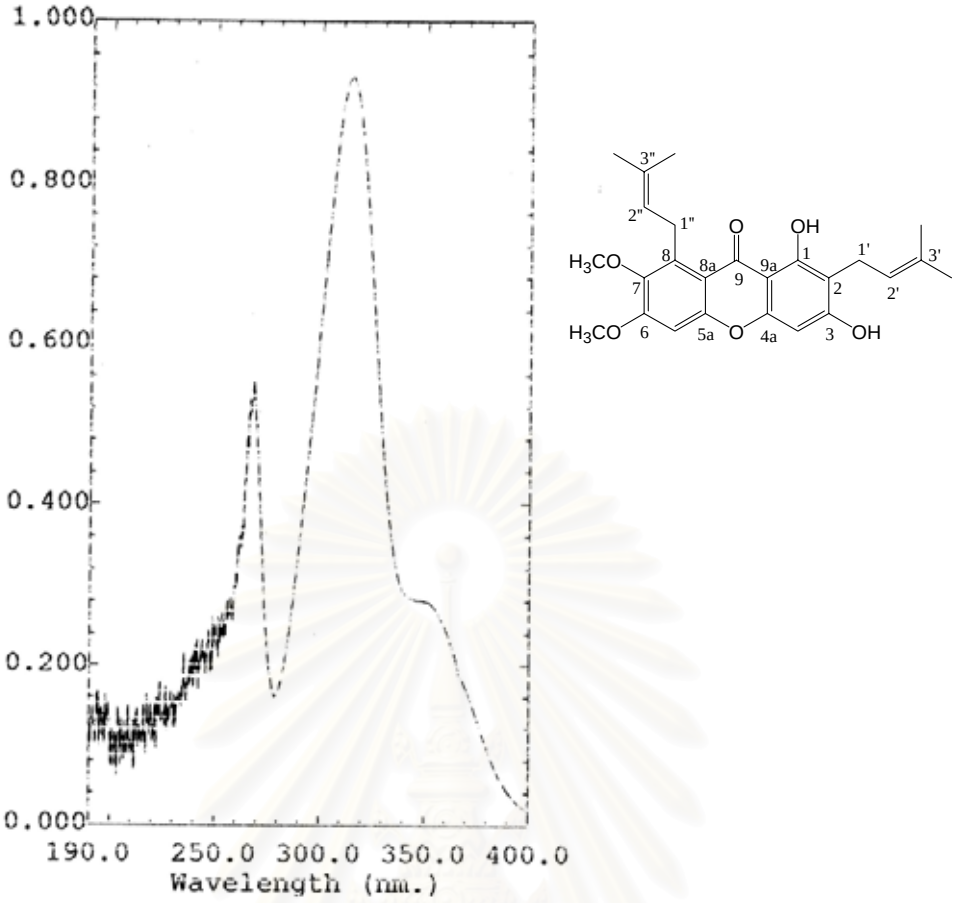


Figure 23. UV Spectrum of Compound CT-03 (methanol)

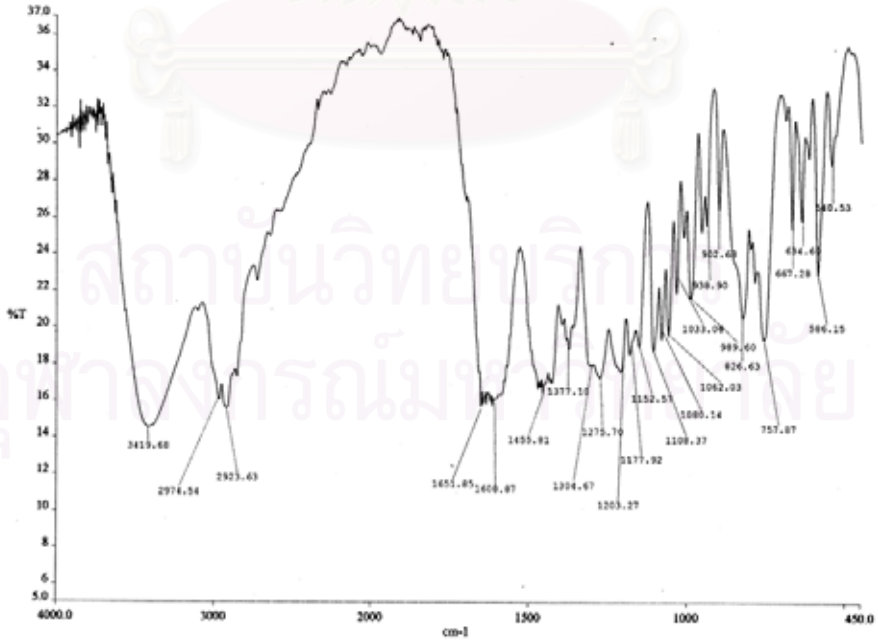


Figure 24. IR Spectrum of Compound CT-03 (KBr disc)

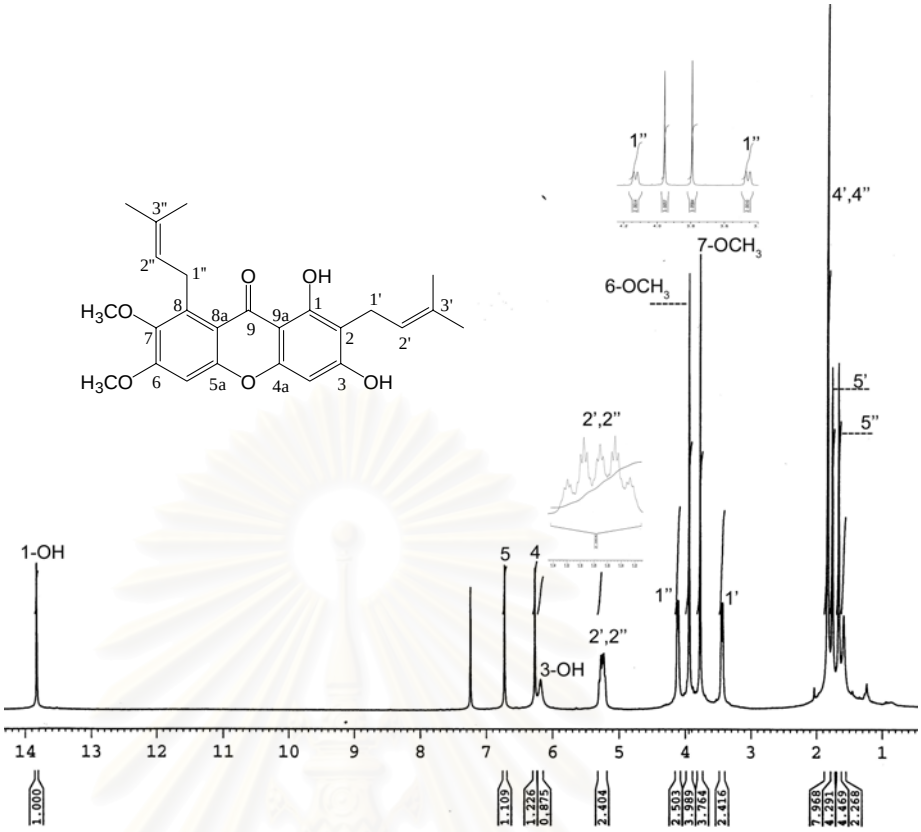


Figure 25. ^{1}H NMR (300 MHz) Spectrum of Compound CT-03 ($CDCl_3$)

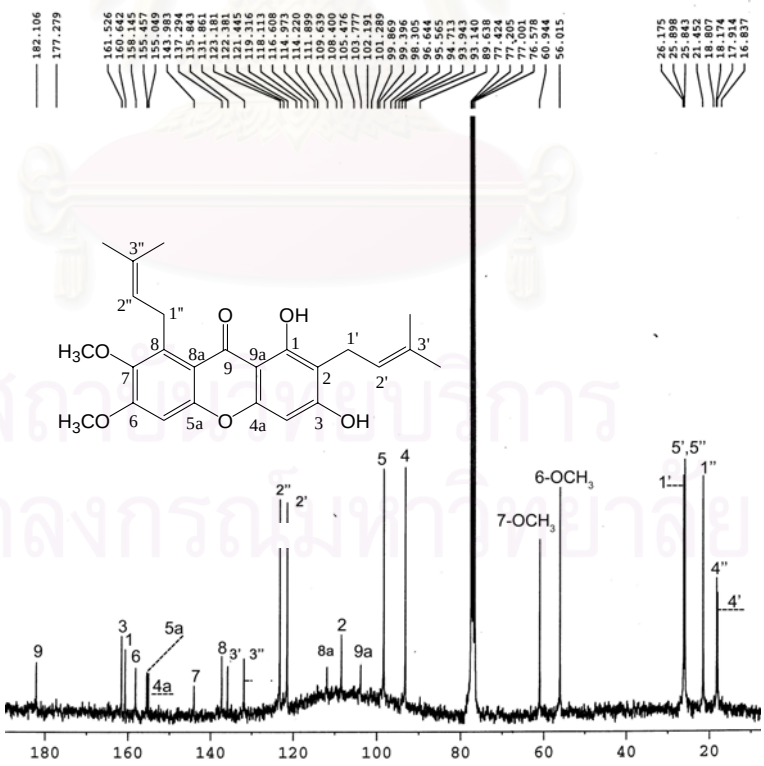


Figure 26. ^{13}C NMR (75 MHz) Spectrum of Compound CT-03 ($CDCl_3$)

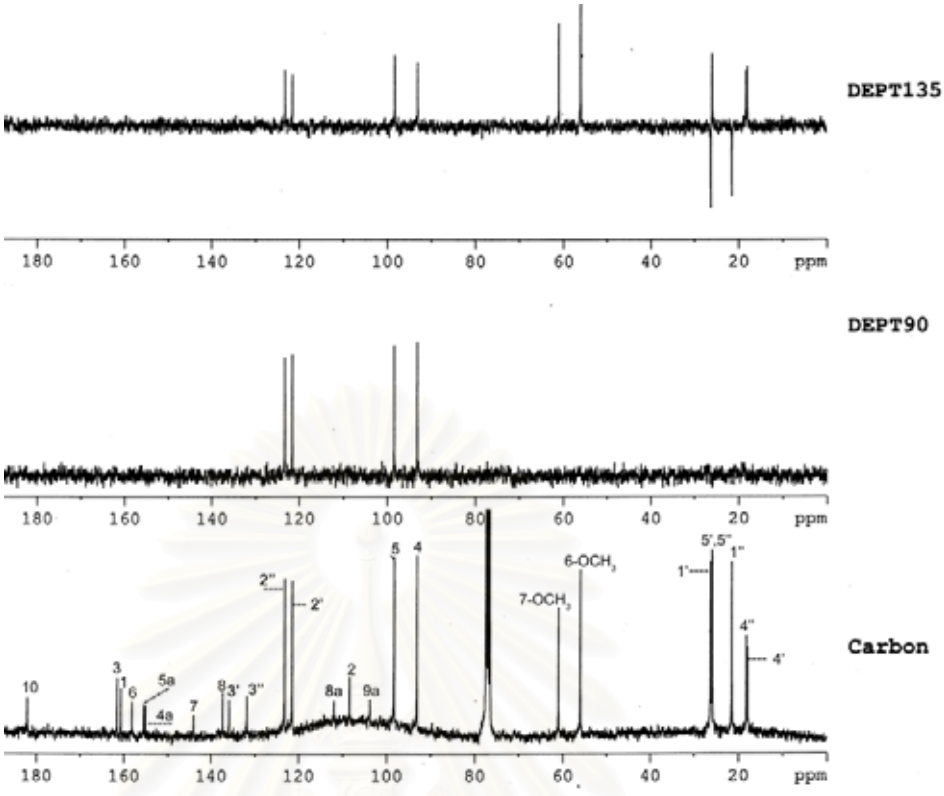


Figure 27. Dept 135, dept 90 and ^{13}C NMR Spectrum of Compound CT 03-($CDCl_3$)

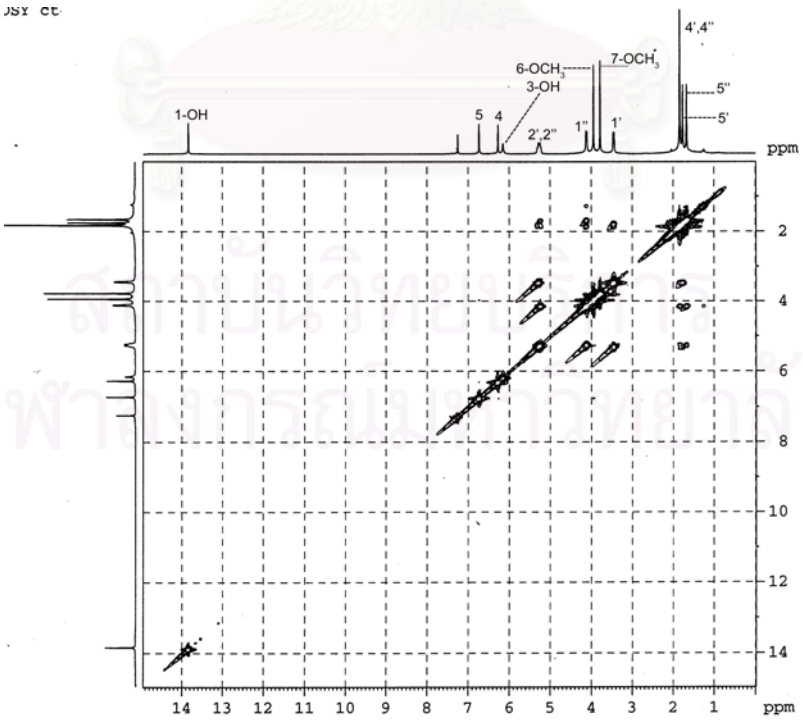


Figure 28. ^{1}H-^{1}H COSY Spectrum of Compound CT-03 ($CDCl_3$)

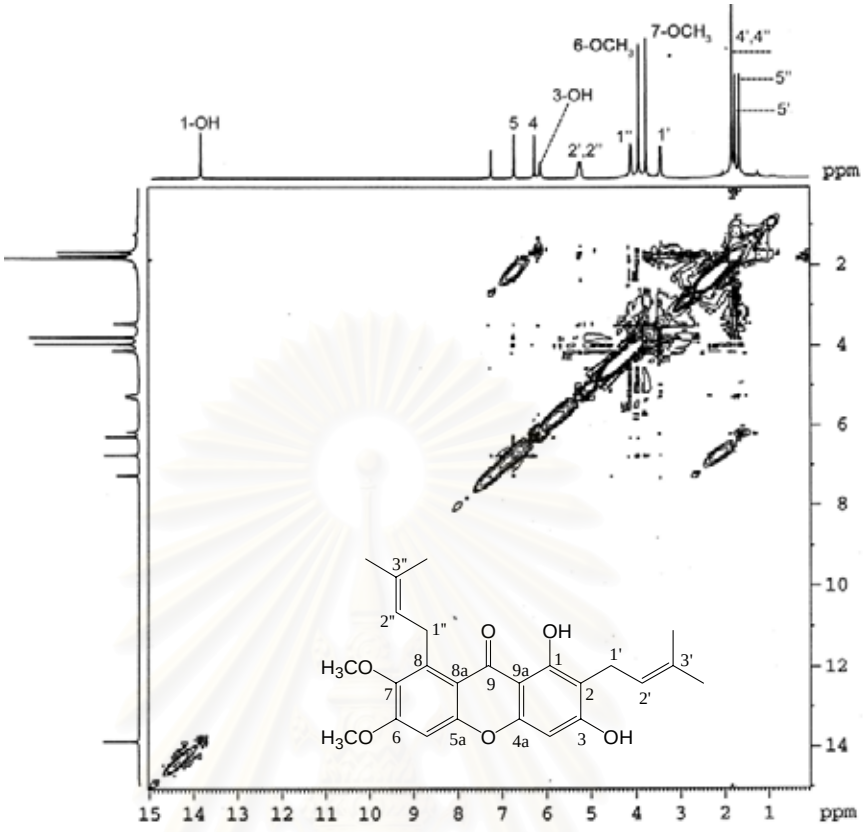


Figure 29. NOESY Spectrum of Compound CT-03 ($CDCl_3$)

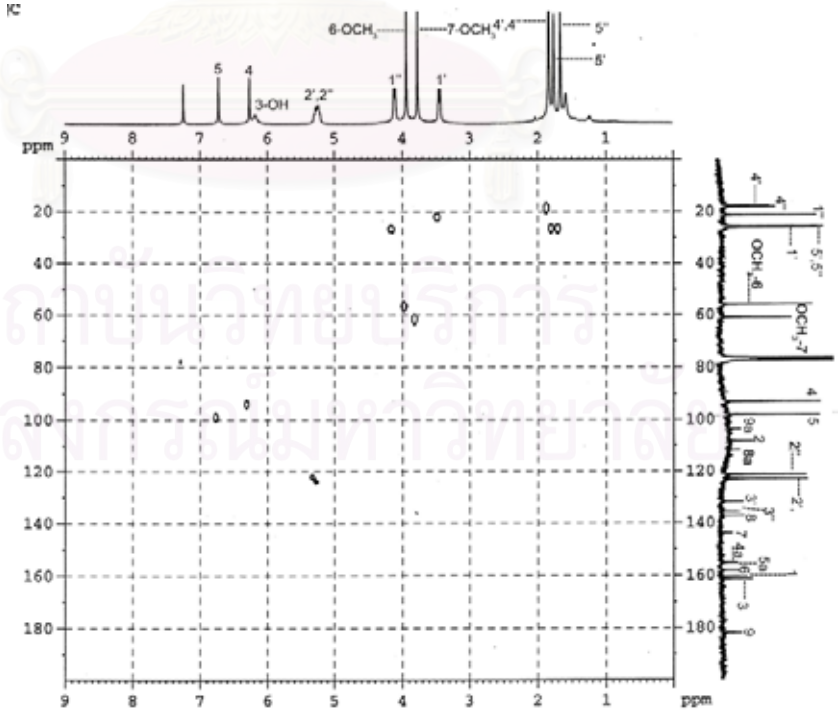


Figure 30. HMQC Spectrum of Compound CT-03 ($CDCl_3$)

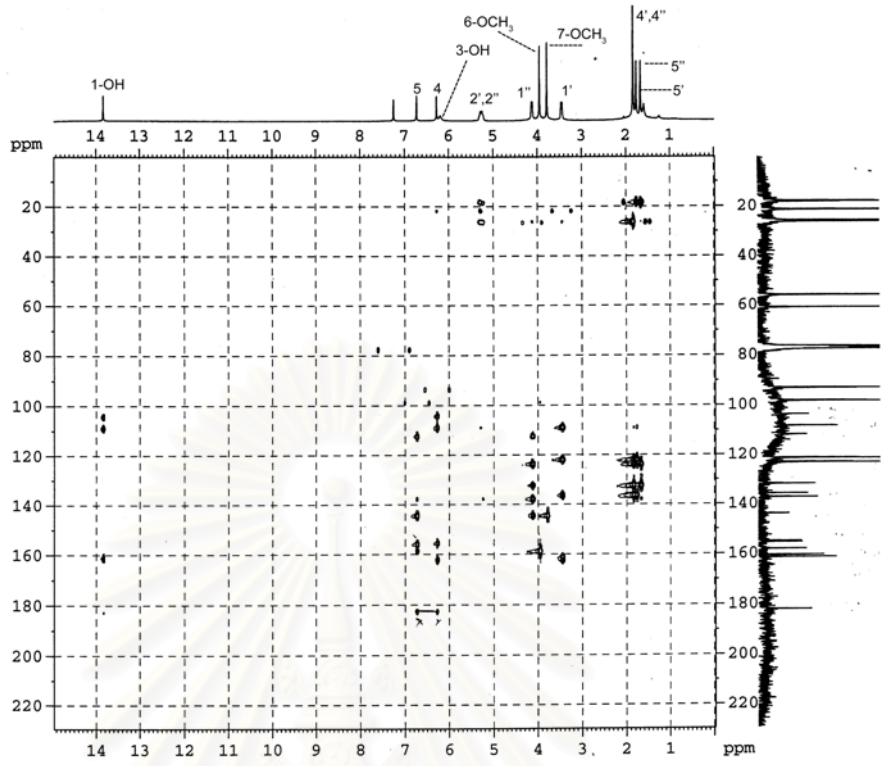


Figure 31. HMBC Spectrum of Compound CT-03 ($CDCl_3$)

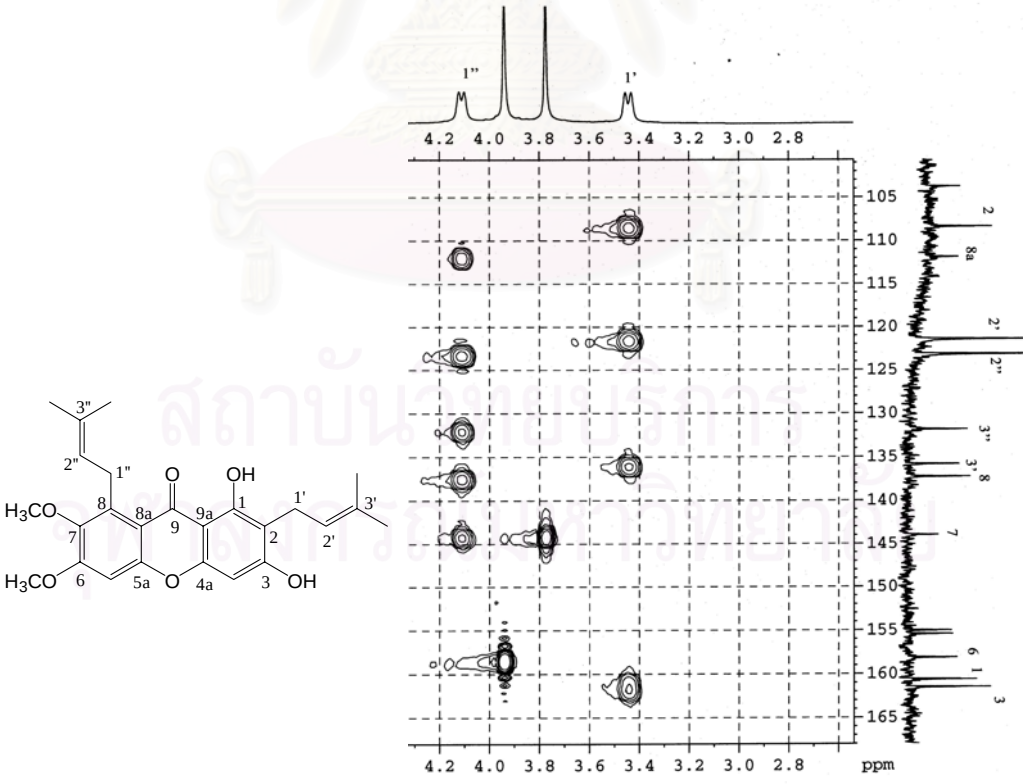


Figure 32. HMBC Spectrum of Compound CT-03 ($CDCl_3$) [δ_H 2.8-4.2 ppm, δ_C105-160 ppm]

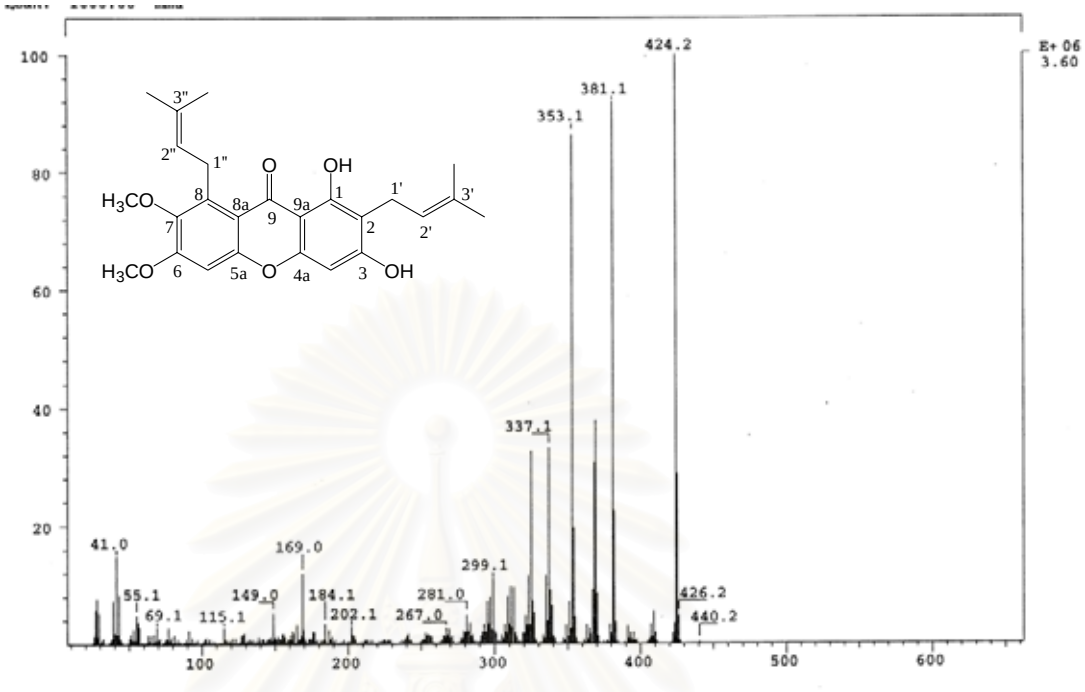


Figure 33. EI Mass Spectrum of Compound CT-03 ($CDCl_3$)

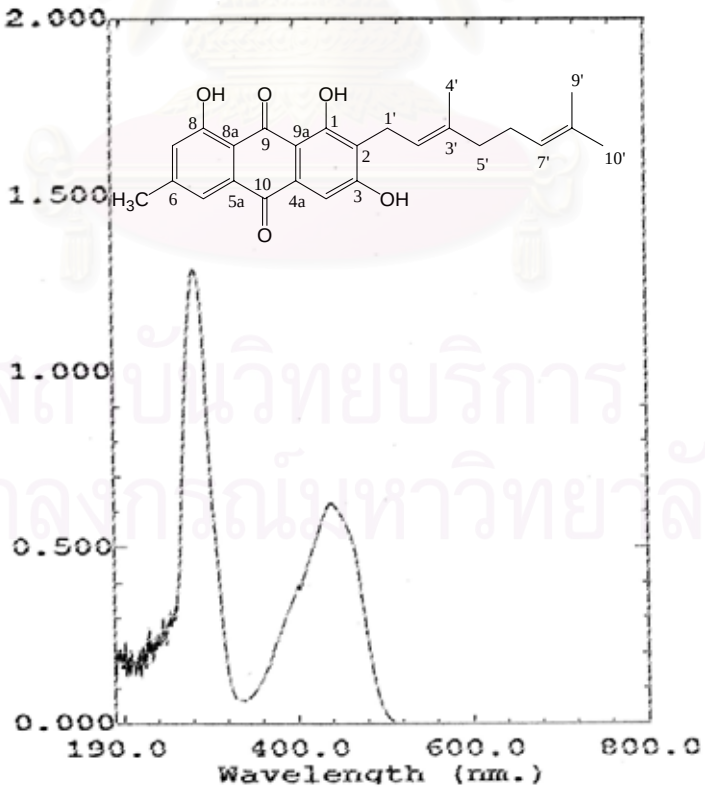


Figure 34. UV Spectrum of Compound CT-04 (methanol)

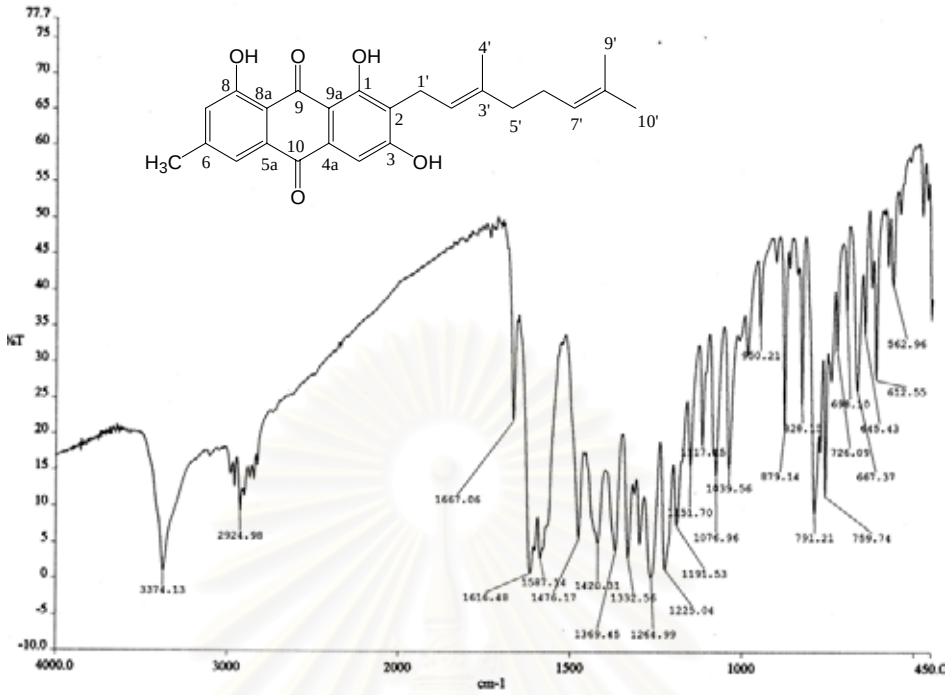


Figure 35. IR Spectrum of Compound CT-04 (KBr disc)

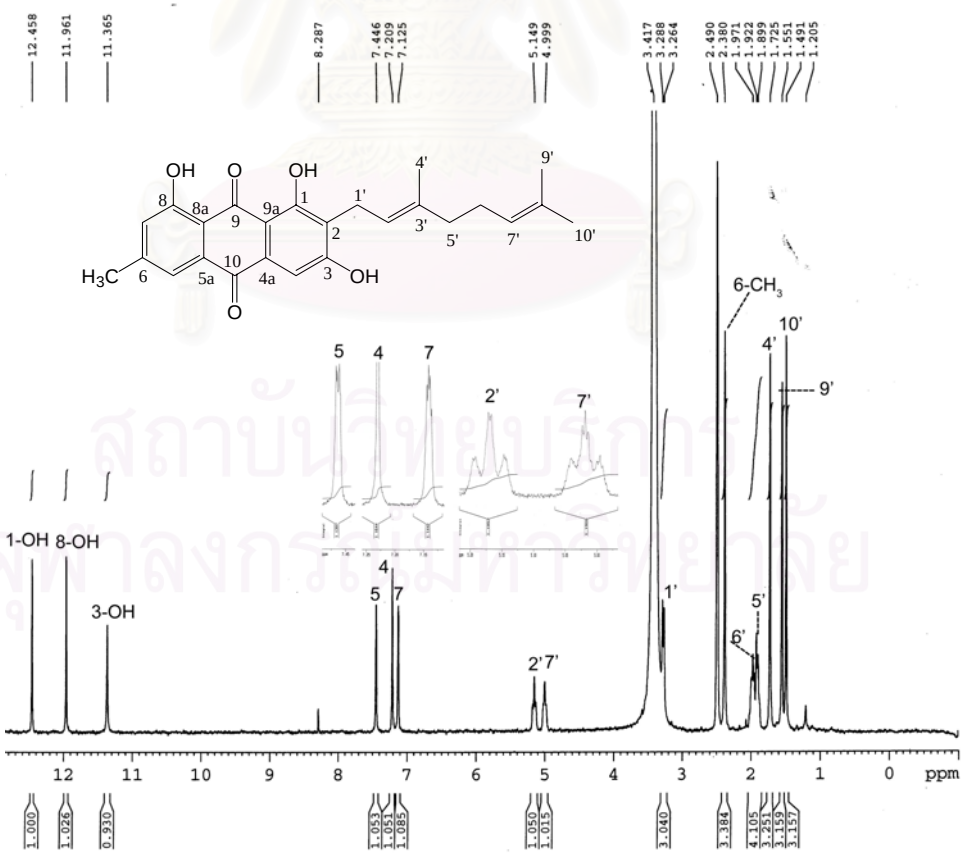


Figure 36. ^{1}H NMR (300 MHz) Spectrum of Compound CT-04 (DMSO-d_6)

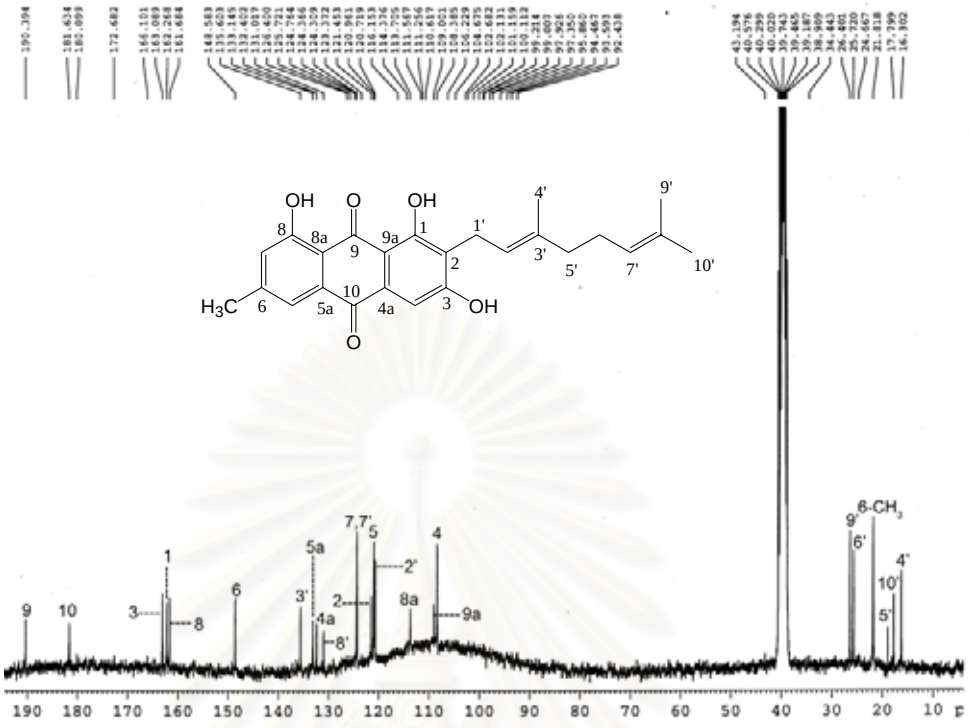


Figure 37. ^{13}C NMR (75 MHz) Spectrum of Compound CT-04 (DMSO-d_6)

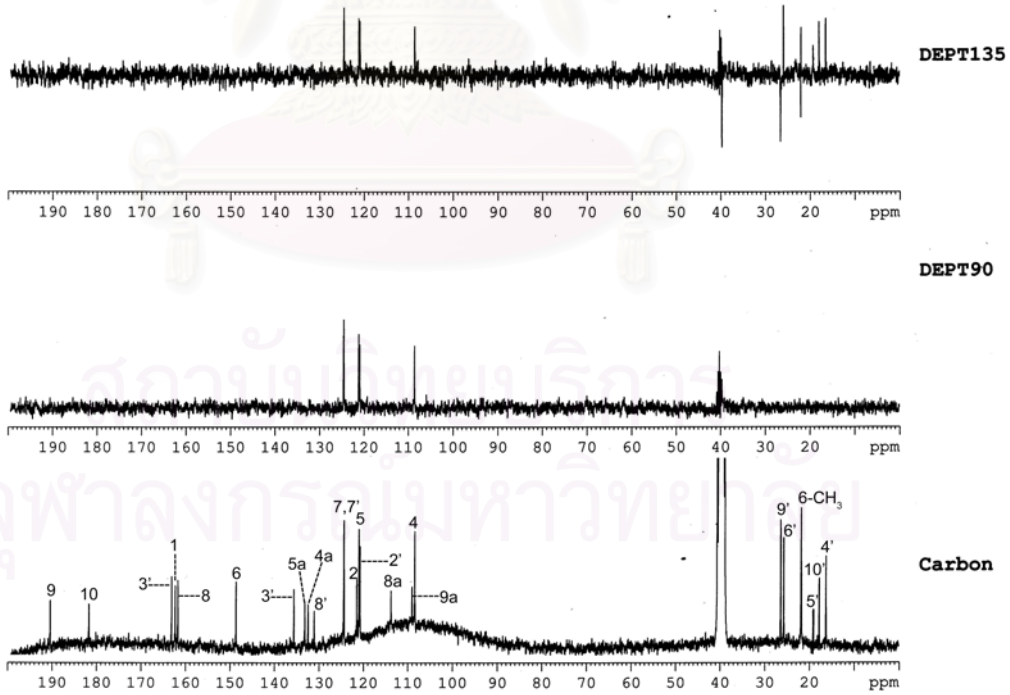


Figure 38. Dept 135, 90 and ^{13}C NMR Spectrum of Compound CT-04 (DMSO-d_6)

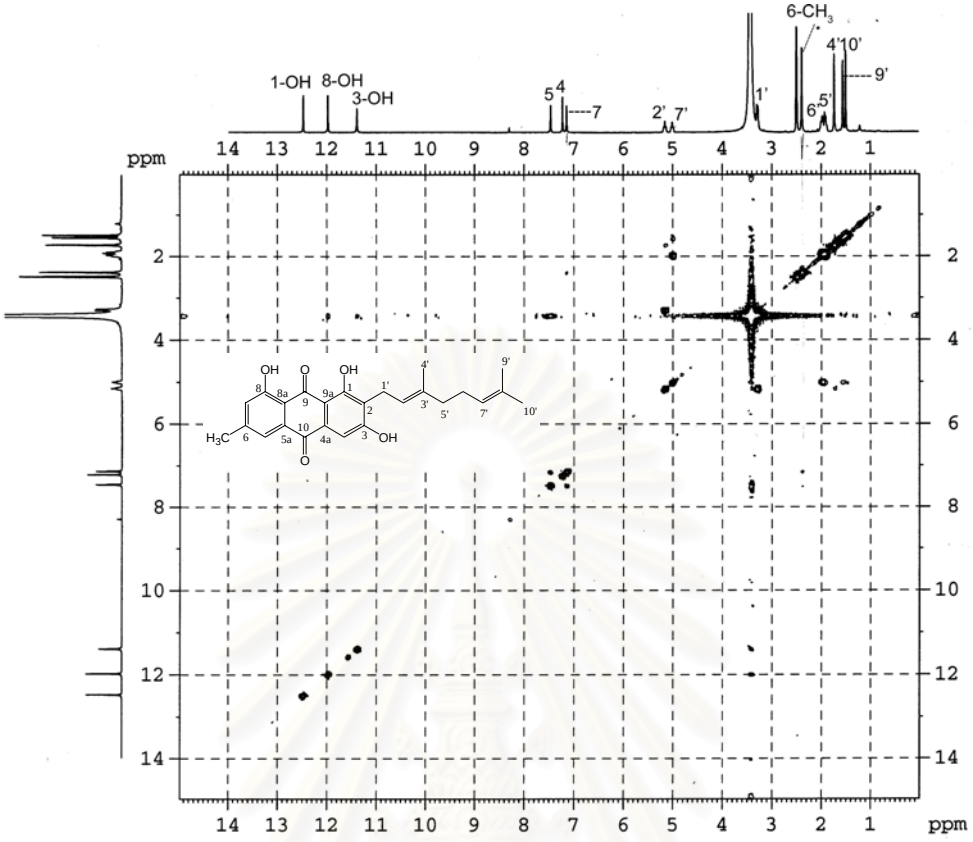


Figure 39. ^{1}H-^{1}H COSY Spectrum of Compound CT-04 (DMSO-d_6)

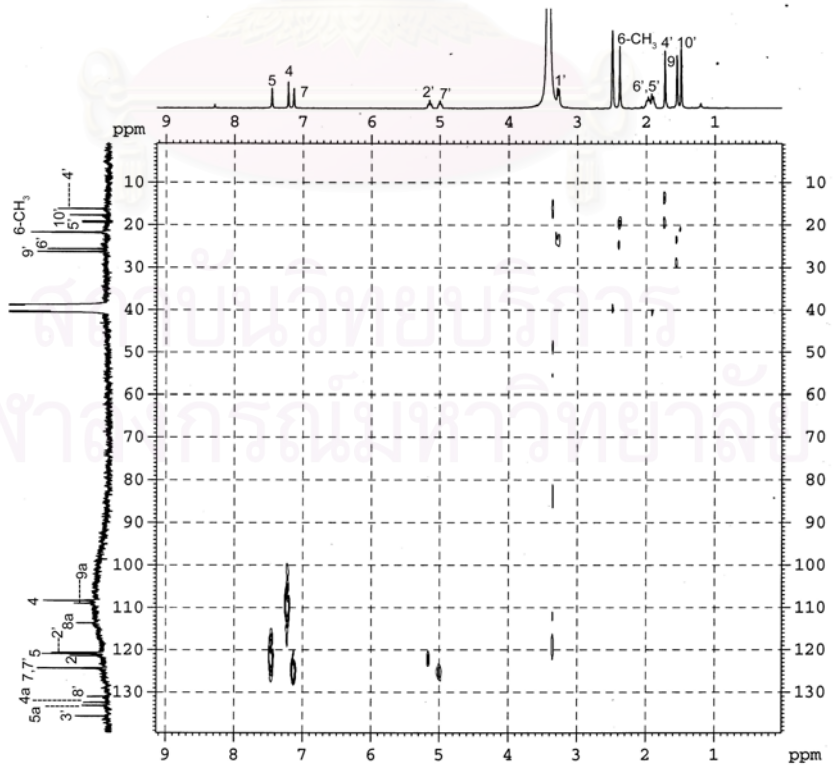


Figure 40. HMQC Spectrum of Compound CT-04 (DMSO-d_6)

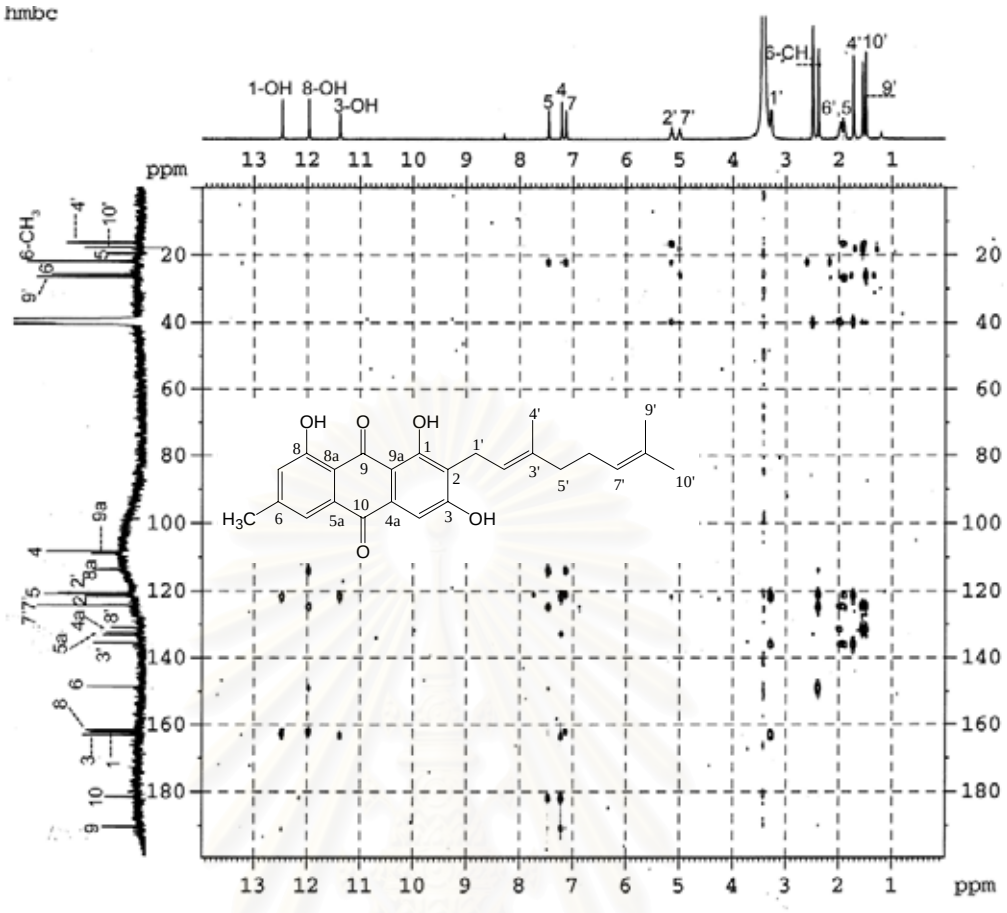


Figure 41. HMBC Spectrum of Compound CT-04 (DMSO-d_6)

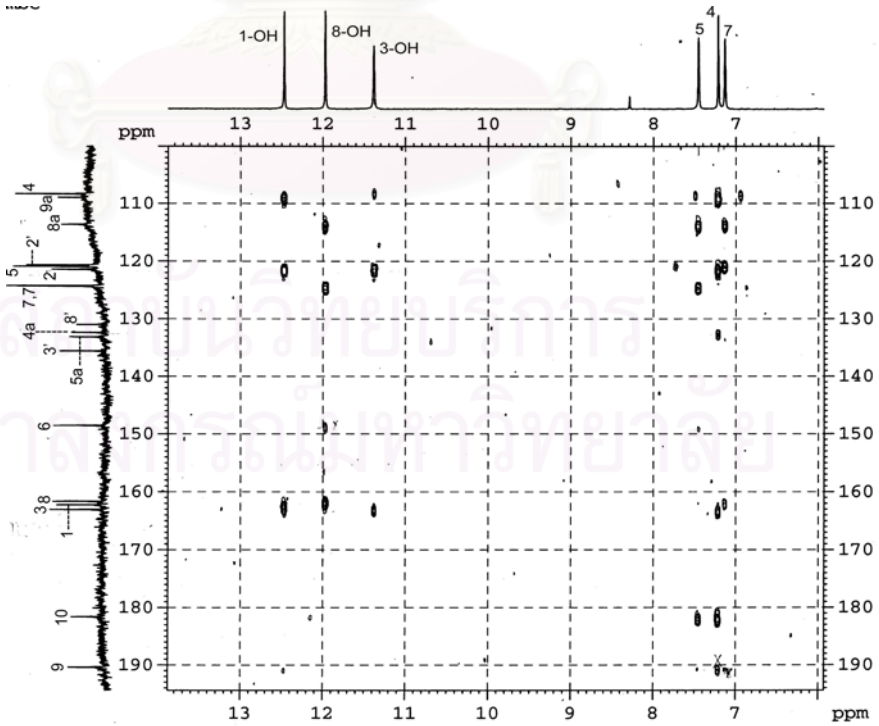


Figure 42. HMBC Spectrum of Compound CT-04 (DMSO-d_6) [δ_H 7.0-13.0 ppm, δ_C110-190 ppm]

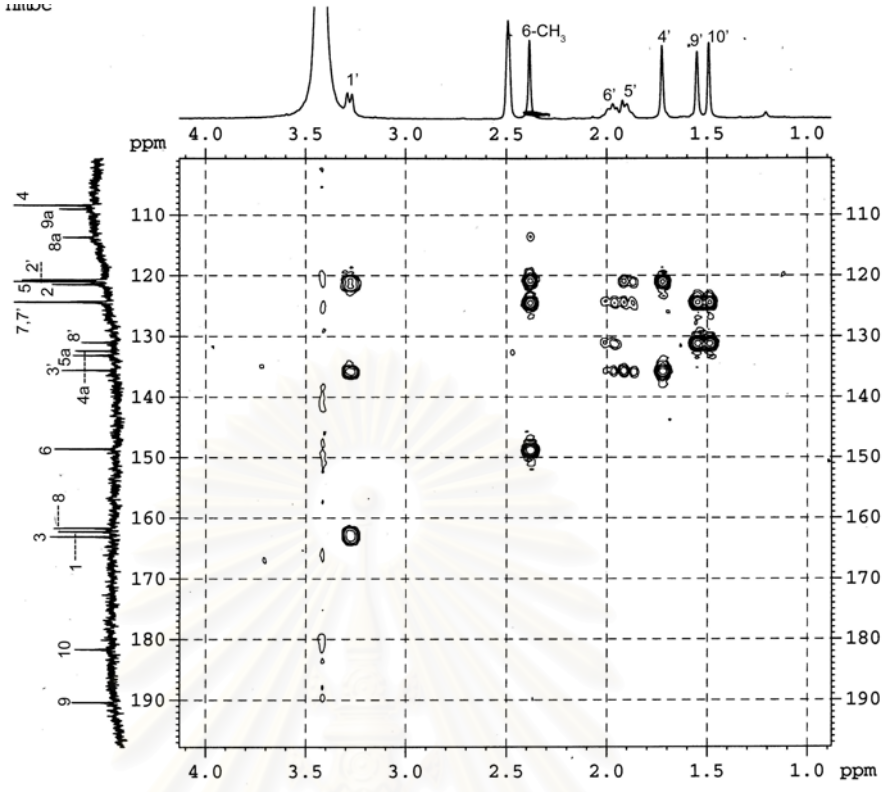


Figure 43. HMBC Spectrum of Compound CT-04 (DMSO-d_6) [δ_H 1.0-4.0 ppm, δ_C110-190 ppm]

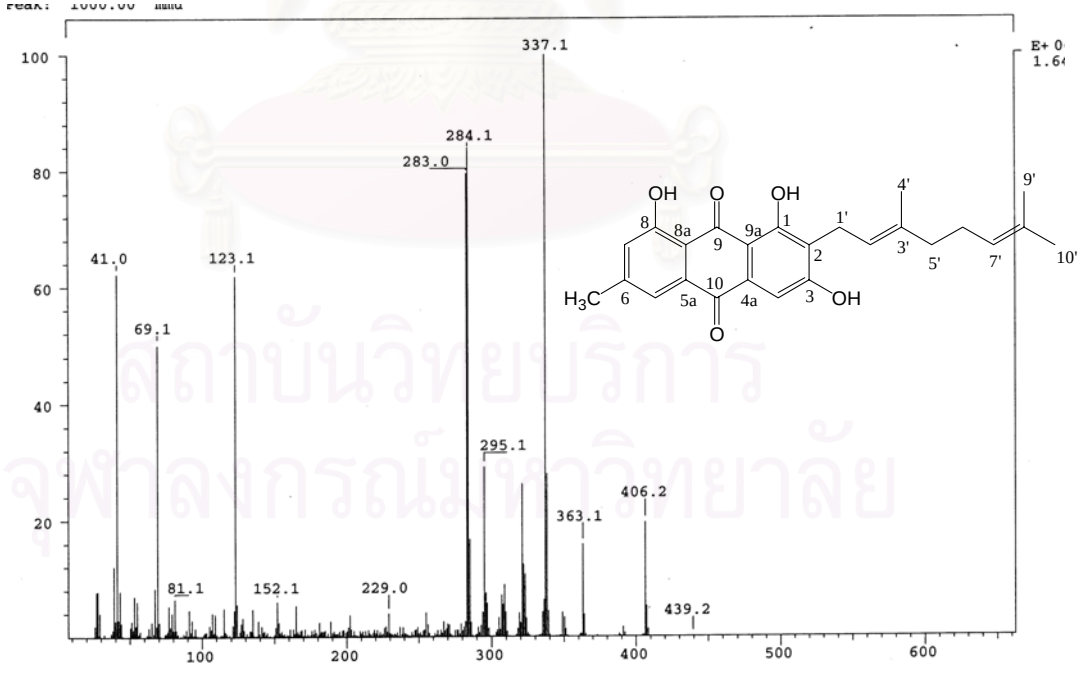


Figure 44. EI Mass Spectrum of Compound CT-04

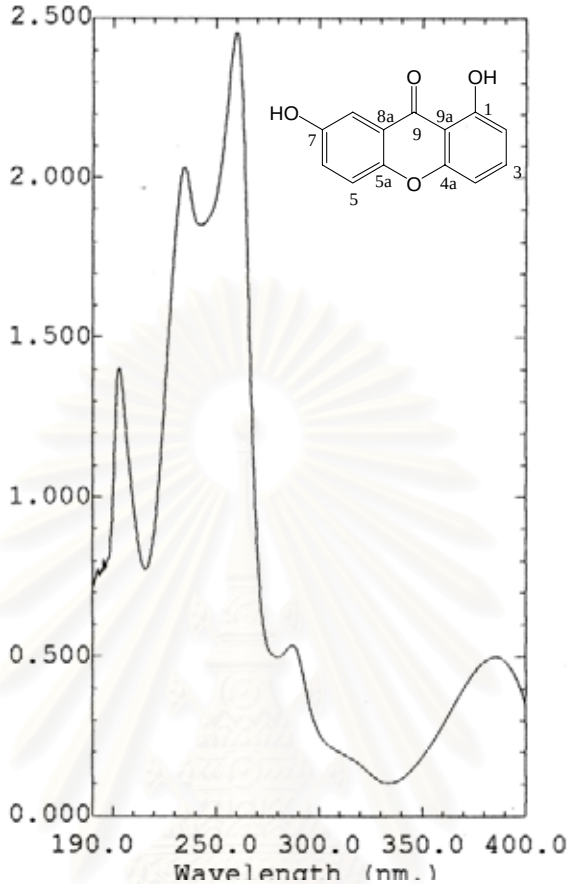


Figure 45. UV Spectrum of Compound CT-05 (methanol)

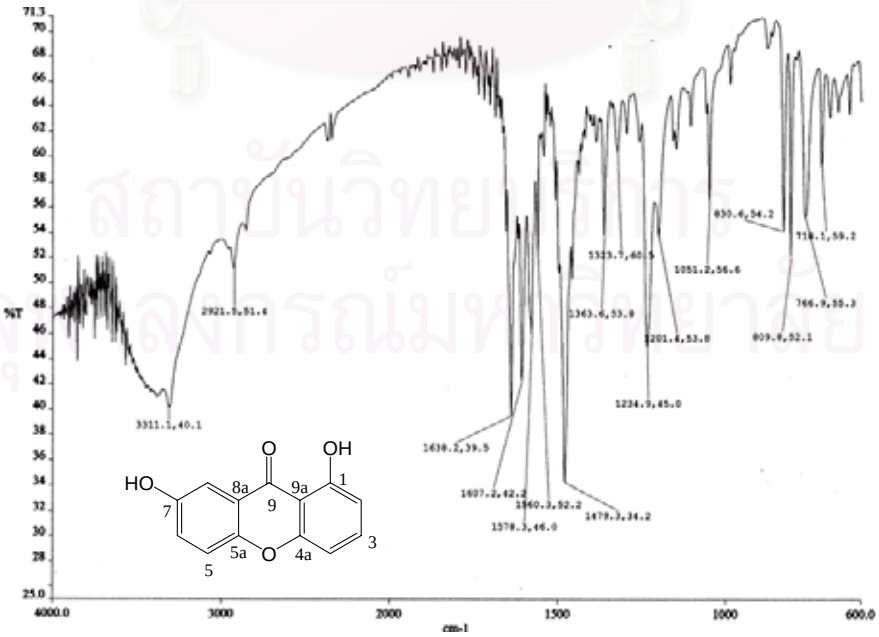


Figure 46. IR Spectrum of Compound CT-05 (KBr disc)

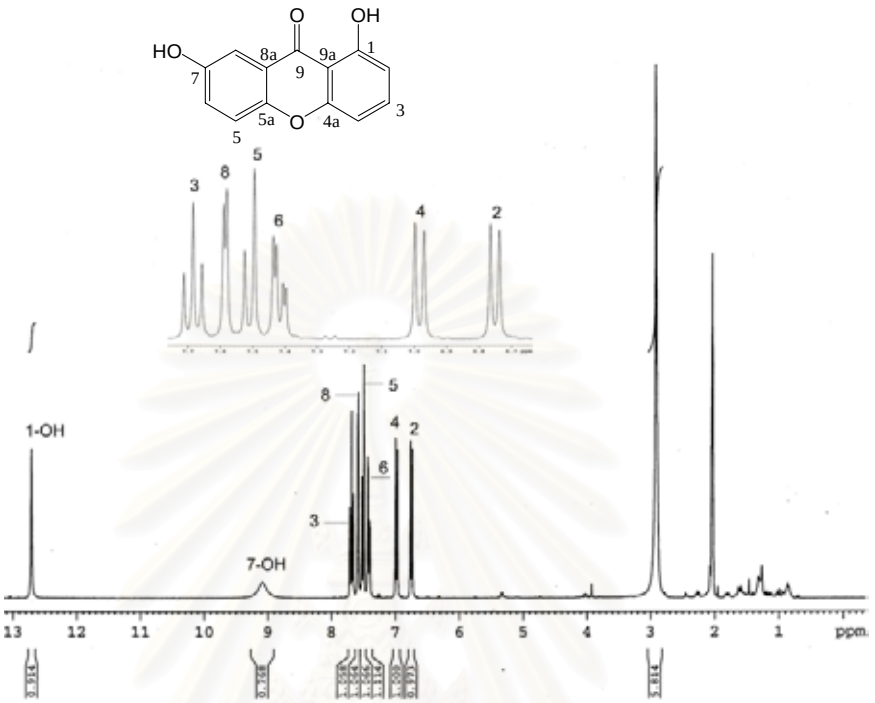


Figure 47. ^{1}H NMR (300 MHz) Spectrum of Compound CT-05 (acetone-d_6)

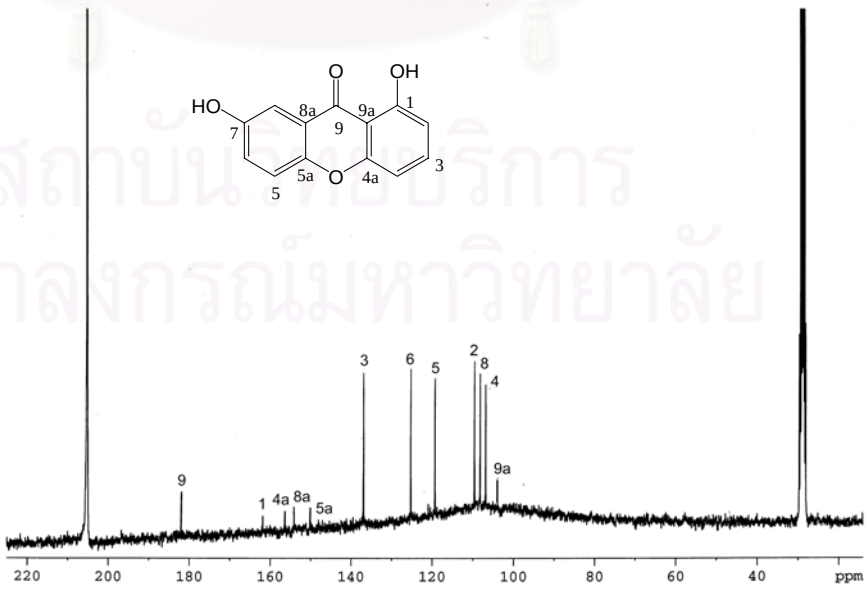


Figure 48. ^{13}C NMR (75 MHz) Spectrum of Compound CT-05 (acetone-d_6)

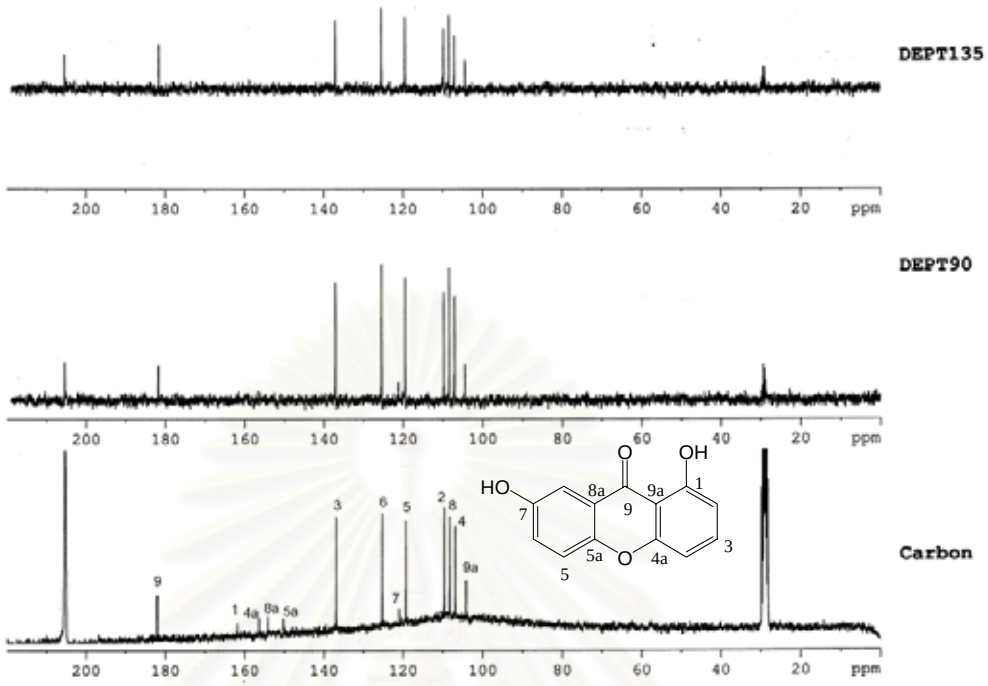


Figure 49. Dept 135, 90 and ^{13}C NMR Spectrum of Compound CT-05 (acetone-d_6)

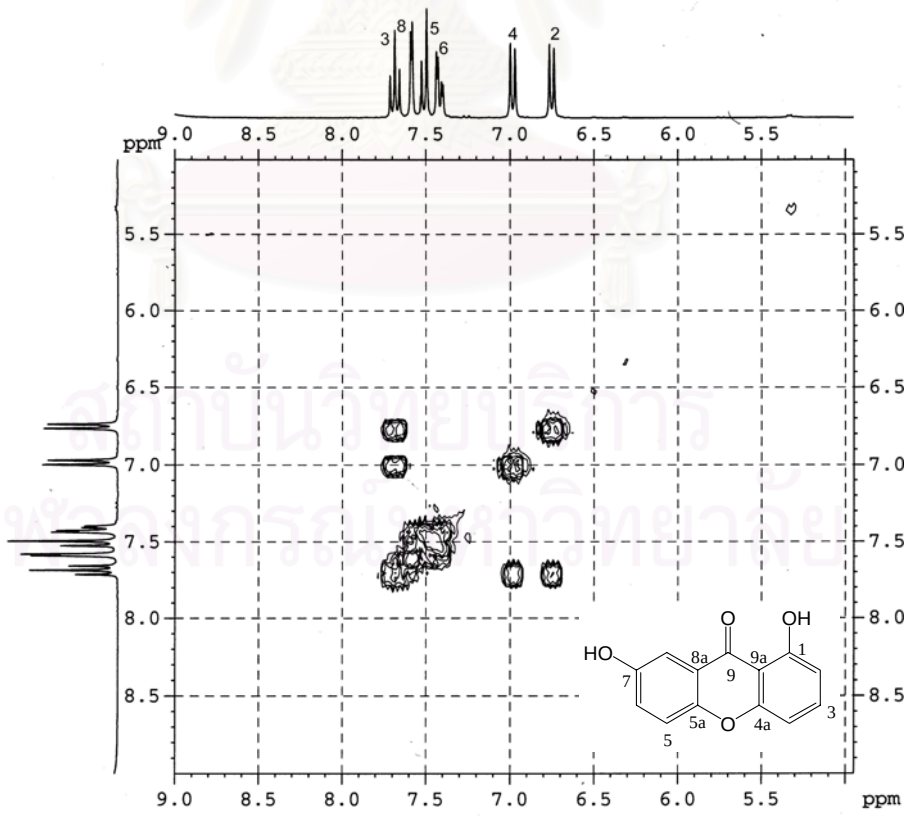


Figure 50. ^{1}H-^{1}H COSY Spectrum of Compound CT-05 (acetone-d_6)

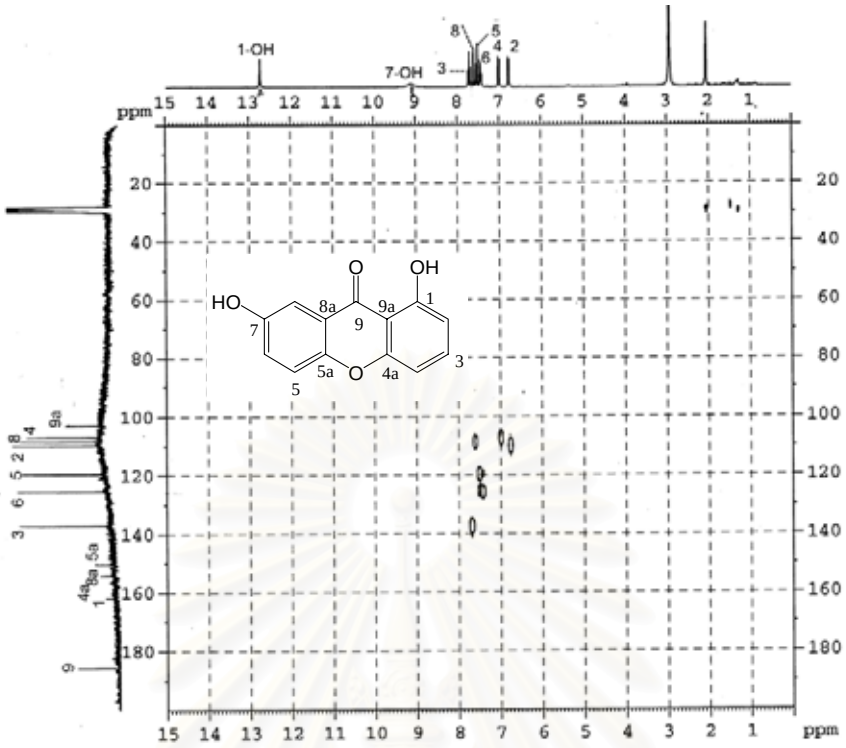


Figure 51. HMQC Spectrum of Compound CT-05 (acetone-d_6)

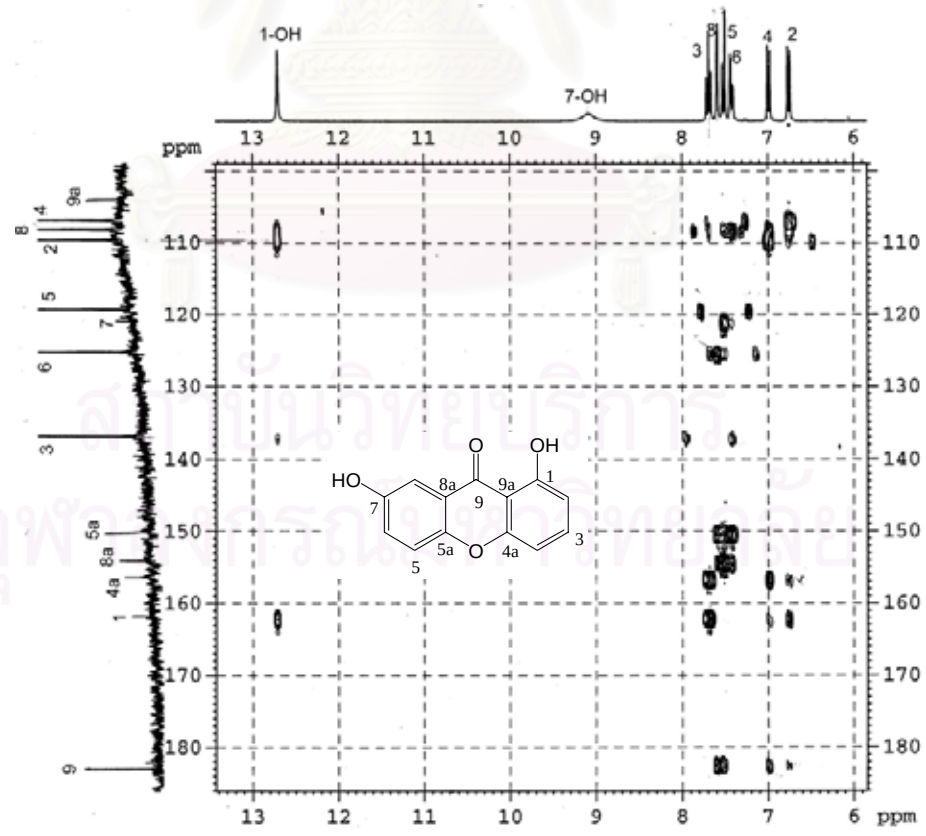


Figure 52. HMBC Spectrum of Compound CT-05 (acetone-d_6)

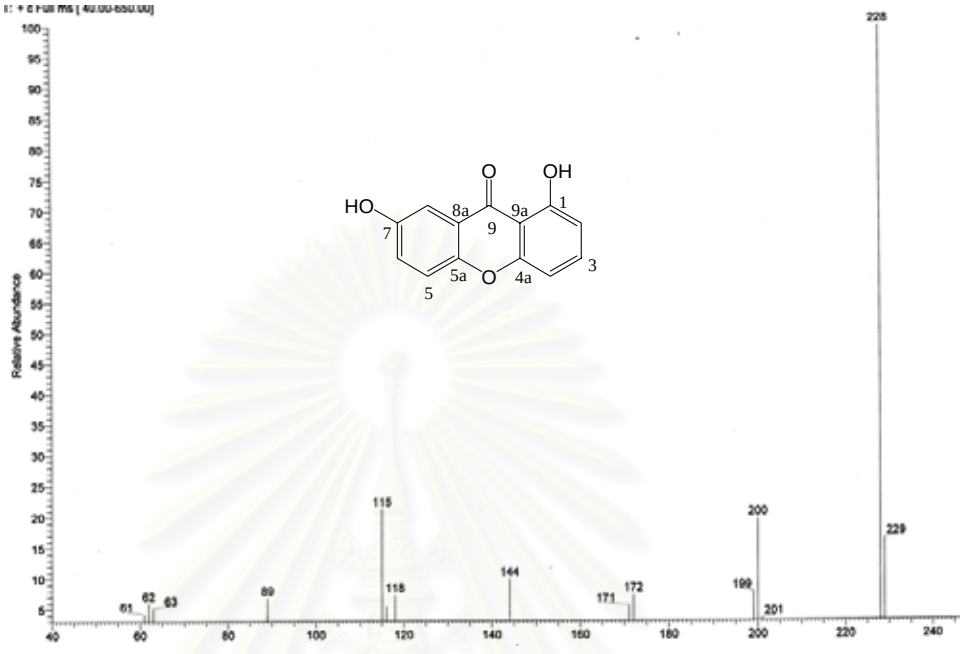


Figure 53. EI Mass Spectrum of Compound CT-05

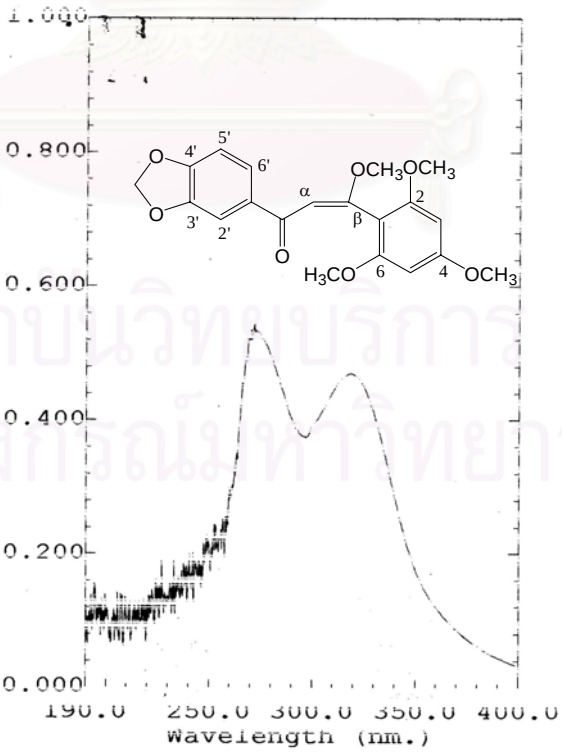


Figure 54. UV Spectrum of Compound MD-01 (methanol)

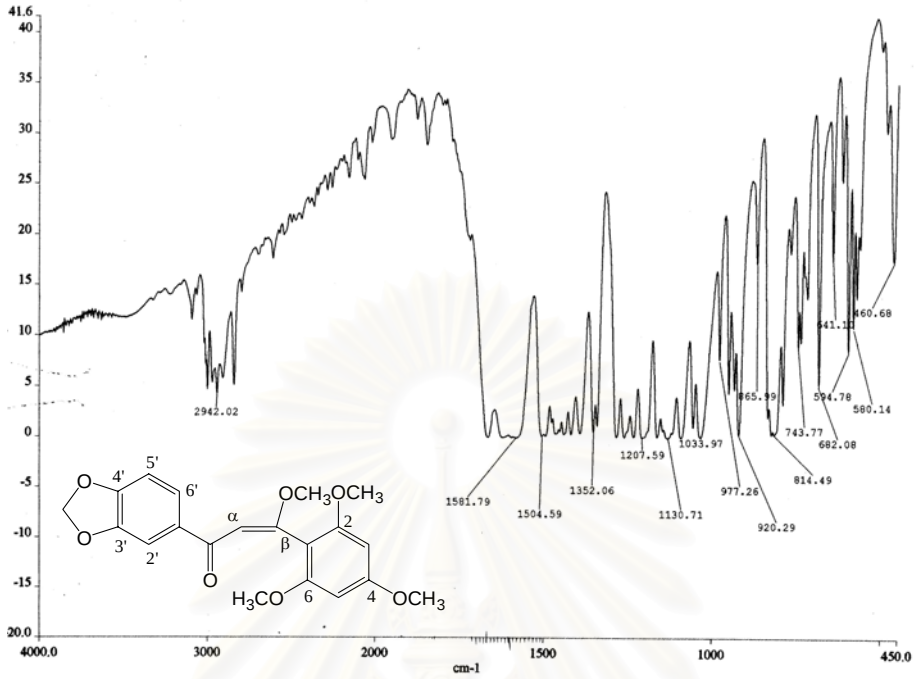


Figure 55. IR Spectrum of Compound MD-01 (KBr disc)

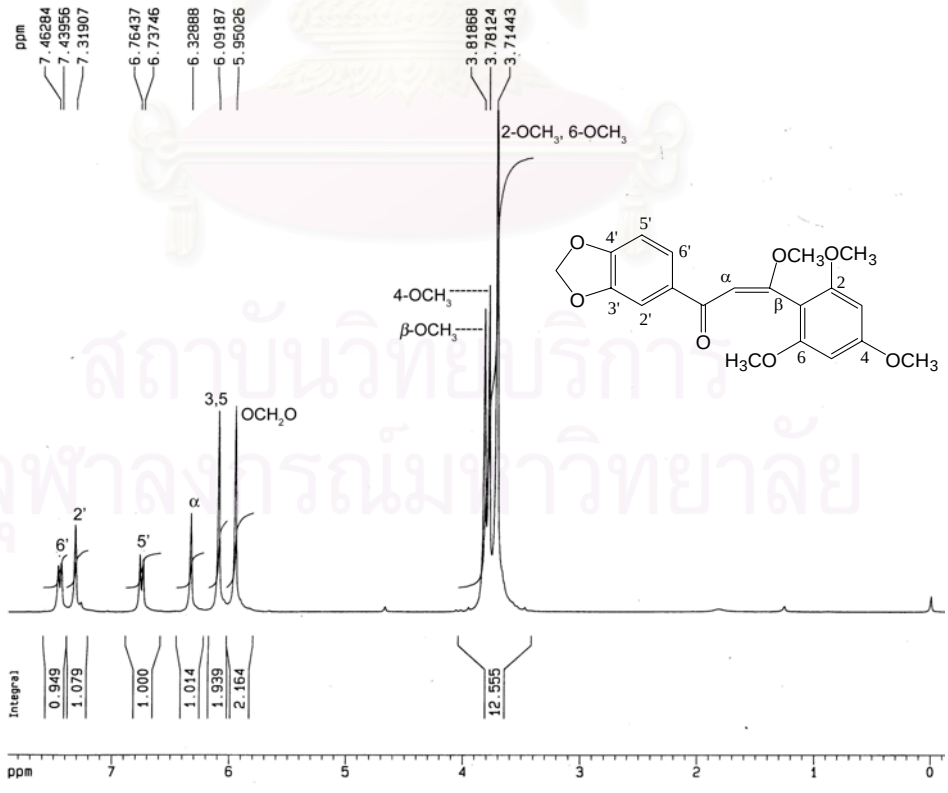


Figure 56. ^{1}H NMR (300 MHz) Spectrum of Compound MD-01 ($CDCl_3$)

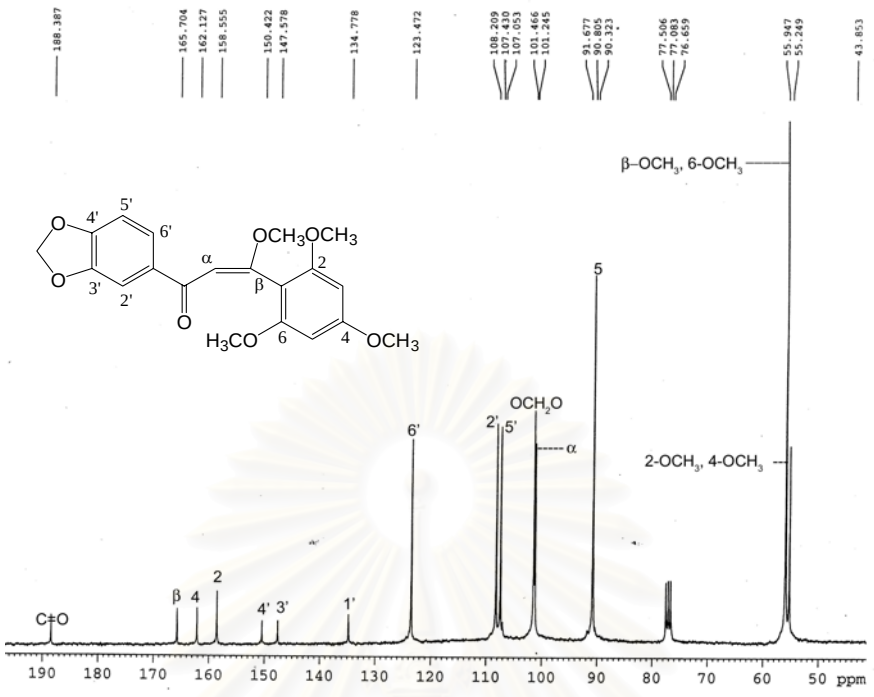


Figure 57. ^{13}C NMR (75 MHz) Spectrum of Compound MD-01 ($CDCl_3$)

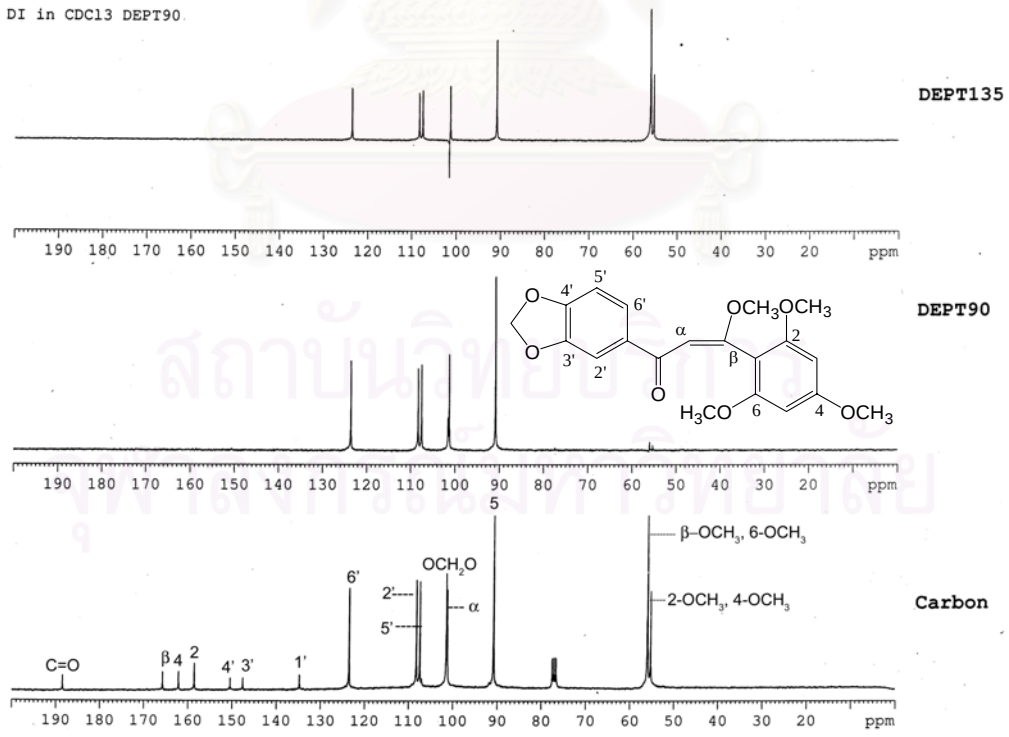


Figure 58. Dept 135, 90 and ^{13}C NMR Spectrum of Compound MD-01 ($CDCl_3$)

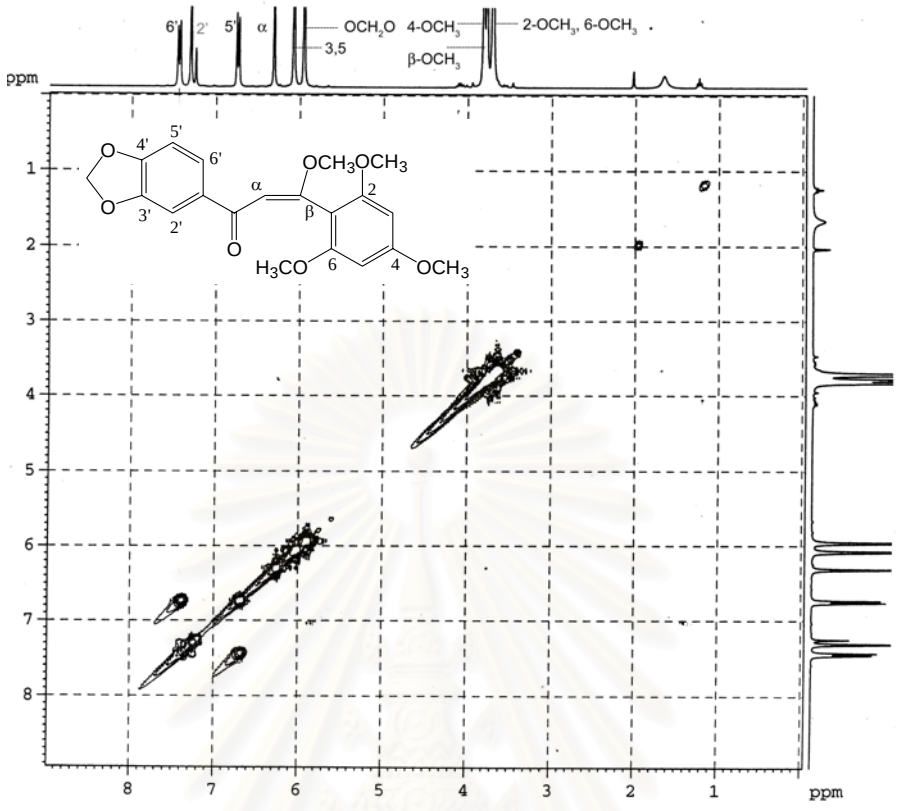


Figure 59. ^{1}H-^{1}H COSY Spectrum of Compound MD-01 ($CDCl_3$)

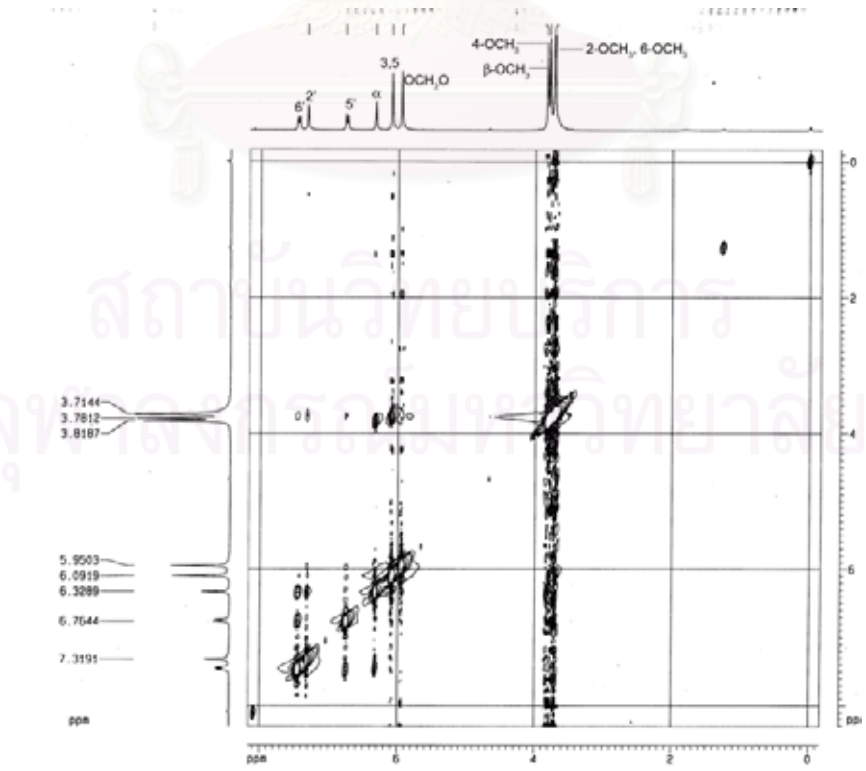


Figure 60. NOESY Spectrum of Compound MD-01 ($CDCl_3$)

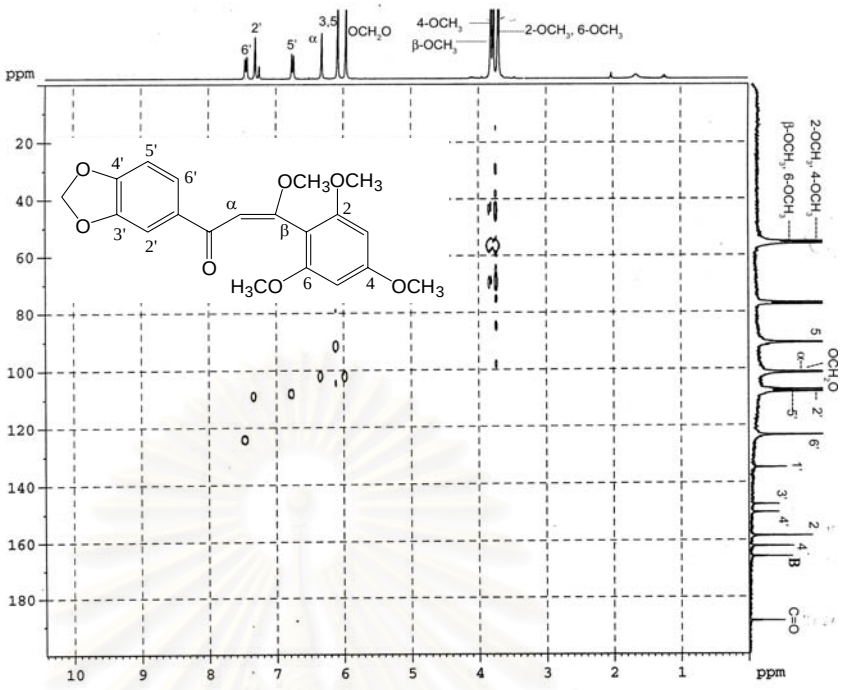


Figure 61. HMQC Spectrum of Compound MD-01 ($CDCl_3$)

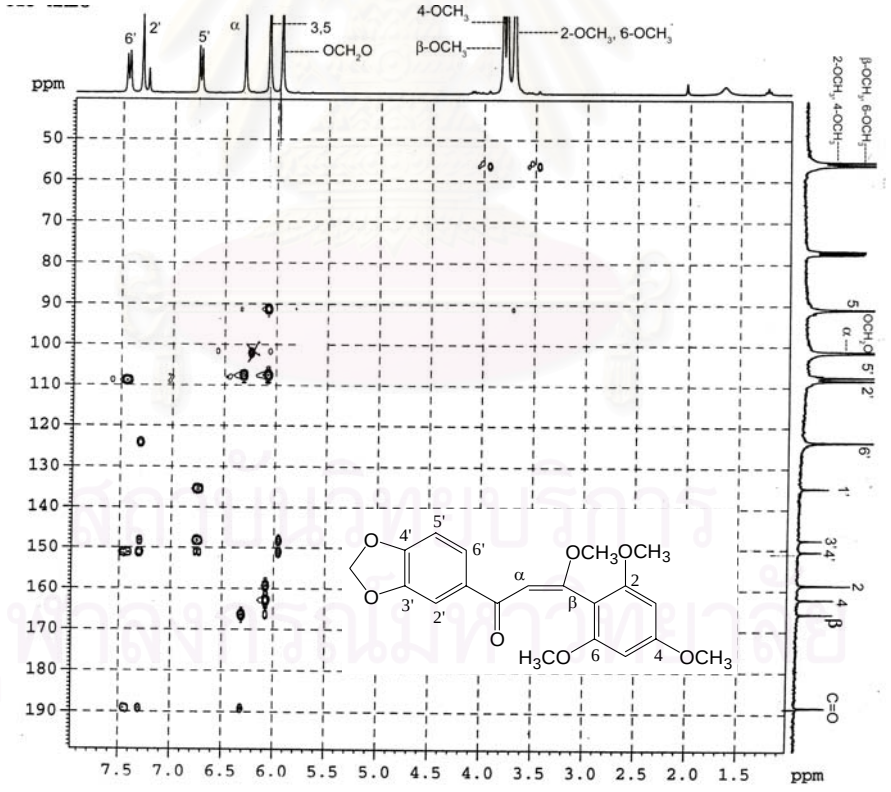


Figure 62. HMBC Spectrum of Compound MD-01 ($CDCl_3$)

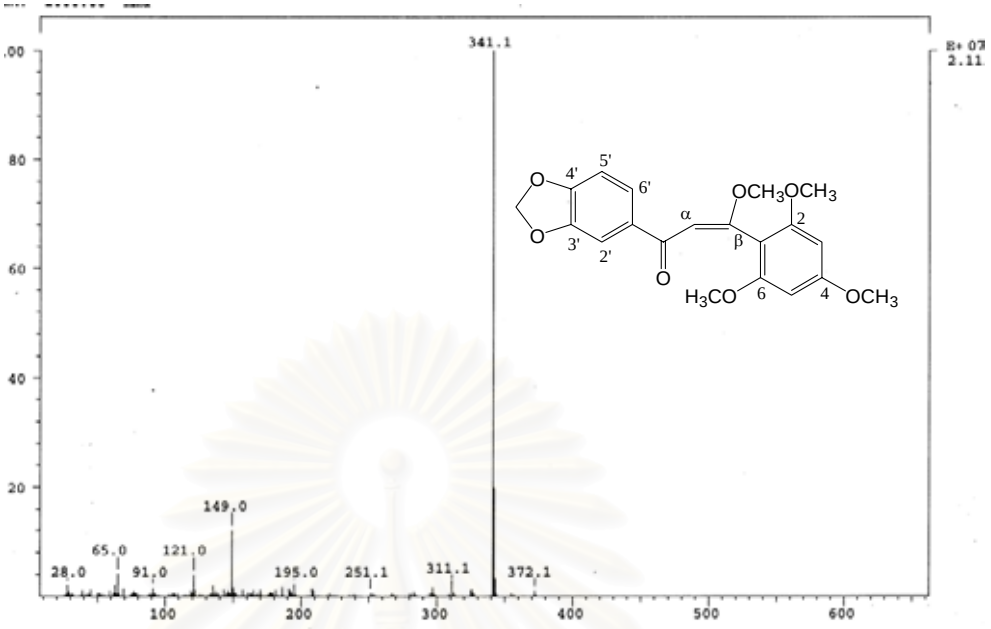


Figure 63. EI Mass Spectrum of Compound MD-01

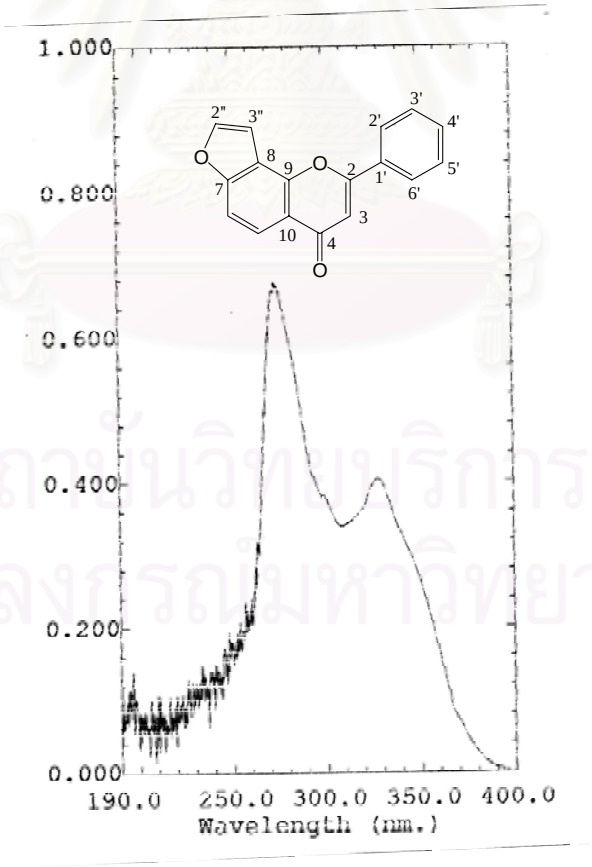


Figure 64. UV Spectrum of Compound MD-02 (methanol)

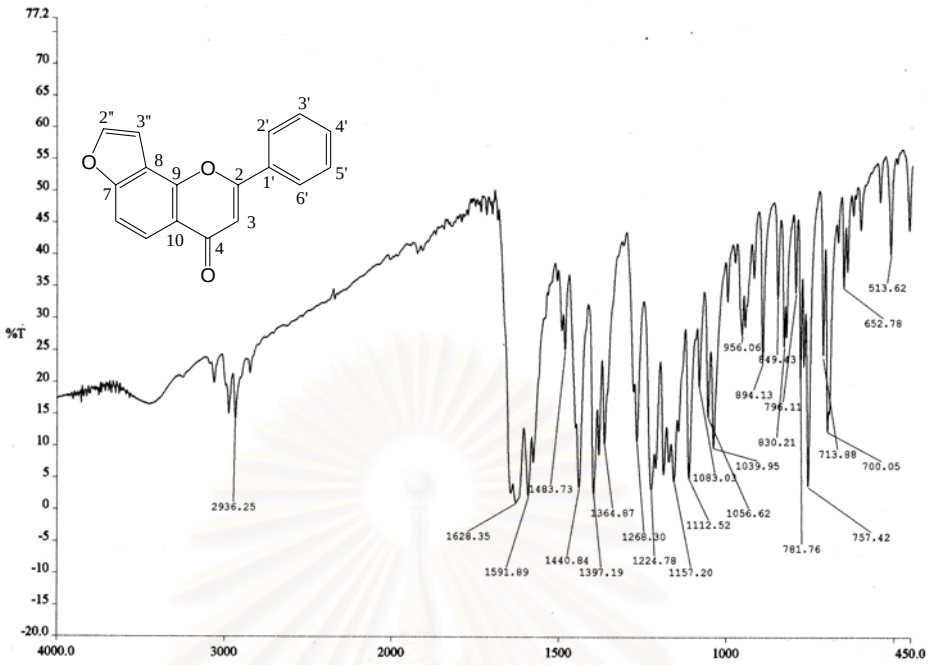


Figure 65. IR Spectrum of Compound MD-02 (KBr disc)

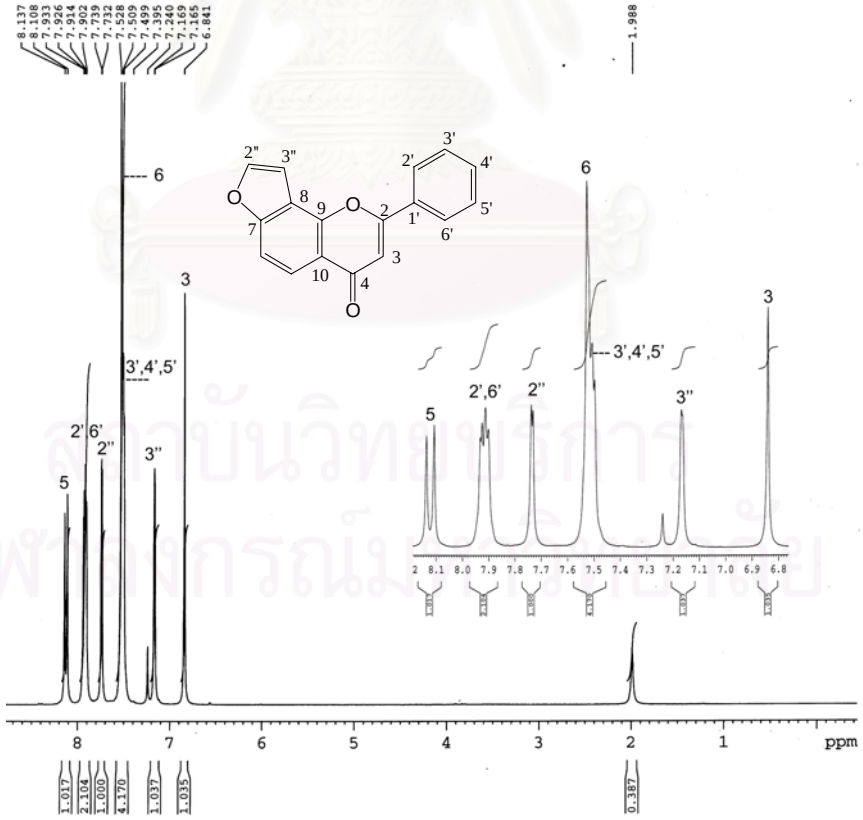


Figure 66. ^{1}H NMR (300 MHz) Spectrum of Compound MD-02 ($CDCl_3$)

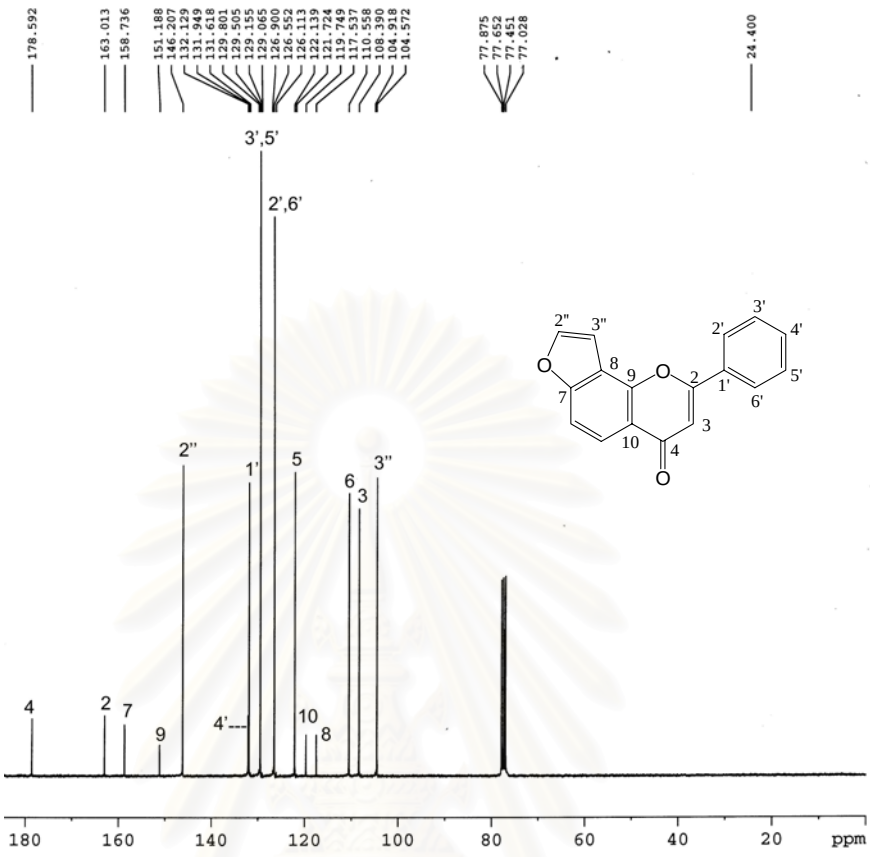


Figure 67. ^{13}C NMR (75 MHz) Spectrum of Compound MD-02 ($CDCl_3$)

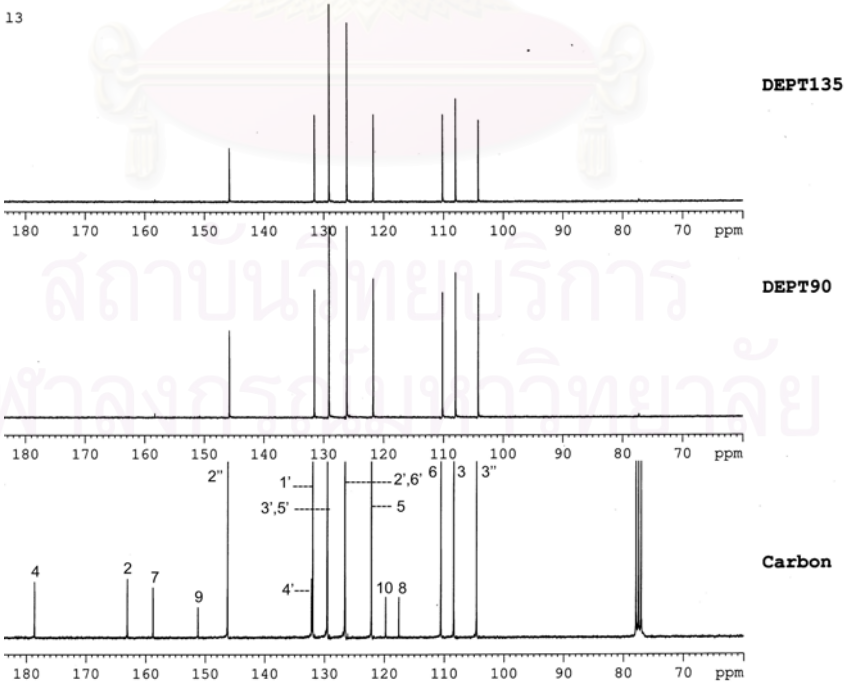


Figure 68. Dept 135, 90 and ^{13}C NMR Spectrum of Compound MD-02 ($CDCl_3$)

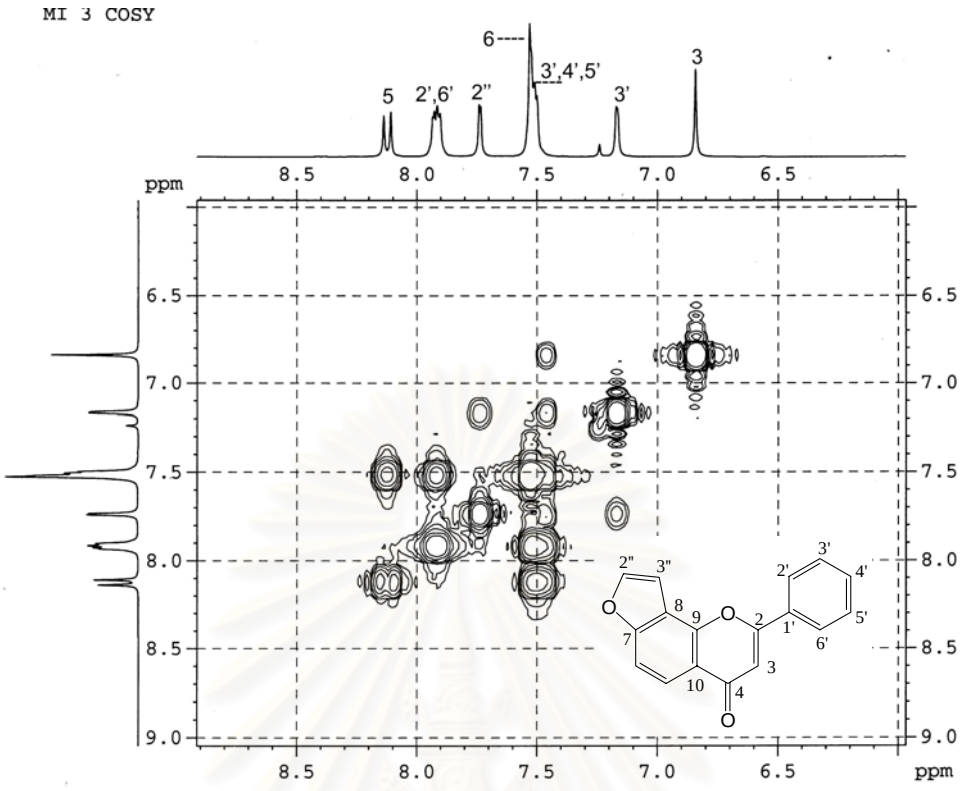


Figure 69. ^{1}H-^{1}H COSY Spectrum of Compound MD-02 ($CDCl_3$)

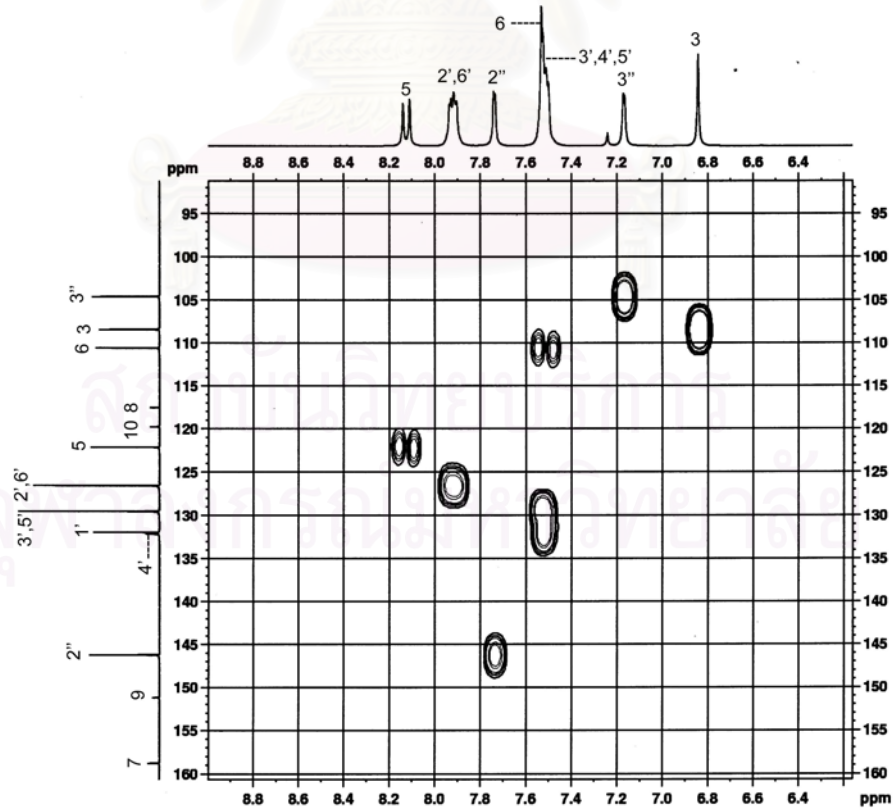


Figure 70. HMQC Spectrum of Compound MD-02 ($CDCl_3$)

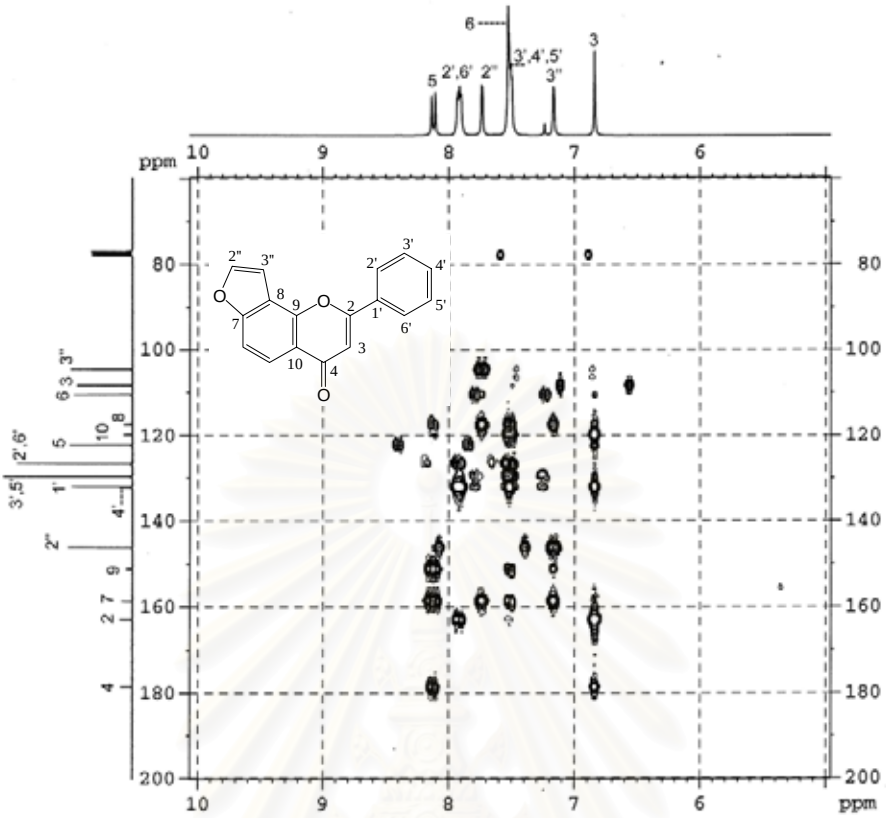


Figure 71. HMBC Spectrum of Compound MD-02 ($CDCl_3$)

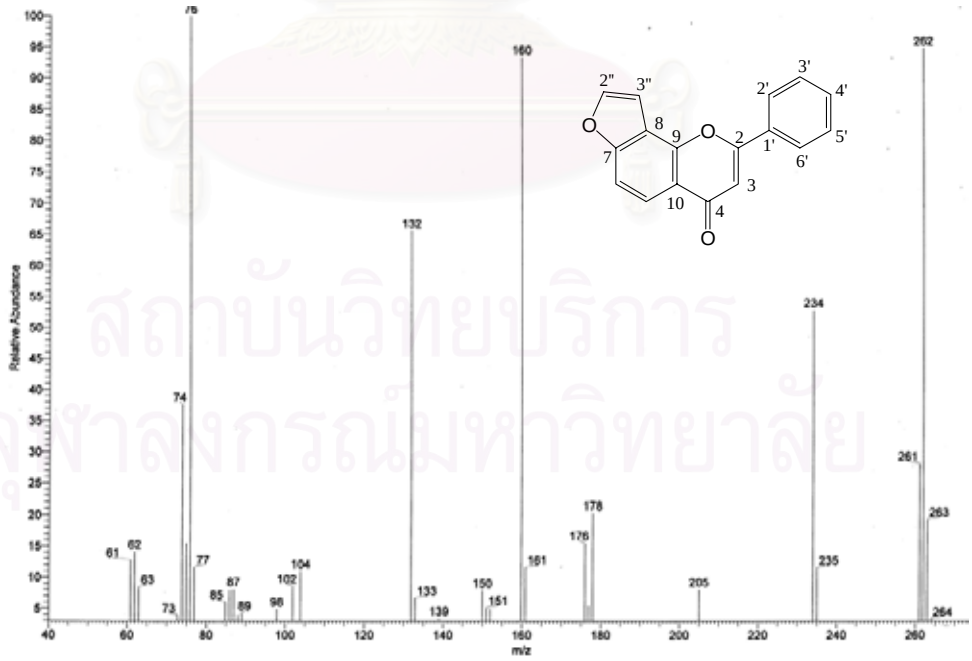


Figure 72. EI Mass Spectrum of Compound MD-02

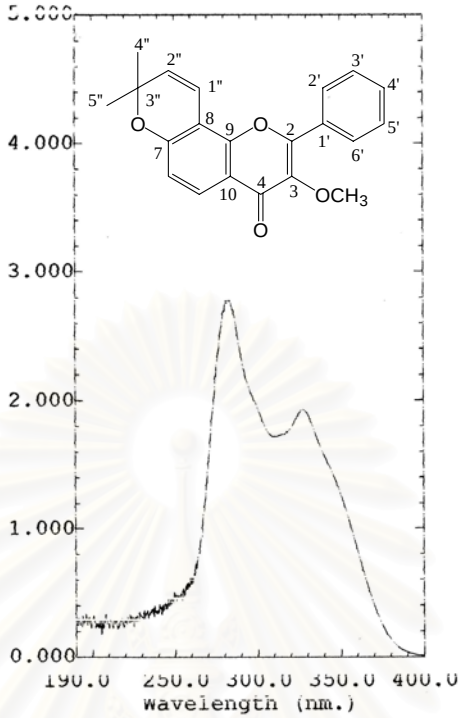


Figure 73. UV Spectrum of Compound MD-03 (methanol)

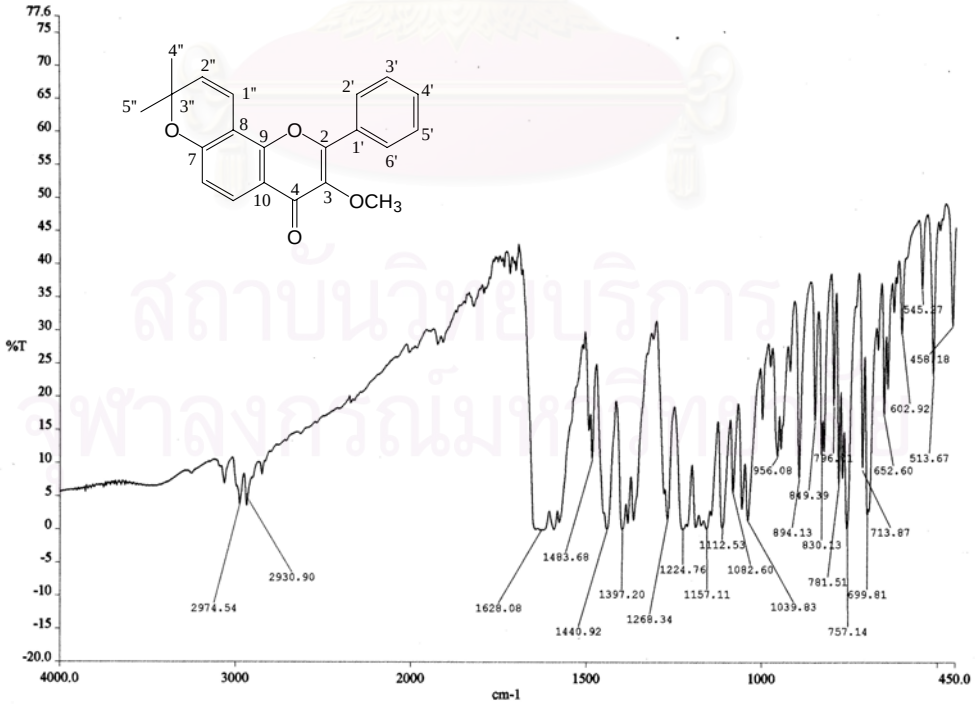


Figure 74. IR Spectrum of Compound MD-03 (KBr disc)

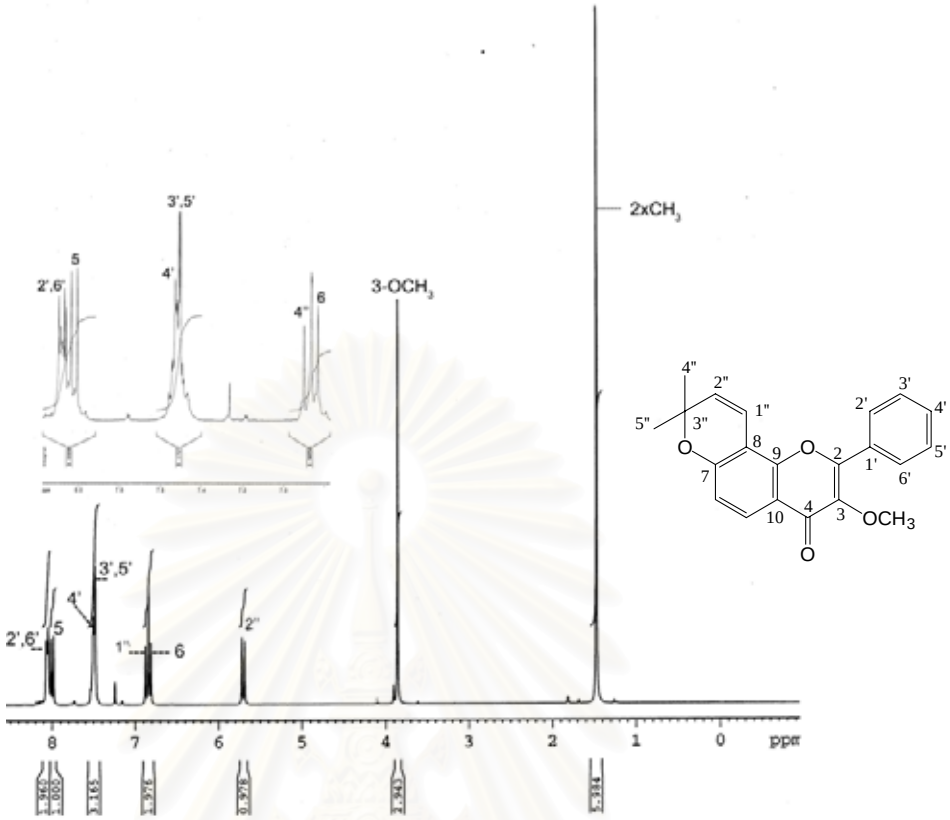


Figure 75. ^{1}H NMR (300 MHz) Spectrum of Compound MD-03 ($CDCl_3$)

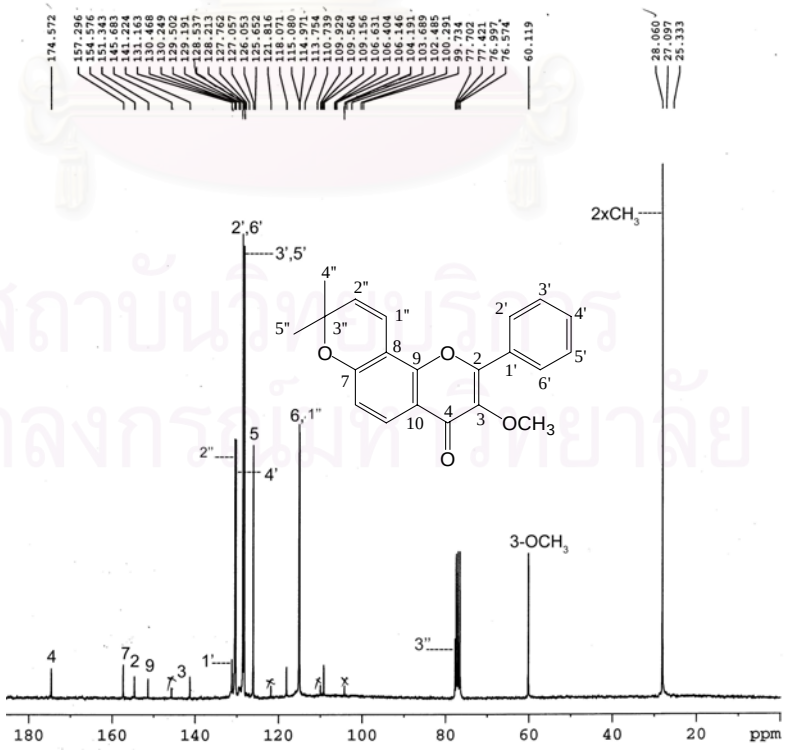


Figure 76. ^{13}C NMR (75MHz) Spectrum of Compound MD-03 ($CDCl_3$)

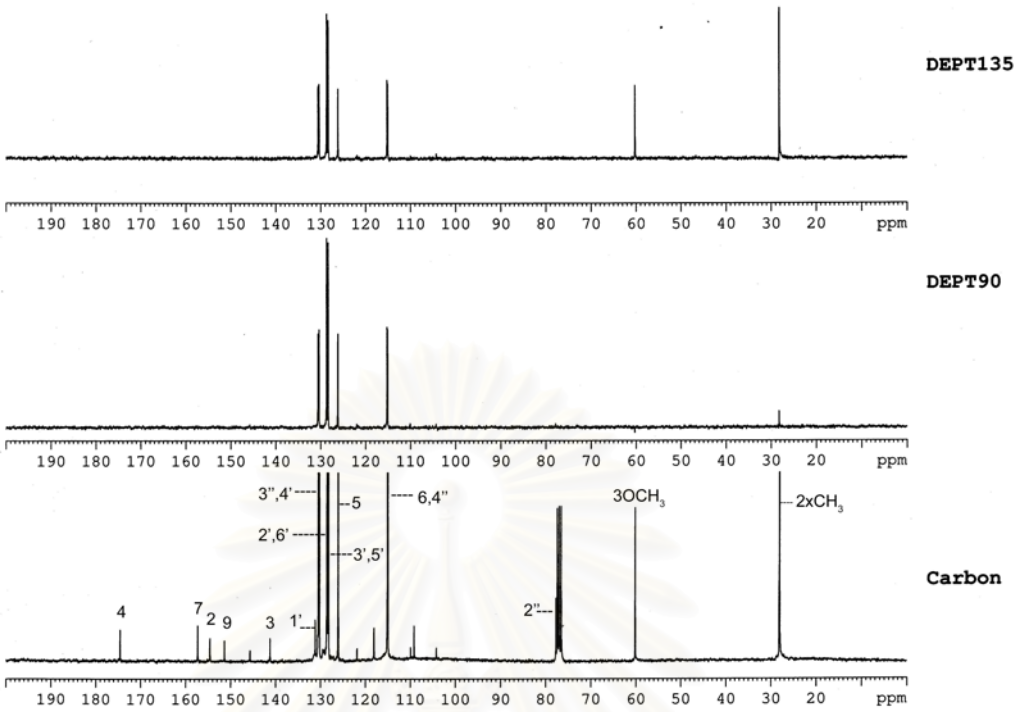


Figure 77. Dept 135, 90 and ^{13}C NMR Spectrum of Compound MD-03 ($CDCl_3$)

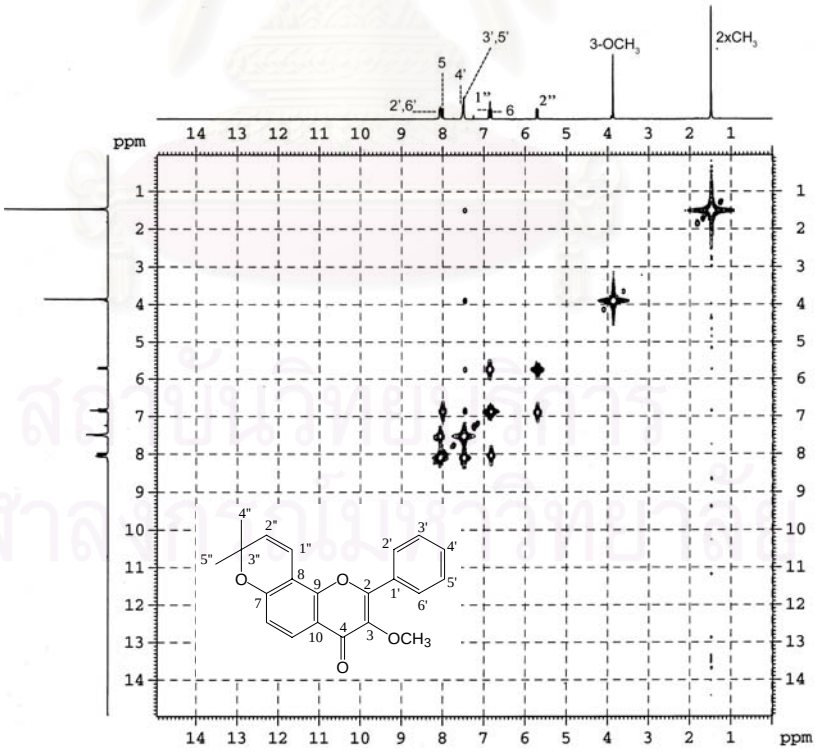


Figure 78. ^{1}H-^{1}H COSY Spectrum of Compound MD-03 ($CDCl_3$)

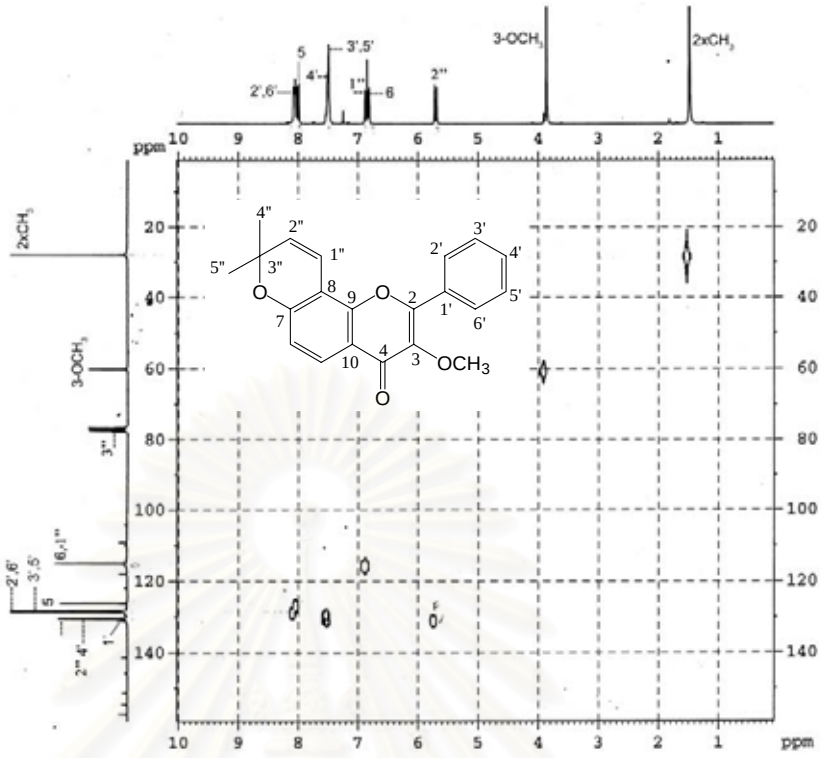


Figure 79. HMQC Spectrum of Compound MD-03 ($CDCl_3$)

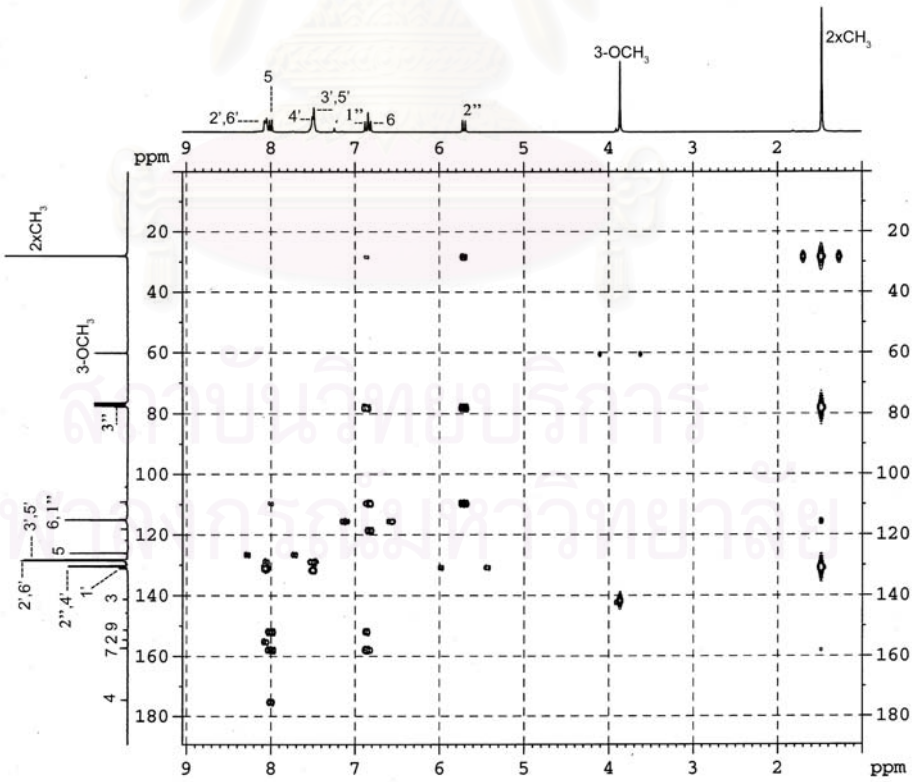


Figure 80. HMBC Spectrum of Compound MD-03 ($CDCl_3$)

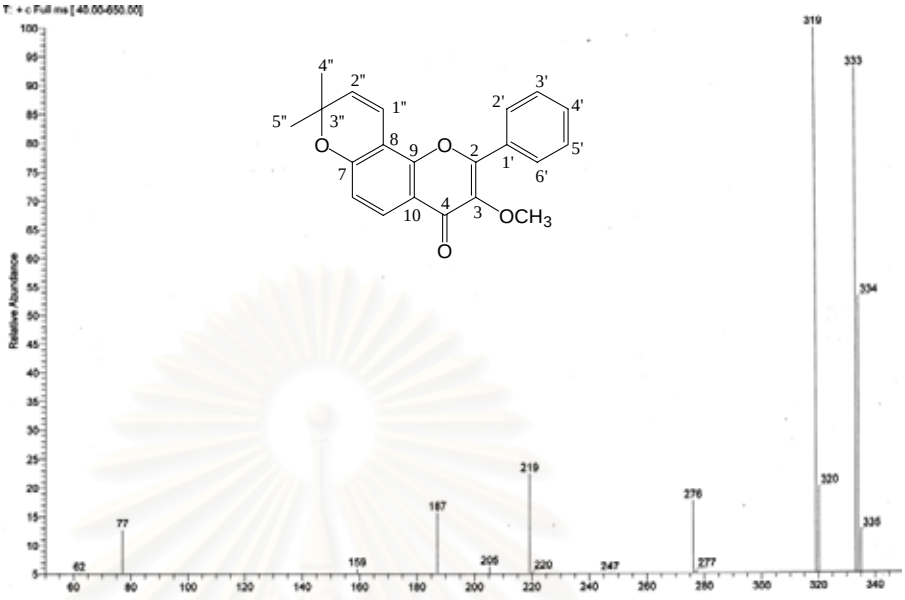


Figure 81. EI Mass Spectrum of Compound MD-03

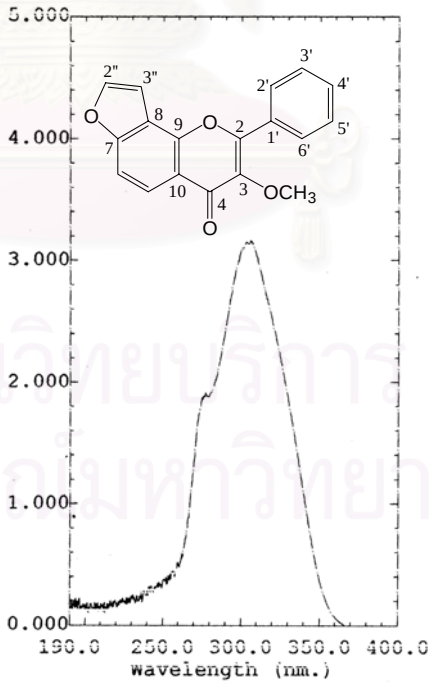


Figure 82. UV Spectrum of Compound MD-04 (methanol)

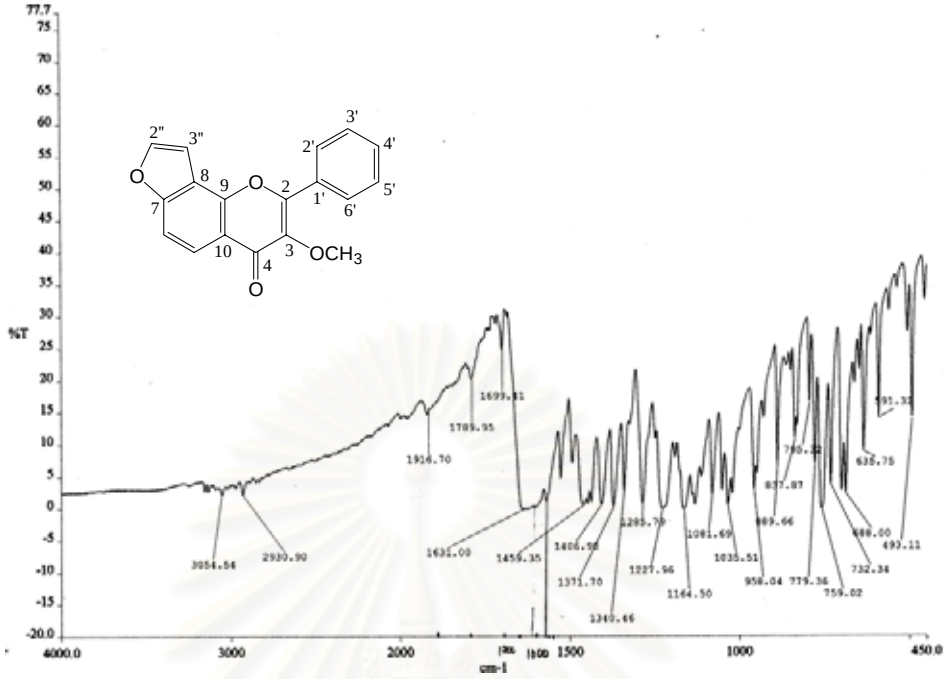


Figure 83. IR Spectrum of Compound MD-04 (KBr disc)

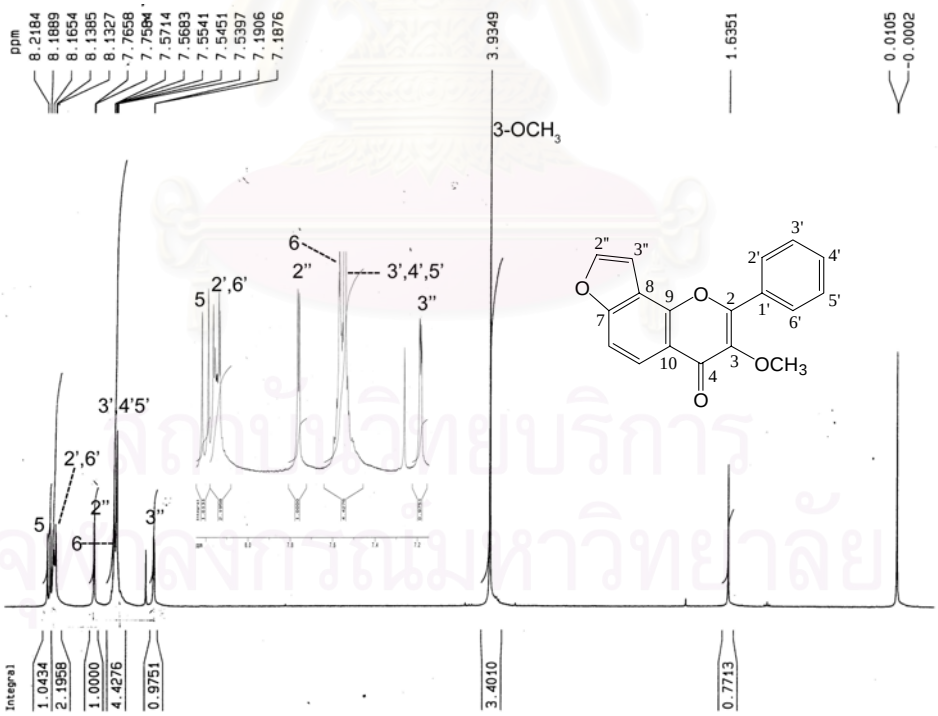


Figure 84. ^{1}H NMR (300 MHz) Spectrum of Compound MD-04 ($CDCl_3$)

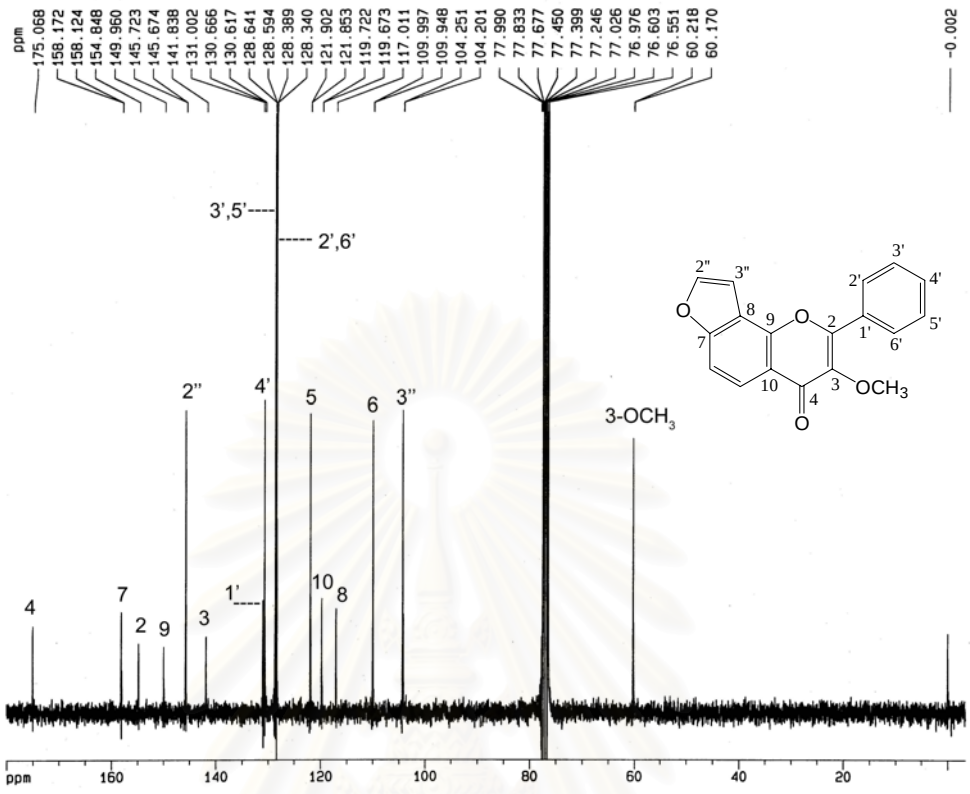


Figure 85. ^{13}C NMR (75 MHz) Spectrum of Compound MD-04 ($CDCl_3$)

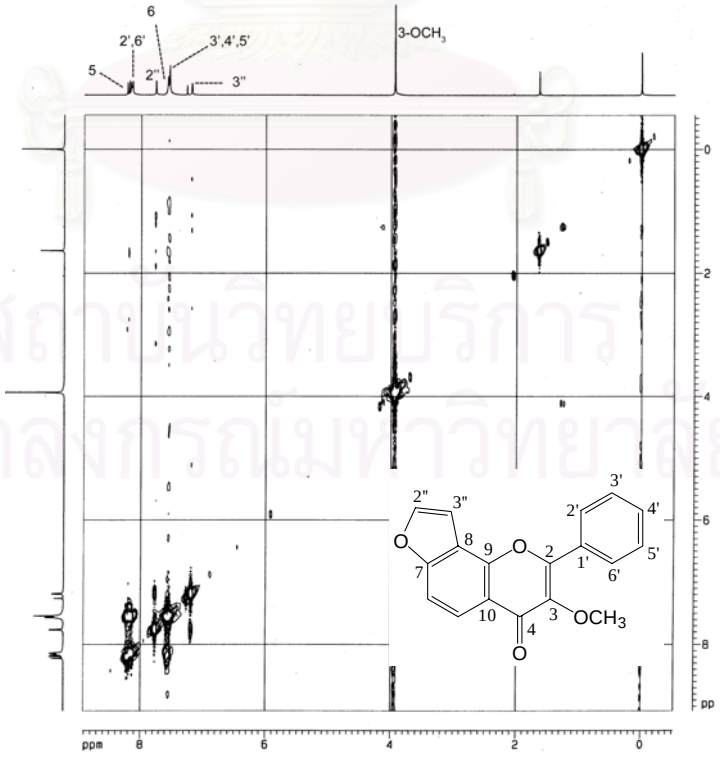


Figure 86. ^{1}H-^{1}H COSY Spectrum of Compound MD-04 ($CDCl_3$)

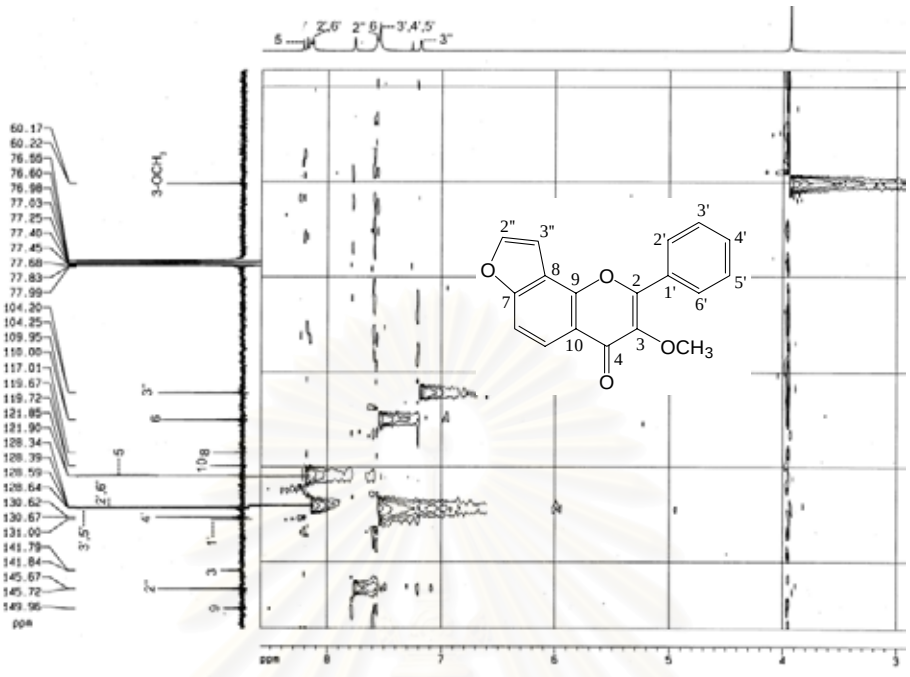


Figure 87. HMQC Spectrum of Compound MD-04 ($CDCl_3$)

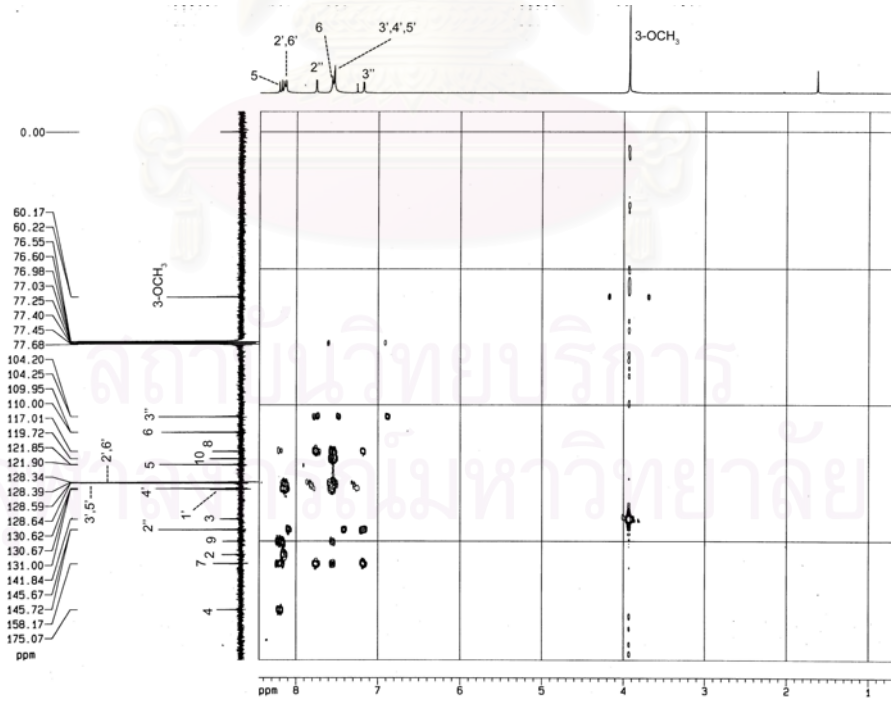


Figure 88. HMBC Spectrum of Compound MD-04 ($CDCl_3$)

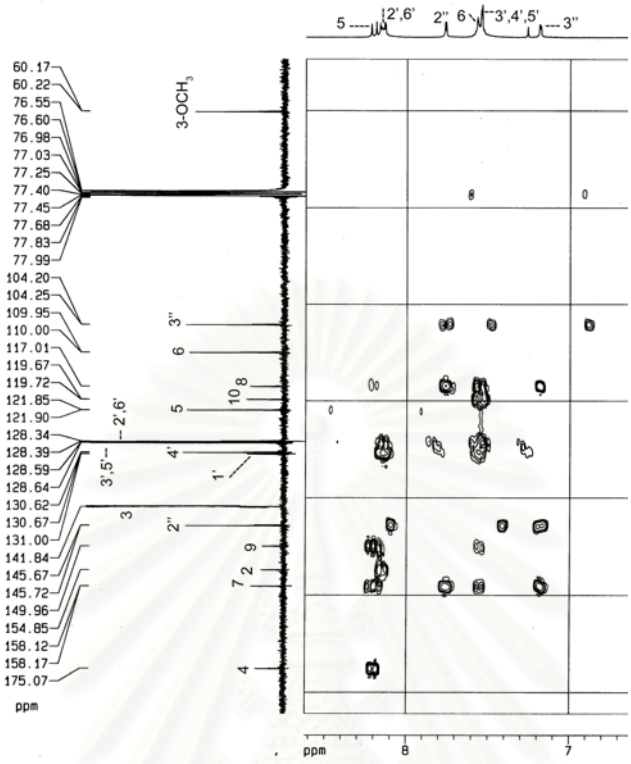


Figure 89. HMBC Spectrum of Compound MD-04 ($CDCl_3$) [δ_H 7.0-8.0 ppm, δ_C 60-175 ppm]

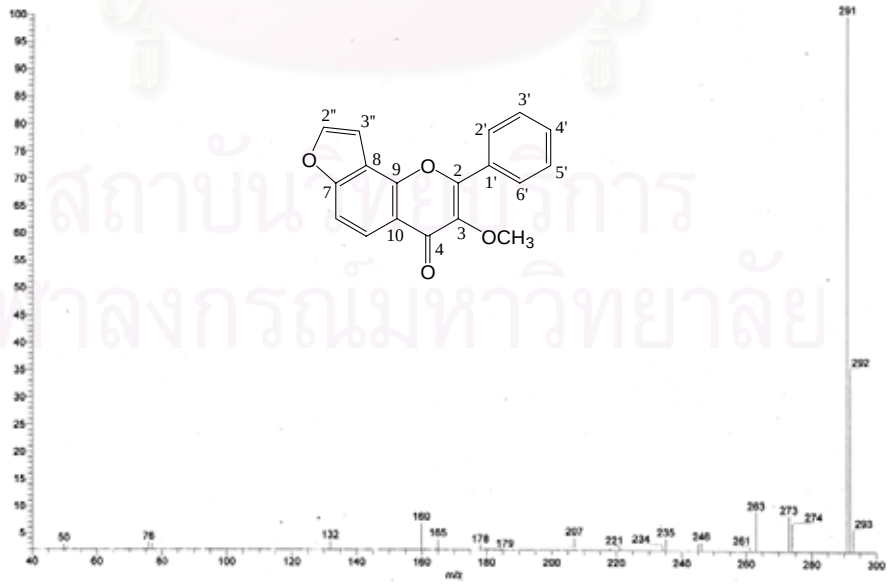


Figure 90. EI Mass Spectrum of Compound MD-04

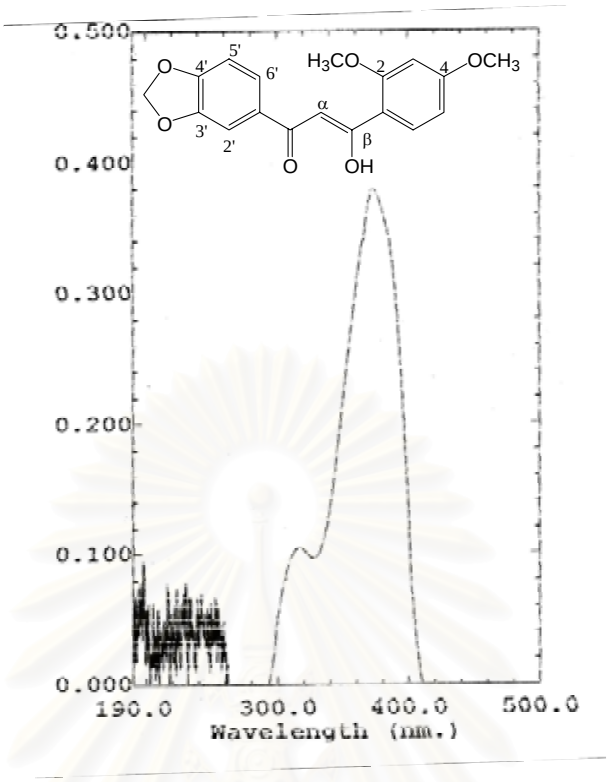


Figure 91. UV Spectrum of Compound MD-05 (methanol)

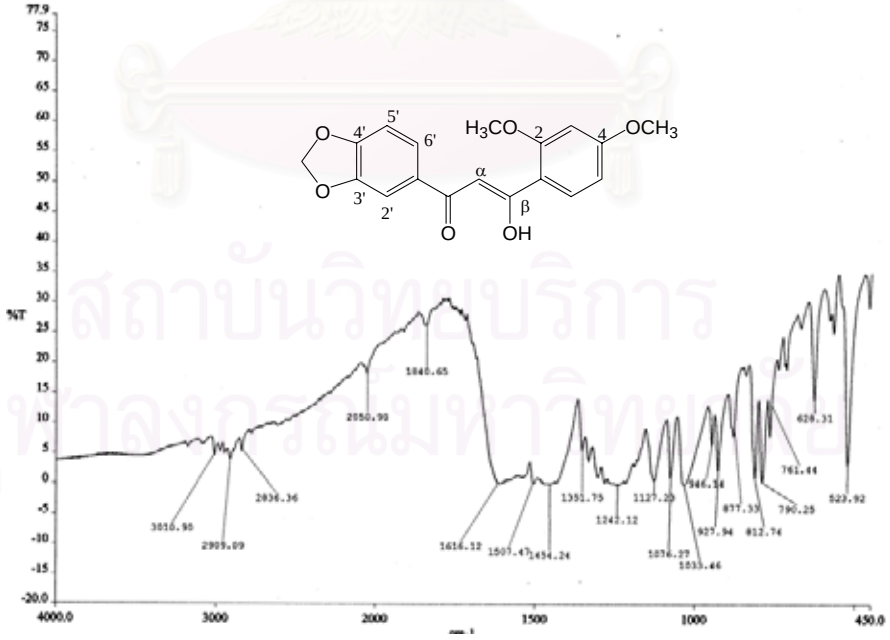


Figure 92. IR Spectrum of Compound MD-05 (KBr disc)

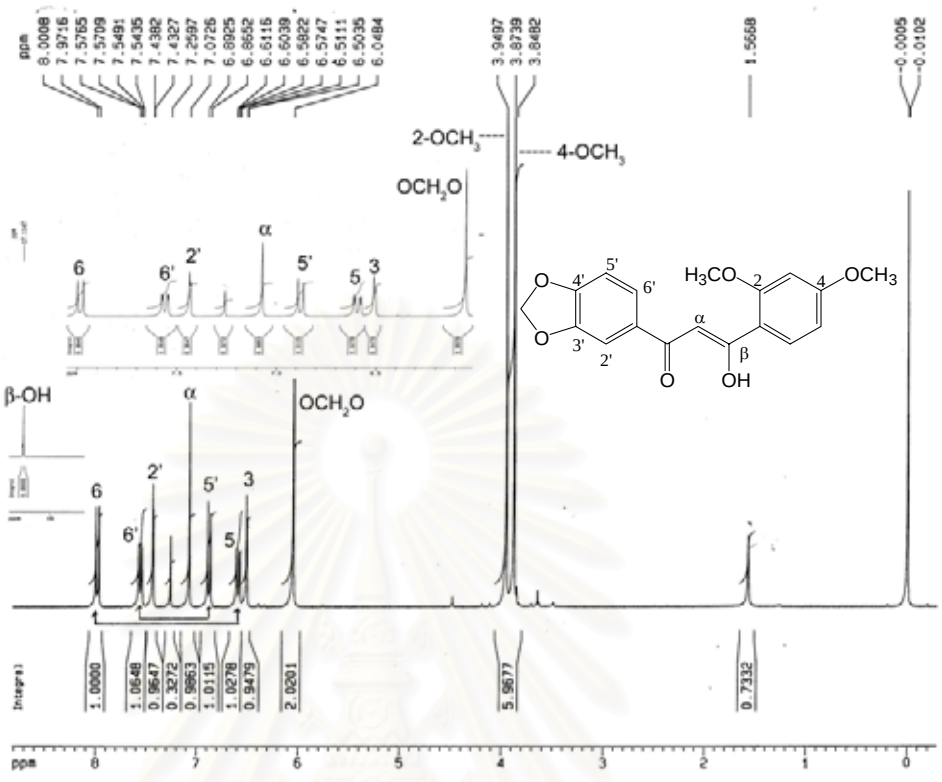


Figure 93. ^{1}H NMR (300 MHz) Spectrum of Compound MD-05 ($CDCl_3$)

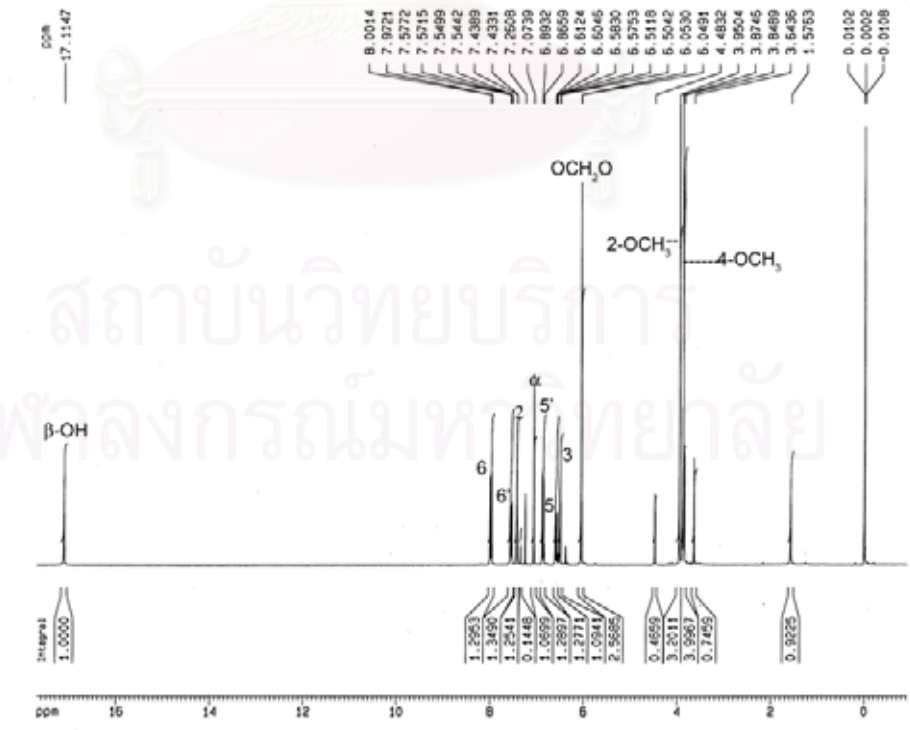


Figure 94. ^{1}H NMR (300 MHz) Spectrum of Compound MD-05 show the mixture of keto and enol form ($CDCl_3$)

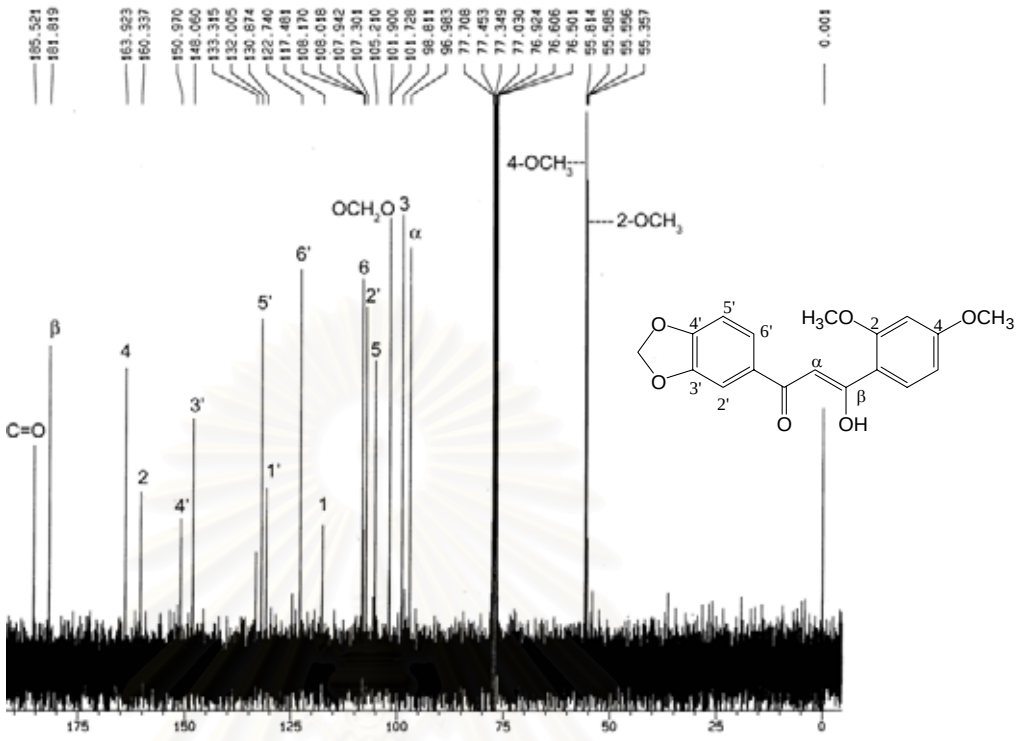


Figure 95. ^{13}C NMR (75 MHz) Spectrum of Compound MD-05 ($CDCl_3$)

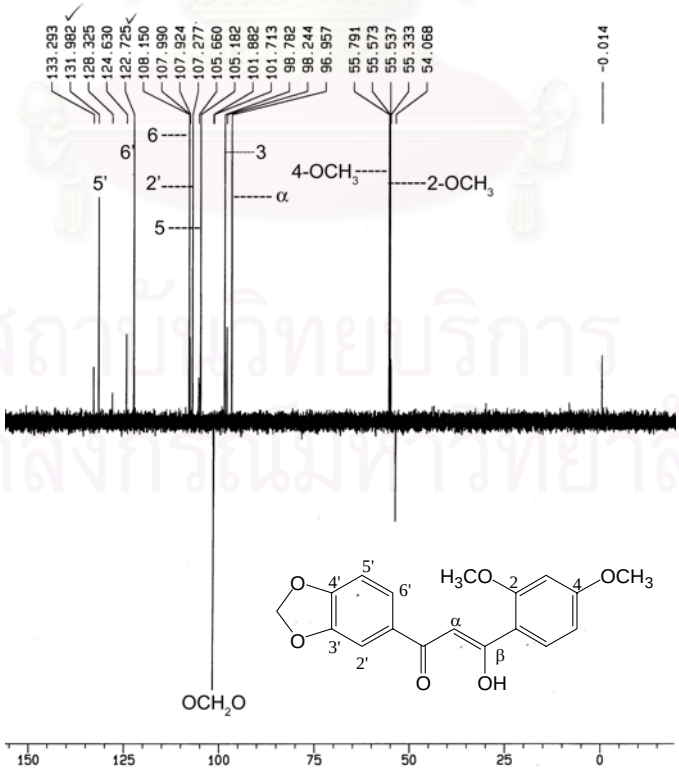


Figure 96. Dept 135 Spectrum of Compound MD-05 ($CDCl_3$)

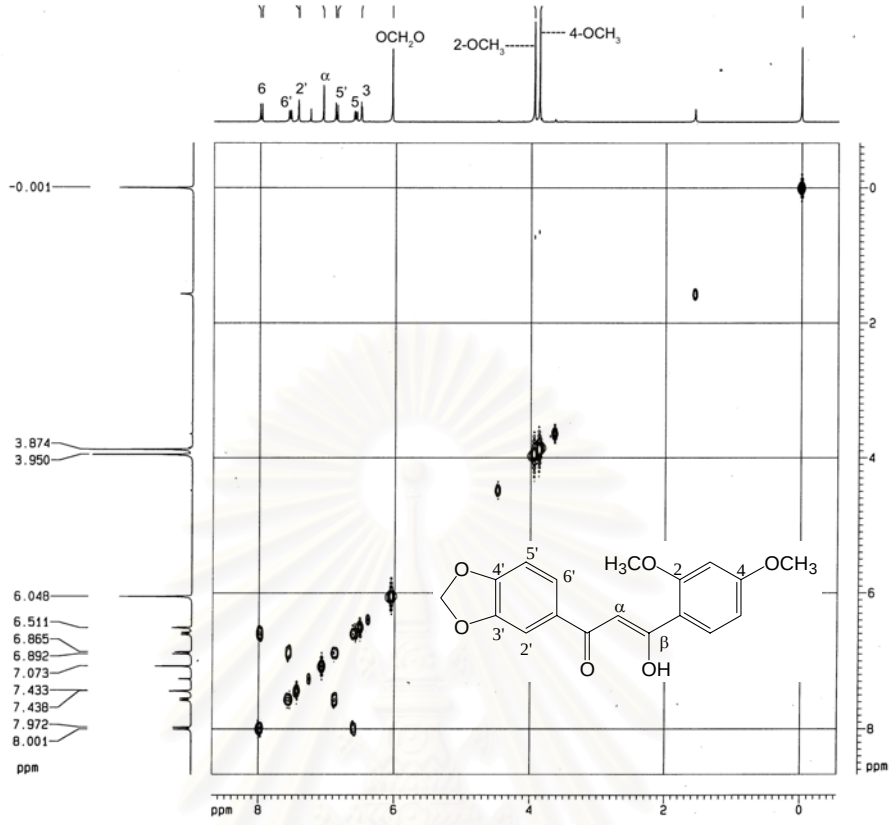


Figure 97. ^{1}H-^{1}H COSY Spectrum of Compound MD-05 ($CDCl_3$)

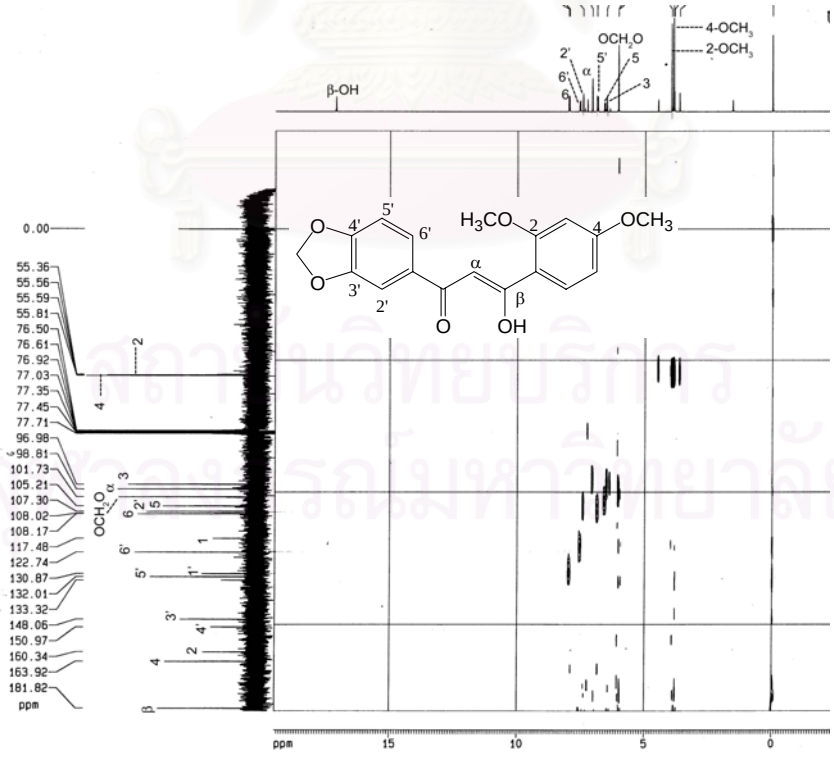


Figure 98. HMQC Spectrum of Compound MD-05 ($CDCl_3$)

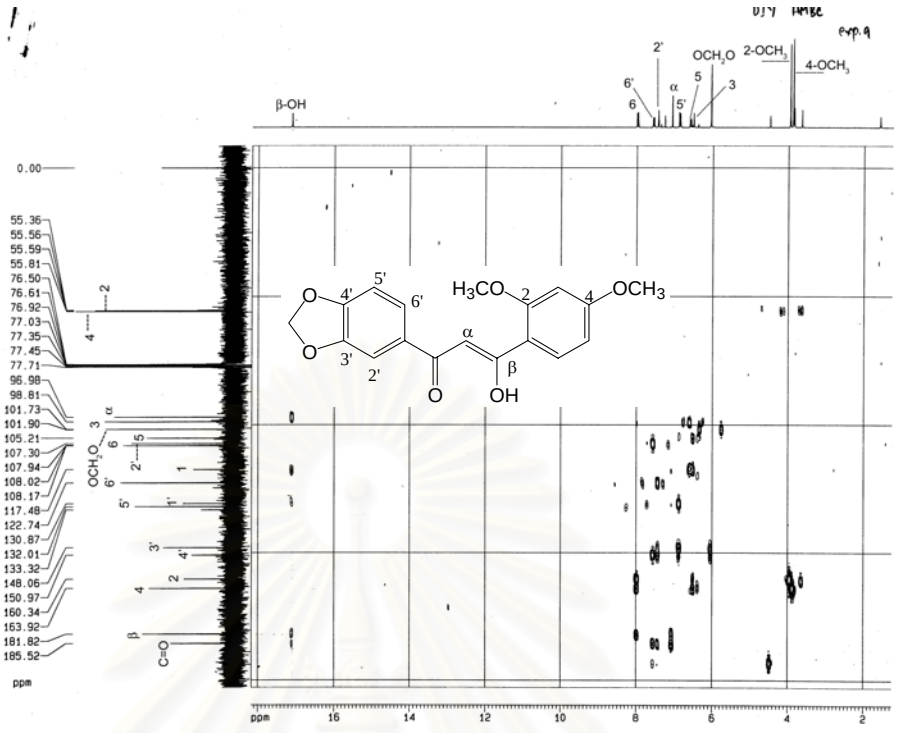


Figure 99. HMBC Spectrum of Compound MD-05 ($CDCl_3$)

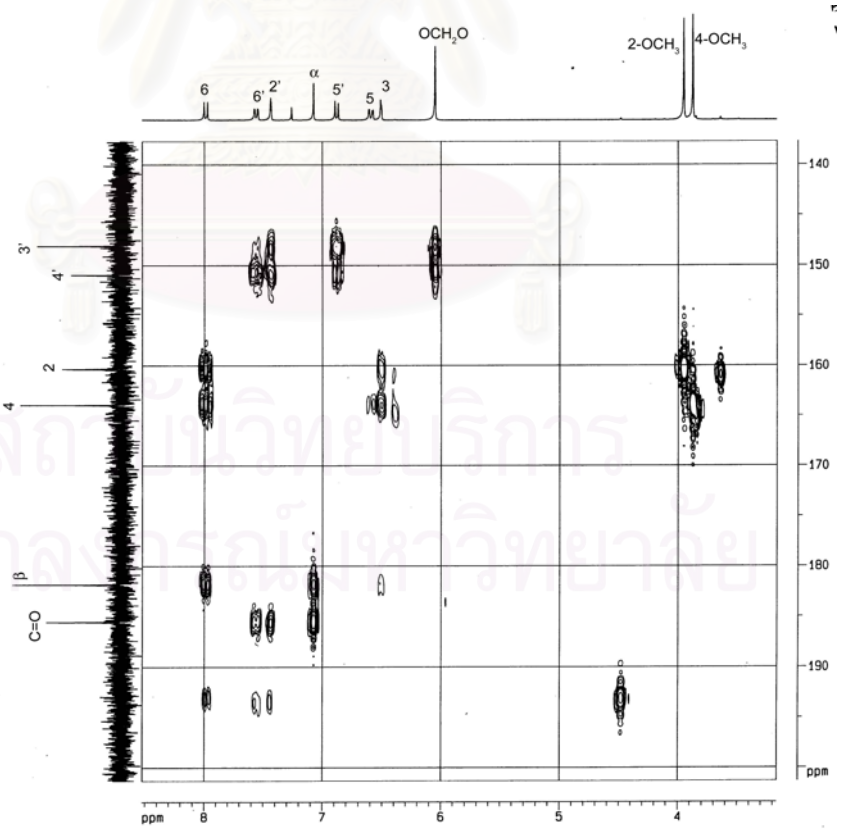


Figure 100. HMBC Spectrum of Compound MD-05 ($CDCl_3$) [δ_H 3.8-8.0 ppm, δ_C 140-200 ppm]

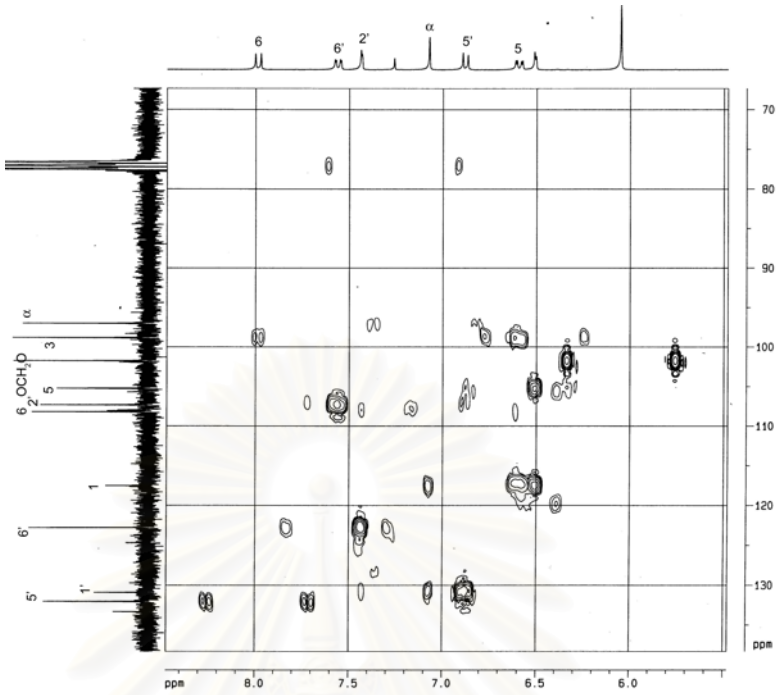


Figure 101. HMBC Spectrum of Compound MD-05 ($CDCl_3$) [δ_H 6.0-8.0 ppm, δ_C 70-135 ppm]

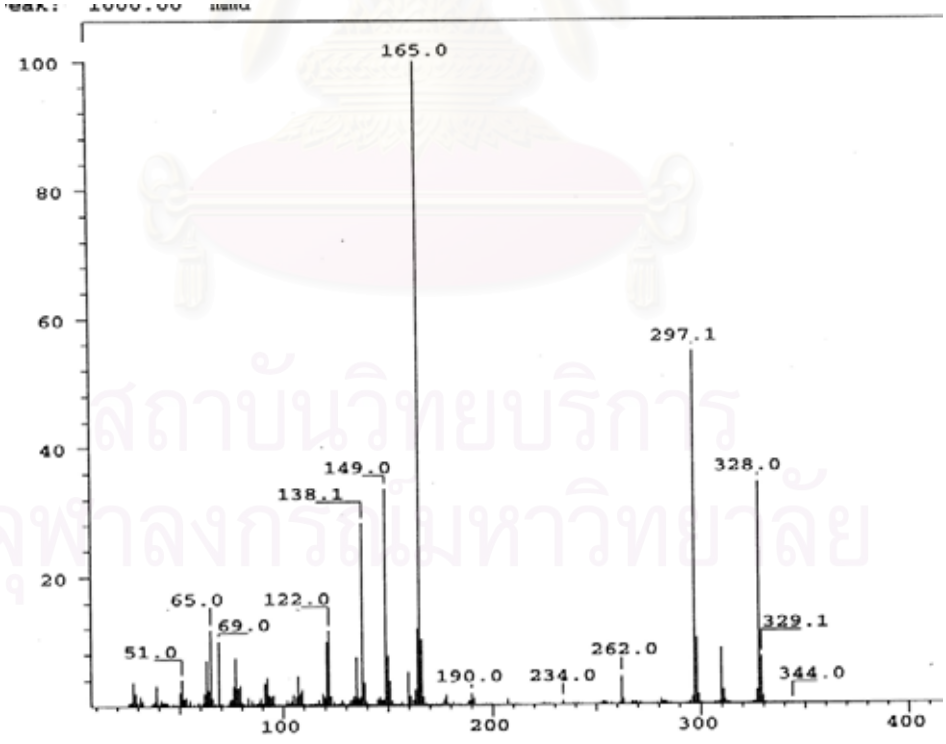


Figure 102. EI Mass Spectrum of Compound MD-05

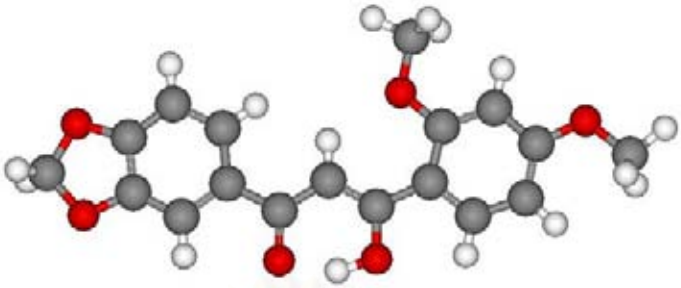


Figure 103. The optimized structure of **46a** in *Cs* symmetry

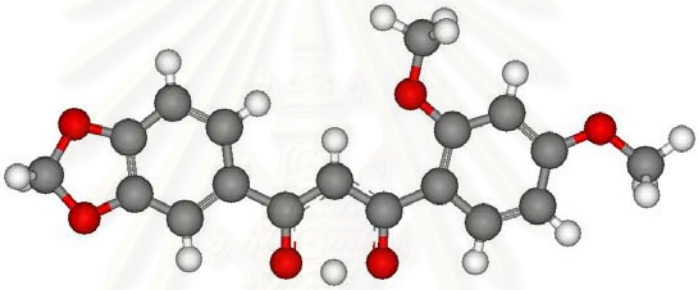


Figure 104. The optimized structure of transition state in *Cs* symmetry

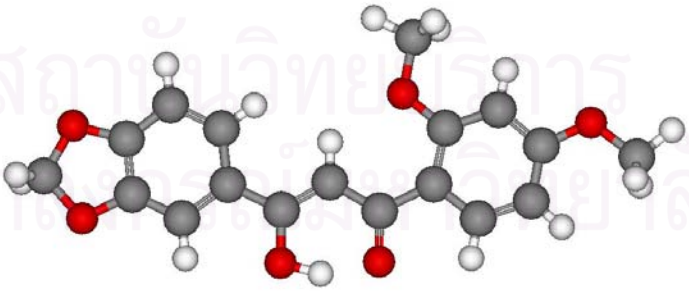


Figure 105. The optimized structure of **46b** in *Cs* symmetry

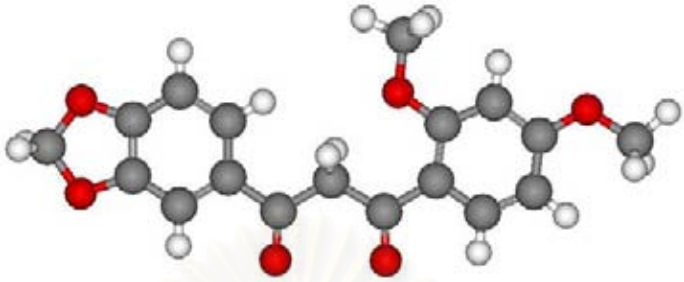


Figure 106. The optimized structure of **46c** without symmetry

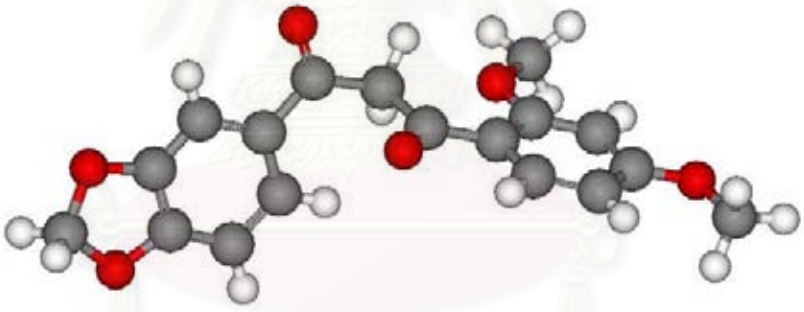


Figure 107. The optimized structure of **46d** in *Cs* symmetry

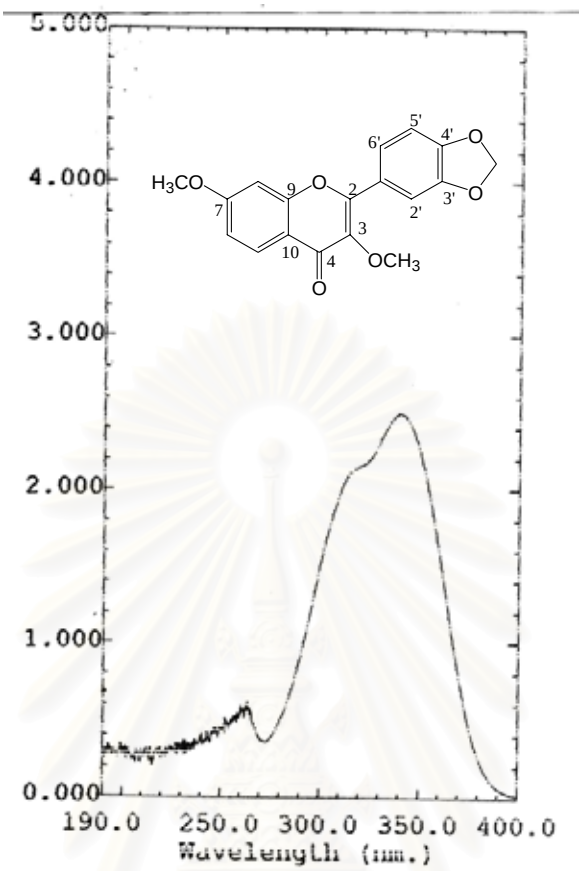


Figure 108. UV Spectrum of Compound MD-06 (methanol)

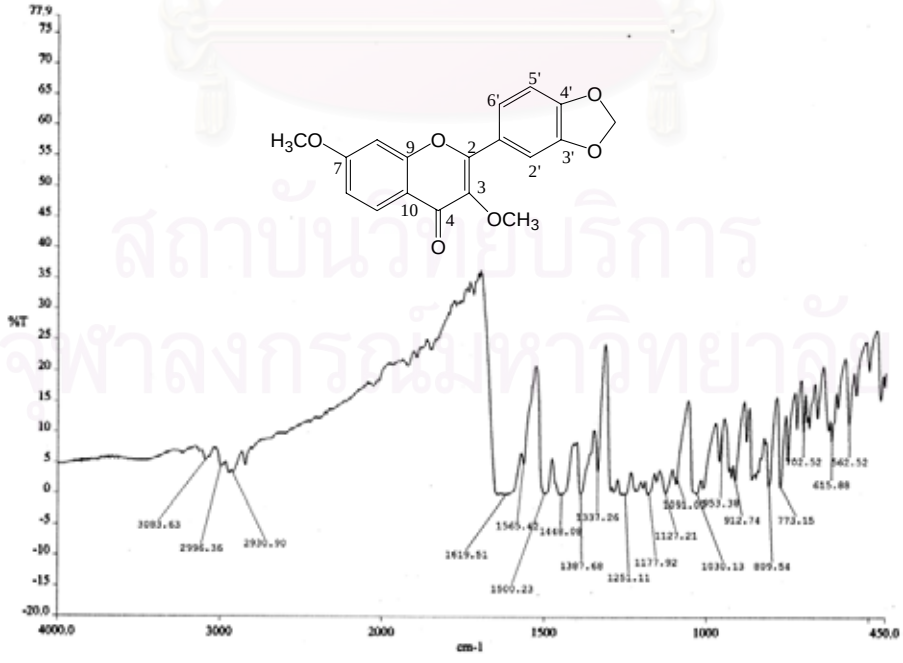


Figure 109. IR Spectrum of Compound MD-06 (KBr disc)

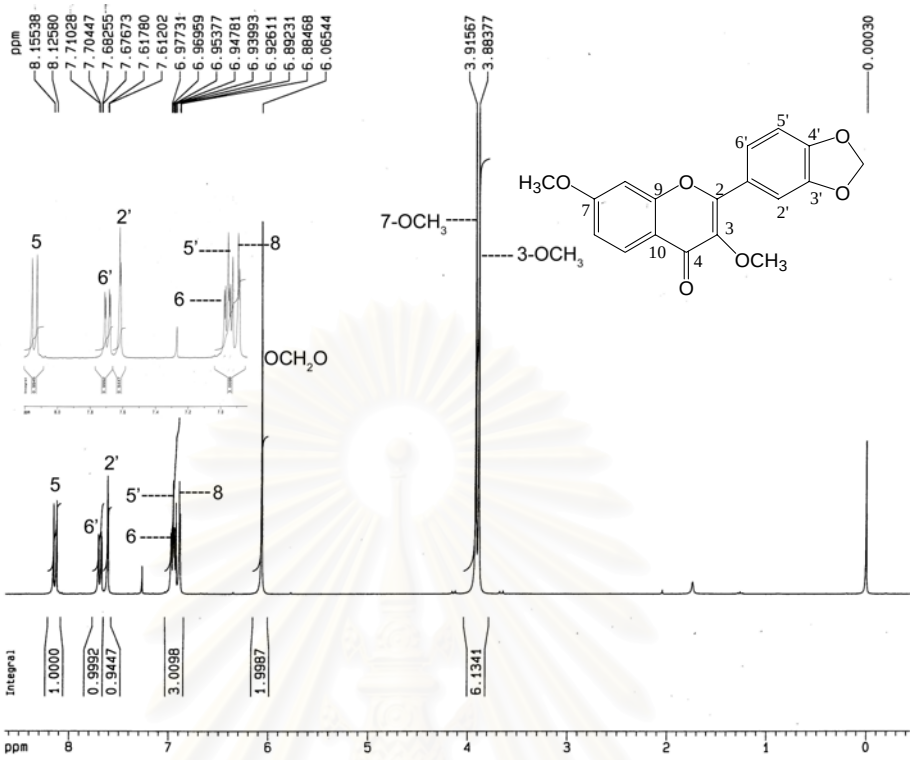


Figure 110. ^{1}H NMR (300 MHz) Spectrum of Compound MD-06 ($CDCl_3$)

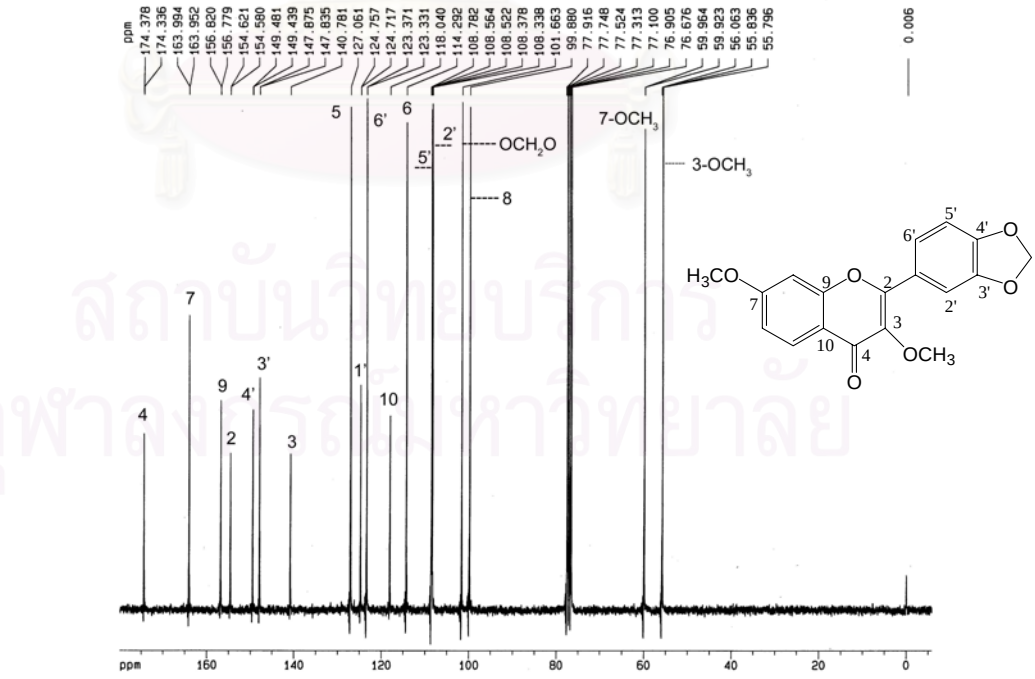


Figure 111. ^{13}C NMR (75 MHz) Spectrum of Compound MD-06 ($CDCl_3$)

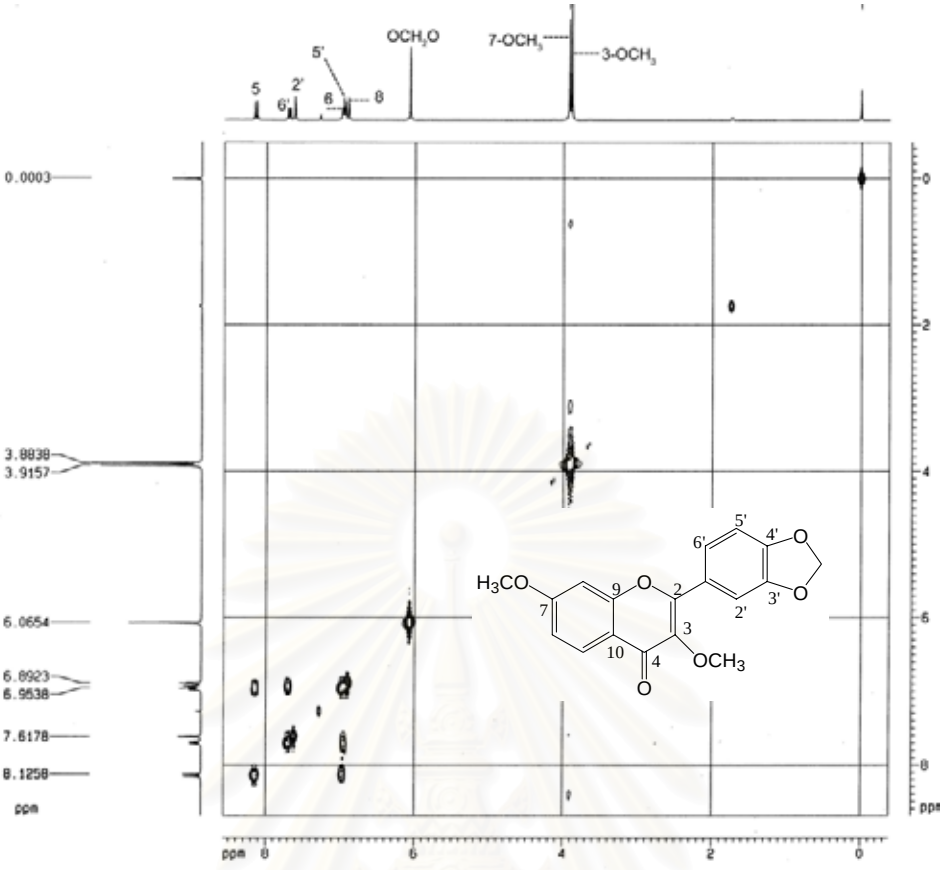


Figure 112. ^{1}H-^{1}H COSY Spectrum of Compound MD-06 ($CDCl_3$)

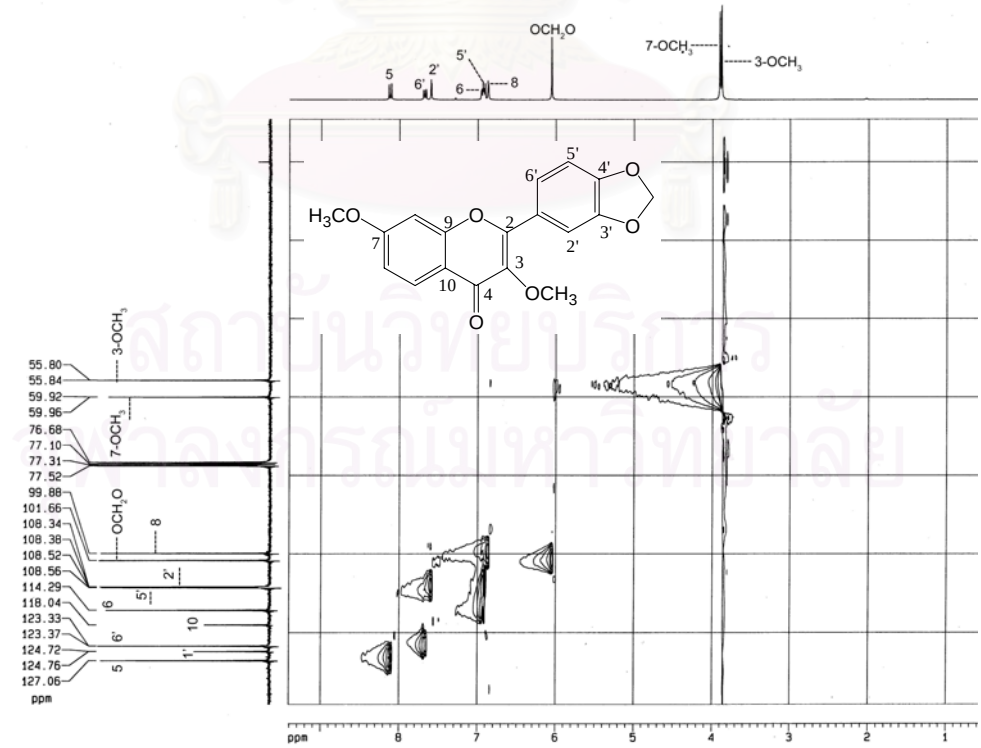


Figure 113. HMQC Spectrum of Compound MD-06 ($CDCl_3$)

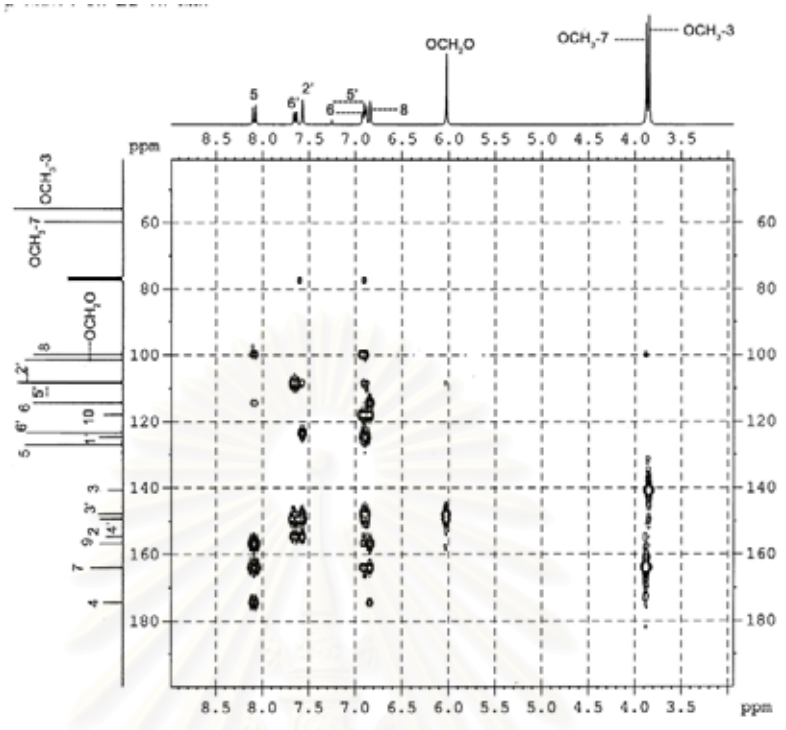


Figure 114. HMBC Spectrum of Compound MD-06 ($CDCl_3$)

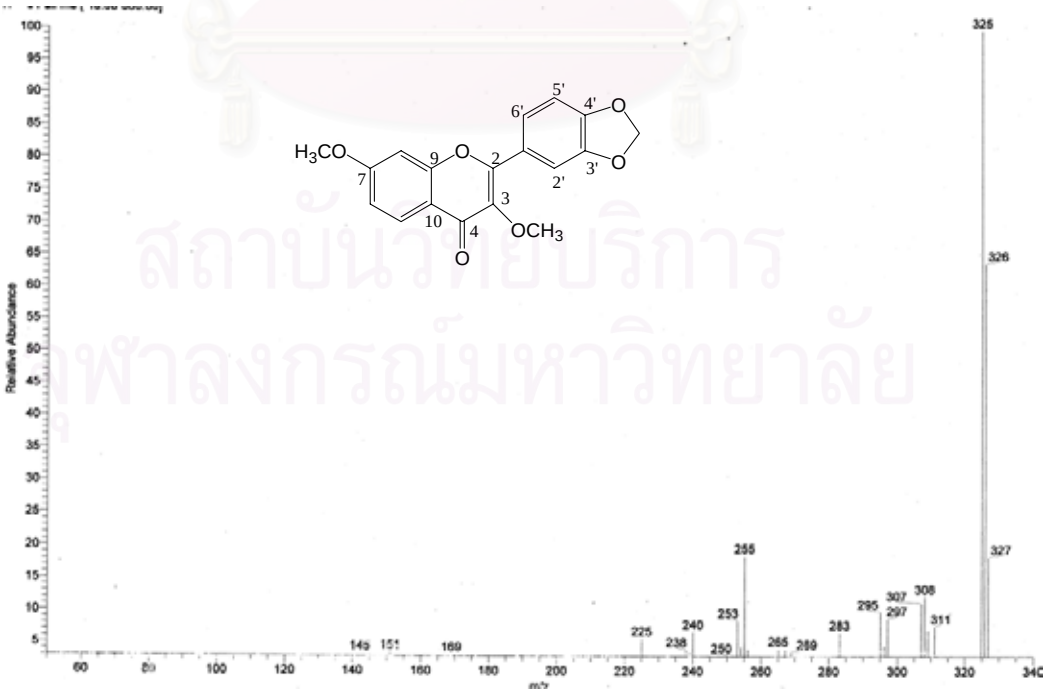


Figure 115. EI Mass Spectrum of Compound MD-06

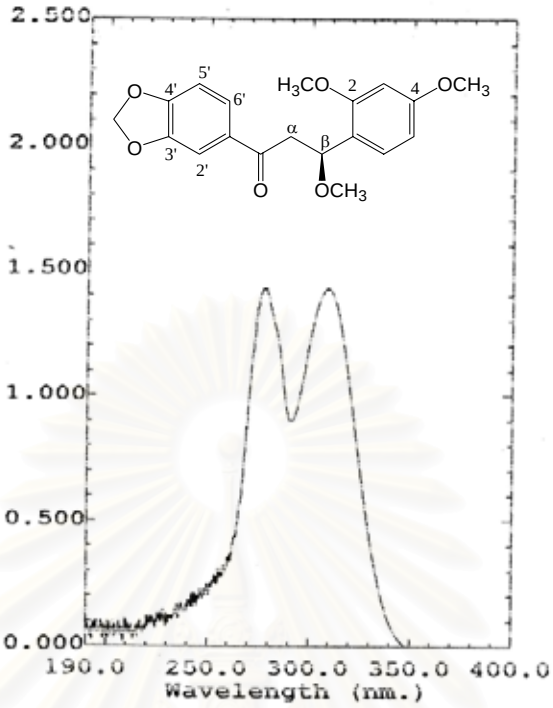


Figure 116. UV Spectrum of Compound MD-07 (methanol)

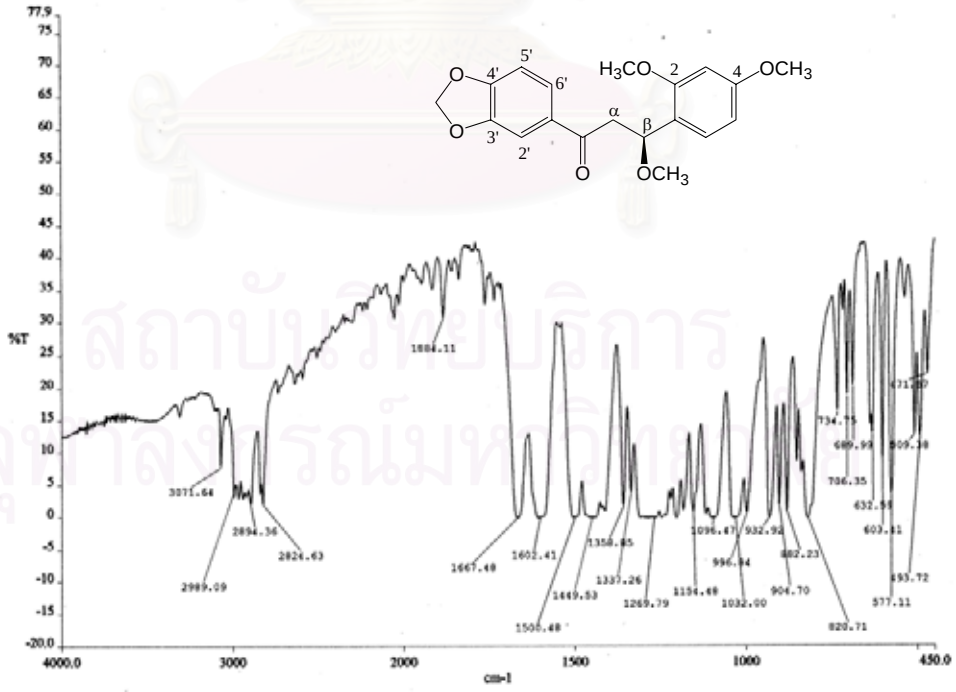


Figure 117. IR Spectrum of Compound MD-07 (KBr disc)

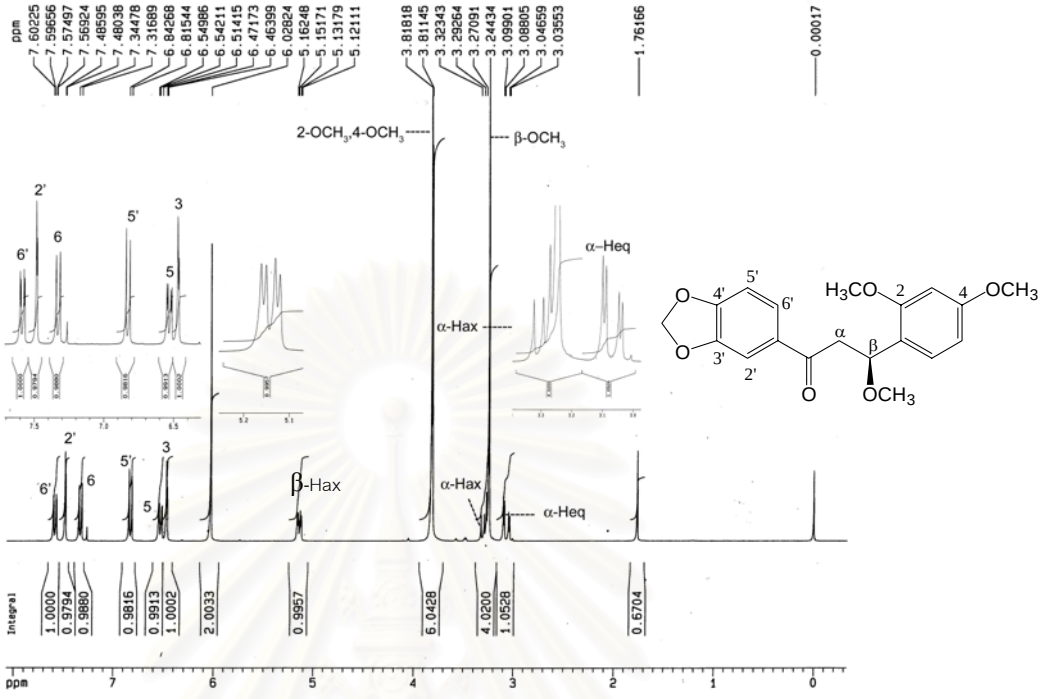


Figure 118. ^{1}H NMR (300 MHz) Spectrum of Compound MD-07 ($CDCl_3$)

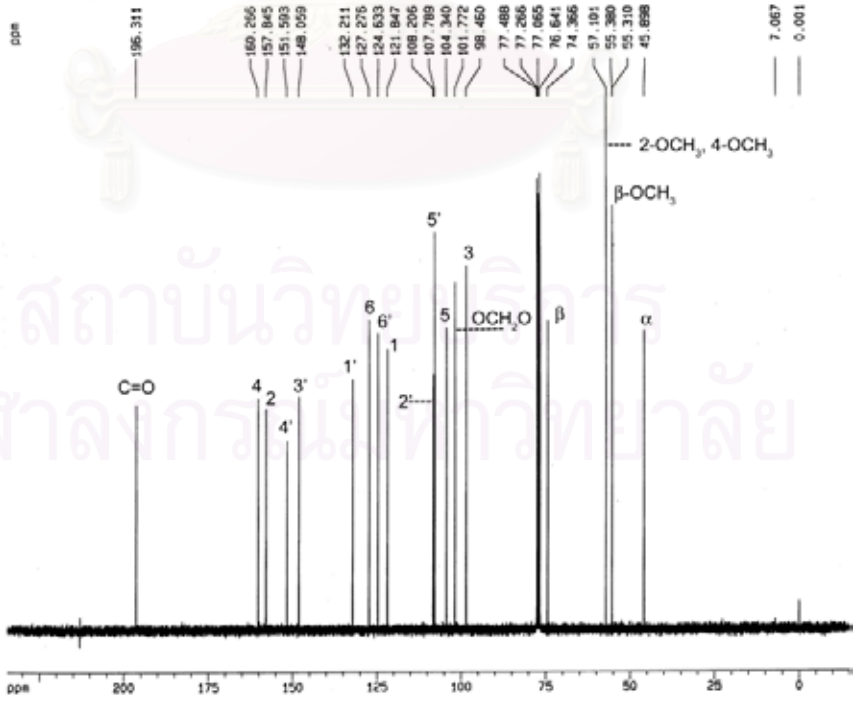


Figure 119. ^{13}C NMR (75 MHz) Spectrum of Compound MD-07 ($CDCl_3$)

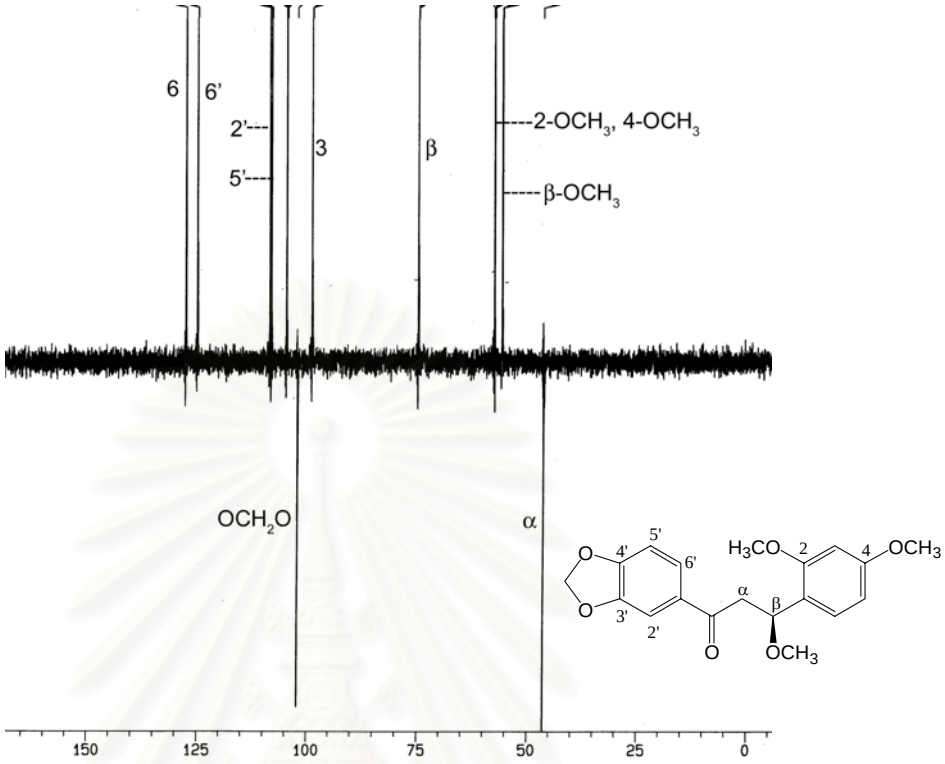


Figure 120. Dept 135 Spectrum of Compound MD-07 ($CDCl_3$)

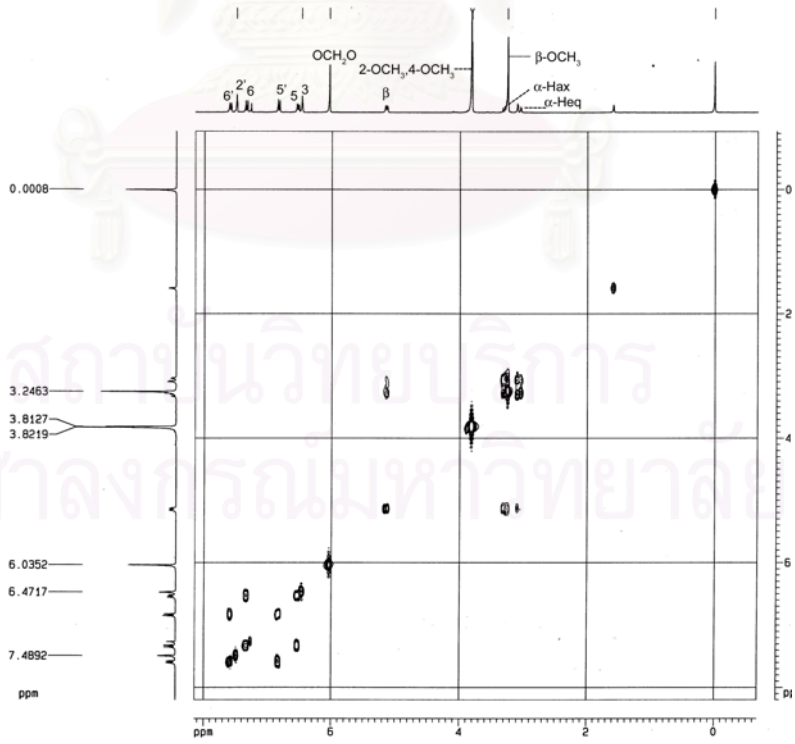


Figure 121. ^{1}H-^{1}H COSY Spectrum of Compound MD-07 ($CDCl_3$)

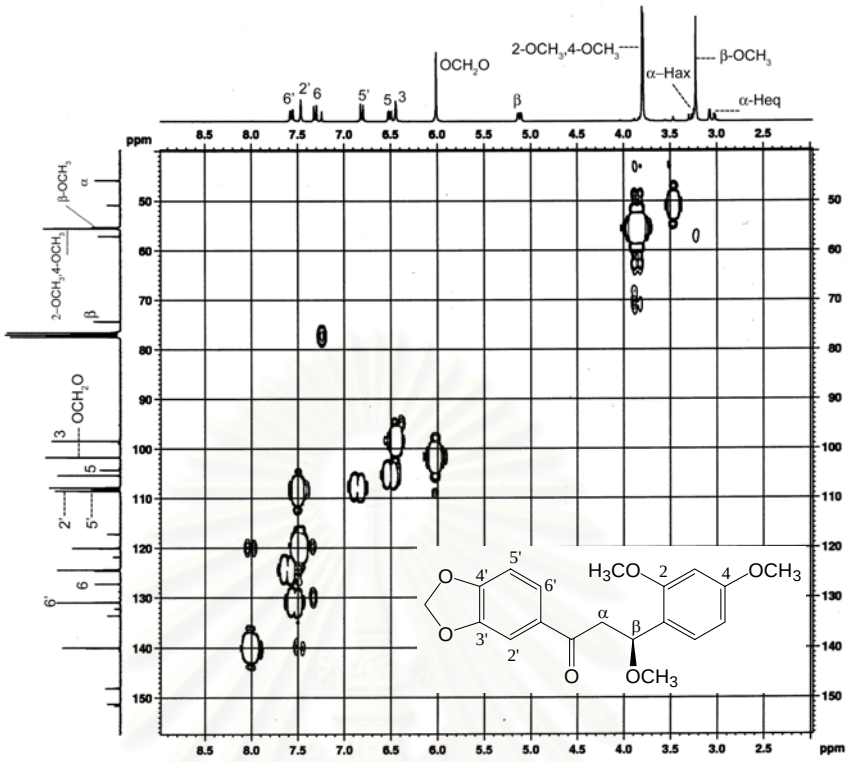


Figure 122. HMQC Spectrum of Compound MD-07 ($CDCl_3$)

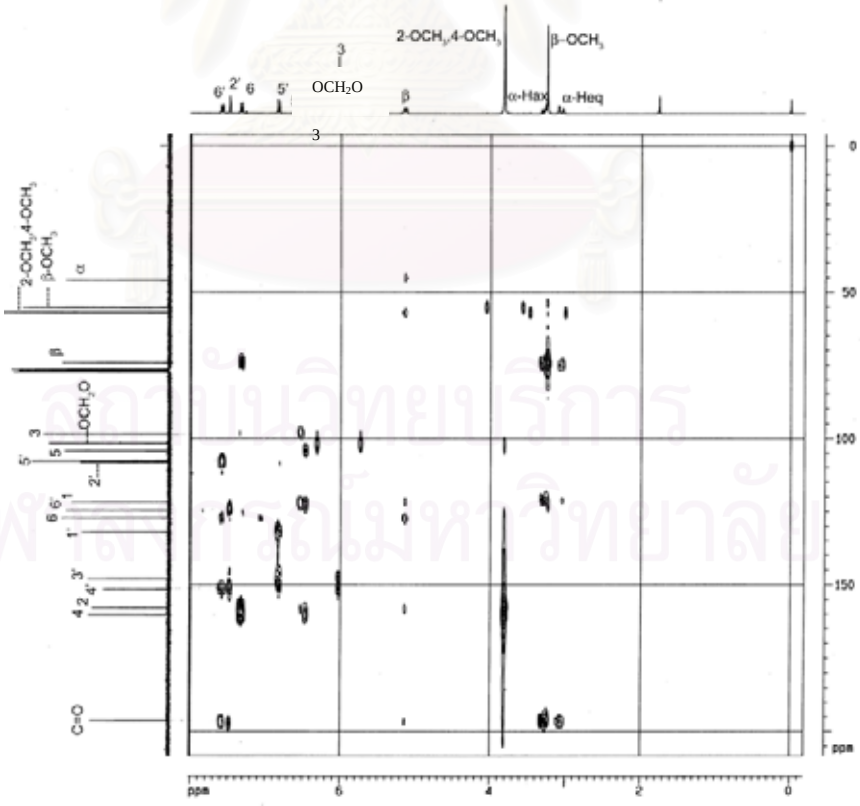


Figure 123. HMBC Spectrum of Compound MD-07 ($CDCl_3$)

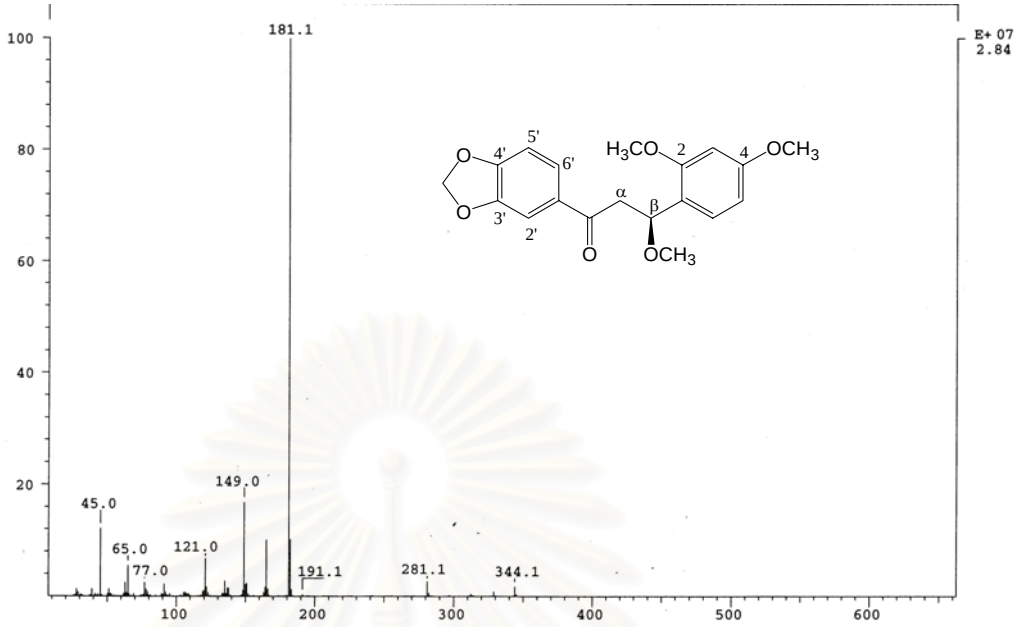


Figure 124. EI Mass Spectrum of Compound MD-07

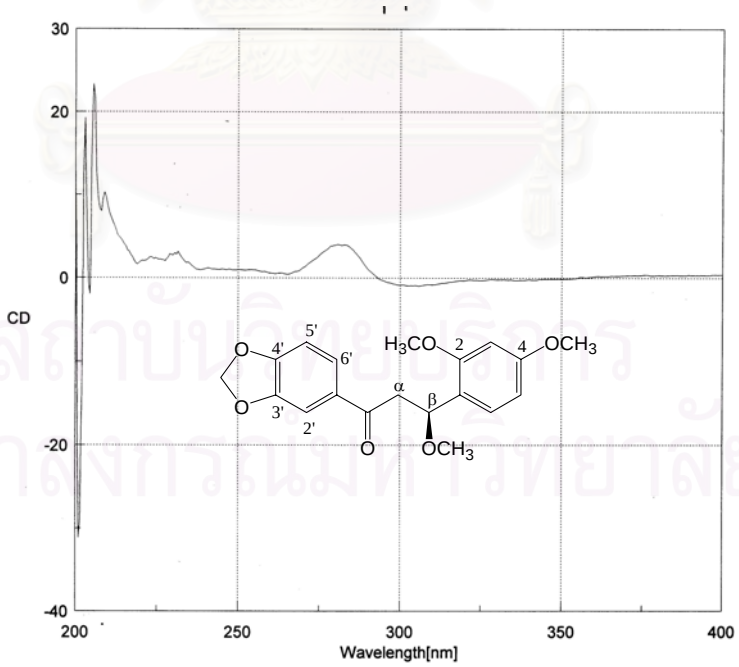


Figure 125. CD Spectrum of Compound MD-07

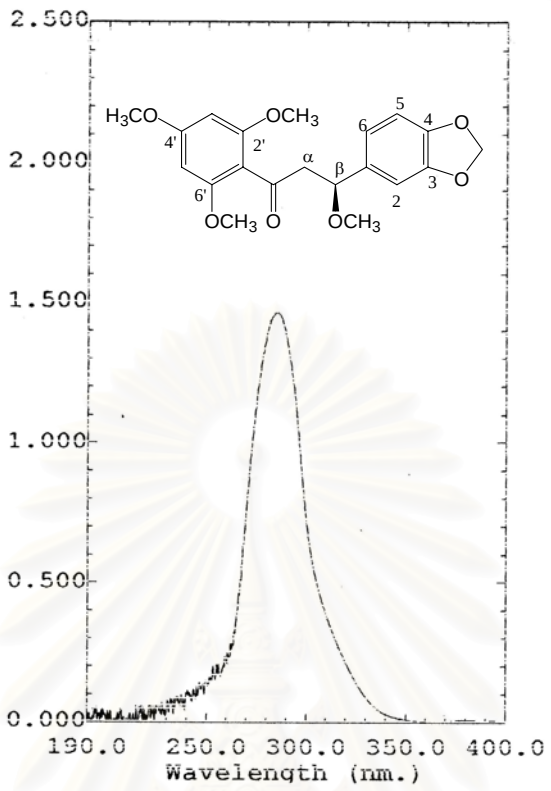


Figure 126. UV Spectrum of Compound MD-08 (methanol)

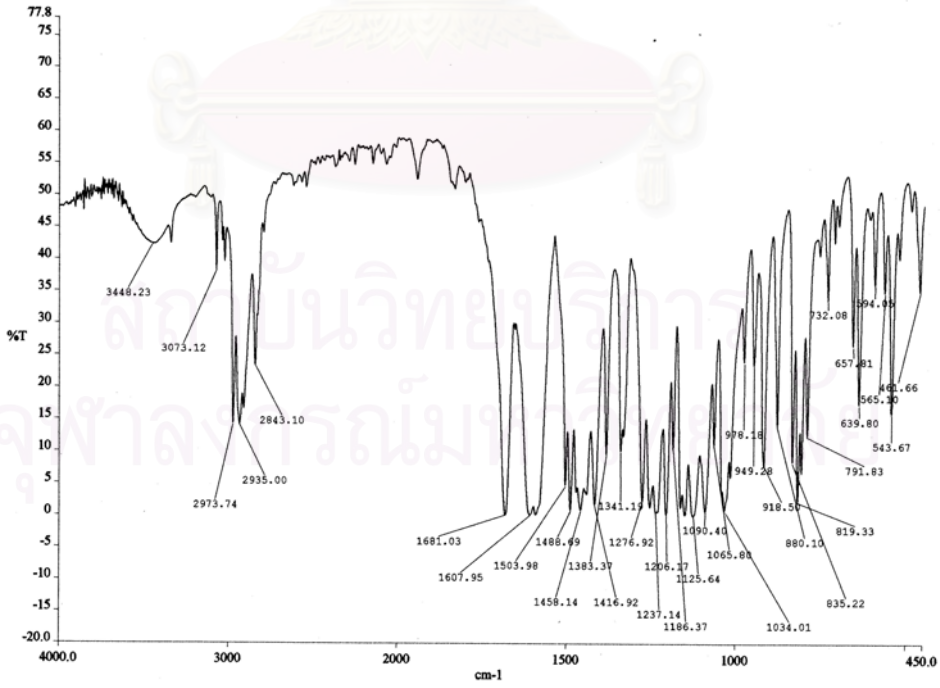


Figure 127. IR Spectrum of Compound MD-08 (KBr disc)

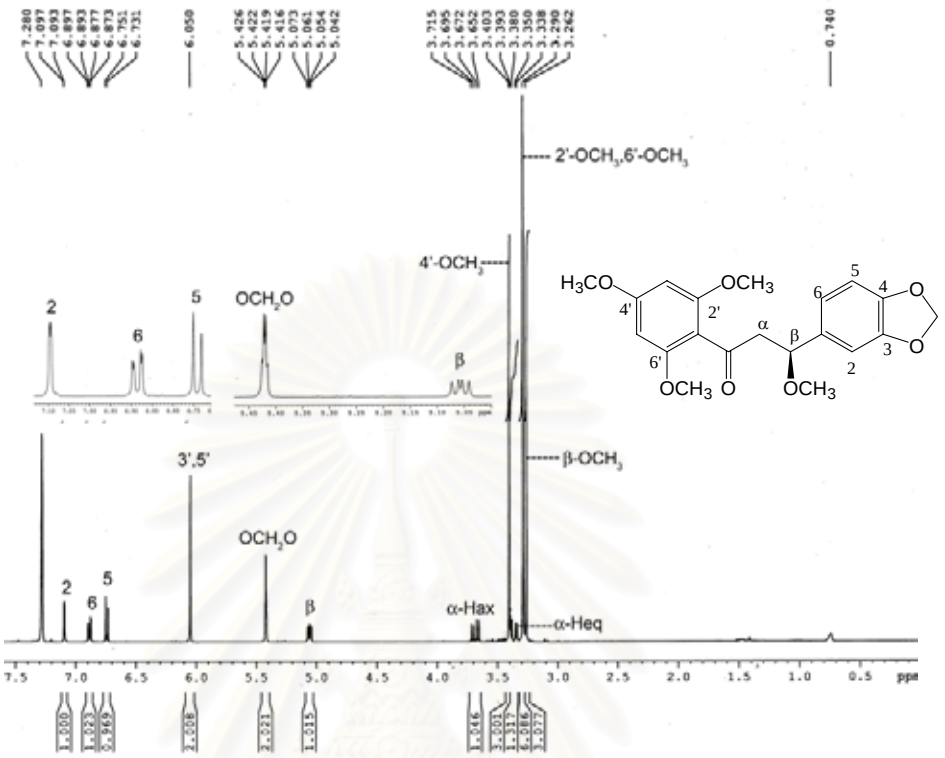


Figure 128. ^{1}H NMR (400 MHz) Spectrum of Compound MD-08 (C_6D_6)

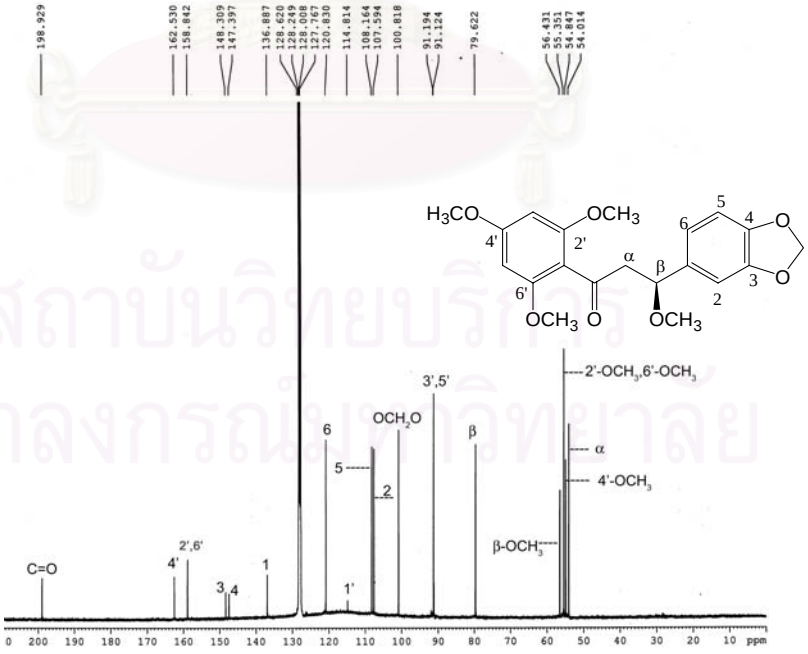


Figure 129. ^{13}C NMR (100 MHz) Spectrum of Compound MD-08 (C_6D_6)

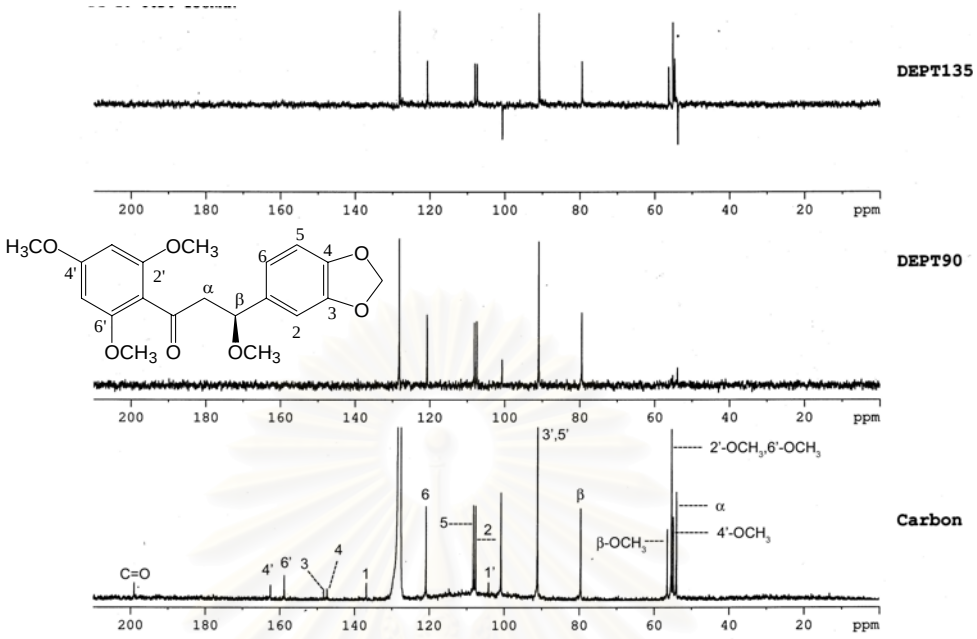


Figure 130. Dept 135 and 90 Spectrum of Compound MD-08 (C_6D_6)

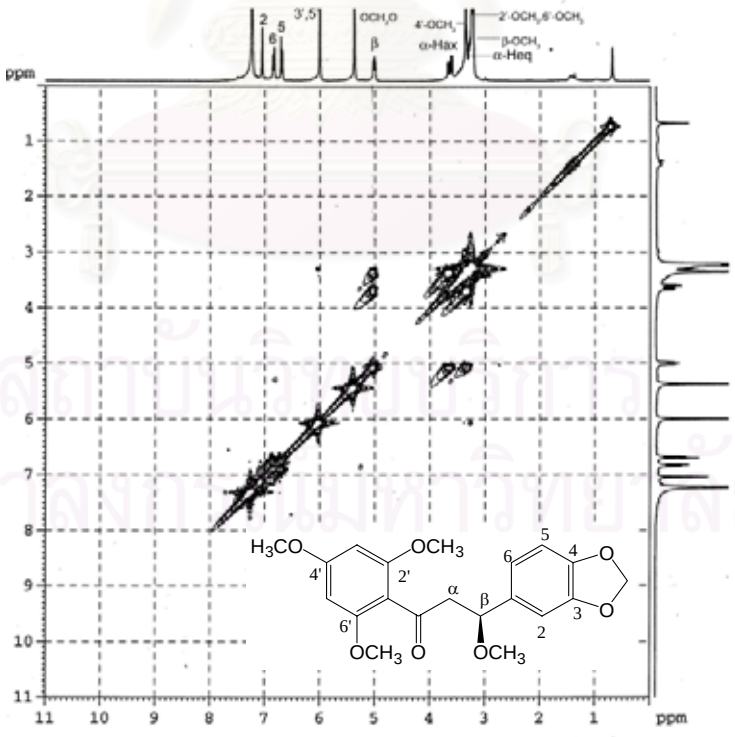


Figure 131. ^{1}H-^{1}H COSY Spectrum of Compound MD-08 (C_6D_6)

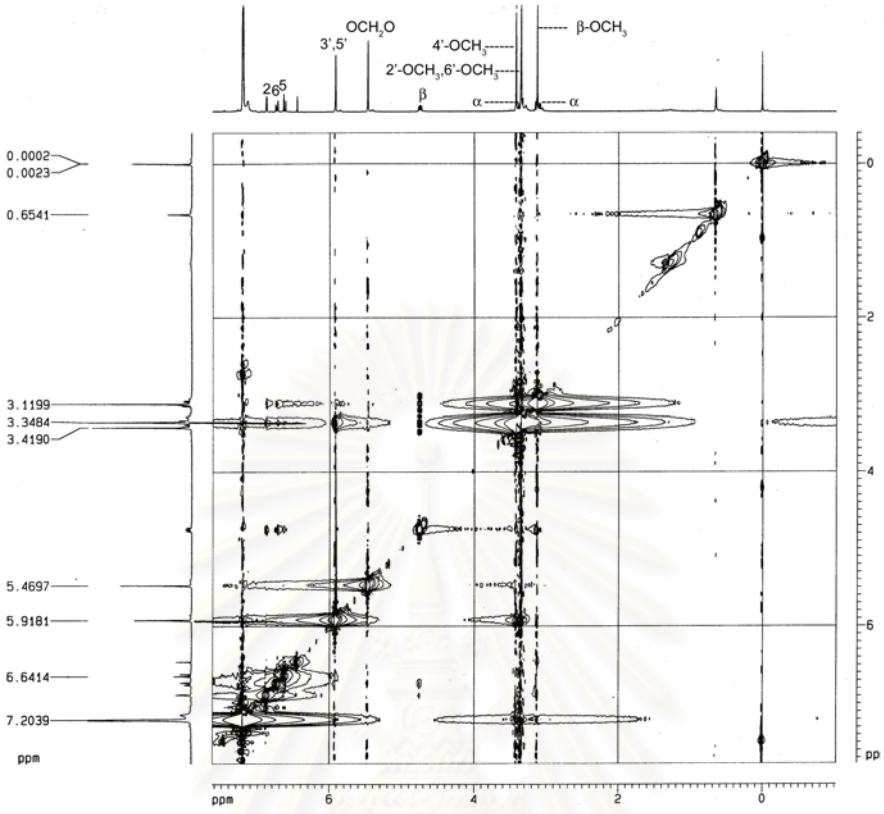


Figure 132. NOESY Spectrum of Compound MD-08 (C_6D_6)

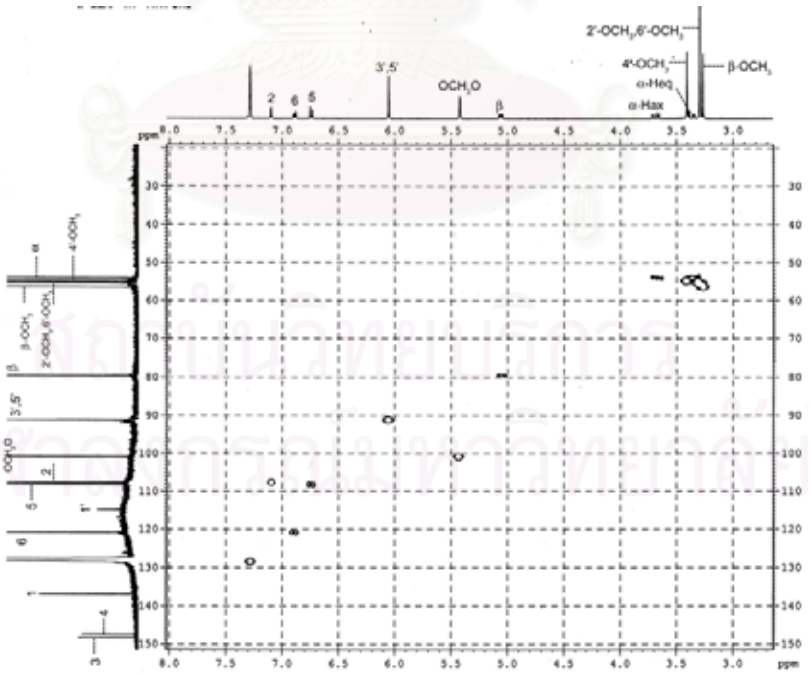


Figure 133. HMQC Spectrum of Compound MD-08 (C_6D_6)

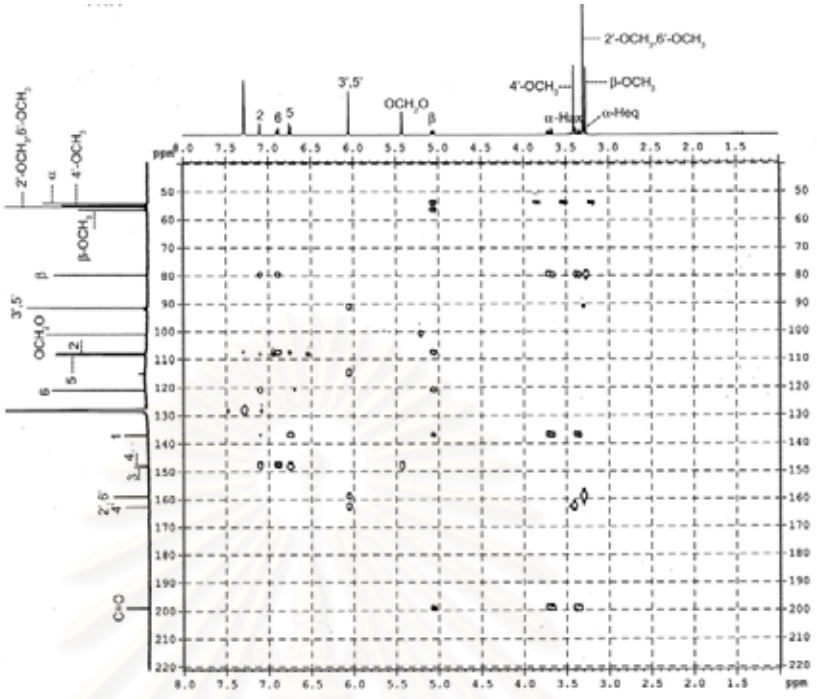


Figure 134. HMBC Spectrum of Compound MD-08 (C_6D_6)

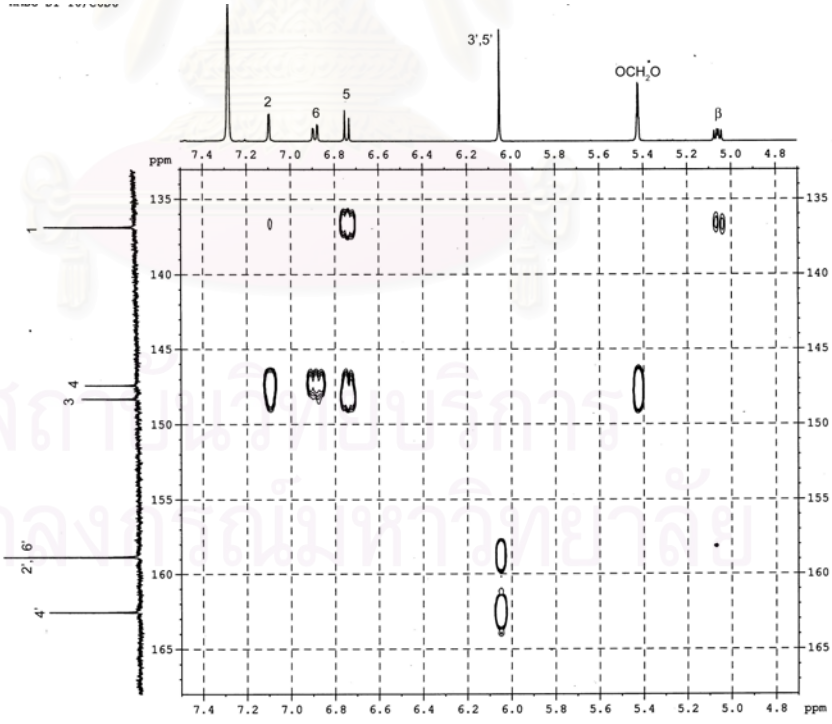


Figure 135. HMBC Spectrum of Compound MD-08 (C_6D_6) [δ_H 5.0-7.4 ppm, δ_C 135-165 ppm]

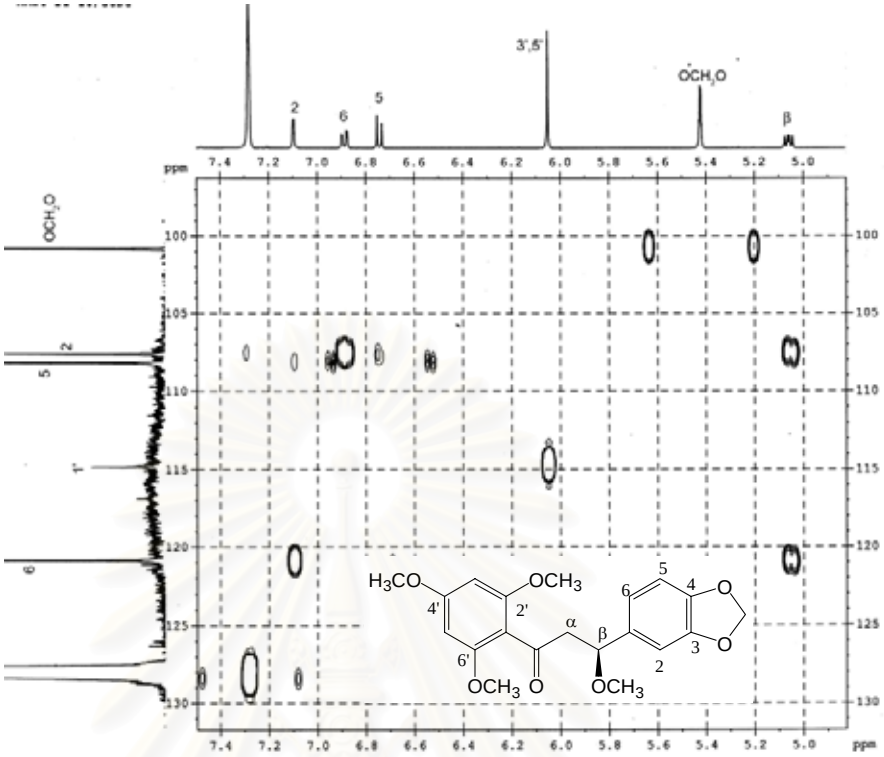


Figure 136. HMBC Spectrum of Compound MD-08 (C_6D_6) [δ_H 5.0-7.4 ppm, δ_C 100-130 ppm]

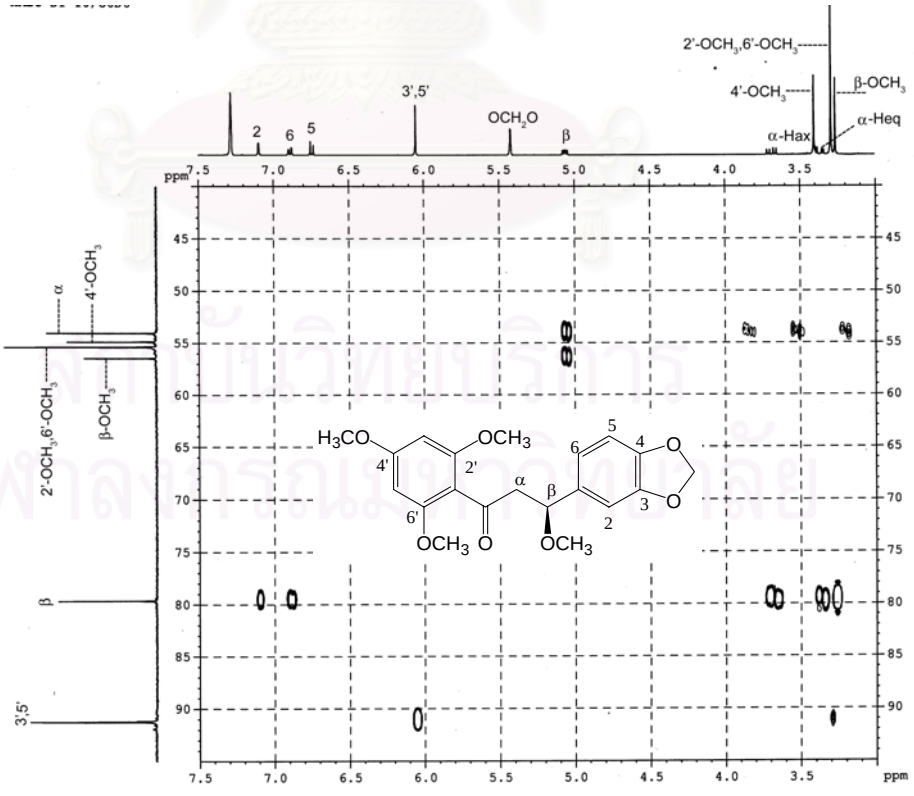


Figure 137. HMBC Spectrum of Compound MD-08 (C_6D_6) [δ_H 3.2-7.5 ppm, δ_C 45-92 ppm]

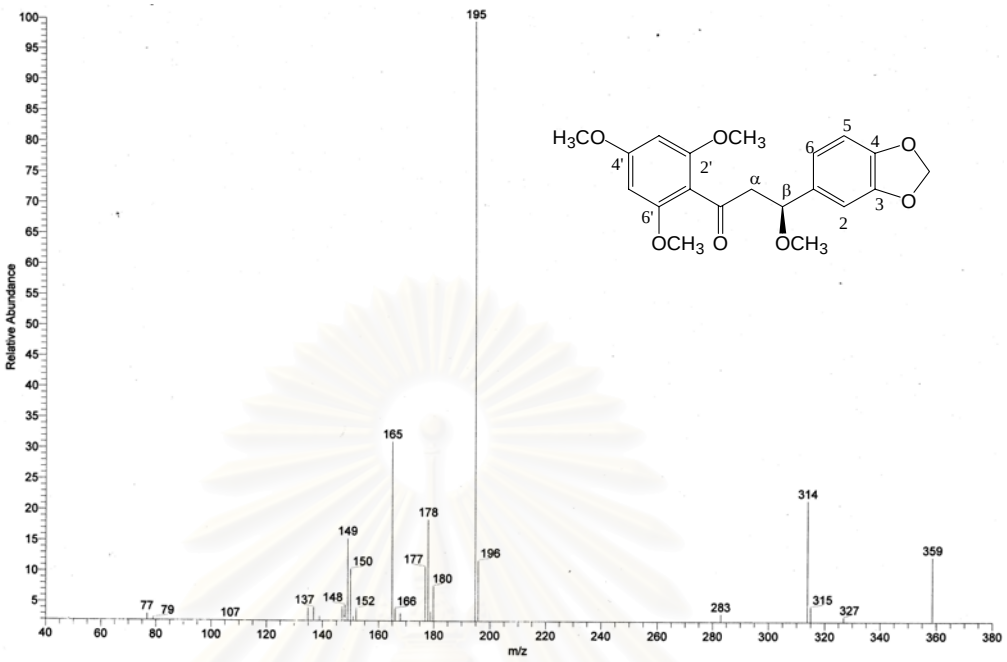


Figure 138. EI Mass Spectrum of Compound MD-08

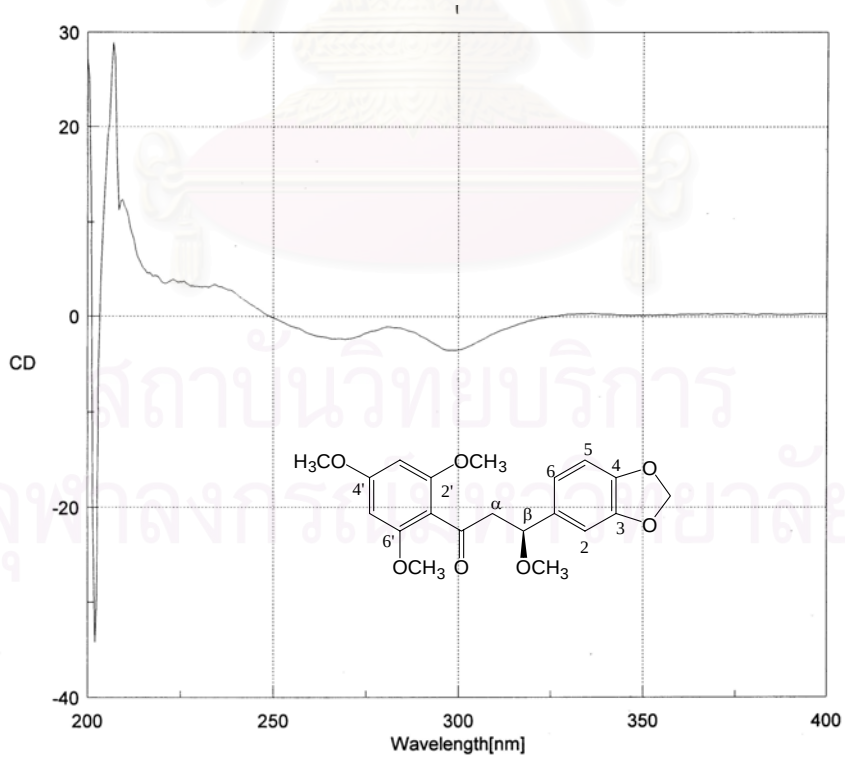


Figure 139. CD Spectrum of Compound MD-08

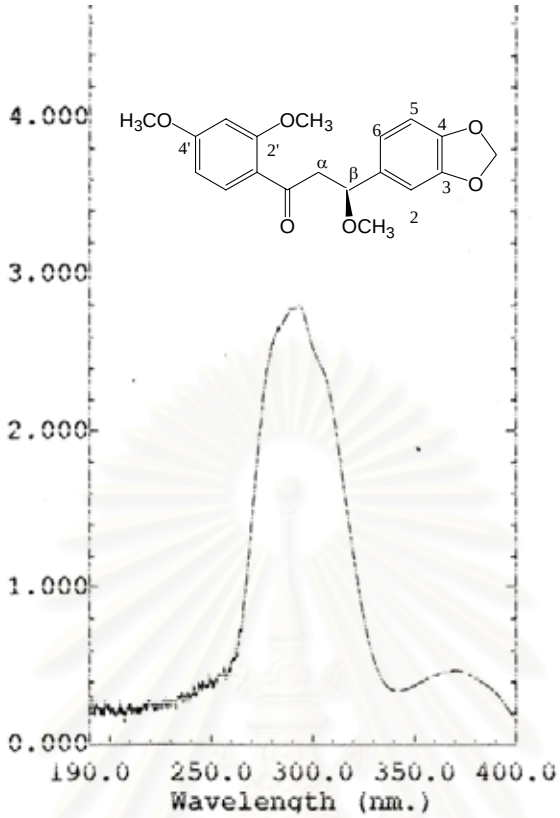


Figure 140. UV Spectrum of Compound MD-09 (methanol)

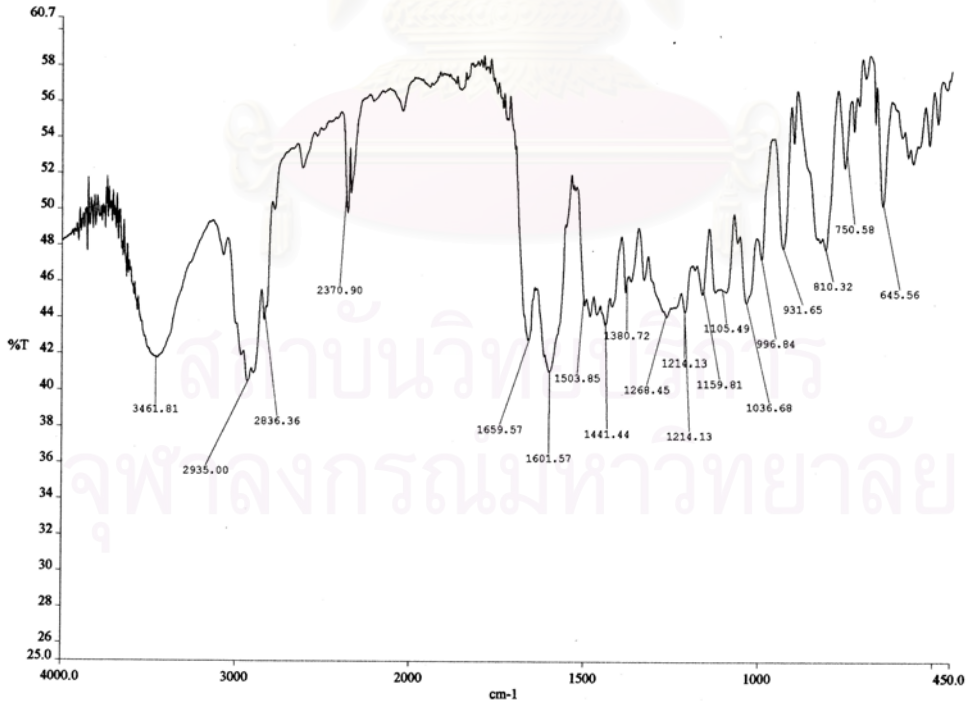


Figure 141. IR Spectrum of Compound MD-09 (KBr disc)

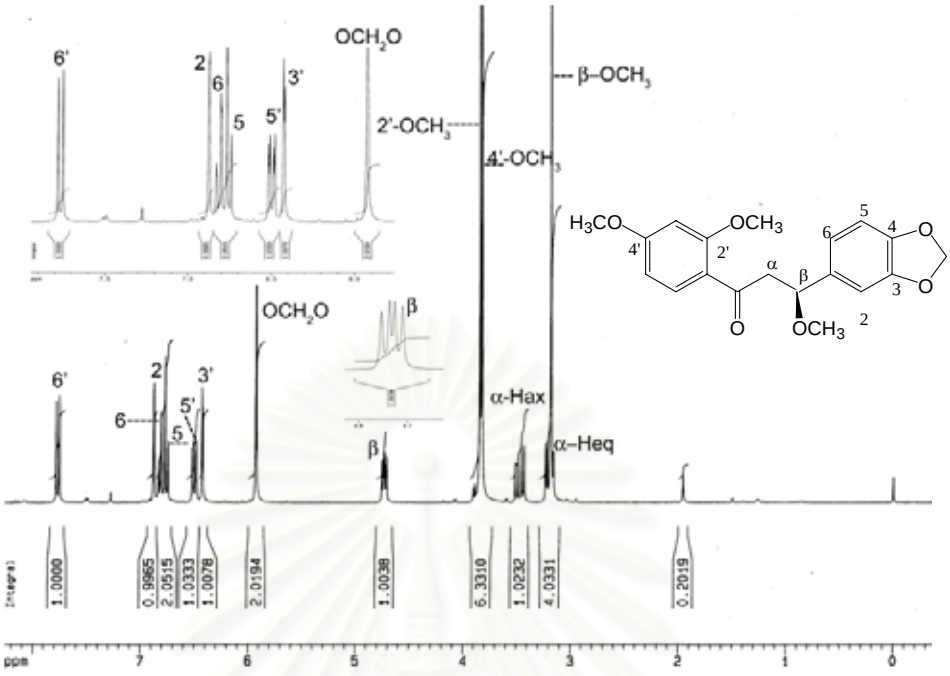


Figure 142. ^{1}H NMR (300 MHz) Spectrum of Compound MD-09 ($CDCl_3$)

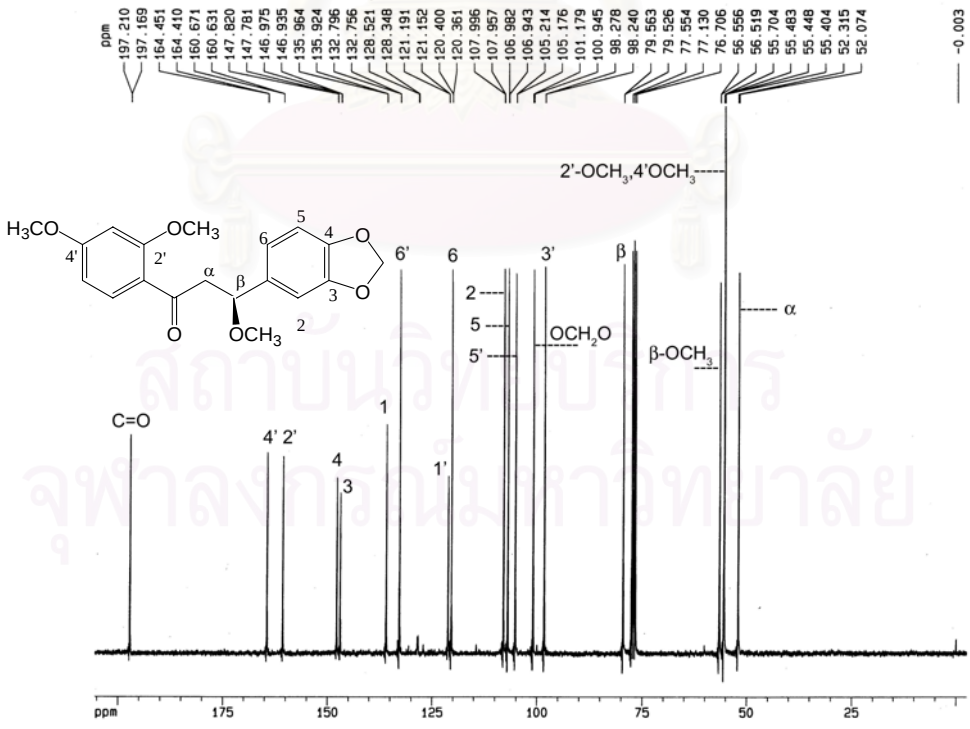


Figure 143. ^{13}C NMR (75 MHz) Spectrum of Compound MD-09 ($CDCl_3$)

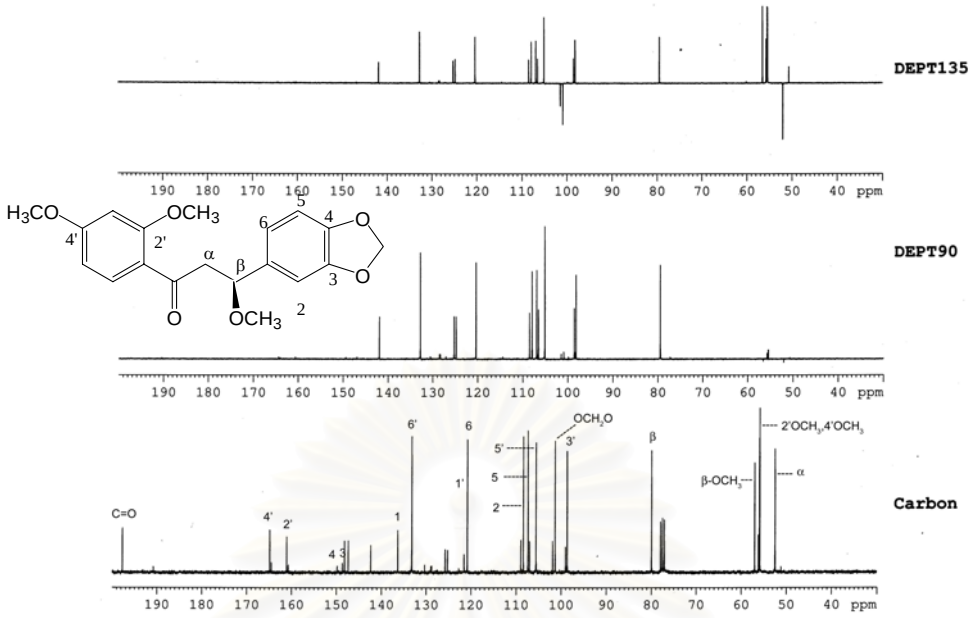


Figure 144. Dept 135, 90 and ^{13}C NMR Spectrum of Compound MD-09 ($CDCl_3$)

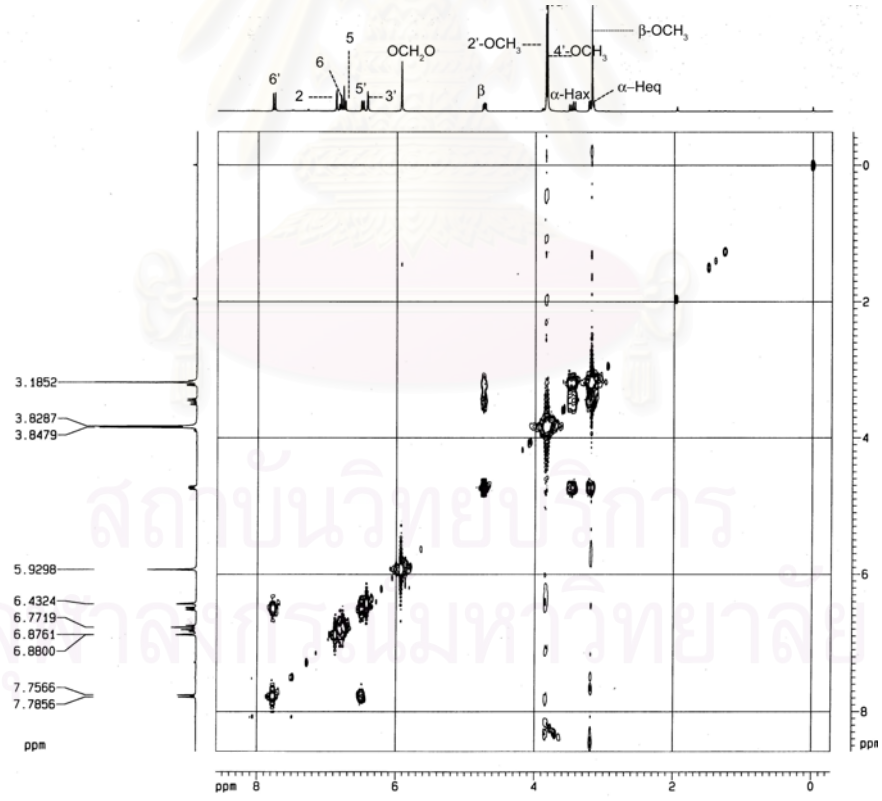


Figure 145. ^{1}H-^{1}H COSY Spectrum of Compound MD-09 ($CDCl_3$)

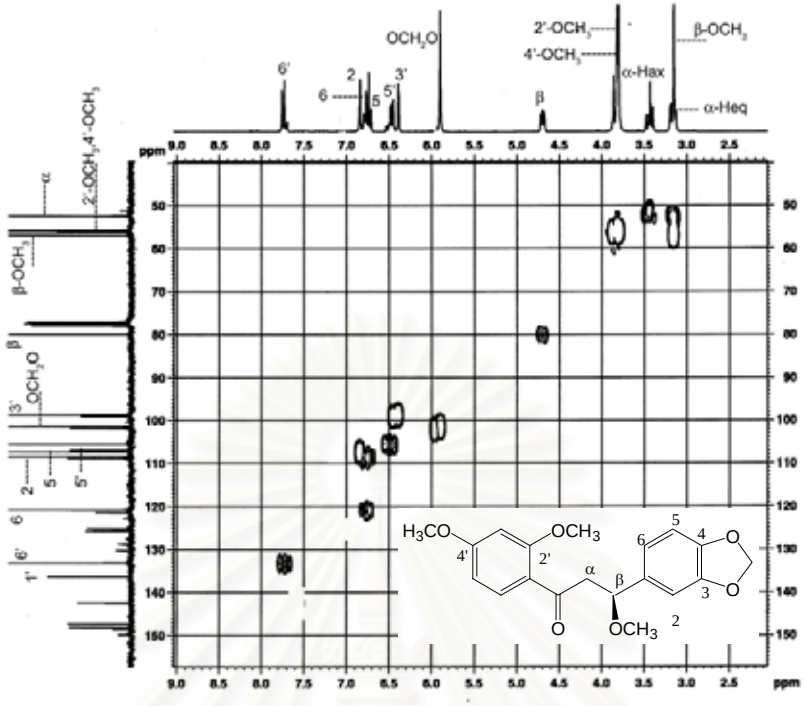


Figure 146. HMQC Spectrum of Compound MD-09 ($CDCl_3$)

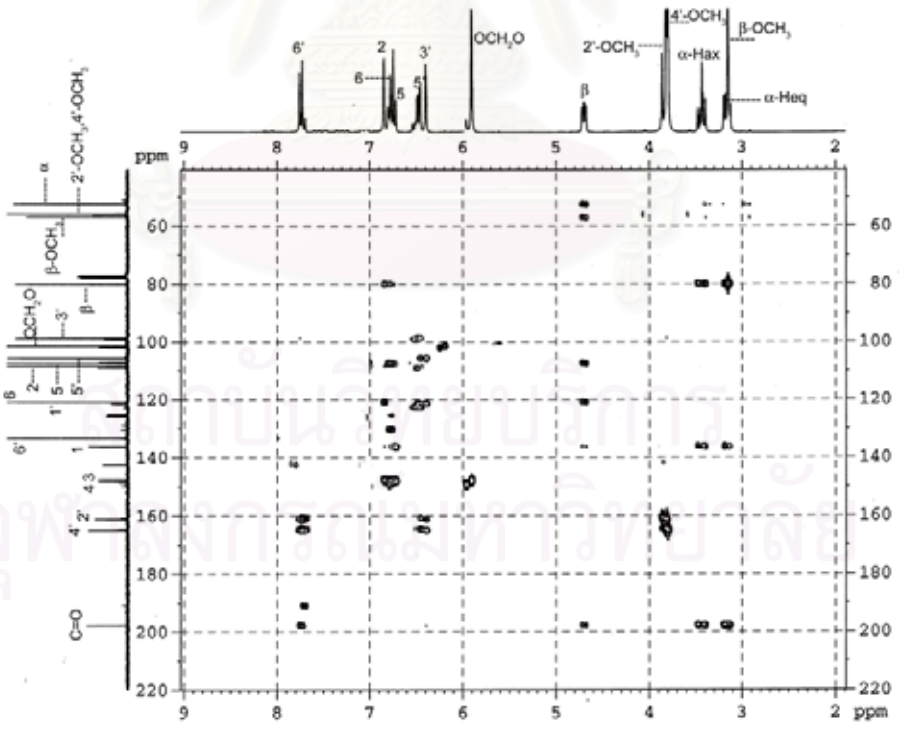


Figure 147. HMBC Spectrum of Compound MD-09 ($CDCl_3$)

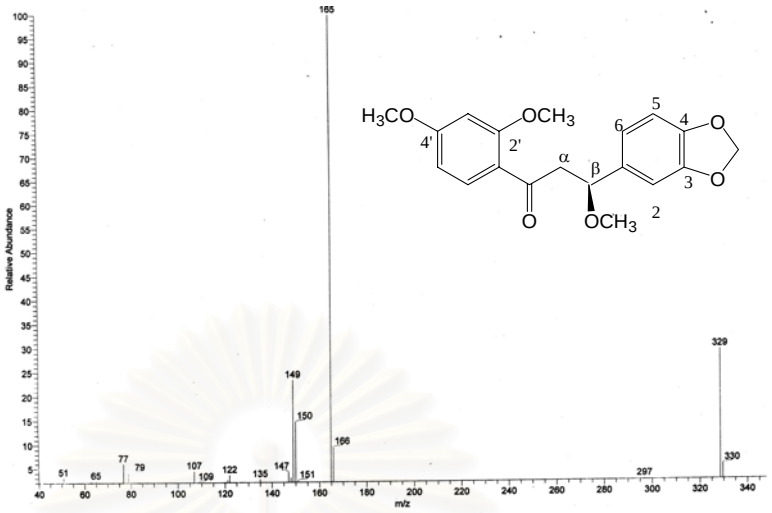


Figure 148. EI Mass Spectrum of Compound MD-09 ($CDCl_3$)

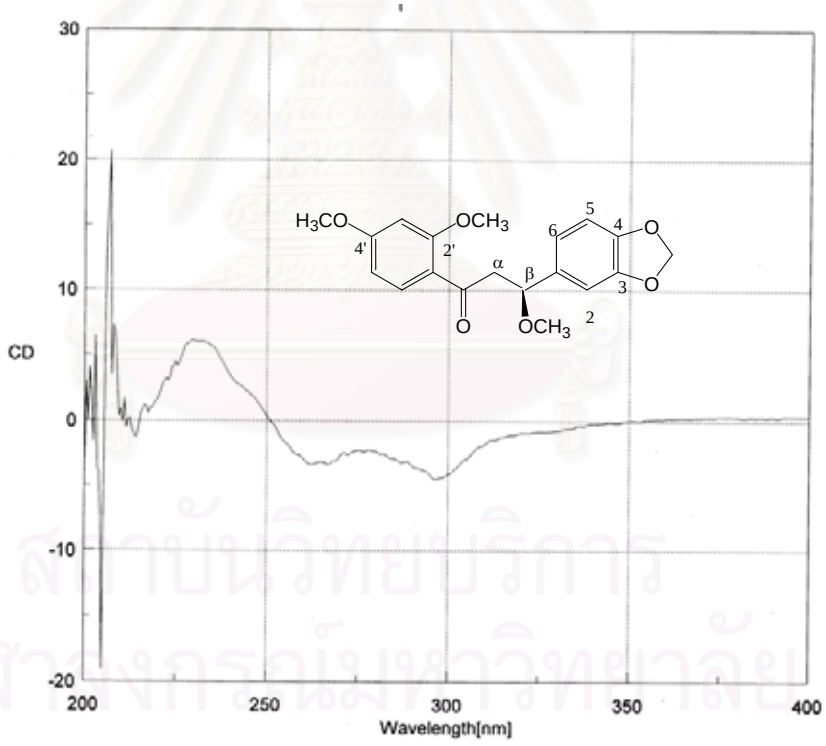


Figure 149. CD Spectrum of Compound MD-09

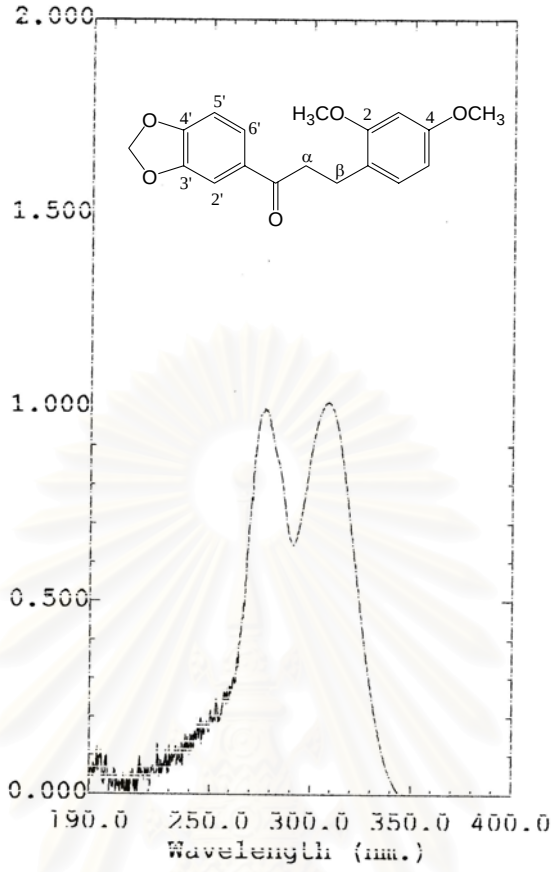


Figure 150. UV Spectrum of Compound MD-10 (methanol)

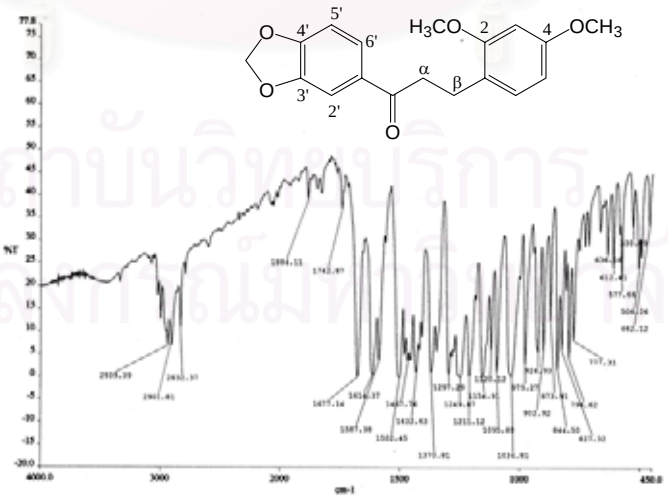


Figure 151. IR Spectrum of Compound MD-10 (KBr disc)

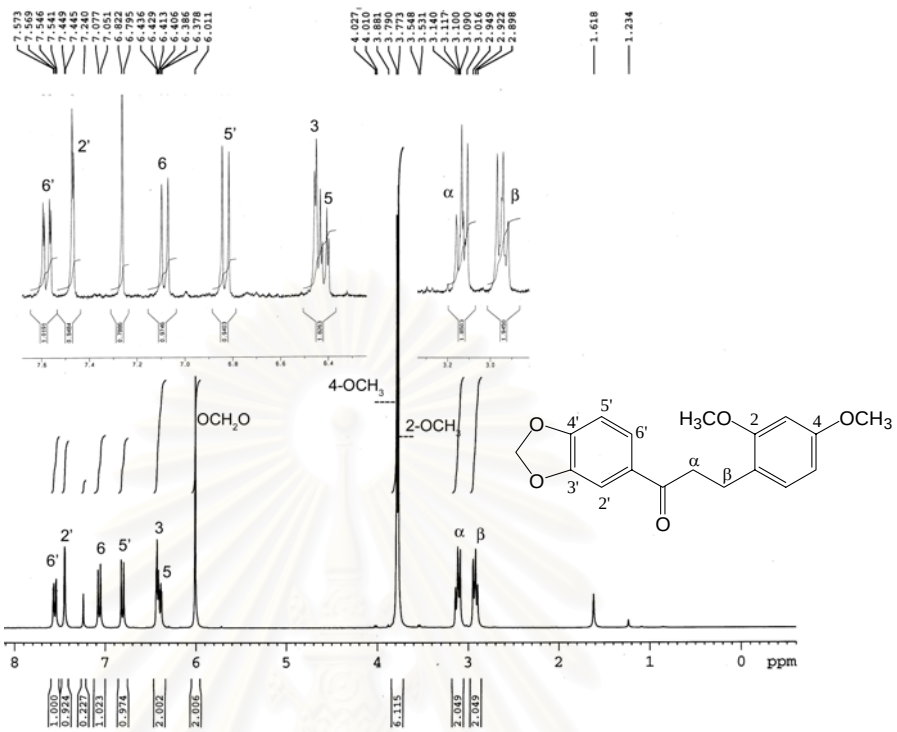


Figure 152. ^{1}H NMR (300 MHz) Spectrum of Compound MD-10 ($CDCl_3$)

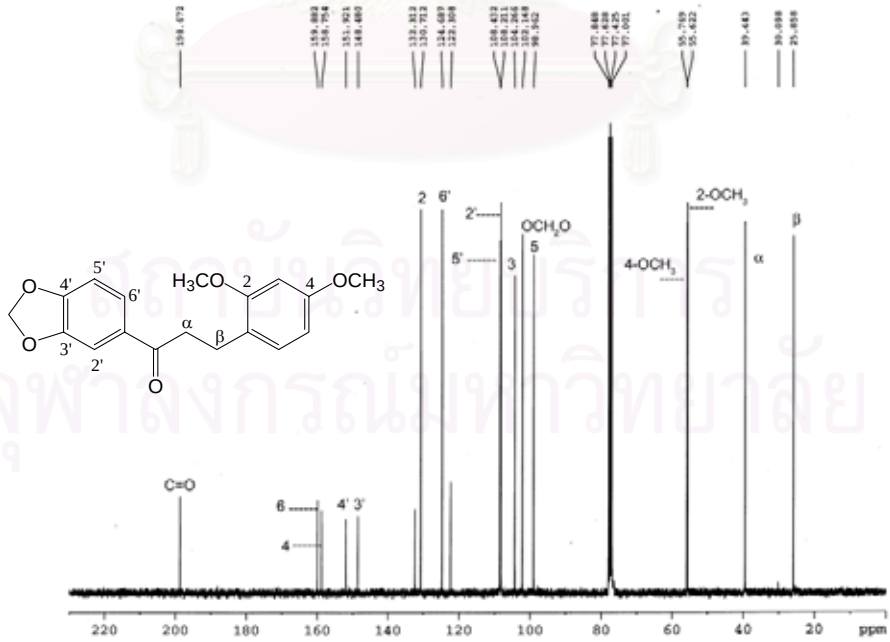


Figure 153. ^{13}C NMR (75 MHz) Spectrum of Compound MD-10 ($CDCl_3$)

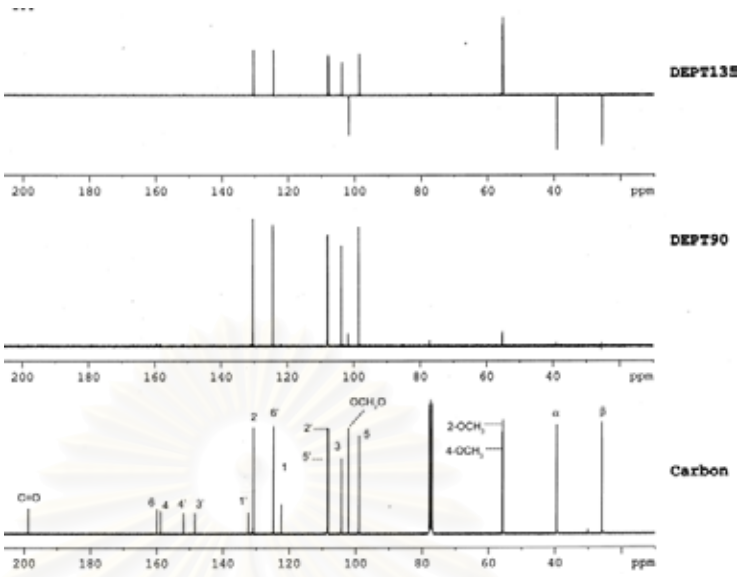


Figure 154. Dept 135, 90 and ^{13}C NMR Spectrum of Compound MD-10 ($CDCl_3$)

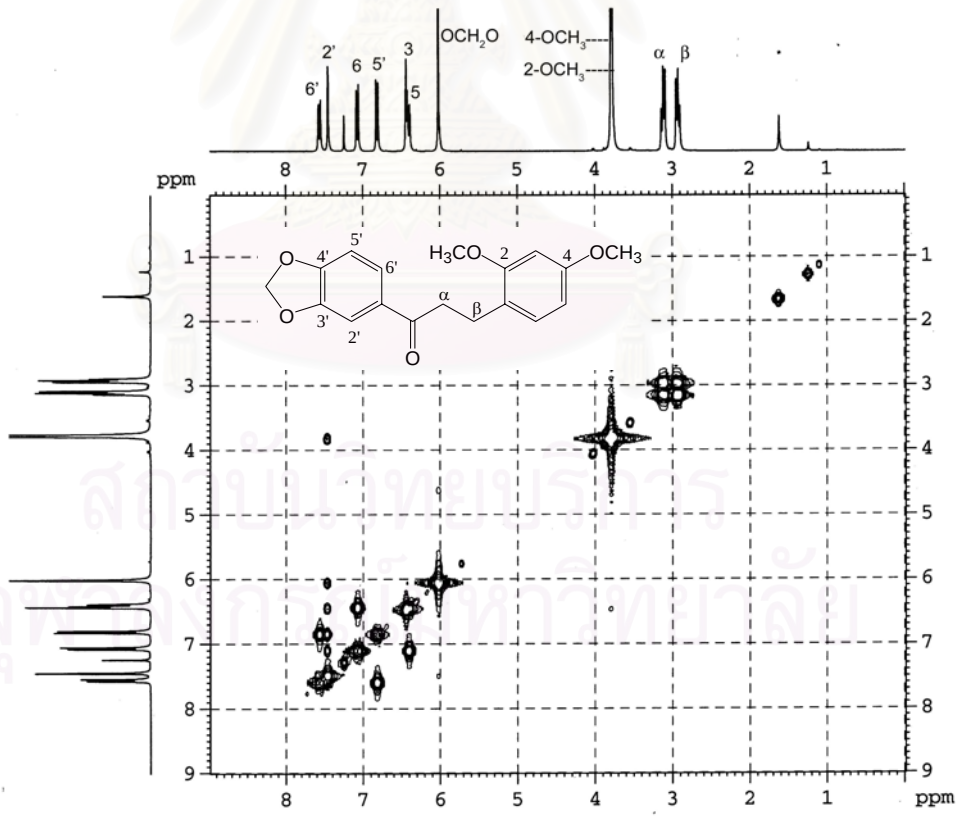


Figure 155. ^{1}H-^{1}H COSY Spectrum of Compound MD-10 ($CDCl_3$)

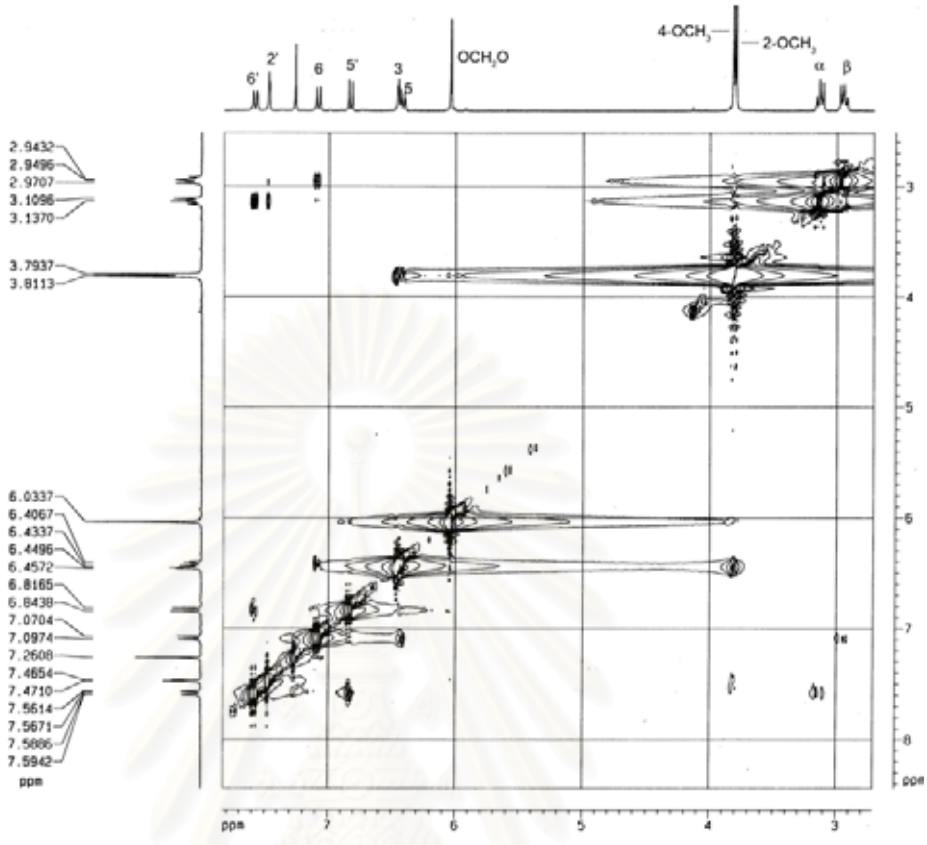


Figure 156. NOESY Spectrum of Compound MD-10 ($CDCl_3$)

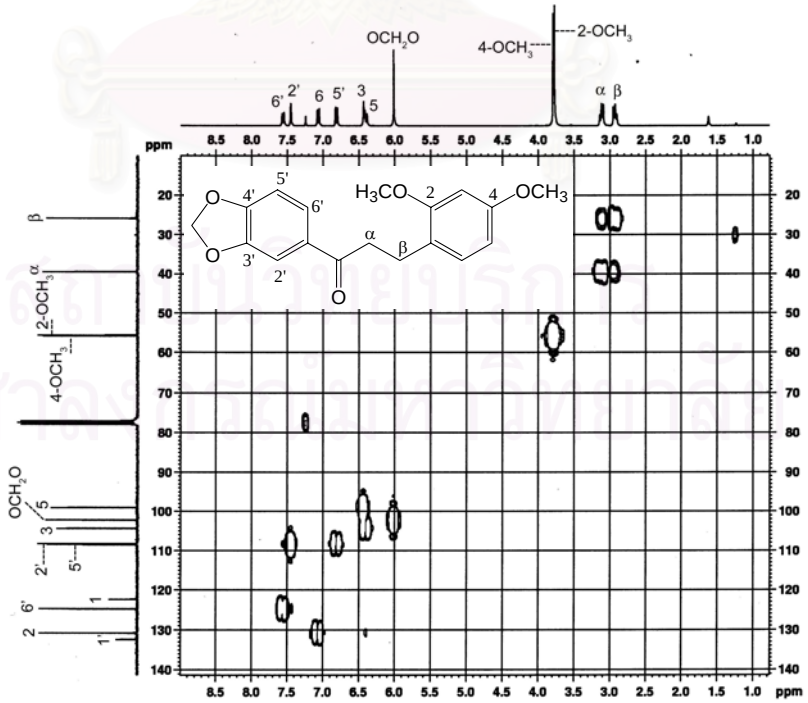


Figure 157. HMQC Spectrum of Compound MD-10 ($CDCl_3$)

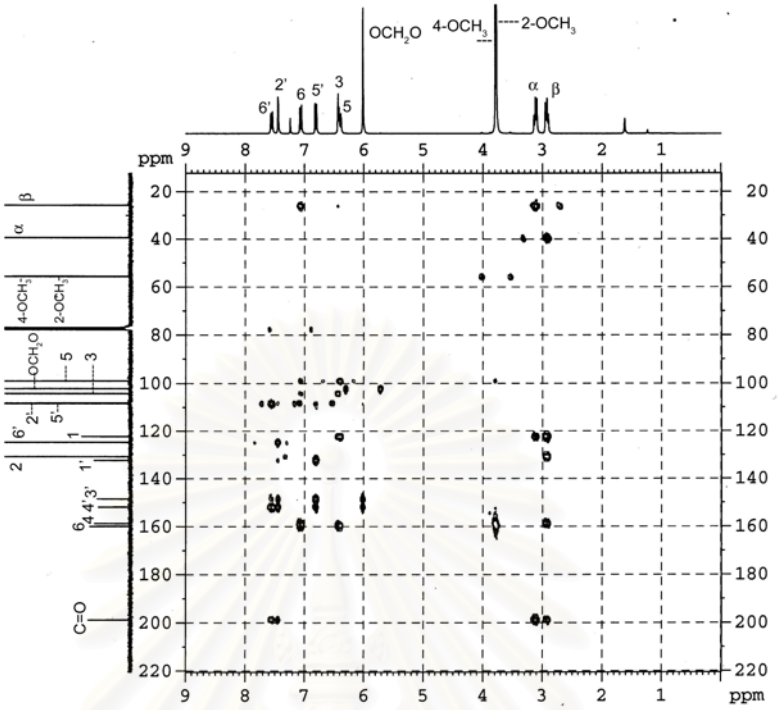


Figure 158. HMBC Spectrum of Compound MD-10 ($CDCl_3$)

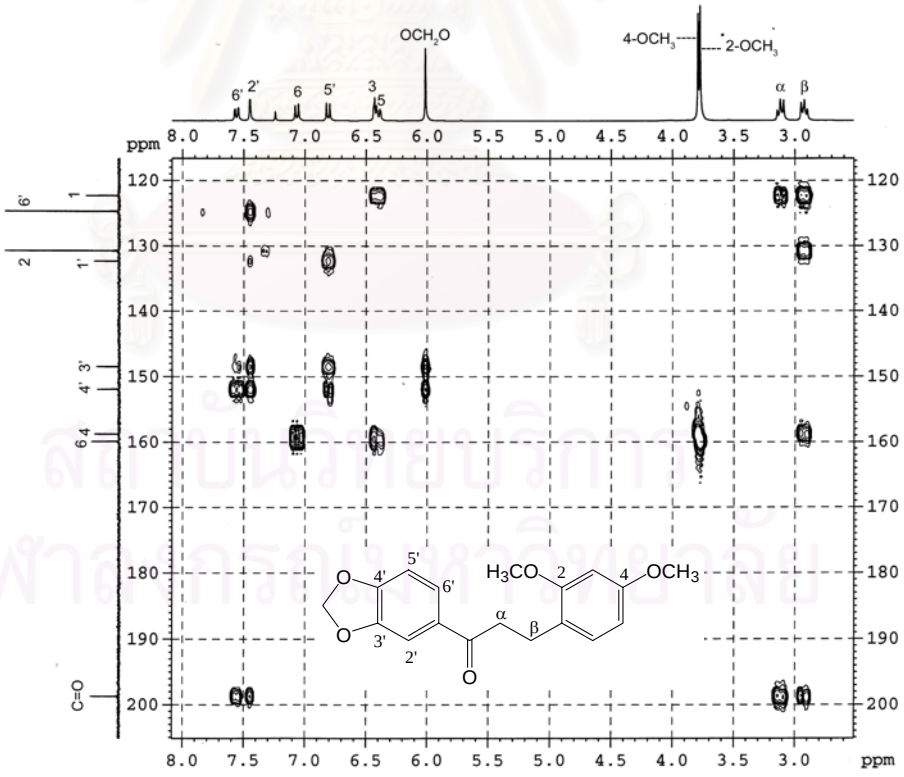


Figure 159. HMBC Spectrum of Compound MD-10 ($CDCl_3$) [δ_H 3.0-7.6 ppm, δ_C 120-200 ppm]

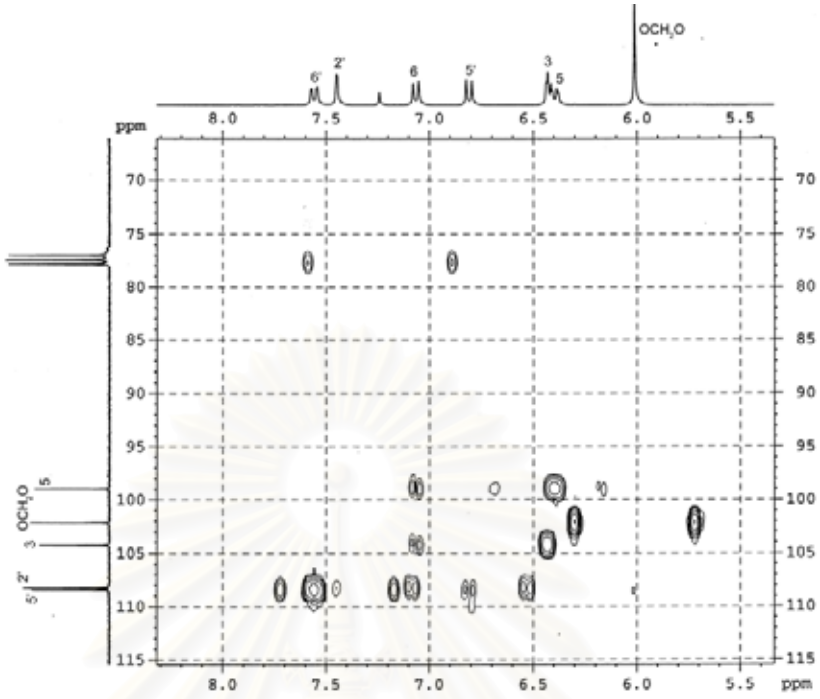


Figure 160. HMBC Spectrum of Compound MD-10 ($CDCl_3$) [δ_H 6.0-7.6 ppm, δ_C 70-110 ppm]

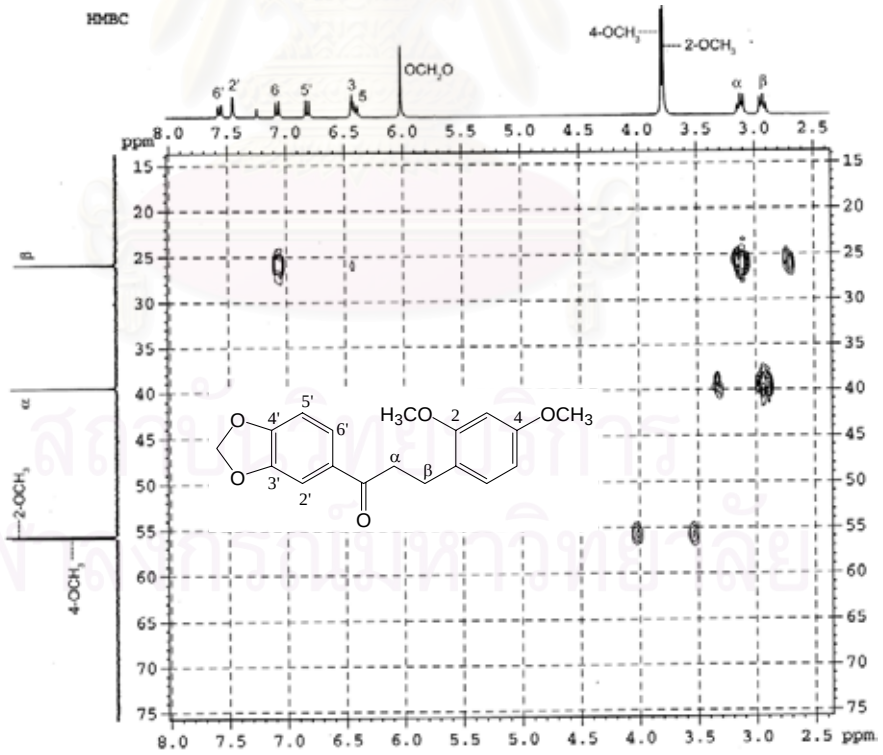


Figure 161. HMBC Spectrum of Compound MD-10 ($CDCl_3$) [δ_H 3.0-7.6 ppm, δ_C 25-55 ppm]

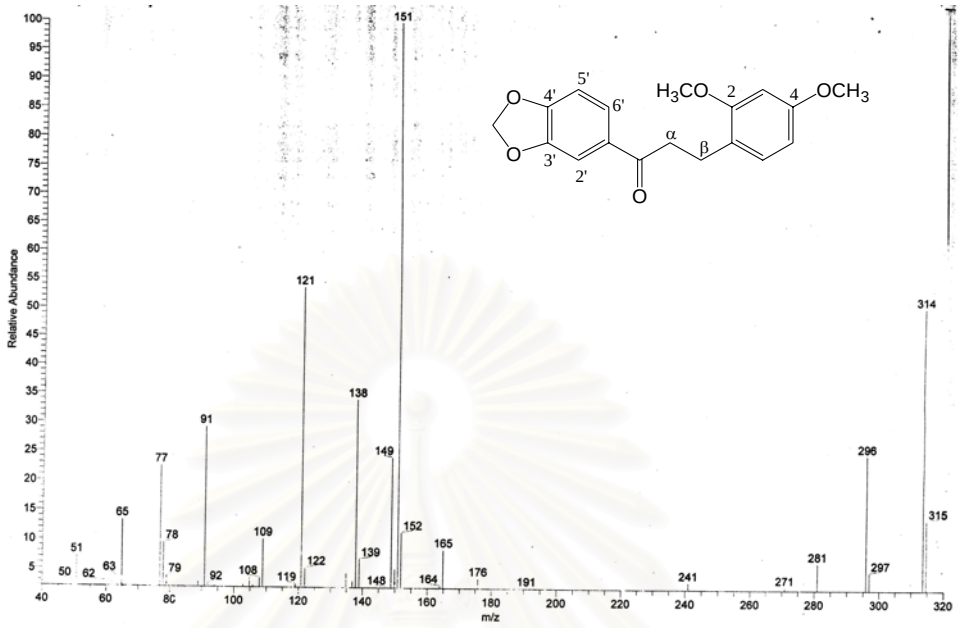


Figure 162. EI Mass Spectrum of Compound MD-10

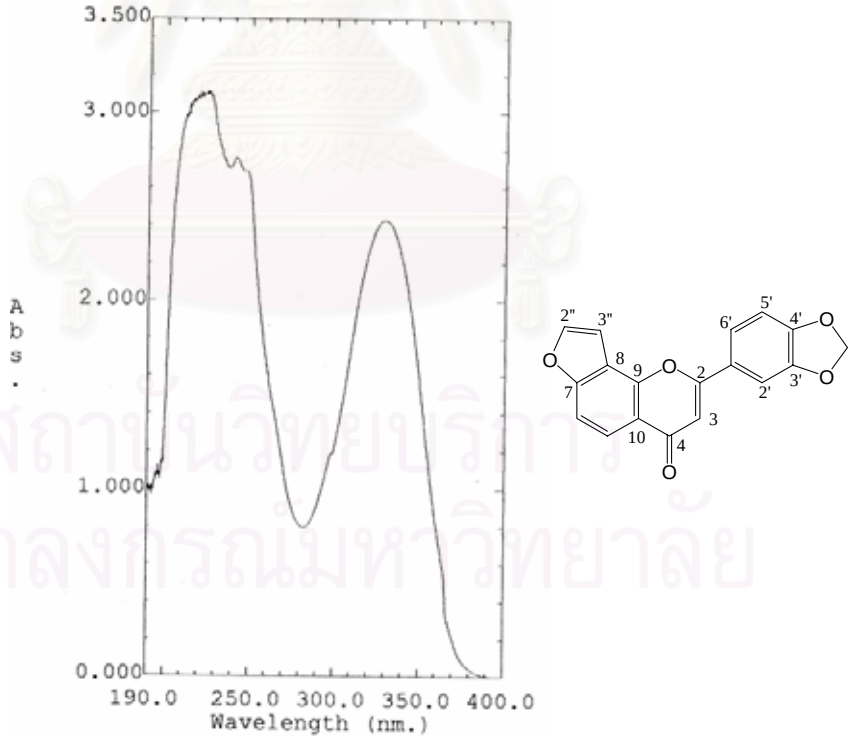


Figure 163. UV Spectrum of Compound MD-11 (methanol)

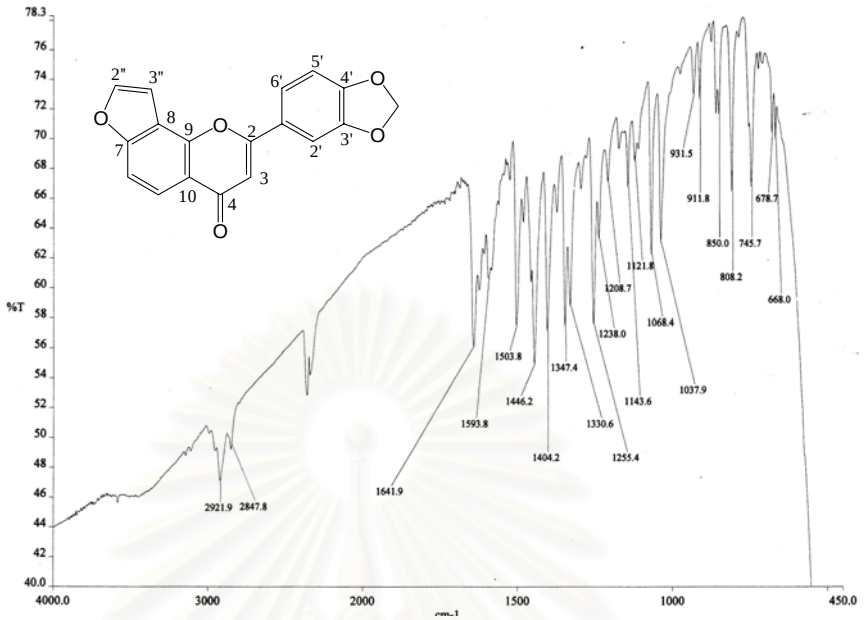


Figure 164. IR Spectrum of Compound MD-11 (KBr disc)

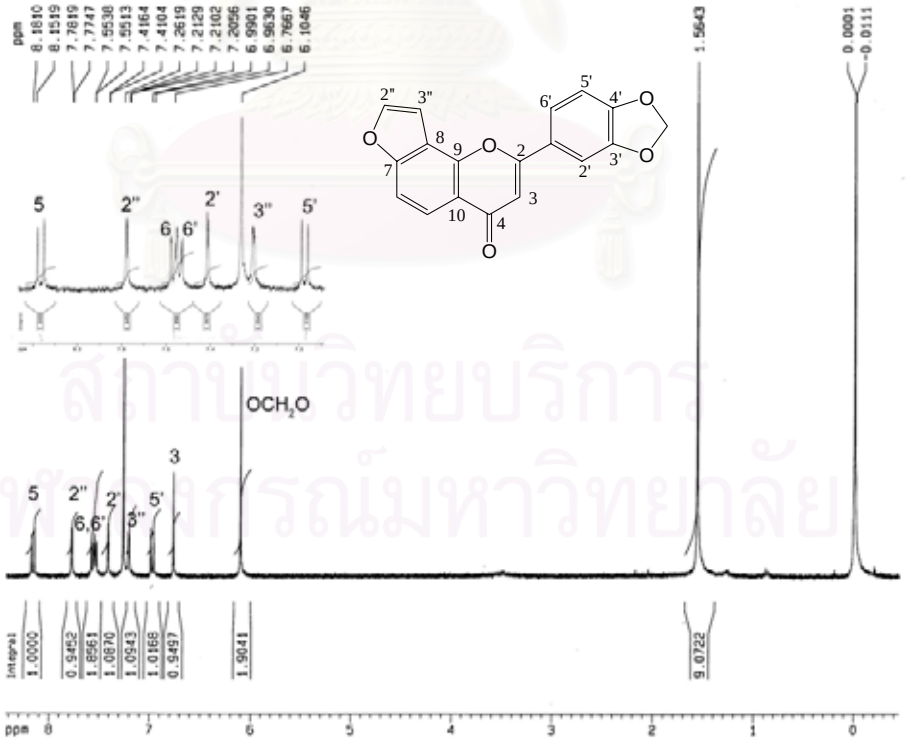


Figure 165. ^{1}H NMR (300 MHz) Spectrum of Compound MD-11 ($CDCl_3$)

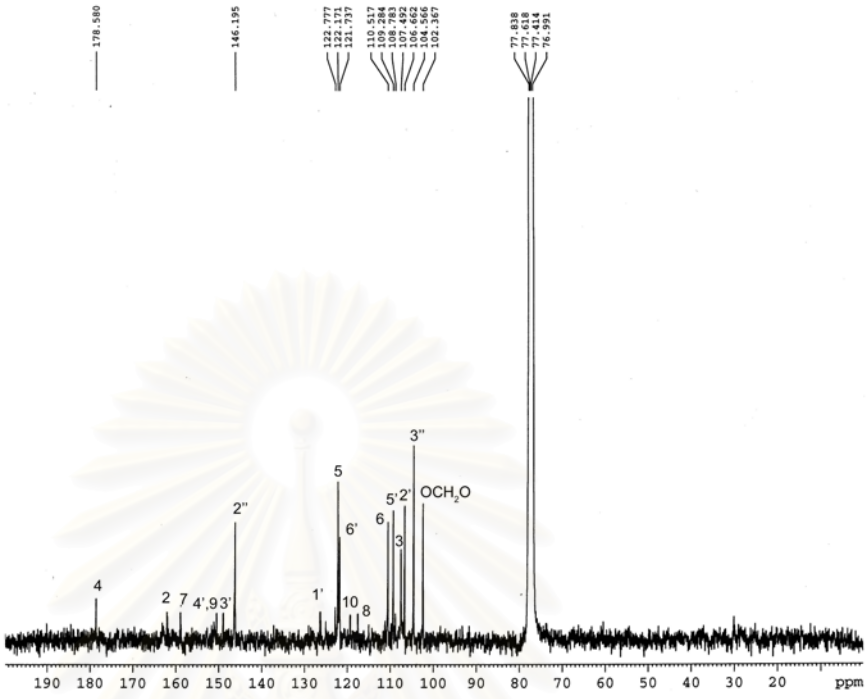


Figure 166. ^{13}C NMR (75 MHz) Spectrum of Compound MD-11 ($CDCl_3$)

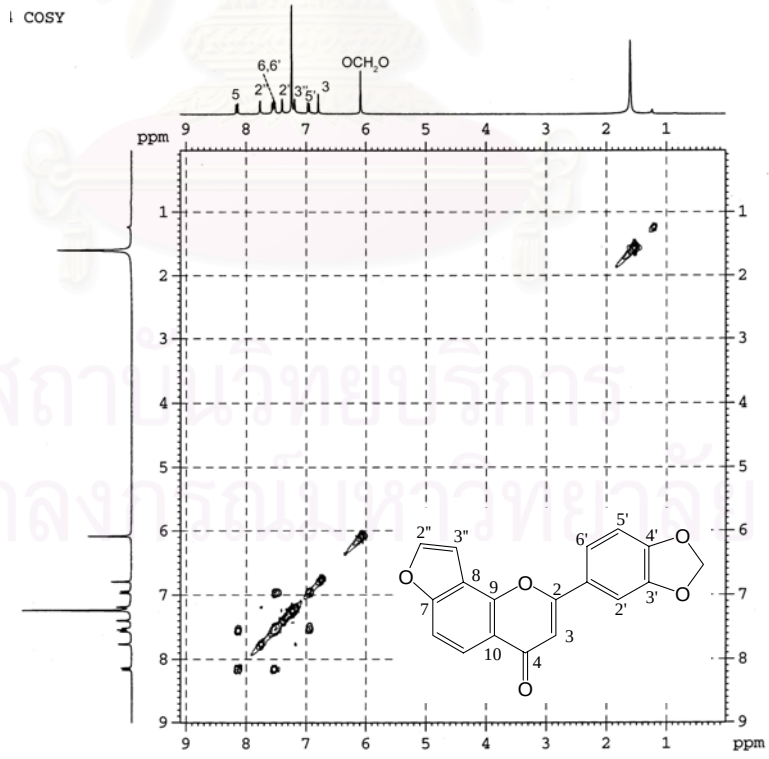


Figure 167. ^{1}H-^{1}H COSY Spectrum of Compound MD-11 ($CDCl_3$)

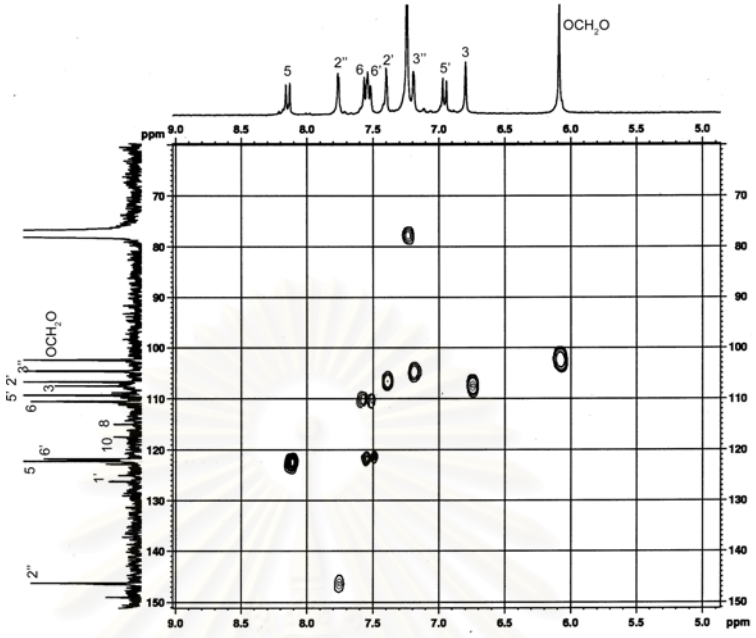


Figure 168. HMQC Spectrum of Compound MD-11 ($CDCl_3$)

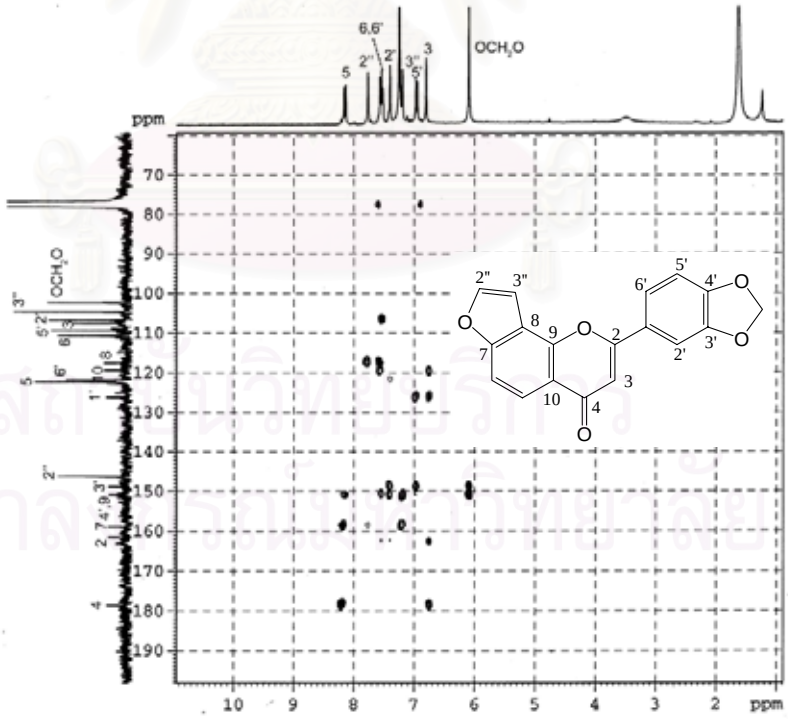


Figure 169. HMBC Spectrum of Compound MD-11 ($CDCl_3$)

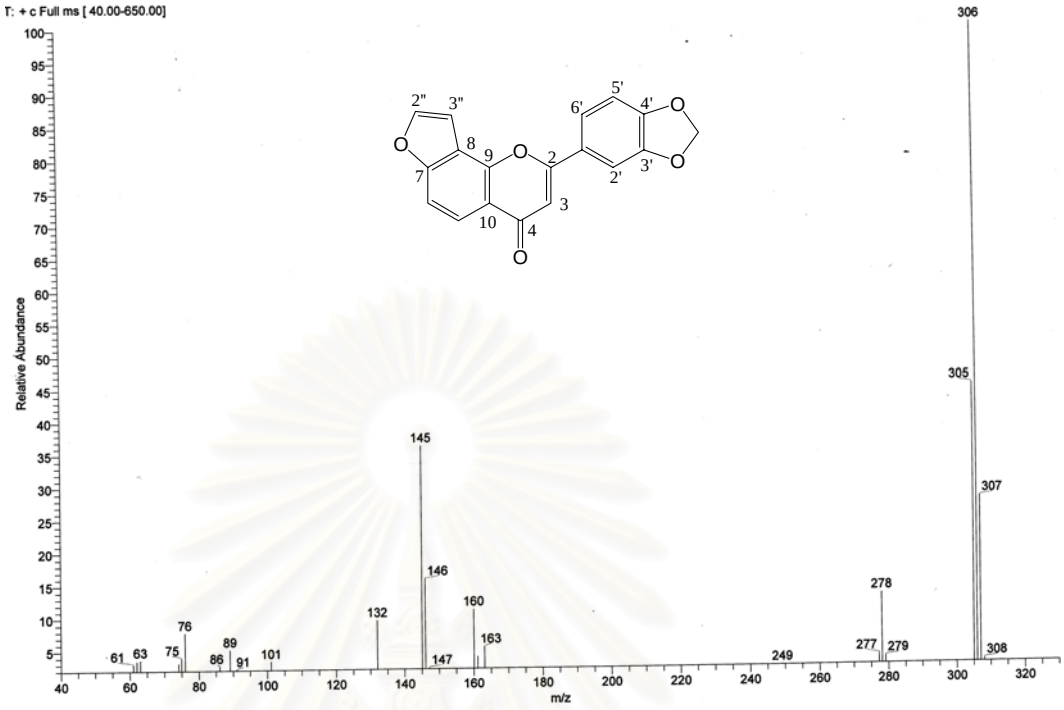


Figure 170. EI Mass Spectrum of Compound MD-11

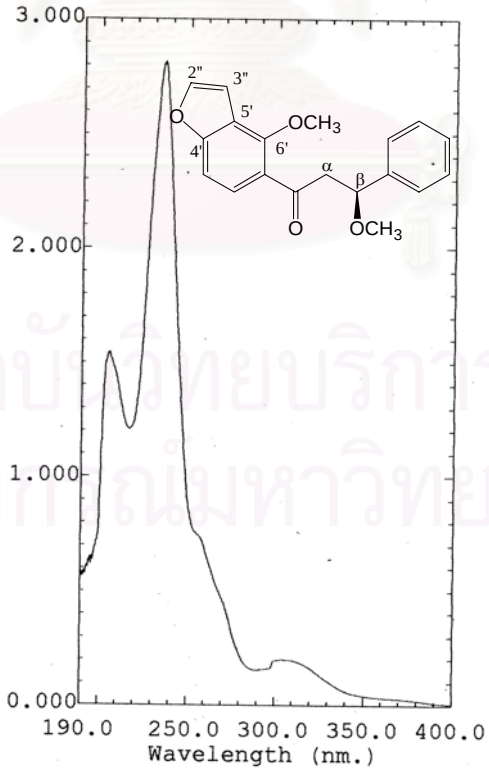


Figure 171. UV Spectrum of Compound MD-12 (methanol)

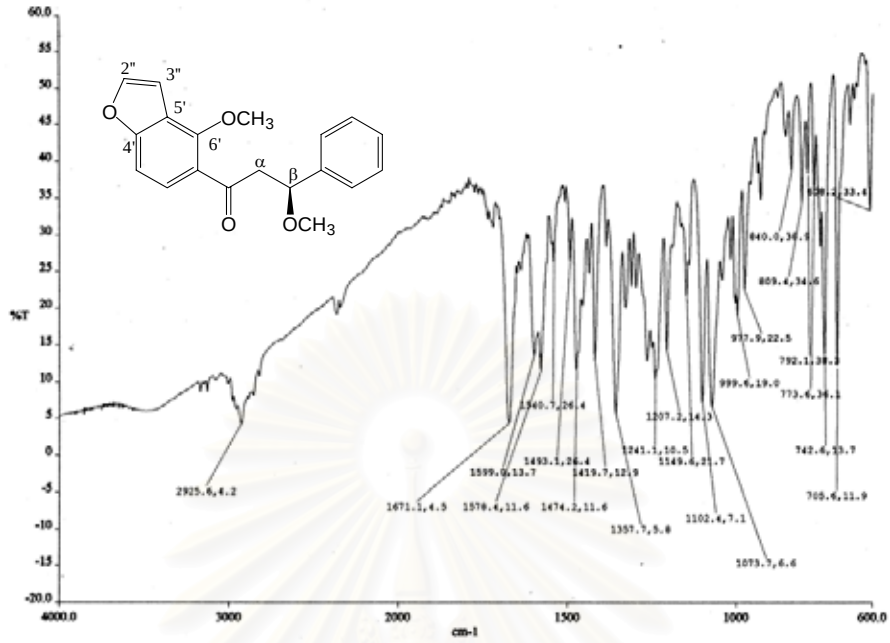


Figure 172. IR Spectrum of Compound MD-12 (KBr disc)

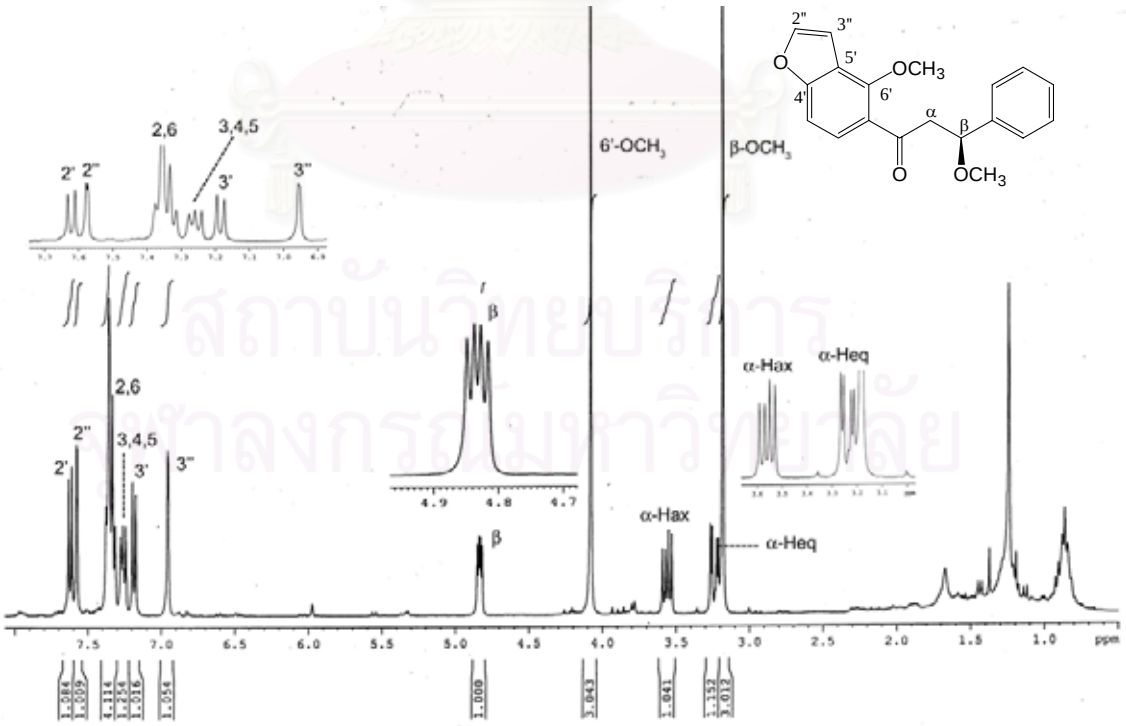


Figure 173. ^{1}H NMR (400 MHz) Spectrum of Compound MD-12 ($CDCl_3$)

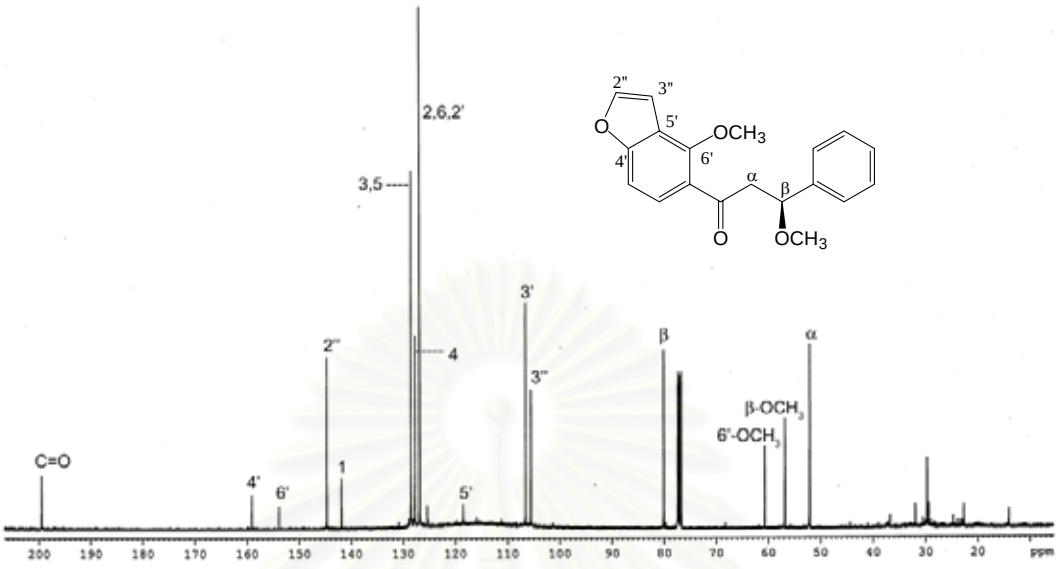


Figure 174. ^{13}C NMR (100 MHz) Spectrum of Compound MD-12 ($CDCl_3$)

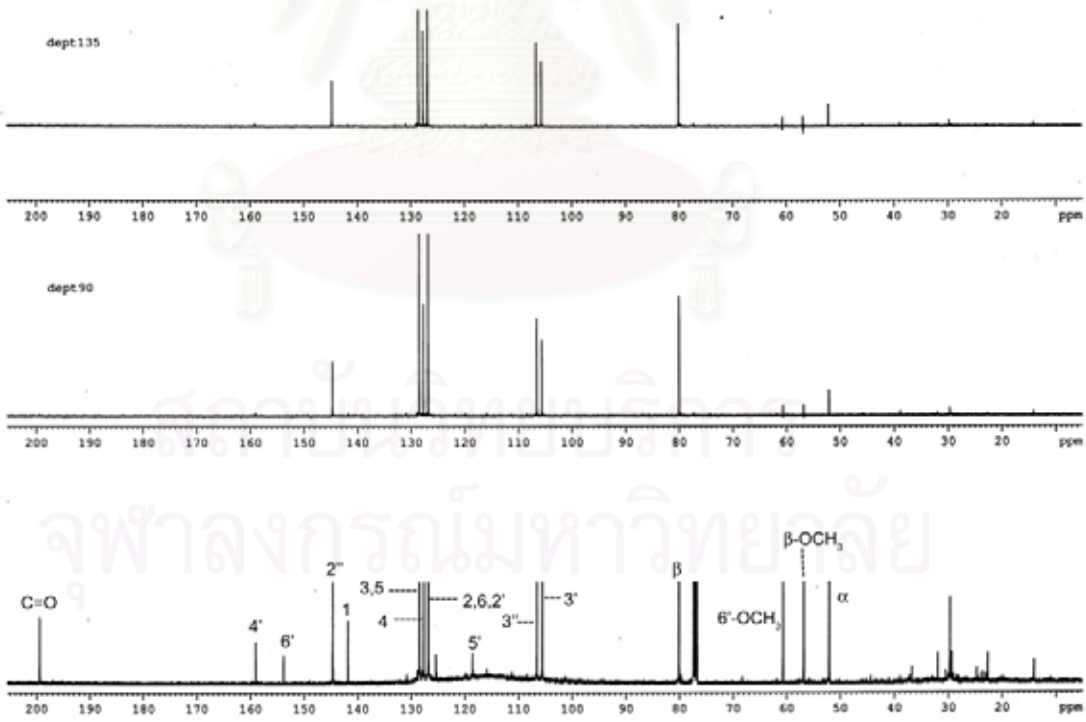


Figure 175. Dept 135, 90 and ^{13}C NMR Spectrum of Compound MD-12 ($CDCl_3$)

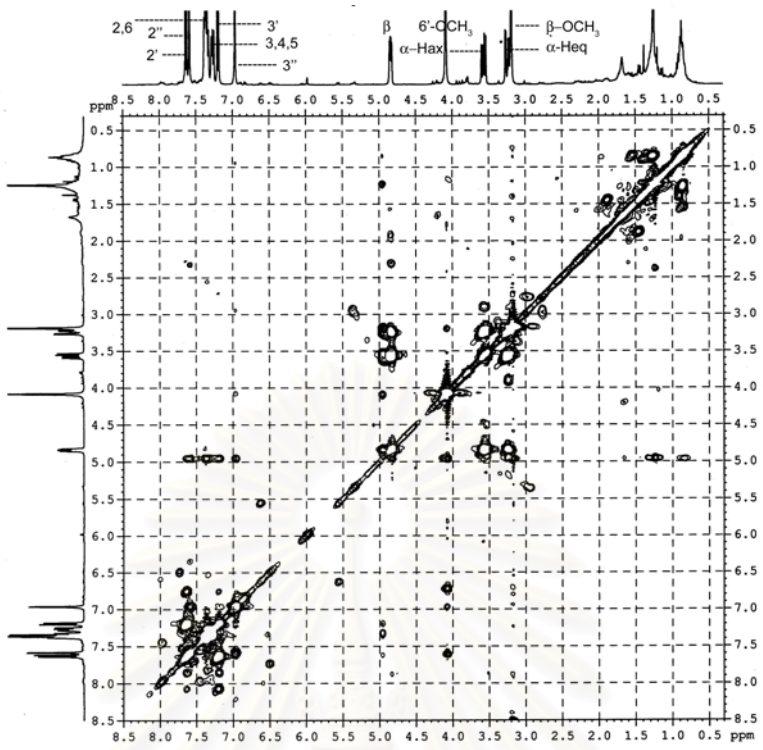


Figure 176. ^{1}H-^{1}H COSY Spectrum of Compound MD12 ($CDCl_3$)

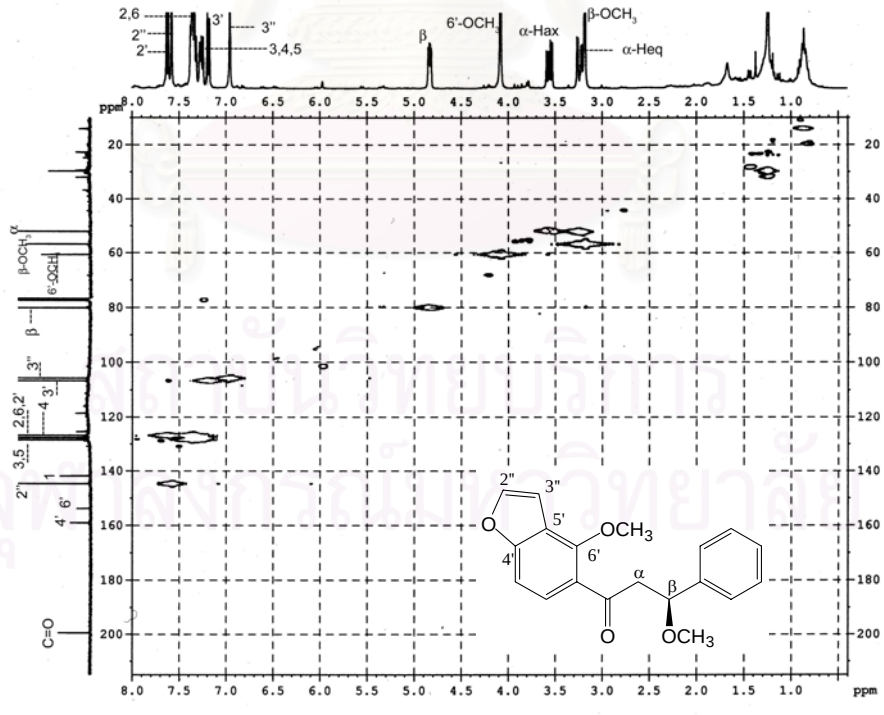


Figure 177. HMQC Spectrum of Compound MD-12 ($CDCl_3$)

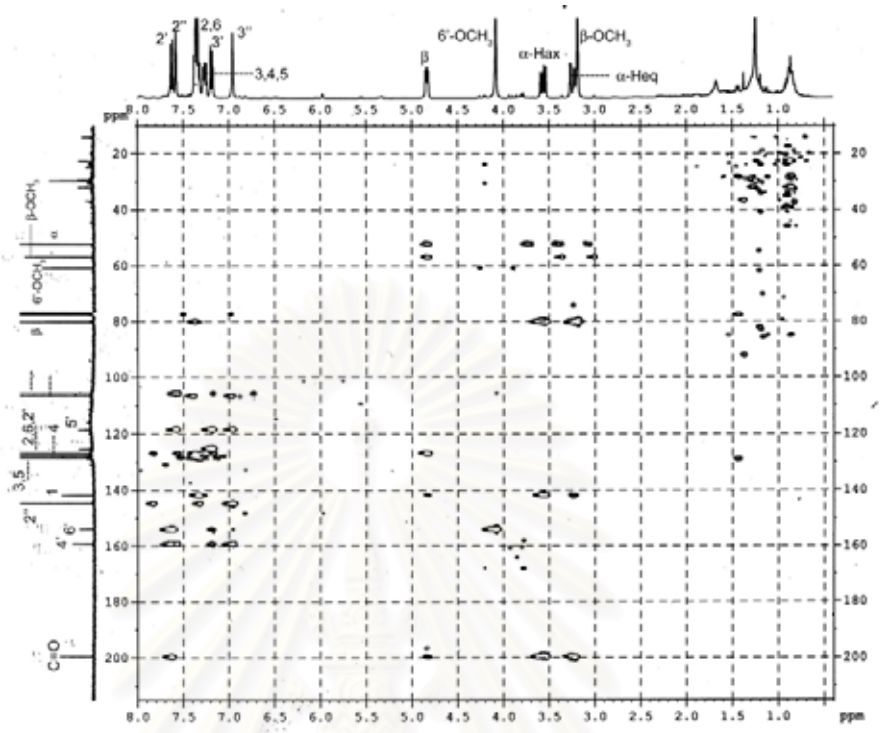


Figure 178. HMBC Spectrum of Compound MD-12 ($CDCl_3$)

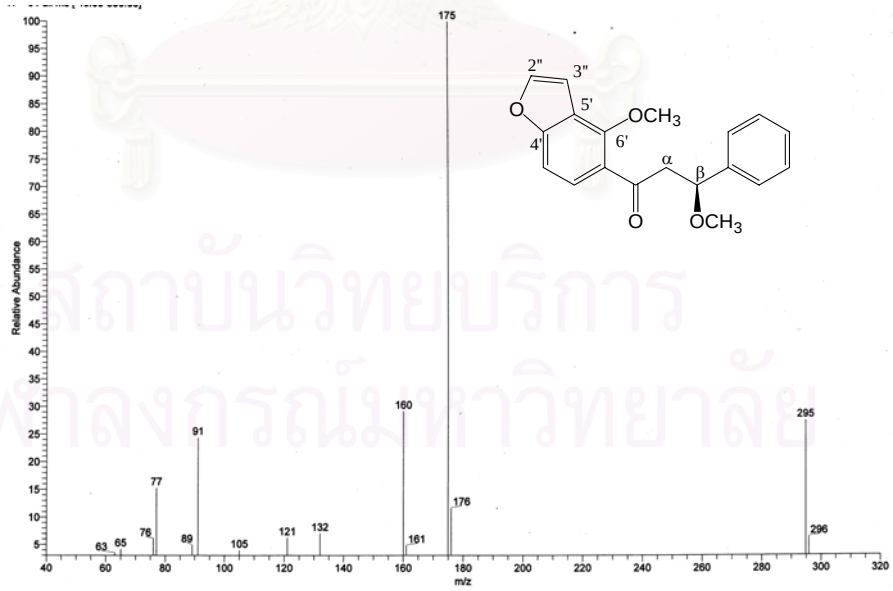


Figure 179. EI Mass Spectrum of Compound MD-12

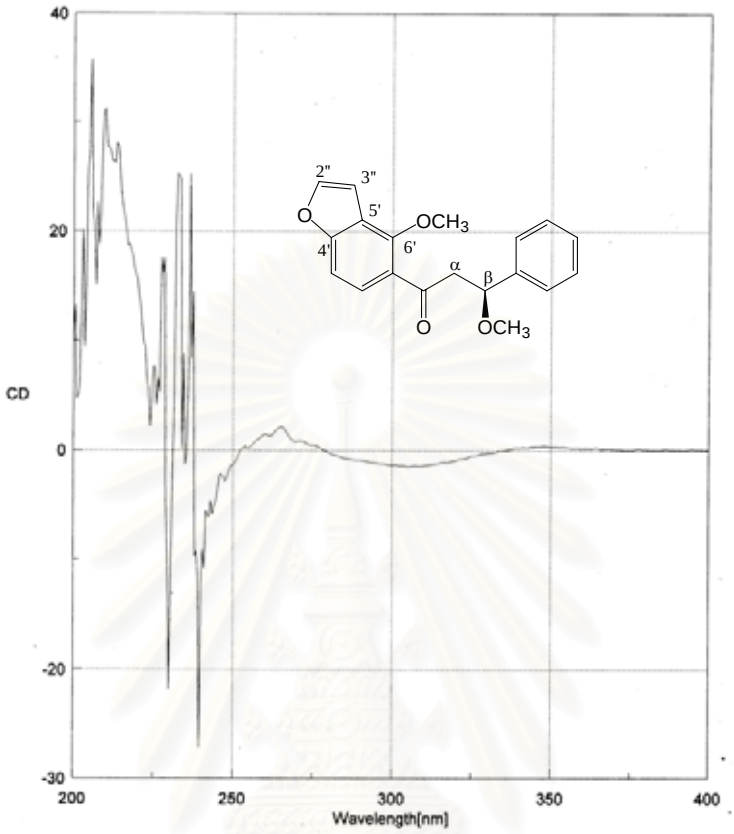


Figure 180. CD Spectrum of Compound MD-12

Table 20: Total energy of **46a**, **46b, 46c**, **46d**, and Transition state structures.

Structure	Total energy (A.U.)*
46a	-1146.85828
46b	-1146.851052
46c	-1146.837588
46d	-1146.846988
Transition state	-1146.848501

*Atomic mass unit

Table 21: The relative energies in kcal/mol of **46a-d**

Structure	Energy (kcal/mol)*
46a**	0
46b-46a	0.49
46c-46a	8.94
46d-46a	3.04
Transition state-**46a**	2.09

*Atomic mass unit energy x 627.51 = kcal/mol

** reference energy

VITA

Mr. Prasert Pattanaprateeb was born on January 22, 1967 in Buriram, Thailand. He received his Bachelor's degree of Science in Chemistry from Ramkhamhaeng University in 1990, and Master's degree of Science in Applied Chemistry from Ramkhamhaeng University in 1994. He was awards a 2001 Royal Golden Jubilee Scholarship from the Thailand Research Fund. He was a lecturer at Department of Chemistry, Faculty of Science, Rangsit University from 1995-1996.

He is a lecturer at Department of Chemistry, Faculty of Science, Srinakharinwirot University, 1996- .

Publication

1. Suksamrarn, A., Pattanaprateeb, P., 1995. Selective acetylation of 20-hydroxyecdysone. Partial synthesis of some minor ecdysteroids and analogues. *Tetrahedron* **51**, 10633-10650.
2. Suksamrarn, A., Pattanaprateeb, P., Tanachatchairatana, T., Haritakun, W., Yingyongnarongkul., Chimnoi N., 2002. Chemical modifications at 22-hydroxyl group of ecdysteroids: alternative structural requirements for high moulting activity. *Insect Biochemistry and Molecular Biology* **32**, 193-197.
3. Pattanaprateeb, P., Ruangrungsi N., Cordell, G. A., 2005. Cytotoxic Constituents from *Cratoxylum arborescens*. *Planta Medica* **71**, 181-183.

Poster Presentations

1. Pattanaprateeb, P., Ruangrugsi, N., and Cordell, G. A., Chemical Constituents of *Millettia decipiens* and *Cratoxylum arborescens*. NRCT-JSPS CORE UNIVERSITY SYSTEM: The sixth NRCT-JSPS Joint Seminar in Pharmaceutical Sciences; Drug Development Through Biopharmaceutical Sciences. December 2-4, 2003, Bangkok, Thailand.

2. Pattanaprateeb, P., Ruangrugsi, N., and Cordell, G. A., Chemical Constituents of *Millettia decipiens.* Asian Symposium on Medicinal Plants, Spices and Other Natural Products XI. October 26-30, 2003, Kunming, China.