สารออกฤทธิ์ทางชีวภาพจากเปล้าเงิน หัสคืน และกระเจาะ

นางสาวจิราภรณ์ ทองตัน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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BIOACTIVE COMPOUNDS
FROM
CROTON KONGENSIS, *CROTON BIRMANICUS* AND *MILLETTIA KANGENSIS*

Miss Jiraporn Thongtan

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Pharmaceutical Chemistry and Natural Products
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จากการศึกษาสารออกฤทธิ์ทางชีวภาพของเปล้าเงิน หัสคืน และ กระเจาะ สามารถแยกสารในกลุ่มไดเทอร์ปีนอยด์ 4 ชนิด ฟลาโวนอยด์ 7 ชนิด และอัลคาลอยด์ 1 ชนิด การพิสูจน์โครงสร้างของสารทั้งหมดที่แยกได้อาศัยการวิเคราะห์เชิงสเปคตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลกับสารที่ทราบโครงสร้างแล้ว พบว่าสารที่แยกได้จากเปล้าเงินประกอบด้วยสารใหม่ที่มีโครงสร้างในกลุ่ม 8,9-secokaurane 2 ชนิดคือ คือ *ent*-8,9-*seco*-7α,11β-diacetoxy kaura-8(14),16-dien-9,15-dione, *ent*-8,9-*seco*-8,14-epoxy-7α-hydroxy-11β-acetoxy-16-kauren-9,15-dione, สารที่เคยมีรายงานแล้ว 1 ชนิดคือ *ent*-8,9-*seco*-7α-hydroxy-11-acetoxy kaura-8(14),16-dien-9,15-dione และสารที่เคยมีรายงานแล้วในกลุ่ม kaurane 1 ชนิด คือ *ent*-7β-hydroxy-15-oxokaur-16-en-18-yl acetate สารที่แยกได้จากหัสคืนประกอบ ด้วยสารที่เคยมีรายงานแล้วในกลุ่ม glutarimide alkaloid 1 ชนิดคือ julocrotine สารที่แยกได้จากกระเจาะประกอบด้วยสารใหม่ในกลุ่ม furanoflavonoids 2 ชนิด คือ 3-methoxy-6-hydroxy-[4″,5″:8,7]-furanoflavone, 2,5,8-trimethoxy-[4″,5″:6,7]-furanoflavanone, pyranoflavonoid 1 ชนิด คือ 3,6-dimethoxy-2″-dimethyl-[5″,6″:8,7]-pyranoflavone และ coumestan 1 ชนิด คือ 4′-hydroxy,5,6,7-trimethoxycoumestan สารที่เคยมีรายงานแล้ว 2 ชนิดคือ karanjin และ 3,6-dimethoxy-[4″,5″:8,7]-furanoflavone และสารที่พบครั้งแรกจากธรรมชาติอีก 1 ชนิด คือ 5,8-dimethoxy-[4″,5″:7,6]-furanoflavone สารที่แยกได้ทั้งหมด 12 ชนิดถูกนำไปทดสอบฤทธิ์ทางชีวภาพได้แก่ฤทธิ์ต้านวัณโรค ฤทธิ์ต้านมาลาเรีย และฤทธิ์ความเป็นพิษต่อเซลล์ พบว่า *ent*-8,9-*seco*-7α,11β-diacetoxykaura-8(14),16-dien-9,15-dione, *ent*-8,9-*seco*-8,14-epoxy-7α-hydroxy-11β-acetoxy-16-kauren-9,15-dione, *ent*-8,9-*seco*-7α-hydroxy-11-acetoxykaura -8(14),16-dien-9,15-dione และ *ent*-7β-hydroxy-15-oxokaur-16-en-18-yl acetate จากเปล้าเงินมีฤทธิ์ต้านวัณโรค ฤทธิ์ต้านมาลาเรีย และฤทธิ์ความเป็นพิษต่อเซลล์ ขณะที่ julocrotine จากหัสคืนและ 5,8-dimethoxy-[4″,5″:7,6]-furanoflavone จากกระเจาะมีฤทธิ์ต้านวัณโรค

ภาควิชา เภสัชเวท	ลายมือชื่อนิสิต……..
สาขาวิชา เภสัชเคมีและผลิตภัณฑ์ธรรมชาติ	ลายมือชื่ออาจารย์ที่ปรึกษา…….....
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JIRAPORN THONGTAN: BIOACTIVE COMPOUNDS FROM *CROTON KONGENSIS, CROTON BIRMANICUS* AND *MILLETTIA KANGENSIS* THESIS ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., THESIS CO-ADVISOR: MR. PRASAT KITTAKOOP Ph.D., 161 pp. ISBN: 974-17-5114-1

Chemical investigation of *Croton kongensis*, *Croton birmanicus* and *Millettia kangensis*, led to the isolation of four diterpenoids, seven flavonoids and an alkaloid. The structure determination of these compounds was accomplished by spectroscopic analyses (UV, IR, MS and NMR) and by comparison with previously reported data of known compounds. *C. kongensis* provided two new 8,9-secokauranes identified as *ent*-8,9-*seco*-7α,11β-diacetoxykaura-8(14),16-dien-9,15-dione and *ent*-8,9-*seco*-8,14-epoxy-7α-hydroxy-11β-acetoxy-16-kauren-9,15-dione, and also gave two known diterpenes, *ent*-8,9-seco-7α-hydroxy-11-acetoxykaura-8(14),16-dien-9,15-dione and *ent*-7β-hydroxy-15-oxokaur-16-en-18-yl acetate. *C. birmanicus* was isolated to yield a known glutarimide alkaloid, julocrotine. Isolation of a crude extract of *M. kangensis* afforded two new furanoflavonoids identified as 3-methoxy-6-hydroxy-[4″,5″:8,7]-furanoflavone and 2,5,8-trimethoxy-[4″,5″:6,7]-furanoflava none, a new pyranoflavonoid, 3,6-dimethoxy-2″-dimethyl-[5″,6″:8,7]-pyranoflavone, a new coumestan (4′-hydroxy,5,6,7-trimethoxycoumestan), a new natural product (5,8-dimethoxy-[4″,5″:7,6]-furanoflavone), together with two known compounds, karanjin and 3,6-dimethoxy-[4″,5″:8,7]-furanoflavone. The isolated compounds were evaluated for their biological activities, including antimycobacterial, antimalarial, and cytotoxic activities. *ent*-8,9-*Seco*-7α,11β-diacetoxykaura-8(14),16-dien-9,15-dione, *ent*-8,9-*seco*-8,14-epoxy-7α-hydroxy-11β-acetoxy-16-kauren-9,15-dione, *ent*-8,9-seco-7α-hydroxy-11-acetoxykaura-8(14),16-dien-9,15-dione and *ent*-7β-hydroxy-15-oxokaur-16-en-18-yl from *C. kongensis* exhibited antimycobacterial, antimalarial, and cytotoxicity activities, while julocrotine from *C. birmanicus* and 5,8-dimethoxy-[4″,5″:7,6]-furanoflavone from *M. kangensis* showed mild antimycobacterial activity.

Field of Study Pharmaceutical Chemistry and Natuaral Products

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Student's signature……...

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LIST OF ABBREVIATIONS AND SYMBOLS

α	=	Alpha
$[\alpha]^{30}_{D}$	=	Specific rotation at 30˚ and sodium D line (589 nm)
β	=	Beta
br d	=	Broad doublet (for NMR spectra)
br t	=	Broad triplet (for NMR spectra)
br s	=	Broad singlet (for NMR spectra)
calcd	=	Calculated
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chloroform
CH_2Cl_2	=	Dichloromethane
cm	=	Centimeter
cm^{-1}	=	Reciprocal centimeter (unit of wave number)
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
d	=	Doublet (for NMR spectra)
dd	=	Doublet of doublets (for NMR spectra)
ddd	=	Doublet of doublet of doublets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
DMSO-*d6*	=	Deuterated dimethyl sulfoxide
δ	=	Chemical shift
ESIMS	=	Electrospray Ionization Mass Spectrometry
ESITOFMS	=	Electrospray Ionization Time of Flight Mass Spectrometry
EtOAc	=	Ethyl acetate
g	=	Gram
^{1}H NMR	=	Proton Nuclear Magnetic Resonance

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

HMBC	=	^{1}H-Detected Heteronuclear Multiple Bond Coherence
HMQC	=	^{1}H-Detected Heteronuclear Multiple Quantum Coherence
HPLC	=	High Perfornance Liquid Chromatography
HRESIMS	=	High Resolution Electrospray Ionization Mass Spectrometry
Hz	=	Hertz
IR	=	Infrared Spectrum
J	=	Coupling constant
Kg	=	Kilogram
L	=	Liter
λ_{max}	=	Wavelength at maximal absorption
ε	=	Molar absorptivity
m	=	Multiplet (for NMR spectra)
MeOH	=	Methanol
mg	=	Milligram
$[M+H]^{+}$	=	Protonated molecular ion
MHz	=	Megahertz
mL	=	Milliliter
MW	=	Molecular weight
m/z	=	Mass to charge ratio
MS	=	Mass Spectrometry
NMR	=	Nuclear Magnetic Resonance Spectroscopy
NOESY	=	Nuclear Overhauser Effect Spectroscopy
o	=	Ortho

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

p	=	Para
ν_{max}	=	Wave number at maximal absorption
s	=	Singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)
TLC	=	Thin Layer Chromatography
UV	=	Ultraviolet
UV-VIS	=	Ultraviolet and Visible Spectrophotometry

CHAPTER I

INTRODUCTION

The genus *Croton* belongs to the family of Euphorbiaceae. It is distributed throughout Thailand and over all tropical countries. This genus consists of about 750 species. They are either trees or shrubs, occasionally rheophytic (one introduced species and annual herb), densely or sparsely clothed with stellate hairs or shinning scales, occasionally subglabrous. Leaves alternate or pseudo-verticillate, petiolate, subentire or crenate or dentate or occasionally lobed, penninerved or sometimes palminerved at the base, biglandular at junction of petiole and lamina; stipules minute or shortly filiform, sometimes obsolete. Flowers are mostly monoecious. Inflorescenses terminal, racemose, androgynaecious. The female flowers sometimes reduced to 1 basal long-pedicelled flower. Male flower: sepals mostly 5, free, imbricate or valvate; petals 5, free, often lanate at the apex; disk-glands small, opposite the sepals; stamens 5-30, mostly lanate at the base, inflexd at the apex in bud; pistillode absence. Female flower: sepals much as in male; petals mostly or vestigial; ovary 3-locular; styles variously divided into 2 or 4 linear or thickened branches or occasionally shortly flabellate. Capsule tricoccous, smooth or shortly muricate; seeds ovoid or ellipsoid, smooth, occasionally sparely stellate-lepidote (Shaw, 1980; Shaw, 1981).

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

Croton acutifolius Esser	จิมิจิยา Chi-mi-chi-ya, เปล้า Plao, เปล้าแพะ Plao pae, มะดอไก่ Madokai (Northern).
C. argyratus Blume	เปล้า Plao (Prachuap Khiri Khun); เปล้าเงิน Plao ngoen (Nong Khai).
C. birmanicus Müll.Arg.	= *C. tiglium* L.
C. bonplandianus Daillon	เปล้าทุ่ง Plao thung (General).

Croton cascarilloides Raeusch.	เปล้าเงิน Plao ngoen (Songkhla); เปล้าน้ำเงิน Plao nam ngoen (Prachuap Khiri Khun).
C. caudatus Geiseler	กระดอหดใบขน Krado hot bai khon (Chanthaburi); โคคลาน Kho khlan (Nakon Ratchasima); ปริก Prik (Trang); โคคลานใบขน Kho khlan bai khon (General)กูเราะปริยะ Ku-ro-pri-ya (Malay-Narathiwat).
C. columnaris Airy Shaw	เปล้าคำ Plao khum (Sukhothai).
C. crassifolius Geiseler	ปังคี Pang khi, พังคี Phang khi (Chiang Mai).
C. cumingii Müll.Arg.	= *C. crassifolius* Geiseler
C. delpyi Gagnep.	เปล้า Plao, เปล้าน้อย Plao noi, นมน้ำเขียว Nom nam khiao (Southeastern).
C. griffithii Hook.f.	จิก Chik, เปล้า Plao (Peninsular).
C. hirtus L.Her.	เปล้าส้มลุก Plao lom luk (Peninsular).
C. hutchinsonianus Hosseus	เปล้า Plao, เปล้าแพะ Plao phae, เปล้าเลือด Plao lueat, แม่ลาเลือด Mae la lueat, เหมือนฮ้อน Mueat hon (Northern).
C. kerii Airy Shaw	เปล้า Plao (General).
C. kongensis Gagnep.	เปล้าเงิน Plao ngoen, เปล้าน้อย Plao noi (Northeastern); เปล้าน้ำเงิน Plao nam ngoen (Eastern); เสปอตุ Se-po-tu (Karen-Chiang Mai).
C. krabas Gagnep.	ทรายขาว Sai khon (Northern); พริกนา Prik na (Central); ฝ้ายน้ำ Fai num (Eastern).
C. lachnocarpus Benth.	ขี้อ้น Khi on (Southwestern).

Croton longissimus Gagnep.	เปล้าน้อย Plao noi {Lampang).
C. mekongensis Gagnep.	เปล้าน้ำเงิน Plao num ngeon, พริกนา Prik na (Northern).
C. oblongifolius Roxb	.= *C. roxburghii* N.P. balakar.
C. poilanei Gagnep.	เปล้าใหญ่ Plao yai (Southeastern).
C. pierri Gagnep.	= *C. cascarilloides* Raeusch.
C. robustus Kurz	เปล้าเลือด Plao lueat (Lampang).
C. rottleri Geiseler Gagnep.	= *C. cascarilloides* Raeusch
C. roxburghii N.P.Balakr.	ควะวู Khwa-wu (Karen-Kanchanaburi), เปล้าใหญ่ Plao yai (Central).
C. santisukii Airy Shaw	เปล้าสันติสุข Plao santisuk (Southwestern).
C. sepalinus Airy Shaw	เปล้าเงิน Plao ngoen (Peninsular).
C. siamensis Craib	= *C. robustus* Kurz
C. stellatopilosus OHba	เปล้าน้อย Plao noi (Prachin Buri, Prachuap Khiri Khan) (Southeastern).
C. thorelii Gagnep.	เปล้าตะวัน Plao tawan (Southeastern).
C. tiglium L.	บะกั้งB a kang (Phare), หัสคืน Has sa Khuen (Northern), สลอด Salot, หมากยอง Mak-yong (Shan-Mae Hong Son).
C. tomentosus Müll.Arg.	= *C. cascarilloides* Raeusch
C. trachycaulis Airy Shaw	กวาวะ Kwa wa, ขี้อ้น Khi on (Prachuap Khiri Khun).
C. wallichii Müll.Arg.	เปล้า Plao, เปล้านา Ploa na (General).

Croton kongensis Gagnep. is an indigeneous plant, commonly known in Thai as ″Plao Ngeon″ or ″Plao Noi″, is frequently used in folk medicine. The leaves of *C. kongensis* are used in Indo-China for various stomach disorders including ulcers, and a decoction is externally applied for furuncles and impetigo. This plant is deciduous

shrub (1.8-3 M height), basal diameter 10-15 mm, bark thin, smooth, brown; male inflo-erect, sepals with scales medially brown, sides greyish petals, filaments pale light green, anthers pale light yellow; blade dull dark green above, silvery-greyish undemeath, small leaves, and creamy white flowers (van Valkenburg and Bunyapraphatsara, 2001).

Croton birmanicus Müll.Arg. is exotic plant (Burma) known in Thai as ˝Has sa Khuen˝, is similar to *Croton tiglium*. This plant is shrub or small tree, 3-6 m high. Leaf simple, alternate, ovate, 4-7 cm wide, 7-10 cm long, brownish green. Inflorescence in axillary raceme, monoecious, pisillate flowers at base, staminate flowers upward. Fruit schizocarp, 3-lobed, 1-3 seeded (Saralamp, Chuakul, Temsiririrkkul *et al.*, 1996).

The plants in genus *Millettia* belongs to Family Leguminosae, 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).

Latin descriptions of *Millettia kangensis* Craib, according to Craib (1927), are as follow:

Millettia kangensis Craib; *species floribus inter maiores cum foliis iuvenilibus orientibus, vexillo basi calloso extra sericeo, ovario pubescente distinguenda.*

Arbor circa I0 m. alta (ex Kerr); ramuli iuventute densius breviter crispatim fulvo-pubescents, mox glabri, cortice brunneo vel cinereo-brunneo obtecti, lenticellis numeriosis prominaentibus. Folia 7-9 foliolata, petiole circa 4 cm, longo incluso circa I7 cm, longa, et rhachi subteretibus vel hoc superne late canaliculato indumento ei ramulorum invenilium simili obtectis; stipulae lineares, circa 3 mm longae; folila opposite, oblonga, oblongooblanceolata vel terminali obovato, apice breviter subito

acuminata, ad 8 cm longa et 4.2 cm lata, chartacea, supra primo sericea, mox adpresse pubescentia, subtus breviter molliter pubescentia, nervis lateralibus utrinque 8-IO supra conspicuis subtus prominulis, reticulatione gracili sub oculo armato subtus conspicus, petiolulo circa 3 mm longo suffulta, terminali a lateralibus fere 2 cm distante, dtipellis filiformibus pubescentibus circa 3.5 mm longis. Paniculae partials in paniculas terminals paucifoliatas vel efoliatas adflores e ramusculis lateralibus ad 3 cm, longis racemosim orti; bracteae angustae, circa 4 mm longae, deciduae; bracteolae binae, ad pedicelli apicem positae, circa 3 mm longae, angustae, deciduae; rhachis, ramuli, et pediceli densius fulvo-tomentelli vel etiam parce pubescentes; pedicelli ad I cm longi, breviter pubescentes. Calyx extra pubescens, ad 6.5 cm longus; lobi postici approximate, breves, laterals et anticus deltoidei, acuti, I.5 mm longi 2 mm lati. Vexillum oblongum, basi cordatulum, bicallosum, I.5 cm longum, 0.8 cm latum, dorso sericeum, ungui 3 mm longo suffultum; alae I4 mm longae, 4 mm latae, basi auriculatae, apicem versus angustatae, obtusae vel rotundatae, extra apicem versus sparse sericeae, ungui 5 mm longo suffultae; carnae petala basi auriculata, I2 mm longa, 4.5 mm lata, extra apice sericea, ungui 5 mm longo suffulta. Stamina monadelpha, vexillari basi tantum ab aliis libero. Ovarium I cm altum, subsessile, sericeum, stylobasi sericeo apicem versus glabro, ovules 7 (Craib, 1927) .

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

Millettia 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 cho, 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.	ขะเจาะหลวง Kha cho luang, ขะเจาะใหญ่

var. *tecta* Craib	Kha cho yai (Narathiwat).
Millettia 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).

Several phytochemical studies on many species of *Croton* and *Millettia* have been reported but none on *Croton kongensis*, *Croton birmanicus*, and *Millettia kangensis* were found.

Our preliminary activity screening showed that a crude CH_2Cl_2 extract from the leaves of *Croton kongensis* exhibited antimalarial at IC_{50} 0.9 μg/mL and antimycobacterial at MIC 12.5 μg/mL activity. A crude CH_2Cl_2 extract from the root of *Croton birmanicus* showed antimycobacterial activity at MIC 100 μg/mL. These crude $CHCl_3$ extract from the leaves and CH_2Cl_2 extract from the twigs of *Millettia kangensis* exhibited antimycobacterial activity at MIC 100 μg/mL. Therefore, these plant extracts were selected for phytochemical investigation. Aims of this research work are as follows:

1. Isolation and purification of compounds from the leaves of *Croton kongensis* Gagnep., the roots of *Croton birmanicus* Müll.Arg., and the leaves and twigs of *Millettia kangensis* Craib.
2. Determination of chemical structures of isolated compounds.
3. Evaluation of biological activities of isolated compounds.

A

B

Figure 1 *Croton kongensis* Gagnep. A) Whole plants, B) Leaves and inflorescence

A

B

Figure 2 *Croton birmanicus* Müll.Arg. A) Whole plant, B) Leaves

A

B

C

D

Figure 3 *Millettia kangensis* Craib A) Whole plant, B) Leaves, C) Whole plant with inflorescene, D) Inflorescene

CHAPTER II

HISTORICAL

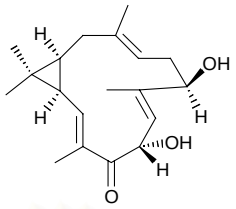
The genus *Croton* belongs to the family Euphorbiaceae, distributed throughout Thailand, and several species have been used as ingredients in traditional medicine. Croton plants are used in folk medicine for antiinflammatory (Bettolo and Scarpati, 1979; Cai, Chen and Phillipson, 1993; Kubo, Asaka and Shibata, 1991; Mazzanti, Bolle, Matinoli *et al.*, 1987), antibacterial (Chen, Cai and Phillipson, 1994), antimicrobial (Peres, Monache, Cruz *et al.*, 1997), gastric ulcer (Craveiro, Andrade, Matos *et al.*, 1980; Roengsumran, Petsom, Kuptiyanuwat *et al.*, 2001), wound healing (Cai *et al.*, 1993; Cai, Evans, Roberts *et al.*, 1991; Milo, Risco, Vila *et al.*, 2002; Pieters, De Bruyne, Mei *et al.*, 1992), cancer (Cai *et al.*, 1993; Cai *et al.*, 1991; Milo *et al.*, 2002), antitumor (Boonyarathanakornkit, Che, Fong *et al.*, 1987; Ferrigni, Puynum, Anderson *et al.*, 1982), dysentery (Milo *et al.*, 2002), purgative (Asuzu, Gray and Waterman, 1988; Mazzanti *et al.*, 1987), bronchitis, fever, malaria (Vigor, Fabre, Fouraste *et al.*, 2002), nervous disturbances (Batatinha, de Souza-Spinosa and Bernardi, 1995), narcotic (Vigor, Fabre, Fouraste *et al.*, 2001), aphrodisiac (Moulis and Fouraste, 1992), antidiabetic (Itokawa, Ichihara, Kojima *et al.*, 1989; Kubo *et al.*, 1991), antilipotropic (Itokawa *et al.*, 1989), hypertension (Puebla, Lopez, Guerrero *et al.*, 2003), syphilis (Babili, Moulis, Bon *et al.*, 1998), hypoglycaemia (Maciel, Pinto, Arruda *et al.*, 2000), and rheumatism (Cai *et al.*, 1991). In addition, these plants showed cytotoxicity, and insecticidal activity (Smitt and Hogberg, 2002).

Croton species contain a number of diterpenes. Typical diterpenes in *Croton* spp. are casbanes, cembranes, clerodanes, cleistanthanes, kauranes, labdanes, pimaranes, and halimanes. In addition, *Croton* species also produce phorbols, polysaccharides, flavonoids, lignans, benzofurans, sesquiterpenes, polyphenols, and alkaloids.

1. Classification and Bioactivities of Diterpenes from some *Croton* species

1.1 Casbane Diterpenes

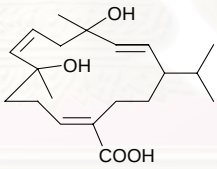
In 1990, Moura and co-workers isolated a new macrocyclic diterpene [**1**] from the stems of *Croton nepetaefolius* (Moura, Monte and Filho, 1990).



[**1**]

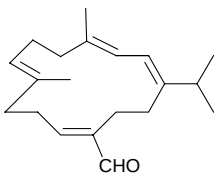
1.2 Cembrane Diterpenes

In 1998, Nareeboon isolated a new cembrane diterpene, namely 1-isopropyl-4,8-dimethylcyclotetradeca-1,4,8-triol-2*E*,6*Z*,11*E*-triene-12-carboxylic acid [**2**], and two new diterpenes, 2*β*,3*β*-dihydroxy-labda-8(17),12(13),14(15)-triene [**3**] and 2*β*,3*β*,11-trihydroxy-16-norlabd-8(17),12(13)-dien-14-one [**4**], from leaves of *Croton joufra* (Nareeboon, 1998).



[**2**]

C. oblongifolius, a Thai medicinal plant, was found as a source of neocrotocembranal [**5**]. The compound **5** inhibited platelet aggregation induced by thrombin (IC_{50} = 47.21 μg/mL), and showed cytotoxicity against P-388 cells *in vitro* (IC_{50} = 6.48 μg/mL) (Roengsumran, Singtothong, Pudhom *et al.*, 1999).



[**5**]

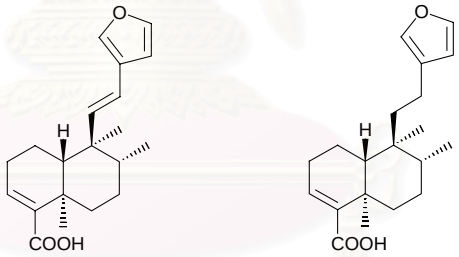
1.3 Clerodane Diterpenes

Croton species is a rich source of clerodane diterpenes and nor-clerodane diterpenes.

In 1972, 11-dehydro-hardwickiciic acid [**6**] was isolated from the stems bark of *Croton oblongifolius* by Aiyar and Seshadri (Aiyar and Seshadri, 1972).

C. californicus Muell. Arg., an herbaceous shrub indigenous to the Sonoran Desert, Arizona, U.S.A., was found to posses an antimalarial (-)-hardwickiic acid [**7**] (Luzbetak, Torrance, Hoffmann *et al.*, 1978).

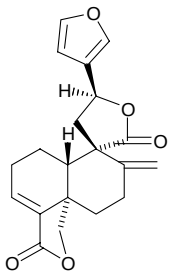
C. aromaticus L. is widely distributed in Sri Lunka, and used in ethnomedical preparations and in traditional agriculture. The air dried roots of this plant provided obtained a bioactive compound, (-)-hardwickiic acid [**7**], which showed insecticidal activity against *Apis craccivora* (Bandara, Wimalasiri and Bandara, 1987).



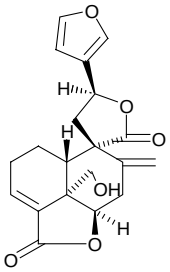
[**6**] [**7**]

Stems of *C. sublyratus* were found to posses plaunolide [**8**] and plaunol B [**9**], which exhibited antipeptic ulcer activity (Takahashi, Kurabayashi, Kiyazawa *et al.*, 1983).

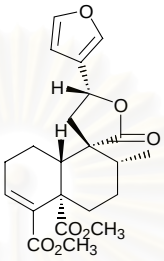
Leaves and barks of *C. haumanianus* are used in folk medicine against gastric ulcer and antihypertensive, and used as an antiepileptic drug. Chemical investigation of the petroleum ether extract of *C. haumanianus* led to the isolation of crotocorylifuran [**10**] and crotohaumanoxide (Tchissambou, Chiaroni, Riche *et al.*, 1990).



[**8**]

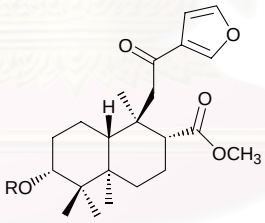


[**9**]



[**10**]

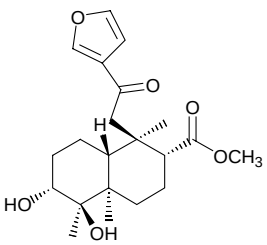
Chiromodine [**11**] and its monoacrtyl derivative [**12**] were isolated from the East African medicinal plant, *Croton megalocarpus* (Weckert, Hummer, Mensah *et al.*, 1992).



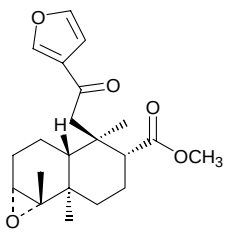
[**11**] : R = H

[**12**] : R = CH_3CO

In 1992, MenSah, I. A. *et al*. isolated chiromodine [**13**] and epoxychiromodine [**14**] from the bark of *C. megalocarpus* (Mensah, Achenbach, Thoithi *et al.*, 1992).

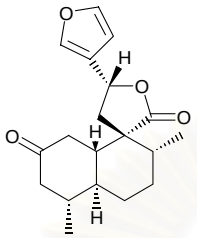


[**13**]

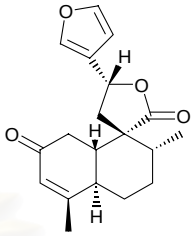


[**14**]

Croton cajucara Benth is a Brazilian medicinal plant, commonly called Sacaca, its cortices are known for their antidiabetic and antilipotropic properties. In 1989, Itokawa *et al.* isolated nor-clerodane diterpenes, *trans*-crotonon [**15**] and dehydrocrotonin [**16**] (Itokawa *et al.*, 1989).



[**15**]

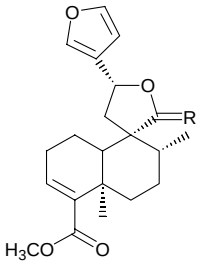


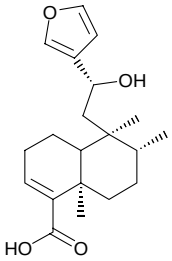
[**16**]

In 1997, Farias *et al*. studied activities of *trans*-dehydrocrotonin [**16**], which was isolated from the bark of *C. cajucara*. Compound **16** demonstrated a significant hypoglycemic activity in alloxan-induced diabetic rats but not in normal rat, at oral dose of 25 and 50 mg/kg body weight (Farias, Rao, Viana *et al.*, 1997). Compound **16** also showed antiulcerogenic activity on human promyelocytic leukaemia cells (Freire, Melo, Aoyama *et al.*, 2002).

C. sonderianus Muell. Arg. is used in folk medicine as a remedy for gastric disturbances. Antimicrobacterial terpenes, sonderianin [**17**], hardwickic acid [**7**], 12-hydroxyhardwickic acid [**18**], and sonderianial [**19**], were isolated from *C. sonderianus* (McChesney and Silveira, 1989).

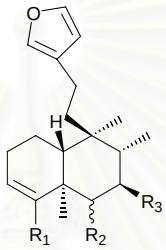
In 1994, Silveira. and McChesney. isolated 6*α*-hydroxyannonence [**20**], 6*α*,7*β*-dihydroxyannonence [**21**], and 6*α*,7*β*-diacetoxyannonence [**22**], from the roots of *C. sonderianus* (Silveira and McChesney, 1994).





[**17**] : R = O [**18**]

[**19**] : R = αH, βOH

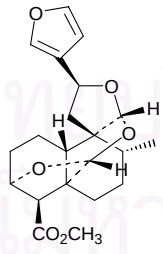


[**20**] : $R_1 = CH_3$, $R_2 = \alpha OH$, $R_3 = H$

[**21**] : $R_1 = CH_3$, $R_2 = \alpha OH$, $R_3 = OH$

[**22**] : $R_1 = CH_3$, $R_2 = \alpha OAc$, $R_3 = OAc$

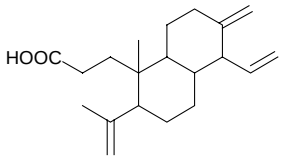
In 1992, Moulis and Fouraste isolated crovalin [**23**], a clerodane diterpene, from the stem bark of *Croton levatii* Guill. (Moulis and Fouraste, 1992).



[**23**]

1.4 Cleistanthane Diterpenes

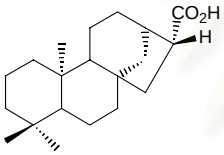
In 1999, Siriwat isolated 3,4-*seco*-cleistantha-4(18),13(17),15-trien-3-ioc acid [**24**] from stem barks of *C. oblongifolius* Roxb. (Siriwat, 1999).



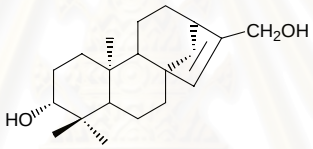
[**24**]

1.5 Kaurane Diterpenes

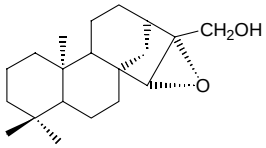
Croton lacciferus Linn. is a medicinally important plant commonly found in Sri Lanka and South India. The roots of *C. lacciferus* furnished three *ent*-kauranoids, 16*α*-H-*ent*-kauran-17-oic acid [**25**], *ent*-15*β*,16-epoxykauran-17-ol [**26**], and *ent*-kauran-15-en-3*β*,17-diol [**27**]. In addition, compounds **26** and **27** showed moderate insecticidal activity against *Apis craccivora* at a dose of 5 ppm per insect against 61% and 62% mortarity, respectively (Bandara, Wimalasiri and Macleod, 1988).



[**25**]

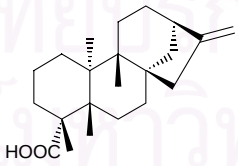


[**26**]



[**27**]

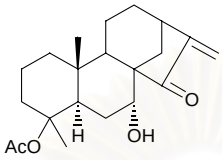
In 1998, Pattamadilok, isolated a kaurane diterpene, *ent*-kaur-16-en-19-oic [28], from the stem barks of *C. oblonifolius*, a Thai medicinal plant (Pattamadilok, 1998).



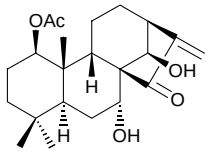
[**28**]

The kaurane diterpene, (-)-*ent*-kaur-16-en-19-oic acid [**28**], showed significant Na^+, K^+-ATPase inhibitory effect (IC_{50} = 2.2x10^{-5} M) (Ngamrojnanich, Sirimongkon, Roengsumran *et al.*, 2003).

The leaves of *Croton tonkinensis* were previously found to have an inhibitory effect on malarial parasites, and yilded an *ent*-kaurane diterpenoid *ent*-7*β*-hydroxy-15oxokaur-16-en-18-yl [**29**]. A novel *ent*-kaurane diterpenoid, *ent*-1*α*-acetoxy-7*β*,14α-dihydroxy-kaur-16-en-15-on [**30**], has been isolated from this plant (Minh, Ngoc, Quang *et al.*, 2003).



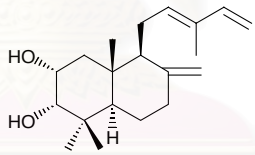
[**29**]



[**30**]

1.6 Labdane Diterpenes

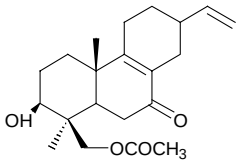
In 2001, Sutthivaiyakit *et al*. isolated a new labdane diterpene, 2*α*,3*α*-dihydroxy-labda-8,12,14-triene [**31**], from a Thai medicinal plant, *C. joufra* (Sutthivaiyakit, Nareeboon, Ruangrangsi *et al.*, 2001).



[**31**]

1.7 Pimarane Diterpenes

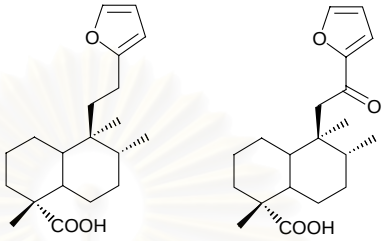
From the $CHCl_3$ extract of leaves of *C. joufra*, 3*β*-hydroxy-19-*O*-acetyl-pimara-8,15-diene-7-one [**32**], was isolated (Sutthivaiyakit *et al.*, 2001).



[**32**]

1.8 Halimane Diterpenes

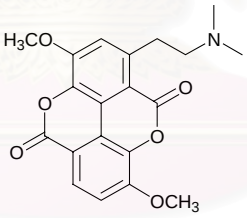
Non-specific strong cytotoxic compounds, crotohalimaneic acid [**33**] and crotohalimoneic acid [**34**], were isolated from *Croton oblongifolius* (Roengsumran, Pornpakakul, Muangsin *et al.*, 2004).



[**33**] [**34**]

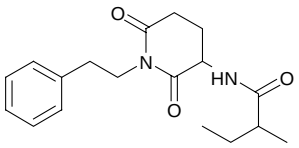
2. Miscellaneous

A cytotoxic alkaloid taspine [**35**] was isolated from South American Dragon's blood (*Croton* spp.) (Pieters *et al.*, 1992).



[**35**]

Julocrotin [**36**], a glutarimide alkaloid, was isolated from *C. humilis* (Stuart, McNeill, Kutney *et al.*, 1973) and *C. membranaceus* (Aboagye, Sam, Massiot *et al.*, 2000; Stuart *et al.*, 1973).



[**36**]

C. kongensis is an indigenous plant, and distributes in the North of Thailand. This plant is a shrub tree, and used as folk medicine. *C. birmanicus* is an exotic plant, similar to *Croton tiglium*. *C. birmanicus* is taller than *C. tiglium*. Several chemical studies on the *Croton* spp. have been reported but none on *C. kongensis* and *C. birmanicus*.

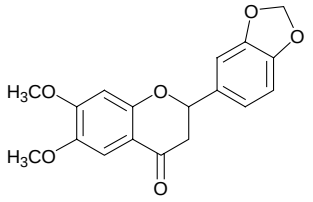
The genus *Millettia* belongs to the family Leguminosae, these plants are used in traditional medicine as a laxative, a blood purifier, a dewormer, an analgesic, a diarrhoea (Irvine, 1961), an anti-plasmodial (Yenesew, Derese, Midiwo *et al.*, 2003), an anthelmintic, and a purgative (Perrett, Whitfield, Sanderson *et al.*, 1995). The *Millettia* spp. exhibits insecticidal (Gupta, Bhattacharyya, Mitra *et al.*, 1983; Hooker, 1973; Singhal, Baruan, Sharma *et al.*, 1983; Singhal, Sharma, Baruan *et al.*, 1982), pesticidal (Gupta *et al.*, 1983; Singhal *et al.*, 1982), fish poison (Dagne and Bekele, 1990; Singhal *et al.*, 1982), molluscicidal, and cercaricidal activities (Perrett *et al.*, 1995).

Previous chemical studies of genus *Millettia* have shown that they are a rich source of flavonoids and isoflavonoids (Hooker, 1973; Mahmoud and Waterman, 1985). Typical metabolites of *Mellettia* are flavanones, isoflavanones, flavanes, isoflavanes, flavones, isoflavones, chalcones, rotenoids, coumarins, and quinones.

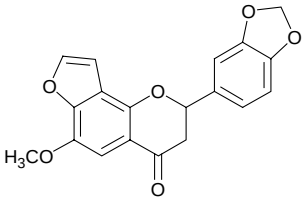
3. Classification and Biological Activities of Flavonoids from *Millettia* Species.

3.1. Flavanones and Isoflavanones.

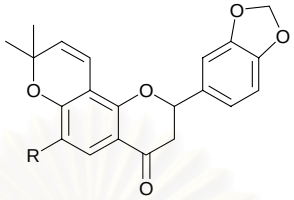
From 1974 to 1980, *Millettia ovalifolia* had been intensively studied for chemical constituents, which led to the isolation of several flavanones, isoflavanones, flavones, isoflavones and chalcones. The flavanones millitenins A [**37**] and B [**38**], the chromenoflavanones, ovalichromenes A [**39**] and B [**40**], the prenylated flavanones, 7-hydroxy-6,8-di-*C*-prenylflavanone [**41**] and 7-hydroxy-8-di-*C*-prenylflavanone [**42**], were isolated from this plant (Gupta and Krishnamurti, 1976; Islam, Gupta and Krishnamurti, 1980; Khan and Zaman, 1974).



[**37**]

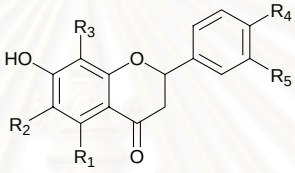


[**38**]



[**39**] R = OCH_3

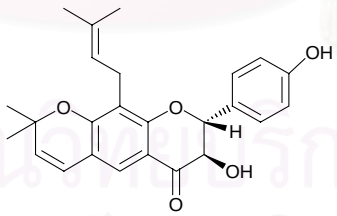
[**40**] R = H



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

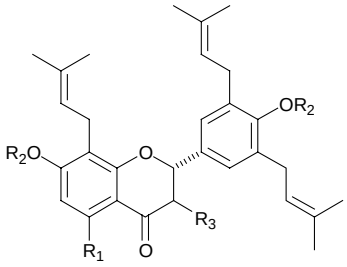
[**42**] $R_1 = R_2 = R_4 = R_5 = H$, $R_3 =$ 

In 1980, *Millettia pachycarpa* was found to possess a prenylated dihydroflavonol [**43**] (Singhal, Sharma, Thyagarajan *et al.*, 1980).



[**43**]

In 1984, Baruah's group isolated a dihydroflavanol, (2*S*)-3,7,4′-trihydroxy-8,3′,5′-triprenylflavanone [**44**], two flavanones, (-)-sophoranone [**45**] and its 5-hydroxy derivative [**46**], and four pterocarpans from *M. pulchra* (Baruah, Baruah, Sharma *et al.*, 1984).

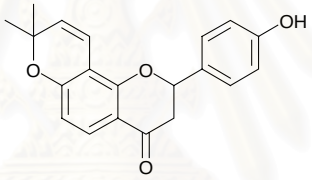


[**44**] $R_1 = R_2 = R_3 = H$

[**45**] $R_1 = OH, R_2 = R_3 = H$

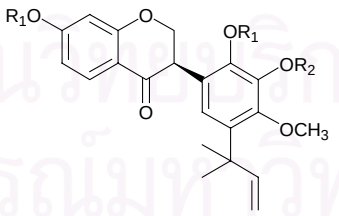
[**46**] $R_1 = H, R_2 = R_3 = OH$

In 1989, *Millettia ferruginea* was chemically explored, and a pyranoflavanone 4′-hydroxyisolonchocarpin [**47**], eight isoflavones, a chalcone, and a pterocarpene, were isolated from this plant (Dagne, Bekele and Waterman, 1989).



[**47**]

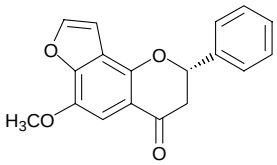
Cytotoxic isoflavanones, pervilleanons [**48**] and its 3′-*O*-demethyl derivative [**49**], were isolated from *M. pervilleana* (Galeffi, Rasoanaivo, Federici *et al.*, 1997).



[**48**] $R_1 = H, R_2 = CH_3$

[**49**] $R_1 = R_2 = H$

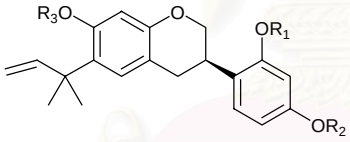
Sritularak's group could isolate 6-methoxy-[2″,3″:7,8]-furanoflavanone [**50**] and 2,5-dimethoxy-4-hydroxy-[2″,3″:7,8]-furanoflavan from *M. erythrocalyx* (Sritularak, Likhitwitayawuid, Conrad *et al.*, 2002a).



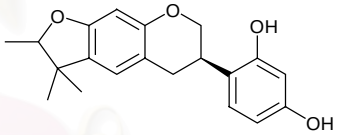
[**50**]

3.2 Flavans and Isoflavans

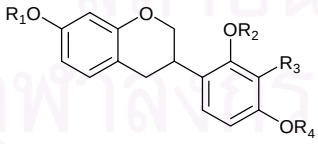
In 1989, Kumar, Krupadanam and Srimannarayana isolated three isoflavans, 3*R*(+)-millinol [**51**], 3*R*(+)-millinol-B [**52**], and 3*R*(+)-cyclomillinol [**53**], from a stem bark of *Millettia racemosa* (Kumar, Krupadanam and Srimannarayana, 1989). In 1994, Rao and Krupadanam isolated compounds **51**, **52**, **53**, 3*R*(+)-isomillinol-B [**54**], 3*R*(-) vestitol [**55**], and 3*R*(-)-laxifloran [**56**], from *M*. *racemosa*. Compounds **53** and **55** showed significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Rao and Krupadanam, 1994). Two prenylated isoflavans, neomillinol [**57**] and millinolol [**58**], were also isolated from *M*. *racemosa* (Rao, Prashant and Krupadanam, 1996).



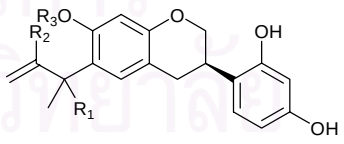
[**51**] $R_1 = R_2 = R_3 = H$
[**52**] $R_1 = CH_3$, $R_2 = R_3 = H$
[**54**] $R_1 = R_3 = H$, $R_2 = CH_3$



[**53**]

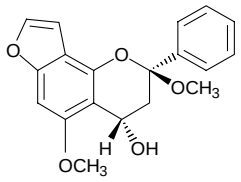


[**55**] $R_1 = R_4 = H$, $R_2 = CH_3$, $R_3 = OCH_3$
[**56**] $R_1 = R_2 = R_3 = H$, $R_4 = CH_3$



[**57**] $R_1 = H$, $R_2 = CH_3$
[**58**] $R_1 = CH_3$, $R_2 = H$

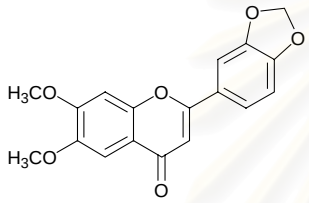
In 2002, Sritularak *et al* isolated 2,5-dimethoxy-4-hydroxy-[2″,3″:7,8]-furanoflavan [**59**] from *M. erythrocalyx* (Sritularak *et al.*, 2002a).



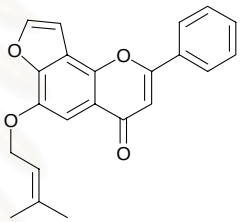
[**59**]

3.3 Flavones and Isoflavones.

In 1974, *Millettia ovalifolia* was isolated, and two flavones, milletenin C [**60**] and ovalifolin A [**61**], were obtained (Khan and Zaman, 1974).

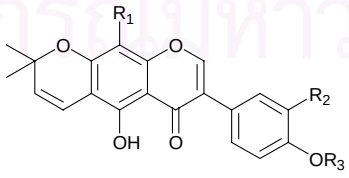


[**60**]



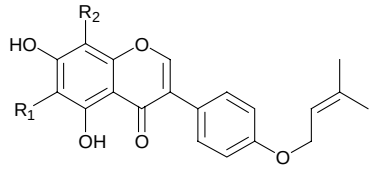
[**61**]

M. auriculata was isolated to yield auriculasin [**62**] and isoauriculasin [**63**] (Minhaj, Khan, Kapoor *et al.*, 1976). Raju and Srimannarayana isolated aurmillone [**64**] from the seeds of *M. auriculata* (Raju and Srimannarayana, 1978), while Gupta's group isolated an isoflavone isoaurmillone [**65**] from the pods (Gupta *et al.*, 1983). Three new prenylated flavonones, 2′-deoxyisoauriculatin [**66**], 2′-*O*-methyliso auriculatin [**67**], and auriculatin [**68**], were isolated from *M. auriculata* (Rao, Prasad and Ganapaty, 1992).



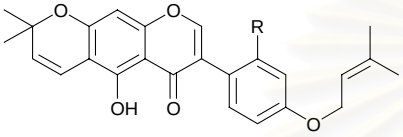
[**63**] R_1 =  , R_2 = OH, R_3 = H,

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



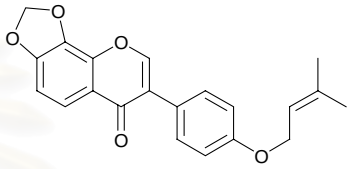
[**62**] R_1 = H, R_2 = OCH_3

[**65**] R_1 = OCH_3, R_2 = H



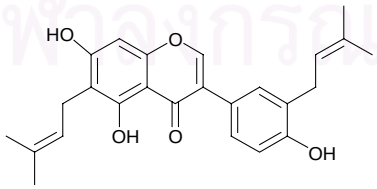
[**66**] R = H

[**67**] R = OCH_3

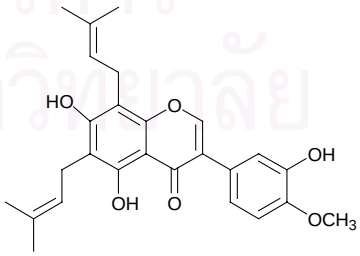


[**68**]

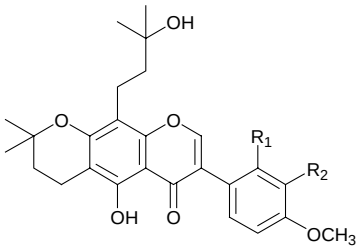
Isolation of the aerial part of *Millettia pachycarpa* Benth. gave a new prenylated isoflavone, 5,7,4′-trihydroxy-6,3′-diprenylaisoflavone [**69**] (Singhal *et al.*, 1980). In 1981, *M. pachycarpa* was chemically explored, and four new prenylated 5-hydroxyisoflavones with 3,3 dimethyl-3-hydroxylpropyl group [**70**] and its isomers [**71**], [**72**] and [**73**] were obtained (Singhal, Sharma, Madhusudanan *et al.*, 1981). In 1983, a new prenylated isoflavone, 5,7,3′-trihydroxy-4′-methoxy-6,8-diprenylated isoflavone [**74**] was isolated from *M. pachycarpa* (Singhal *et al.*, 1983).

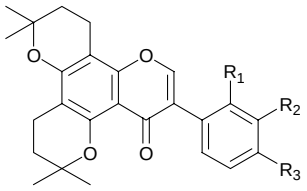


[**69**]



[**74**]





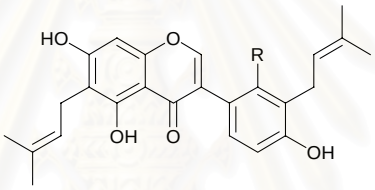
[**70**] R_1 = H, R_2 = OCH_3

[**71**] R_1 = OCH_3, R_2 = H

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

[**73**] R_1 = OH, R_2 =H, R_3 = OCH_3

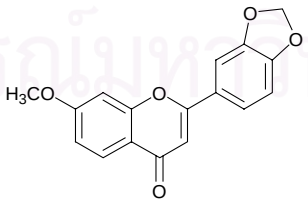
In 1984, *Millettia pulchara* was chemically investigated to afford two new ptercarpans and two new prenylated isoflavones 5,7,2′,4′-tetrahydroxy-6,3′-diprenylisoflavone, together with its derivatives **75** and **76** (Baruah *et al.*, 1984).



[**75**] R = OH

[**76**] R = OCH_3

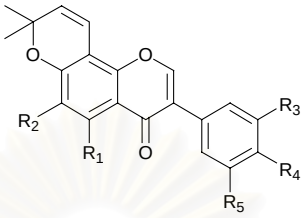
M. hemsleyana, collected from the South of Thailand, was isolated by Mahmound and Waterman. The stem bark of *M. hemsleyana* has yielded a methylenedioxyflavone, 3′,4′-methylenedioxy-7-methoxyflavone [**77**] and three chalcones (Mahmoud and Waterman, 1985).



[**77**]

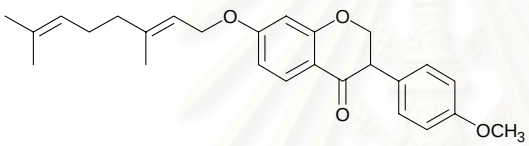
The barks and seed pods of *M. ferruginea* (Hochst.) Bak.subsp. *ferruginea* and *darassana*, endemic to Ethiopia, provided two new isoflavones [**78,79**], a new chalcone, a new flavanone and a new pterocarpene (Dagne *et al.*, 1989). In 1990,

O-geranylated [**80**] and *O*-prenylated flavonoids [**81**] were isolated from *Millettia ferruginea* (Dagne, Bekele, Noguchi *et al.*, 1990). Dagne *et al*. also isolated three novel C-prenylated isoflavonoids [**82-84**] from the seeds of *M. ferruginea* (Dagne *et al.*, 1990).

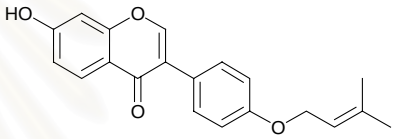


[**78**] $R_1 = R_2 = OCH_3$, $R_3R_4 = -OCH_2O-$, $R_5 = H$

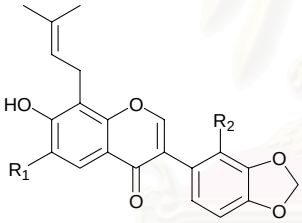
[**79**] $R_1 = R_2 = H$, $R_3 = OH$, $R_4\ R_5 = -OCH_2O-$



[**80**]

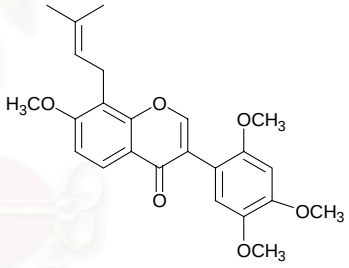


[**81**]



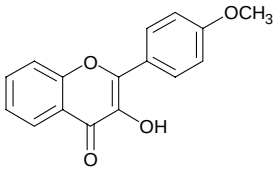
[**82**] $R_1 = H$, $R_2 = OCH_3$

[**83**] $R_1 = OCH_3$, $R_2 = H$



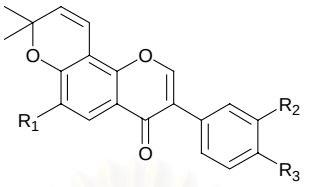
[**84**]

3-Hydroxy-4′-methoxyflavone [**85**] was isolated from the flowers of *M. zechiana* by Parvez and Ogbeide (Parvez and Ogbeide, 1989).



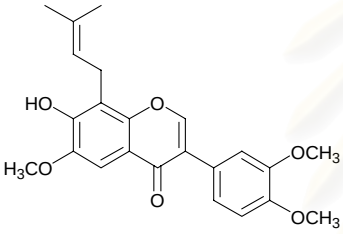
[**85**]

Isolation of seed pods of *Millettia dura* yielded four new isoflavones, durallone [**86**], 6-demethyldurallone [**87**], predurallone [**88**] and isoerythrinin-A 4′-(3-methylbuty-2-enyl)ether [**89**] (Yanesew, Midiwo and Waterman, 1996).

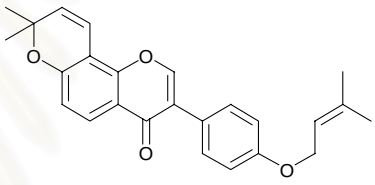


[**86**] $R_1 = OCH_3$, $R_2 = R_3 = CH_3$

[**87**] $R_1 = R_2 = H$, $R_3 =$ 

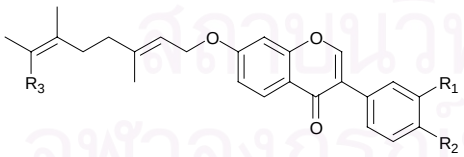


[**88**]

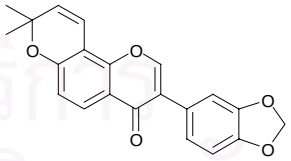


[**89**]

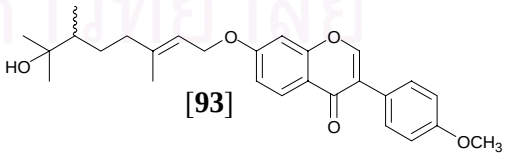
A root bark of *M. griffoniana* was chemically investigated, and five new isoflavonoids [**89-93**], a new courmarin, and a new rotenoid, were isolated (Yanesew *et al.*, 1996; Yankep, Fomun, Bisrat *et al.*, 1998; Yankep, Mbafor, Fomun *et al.*, 2001; Yankep, Njamen, Fotsing *et al.*, 2003).



[**89**] $R_1R_2 = -OCH_2O-$, $R_3 = H$
[**90**] $R_1 = H$, $R_2 = OCH_3$, $R_3 = CH_2OH$
[**91**] $R_1 = R_2 = OH$, $R_3 = CH_3$

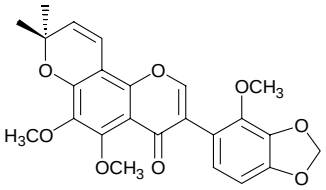


[**92**]

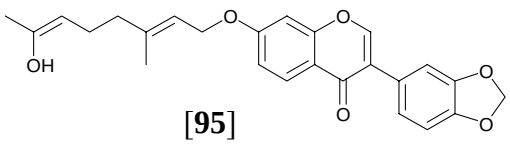


[**93**]

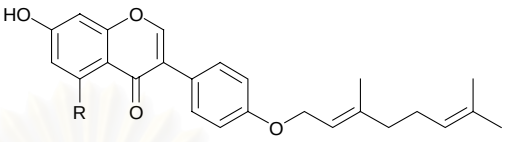
Conrauinones A, B, C and D [**94-97**] were isolated from the stems bark of *M. conraui* (Fuendjiep, Nkengfack, Fomun *et al.*, 1998a;b).



[**94**]

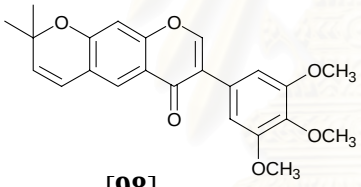


[**95**]

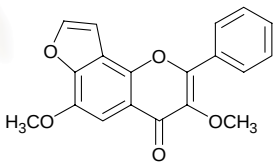


[**96**] R = OCH_3
[**97**] R = H

In 1998, Kamperdick *et al*. isolated a new furanoflavone [**98**] and a new pyranoisoflavonoid [**99**] from the leaves of *Millettia ichyochtona* (Kamperdick, Phuong, Sung *et al.*, 1998).

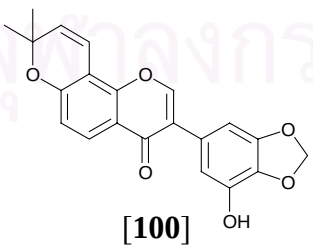


[**98**]

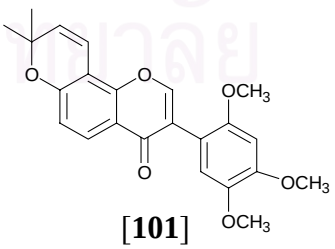


[**99**]

From the stem bark of *M. usaramensis* subsp. *usaramensis*, a new isoflavone norisojamicin [**100**] and anti-plasmodial compound [**101**] were isolated (Yanesew, Midiwo and Waterman, 1998; Yenesew *et al.*, 2003).

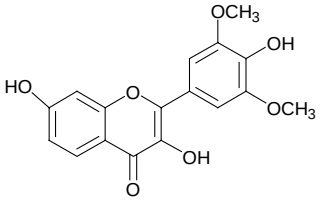


[**100**]



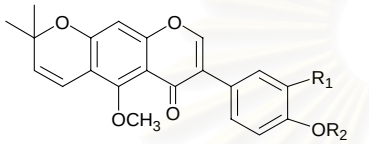
[**101**]

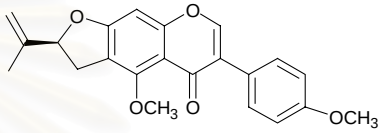
In 1999, a new flavonol laurentiol [**102**] was isolated from the heart wood of *M. laurentii* (Kammaing, Free, Nkengfack *et al.*, 1999).



[**102**]

The novel pyranoisoflavones **103 and 105** were isolated from *Millettia thonningii* (Olivares, Lwande, Monache *et al.*, 1982).



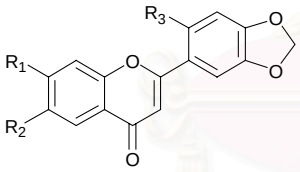


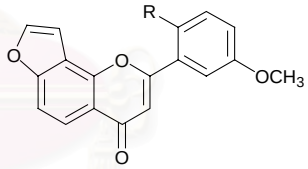
[**103**] R_1 = OH, R_2 = 

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

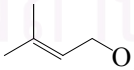
[**105**]

M. erythrocalyx afforded the new flavones, millettocalyxins A-C [**106-108**], and pongol methyl ether [**109**] (Sritularak, Likhitwitayawuid, Conrad *et al.*, 2002b).





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

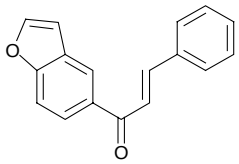
[**107**] R_1 =  , R_2 = OCH_3, R_3 = H

[**108**] R = OCH_3

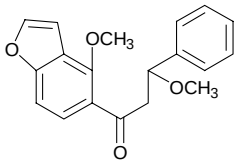
[**109**] R = H

3.4 Chalcones

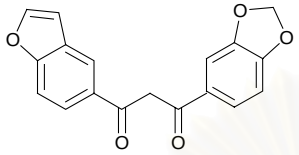
Chalcone derivatives, ovalitenins A-D [**110-113**], were isolated from the seeds of *Millettia ovalifolia* (Gupta and Krishnamurti, 1977; Islam *et al.*, 1980). A new chalcone monoethoxychalcone [**114**] was obtained from *Millettia pachcarpa* (Singhal *et al.*, 1983).



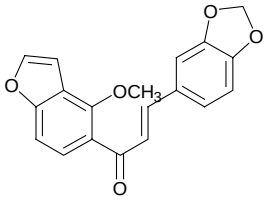
[**110**]



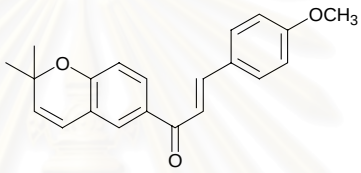
[**111**]



[**112**]

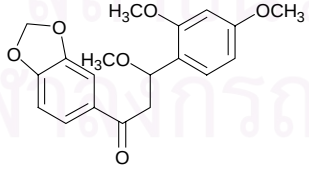


[**113**]

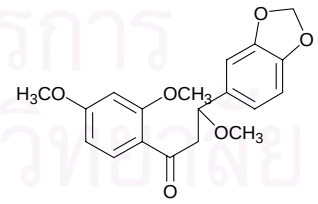


[**114**]

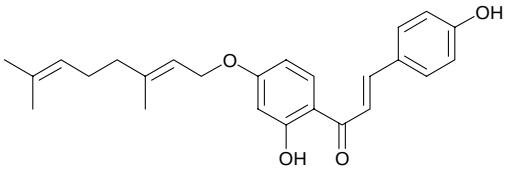
Two novel chalcones, dihydromillellltenone methyl ether [**115**] and dihydroisomilletenone methyl ether [**116**] (Mahmoud and Waterman, 1985), were isolated from the stem bark of *M. hemsleyana*, while a novel 4′-*O*-geranylisoliquiritigenin [**117**] (Yankep, Fomun and Dagne, 1997) and a known chalcone [**118**] were isolated from bark and seed pods of *M. ferruginea* (Dagne *et al.*, 1989). Compound **117** was also found from the extract of *M. griffoniana*.



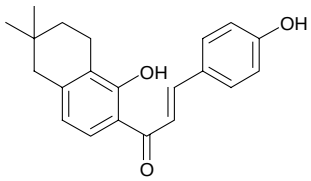
[**115**]



[**116**]

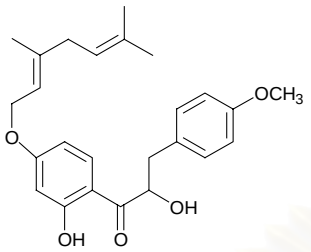


[**117**]

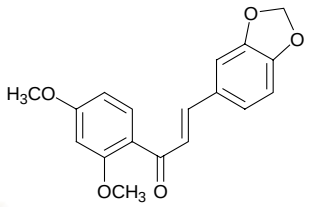


[**118**]

A new *α*-hydroxydihydrochalcone [**119**] was isolated from the stem bark of *Mllettia usaramensis* subsp. *usaramensis* (Yanesew *et al.*, 1998), while a new chalcone [**120**] was isolated from *M. erythrocalyx* (Sritularak *et al.*, 2002a).

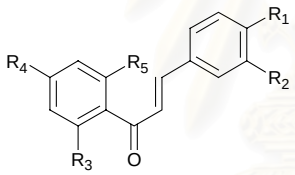


[**119**]

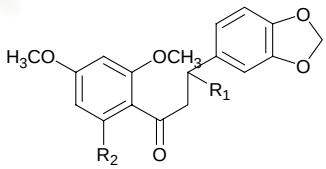


[**120**]

In 2003, Phrutivorapongkul *et al* isolated anti-Herpes Simplex Virus (HSV) compounds [**121** and **122**], and cytotoxic compounds [**123** and **124**] from the stem barks of *Millettia leucantha* (Phrutivorapongkul, Lipipun, Ruangrungsi *et al.*, 2003).



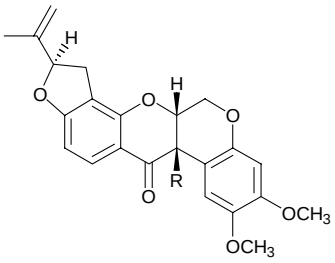
[**121**] R_1R_2 -OCH_2O-, R_3 = H, R_4= R_5 = OCH_3
[**122**] R_1R_2 -OCH_2O-, R_3 = R_4 = R_5 = OCH_3



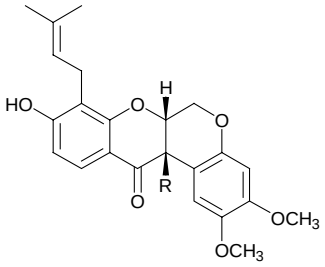
[**123**] R_1 = H, R_2 = OCH_3
[**124**] R_1 = OCH_3, R_2 = H

3.5 Rotenoids

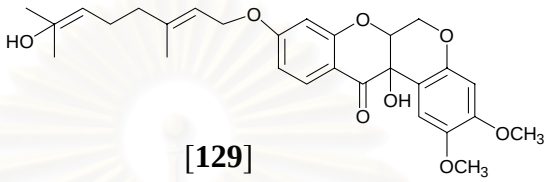
The roots of *Millettia pachycarpa* furnished a new rotenone, *cis*-12a-hydroxyrot-2-enoic acid [**125**], and three known compounds [**126-128**]. A new rotenone griffonianone [**129**] was isolated from the root barks of *M. griffoniana* (Singhal *et al.*, 1982; Yankep *et al.*, 2001).



[**125**] R = OH
[**126**] R = H

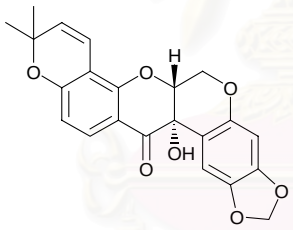


[**127**] R =H
[**128**] R = OH

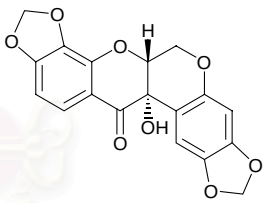


[**129**]

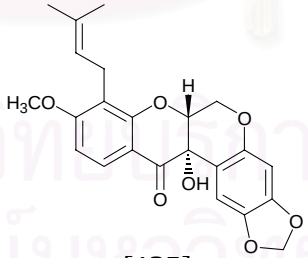
The stem bark of *M. usaramensis* subsp. *usaramensis* provided four new rotenones, (+)-12a-epimillettosin [**130**], (+)-usararotenoid-A [**131**], (+)-12-dihydrousararotenoid-A [**132**], and (+)-usararotenoid-B [**133**], and an anti-plasmodial rotenoid usararotenoid C [**134**] (Yanesew *et al.*, 1998; Yenesew *et al.*, 2003).



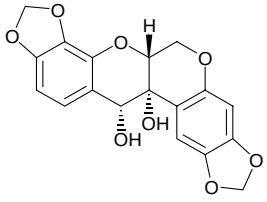
[**130**]



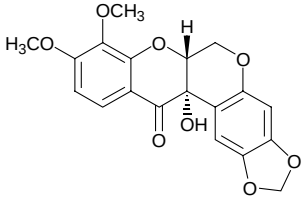
[**131**]



[**135**]



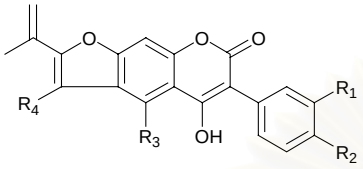
[**132**]



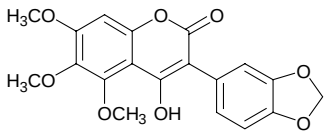
[**134**]

3.6 Coumarins

The seeds of *Millettia thonningii* have yielded the novel 3-phenylcoumarins, thonningine A [**136**] and thonningine B [**137**] (Khalid and Waterman, 1983). A new 3-phenylcoumarin [**138**] was isolated from *M. griffoniana* (Yankep *et al.*, 1998).



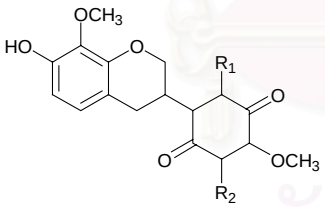
[**136**] R_1R_2 = -OCH_2O-, R_3 = R_4 = H
[**137**] R_1= H, R_2 = OCH_3, R_3 = R_4 = H



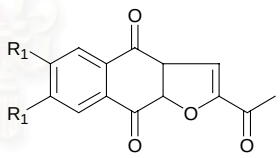
[**138**]

3.7 Quinones

A new isoflavan-quinone laurentiquinone [**139**] and its isomer [**140**] were isolated from the heartwood of *M. laurentii* (Kammaing *et al.*, 1999). An anti-inflammatory quinone [**141**] along with two known quinones **142** and **143** were isolated from the stem bark of *M. versicolor* (Fotsing, Yankep, Njamen *et al.*, 2003).



[**139**] R_1 = OCH_3, R_2 = H
[**140**] R_1 = H, R_2 = OCH_3

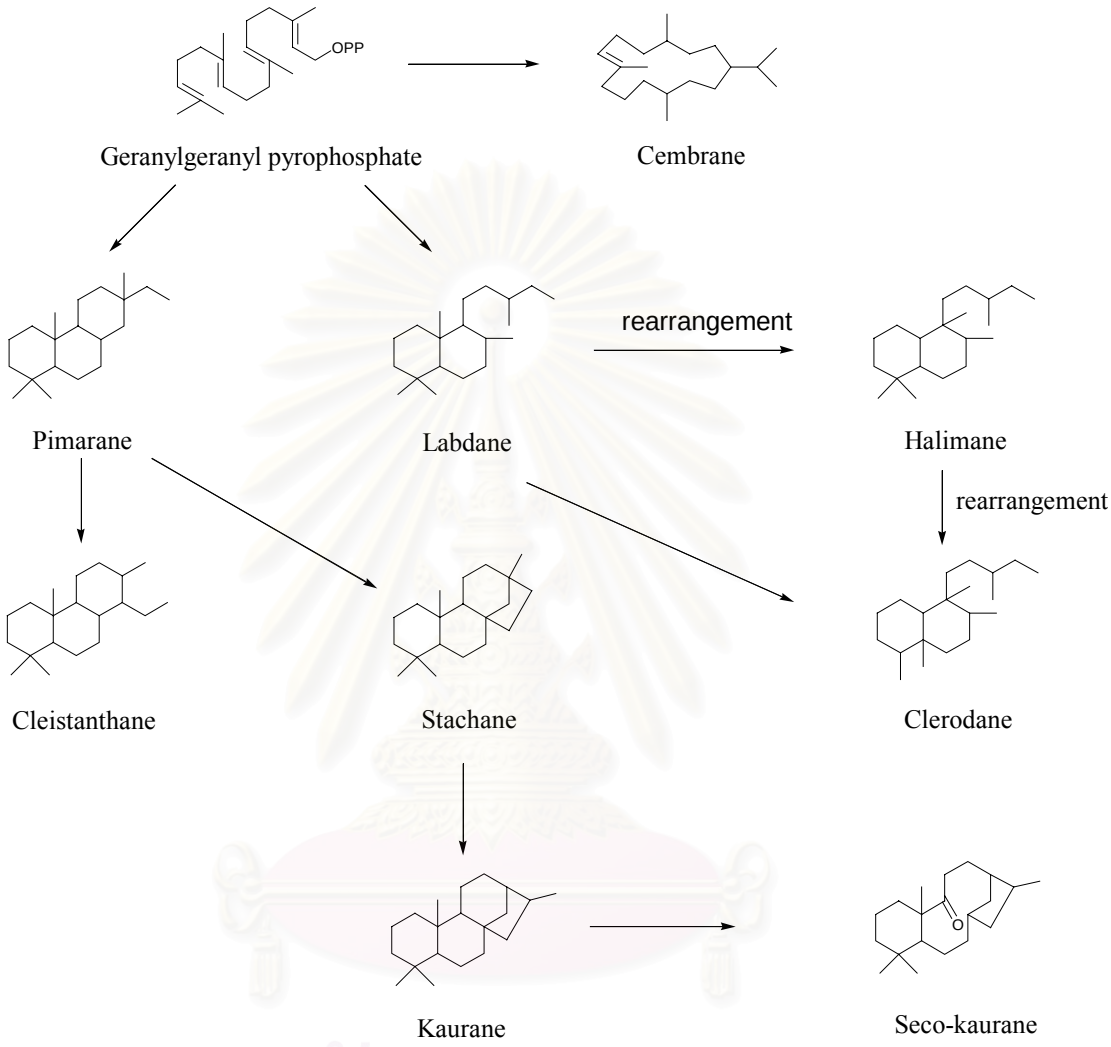


[**141**] R_1 = H, R_2 = OCH_3
[**142**] R_1 = R_2 = H
[**143**] R_1 = OCH_3, R_2= H

4. Biosynthetic Relationship of Diterpenoids in *Croton* spp.

The diterpenes possess twenty carbon atoms in their molecules. They are biogenetically derived from geranylgeranyl pyrophosphate (GGPP). The diterpene skeleton is the fascinating variation encountered in their core structure, these compounds could be classified into several types, such as mono-, bi-, tri-, tetra-, and pentacyclic diterpenes. The typical diterpenes in *Croton* spp. are casbane, cembrane,

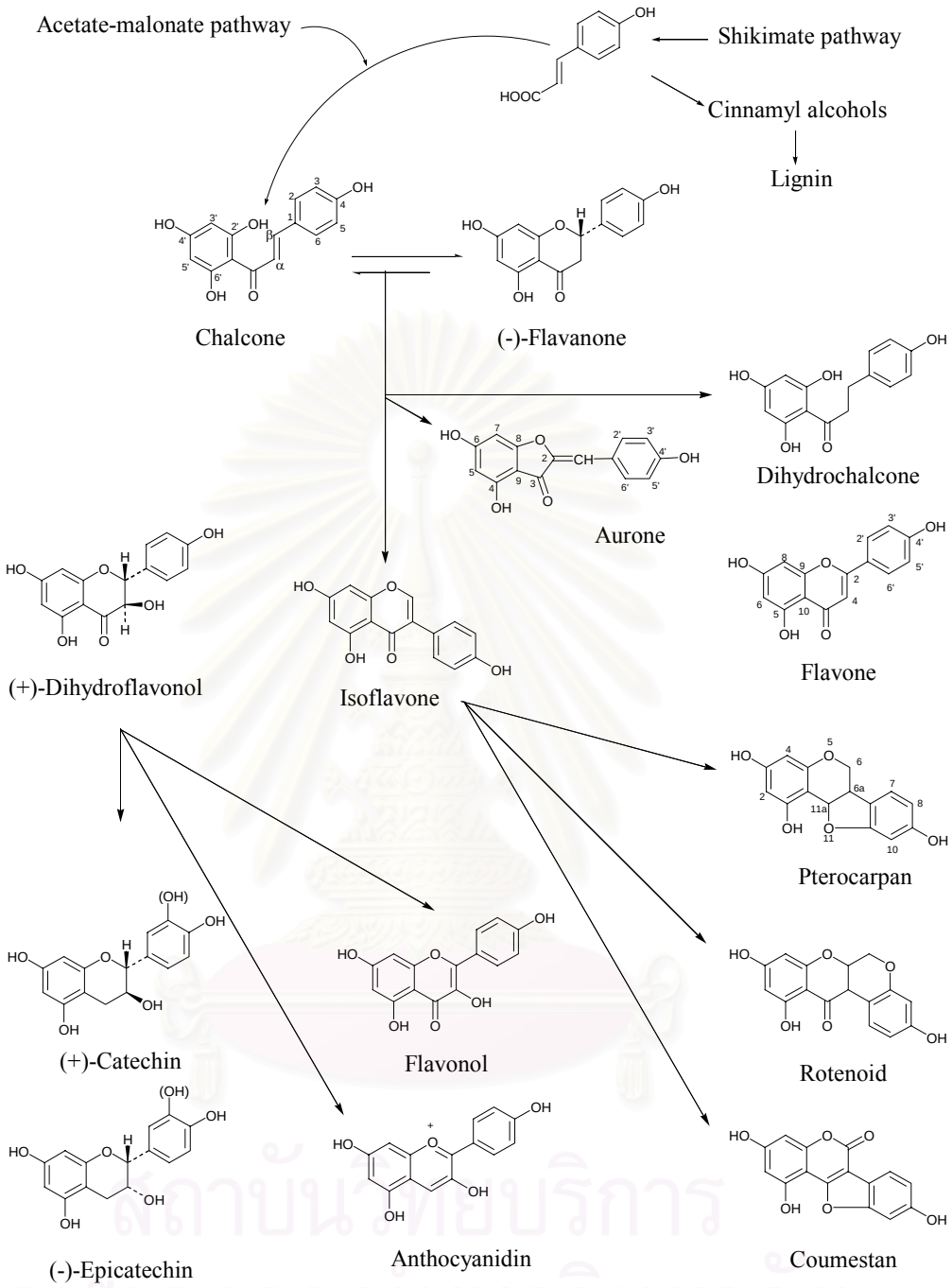
clerodane, cleistanthane, kaurane, labdane, pimarane, and halimane. The relationship of diterpenes is displayed in scheme 1. In addition, the biosynthetic is also proposed (Devon and Scott, 1972).



Scheme 1 Biosynthetic relationship of diterpenes in *Croton* spp.

5.Biosynthetic Relationship of Flavonoids in *Millettia* spp.

Flavonoids possess fifteen carbon atoms in their basic skeleton, which are derived from shikimate and acetate-malonate pathway. The typical flavonoids in *Millettia* spp. are flavanones, isoflavanones, flavanes, isoflavanes, flavones, isoflavones, chalcones, rotenoids, coumarins, and quinines. The relationship of flavonoids is displayed in **scheme 2**. (Markham, 1982).



Scheme 2 Currently proposed interrelationships between flavonoid monomer

CHAPTER III
EXPERIMENTAL

1. Source of Material

The leaves of *Croton kongensis* Gagnep. and the roots of *Croton birmanicus* Müll.Arg. were collected from Maetang District, Chiangmai Province, Northern Thailand, in November 2001. The voucher specimens of *C. kongensis* (No. NR 1291951) and *C. birmanicus* (No. NR 2291951) have been deposited at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

The leaves and twigs of *Millettia kangensis* Craib were collected from Maerim District, Chiangmai Province, Northern Thailand, in January 2002. The voucher specimen of *M. kangensis* (No. NR 3291951) has been deposited at the Faculty of Pharmaceutical Sciences, 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.

2.2.3 Gel Filtration Chromatography

Gel filter	:	Sephadex LH 20 (Phamacia).
Packing method	:	Gel filter was suspended in the eluent and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.
Sample loading	:	The sample was dissolved in a small volume of

eluent and applied on top of the column.

2.2.4 High Performance Liquid Chromatography

High pressure pump	:	Waters 600
Detector	:	Waters 996 Photodiode array detector.
Column	:	1. LiChroCart 250-10 HPLC-Cartridge
		2. PrepNova-Pak cartridge 40x100mm, 6μm 60˚A

2.3 Spectroscopy

2.3.1 Infrared (IR) Absorption Spectra

IR spectra (KBr disc and neat film) were obtained on a Bruker vector 22 spectrophotometer (National Center for Genetic Engineering and Biotechnology, BIOTEC, NSTDA, Thailand Science Park, Pathumthani, Thailand).

2.3.2 Ultraviolet (UV) Absorption Spectra

UV (in methanol and chloroform) spectra were recorded on a Cary 1E UV-visible spectrophotometer UVIDEC-650 (National Center for Genetic Engineering and Biotechnology, BIOTEC, NSTDA, Thailand Science Park, Pathumthani, Thailand).

2.3.3 Mass Spectra

Electrospray ionization mass spectra (ESIMS) were measured on a mass spectrometer LCT (LCMS) Micromass (National Center for Genetic Engineering and Biotechnology, BIOTEC, NSTDA, Thailand Science Park), and LCMS spectra were recorded on BRUKER mass spectrometer (Department of Chemistry, Faculty of Sciences, Mahidol University, Bangkok, Thailand).

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

^{1}H NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained on a BRUKER AV500D spectrometer, and in some experiments the spectra were obtained on a BRUKER DRX400 spectrometer (National Center for Genetic Engineering and Biotechnology, BIOTEC, NSTDA, Thailand Science Park, Pathumthani, Thailand).

2.4 Physical Properties

2.4.1 Optical Rotations

Optical Rotations were recorded in methanol and chloroform with sodium D line (589 nm) on a JASCO DIP-370 digital polarimeter (Department of Chemistry, Faculty of Sciences, Mahidol University, Bangkok, Thailand).

2.4.2 X-ray Crystallography

X-ray Crystallographic data were measured at room temperature on a Bruker Nonius kappa CCD diffractometer (Department of Chemistry, Faculty of Sciences, Mahidol University, Bangkok, Thailand).

2.5 Solvents

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

3. Extraction and Isolation

3.1 Extraction and Isolation of Compounds from *Croton kongensis*

3.1.1 Extraction

The dried powder leaves of *C. kongensis* (1 kg) were macerated in CH_2Cl_2 (2x4L). The extracts were filtered and evaporated under reduced pressure, to give green gummy crude extract (11.11 g).

3.1.2 Isolation of Compounds from CH_2Cl_2 Extract

The CH_2Cl_2 extract was dissolved in a lot of volumn of MeOH, filtered by filter paper, and then applied on the top of column. The fraction was isolated by gel filtration chromatography (on Sephadex LH-20), MeOH as eluent. Nine fractions (80 mL each) were collected. Fraction 5 was repeatedly chromatographed on a Sephadex LH-20 and a preperative HPLC (reversed-phase C_{18} column), to yield compounds CK01 and CK 02. Fraction 4 was subjected to Sephadex LH-20 chromatography, MeOH as eluent, to furnish compound CK04. Fraction 2 was re-chromatographed on Sephadex LH-20 and silica gel chromatography, furnishing compound CK02. Detail of isolation of the CH_2Cl_2 extract of *C. kongensis* are demonstrated in **Scheme 3**.

3.1.3 Isolation of Compounds CK 01 and CK 03

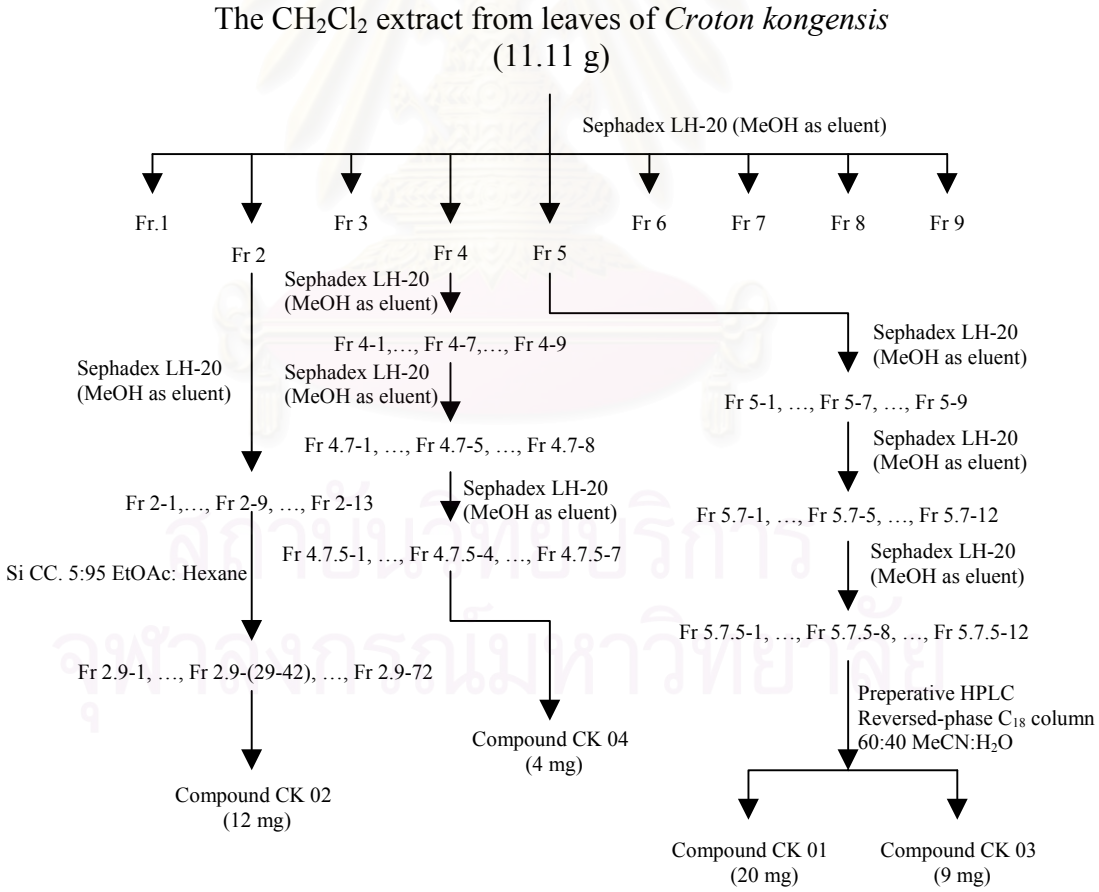
Fraction 5 was subjected to Sephadex LH-20 using MeOH as eluent, to afford nine fractions (50 mL per fraction). Fraction 7 was repeatedly chromatographed on a Sephadex LH-20 with MeOH, yielding twelve fractions. Fraction 5 was re-chromatographed on Sephadex LH-20 also using MeOH as eluent, to give twelve fractions. Isolation of fraction 8 by reverse phase C_{18} HPLC with 60:40 acetonitrile and water gave compounds CK 01 (20 mg) and CK 03 (9 mg).

3.1.4 Isolation of Compound CK 02

Fraction 2 was re-chromatographed on a Sephadex LH-20, MeOH as eluent. Fraction 9 was subjected to column chromatography using silica gel 60 (No. 9385) as adsorbent, 5:95 of ethyl acetate and hexane as mobile phase to furnish compound CK 02 (12 mg).

3.1.5 Isolation of Compound CK 04

Fraction 4 was subject on Sephadex LH-20, MeOH as eluent gave nine fractions (50 mL per fraction). Fraction 7 was repeatedly chromatographed on a Sephadex LH-20 with MeOH, yielding eight fractions. Purification fraction 4 by Sephadex LH-20 (MeOH as mobile phase) furnished compound CK 04 (4 mg).



Scheme 3 Separation of a CH_2Cl_2 extract from the leaves of *Croton kongensis*

3.2 Extraction and Isolation of Compounds from *Croton birmanicus*

3.2.1 Extraction

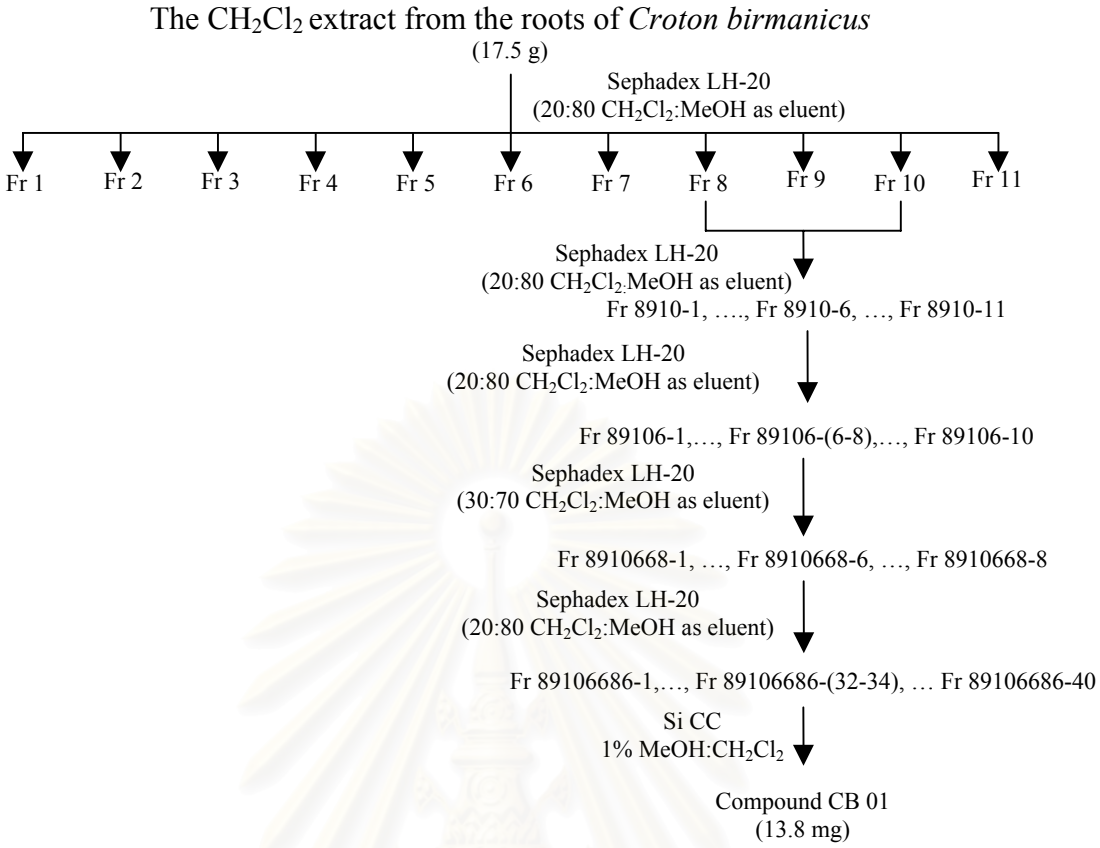
The dried roots of *C. birmanicus* (2 kg) were milled and macerated in CH_2Cl_2 (2x5L) and MeOH (2x5L). The extract was filtered and evaporated to dryness to give crude extract gummy (17.5 g for CH_2Cl_2 extract and 112.0 g for MeOH extract).

3.2.2 Isolation Compounds from CH_2Cl_2 Extract

The CH_2Cl_2 extract was dissolved in CH_2Cl_2:MeOH (20:80), filtered, and then applied to Sephadex LH-20 (MeOH as eluent), to give eleven frations (100 mL each). Fraction 4 was re-chromatographed on Sephadex LH-20 (MeOH as eluent), furnishing compound CB 01 as shown in **Scheme 4**.

3.2.3 Isolation of Compound CB 01

Fractions 8-10 were combined and further isolatated by Sephadex LH-20, 20:80 of MeOH and CH_2Cl_2 as eluent to yield eleven fractions. Fraction 6 was separated by Sephadex LH-20 with 20:80 of MeOH and CH_2Cl_2 as mobile phase, yielding eight fractions. Fraction 6-8 were combined and re-chromatographed on Sephadex LH-20 (30:70 of MeOH and CH_2Cl_2 as eluent), gave fourty fractions (10 mL per fraction). Fraction 32-34 were purified by silica gel 60 (No. 7734) as adsorbent, 1% MeOH/CH_2Cl_2 as mobile phase to furnish compound CB 01 (13.8 mg).



Scheme 4 Separation of a CH_2Cl_2 extract from the roots of *Croton birmanicus*

3.3 Extraction and Isolation of Compounds from *Millettia kangensis*

3.3.1 Extraction and Isolation of Compounds from the Leaves of *Millettia kangensis*

3.3.1.1 Extraction

The dried powder leaves (4.3 kg) of *M. kangensis* was extracted with $CHCl_3$ (2x15L), filtered and evaporated, yielding deep green gum (52 g).

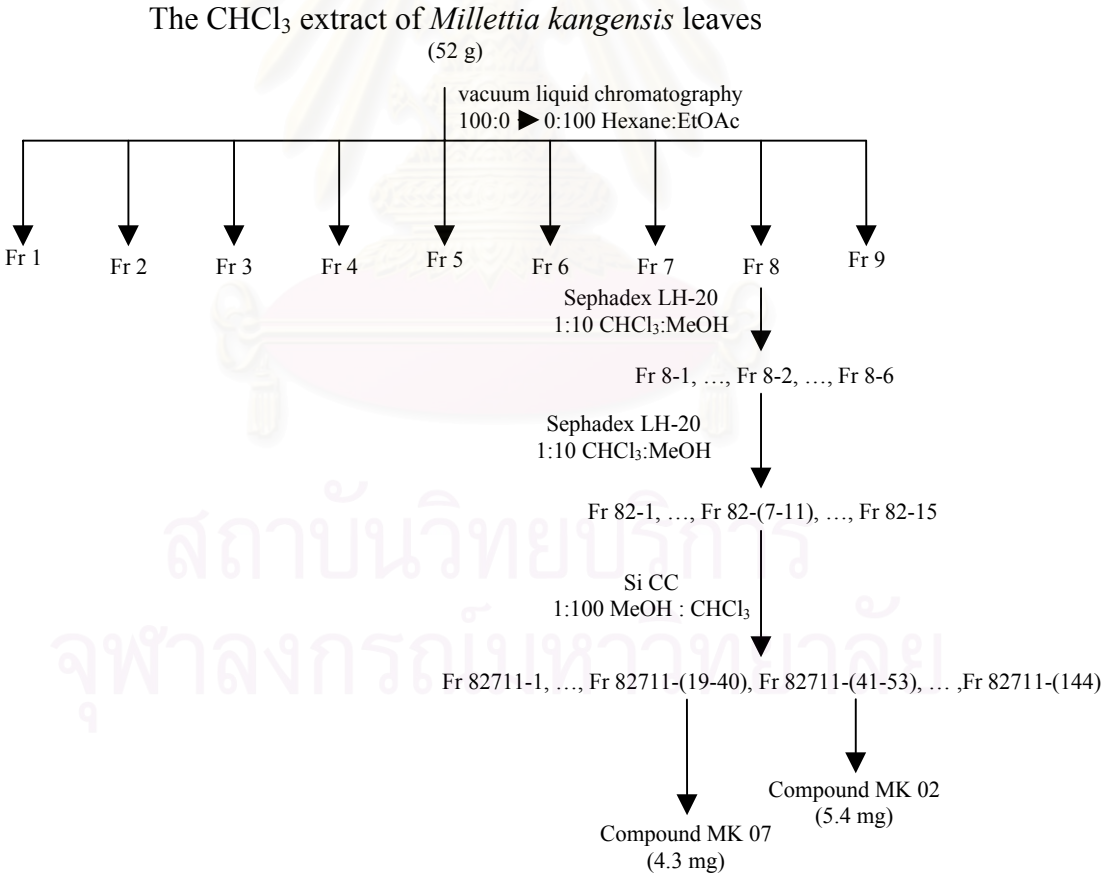
3.3.1.2 Isolation of compounds from $CHCl_3$ extract

The $CHCl_3$ extract (52 g) was dissolved in a small volume of $CHCl_3$, triturated with silica gel 60 (No. 7734), and dried under vacuum. It was separated by vacuum liquid column chromatography using a sintered glass filter

column of silica gel 60 (No. 7734). Fractions were collected (250 ml). Elution was performed in a polarity gradient manner with mixtures of hexane and ethyl acetate (100:0 to 0:100). Sixty fractions were collected, and combined similar fractions by TLC, yielding nine fractions. Fraction 8 was isolated, yielding compounds MK 02 and 07.

3.3.1.3 Isolation of compounds MK 02 and MK 07

Fraction 8 was subjected to Sephadex LH-20 (1:10 $CHCl_3$:MeOH as eluent), yielding six fractions (50 mL per fraction). Fraction 2 was re-chromatographed on Sephadex LH-20 (1:10 $CHCl_3$:MeOH as eluent), which gave fifteen fractions (25 mL per fraction). Fractions 7-11 were isolated, yielding compounds MK 02 (5.4 mg) and 07 (.3 mg).



Scheme 5 Separation of a $CHCl_3$ extract from the leaves of *Millettia kangensis*

3.3.2 Extraction and Isolation of Compounds from the Twigs of *Millettia kangensis*

3.3.2.1 Extraction

The dried twigs (0.8 Kg) of *M. kangensis* were macerated in CH_2Cl_2 (2x3L). The extracts were filtered and evaporated under reduced pressure, to yield a deep green gum (11.4 g).

3.3.2.2 Isolation of compounds of CH_2Cl_2 extract

The CH_2Cl_2 extract was fractionated by Sephadex LH-20 (MeOH as eluent), yielding eight fractions (each 100 mL). Recrytalization of fraction 5 afforded compound MK 06. Fraction 6 was isolated by Sephadex LH-20 and HPLC, yielding compounds MK 03 and MK 04. Fraction 7 was refractionated with Sephadex LH-20 and HPLC to give compounds MK 03 and MK 06. Fraction 8 was isolated with Sephadex LH-20 and HPLC, yielding compounds MK 03, MK 05 and MK 01.

3.3.2.3 Isolation of compound MK 01

Fraction 8 was fractionated by Sephadex LH-20 (MeOH as eluent), yielding eight fractions (50 mL per fraction). Fraction 4 was isolated by RP-C_{18} HPLC with 40:60 MeCN:H_2O, to yield compounds MK 01 (1.7 mg), MK 03 (2.6 mg), and MK 06 (6.8 mg).

3.3.2.4 Isolation of compound MK 03

Fraction 7 was isolated by Sephadex LH-20 (MeOH as eluent), obtaining as nine fractions (50 mL per fraction). Fraction 7 was purified by RP-C_{18} HPLC (35:65 MeCN:H_2O) to yield three fractions, while fraction 1 gave compound MK 03 (12.2 mg). Fraction 2 was isolated by RP-C_{18} HPLC (40:60 MeCN:H_2O), furnished compounds MK 03 (1.3 mg) and MK 05 (6.4 mg).

Fraction 8 was fractionated by Sephadex LH-20 (MeOH as eluent), yielding eight fractions (50 mL per fraction). Fraction 4 was isolated by RP-C_{18} HPLC with 40:60 MeCN:H_2O, to yield compounds MK 01 (1.7 mg), MK 06 (6.8 mg), and MK 03 (2.6 mg).

3.3.2.5 Isolation of compound MK 04

Fraction 6 was isolated by Sephadex LH-20 (MeOH as eluent), yielding nine fractions (30 mL per fraction). Fraction 5 was purified by RP-C_{18} HPLC with 25:75 MeOH:H_2O, to yield compounds MK 04 (12.2 mg) and MK 03 (26.4).

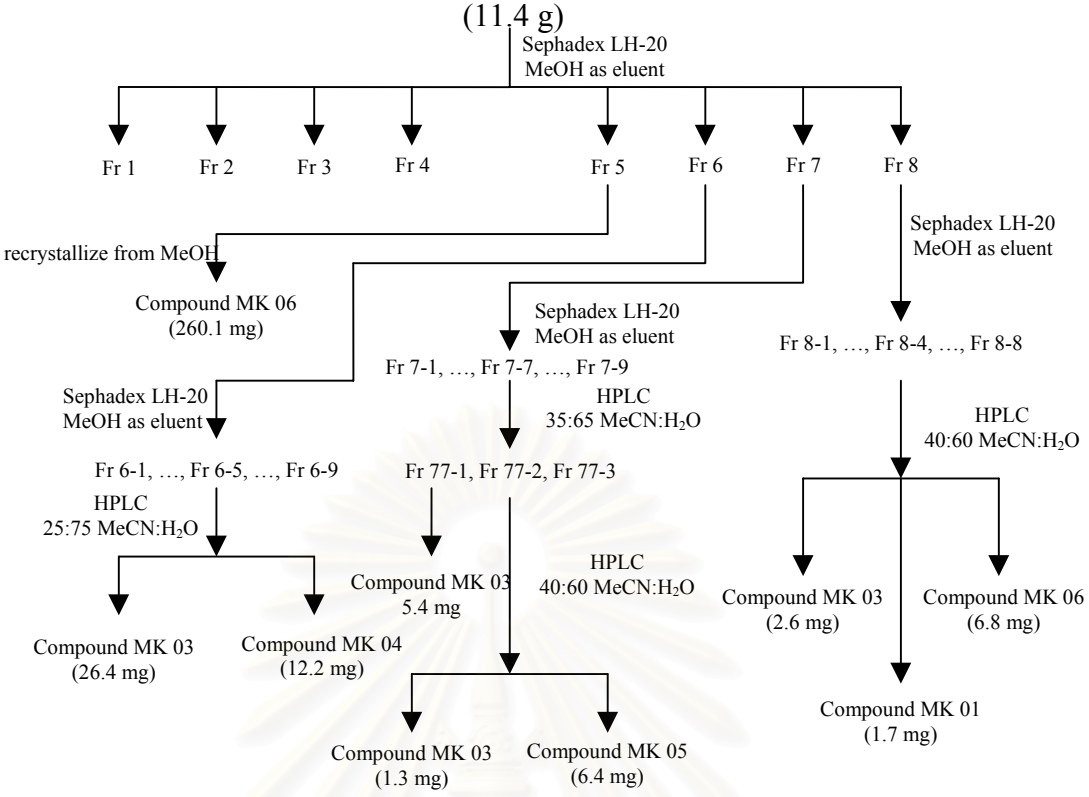
3.3.2.5 Isolation of compound MK 05

Fraction 7 was isolated by Sephadex LH-20 (MeOH as eluent), obtaining as nine fractions (50 mL per fraction). Fraction 7 was purified by RP-C_{18} HPLC (35:65 MeCN:H_2O) to yield three fractions,while fraction 1 gave compound MK 03 (12.2 mg). Fraction 2 was isolated by RP-C_{18} HPLC (40:60 MeCN:H_2O), which furnished compounds MK 05 (6.4 mg) and MK 03 (1.3 mg).

3.3.2.5 Isolation of compound MK 06

Fraction 5 was recrystallized from MeOH, yielding compound MK 05 (260.1 mg).

Fraction 8 was fractionated by Sephadex LH-20 (MeOH as eluent), yielding eight fractions (50 mL per fraction). Fraction 4 was isolated by RP-C_{18} HPLC with 40:60 MeCN:H_2O, to yield compounds MK 06 (6.8 mg), MK 01 (1.7 mg), and MK 03 (2.6 mg).



Scheme 6 Separation of CH_2Cl_2 extract from the twigs of *Millettia kangensis*

4. Physical and Spectral Data of Isolated Compounds

4.1 Compound CK 01

Compound CK 01 was obtained as colourless oil, soluble in $CHCl_3$ (20.0 mg, 2.0 x 10^{-3}% based on dried weight of leaves).

UV : λ_{max} nm (log ε), in $CHCl_3$; 239.2 (4.46), see **Figure 4**

IR : ν_{max} cm^{-1}, neat ($CHCl_3$); 3444, 1647, see **Figure 5**

EITOFMS : *m/z*; 375.2412 $[M+H]^+$, see **Figure 6**

$[\alpha]^{30}_D$: -54.4° (*c* 0.45, $CHCl_3$)

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$, see **Table 2**, and **Figure 7**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$, see **Table 2**, and **Figure 8**

4.2 Compound CK 02

Compound CK 02 was obtained as brown oil, soluble in $CHCl_3$ (12.0 mg, 1.2 x 10^{-3}% based on dried weight of leaves).

UV : λ_{max} nm (log ε), in $CHCl_3$; 241.4 (4.07) , see **Figure 13**

IR : ν_{max} cm^{-1}, neat ($CHCl_3$); 2928, 1743, 1704, 1653, 1624, 1370, 1231, 1022, 932, see **Figure 14**

EITOFMS : *m/z* ; 439.2089 $[M+Na]^+$, see **Figure 15**

$[\alpha]^{30}_D$: -147.6° (*c* 0.575, $CHCl_3$)

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$, see **Table 3**, and **Figure 16**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$, see **Table 3**, and **Figure 17**

4.3 Compound CK 03

Compound CK 02 was obtained as brown oil, soluble in $CHCl_3$ (9.0 mg, 9.0 x 10^{-4} % based on dried weight of leaves).

UV : λ_{max} nm (log ε), in $CHCl_3$; 233.2 (3.48) nm, see **Figure 22**

IR : ν_{max} cm^{-1}, neat ($CHCl_3$); 3445, 1748, 1733, 1697, 1684, 1646, 1636, 1558, 1540, 1374, 1219, see **Figure 23**

EITOFMS : *m/z* ; 413.1969 $[M+Na]^+$, see **Figure 24**

$[\alpha]^{30}_D$: -16.7° (*c* 0.45, $CHCl_3$)

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$, see **Table 4**, and **Figure 25**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$, see **Table 4**, and **Figure 26**

4.4 Compound CK 04

Compound CK 04 was obtained as colourless oil, soluble in $CHCl_3$ (4.0 mg, 4.0 x 10^{-4}% based on dried weight of leaves).

UV : λ_{max} nm (log ε), in $CHCl_3$; 242.8 (3.68) , see **Figure 31**

IR : ν_{max} cm^{-1}, neat ($CHCl_3$); 3443, 1635 , see **Figure 32**

EITOFMS : *m/z* ; 383 $[M+Na]^+$, see **Figure 33**

$[\alpha]^{30}_D$: -4.0˚ (*c* 1.0, $CHCl_3$)

^1H NMR : δ ppm, 500 MHz, in $CDCl_3$, see **Table 5**, and **Figure 34**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$, see **Table 5**, and **Figure 35**

4.5 Compound CB 01

Compound CK 05 was obtained as brown oil, soluble in $CHCl_3$ (13.8 mg, 6.9 x 10^{-4}% based on dried weight of roots).

UV : λ_{max} nm (log ε), in $CHCl_3$; 214 (2.47) , see **Figure 40**

IR : ν_{max} cm^{-1}, film; 350, 3063, 2931, 2856, 1726 and 1682 , see **Figure 41**

ESIMS : *m/z* ; 339.2 $[M+Na]^+$, see **Figure 42**

$[\alpha]^{30}_D$: -20.6˚ (*c* 0.825, $CHCl_3$)

^1H NMR : δ ppm, 400 MHz, in $CDCl_3$, see **Table 6**, and **Figure 43**

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$, see **Table 6**, and **Figure 44**

4.6 Compound MK 01

Compound MK 01 was obtained as colourless crystal, soluble in $CHCl_3$ (1.7 mg, 1.7 x 10^{-4}% based on dried weight of twigs).

UV : λ_{max} nm (log ε), in MeOH; 203 (3.16), 217 (3.25), 260 (3.13) and 303 (2.95), see **Figure 49**

IR : ν_{max} cm^{-1}, film; 3450, 2927,1637,1624 and 1458, see **Figure 50**

EITOFMS : *m/z* ; 293.0818 $[M+H]^+$, see **Figure 51**

^1H NMR : δ ppm, 400 MHz, in $CDCl_3$, see **Table 7**, and **Figure 52**

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$, see **Table 7**, and **Figure 53**

4.7 Compound MK 02

Compound MK 02 was obtained as colourless plates, soluble in DMSO (5.4 mg, 1.3 x 10^{-4}% based on dried weight of leaves).

UV : λ_{max} nm (log ε), in MeOH; 280 (3.02) and 308 (3.17), see **Figure 58**

IR : ν_{max} cm^{-1}, film; 3265, 1593, 15661, 1483, 1466, 1396, 1358, 1316, 1229 and 1136, see **Figure 59**

ESIMS : *m/z* ; 331.3 $[M+Na]^+$, see **Figure 60**

^{1}H NMR : δ ppm, 400 MHz, in DMSO-*d6*, see **Table 8**, and **Figure 61**

^{13}C NMR : δ ppm, 100 MHz, in DMSO-*d6*, see **Table 8**, and **Figure 62**

4.8 Compound MK 03

Compound MK03 was obtained as colourless plates, soluble in $CHCl_3$ (35.7 mg, 3.6 x 10^{-3}% based on dried weight of twigs).

UV : λ_{max} nm (log ε), in MeOH; 203 (2.26), 206 (2.61) and 306 (2.48), see **Figure 67**

IR : ν_{max} cm^{-1}, film; 2918, 2849, 1753 and 1473 cm^{-1}, see **Figure 68**

EITOFMS : *m/z* ; 345.48 $[M+Na]^+$, see **Figure 69**

^{1}H NMR : δ ppm, 400 MHz, in $CDCl_3$, see **Table 9**, and **Figure 70**

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$, see **Table 9**, and **Figure 71**

4.9 Compound MK 04

Compound MK04 was obtained as colourless plates, soluble in $CHCl_3$ (12.2 mg, 1.2 x 10^{-3}% based on dried weight of twigs).

UV : λ_{max} nm (log ε), in $CHCl_3$; 282 (2.77), 312 (2.31) and 348 (2.04), see **Figure 76**

IR : ν_{max} cm^{-1}, film; 2919, 2845, 1735, 1473, 1463 and 1372, see **Figure 77**

EITOFMS : *m/z* ; 323.0919 $[M+H]^+$, see **Figure 78**

^{1}H NMR : δ ppm, 400 MHz, in $CDCl_3$, see **Table 10**, and **Figure 79**

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$, see **Table 10**, and **Figure 80**

4.10 Compound MK 05

Compound MK05 was afforded as colourless crystals, soluble in $CHCl_3$ (6.4 mg, 6.4 x 10^{-4}% based on dried weight of twigs).

UV : λ_{max} nm (log ε), in $CHCl_3$; 203 (2.11), 271 (2.62), 309 (2.05), see **Figure 85**

$[\alpha]^{30}_D$ -12.4° (*c* 0.50, $CHCl_3$)

^{1}H NMR : δ ppm, 400 MHz, in $CDCl_3$, see **Table 11**, and **Figure 86**

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$, see **Table 11**, and **Figure 87**

4.11Compound MK 06

Compound MK06 was obtained as colourless needles, soluble in $CHCl_3$ (266.9 mg, 2.7 x 10^{-2}% based on dried weight of leaves).

UV : λ_{max} nm (log ε), in $CHCl_3$; 214 (2.56), 246 (2.93), 280 (2.75) and 340 (2.71), see **Figure 92**

IR : ν_{max} cm^{-1}, film; 2923 and1615, see **Figure 93**

EITOFMS : *m/z* ; 387.1204 $[M+Na]^+$, see **Figure 94**

^{1}H NMR : δ ppm, 400 MHz, in $CDCl_3$, see **Table 12**, and **Figure 95**

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$, see **Table 12**, and **Figure 96**

4.12 Compound MK 07

Compound MK07 was displayed as colourless plates, soluble in DMSO (4.3 mg, 1.0 x 10^{-4}% based on dried weight of leaves).

UV : λ_{max} nm (log ε), in $CHCl_3$; 280 (2.55), 302 (2.84), 343 (2.94) and 358 (3.16) , see **Figure 101**

IR : ν_{max} cm^{-1}, film; 3374, 1731, 1621 and 1469, see **Figure 102**

EITOFMS : *m/z* ; 365.2 $[M+Na]^+$, see **Figure 103**

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$, see **Table 13**, and **Figure 104**

^{13}C NMR : δ ppm, 125 MHz, in $CDCl_3$, see **Table 13**, and **Figure 105**

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, Canfield, Haynes *et al.*, 1979).

5.3 Cytotoxic Activity

Cytotoxicity was determined by employing the colorimetric method described by Skehan and co-workers. The reference compound, ellipticine, exhibited activity toward Vero, KB and BC cell lines with the IC_{50} ranges of 0.2-0.3 μg/mL (Skehan, Storeng, Scudiero *et al.*, 1990).

CHAPTER IV

RESULTS AND DISSCUSSION

Preliminary bioactivity screening revealed that *Croton kongensis*, *Croton birmanicus*, and *Millettia kangensis* exhibited antimycobacterial and antimalarial activities. These results of bioactivities are summarized in **Table 1**.

Table 1 Antimycobacterial and antimalarial activities of the crude extract

Crude extract	Antimycobacterial activity MIC (μg/mL)	Antimalarial activity IC_{50} (μg/mL)
C. kongensis		
The CH_2CL_2 leaves extract	12.50	0.90
The MeOH leaves extract	100.00	5.79
C. birmanicus		
The CH_2Cl_2 roots extract	100.00	inactive
M. kangensis		
The CH_2Cl_2 leaves extract	100.00	inactive
The CH_2Cl2 twigs extract	100.00	inactive

The CH_2Cl_2 leaves extract of *C. kongensis* were isolated, yielding three 8,9-secokauranes (**CK 01-03**) and a kaurane (**CK 04**). Compounds CK 01 and CK 02 are new compounds. The CH_2Cl_2 roots extract of *C. birmanicus* was isolated, furnishing a glutarimide alkaloid (**CB 01**). The CH_2Cl_2 leaves and twigs extracts from *M. kangensis* afforded five furanoflavonoids (**MK 01-05**), a pyranoflavonoid (**MK 06**), and a coumestan (**MK 07**). Compounds MK 03, 05, 06 and 07 are new compounds, while compound MK 04 is a new natural product. Structure elucidation of these compounds was performed by interpretion of their UV, IR, NMR, MS, and X-ray crystallographic data, and by comparison with previous reports. In addition, their antimycobacterial, antimalarial, and cytotoxic activities are displayed in **Table 14**.

Although there have been various classes of diterpenoids isolated from genus *Croton*, the presence of 8,9-secokauranes has never been before reported. The 8,9-secokauranes have been reported from two liverwort species and from several species

in the higher plant genus *Rabdisia* (Family Lamiaceae). This is the first report on the presence of 8,9-secokauranes in the plant genus *Croton*. Additionally, the presence of coumestan, the rare derivative of isoflavonoid, has never been before reported from genus *Millettia*. Coumestan, erosnin, has been reported from *Pachyrrhizus erosus* in 1977. This is the first report of coumestan skeleton in the plant genus *Millettia*.

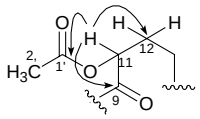
1. Structure Elucidation of Compounds Isolated from *Croton kongensis*

1.1 Structure Elucidation of Compound CK 01

A compound CK 01 was obtained as colourless oil. CK 01 possessed a molecular formula $C_{22}H_{30}O_5$, as revealed by the ESITOFMS spectrum, showing a prominent peak at *m/z* 375.2412 $[M+H]^+$(**Figure 6**). The IR spectrum displayed OH stretching at ν 3444 cm^{-1}, and C=O stretching at ν 1647 cm^{-1}**(Figure 5)**. The UV absorption showed λ_{max} at 243 nm **(Figure 4)**.

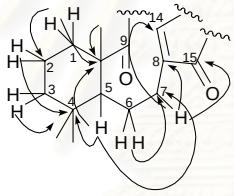
The ^{1}H-NMR spectrum ($CDCl_3$) (**Figure 7**) of compound CK01 showed signals of four methyl singlets at δ_H 0.88 (3H), 1.05 (3H), 1.17 (3H), and 2.0 (3H), two singlet signals of exocyclic methylene at δ_H 5.33 (1H) and 5.95 (1H), a broad singlet signal of olefinic proton at δ_H 7.28 (1H), two doublet of doublet signals of δ_H 4.72 (1H, J = 11.9, 4.6 Hz) and δ_H 5.25 (1H, J = 5.4, 1.2 Hz), a broad singlet signal of methine proton at δ_H 3.6 (1H), doublet of quartet and doublet of doublet of doublet signals of methylene protons at δ_H 2.34 (1H, J = 14.7, 2.7 Hz) and δ_H 2.95 (1H, J = 14.7, 5.0, 1.2 Hz), and four methylene protons at δ_H 1.95-1.15.

The ^{13}C-NMR spectrum (**Figure 8**) of compound CK01 revealed 22 signals, while DEPT135 spectrum **(Figure 8)** revealed four methyl carbons, six methylene carbons, five methine, and seven quaternary carbons. A carbonyl carbon and a methyl group were resonanced at δ_C 172.0 and 22.0, a characteristic of an acetate group. The downfield shift at δ_H 5.25 (H-11) and the HMBC **(Figure 12)** spectrum demonstrated that correlation from H-11 to δ_C 172.0 (C-1′), 31.0 (C-12), and 213 (C-9), establishing the first substructure of CK 01 as shown below.



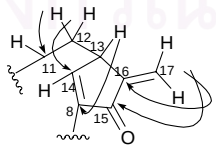
→ ^{1}H-^{13}C HMBC

The ^{1}H-^{1}H COSY spectrum (**Figure 9**) of compound CK01 displayed cross peaks from H-2 (δ_H 1.50) to H-1 (δ_H 1.60) and δ_H H-3 (1.15), from H-5 (δ_H 1.80) to H-6 (δ_H 1.92), while the HMBC spectrum revealed correlations from CH_3-20 (δ_H 1.05) to C-10 (δ_C 34.0), from H-1 (δ_H 1.26) to C-10 (δ_C 34.0), from H-1 (δ_H 1.60) to C-2 (δ_C 17.7), from H-2 (δ_H 1.5) to δ_C C-4 (54.0), from H-3 (δ_H 1.51) to C-4 (δ_C 54.0), from CH_3-19 (δ_H 1.17) to C-4 (δ_C 54.0), from H-5 (δ_H 1.8) to C-7 (δ_C 64.0), C-4 (δ_H 54.0), C-10 (δ_C 34.0), from H-5 (δ_H 1.8) to C-7 (δ_C 64), C-4 (δ_C 54), and C-10 (δ_C 34), from H-6 methylene (δ_H 1.95 and 1.52) to C-5 (δ_C 41.0), C-10 (δ_C 34), and C-7 (δ_C 64), and from H-7 (δ_H 4.72) to C-14 (δ_C 160), C-8 (δ_C 148.5), and C-15 (δ_C 194.5). Based on these spectral data, the second substructure was created as shown.



→ ^{1}H-^{13}C HMBC

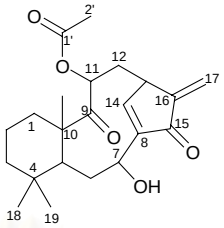
A typical of exocyclic methylene was found in CK 01, exhibiting two singlet resonances at δ_H 5.33 (1H, *s*) and δ_H 5.95 (1H, *s*). The HMBC revealed the correlation from H-17 (δ_H 5.33 and δ_H 5.95) to C-16 (δ_C 148), and C-15 (δ_C 194.5), from H-12 (δ_H 2.35 and 2.95) to C-14; from H-13 (δ_H 3.6) to C-8 (δ_C 148.5); and from H-12 to C-11. These spectral data established the third partial structure of CK 01.



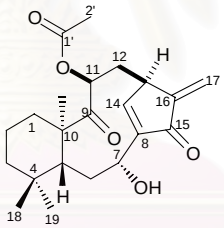
→ ^{1}H-^{13}C HMBC

Combination of the first, second and the third fragments well assembled a gross structure of CK 01. Therefore compound CK 01 was identified as *ent*-8,9-*seco*-7α-hydroxy-11-acetoxykaura-8(14),16-dien-9,15-dione, which is a known compound

previously isolated from a New Zealand liverwort, *Lepidolaena taylorii* (Perry, Burgess, Baek *et al.* 1999).



CK 01 exhibited negative optical rotation ($[\alpha]^{30}_{D}$ -54.4˚, *c* 0.45, $CHCl_3$) similar to *ent*-8,9-*seco*-7*α*-hydroxy-11-acetoxykaura-8(14),16-dien-9,15-dione, and therefore compound CK 01 possessed the same stereochemistry as that of *ent*-8,9-*seco*-7*α*-hydroxy-11-acetoxykaura-8(14),16-dien-9,15-dione. Proton and carbon of compound CK 01 were completely assigned by analyses of ^{1}H-^{1}H COSY (**Figure 9**), NOESY (**Figure 10**), HMQC (**Figure 11**) and HMBC (**Figure 12**) spectral data as shown in **Table 2**.



Compound CK 01

Table 2: The ^{1}H and ^{13}C-NMR Spectral Data of *ent*-8,9-*seco*-7*α*-Hydroxy-11-acetoxykaura-8(14),16-dien-9,15-dione and Compound CK 01 in $CDCl_3$

Position	*ent*-8,9-*seco*-7*α*-hydroxy-11-aceto xykaura-8(14),16-dien-9,15-dione (Perry *et al.*, 1999).		Compound CK 01	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	No report	31.8	1.26 (1H, *m*) 1.60 (1H, *m*)	31.6
2	No report	17.9	1.50 (1H, *m*) 1.65 (1H, *m*)	17.7
3	No report	41.5	1.15 (1H, *m*) 1.51 (1H, *m*)	41.4
4	-	34.3	-	34.0
5	1.73 (*dd*, 6, 2)	40.6	1.8 (1H, *br d*, 6.1)	41.0
6 (*S*) 6 (*R*)	1.90 (*ddd*, 13, 6, 5) 1.45	32.4	1.95 (1H, *br d*, 1.0) 1.52 (1H, *br d,* 1.6)	31.0
7	4.71 (*dd*, 12, 4)	63.8	4.72 (1H, *dd*, 11.9, 4.6)	64.0
8	-	148.5	-	148.5
9	-	212.2	-	213.0
10	-	54.7	-	54.0
11	5.23 (*dd*, 5, 1)	77.7	5.25 (1H, *dd*, 5.5, 1.2)	76.0
12 (*R*) 12 (*S*)	2.91 (*ddd*, 14, 5, 2) 2.32 (*ddd*, 15, 6, 3)	37.1	2.95 (1H, *ddd*, 14.7, 5.0,1.2) 2.34 (1H, *dq*, 14.7, 2.7)	37.0
13	3.57 (*br m*)	41.0	3.60 (1H, *br s*)	41.0
14	7.25 (*br d*, 3)	159.1	7.28 (1H, *d*, 2.8)	160.0
15	-	194.7	-	194.5
16		148.2	-	148.0
17 (*E*) (*Z*)	5.24 (*br s*) 5.88 (*br s*)	113.0	5.33 (1H, *s*) 5.95 (1H, *s*)	112.0
18	(*ax*) 0.95 (*s*)	34.1	0.88 (3H, *s*)	34.0
19	(*eq*) 1.03 (*s*)	22.2	1.17 (3H, *s*)	20.7
20	1.01 (*s*)	18.3	1.05 (3H, *s*)	18.0
1′	-	169.1	-	172.0
2′	-	20.8	2.0 (3H, *s*)	22.0

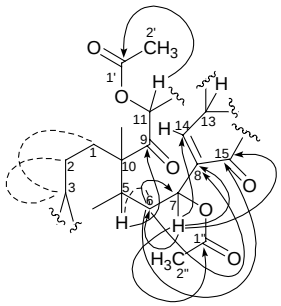
1.2 Structure Elucidation of Compound CK 02

A compound CK 02 was obtained as brown oil. A molecular formula of $C_{24}H_{32}O_6$, [*m/z* 439.2089 $[M+Na]^+$, calculated for 439.2097] was obtained from the ESITOFMS (**Figure 15**). The IR absorption (**Figure 14**) showed bands of C=O stretching at *v* 1743 and 1704 cm^{-1}, and the UV spectrum (**figure 13**) displayed λ_{max} at 214 nm. The optical rotation of CK02 was negative, $[\alpha]^{30}_D$ -147.6˚ (*c* 0.575, $CHCl_3$).

The ^{1}H-NMR spectrum ($CDCl_3$) (**Figure 16**) of compound CK02 displayed signals of five methyl singlets at δ_H 1.14 (3H, *s*), 0.97 (3H, *s*), 1.06 (3H, *s*), 2.05 (3H, *s*) and 2.02 (3H, *s*), three methine doublet of doublets at δ_H 2.12 (1H, *dd*, *J* = 7.2, 0.2 Hz), δ_H 5.55 (1H, *dd*, *J* = 12.2, 4.5 Hz), δ_H 5.28 (1H, *dd*, *J* = 5.5, 1.3), a broad singlet methine at δ_H 3.61 (1H, *br s*), two methylene doublet of doublet of doublets at δ_H 2.36 (1H, *ddd*, *J* = 14.6, 5.4, 2.8 Hz) and δ_H 2.95 (1H, *ddd*, *J* = 14.6, 5.0, 1.3 Hz), two olefinic broad singlets at δ_H 5.31 (1H, *br s*) and δ_H 5.94 (1H, *br s*), and five methylene multiplets at δ_H 1.26-0.98.

The ^{13}C NMR spectrum (**Figure 17**) of compound CK02 revealed 24 signals, and the DEPT135 (**Figure 17**) spectral data revealed the presence of five methyl carbons, six methylene carbons, five methine carbons, and eight quaternary carbons. Two acetate groups were found in CK 02, having two singlet resonances of carbonyl at C-1′ (δ_C 169.0) and C-1″ (δ_C 169.7), two singlet resonances of methyl groups at C-2′ (δ_C 20.6) and C-2″ (δ_C 20.9). In addition, the HMBC spectral data (**Figure 21**) demonstrated the correlation from H-11 (δ_H 5.28) to C-1′ (δ_C 169.0), and from H-7 (δ_H 5.55) to C-1″ (δ_C 169.7), placing an acetate at C-11 and C-7, respectively.

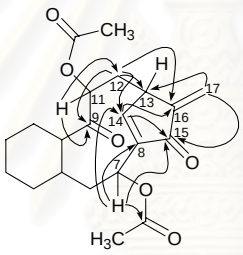
The ^{1}H-^{1}H COSY spectrum **(Figure 18)** of compound CK 02 revealed correlations from H-1(δ_H 1.26) to H-3 (δ_H 1.59), from H-2 (δ_H 1.48) to H-3 (δ_H 1.59), from H-5 (δ_H 1.81) to H-6 (δ_H 1.98), and from H-7 (δ_H 5.55) to H-6 (δ_H 1.45 and 1.98). The HMBC spectrum of CK 02 demonstrated correlations from H-5 to C-9 (δ_C 212.1), from H-7 to C-8 (δ_C 145), C-14 (δ_C 159.3), C-1″(δ_C 169.7), C-6 (δ_C 32.3), and C-15 (δ_C 193.5), and from H-6 to C-8 (δ_C 145.0), C-7 (δ_C 66.3). Therefore, the first substructure of CK 02 was assembled as shown



^{1}H-^{1}H COSY

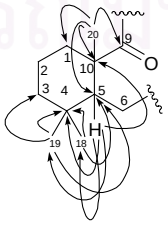
^{1}H-^{13}C HMBC

The ^{1}H-NMR spectrum of CK 02 displayed two broad singlet signals at δ_H 5.31 and δ_H 5.94, attributable to an exocyclic methylene. The HMBC correlations from H-7 (δ_H 5.55) to C-8, C-14, C15 and C-1′, from H-11 (δ_H 5.28) to C-9 and C-11, from H-12 (δ_H 2.36 and 2.95) to C-9, C-13, C-14 and C-16, from H-14 (δ_H 7.23) to C-15 and C-16, and from H-17 (δ_H 5.31 and 5.94) to C-13 and C-16. The construction of the second partial structure was by analyses of the above spectral data..



^{1}H-^{13}C HMBC

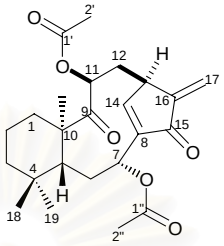
The HMBC spectrum displayed correlations from CH_3-18 (δ_H 1.14) to C-4 (δ_C 34.2), C-3 (δ_C 41.4) and C-5 (δ_C 40.4), from CH_3-19 (δ_H 0.97) to C-4 (δ_C 34.2), C-3 (δ_C 41.4) and C-5 (δ_C 40.4), from CH_3-20 (δ_H 1.06) to C-1 (δ_C 32.3), C-5 (δ_C 40.4), C-9 (δ_C 212.1), and C-10 (δ_C 54.6), and from H-5 (δ_H 1.81) to C-4 (δ_C 34.2), and C-5 (δ_C 40.4). Therefore, the third partial structure is created.



^{1}H-^{13}C HMBC

On the basis of these spectral data compound CK 02 was assigned as an acetate derivative of the known *ent*-8,9-*seco*-7α-hydroxy-11-acetoxykaura-8(14),16-dien-

9,15-dione and identified as *ent*-8,9-*seco*-7α,11β-diacetoxykaura-8(14),16-dien-9,15-dione (compound CK 01). Proton and carbon of compound CK 02 were completely assigned by analyses of ^{1}H-^{1}H COSY (**Figure 18**), NOESY (**Figure 19**), HMQC (**Figure 20**) and HMBC (**Figure 21**) spectral data as shown in **Table 2**.



Compound CK 02

Compound CK 02 exhibited a negative rotation similar to that of compound CK 01, so it is therefore reasonable to assume that the absolute configuration of compound CK 02 is the same as that of compound CK 01. Moreover, the coupling constants at H-7 (δ_H 5.55, *dd*, 12.2 and 4.5 Hz of CK 02; δ_H 4.71, *dd*, 12 and 4 Hz of CK 01) and H-11 (δ_H 5.28, *dd*, 5.5 and 1.3 Hz of CK 02; δ_H 5.23, *dd*, 5 and 1 Hz of CK 01) of compound CK 02 were also relatively close to those of CK01.

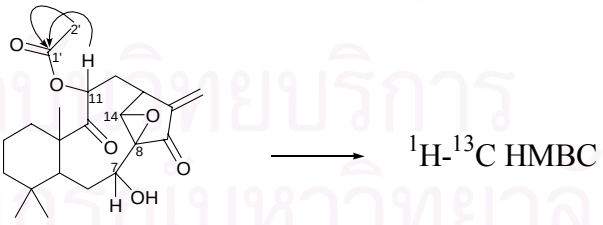
Table 3: The ^{1}H and ^{13}C-NMR Spectral Data of Compound CK 02 in $CDCl_3$

Position	δ_H (ppm), J (Hz)	δ_C (ppm)
1	1.26 (1H, *m*) 1.57 (1H, *m*)	32.3
2	1.48 (1H, *m*) 1.60 (1H, *m*)	17.8
3	1.51 (1H, *m*) 1.59 (1H, *m*)	41.4
4	-	34.2
5	1.81 (1H, *dd*, 6.1, 1.2)	40.4
6	1.45 (1H, *m*) 1.98 (1H, *m*)	32.3
7	5.55 (1H, *dd*, 12.2, 4.5)	66.3
8	-	145.0
9	-	212.1
10	-	54.6
11	5.28 (1H, *dd*, 5.5, 1.3)	77.6
12	(α) 2.36 (1H, *ddd*, 14.6, 5.4, 2.8) (β) 2.95 (1H, *ddd*, 14.6, 5.0, 1.3)	31.7
13	3.61 (1H, *br s*)	41.1
14	7.23 (1H, *d*, 2.7)	159.3
15	-	193.5
16	-	147.8
17 (*E*) (*Z*)	5.31 (1H, *br s*) 5.94 (1H, *br s*)	112.9
18	1.14 (3H, *s*)	33.8
19	0.97 (3H, *s*)	22.0
20	1.06 (3H, *s*)	18.2
1′	-	169.0
2′	2.08 (3H, *s*)	20.6
1″	-	169.7
2″	2.02 (3H, s)	20.9

1.3 Structure Elucidation of Compound CK 03

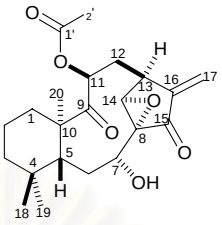
A compound CK 03 was obtained as brown oil. A molecular formula of $C_{22}H_{30}O_6$ [*m/z* 413.1969 $[M + Na]^+$, calculated for $[C_{22}H_{30}O_6 + Na]^+$, 413.1940] was obtained from the ESITOFMS spectrum **(Figure 24)**. The IR absorption **(Figure 23)** showed bands of OH-strecthing at ν 3445 cm^{-1}, C=O stretching at ν 1748 and 1733 cm^{-1}, and the UV spectrum (**Figure 22**) displayed λ_{max} at 233 nm. The optical rotation of CK 03 was negative, $[\alpha]^{30}_D$ -16.7° (*c* 0.45, $CHCl_3$).

The ^1H and ^{13}C NMR ($CDCl_3$) spectra of CK 03 **(Figures 25 and 26)** resembled those of the 8,9-secokauranes CK 01 and CK 02, but an olefinic proton signal (δ_H 7.23) in CK 01 and CK 02 were replaced by an oxygenated methine signal (δ_H 3.84) in CK 03. These ^1H and ^{13}C NMR spectral data, together with the evidence from the ESITOFMS spectrum, indicated that compound CK 03 is an oxidized form of CK 01 in which the double bond C-8/C-14 (δ_C 64.7/60.9) is epoxidized. The HMBC spectrum **(Figure 30)** helped place an acetate ester at C-11 (δ_C 77.6), showing the correlations from H-11(δ_H 5.39) to C-1′ (δ_C 168.8) and from the singlet methyl (at δ_H 2.08, H-2′) to C-1′. Based upon these spectral data, compound CK 03 was identified as *ent*-8,9-*seco*-8,14-epoxy-7α-hydroxy-11β-acetoxy-16-kauren-9,15-dione. The protons and carbons in CK 03 were completely assigned by analysis of its 2D NMR spectra **(Table 4)(Figures 25, 26, 27, 28, 29 and 30)**.



The absolute stereochemistry of compound CK 03 was assumed to be the same as that of compounds CK 01 and CK 02 due to the similarity of negative rotations observed. The orientation of the epoxide in compound CK 03 was evident from the NOESY spectrum **(Figure 28)** whereupon H-13 (δ_H 3.28) showed a more intense cross peak with H-12α (δ_H 2.27) than with H-12β (δ_H 2.94), while the H-14 (δ_H 3.84) epoxy proton exhibited a cross peak with H-12β (δ_H 2.94). These spectral data implied

that H-14 in compound CK 03 is β, thus the oxirane ring is α-oriented. The stereochemistry of the 8,14-epoxide ring in 8,9-secokauranes has been oriented in the same manner as that previously reported (Perry, Burgess and Tangney 1996).



CK 03

Table 4: The ^{1}H and ^{13}C-NMR Spectral Data of Compound CK 03 in $CDCl_3$

Position	δ_H (ppm), J (Hz)	δ_C (ppm)
1	1.34 (1H, *m*) 1.66 (1H, *m*)	32.0
2	1.52 (1H, *m*) 1.63 (1H, *m*)	17.7
3	1.51 (1H, *m*) 1.56 (1H, *m*)	41.5
4	-	34.5
5	2.12 (1H, *dd*, 7.2, 0.8)	39.0
6	1.20 (1H, *m*) 1.95 (1H, *m*)	34.2
7	4.73 (1H, *dd*, 11.8, 3.4)	61.8
8	-	64.7
9	-	211.6
10	-	54.3
11	5.39 (1H, *dd*, 6.1, 1.4)	77.6
12	(α) 2.27 (1H, *ddd*, 15.1, 6.1, 3.6) (β) 2.94 (1H, *ddd*, 15.1, 4.6, 1.6)	30.5
13	3.28 (1H, *br t*, 1.5)	38.7
14	3.84 (1H, *s*)	60.9
15	-	195.8
16	-	146.8
17 (*E*) (*Z*)	5.36 (1H, *d*, 1.6) 6.01 (1H, *br s*)	118.2
18	1.12 (3H, *s*)	33.9
19	1.00 (3H, *s*)	21.6
20	1.08 (3H, *s*)	18.1
1′	-	168.8
2′	2.08 (3H, *s*)	20.6

1.4 Structure Elucidation of Compound CK 04

Compound CK 04 was obtained as colorless oil. It was assigned the molecular $C_{22}H_{32}O_4$ by ESITOFMS [*m/z* 383 $[M + Na]^+$**(Figure 33)**. The IR adsorption bands **(Figure 32)** showed OH-stretching at *v* 3443 cm^{-1}, and C=O stretching at *v* 1635 cm^{-1}. The UV spectrum **(Figure 31)** of CK 04 showed λ_{max} at 230 nm, and the optical rotation of CK 03 was negative, $[\alpha]^{30}_D$ -4.0° (*c* 1.0, $CHCl_3$).

The ^{1}H-NMR spectrum ($CDCl_3$) (**Figure 34**) of compound CK 04 displayed signals of three methyl singlets at δ_H 0.84 (3H, *s*), 1.15 (3H, *s*), and 2.11 (3H, *s*), five methylene multiplets at δ_H 2.08 (2H, *m*), δ_H 1.56 (2H, *m*), 1.50 (2H, *m*), 1.65 (2H, *m*), and δ_H 1.72 (2H, *m*) , a methylene doublet of doublet of triplet at 1.92 (2.6, 6.2, 13.3), a methylene triplet of doublet and doublet of triplet at δ_H 1.81 (1H, *dt*, 3.2, 12.8) and δ_H 0.75 (1H, *td*, 3.2, 13.1), a methylene singlet at δ_H 5.3 (1H, *s*) and δ_H 3.88 (1H, *s*), a methylene doublet at δ_H 3.67 (1H, *d*, 11.1) and δ_H 3.88 (1H, *d*, 11.2), a methylene singlets at δ_H 5.3 (1H, *s*) and δ_H 6.0 (1H, *s*), a methine doublet of doublet at δ_H 4.05 (1H, *dd*, 11.8, 4.4), a methine doublet at δ_H 1.25 (1H, *d*, 8.5), two methine broad singlets at δ_H 1.30 (1H, *br s*) and δ_H 3.12 (1H, *br s*), and a methine doublet of doublet at 4.05 (1H, *dd*, 11.8, 4.4).

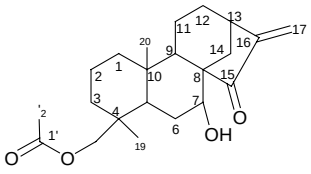
The ^{13}C-NMR spectral data **(Figure 35)** revealed three methyl carbons at δ_C 17.6 (19-CH_3), δ_C 18.2 (20-CH_3), and δ_C 21.2 (2′-CH_3), nine methylene carbons at δ_C 17.4 (C-2), δ_C 18.0 (C-11), δ_C 27.7 (C-6), δ_C 28.0 (C-14), δ_C 32.8 (C-12), δ_C 35.5 (C-3), δ_C 39.0 (C-1), δ_C 72.4 (C-18), and δ_C 115.0 (C-17), four methine carbons at δ_C 37.6 (C- 13), δ_C 46.3 (C-5), δ_C 71.0 (C-7), and δ_C 51.8 (C-9), and six quaternary carbons at δ_C 36.4 (C-4), δ_C 39.7 (C-10), δ_C 58.4 (C-8), and δ_C 149.2 (C-16), δ_C 171.3 (C-1′) and δ_C 209.8 (C-15).

The ^{1}H-NMR spectral data **(Figure 34)** exhibited resonances of an acetate group at δ_H 2.10 (3H, *s*), an oxygenated methine proton at δ_H 4.05 (1H, *dd*, 11.8, 4.4), and a methylene proton bearing oxygen at δ_H 3.67 (1H, *d*, 11.1) and δ_H 3.88 (1H, *d*, 11.2). The ^{1}H-^{1}H COSY spectrum **(Figure 36)** demonstrated for the cross peak of methylene proton from δ_H 0.75 (H-1) to δ_H 1.52 and 1.65 (H-2), from δ_H 1.52 (H-2) to δ_H 1.45 (H-3), and from δ_H 4.05 (H-7) to δ_H 1.72 (H-6). The HMBC spectrum **(Figure**

39) showed correlations from δ_H 0.84 (18-CH_3) to δ_C 36.4 (C-4), from δ_H 3.67 and δ_H 3.88 (H-18) to δ_C 171.3 (C-1´), from δ_H 2.11 (2´-CH_3) to δ_C 171.3 (C-1´), from δ_H 1.25 (H-5) to δ_C 39.0 (C-1) and δ_C 51.8 (C-9), from δ_H 1.15 (20-CH_3) to δ_C 39.7 (C-10), and from δ_H 1.72 (H-6) to δ_C 71.0 (C-7) and δ_C 58.4 (C-8). Based on these spectral data the first substructure of compound CK04 is propossed as shown below.

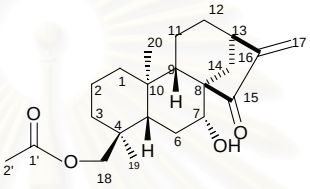
The ^{1}H-^{1}H COSY spectrum **(Figure 36)** of compound CK 04 displayed a correlation between δ_H 3.12 (H-13) to δ_H 2.08 (H-14), while the HMBC spectrum of compound CK04 showed the correlations from δ_H 4.05 (H-7) to δ_C 209.8 (C-15), from δ_H 2.08 (H-14) to δ_C 58.4 (C-8), δ_C 209.8 (C-15), δ_C 149.2 (C-16), and from δ_H 5.3 and δ_H 6.0 (H-17) to δ_C 149.2 (C-16), δ_C 37.6 (C-13), δ_C 209.8 (C-15) and δ_C 58.4 (C-8), therefore the second fragment of compound CK 04 is assembled as shown.

Combination of the first and the second fragments established a gross structure of CK 04. Therefore compound CK 04 was identified as *ent*-7β-hydroxy-15-oxokaur-16-en-18-eyl acetate, which is a known compound previously isolated from a Vietnamese folk medicine, *Croton tonkinensis* (Son, Giang and Taylor 2000).



Compound CK 04

Compound CK 04 exhibited negative optical rotation ($[\alpha]^{30}_{D}$ -4.0˚, *c* 1.0, $CHCl_3$) similar to *ent*-7*β*-hydroxy-15-oxokaur-16-en-18-eyl acetate, and therefore compound CK 04 possibly possessed the same stereochemistry as that of *ent*-7*β*-hydroxy-15-oxokaur-16-en-18-eyl acetate (Son *et al.* 2000). Proton and carbon of compound CK 04 were completely assigned by analyses of ^{1}H-^{1}H COSY (**Figure 36**), NOESY (**Figure 37**), HMQC (**Figure 38**) and HMBC (**Figure 39**) spectral data as shown in **Table 5**.



Compound CK 04

Table 5: The ^{1}H and ^{13}C-NMR Spectral Data of *ent*-7*β*-Hydroxy-15-oxokaur-16-en-18-eyl acetate (Son *et al.* 2000) and Compound CK 04 in $CDCl_3$

Position	*ent*-7*β*-Hydroxy-15-oxokaur-16-en-18-eyl acetate ($CDCl_3$, 400MHz) (Son *et al.* 2000)		Compound CK 04 ($CDCl_3$, 500 MHz)	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	*α* 1.79 (*ddd*, 3.5, 3.5, 13.0) *β* 0.74 (*tdq*, 13.0, 4.0, 0.9)	38.9	*α* 1.81 (1H, *dt*, 3.2, 12.8) *β* 0.75(1H, *td*, 3.2, 13.1)	39.0
2	*α* 1.65 (*m*) *β* 1.50 (*m*)	17.5	1.52 (1H, *m*) 1.49 (1H, *m*)	17.4
3	*α* 1.38 (*br d*, 4.2) *β* 1.35 (*m*)	35.4	1.45 (1H, *m*) 1.35 (1H, *m*)	35.4
4	-	36.4	-	36.4
5	1.28 (*dd*, 12.6, 1.8)	46.3	1.30 (1H, *d*, 12.5)	46.3
6	*α* 1.45 (*q*, 12.0) *β* 1.70 (*ddd*, 12.0, 4.4, 1.6)	27.7	1.72 (2H, *m*)	27.7
7	4.05 (*dd*, 4.4, 12.0)	70.8	4.05 (1H, *dd*, 11.8, 4.4)	71.0
8	-	58.3	-	58.4
9	1.23 (br d, 8.5)	51.8	1.25 (1H, d, 8.5)	51.8
10	-	39.6	-	39.7
11	*α* 1.70 (*m*) *β* 1.47 (*m*)	18.0	1.65 (1H, *m*) 1.50 (1H, *m*)	18.0
12	*α* 1.96 (*tdd*, 13.0, 6.2, 2.7) *β* 1.70 (*m*)	32.8	1.98 (2H, *ddt*, 2.6, 6.2, 13.3)	32.8
13	3.10 (*m*)	37.6	3.12 (1H, *br s*)	37.6
14	2.07 (br d)	27.9	2.08 (2H, *m*)	27.8
15	-	209.7	-	209.8
16	-	149.2	-	149.2
17	5.29 (*t*, 1.1) 5.97 (*t*, 1.1)	115.0	5.3 (1H, *s*) 6.0 (1H, *s*)	115.0
18	3.66 (*d*, 10.8) 3.87 (*d*, 10.8)	72.3	3.67 (1H, *d*, 11.1) 3.88 (1H, *d*, 11.2)	72.4
19	0.84 (*s*)	17.5	0.84 (3H, *s*)	17.6
20	1.14 (*d*, 0.9)	18.2	1.15 (3H, *s*)	18.2
1′	-	171.2	-	171.3
2′	2.10 (*s*)	21.1	2.11 (3H, *s*)	21.2

2. Structure Elucidation of Compounds Isolated from *Croton birmanicus*

2.1 Structure Elucidation of Compound CB 01

Compound CB 01 was obtained as brown oil. A molecular formula of $C_{18}H_{24}N_2O_3$ *m/z* 339.2 $[M+Na]^+$ was obtained from the ESIMS spectrum **(Figure 42)**. The IR spectrum **(Figure 41)** showed bands of NH stretching at *v* 3350 cm^{-1}, CH_3 stretching at *v* 3063 cm^{-1}, CH_2 stretching at *v* 2931 cm^{-1}, C=O stretching at *v* 1726 and 1682 cm^{-1}, and the UV spectrum **(Figure 40)** displayed λ_{max} at 214 nm. The optical rotation of CB 01 was negative, $[\alpha]^{30}_D$ -20.6° (*c* 0.825, $CHCl_3$).

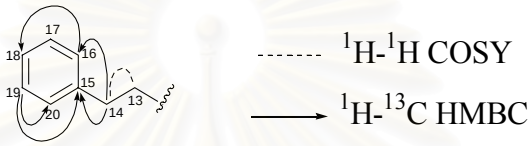
The ^1H NMR ($CDCl_3$) spectral data **(Figure 43)** of CB 01 displayed the signals of five non- equivalent methylene protons at δ_H 2.77 (H-14, *t*, 7.7), δ_H 3.94 (H-13, *m*), δ_H 2.66 (H-4, *dd*, 5.2), δ_H 2.45 and δ_H 1.62 (H-5, *m*), and δ_H 1.41 and δ_H 1.62 (H-10, *m*), two methine protons at δ_H 4.41 (H-6, *m*), and δ_H 2.14 (H-9, *m*), five aromatic protons at δ_H 7.14 (H-16 and H-20, *m*), δ_H 7.22 (H-17 and H-19, *m*), and δ_H 7.15 (H-18, *m*), and an exchangeable NH at δ_H 6.20 (H-7, *br d*, 5.0).

The ^{13}C NMR and DEPT135 spectral data **(Figure 44)** of CB 01 revealed 16 signals of two methyl carbons at δ_C 11.9 (C-11) and δ_C 17.4 (C-12); five methylene carbons at δ_C 24.6 (C-5), δ_C 27.4 (C-10), δ_C 31.8 (C-4), δ_C 34.1 (C-14), and δ_C 41.8 (C-13), two methine carbons at δ_C 51.4 (C-6) and δ_C 43.1 (C-9); six aromatic carbons at δ_C 126.7 (C-18), δ_C 128.6 (2xC, C-17 and C-19), δ_C 129.1 (2xC, C-16 and C-20) and δ_C 138.3 (C-15); and three carbonyl carbons at δ_C 171.1 (C-3), δ_C 172.0 (C-1), and δ_C 177.0 (C-8).

The ^1H-^1H COSY spectrum **(Figure 46)** of CB 01 displayed cross peak from δ_H 2.45 to δ_H 1.62, from δ_H 2.74 to δ_H 2.66, and from δ_H 1.62 to δ_H 1.43, while the HMQC spectrum **(Figure 47)** of CB 01 showed correlations from 2.45 and 1.62 (H-4) to 24.6 (C-4), from δ_H 2.74 and δ_H 2.66 (H-5) to 31.8 (C-5), and from δ_H 1.62 and δ_H 1.43 (H-10) to 27.4 (C-10).

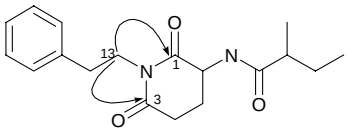
A typical resonance of 1-substituted benzene ring in CB 01 could be observed, exhibiting three multiplet resonances at δ_H 7.14 (2H, *m*), δ_H 7.15 (1H, m), and δ_H 7.22

(2H, m). The ^{13}C and HMQC spectral data indicated signals of 1-substituted benzene ring at δ_C 129.1 (2xC), 128.6 (2xC), 126.7 (1xC) and 138.0 (1xC) assignable to C-16 (C-20), C-17 (C-19), C-18 and C-15, respectively. The ^1H-^1H COSY spectrum of CB 01 displayed cross peak from δ_H 3.98 (H-14) to δ_H 2.77 (H-13), while the ^1H-^{13}C correlation of HMBC**(Figure 48)** demonstrated the correlations from H-16 (H-20) to C-18 and C-14, from H-17 (H-19) to C-16 (C-20) and C-15, from H-14 to C-13, C-15 and C-16 (C-20), and from H-13 to C-15. Based on these spectral data, a mono-substituted aromatic ring in CB 01 is constructed as shown below.



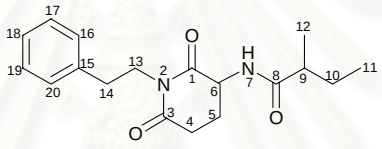
The ^1H-^1H COSY spectrum of CB 01 revealed the cross peaks from δ_H 2.74 and 2.66 (H-5) to δ_H 2.45 and 1.62 (H-4), from δ_H 4.41 (H-6) to δ_H 2.74 and 2.66 (H-5) and δ_H 6.2 (H-7), from δ_H 1.62 and 1.43 (H-10) to δ_H 1.10 (12-CH_3), and from δ_H 2.15 (H-9) to δ_H 1.10 (11-CH_3). The HMBC spectrum of CB 01 showed the correlations from H-4 to δ_C 171.1 (C-3), from H-6 to δ_C 172.0 (C-1), from H-9 to δ_C 177 (C-8), and from H-10 to δ_C 11.9 (C-11), from 11-CH_3 to δ_C 27.4 (C-10) and δ_C 43.1 (C-9), and from 12-CH_3 to δ_C 43.1 (C-9) and δ_C 27.4 (C-10). The ^1H-^1H COSY spectrum of CB 01 revealed the correlation of NH proton and H-6, and the downfield resonance of H-6 suggested the presence of amide linkage in CB 01. Therefore the second partial structure of compound CB 01 is assembled as shown.

The HMBC spectrum of CB 01 assisted in placing the connection between the first partial and the second partial structure, showing the correlation from H-13 to C-1 and C-3.



^{1}H-^{13}C HMBC

Based on these spectral data, the gross structure of compound CB 01 was identified as julocrotine, a glutarimide alkaloid, which was previously isolated from *Julocroton montevidensis* Klotzsch (Nakano, Djerassi, Corral *et al.* 1961), *Croton humilis* (Stuart, McNeill, Kutney *et al.* 1973), and *Croton membranaceus* (Aboagye, Sam, Massiot *et al.* 2000). Proton and carbon of compound CB 01 were completely assigned by analyses of ^{1}H-^{1}H COSY (**Figure 45**), NOESY (**Figure 46**), HMQC (**Figure 47**) and HMBC (**Figure 48**) spectral data as shown in **Table 6**.



Compound CB 01

Table 6: The ^{1}H and ^{13}C-NMR Spectral Data of Julocrotine (Aboagye *et al.* 2000) and Compound CB 01 in $CDCl_3$

Position	Julocrotoine (300 MHz, $CDCl_3$) (Aboagye *et al.* 2000)		CB 01 (400 MHz, $CDCl_3$)	
	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	-	171.7	-	172.0
2	-	-	-	-
3	-	170.9	-	171.1
4	2.72 (*m*)	31.6	2.74 (1H, *m*) 2.66 (1H, *m*)	31.8
5	2.51 (*m*) 1.71 (*m*)	24.3	2.45 (1H, *m*) 1.62 (1H, *m*)	24.6
6	4.52 (*dd*)	51.0	4.41(1H, *td*, 7.8, 5.4)	51.4
7 (NH)	6.38 (*br s*)	-	6.20 (1H, *br d*, 5.0)	-
8	-	176.6	-	177.0
9	2.23	42.8	2.15 (1H, *q*, 6.7)	43.1
10	1.48 (*m*) 1.71 (*m*)	27.1	1.43 (1H, *m*) 1.62 (1H, *m*)	27.4
11	0.95 (*dd*)	11.7	0.87 (3H, *t*, 8.5)	11.9
12	1.19 (*d*)	17.1	1.10 (3H, *d*, 6.9)	17.4
13	4.01 (*m*)	41.4	3.94 (2H, *m*)	41.8
14	2.82 (*t*)	33.6	2.77 (2H, *t*, 7.8)	34.1
15	-	138.0	-	138.0
16	7.21 (*m*)	128.8	7.14 (1H, *m*)	129.1
17	7.29 (*m*)	128.3	7.22 (1H, *m*)	128.6
18	7.29 (*m*)	126.5	7.15 (1H, *m*)	126.7
19	7.29 (*m*)	128.3	7.22 (1H, *m*)	128.6
20	7.21 (*m*)	128.8	7.14 (1H, *m*)	129.1

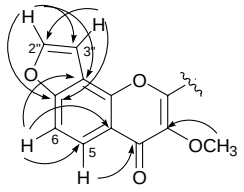
3. Structure Elucidation of Compounds Isolated from *Millettia kangensis*

3.1 Structure Elucidation of MK 01

The compound MK 01 was obtained as colourless crystal. The molecular formular $C_{18}H_{12}O_4$ was determined by ESITOFMS **(Figure 51)**, showing $[M+H]^+$ peak at *m/z* 293.0818 (*calc*. for $C_{18}H_{13}O_4$ 293.0814). The IR spectrum **(Figure 50)** of MK 01 revealed the presence of a conjugated carbonyl stretching at *v*1637, and aromatic ring stretching at *v* 1624 and 1458 cm^{-1}. The UV spectrum **(Figure 49)** demonstrated the absorption at λ_{max} at 303, 260, 217, 203 nm.

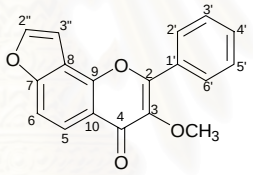
The ^{1}H-NMR ($CDCl_3$) spectral data of MK 01 **(Figure 52)** displayed a characteristic of a1-substituted aromatic ring, showing a doublet of doublet signal at δ_H 8.16 (2H, *dd*, *J* = 8.0, 2.1 Hz), and multiplet signal at δ_H 7.56 (3H, *m*). The ^{13}C and HMQC spectrum **(Figures 53 and 56)** of MK 01 showed signals of mono-substituted aromatic ring at δ_C 130.6 (C-1′), 128.0 (C-2′ and C-6′), 128.2 (C-3′ and C-5′), and 130.2 (C-4′). The HMBC spectrum **(Figure 57)** of MK 01 further revealed the correlation from H-2′ to C-3′, C-4′, C-1′, and C-2. Based upon these spectral data, the first substructure was constructed.

Typical signals of an anellated furan ring on the ^{1}H NMR spectrum of MK 01, showing two doublets at δ_H 7.19 (1H, *d*, 2.2 Hz) and at δ_H 7.76 (1H, *d*, 2.2 Hz). The coupling constant of 8.8 Hz for δ_H 8.21 (1H, *d*, 8.8 Hz) and δ 7.56 (1H, *d*, 8.8 Hz) indicated *ortho* coupling between H-5 and H-6, while the HMQC spectrum showed the attachment of H-5 (δ_H 8.81) to C-5 δ_C (121.5), and H-6 (δ_H 7.56) to C-6 (δ_C 109.57). The HMBC spectrum of MK 01 exhibited correlations from H-6 to C-8, C-10, and C-5, from H-5 to C-4, and from 3-OCH_3 protons to C-3. The assignment of the second partial structure of MK 01 was established as shown.



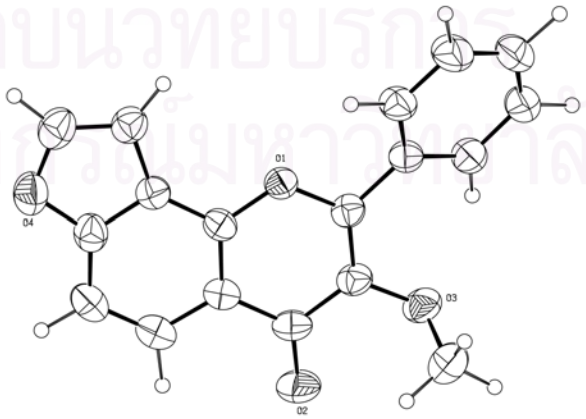
^{1}H-^{13}C HMBC

Combination of the two fragments as described earlier led to the construction of a gross structure of MK 01. Finally, the structure of MK 01 was confirmed by X-ray crystallography. Compound MK 01 was therefore identified as 3-hydroxy-[4″,5″:8,7]-furanoflavone (Karanjin), which is a known agent previously isolated from *Millettia leucantha* (Phrutivorapongkul, Lipipun, Ruangrungsi *et al.* 2003). Proton and carbon of compound MK 01 were completely assigned by analyses of ^{1}H-^{1}H COSY (**Figure 54**), NOESY (**Figure 55**), HMQC (**Figure 56**) and HMBC (**Figure 57**) spectral data as shown in **Table 7**.



MK 01

The structure of compound MK 01 is confirmed by single-crystal X-ray diffraction analysis.



ORTEP PLOT of MK 01

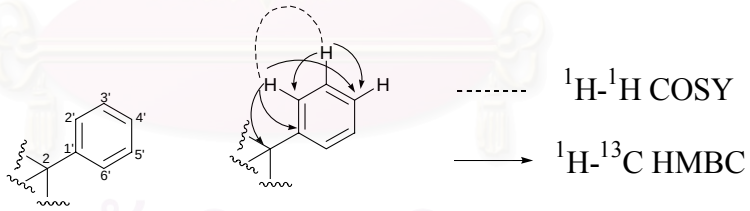
Table 7: ^{1}H and ^{13}C-NMR Spectral Data of Karanjin and Compound MK 01

Position	Karanjin ($CDCl_3$, 400 Hz) (Phrutivorapongkul *et al.,* 2003)		Compound MK 01 ($CDCl_3$, 400 Hz)	
	δ_H (ppm), J (Hz)	δ_C (ppm)	δ_H (ppm), J (Hz)	δ_C (ppm)
2	-	154.9	-	153.4
3	-	141.8	-	141.3
4	-	175.3	-	174.8
5	8.21 (1H, *d*, 8.8)	121.8	8.21 (1H, *d*, 8.8)	121.5
6	7.56 (1H, m)	110.0	7.56 (1H, *m*)	109.6
7	-	158.2	-	157.5
8	-	117.0	-	116.7
9	-	150.0	-	148.3
10	-	119.7	-	118.3
1′	-	131.1	-	130.6
2′	8.15 (1H, *m*)	128.4	8.16 (1H, *dd*, 8.0, 2.1)	128.0
3′	7.56 (1H, *m*)	128.7	7.56 (1H, *m*)	128.2
4′	7.56 (1H, *m*)	130.7	7.56 (1H, *m*)	130.2
5′	7.56 (1H, *m*)	128.7	7.56 (1H, *m*)	128.2
6′	8.15 (1H, *m*)	128.4	8.11 (1H, *dd*, 8.0, 2.1)	128.0
2″	7.77 (1H, *d*, 2.4)	145.6	7.76 (1H, *d*, 2.2)	145.3
3″	7.19 (1H, *dd*, J = 2.4, 1.2)	104.2	7.19 (1H, *d*, 2.2)	103.8
3-OCH_3	3.93 (3H, *s*)	60.3	3.93 (3H, *s*)	59.8

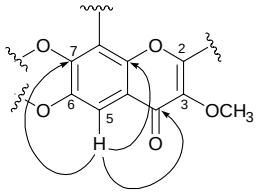
3.2 Structure Elucidation of MK 02

Compound MK 02 was obtained as colourless plate. The ESIMS **(Figure 60)** exhibited a peak of $[M+Na]^+$ at *m/z* 331.3, calculated for $C_{18}H_{12}O_5$. The UV absorptions **(Figure 58)** bands appeared at λ_{max} 308 and 208 nm. The IR spectrum **(Figure 59)** showed conjugated carbonyl stretching at v 1592.5 cm^{-1} and OH stretching at v 3265 cm^{-1}.

The ^{1}H-NMR spectrum (DMSO-d_6) **(Figure 61)** exhibited typical signals of 1-substituted benzene ring showing a multiplet signal at δ_H 7.59 (3H, *m*) and a doublet of doublet signal at δ_H 8.11 (2H, *dd, J* = 1.85, 7.98 Hz). The ^{13}C and HMQC spectral data indicated signals of 1-substituted benzene ring at δ_C 128.20 (2xC), 128.80 (2xC), 130.72 (1xC) and 130.68 (1xC) assignable to C-2′ (C-6′), C-3′(C-5′), C-4′ and C-1′, respectively. The ^{1}H-^{1}H COSY **(Figure 63)** showed a correlation between δ_H 7.59 (H-4′, H-6′) and 8.11 (H-2′, H-5′), while the ^{1}H-^{13}C correlation of HMBC **(Figure 66)** demonstrated the correlations from H-2′ to C-1′, C-4′ and C-2, and from H-3′ to C-2′ and C-4′. Based on these spectral data, a mono-substituted aromatic ring in MK 02 was constructed as shown below.

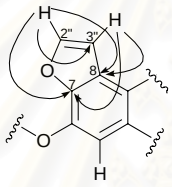


The singlet signal at δ_H 7.33 was assigned to H-5, and the HMBC spectrum showed the correlations from H-5 to C-7 (δ_C 147.5 ppm), C-10 (δ_C 120.0 ppm) and C=O (δ_C 173.6 ppm). Chemical shifts of ^{13}C-NMR spectrum **(Figure 62)** of MK 02 at δ_C 140.65 (C-6), 147.5 (C-7), 147.2 (C-2″), 143.3 (C-9), 153.9 (C-2) and 140.7 (C-3) indicated that these carbons were oxygenated double bonds. Based on these spectral data, the second partial structure of MK 02 was created as shown.



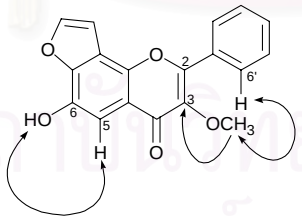
$\longrightarrow$ ^{1}H-^{13}C HMBC

The ^{1}H-NMR spectrum of MK 02 showed the characteristic of anellated furan ring with the signals at δ_H 8.19 (H-2″, *d*, *J* = 2.17 Hz) and δ_H 7.44 (H-3″, *d*, *J*= 2.09 Hz). The HMQC spectrum **(Figure 65**) revealed the attachment of furan protons to its corresponding carbons at δ_C 147.2 (C-2″) and δ_C 104.9 (C-3″). The ^{1}H-^{1}H COSY spectrum showed the correlation between H-3″ and H-2″, while the HMBC spectrum showed the correlations from H-2″ to C-3″, C-7 and C-8, and from H-3″ to C-7 and C-8. The third fragment of MK 02 is shown below.



$\longrightarrow$ ^{1}H-^{13}C HMBC

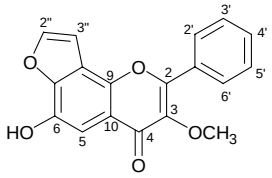
The NOESY spectrum **(Figure 64)** of MK 02 demonstrated the correlations between 6-OH and H-5, and between 3-OCH_3 and H-6′. The HMBC spectrum exhibited the correlation from 3-OCH_3 protons to C-3.



$\longrightarrow$ ^{1}H-^{13}C HMBC

$\longleftrightarrow$ NOESY

On the basis of these spectral data, a gross structure of MK 02 was established as shown below. Compound MK 02 was therefore identified as 3-methoxy-6-hydroxy-[4″,5″:8,7]-furanoflavone. MK 02 is an oxidized form of MK 01, and it is a new compound. Assignment of protons and carbons of MK 02 is in **Table 8**.



MK 02

Table 8: The ^{1}H and ^{13}C-NMR Spectral Data of Compound MK 02 in DMSO-d_6

Position	δ_H (ppm), J (Hz)	δ_C (ppm)
2	-	153.9
3	-	141.7[a]
4	-	173.6
5	7.33 (1H, *s*)	102.6
6	-	140.6[a]
7	-	147.5
8	-	118.8
9	-	143.2[a]
10	-	120.0
1′	-	130.6
2′	8.11 (1H, *dd*, 7.98, 2.14)	128.2
3′	7.58 (1H, *m*)	128.8
4′	7.59 (1H, *m*)	130.7
5′	7.58 (1H, *m*)	128.8
6′	8.11 (1H, *dd*, 7.98, 2.14)	128.2
2″	8.19 (1H, *d*, 2.17)	147.2
3″	7.45 (1H, *d*, 2.09)	104.9
3-OCH_3	3.83 (3H, *s*)	59.7
6-OH	10.80 (1H, *br s*)	-

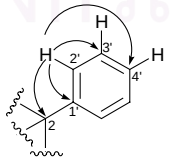
[a] Assignments may be exchangeable.

3.3 Structure Elucidation of MK 03

Compound MK 03 was obtained as colourless plate. The molecular formular was determined as $C_{19}H_{14}O_5$ by ESITOFMS **(Figure 69)**, observing for $[M+Na]^+$ at *m/z* 345.48. The IR spectrum **(Figure 68)** of MK 03 revealed the presence of a conjugated carbonyl at *v* 1753 cm^{-1}, and the UV spectrum **(Figure 67)** exhibited absorptions at λ_{max} 203, 206 and 306 nm.

The ^{1}H-NMR ($CDCl_3$) spectrum **(Figure 70)** of MK 03 showed signals at δ_H 3.92 (3H, *s*), 4.10 (3H, *s*), 7.18 (1H, *d*, *J* = 2.3 Hz), 7.54 (3H, *m*), 7.55 (1H, s), 7.75 (1H, *d*, *J* = 2.0 Hz) and 8.13 (2H, *dd*, *J* = 7.9, 1.8 Hz) ppm. The ^{13}C-NMR spectrum **(Figure 71)** of MK 03 demonstrated signals at δ_C 56.6, 60.3, 100.1, 104.9, 118.9, 126.8, 128.5, 128.8, 130.7, 131.2, 141.7, 145.2, 146.1, 148.3, 154.8 and 175, while the DEPT135 spectrum**(Figure 71)** revealed the presence of eight methine, two methyl, and nine quaternary carbons. Evidence from IR spectrum and ^{13}C NMR data suggested that compound MK 03 is a flavonoid.

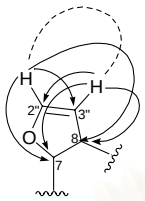
The pattern with doublet of doublet at δ_H 8.13 (2H, *dd*, *J* = 7.9, 1.8 Hz) and multiplet at δ_H 7.54 (3H, *m*) were typical signals of a mono-substituted aromatic ring. The ^{13}C and HMQC spectra **(Figures 71 and 74)** indicated signals of 1-substituted benzene ring at δ_C 128.5 (2xC), 128.8 (2xC), 130.7 (1xC), and 131.2 (1xC) assignable to C-2′ (or C-6′), C-3′ (or C-5′), C-4′, and C-1′, respectively. The HMBC spectrum **(Figure 75)** of MK 03 revealed the correlation from H-2′ (or H-6′) to C-1′, C-3′ (or C-5′), C-4′, and C-2. These spectral data assisted in the construction of the first partial structure of MK 03 as shown.



⟶ ^{1}H-^{13}C HMBC

The ^{1}H NMR spectrum of MK 03 further exhibited two doublet signals at δ_H 7.18 (1H, *d*, 2.3 Hz) and δ_H 7.55 (1H, *d*, 2.0 Hz), while the ^{1}H-^{1}H COSY spectrum **(Figure 72)** displayed the correlation between these two protons (H-2″ and H-3″). The

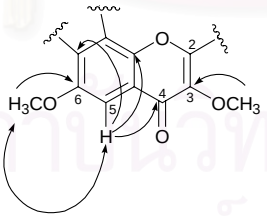
HMBC spectrum of MK 03 showed the correlation from H-2″ to C-7 (δ_C 148.3), C-8 (δ_C 118.9), and C-3″ (δ_C 104.9), and from H-3″ to C-7, C-8, and C-2″. The above spectral data led to the construction of the second partial structure of MK 03 as shown.



^{1}H-^{1}H COSY

^{1}H-^{13}C HMBC

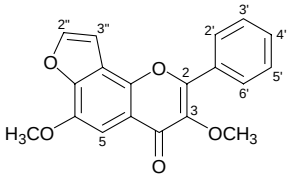
The ^{1}H NMR spectrum of MK 03 displayed two methoxy singlets at δ_H 3.92 and δ_H 4.10, while the ^{1}H-^{13}C correlation of HMBC spectrum showed the correlation from 3-OCH_3 protons to C-3 and from 6-OCH_3 protons to C-6, establishing the attachment of 3-OCH_3 and 6-OCH_3. A singlet methine signal at δ_H 7.55 (1H, *s*) on aromatic ring A was assignable to H-5 by the HMBC correlations from H-5 to C-7 (δ_C 148.3), C-9 (δ_C 145.2), and C-4 (δ_C 175.0). Chemical shifts of ^{13}C-NMR spectrum of MK 03 at δ_C 154.8 (C-2), 141.7 (C-3), 175 (C-4), 145.2 (C-6), 148.3 (C-7), 145.2 (C-9), and 146.1 (C-2″) indicated that these carbons were oxygenated double bonds. The NOESY spectrum **(Figure 73)** of MK 03 demonstrated the correlations between 6-OCH_3 and H-5. Analysis of these spectral data assisted in the construction of the third substructure of MK 03 as shown.



^{1}H-^{13}C HMBC

NOESY

Combination of the three fragments mentioned above led to the assignment of a gross structure of MK 03 **(Table 9)**. Therefore compound MK 03 was identified as 3,6-dimethoxy-[4″,5″:8,7]-furanoflavone, which is a known substance previously isolated from *M. ichthyochtona* (Kamperdick 1998). Protons and carbons of MK 06 were assigned as shown in **Table 9**.



MK 03

Table 9: The ^{1}H and ^{13}C-NMR Spectral Data of 3,6-Dimethoxy-[4″,5″:8,7]-furanoflavone and Compound MK 03

Position	3,6-Dimethoxy-[4″,5″:8,7]-furano flavone ($CDCl_3$, 300 MHz) (Kamperdick 1998)		Compound MK 03 ($CDCl_3$, 400 MHz)	
	δ_H (ppm), J (Hz)	δ_C (ppm)	δ_H (ppm), J (Hz)	δ_C (ppm)
2	-	154.6	-	154.8
3	-	141.5	-	141.7
4	-	174.8	-	175.0
5	7.56 (1H, *s*)	99.8	7.55 (1H, *s*)	100.1
6	-	144.1	-	145.2
7	-	148.1	-	148.3
8	-	118.7	-	118.9
9	-	144.9	-	145.2
10	-	120.4	-	126.8
1′	-	131.0	-	131.2
2′	8.14 (1H, *dd*, 7.9, 1.2)	128.3	8.13 (1H, *dd*, 7.9, 1.8)	128.5
3′	7.56 (1H, *m*)	128.6	7.54 (1H, *m*)	128.8
4′	7.56 (1H, *m*)	130.5	7.54 (1H, *m*)	130.7
5′	7.56 (1H, *m*)	128.6	7.54 (1H, *m*)	128.8
6′	8.14 (1H, *dd*, 7.9, 1.2)	128.6	8.13 (1H, *dd*, 7.9, 1.8)	128.5
2″	7.77 (1H, *d*, 1.9)	145.9	7.75 (1H, *d*, 2.0)	146.1
3″	7.18 (1H, *d*, 1.9)	104.7	7.18 (1H, *d*, 2.3)	104.9
3-OCH_3	3.93 (3H, *s*)	60.2	3.92 (3H, *s*)	60.3
6-OCH_3	4.11 (3H, *s*)	56.5	4.10 (3H, *s*)	56.6

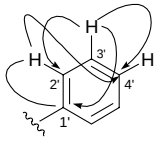
3.4 Structure Elucidation of MK 04

The compound MK 04 was obtained as colourless plate. A molecular formula of $C_{19}H_{14}O_5$ for MK04 was deduced from the ESITOFMS spectrum **(Figure 78)**, $[M+Na]^+$ observed at *m/z* = 345.0742. The IR spectrum **(Figure 77)** of MK 04 revealed the presence of a conjugated carbonyl at *v* 1735 cm^{-1}, and the UV spectrum **(Figure 76)** exhibited absorptions at λ_{max} 282, 312 and 348 nm.

The ^{1}H-NMR ($CDCl_3$) spectrum **(Figure 79)** of MK 04 prominently exhibited signals of a methoxyl group at δ_H 4.10 and 4.26, a singlet signal of aromatic proton at δ_H 6.74 (1H, H-3), multiplet signals of five aromatic protons at δ_H 7.53 (2H, H-3′ and H-5′), 7.54 (1H, H-4′) and 7.98 (2H, H-2′ and H-6′), two doublet signals typically for furan ring at δ_H 7.04 (1H, *d*, *J* = 2.2 Hz) and 7.65 (1H, *d*, *J* = 2.3 Hz) as H-3″ and H-2″.

Analysis of ^{13}C-NMR and DEPT135 spectral **(Figure 80)** data of MK 04 revealed the presence of nine quaternary, eight methine, and two methyl carbons. Compound MK 04 possessed 1-substituted benzene ring, as revealed by analysis of its spectral data.

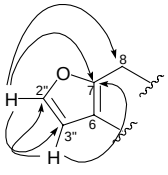
The ^{1}H-^{1}H COSY spectrum **(Figure 81)** exhibited the correlation of H-2′ (or H-6′) and H-3′ (or H-5′), while the ^{1}H-^{13}C HMQC spectrum **(Figure 83)** showed the attachment between H and C, e.g. H-2′ (H-6′) to C-2′ (C-6′), and H-3′ (H-5′) to C-3′ (C-5′). The long range ^{1}H-^{13}C signals on the HMBC spectrum **(Figure 84)** were observed from H-3′ to C-4′, C-2′ and C-1′, and from H-2′ to C-4′ and C-1′. These spectral data assisted in the construction of the first partial structure of MK 04 as shown.



$\longrightarrow$ ^{1}H -^{13}C HMBC

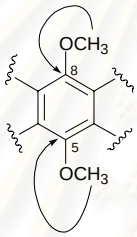
The downfield shift and coupling constant of 2 Hz for H-2″ and H-3″ are typical to an anellated furan ring. The HMBC correlations were seen from H-2″ to C-

3″, C-7 and C-8, and from H-3″ to C-2″ and C-7, leading to the assignment of furan ring in MK 04.



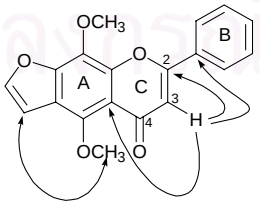
^{1}H -^{13}C HMBC

Two downfield singlet methoxy signals at δ_H 4.10 (5-OCH_3) and 4.26 (8-OCH_3) demonstrated the HMBC correlations to carbons at respective δ_C 147.1 (C-5) and 131.8 (C-8), indicating that the methoxy groups situated at C-5 and C-8. These spectral data implied the presence of the third fragment in MK 04 as shown.



^{1}H-^{13}C HMBC

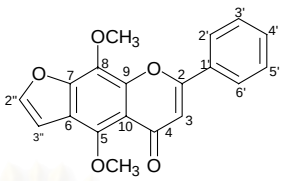
A singlet signal at δ_H 6.75 (H-3) correlated to C-2, C-1′ and C-9, as observed on the HMBC spectrum of MK 04. The NOESY spectrum **(Figure 82)** of MK 04 demonstrated the correlations between 5-OCH_3 and H-3″. The carbonyl carbon of MK 04 exhibited a signal at δ_C 178.7 which is a characteristic of a flavonoid skeleton. Combination of all partial structures mentioned earlier led to the assemble a gross structure for MK 04.



^{1}H -^{13}C HMBC

NOESY

Based upon these spectral data, compound MK 04 is a new natural product, and identified as 5,6-dimethoxy-[4″, 5″;6,7]-furanoflavone. Protons and carbons of MK 04 were assigned as shown in **Table 10**.



MK 04

Table 10: The ^{1}H and ^{13}C-NMR Spretral Data of Compound MK 04 in $CDCl_3$

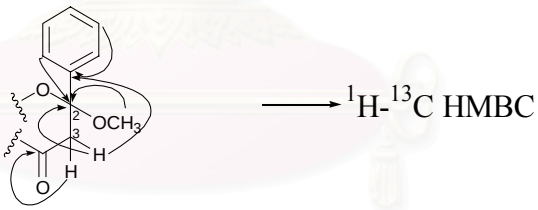
Position	δ_H (ppm), J (Hz)	δ_C (ppm)
2	-	161.7
3	6.77 (1H, *s*)	107.4
4	-	178.7
5	-	147.6
6	-	119.8
7	-	149.5
8	-	131.7
9	-	147.1
10	-	114.0
1′	-	130.5
2′	7.98 (1H, *m*)	126.3
3′	7.53 (1H, *m*)	129.2
4′	7.53 (1H, *m*)	131.7
5′	7.53 (1H, *m*)	129.2
6′	7.98 (1H, *m*)	126.3
2″	7.65 (1H, *d*, 2.3)	145.8
3″	7.04 (1H, *d*, 2.2)	105.4
5-OCH_3	4.10 (3H, *s*)	62.6
8-OCH_3	4.26 (3H, *s*)	61.8

3.5 Structure Elucidation of MK 05

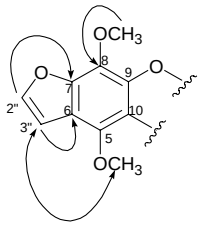
A compound MK 05 was obtained as colourless crystal. The UV spectrum (**Figure 85**) showed λ_{max} at 203, 271 and 309 nm.

The ^{1}H and ^{13}C NMR ($CDCl_3$) spectral (**Figures 86** and **87**) of MK 05 revealed that compound MK 05 was flavonoid derivative. A typical flavonoid carbonyl carbon was observed at δ_C 189.0. The characteristics of 1-phenyl substitution were also observed in MK 05, showing δ_H at 7.64 (*br d*, J = 7.3 Hz, H-2′or H-6′), 7.47 (*m*, H-3′ or H-5′) and 7.41 (*m*, H-4′) with δ_C at 125.5 (2xC, (C-2′ or C-6′)), 128.2 (2xC, (C-3′ or C-5′)), and 128.4 (1xC, (C-4′)).

Interestingly, there was a low field methoxy group (δ_H 3.05) and non-equivalent methylene protons at δ_H 3.00 (1H, *d*, J = 16.1 Hz) and δ_H 3.08 (1H, *d*, J = 16.1 Hz). The HMBC (**Figure 91**) well established the substructure shown below, correlations were observed from H-3′ (or H-5′) to C-1′, H-2′ (or H-6′) to C-2; H-3 to C-2, C-4, and C-1′, and 2-OCH_3 protons to C-2.



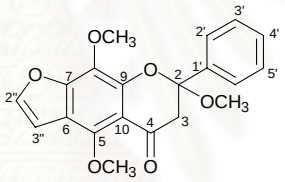
The other partial structure of MK 05 was constructed as shown. The furan on the aromatic ring showed typical resonances at δ_H 6.96 (*d*, J = 2.3 Hz) and 7.55 (*d*, J = 2.3 Hz) with ^{13}C resonances at respective δ_C 105.3 and 143.9. The NOESY spectrum (**Figure 89**) of MK 05 showed the correlation between 5-OCH_3 protons and H-3″, placing the position 5-OCH_3 and the furan on the aromatic ring as shown. The HMBC spectrum showed the correlation from 8-OCH_3 protons to a carbon with δ_C at 130.0 (C-8), and this upfield resonance of C-8 was from the shielding effect of the adjacent oxygen atoms at C-7 and C-9, readily confirming the presence of oxygenated C-7, C-8, and C-9 in MK 05.



^{1}H-^{13}C HMBC

NOESY

A gross structure of MK 05 was assembled by combination of the two partial structures, leading to a flavanone structure uniquely decorated with a methoxy group at C-2. On the basis of these spectral data, compound MK 05 was identified as a new compound, 2,5,8-trimethoxy-[4″, 5″:6, 7]-furanoflavanone. Proton and carbon of compound CK 04 were completely assigned by analyses of ^{1}H-^{1}H COSY (**Figure 88**), NOESY (**Figure 89**), HMQC (**Figure 90**) and HMBC (**Figure 91**) spectral data as shown in **Table 11**.



MK 05

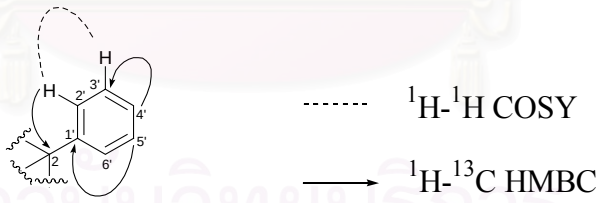
Table 11: The ^{1}H and ^{13}C-NMR Spectral Data of Compound MK 05 in $CDCl_3$

Position	δ_H (ppm), J (Hz)	δ_C (ppm)
2	-	104.5
3	3.00 (1H, *d*, *J* = 16.1) 3.08 (1H, *d*, *J* = 16.1)	51.1
4	-	189.0
5	-	149.2
6	-	130.0
7	-	151.6
8	-	115.6
9	-	146.9
10	-	111.0
1′	-	138.4
2′	7.67 (1H, *br d*, *J* = 7.3)	125.5
3′	7.47 (1H, *m*)	128.3
4′	7.41 (1H, *m*)	128.4
5′	7.47 (1H, *m*)	128.3
6′	7.67 (1H, *br d*, *J* = 7.3)	125.5
2″	7.55 (1H, *d*, 2.3)	143.9
3″	6.92 (1H, *d*, 2.3)	105.3
2-OCH_3	3.05 (3H, *s*)	50.4
5-OCH_3	4.09 (3H, *s*)	61.2
6-OCH_3	4.06 (3H, *s*)	61.1

3.6 Structure Elucidation of Compound MK 06

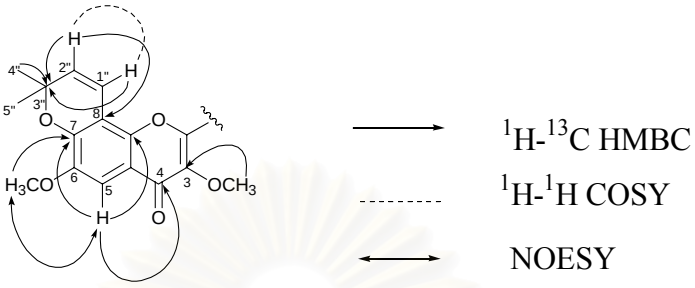
Compound MK 06 was obtained as a colorless needle. The molecular formula was determined as $C_{22}H_{20}O_5$ by ESITOFMS $[M+Na]^+$ (**Figure 94**) for *m/z* 387.1204 (387.1240). The IR bands (**Figure 93**) showed C-O stretching of conjugated carbonyl at *v* 1615 cm^{-1}, and C-H stretching of CH_3 at *v* 2923 cm^{-1}. The UV spectrum (**Figure 92**) showed λ_{max} at 350, 341, and 285 nm.

The ^{1}H-NMR spectrum (**Figure 95**) (DMSO-d_6) of MK 06 exhibited typical signals of 1-substituted aromatic ring, showing a doublet of doublet for H-2′ (H-6′) at δ_H 8.04 (2H, *dd*, 7.9, 1.8), a multiplet for H-3′ (H-5′) at δ_H 7.58 (2H, *m*), and a multiplet for H-4′ at δ_H 7.58 (1H, *m*). The ^{13}C-NMR and HMQC spectral (**Figures 96** and **99**) data also confirmed the presence 1-substituted benzene ring, showing signals at δ_C 130.7 (1xC), 128.1 (2xC), 128.8 (2xC), and 130.3 (1xC) assignable to C-4′, C-2′ (C-6′), C-3′ (C-5′), and C-1′, respectively. The ^{1}H-^{1}H COSY (**Figure 97**) showed a correlation between H-2′ (or H-4′) and H-3′ (or H-5′), while the HMBC spectrum (**Figure 100**) showed correlation from H-2′ to C-2, and from H-5′ to C-1′ Based on these spectral data, the first partial structure of compound MK 06 was created as shown.

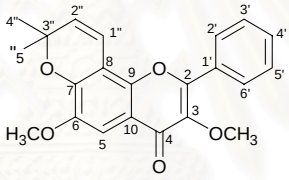


The ^{1}H-NMR spectrum further revealed a methyl singlet at δ_H 1.46 (6H, *s*) deduced to geminal methyl groups (H-4″ and H-5″) and two doublet signals at δ_H 5.95 (1H, *d*, *J* = 10.0 Hz) and δ_H 6.90 (1H, *d*, *J* = 10.0 Hz). The HMBC spectrum displayed correlations from two methyl groups (H-4″ and H-5″) to C-2″ and C-3″, from H-2″ to C-3″ and C-8, and from H-1″ to C-3″, C-7 and C-9, leading to the assignment of an anellated pyrano ring attactched at C-7 and C-8. The HMBC spectral data showed correlations from H-5 to carbons at δ_C 145.7 (C-7), δ_C 146.6 (C-9), and δ_C 172.9 (C-4), from δ_H 3.88 (3H, s) to δ_C 146.8 (C-6), and from δ_H 3.81 (3H, s) to δ_C 140.4 (C-3).

The NOESY spectrum (**Figure 98**) of MK 06 showed the correlation between 6-OCH_3 protons and H-5. On the basis of these spectral data, the second partial structure was assigned as shown.

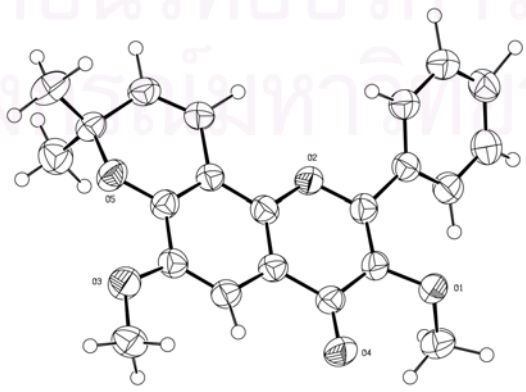


Combination of the first and second fragment led to the construction of a gross structure of MK 06. Compound MK 06 was therefore identified as 3,6-dimethoxy-2″-dimethyl-[5″,6″:8,7]-pyranoflavone.



MK 06

The spectrum of compound MK 06 is confirmed by single-crystal X-ray diffraction analysis. Protons and carbons of MK 06 were assigned as shown in **Table 12**.



ORTEP PLOT of MK 06

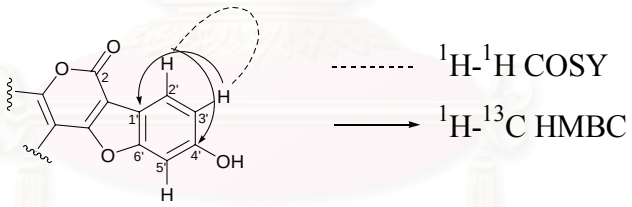
Table 12: The ^{1}H and ^{13}C-NMR Spectral Data of Compound MK 06 in DMSO-d_6

Position	δ_H (ppm), J (Hz)	δ_C (ppm)
2	-	153.9
3	-	140.4
4	-	172.9
5	7.34 (1H, *s*)	103.9
6	-	146.8
7	-	145.7
8	-	109.9
9	-	146.6
10	-	116.7
1′	-	130.3
2′	8.04 (1H, *dd*, J = 7.9, 1.7)	128.1
3′	7.58 (1H, *m*)	128.8
4′	7.57 (1H, *m*)	130.7
5′	7.58 (1H, *m*)	128.8
6′	7.57 (1H,*m*)	128.1
1″	6.90 (1H, *d*, J = 10.0)	114.7
2″	5.95 (1H, *d*, J = 10.0)	131.3
3″	-	78.2
2xCH_3	1.46 (6H, *s*)	27.6
3-OCH_3	3.81 (3H, *s*)	59.7
6-OCH_3	3.88 (3H, *s*)	55.8

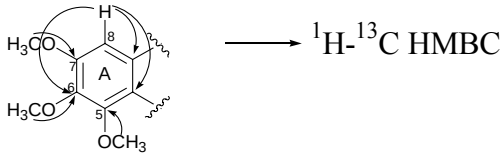
3.7 Structure Elucidation of MK 07

The compound MK07 was obtained as colourless plate. The ESIMS (**Figure 103**) suggested molecular formular of MK 07 as $C_{18}H_{14}O_7$, showing a peak of $[M+Na]^+$ at *m/z* 365.2. The IR bands (**Figure 102**) revealed signals of OH stretching at *v* 3374 cm^{-1}, *α,β* conjugated lactone ring at *v* 1731 cm^{-1}, and aromatic moiety at *v* 1621 and 1469 cm^{-1}. The UV spectrum (**Figure 101**) showed λ_{max} at 208, 302 and 358 nm.

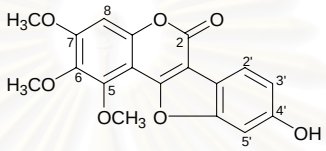
The ^{1}H-NMR spectrum (DMSO-d_6) of MK 07 (**Figure 104**) showed three signals on an aromatic ring at δ_H 7.82 (1H, *d*, 8.60), 6.92 (1H, *dd*, 8.59, 2.06) and 6.87(1H, *d*, 2.05), a typical for an ABX aromatic apin system. The ^{1}H-^{1}H COSY spectrum (**Figure 106**) showed connectivities between H-2′ and H-3′, and between H-3′ and H-5′. The ^{13}C-NMR spectrum (**Figure 105**) displayed a C-4′ hydroxyl carbon at δ_C 160.3. The upfield carbonyl carbon at δ_C 161.4 ppm suggested the presence of conjugated cyclic ester, and the HMBC (**Figure 109**) correlations could be well observed from H-2′ to C-4′, and from H-3′ to C-1′. Based on spectral data substructure of MK 07 was created as shown.



The HMQC (**Figure 108**) data revealed that three methoxyl protons located at δ_H 3.78, 3.88 and 3.89 ppm, belong to δ_C 56.3, 61.4 and 63.2. The ^{1}H-^{13}C HMBC spectrum showed correlations from δ_H 3.88(5-OCH_3) to δ_C 147.1 (C-5), δ_H 3.78 (6-OCH_3) to δ_C 140.7 (C-6), and δ_H 3.89 (7-OCH_3) to δ_C 156.6 (C-5). The HMBC spectrum also showed the correlation from H-8 to C-10, C-9, and C-6. Thus, the combination of HMBC and HMQC spectral data demonstrated that three methoxyl groups and H-8 were located on ring A.



Based on these spectral data, MK 07 was identified as 4′-hydroxy,5,6,7-trimethoxycoumestan, this is the fisrt report of a coumestan skeleton from *Millettia* spp. Proton and carbon of compound MK 07 were completely assigned by analyses of ^{1}H-^{1}H COSY (**Figure 106**), NOESY (**Figure 107**), HMQC (**Figure 108**) and HMBC (**Figure 109**) spectral data as shown in **Table 13**.



MK 07

Table 13: The ^{1}H and ^{13}C-NMR Spectral Data of Compound MK 07 in DMSO-d_6

Position	δ_H (ppm), J (Hz)	δ_C (ppm)
2	-	161.4
3	-	102.7
4	-	156.7
5	-	147.1
6	-	140.7
7	-	153.6
8	7.32 (1H, *s*)	93.3
9	-	151.8
10	-	110.4
1′	-	104.2
2′	7.82 (1H, *d*, 8.60)	123.3
3′	6.92 (1H, *dd*, 8.59, 2.06)	114.2
4′	-	160.3
5′	6.87 (1H, *d*, 2.05)	103.0
6′	-	155.1
5-OCH_3	3.88 (3H, *s*)	61.4
6-OCH_3	3.78 (3H, *s*)	56.8
7-OCH_3	3.89 (3H, *s*)	63.2

4. Biological Activities

The results of biological activities including antimycobacterial, antimalarial, and cytotoxicity are shown in **Table 14**.

4.1 Bioactive Compounds from *Croton kongensis*

Compounds CK 01, 02, 03 and 04 exhibited significant antimalarial (*Plasmodium falciparum* K1), antimycobacterial (*Mycobacterium tuberculosis* H37Ra) and cytotoxicity (KB cell, BC cell and NCI-H187) activities. These results are demonstrated in **Table 14**.

4.2 Bioactive Compound from *Croton birmanicus*

Compound CB 01 has displayed mild antimycobacterial activity against *Mycobacterium tuberculosis*, and this result is in **Table 14**. CB 01 did not exhibit cytotoxicity.

4.3 Bioactive Compounds from *Millettia kangensis*

Compounds MK 01, 02, 03, 05, 06, and 07 did not possess antimycobacterial and antimalarial activities (**Table 4**), while compound MK 04 exhibit only mild antimycobacterial activity. All isolated compounds had no cytotoxicity.

Table 14 Biological Activities of Compounds from *C. kongensis, C. birmanicus* and *M. kangensis*

Compound	Antimalarial[a] activity IC_{50} (μg/mL)	Antimycobacterial[b] activity MIC (μg/mL)	Cytotoxicity IC_{50} (μg/mL)*			
			Vero cell	KB cell[c]	BC cell[d]	NCI-H187[e]
CK 01	2.1	6.25	0.90	1.25	1.13	0.32
CK 02	2.8	25.0	3.16	13.84	inactive	1.10
CK 03	2.7	6.25	0.99	3.39	2.16	0.42
CK 04	1.3	6.25	N.D.	N.D.	inactive	inactive
CB 01	inactive	100	>50	inactive	inactive	inactive
MK 01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
MK 02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
MK 03	inactive	inactive	>50	inactive	inactive	inactive
MK 04	inactive	200	>50	inactive	inactive	inactive
MK 05	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
MK 06	inactive	inactive	>50	inactive	inactive	inactive
MK 07	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a] Antimalarial activity against *Plasmodium falciparum*, K 1 multi-drug resistant strain.

[b] Antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra.

[c] KB cell, Human epidermoid carcinoma cell lines of nasopharynx.

[d] BC cell, Human breast cancer cell lines.

[e] NCI-H187, Human small cell lung cancer cell lines.

N.D.; Not determined.

* IC_{50} (μg/mL) > 20; inactive
>10-20; weakly active
5-10; moderately active
< 5; strongly active

CHAPTER V

CONCLUSION

Two new 8,9 secokauranes namely, *ent*-8,9-*seco*-7α,11β-diacetoxykaura-8(14),16-dien-9,15-dione (**CK 02**) and *ent*-8,9-*seco*-8,14-epoxy-7α-hydroxy-11β-acetoxy-16-kauren-9,15-dione (**CK 03**), were isolated from *Croton kongensis* leaves along with two known compounds *ent*-8,9-*seco*-7α-hydroxy-11-acetoxykaura-8 (14),16-dien-9,15-dione (**CK 01**), and compound *ent*-7β-hydroxy-15-oxokaur-16-en-18-yl acetate (**CK 04**). A known glutarimide alkaloid, julocrotine (**CB 01**), was isolated from the roots of *Croton birmanicus*. Two new furanoflavoniods as 3-methoxy-6-hydroxy-[4″,5″:8,7]-furanoflavone (**MK 02**) and 2,5,8-trimethoxy-[4″,5″:6,7]-furanoflavanone (**MK 05**), a novel pyranoflavonoid, 3,6-dimethoxy-2″-dimethyl-[5″,6″:8,7]-pyrano flavone (**MK 06**), a new coumestan, 4′-hydroxy,5,6,7-trimethoxycoumestan (**MK 07**), together with a new natural product (synthetically known) 5,8-dimethoxy-[4″,5″:7,6]-furanoflavone (**MK 04**), and two known compounds, karanjin (**MK 01**) and 3,6-dimethoxy-[4″,5″:8,7]-furanoflavone (**MK 03**), were isolated from the leaves and twigs of *Millettia kangensis*. *Ent*-8,9-*seco*-7α,11β-diacetoxykaura-8(14),16-dien-9,15-dione (**CK 02**), *ent*-8,9-*seco*-8,14-epoxy-7α-hydroxy-11β-acetoxy-16-kauren-9,15-dione (**CK 03),** *ent*-8,9-seco-7α-hydroxy-11-acetoxykaura-8(14),16-dien-9,15-dione (**CK 01**), *ent*-7β-hydroxy-15-oxokaur-16-en-18-yl acetate (**CK 04**) showed significant antimalarial activity against *Plasmodium falciparum* (at IC_{50} 2.8, 2.7, 2.1, and 1.3 μg/mL, respectively), antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra (at MIC 25.0, 6.25, 6.25, and 6.25 μg/mL, respectively). In addition, CK 01, CK 02 and CK 03 exhibited strongly active toward Vero and NCI-H187 cell lines, and compounds CK 01 and CK 03 showed strongly active against BC cell line. Julocrotine (CB 01) from the roots of *C. birmanicus* showed mild antimycobacterial against *Mycobacterium tuberculosis* H37Ra at MIC 100 μg/mL. A new natural product 3,6-dimethoxy-[4″,5″:8,7]-furanoflavone (**MK 04**) showed only mild antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra at MIC 200 μg/mL, whereas 3,6-dimethoxy-2″-dimethyl-[5″,6″:8,7]-pyranoflavone (**MK 06**) and 3,6-dimethoxy-[4″,5″:8,7]-furanoflavone (**MK 03**) possessed no antimycobacterial and cytotoxic activities.

REFFERENCES

Aboagye, F. A., Sam, G. H., Massiot, G. and Lavaud, C., 2000. Julocrotine, a glutarimide alkaloid from *Croton membranaceus*. Fitoterapia, 71: 461-462.

Aiyar, V. N. and Seshadri, T. R., 1972. 11-Dehydro(-)-hardwickic acid fron *Croton oblongifolius*. Phytochemistry, 11: 1473-1476.

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

Asuzu, I.U., Gray, A. I. and Waterman, P. E., 1988. The extraction, isolation and identification of the purgative component of *Croton penduliflorus* seed oil. J. Ethnopharmacology, 23: 267-271.

Babili, F. E., Moulis, C., Bon, M., Respaud, M. J. and Fouraste, I., 1998. Three furano-diterpenes from the bark of *Croton campestris*. Phytochemistry, 48(1): 165-169.

Bandara, B. M. R., Wimalasiri, W. R. and Bandara, K. A. N. P., 1987. Isolation and insecticidal activity of (-)-hardwickiic acid from *Croton aromaticus.* Planta Med., 53: 575.

Bandara, B. M. R., Wimalasiri, W. R. and Macleod, J. K., 1988. Ent-kauranes and oleananes from *Croton Lacciferus*. Phytochemistry, 27(3): 869-871.

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.

Batatinha, M .J. M., de Souza-Spinosa, H. and Bernardi, M. M., 1995. *Croton zehntneri*: possible central nervous system effects of the essential oil in rodents. J. Ethnopharmacology, 45: 53-57.

Bettolo, R. M. and Scarpati, M. L., 1979. Alkaloids of *Croton draconoides*. Phytochemistry, 18: 520.

Boonyarathanakornkit, L., Che, C., Fong, H. H. S. and Farnsworth, N. R., 1987. Constituents of *Croton crassifolius* roots. Planta Med., 54: 61-62.

Cai, Y., Chen, Z. P., and Phillipson, J. D., 1993. Diterpenes from *Croton lechleri*. Phytochemistry, 32(3): 755-760.

Cai, Y., Evans, F. J., Roberts, M. F., Phillipson, J.D., Zenk, M. H. and Gleba, Y. Y., 1991. Polyphenolic compounds from *Croton lechleri*. Phytochemistry, 30(6): 2033-2040.

Chen, Z. P., Cai, Y. and Phillipson, J. D., 1994. Studies on the anti-tumour, anti-bacterial, and wound-healing properties of dragon's blood. Planta Med., 60: 541-545.

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

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.

Craib, W. G., 1927. Contributions to the flora of Siam. Additameutum XX, 56-72, Bull. Misc. Inform., Kew, London.

Craveiro, A. A., Andrade, C. H. S., Matos, F. J. A., Alencar, J. W. and Dantas, T. N. C., 1980. Fixed and volatile constituents of *Croton aff. nepetifolius*. J. Nat. Prod., 43(6): 756-757.

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.

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.

Devon, T. K. and Scott, A. I., 1972. Handbook of naturally occurring compounds: New York and London: Academic press.

Farias, R. A. F., Rao, V. S. N., Viana, G. S. G., Sileria, E. R., Maciel, M. A. M. and Pinto, A.C., 1997. Hypoglycemic effect of *trans*-dehydrocrotonin, a nor-clerodane diterpene from *Croton cajucara*. Planta Med., 63: 558-560.

Ferrigni, N. R., Puynum, J. E., Anderson, B., Jacobsen, L. B., Nichols, D. E., Moore, D. S. and McLaughlin, J. L., 1982. Modification and evalutation of the potato disc assay and antitumor screening of Euphorbiaceae seeds. J. Nat. Prod., 45(6): 679-686.

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.

Freire, A. C. G., Melo, P. S., Aoyama, H., Haun, M., Durán, N. and Ferreira, C. V., 2002. Cytotoxic effect of the diterpene lactone dehydrocrotonin from *Croton cajucara* on human promyelocytic leukemia cells. Planta Med., 69: 67-69.

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.

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

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

Gupta, R. K. and Krishnamurti, M., 1976b. Chromenoflavanones from *Millettia ovalifolia*. Phytochemistry, 15: 2011.

Hooker, J. D., 1973. The flora of British India; vol II Sabiaceae to Cornaceae. Jayyed Press, New Delhi.

Irvine, F. R., 1961. Woody plants of Ghana: Oxford University Press, London.

Islam, A., Gupta, B. B. and Krishnamurti, M., 1980. Furanochalcone and prenylated flavanones from *Millettia ovalifolia* seeds. Phytochemistry, 19: 1558-1589.

Itokawa, H., Ichihara, Y., Kojima, H., Watanabe, K. and Takeya, K., 1989. Nor-clerodane diterpenes from *Croton cajucara*. Phytochemistry, 28(6): 1667-1669.

Kammaing, 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.

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.

Krishnamurti, M., Sambhy, Y.R. and Seshadri, T.R., 1970. Chemical study of Indian yam beans (*Pachyrrhizus erosus*):isolation of two new rotenoids; 12a-hydroxydolineone and 12a-hydroxypachyrrhizone. Tetrahedron, 26: 3023-3027.

Kubo, I., Asaka, Y. and Shibata, K., 1991. Insect growth inhibitory *nor*-diterpenes, *cis*-dehydrocrotonin and trans-dehydrocrotonin, from *Croton cajucara*. Phytochemistry, 30(8): 2545-2546.

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

Luzbetak, D. J., Torrance, S. J., Hoffmann, J. J. and Cole, J. R., 1978. Isolation of (-)-hardwickiic acid and 1-triacontanol from *Croton californicus*. J. Nat. Prod., 42 (2): 315-316.

Maciel, M. A. M., Pinto, A. C., Arruda, A. C., Pamplona, S. G. S. R., Vanderlinde, F. A., Lapa, A. J., Echevarria, A., Grynberg, N. F., Côlus, I. M. S., Farias, R. A. F., Costa, A. M. L., Rao, V. S. N., 2000. Ethnopharmacology, phytochemistry and pharmacology: a successful combination in the study of *Croton cajucara.* J. Ethnopharmacology, 70: 41-45.

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

Manitto, P., 1981. Biosynthesis of natural products: Ellis Horwood Ltd., England.

Markham, K. R., 1982. Techniques of Flavonoid Identification. Academics press, New York.

Mazzanti, G., Bolle, P., Matinoli, L., Piccinelli, D., Grgurina, I., Animati, F. and Mugne, Y., 1987. *Croton macrostachys*, a plant used in traditional medicine: purgative and inflamatory activity. J. Ethnopharmacology, 19: 213-219.

McChesney, J. D. and Silveira, E. R., 1989. 12-Hydroxyhardwickic acid and sonderianial, neo-clerodanes from *Croton sonderianus*. Phytochemistry, 28 (12): 3411-3414.

Mensah, I. A., Achenbach, H., Thoithi, G. N., Waibel, R. and Mwangi, J. W., 1992. Epoxychiromodine and other constituents of *Croton megalocarpus.* Phytochemistry, 31(6): 2055-2058.

Milo, B., Risco, E., Vila, R., Iglesias, J. and Cañigueral, S., 2002. Characterization of a fucoarabinogalactan, the main polysaccharide from the gum exudate of *Croton urucurana*. J. Nat. Prod., 65: 1143-1146.

Minh, P. T. H., Ngoc, P. H., Quang, D. N., Hashimoto, T., Takaoka, S. and Asakawa, Y., 2003. A novel ent-kaurane diterpenoid from *Croton tonkinensis* Gagnep. Chem. Pharm. Bull., 51(5): 590-591.

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

Moulis, C. and Fouraste, I., 1992. Levatin, an 18-norclerodane diterpene from *Croton levatii*. J. Nat. Prod., 55(4): 445-449.

Moura, V. L. A., Monte, F. J. O. and Filho, R. B., 1990. A new casbane-type diterpenoid from *Croton nepetaefolius*. J. Nat. Prod., 53(6): 1566-1571.

Nakano, T., Djerassi, C., Corral, R. A. and Orazi, O. O., 1961. Structure of julocrotine. J. Org. Chem., 26: 1184-1191.

Nareeboon, P., 1998. Chemical constituents and some bioactivities of leaves of *Croton joufra*. Master's Thesis, Department of Chemistry, Graduate School, Ramkhamhaeng University.

Ngamrojnanich, N., Sirimongkon, S., Roengsumran, S., Petsom, A. and Kamimura, H., 2003. Inhibition of Na^+, K^+-ATPase activity by (-)-*ent*-kaur-16-en-19-oic acid and its derivatives. Planta Med., 69: 555-556.

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.

Parvez, M. and Ogbeide, O. N., 1989. 3-Hydroxy-4'-methoxyflavone from *Millettia zechiana*. Phytochemistry, 29(6): 2043-2044.

Pattamadilok, D., 1998. Chemical constituents of *Croton oblongifolius* stem bark from Chinat. Master's Thesis, Department of Chemistry, Graduate School, Chulalongkorn University.

Peres, M. T. L. P., Monache, F. D., Cruz, A. B., Pizzolatti, M. G. and Yunes, R. A., 1997. Chemical composition and antimicrobial activity of *Croton urucurana* Baillon (Euphorbiaceae). J. Ethnopharmacology, 56: 223-226.

Perrett, S., Whitfield, P. J., Sanderson, L. and Bartlett, A., 1995. The plant molluscicide *Millettia thonningii* (Leguminosae) as a topical antischistosomal agent. J. Ethnopharmacology, 47: 49-54.

Perry, N. B., Burgess, E. J., Baek, S. H., Weavers, R. T., Geis, W. and Mauger, A. B., 1999. 11-Oxygenated cytotoxic 8,9 secokauranes from a New Zealand liverwort, *Lepidolaena taylorii*. Phytochemistry, 50: 423-433.

Perry, N. B., Burgess, E. J. and Tangney, R. S., 1996. Cytotoxic 8,9-secokaurane diterpenes from a New Zealand Liverwort, *Lipidolaena taylorii*. Tetrahedron Lett., 37: 9387-9390.

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.

Pieters, L., De Bruyne, T., Mei, G., Lemière, G., Berghe, D. V. and Vlietinck, J., 1992. *In vitro* and *in vivo* biological activity of South American dragon's blood and its constituents. Planta Med., 58(1): 582-583.

Pieters, L., De Bruyne, T., Claeys, M., Vlietinck, A., Calomme, M., and Berghe, D. V., 1993. Isolation of a dihydrobenzofuran lignan from South America dragon's blood (*Croton* spp.) as an inhibitor of cell proliferation. J. Nat. Prod., 56(6): 899-906.

Puebla, P., López, J. L., Guerrero, M., Carrón, R., Martín, M. L., Román, L. S. and Feliciano, A. S., 2003. *Neo*-clerodane diterpenoids from *Croton schiedeanus*. Phytochemistry, 62: 551-555.

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.

Roengsumran, S., Petsom, A., Kuptiyanuwat, N., Vilaivan, T., Ngamrojnanich, N., Chaichantiyuth, C. and Phuthong, S., 2001. Cytotoxic labdane diterpenoids from *Croton oblongifolius*. Phytochemistry, 56: 103-107.

Roengsumran, S., Pornpakakul, S., Muangsin, N., Sangvanich, T., Singtothong, P., Chaichit, N., Puthong, S. and Petsom, A., 2004. New halimane diterpenoids from *Croton oblongifolius*. Planta Med., 70: 87-89.

Roengsumran, S., Singtothong, P., Pudhom, K., Ngamrojnanich, N., Petsom, A. and Chaichantiyuth, C., 1999. Neocrotocembranal from *Croton oblonggifolius*. J. Nat. Prod., 62: 1163-1164.

Saralamp, P., Chuakul, W., Temsiririrkkul, R. and Clayton, T., 1996. Medicinal Plants in Thailand Volumn 1: 77, Amarin Printing and Publihing Public Co., Ltd., Bangkok.

Shaw, H. K. A., 1980. The Euphorbiaceae platrobeae of Australia. Kew Bulletin, 35 (3): 614.

Shaw, H. K. A., 1981. The Euphorbiaceae of Sumata. Kew Bulletin, 36(2): 283.

Silveira, E. R. and McChesney, J. D., 1994. 6,7-Oxygenated neo-clerodane furan diterpenes from *Croton sonderianus*. 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., Baruan, J. N., Govindan, S. V. and Herz, W., 1982. Rotenoids from roots of *Millettia pachycarpa*. Phytochemistry, 21(4): 946-951.

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

Siriwat, K., 1999. Chemical constituents and biological activity from stem barks of *Croton oblongifolius* Roxb. from Amphur Dan Sai, Loei province. Master's Thesis, Department of Chemistry, Graduate School, Chulalongkorn University.

Skehan, 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.

Smitt, O. and Hogberg, H. E., 2002. Syntheses of a prenylbisabolane diterpene, a natural insecticide from *Croton linearis*, and of the bisabolane sesquiterpenes (-)-delobanone and (-)-epi-delobanone. Tetrahedron, 58: 7691-7700.

Son, P. T., Giang, P. M. and Taylor, W. C., 2000. An *ent*-kaurane diterpenoid from *Croton tonkinensis* Gagnep. Aust. J. Chem., 53: 1003-1005.

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.

Stuart, K. L., McNeill, D., Kutney, J. P., Eigendorf, G. and Klein, K. F., 1973. Isolation and synthesis of glutamine and glutarimide derivatives from *Croton humilis*. Tetrahedron, 29: 4071-4075.

Sutthivaiyakit, A., Nareeboon, P., Ruangrangsi, N., Ruchirawat, S., Pisutjaroenpong, S. and Mahidol, C., 2001. Labdane and pimarane diterpenes from *Croton joufra*. Phytochemistry, 56: 811-814.

Takahashi, S., Kurabayashi, M., Kiyazawa, E., Haruyama, H. and Ogiso, A., 1983. Plaunolide, a furanoid diterpene from *Croton sublyratus*. Phytochemistry, 22 (1): 302-303.

Tchissambou, L., Chiaroni, A., Riche, C. and Khuong-Huu, F., 1990. Crotocorylifuran and crotohaumanoxide, new diterpenes from *Croton Haumanianus* J. Leonard. Phytochemistry, 46(15): 5199-5202.

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

van Valkenburg, J. L. C. H. and Bunyapraphatsara, N., 2001. Medicinal and poisonous plants 2. Plant resources of South-East Asia, 12(2): 198. Backhuys Publishers, Leiden.

Vigor, C., Fabre, N., Fourasté, I. and Moulis, C., 2001. Three clerodane diterpenoids from *Croton eluteria* Bennett. Phytochemistry, 57: 1209-1212.

Vigor, C., Fabre, N., Fouraste, I., and Moulis, C., 2002. Neoclerodane diterpenoids from *Croton eluteria*. J. Nat. Prod., 65: 1180-1182.

Weckert, E., Hummer, K., Mensah, I. A. and Achenbach, H., 1992. The absolute configuration of chiromodine. Phytochemistry, 31(6): 2170-2172.

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

Yanesew, 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.

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*1. J. Nat. Prod., 66(9).

APPENDIX

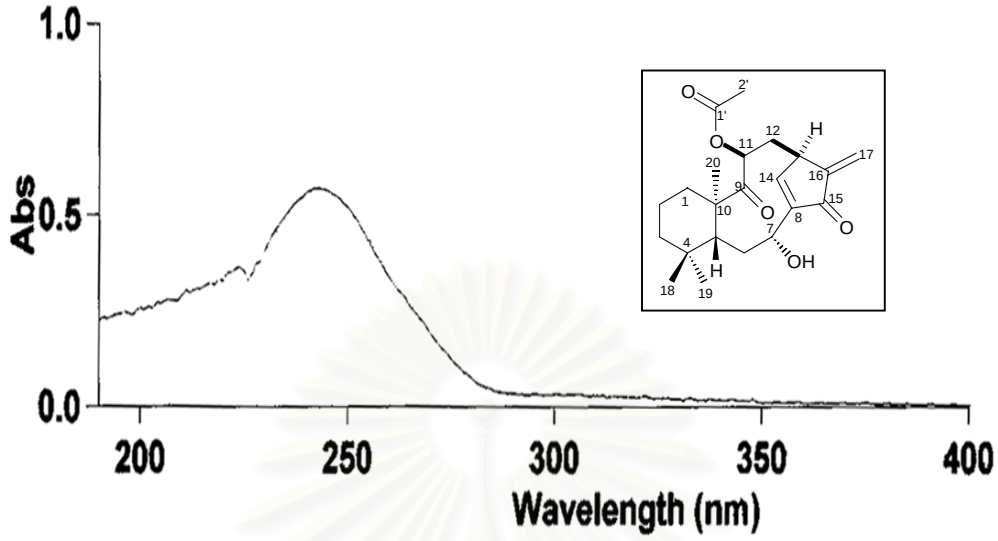


Figure 4 UV Spectrum of Compound CK 01 (chloroform)

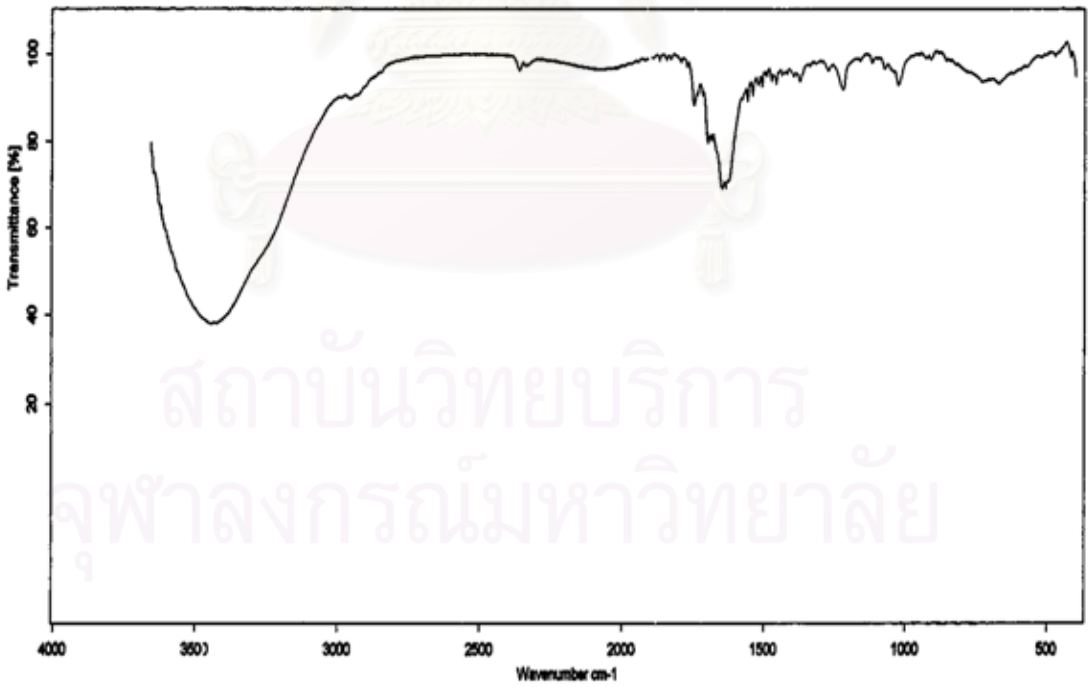


Figure 5 IR Spectrum of Compound CK 01 (neat)

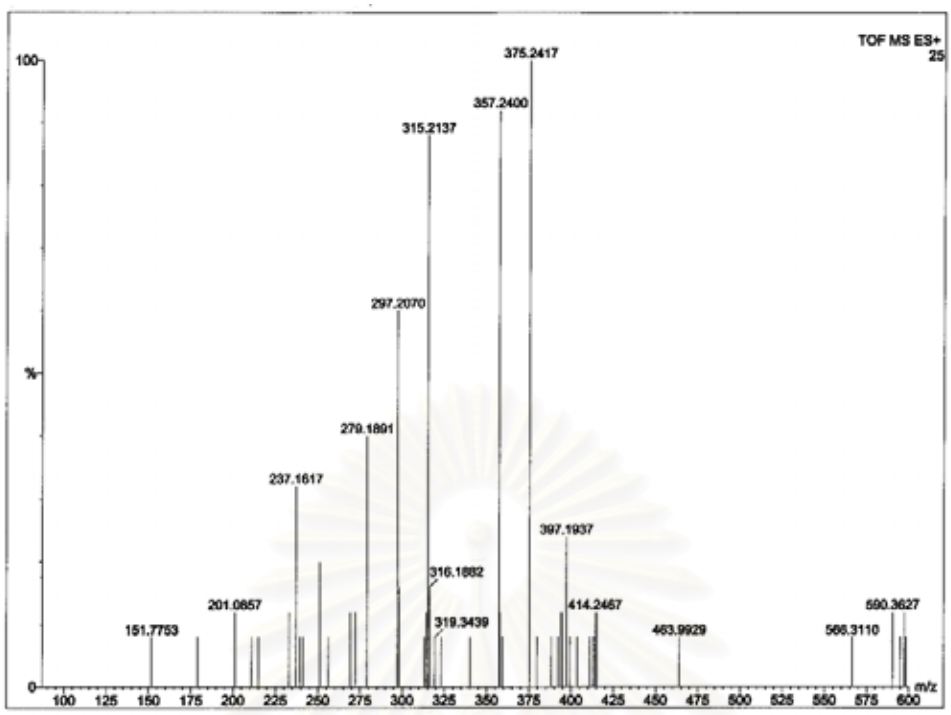


Figure 6 MS Spectrum of Compound CK 01

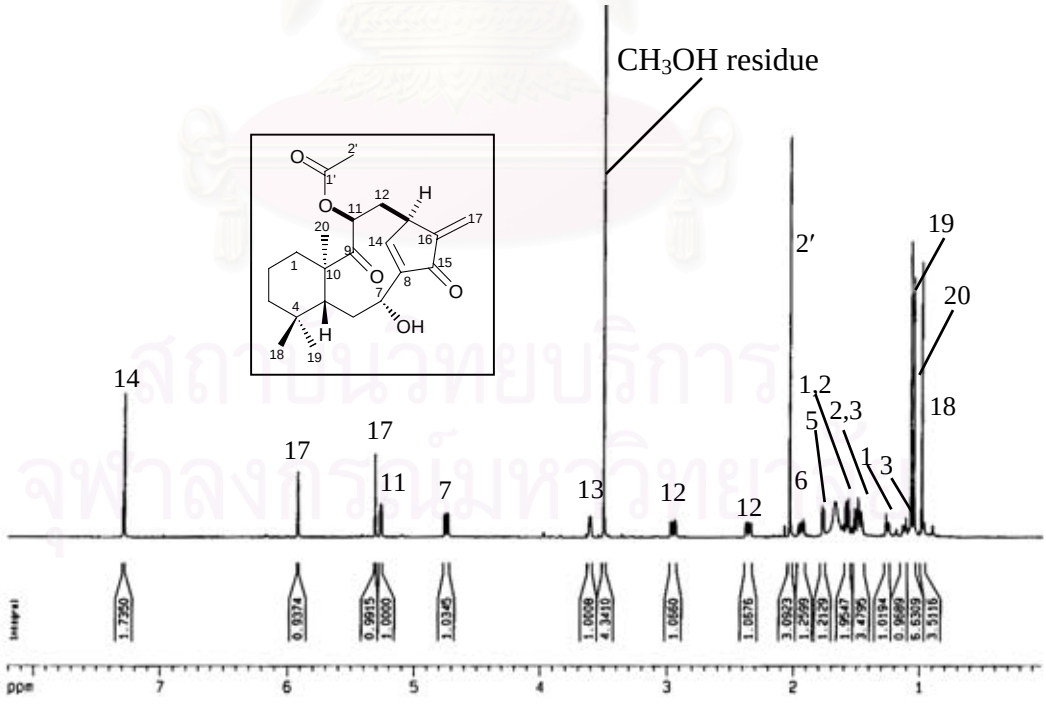


Figure 7 ^{1}H- NMR Spectrum ($CDCl_3$) of Compound CK 01

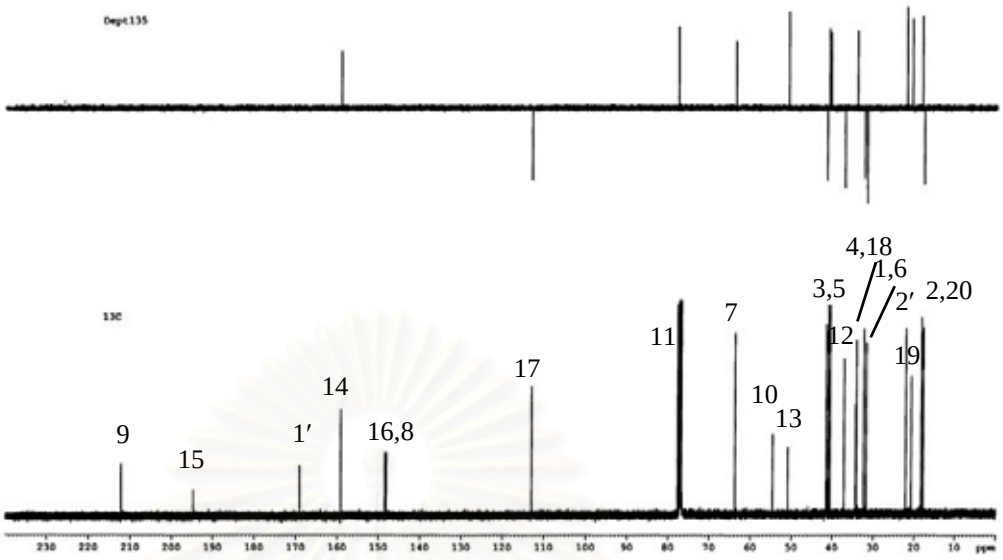


Figure 8 ^{13}C NMR and DEPT Spectra of ($CDCl_3$) Compound CK 01

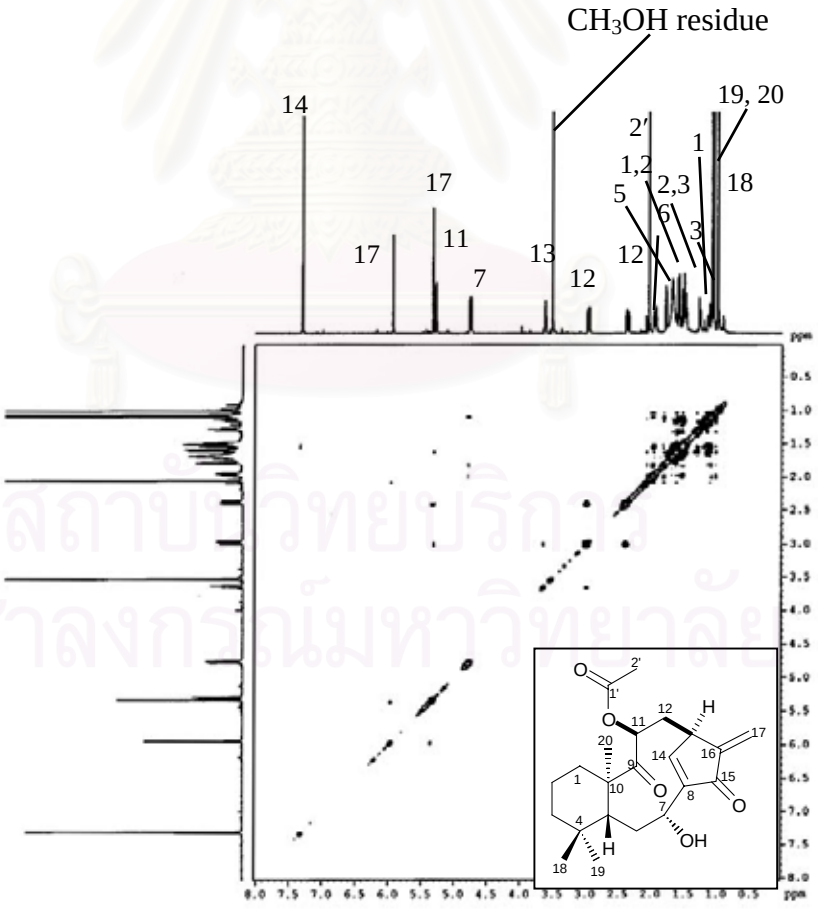


Figure 9 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Compound CK 01

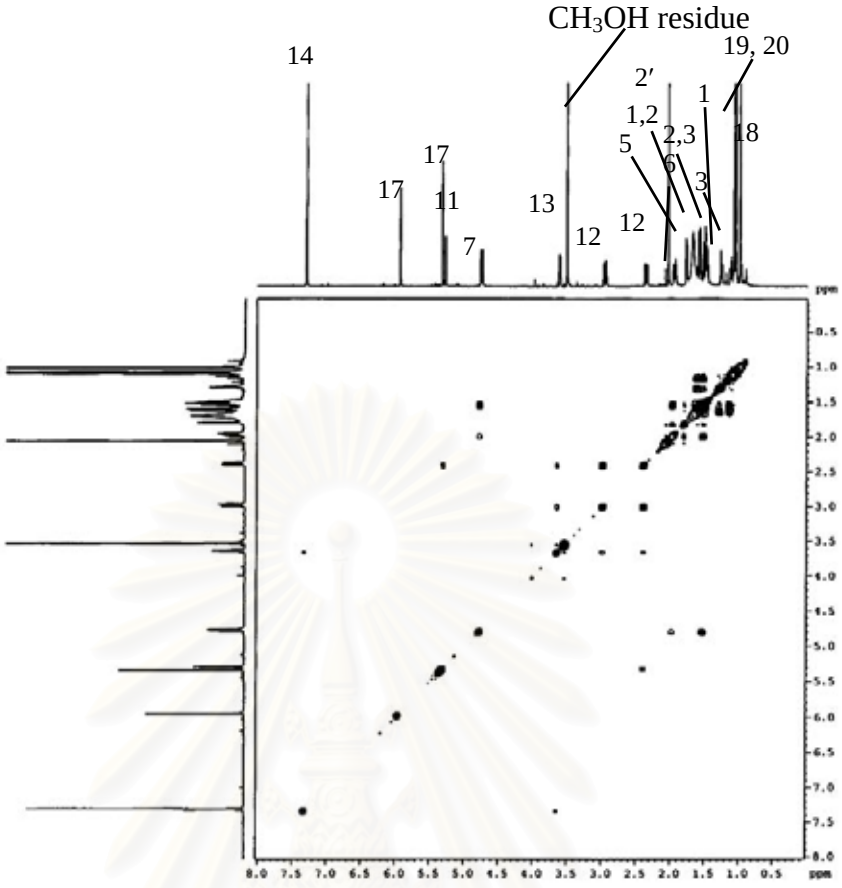


Figure 10 NOESY Spectrum ($CDCl_3$) of Compound CK 01

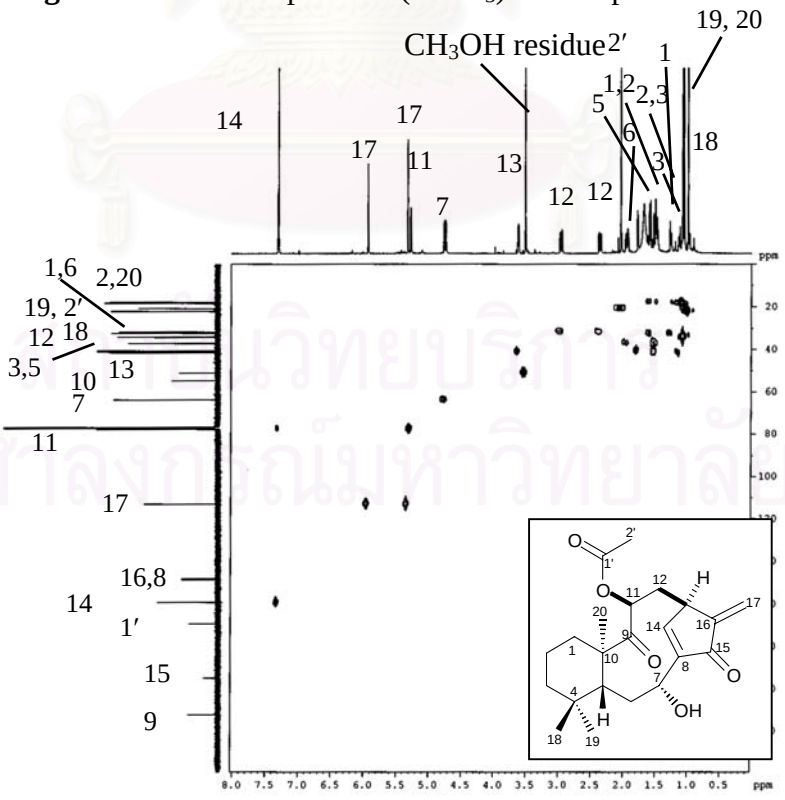


Figure 11 HMQC Spectrum ($CDCl_3$) of Compound CK 01

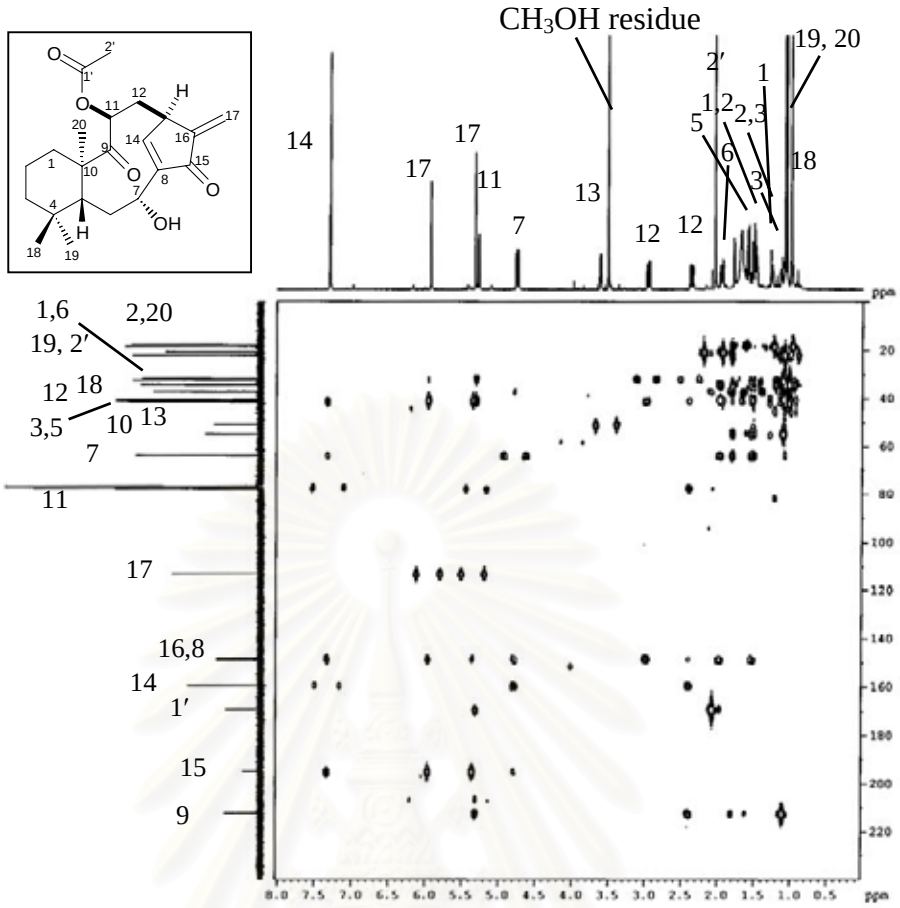


Figure 12 HMBC Spectrum ($CDCl_3$) of Compound CK 01

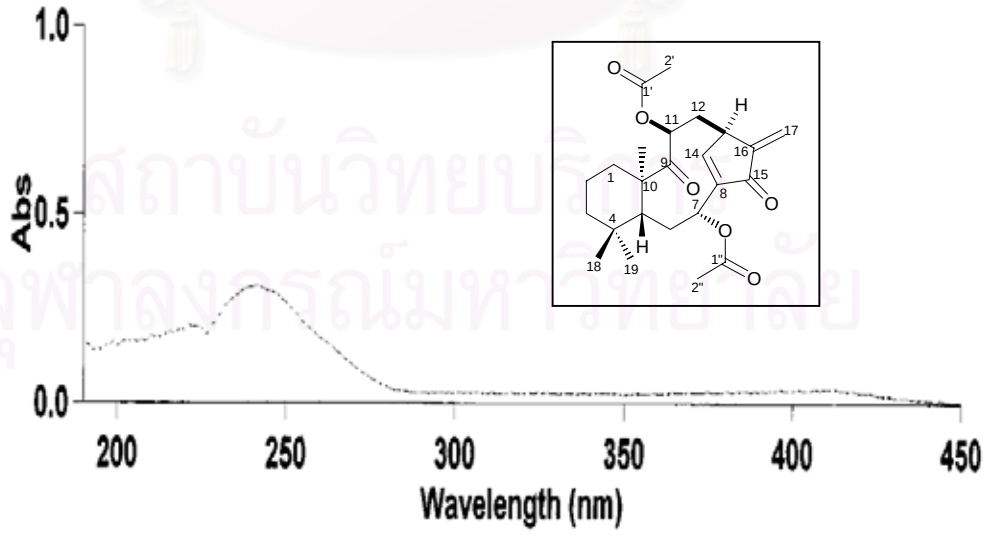


Figure 13 UV Spectrum of Compound CK 02 (chloroform)

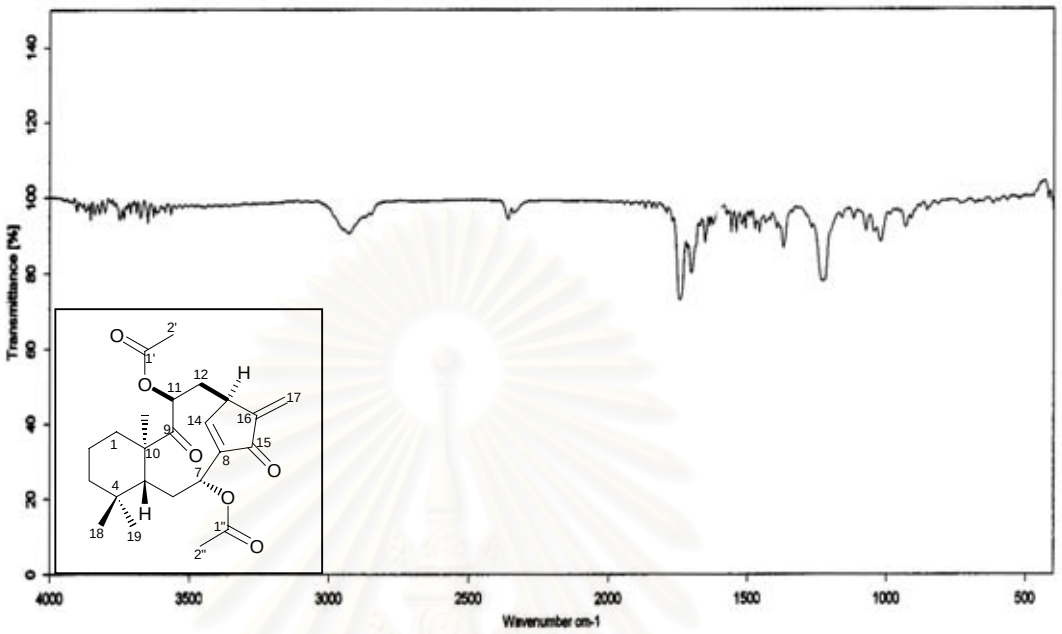


Figure 14 IR Spectrum of Compound CK 02 (neat)

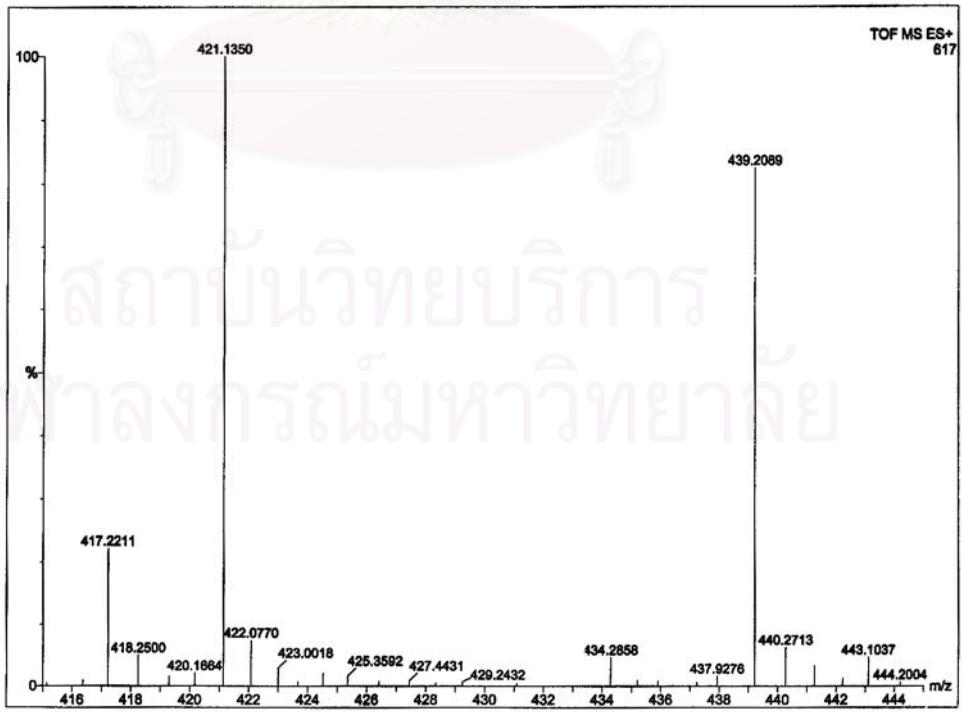


Figure 15 MS Spectrum of Compound CK 02

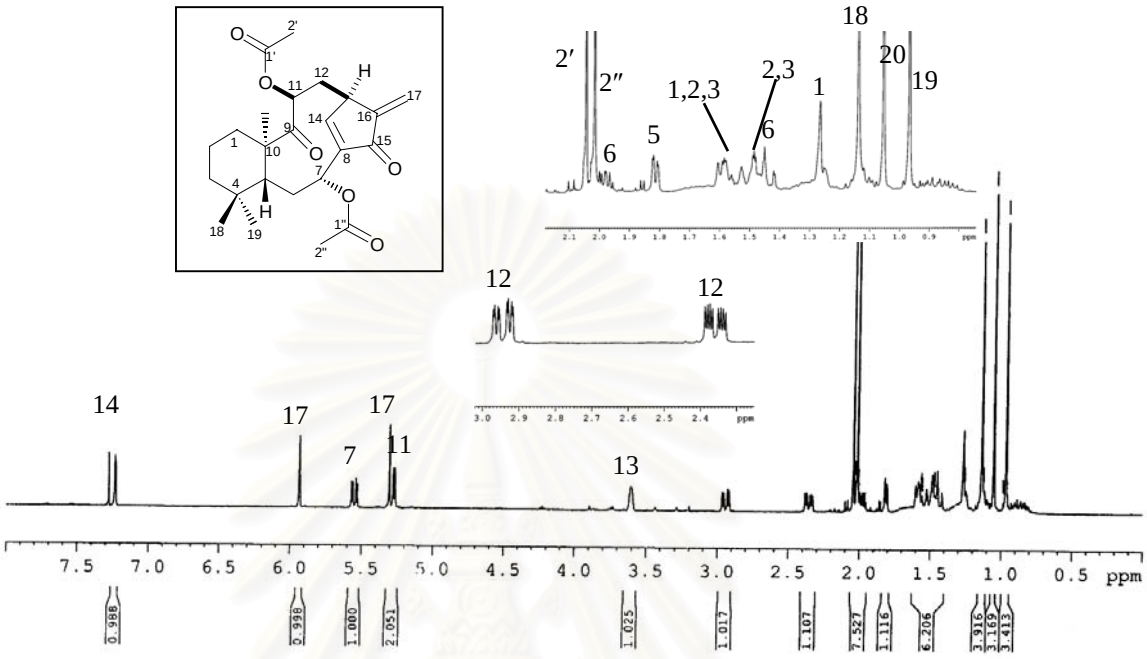


Figure 16 ^{1}H- NMR Spectrum ($CDCl_3$) of Compound CK 02

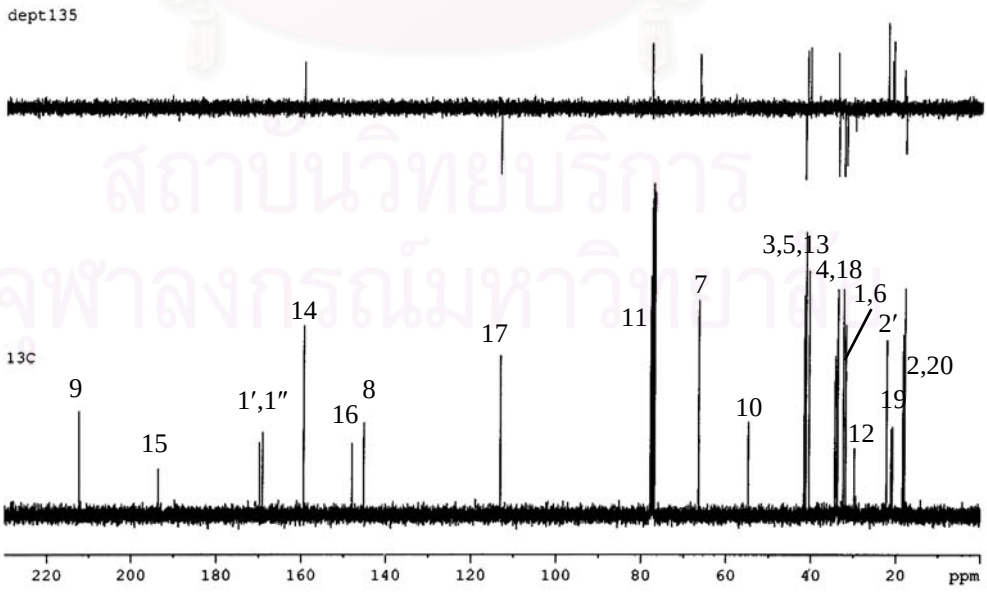


Figure 17 ^{13}C NMR and DEPT Spectra ($CDCl_3$) of Compound CK 02

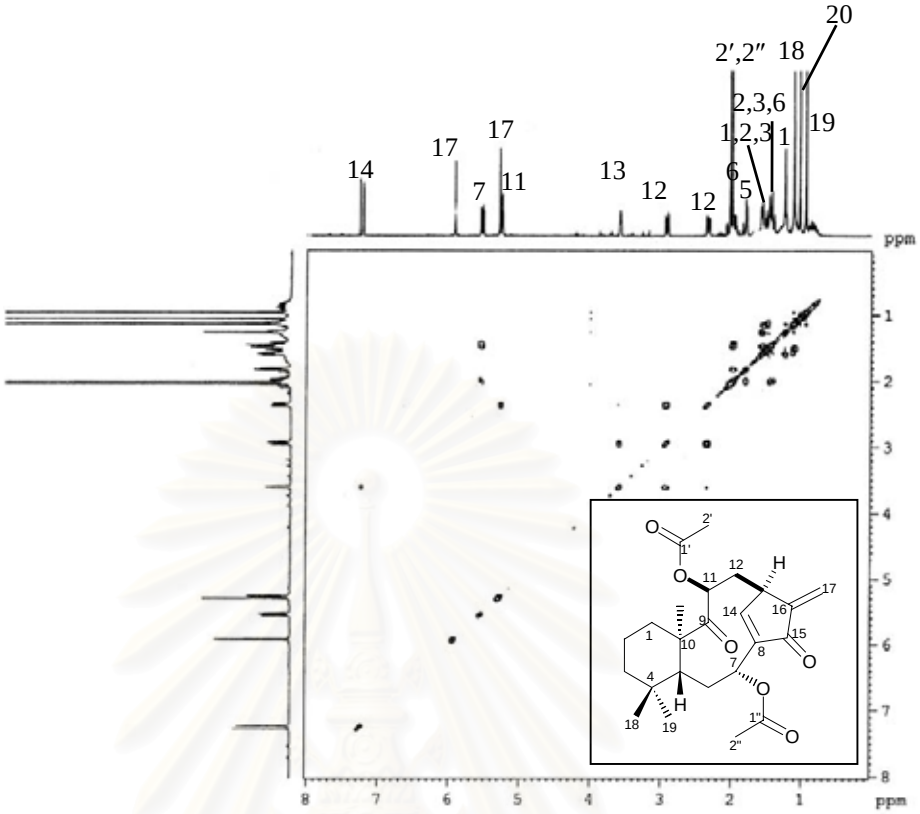


Figure 18 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Compound CK 02

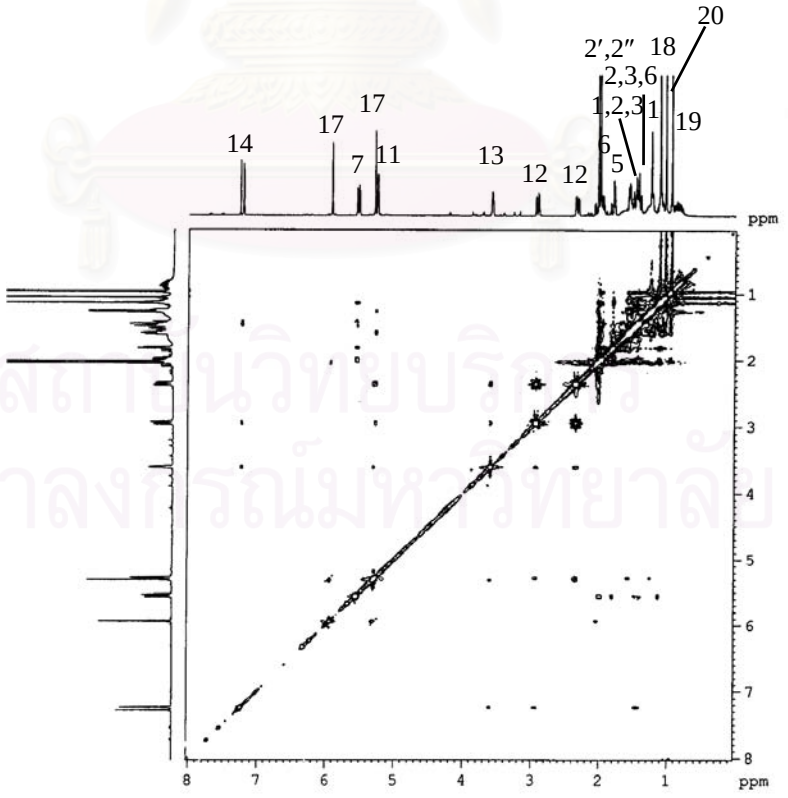


Figure 19 NOESY Spectrum ($CDCl_3$) of Compound CK 02

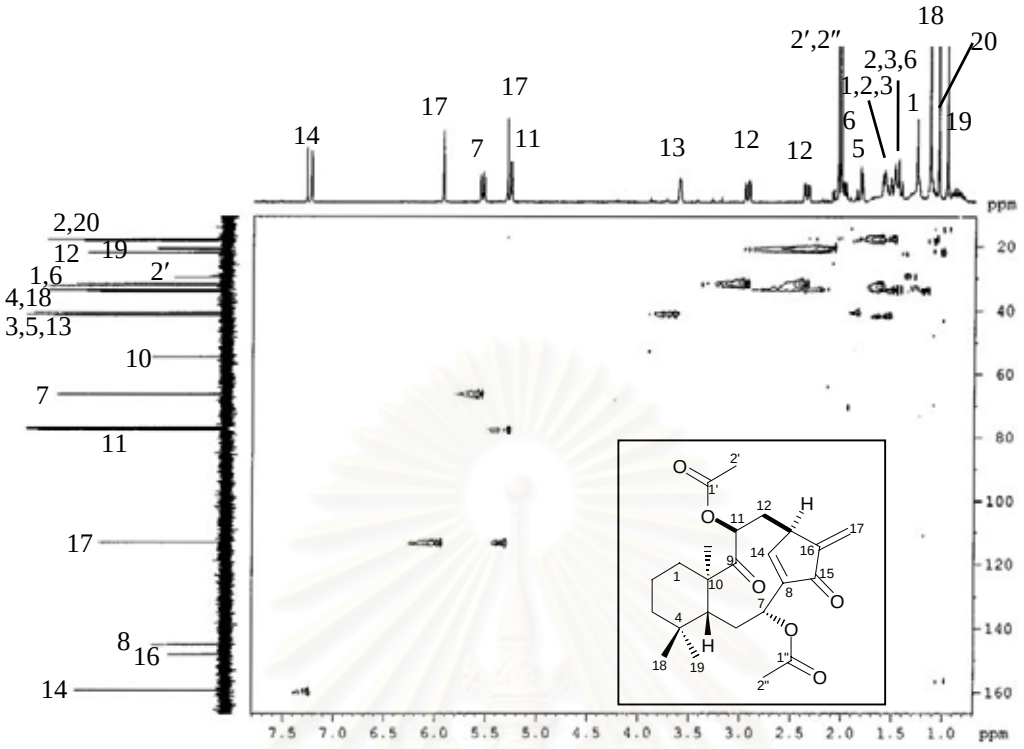


Figure 20 HMQC Spectrum ($CDCl_3$) of Compound CK 02

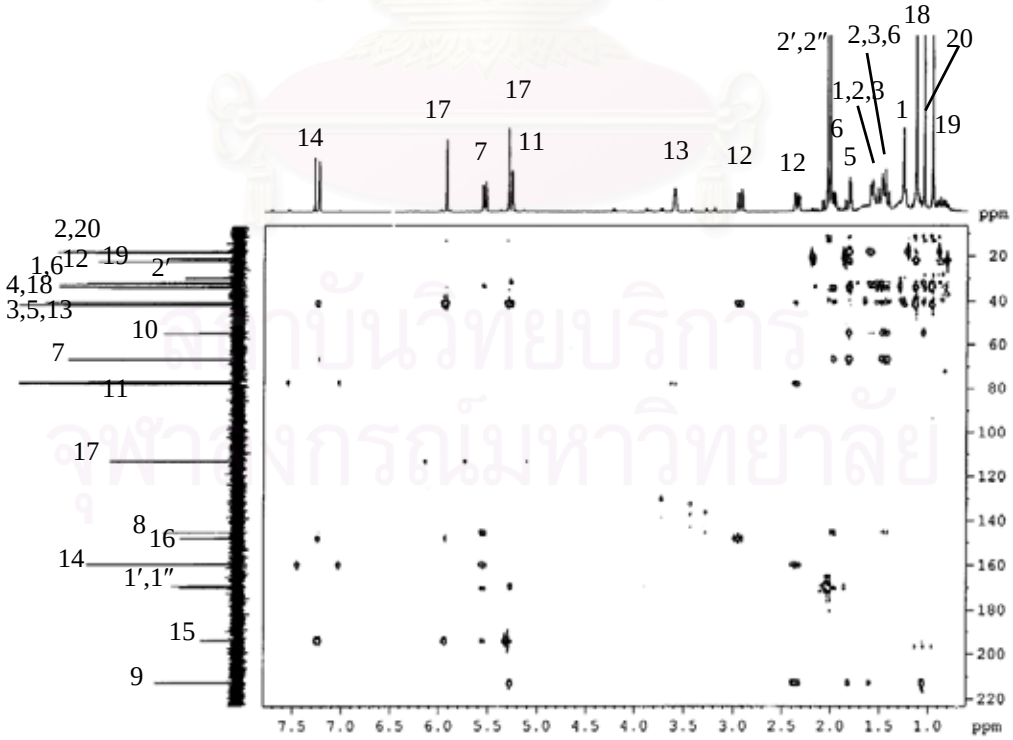


Figure 21 HMBC Spectrum ($CDCl_3$) of Compound CK 02

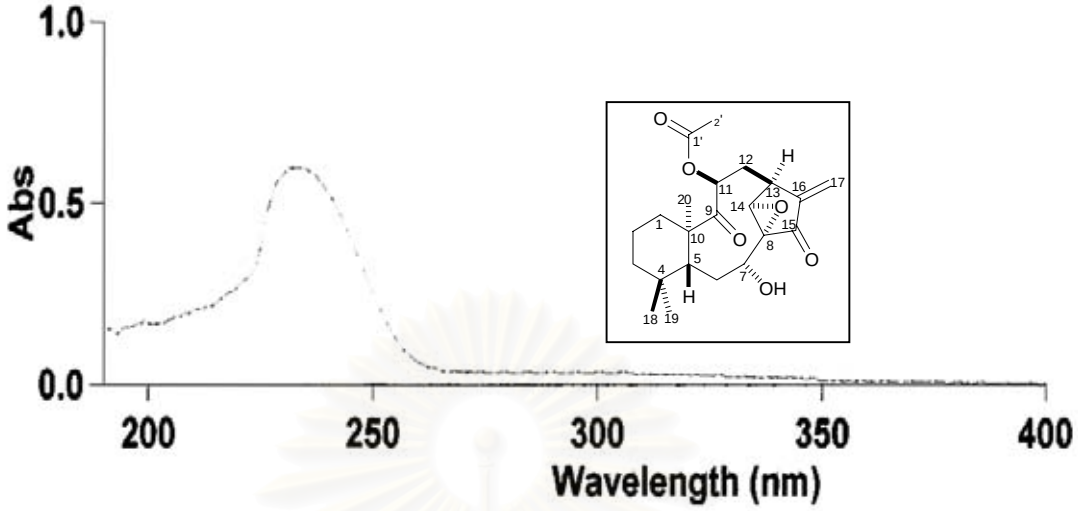


Figure 22 UV Spectrum of Compound CK 03 (chloroform)

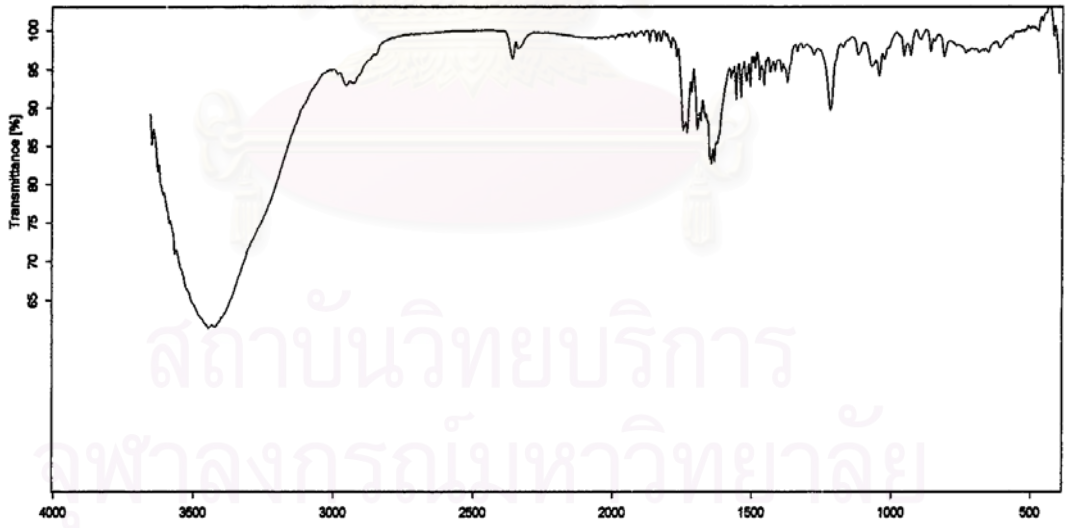


Figure 23 IR Spectrum of Compound CK 03 (neat)

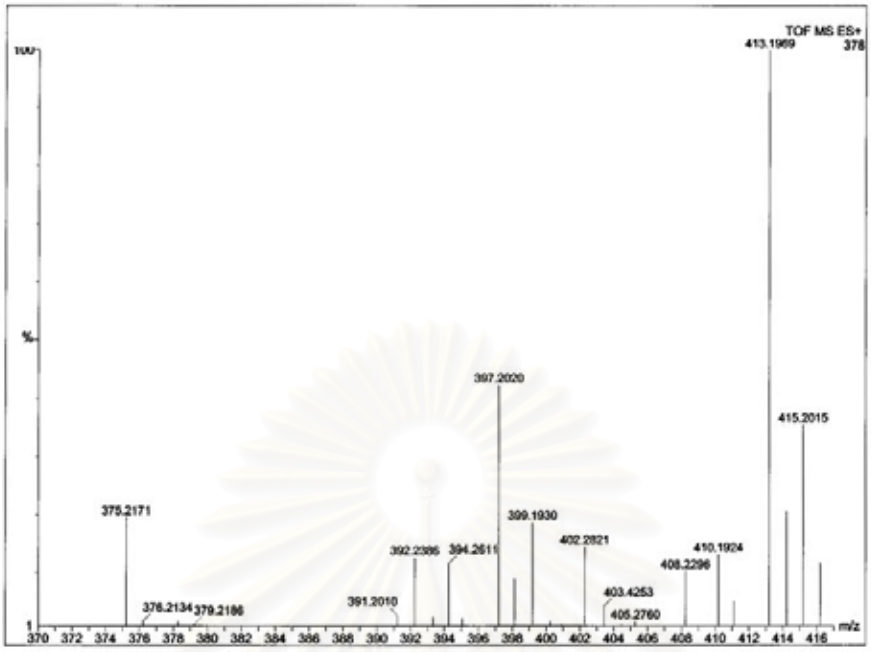


Figure 24 MS Spectrum of Compound CK 03

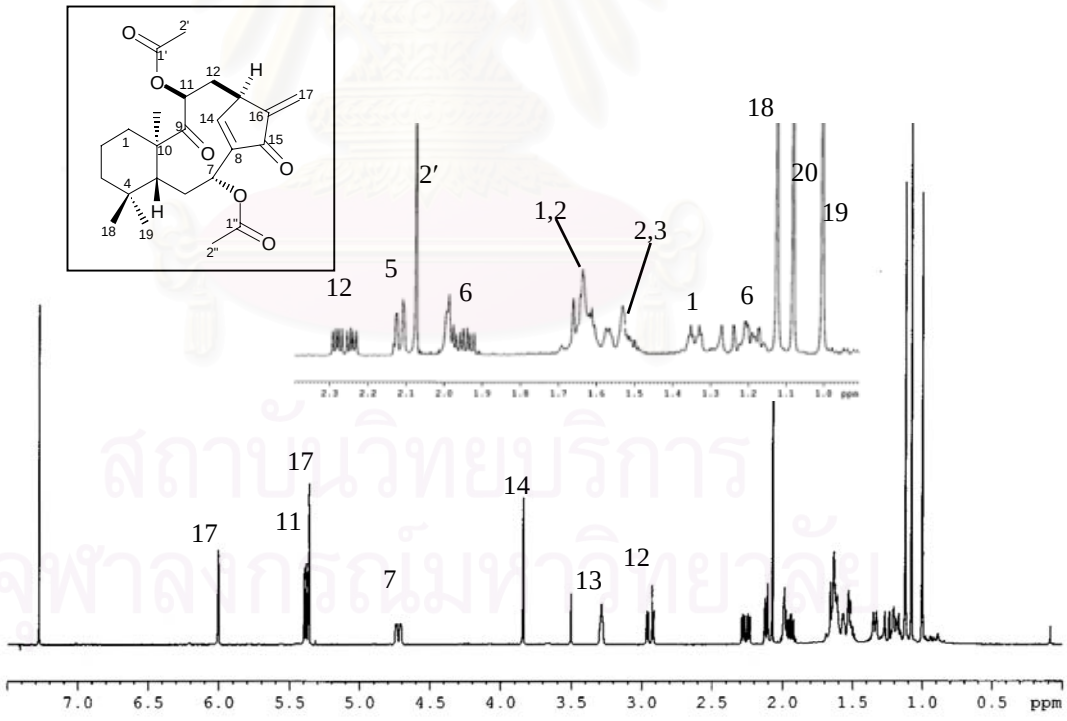


Figure 25 ^{1}H- NMR Spectrum ($CDCl_3$) of Compound CK 03

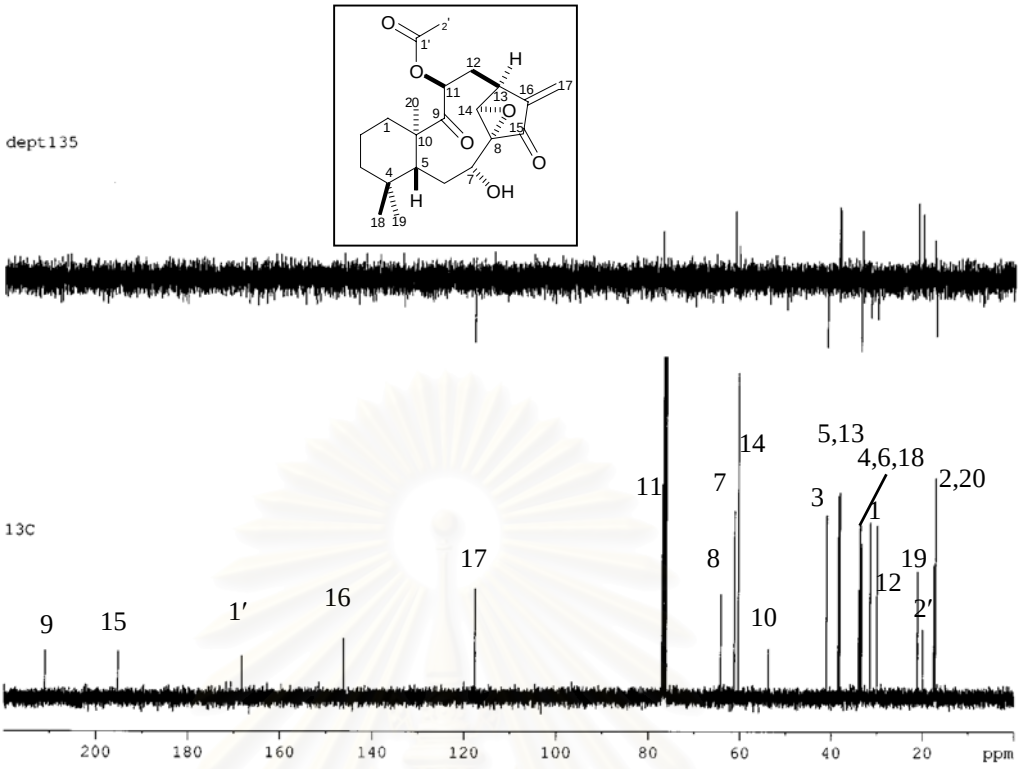


Figure 26 ^{13}C NMR and DEPT Spectra ($CDCl_3$) of Compound CK 03

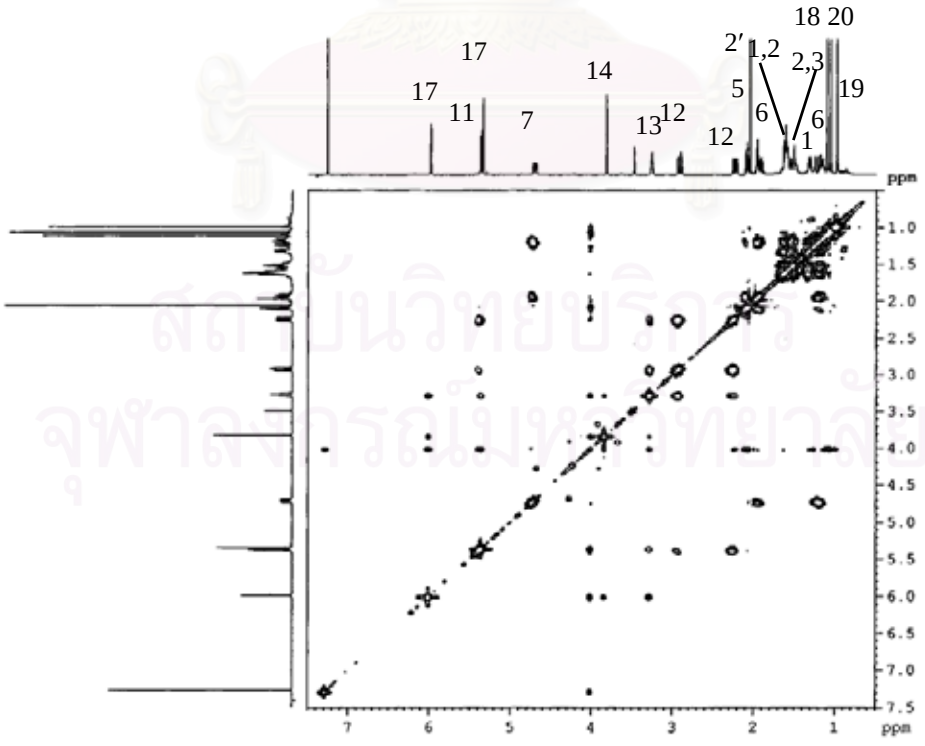


Figure 27 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Compound CK 03

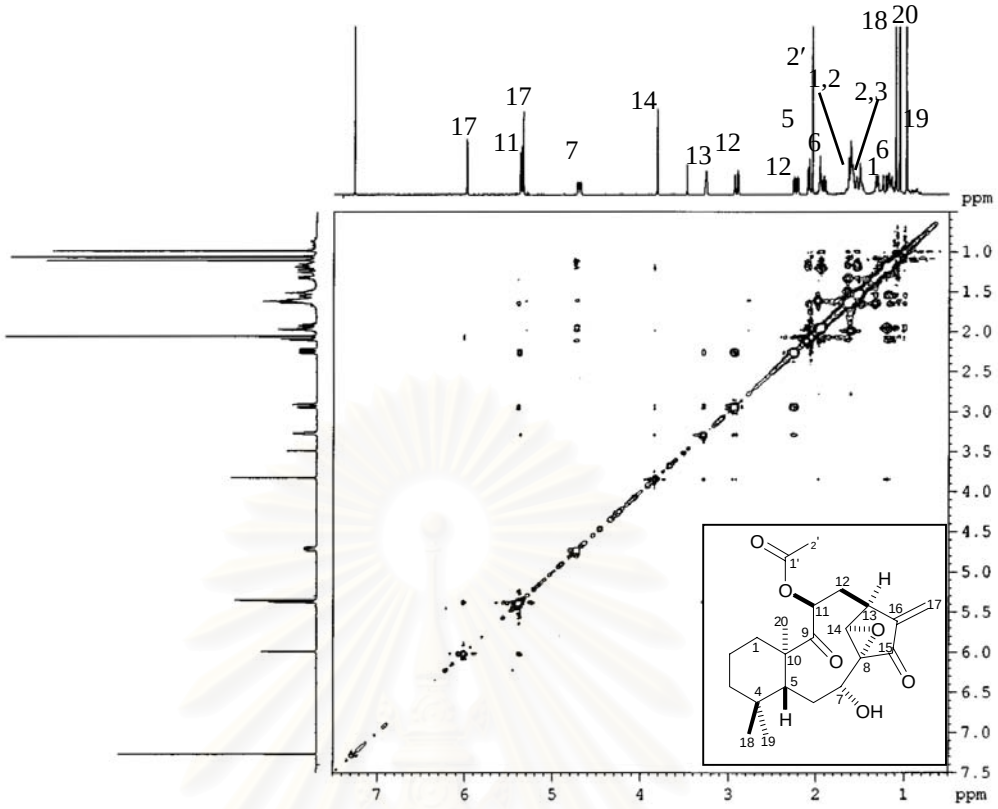


Figure 28 NOESY Spectrum ($CDCl_3$) of Compound CK 03

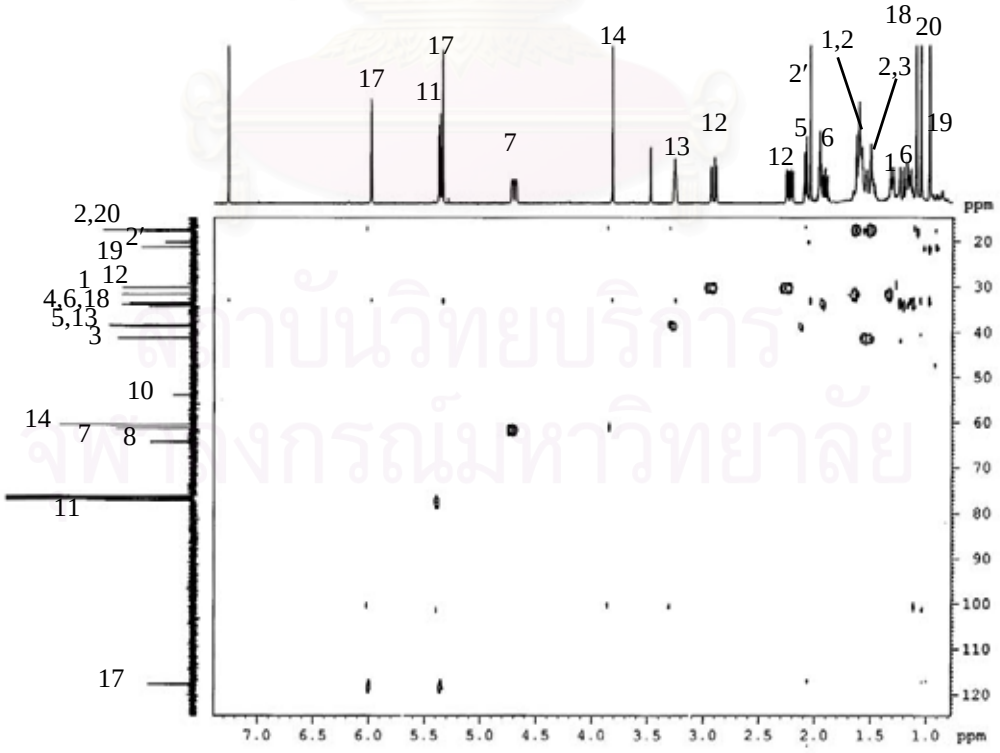


Figure 29 HMQC Spectrum ($CDCl_3$) of Compound CK 03

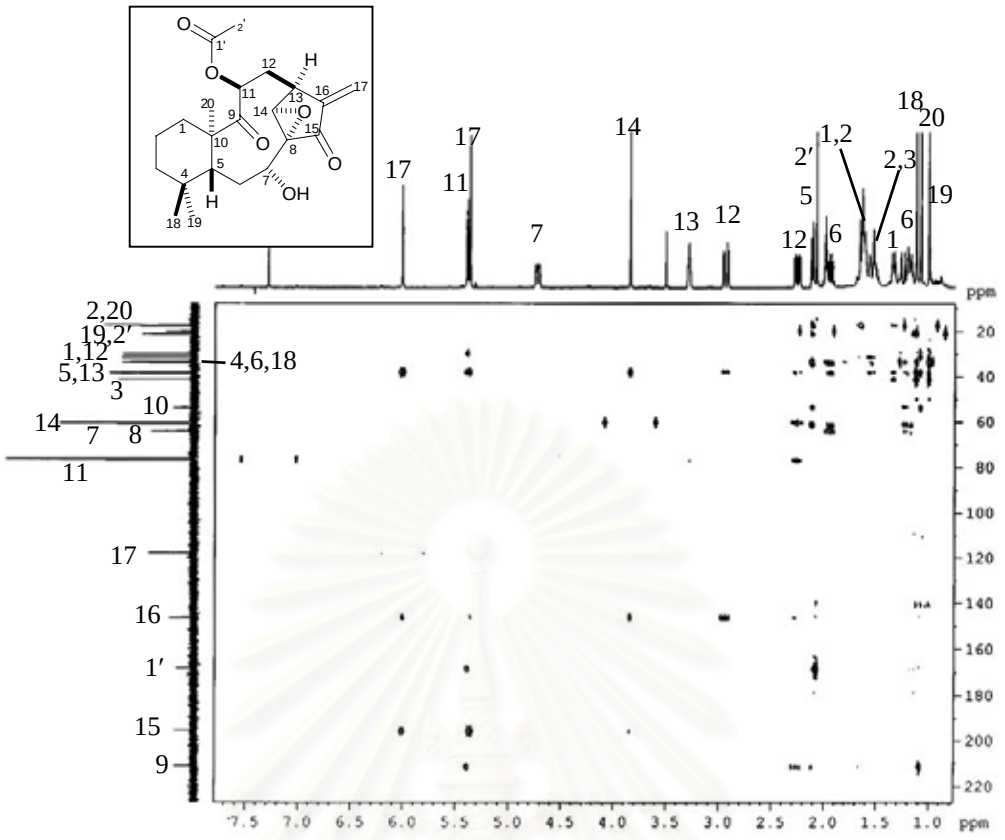


Figure 30 HMBC Spectrum ($CDCl_3$) of Compound CK 03

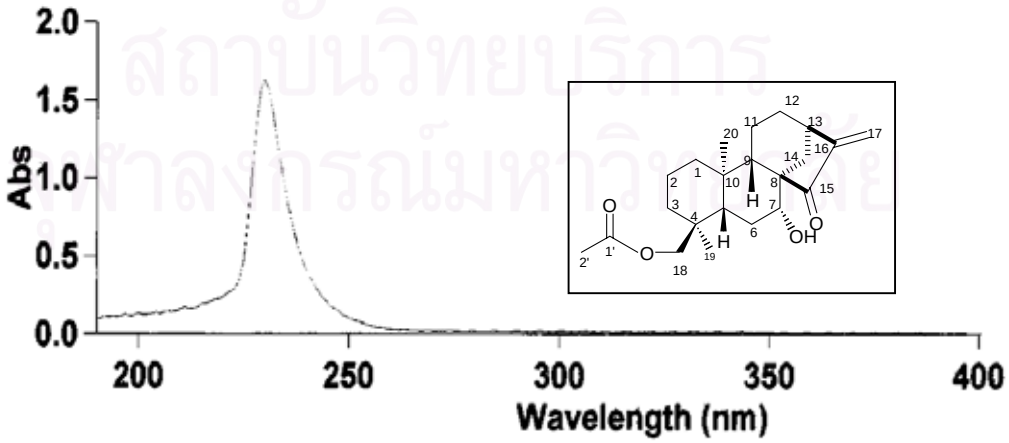


Figure 31 UV Spectrum of Compound CK 04 (chloroform)

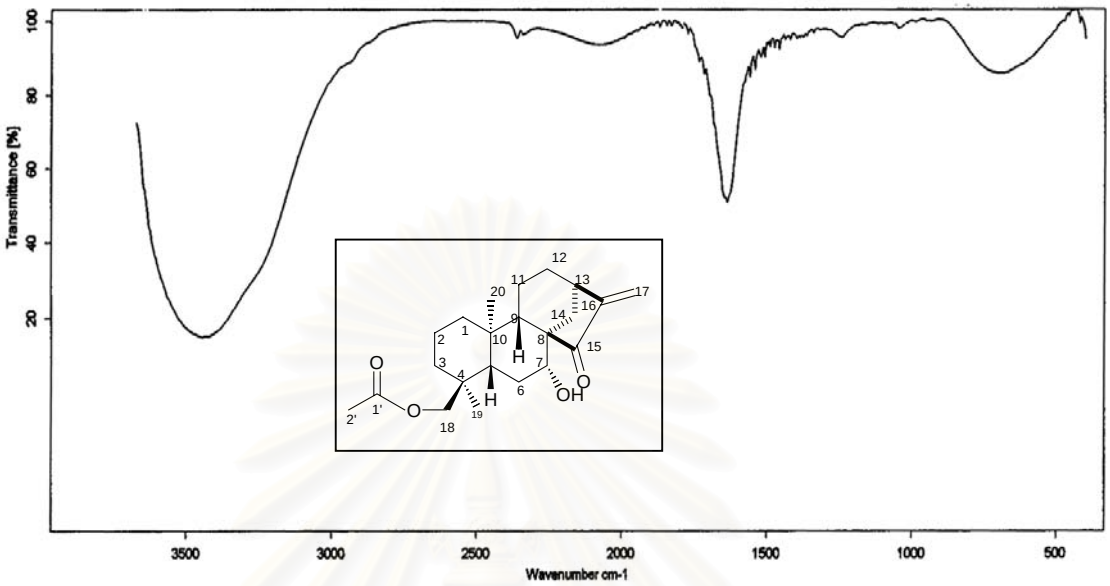


Figure 32 IR Spectrum of Compound CK 04 (neat)

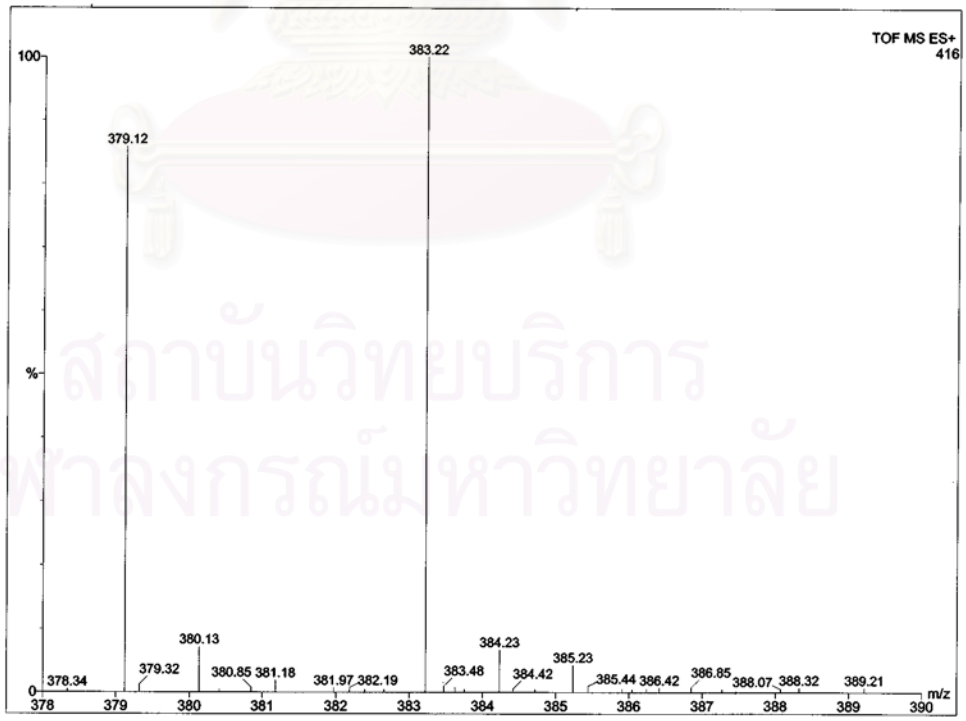


Figure 33 MS Spectrum of Compound CK 04

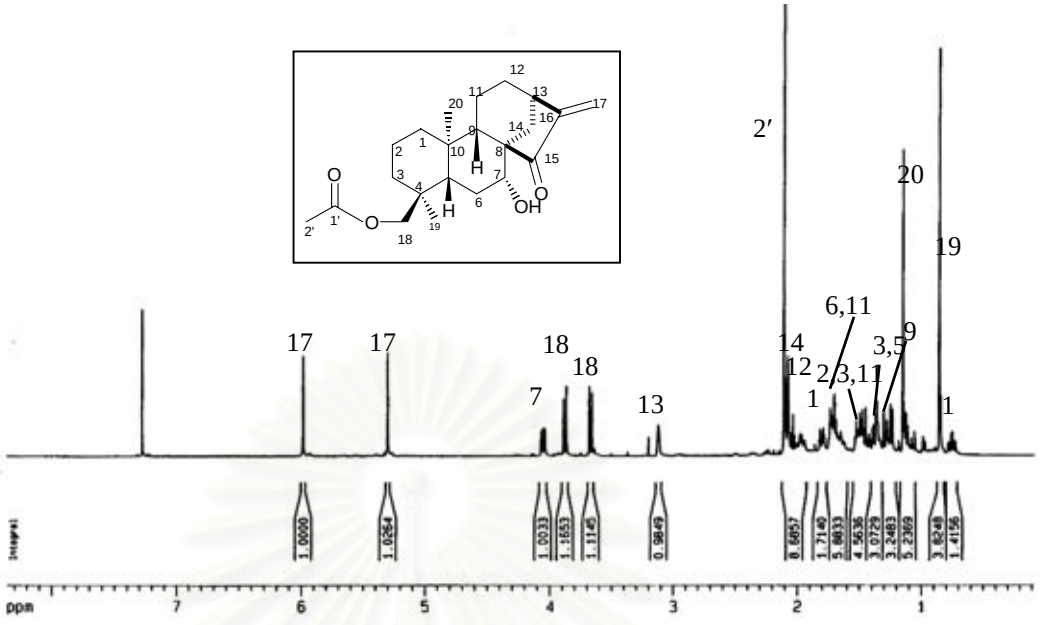


Figure 34 ^{1}H- NMR Spectrum ($CDCl_3$) of Compound CK 04

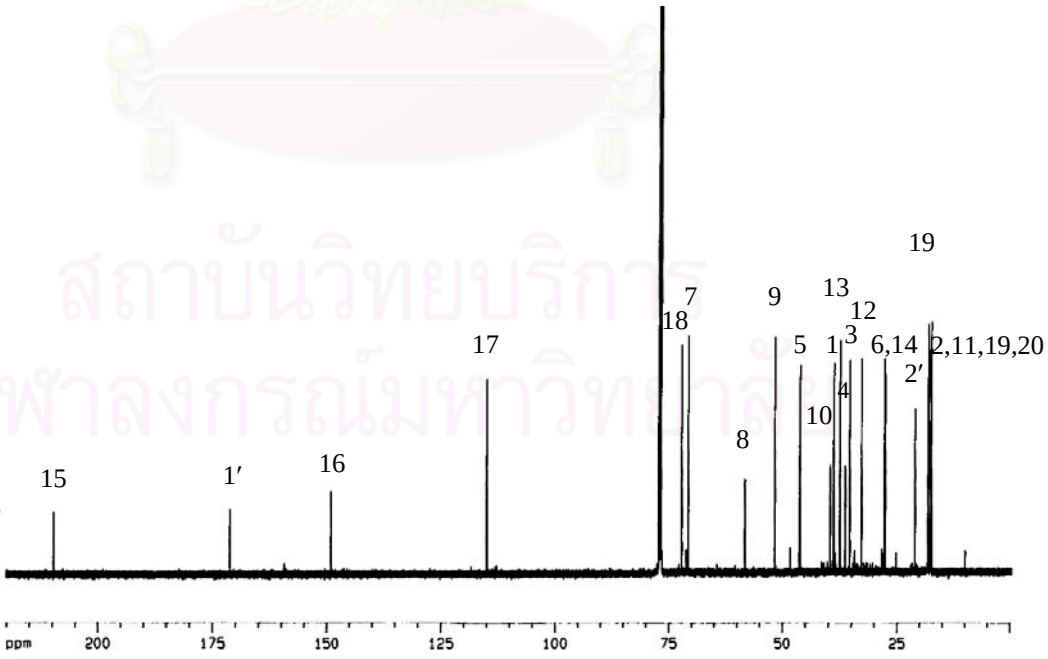


Figure 35 ^{13}C-NMR Spectrum ($CDCl_3$) of Compound CK 04

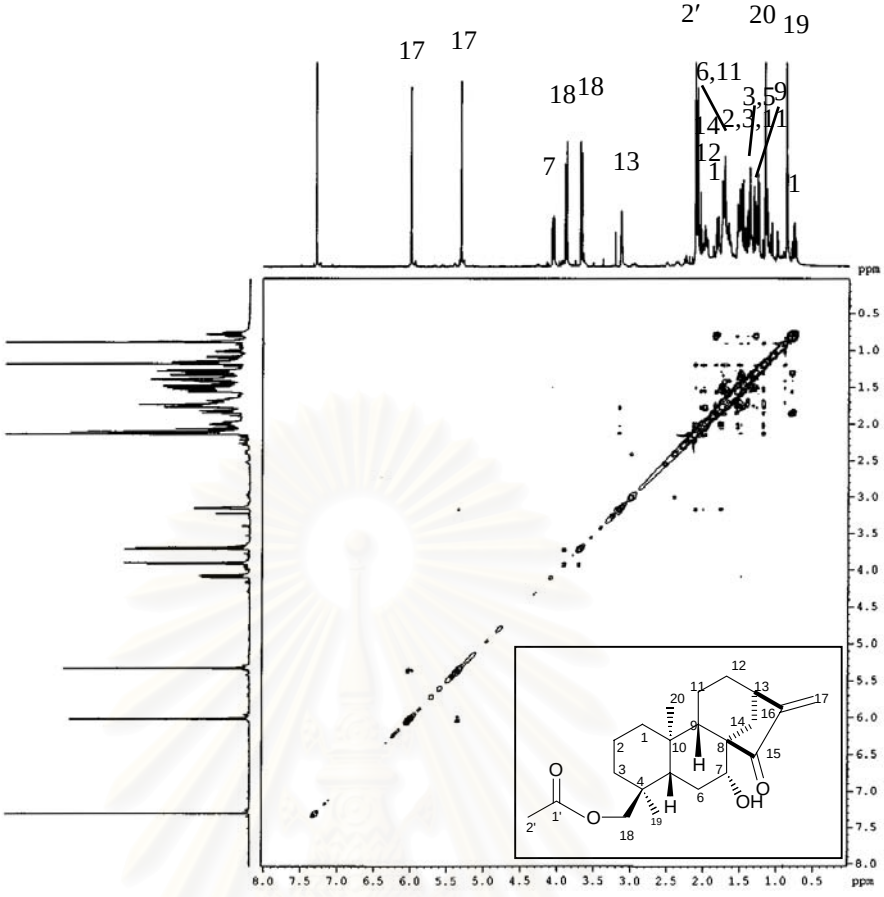


Figure 36 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Compound CK 04

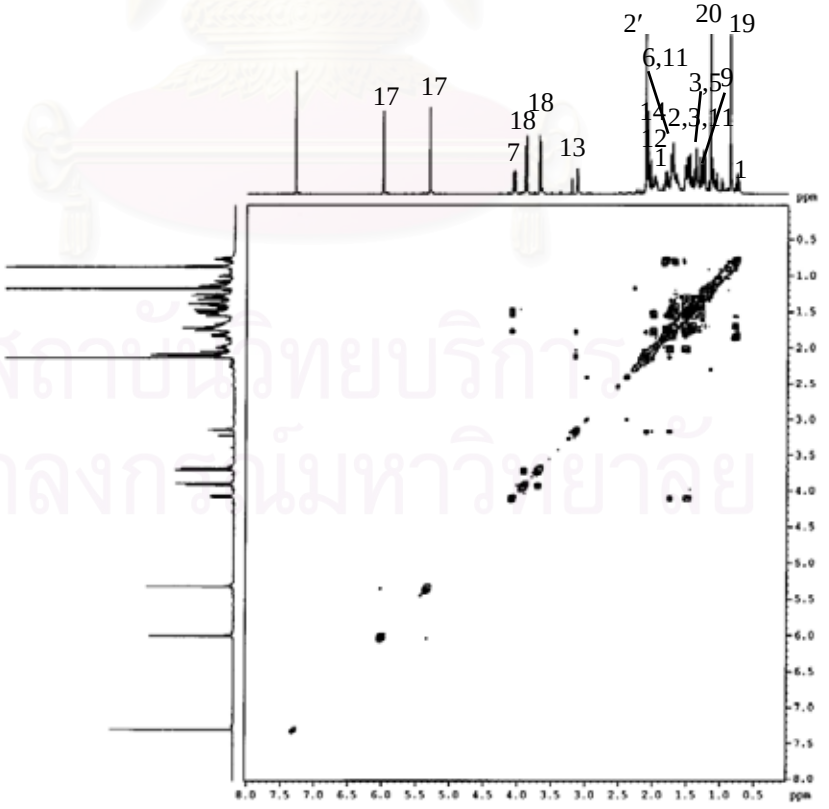


Figure 37 NOESY Spectrum ($CDCl_3$) of Compound CK 04

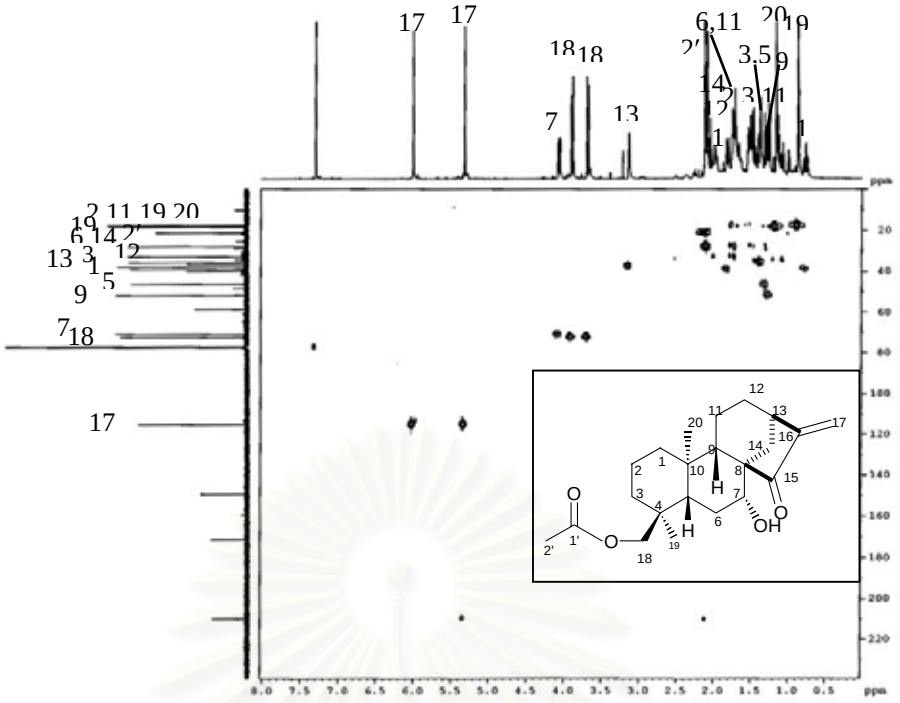


Figure 38 HMQC Spectrum ($CDCl_3$) of Compound CK 04

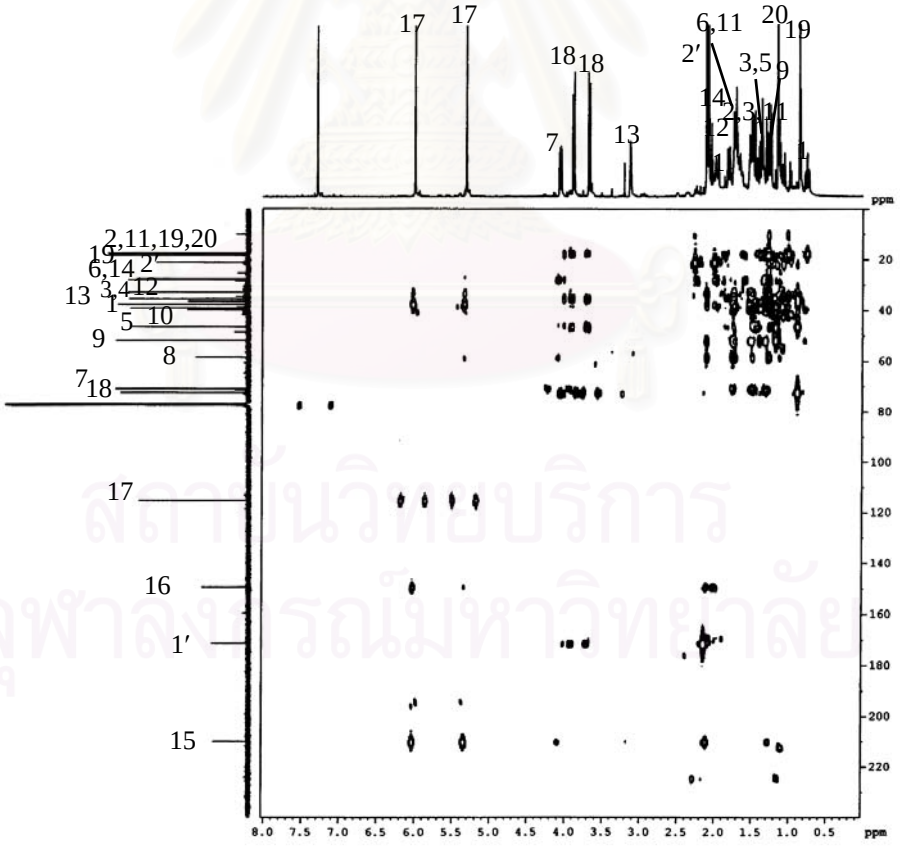


Figure 39 HMBC Spectrum ($CDCl_3$) of Compound CK 04

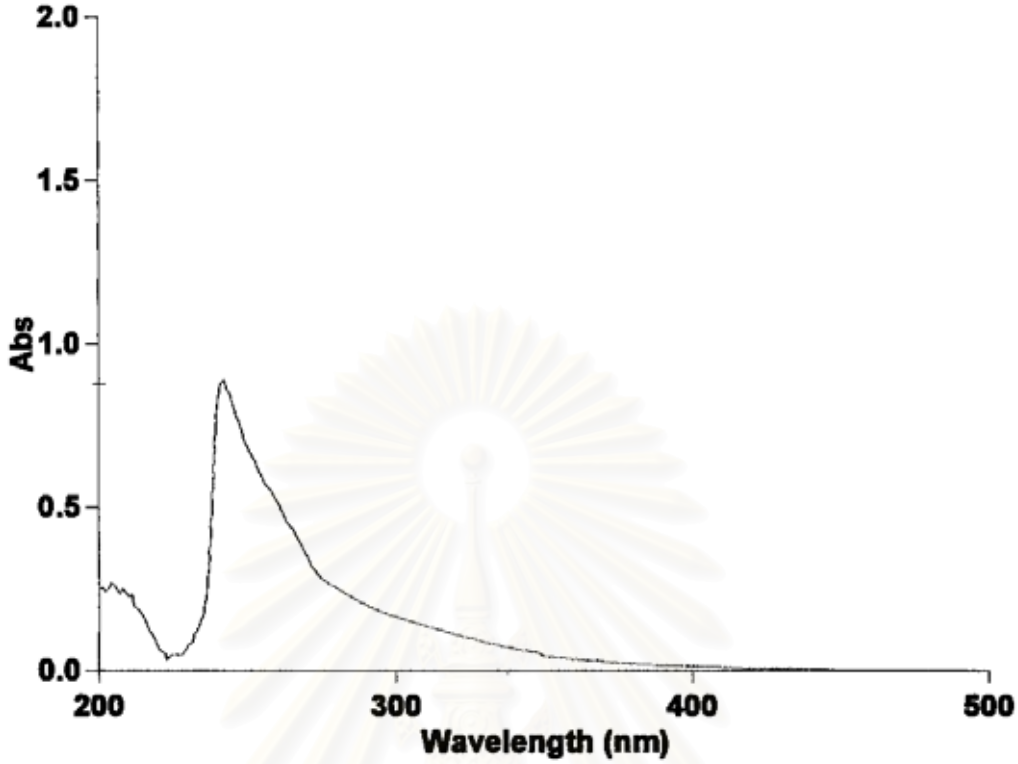


Figure 40 UV Spectrum of Compound CB 01 (chloroform)

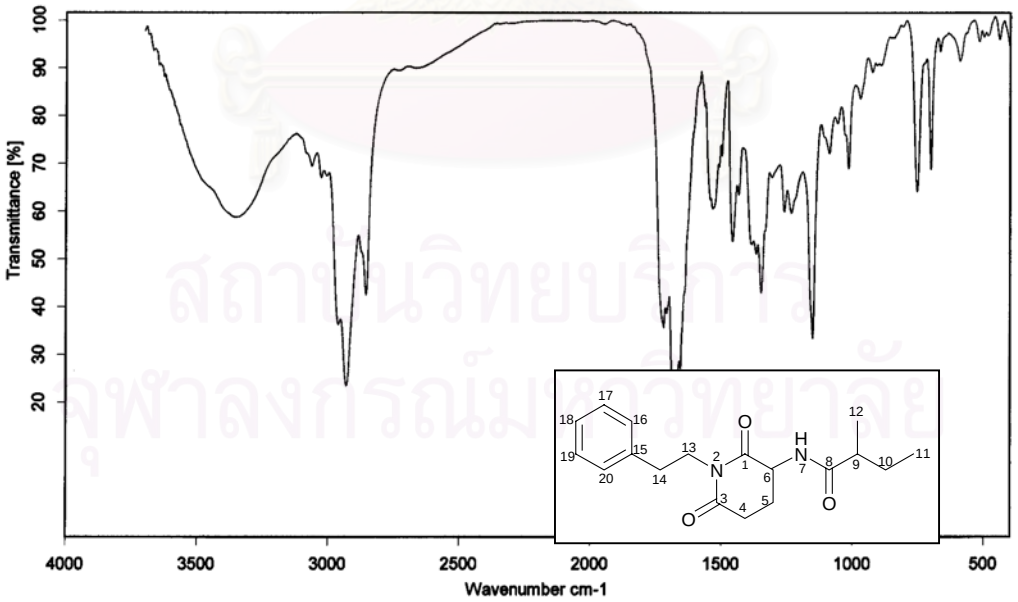


Figure 41 IR Spectrum of Compound CB 01 (film)

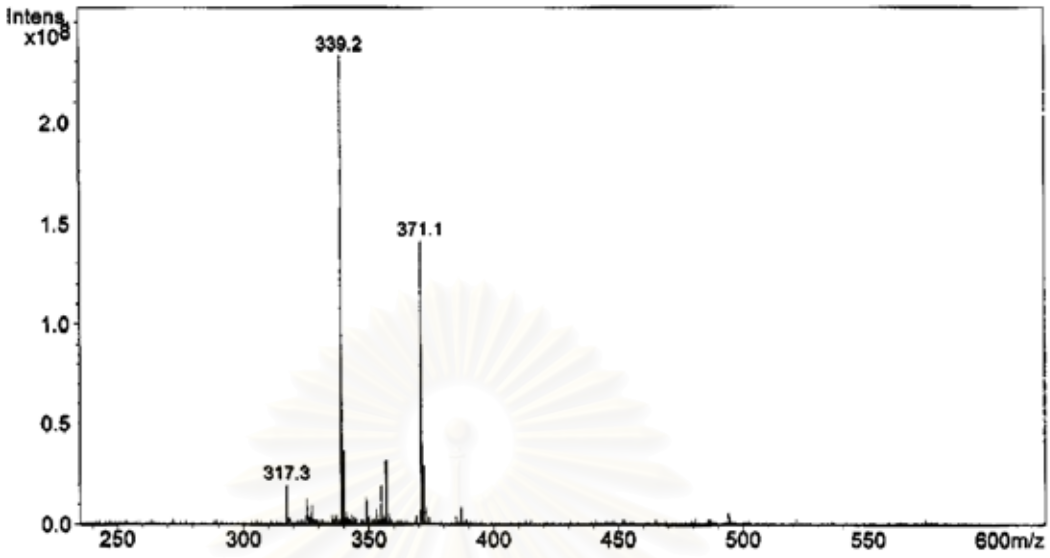


Figure 42 MS Spectrum of Compound CB 01

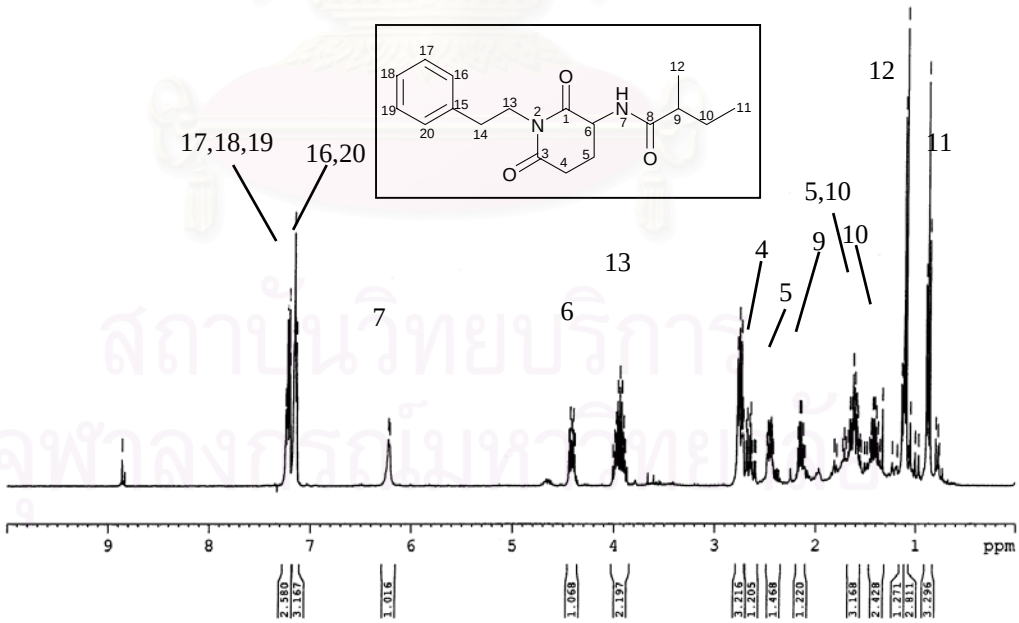


Figure 43 ^{1}H- NMR Spectrum ($CDCl_3$) of Compound CB 01

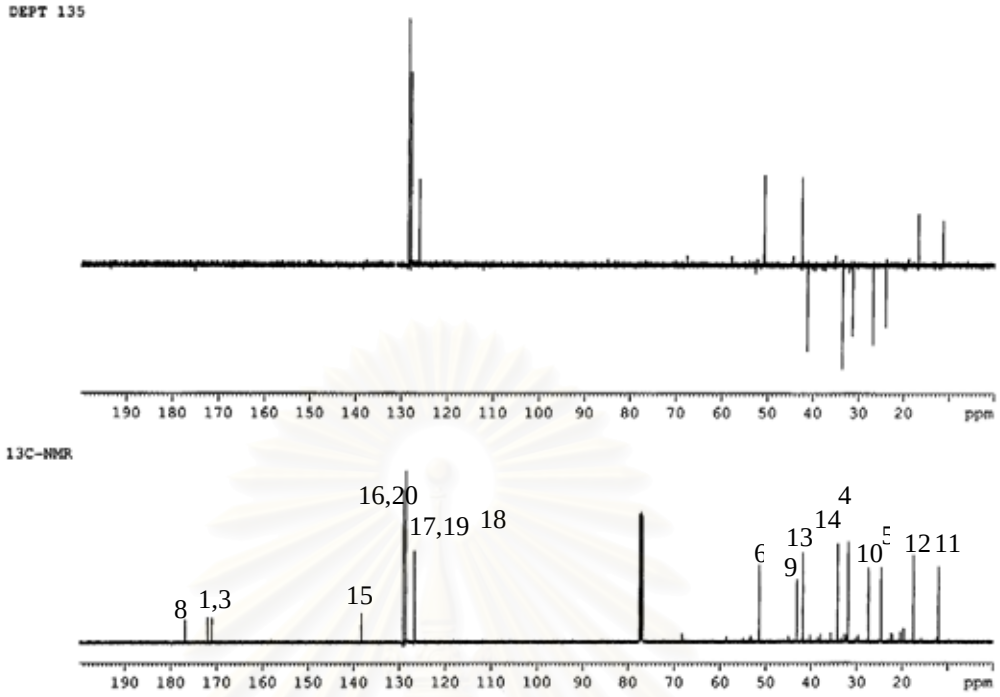


Figure 44 ^{13}C NMR and DEPT Spectra ($CDCl_3$)of Compound CB 01

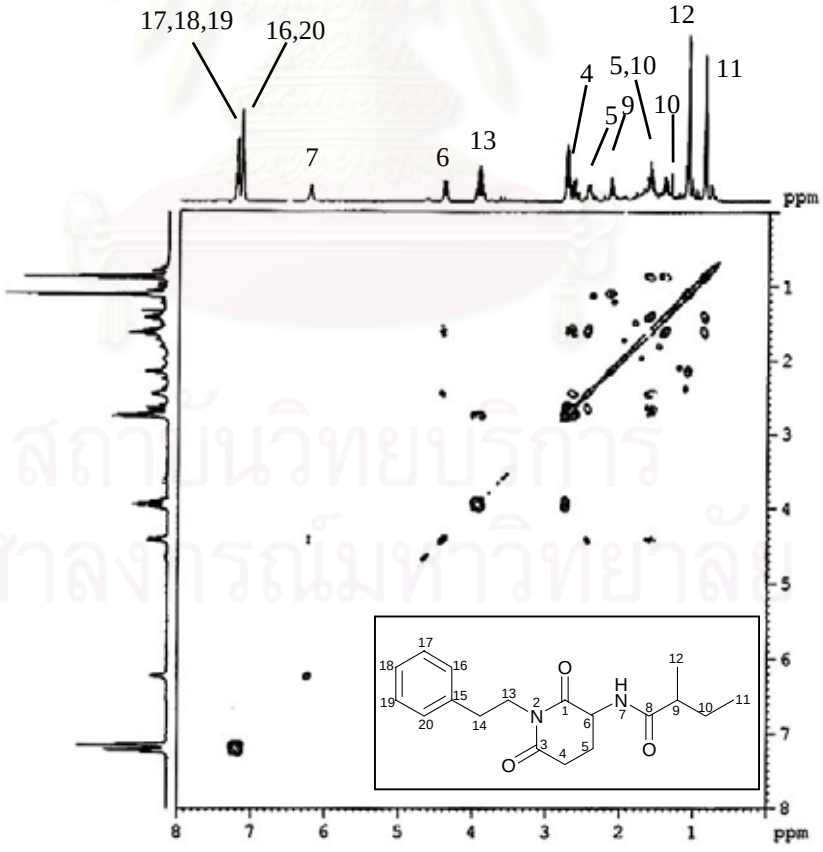


Figure 45 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Compound CB 01

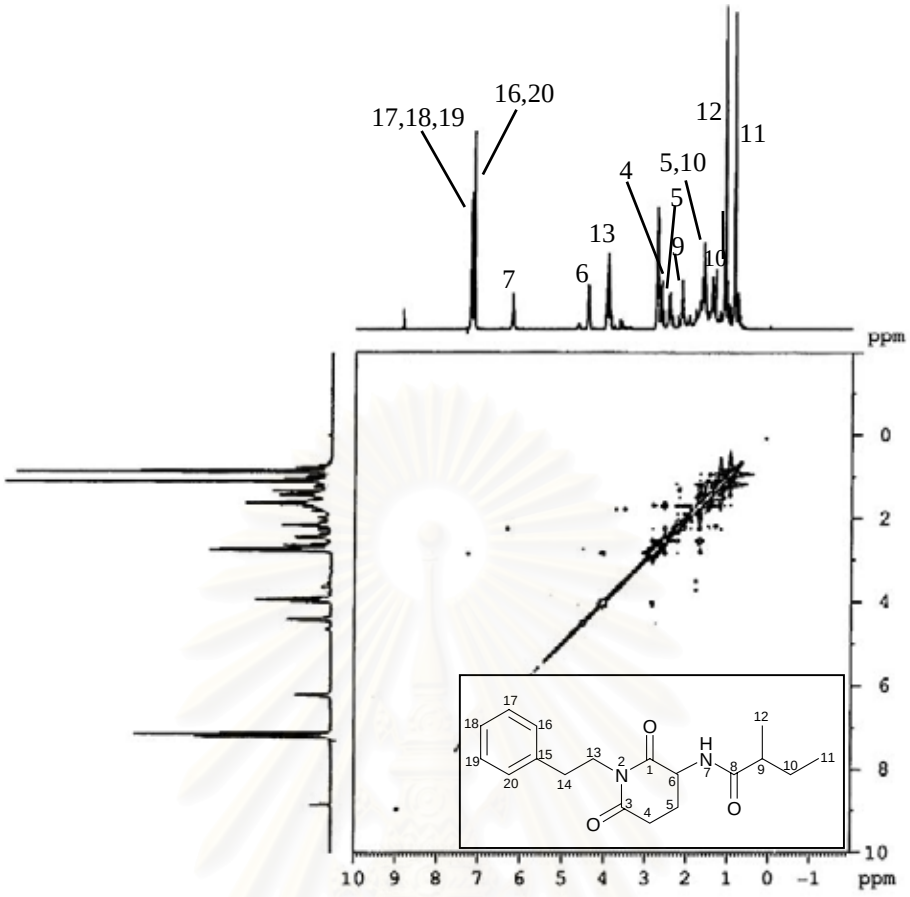


Figure 46 NOESY Spectrum ($CDCl_3$) of Compound CB 01

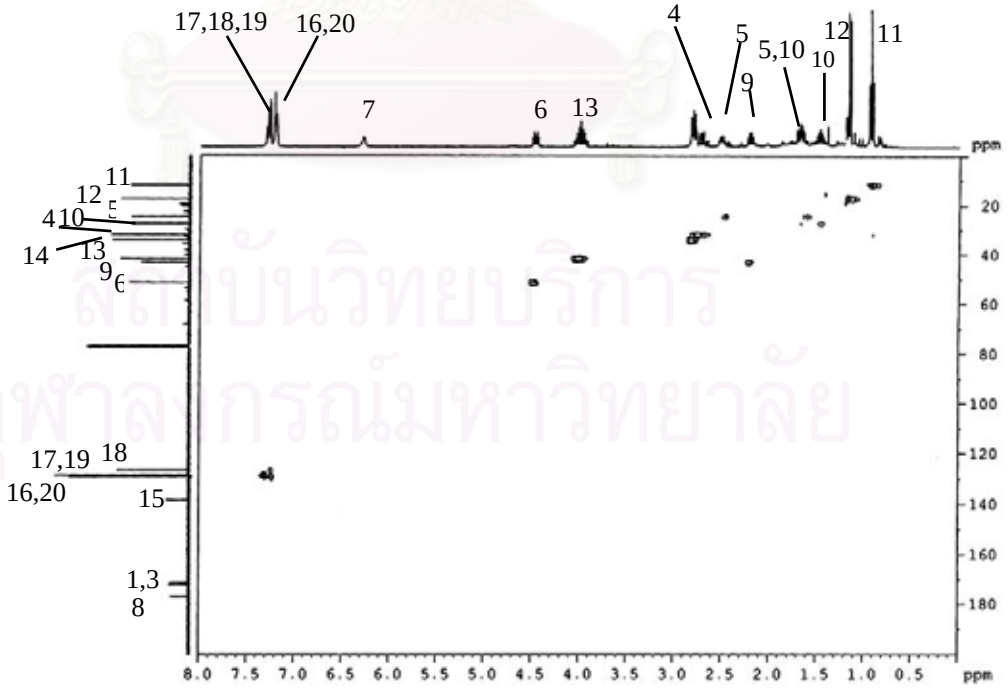


Figure 47 HMQC Spectrum ($CDCl_3$) of Compound CB 01

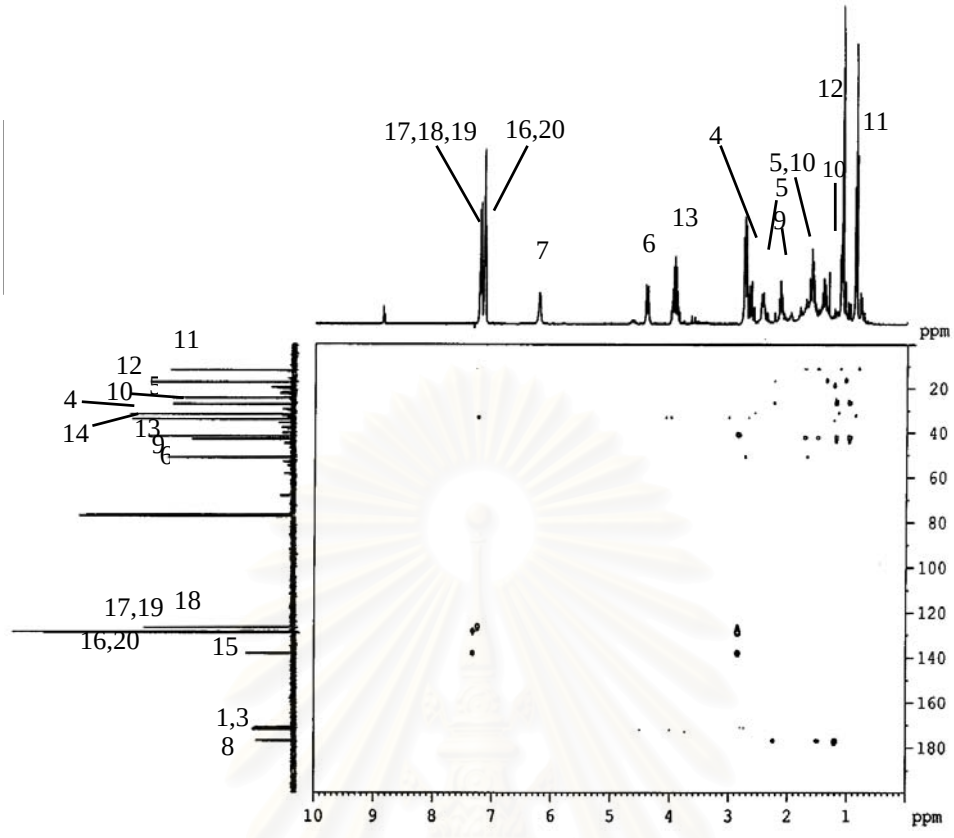


Figure 48 HMBC Spectrum ($CDCl_3$) of Compound CB 01

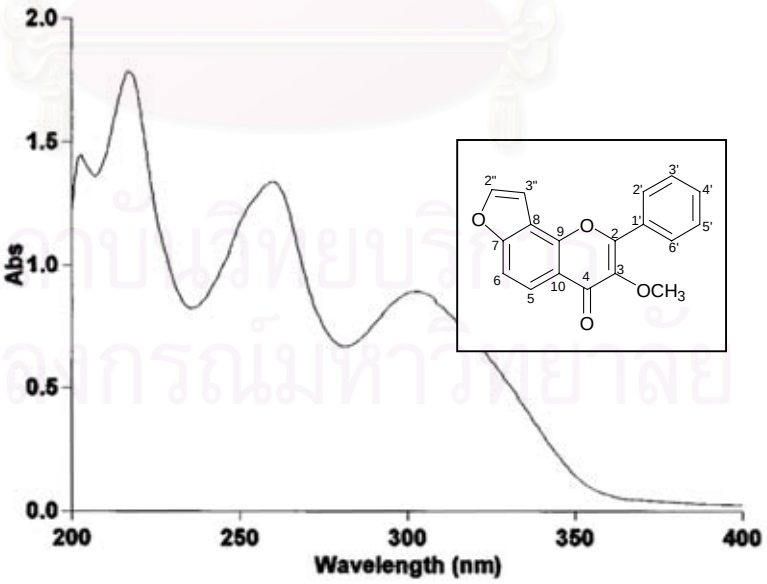


Figure 49 UV Spectrum of Compound MK 01 (methanol)

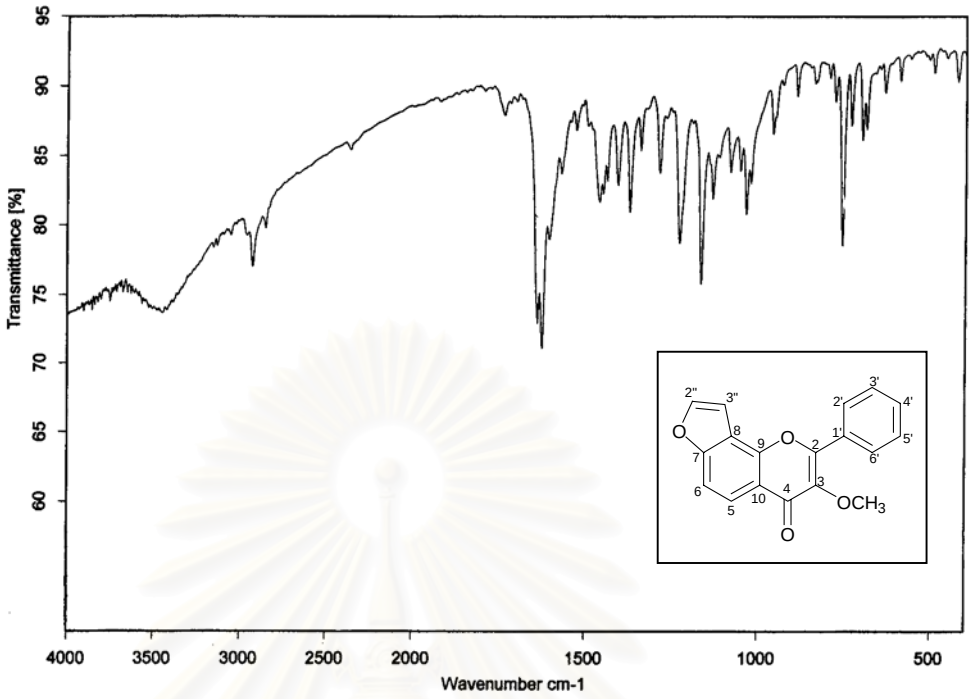


Figure 50 IR Spectrum of Compound MK 01 (film)

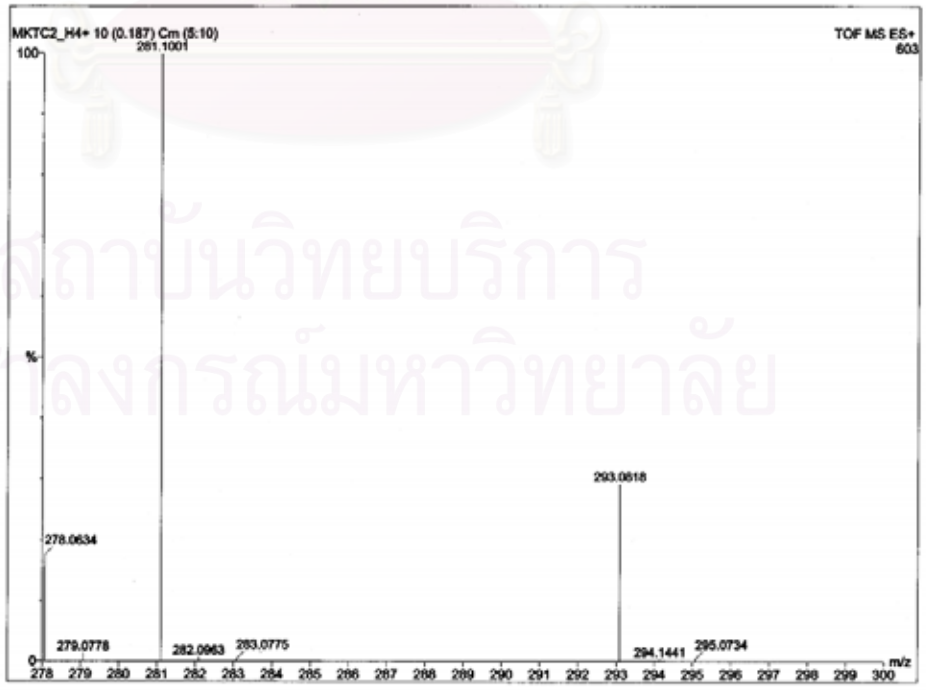


Figure 51 MS Spectrum of Compound MK 01

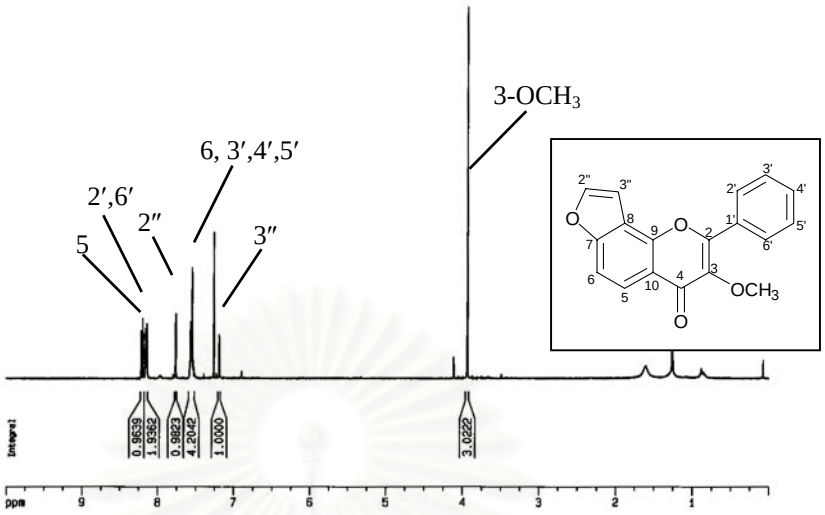


Figure 52 ^{1}H- NMR Spectrum ($CDCl_3$) of Compound MK 01

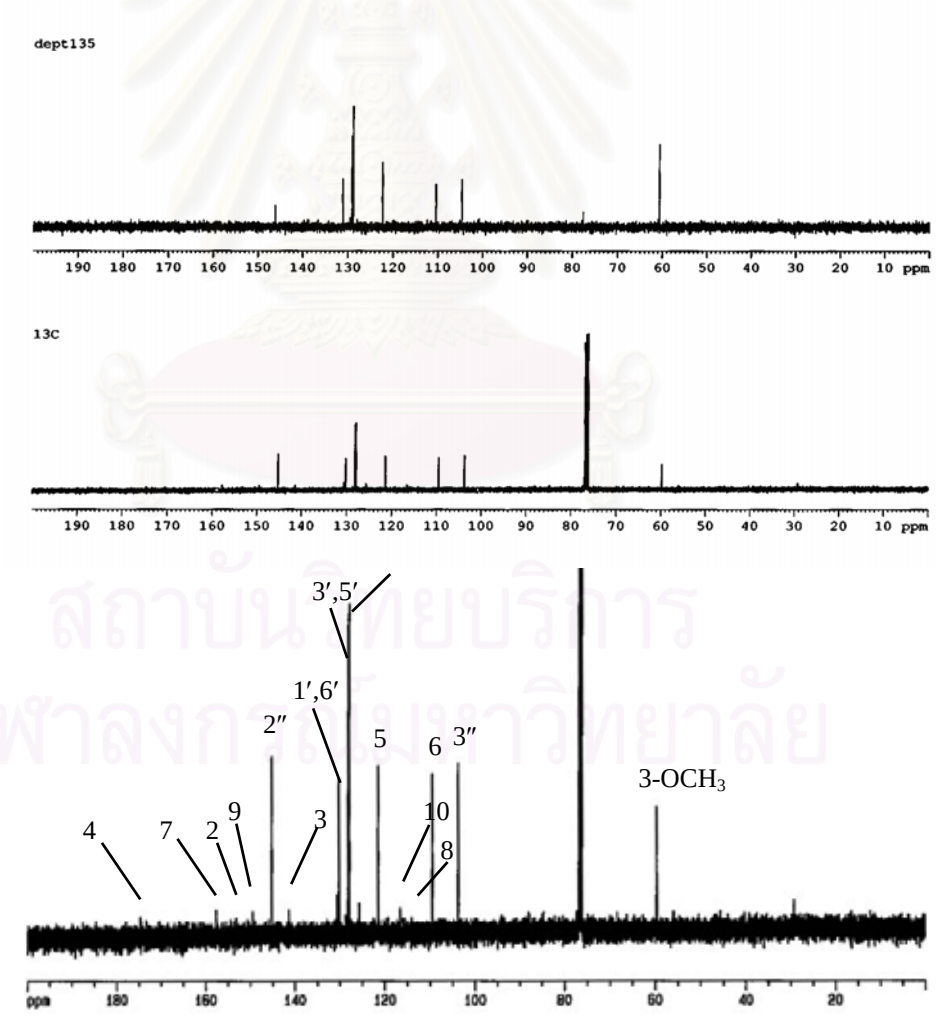


Figure 53 ^{13}C NMR and DEPT Spectra ($CDCl_3$) of Compound MK 01

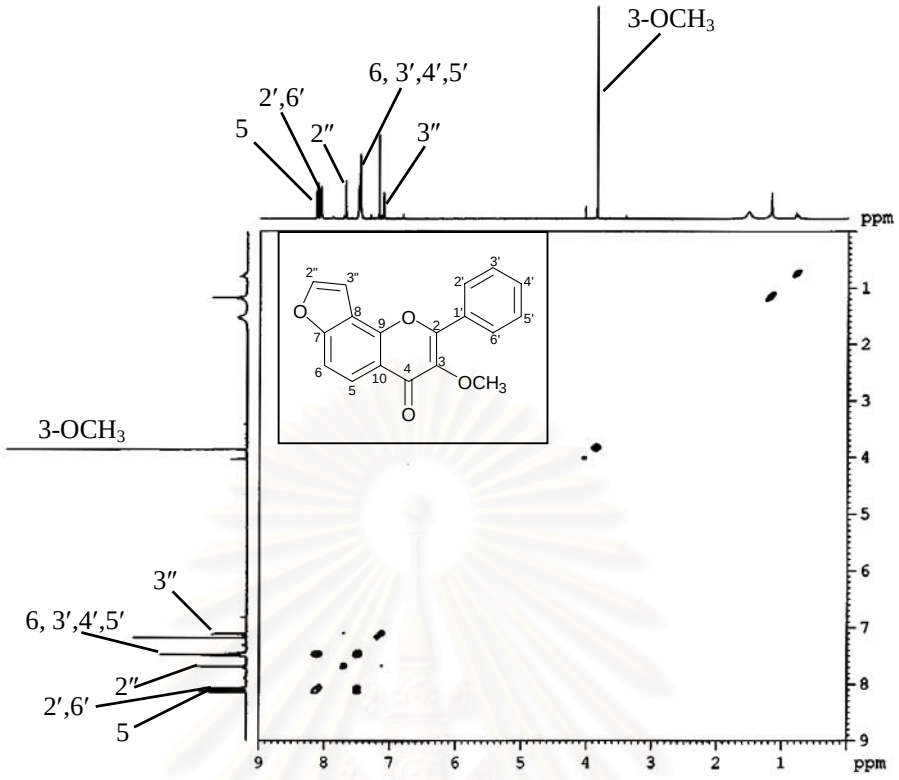


Figure 54 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Compound MK 01

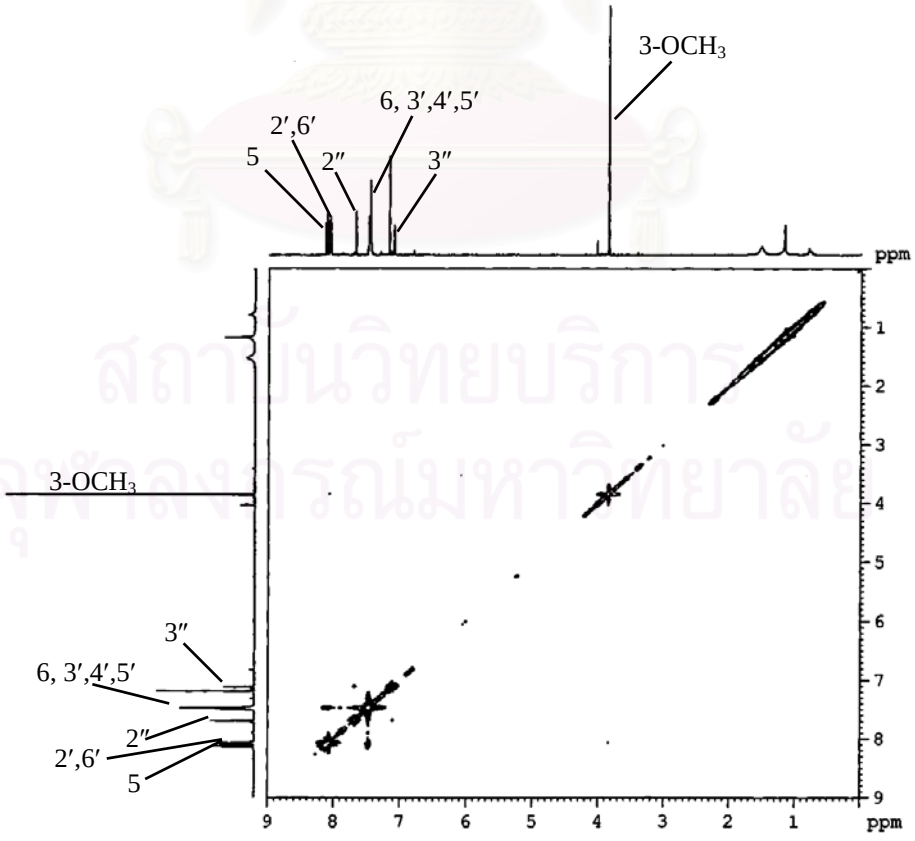


Figure 55 NOESY Spectrum ($CDCl_3$) of Compound MK 01

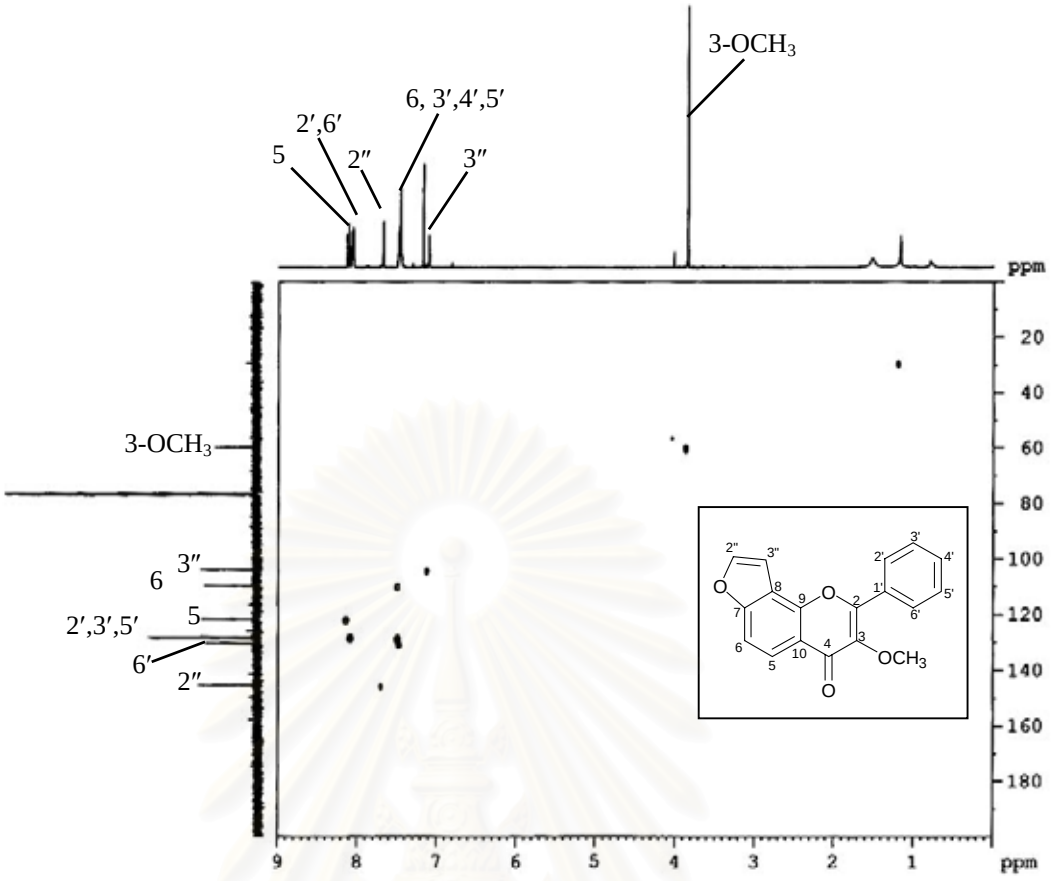


Figure 56 HMQC Spectrum ($CDCl_3$) of Compound MK 01

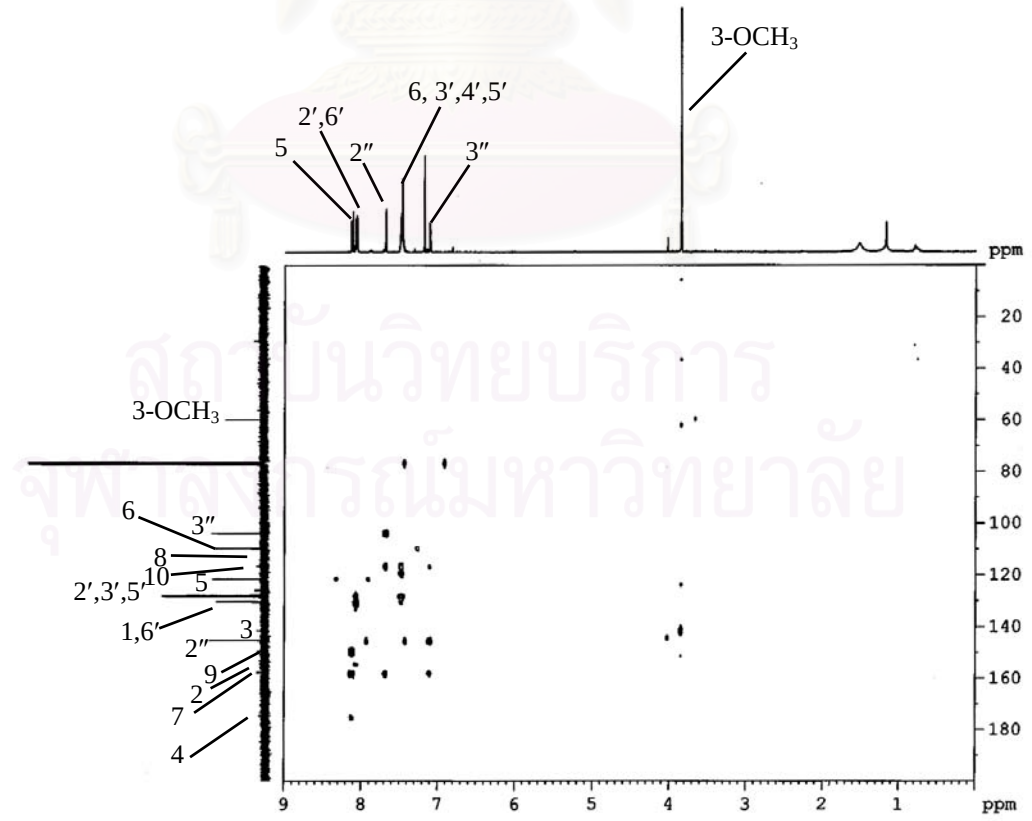


Figure 57 HMBC Spectrum ($CDCl_3$) of Compound MK 01

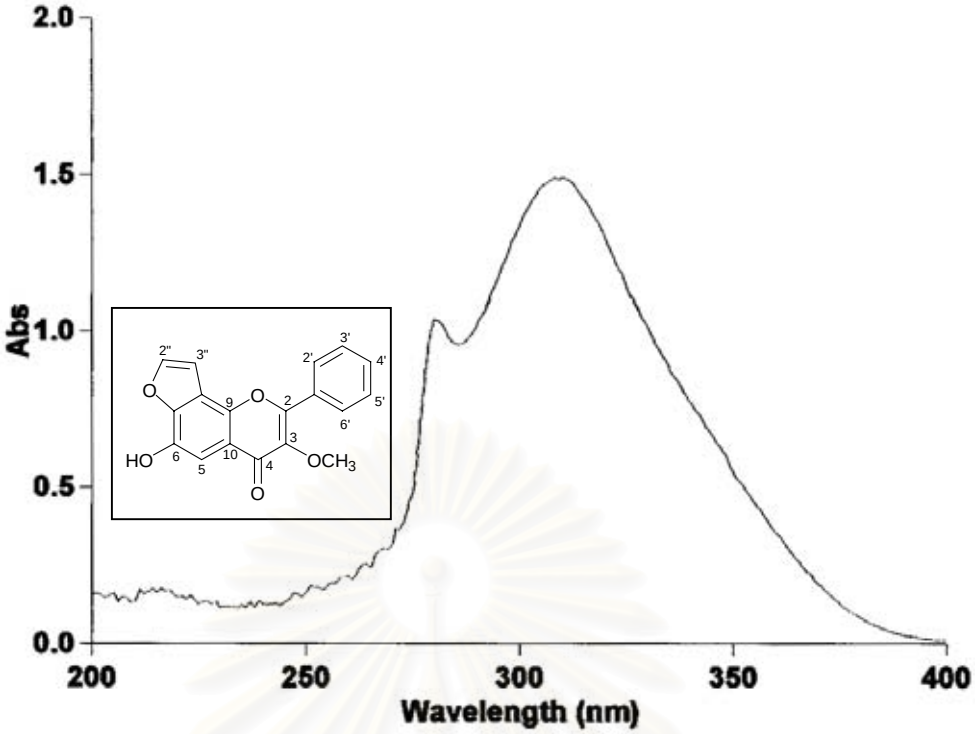


Figure 58 UV Spectrum of Compound MK 02 (methanol)

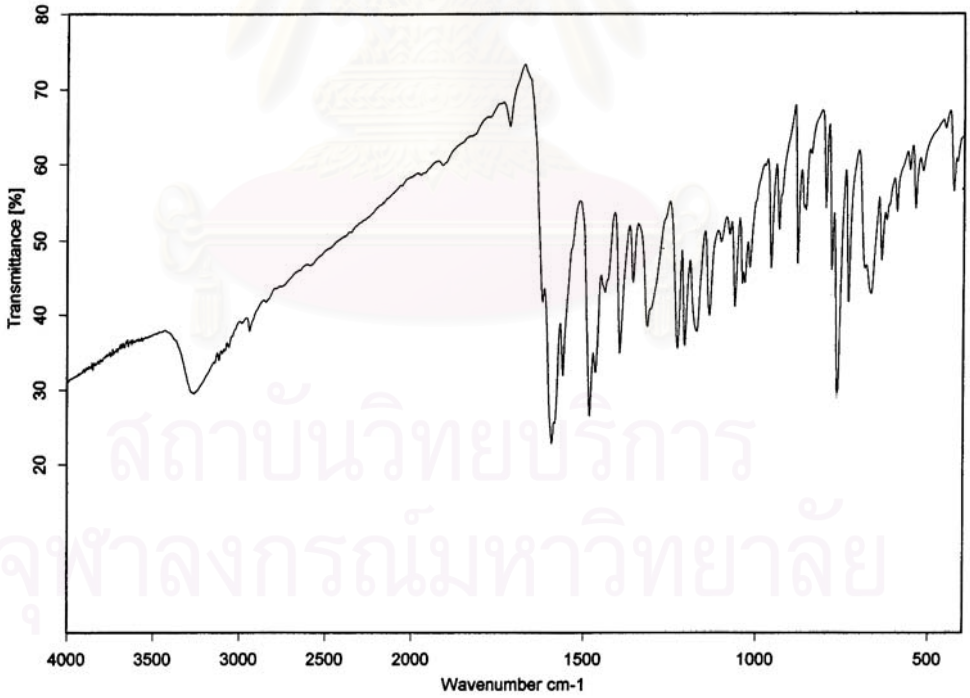


Figure 59 IR Spectrum of Compound MK 02 (film)

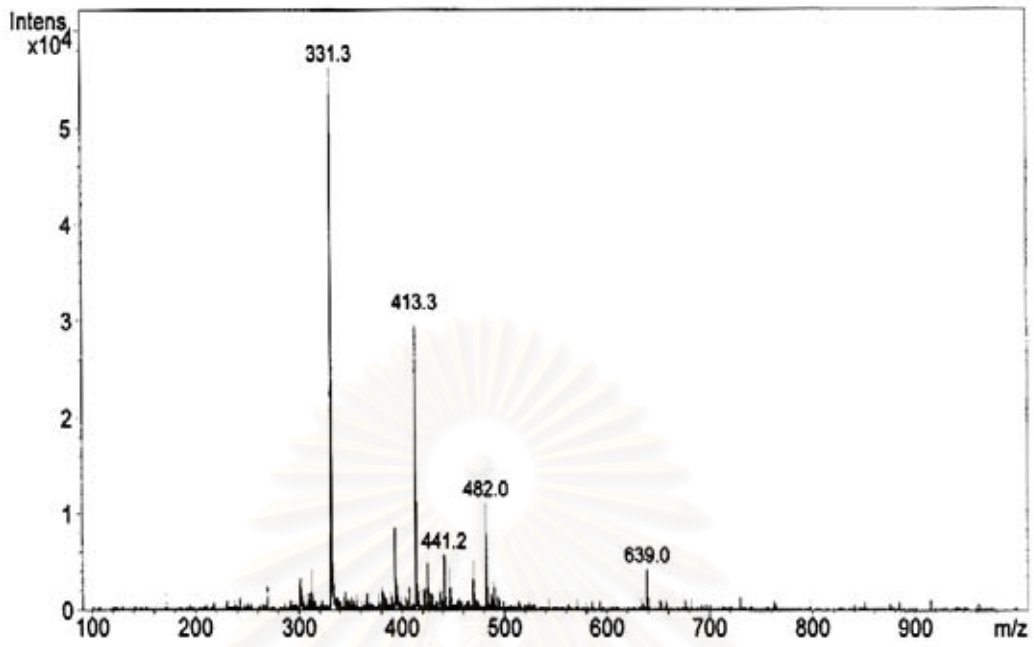


Figure 60 MS Spectrum of Compound MK 02

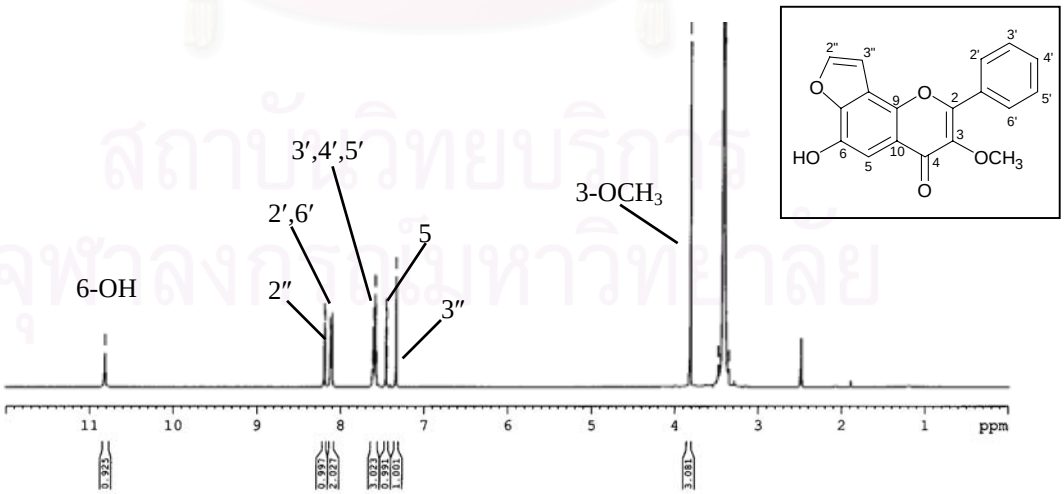


Figure 61 ^{1}H- NMR Spectrum (DMSO-d_6) of Compound MK 02

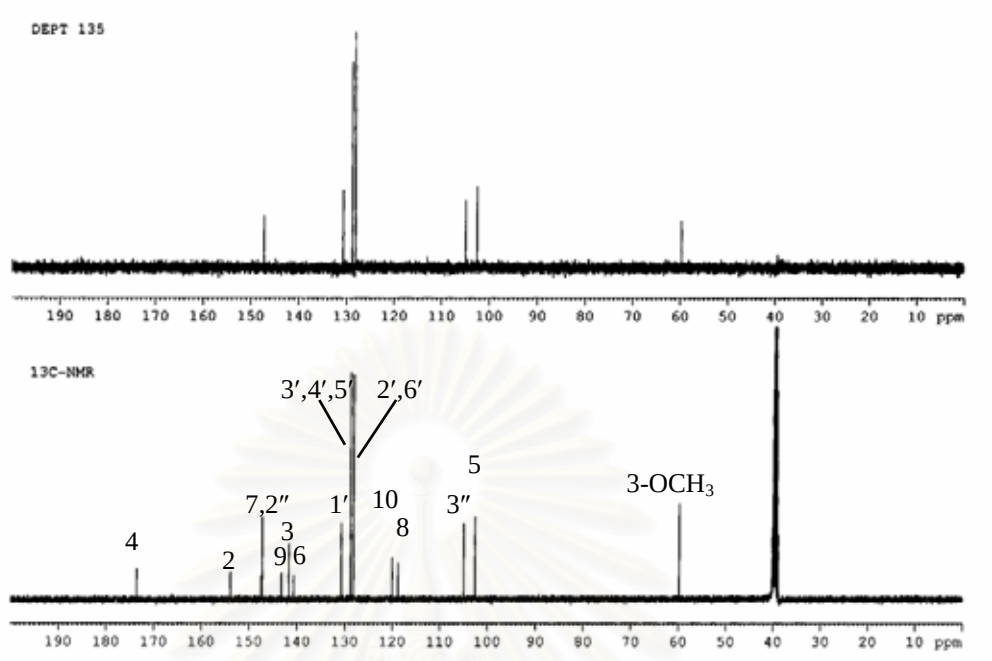


Figure 62 ^{13}C NMR and DEPT Spectra (DMSO-d_6) of Compound MK 02

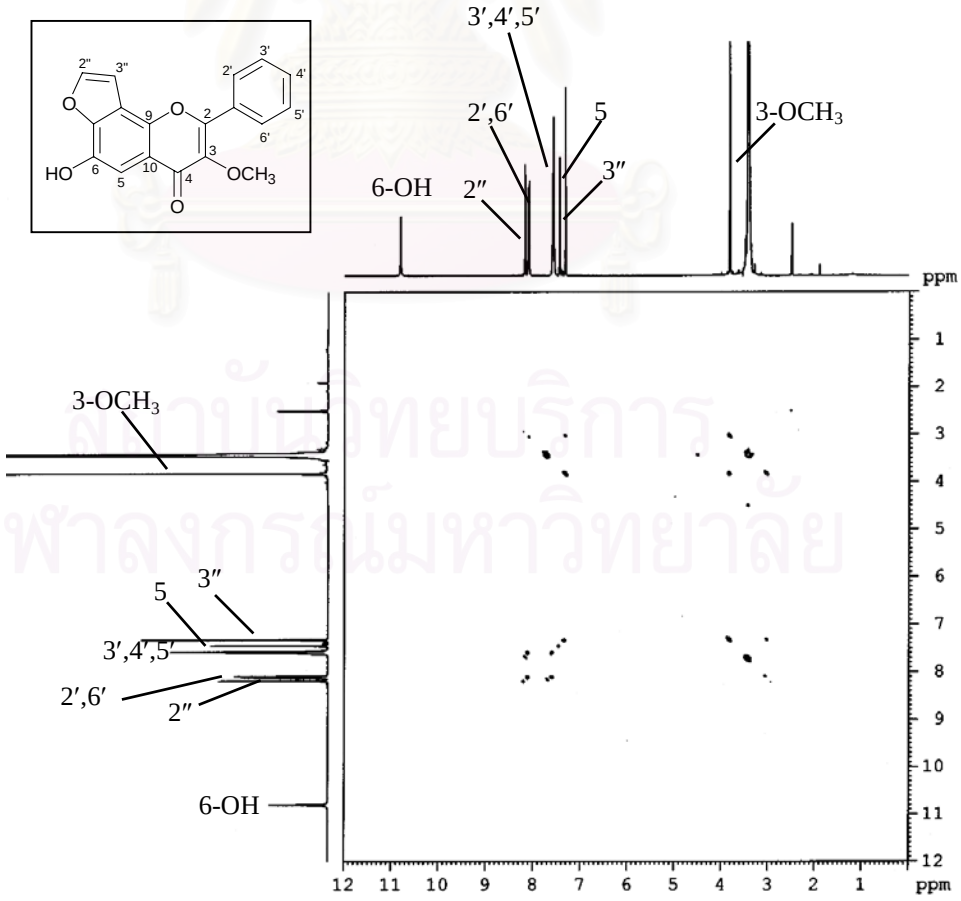


Figure 63 ^{1}H-^{1}H COSY Spectrum (DMSO-d_6) f Compound MK 02

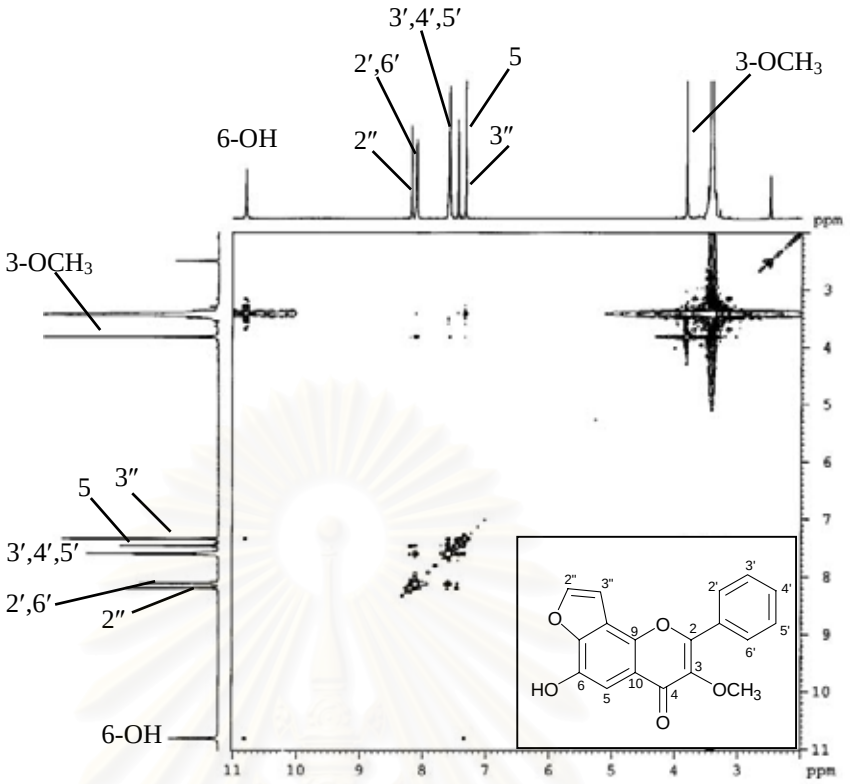


Figure 64 NOESY Spectrum (DMSO-d_6) of Compound MK 02

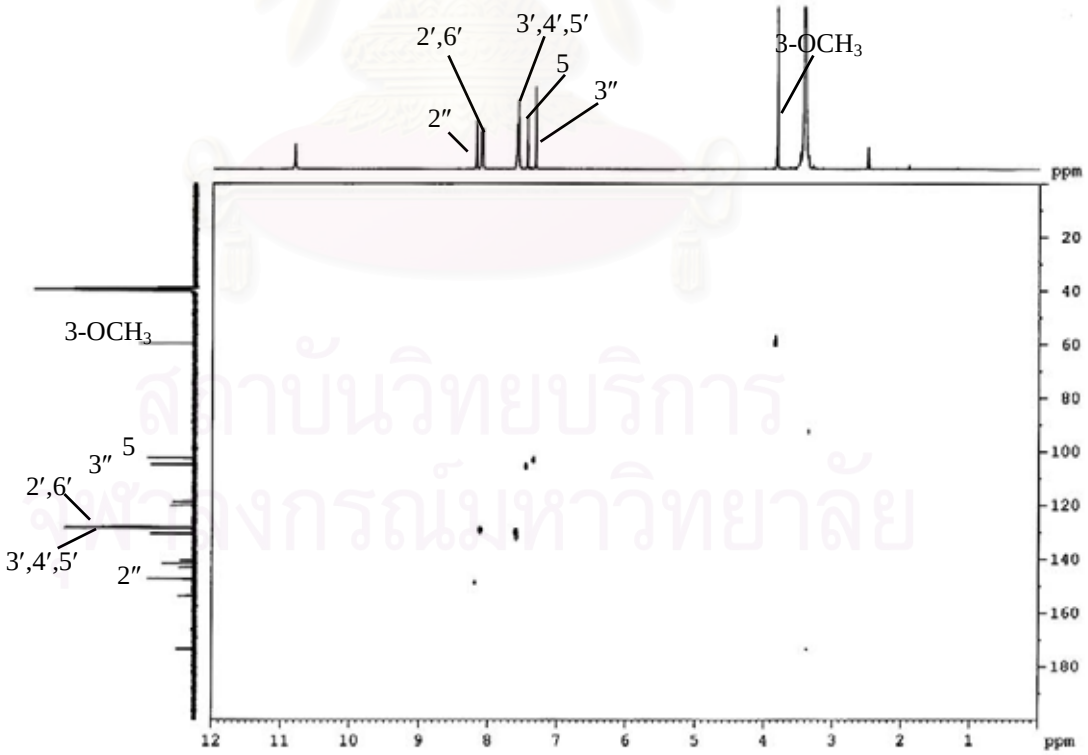


Figure 65 HMQC Spectrum (DMSO-d_6) of Compound MK 02

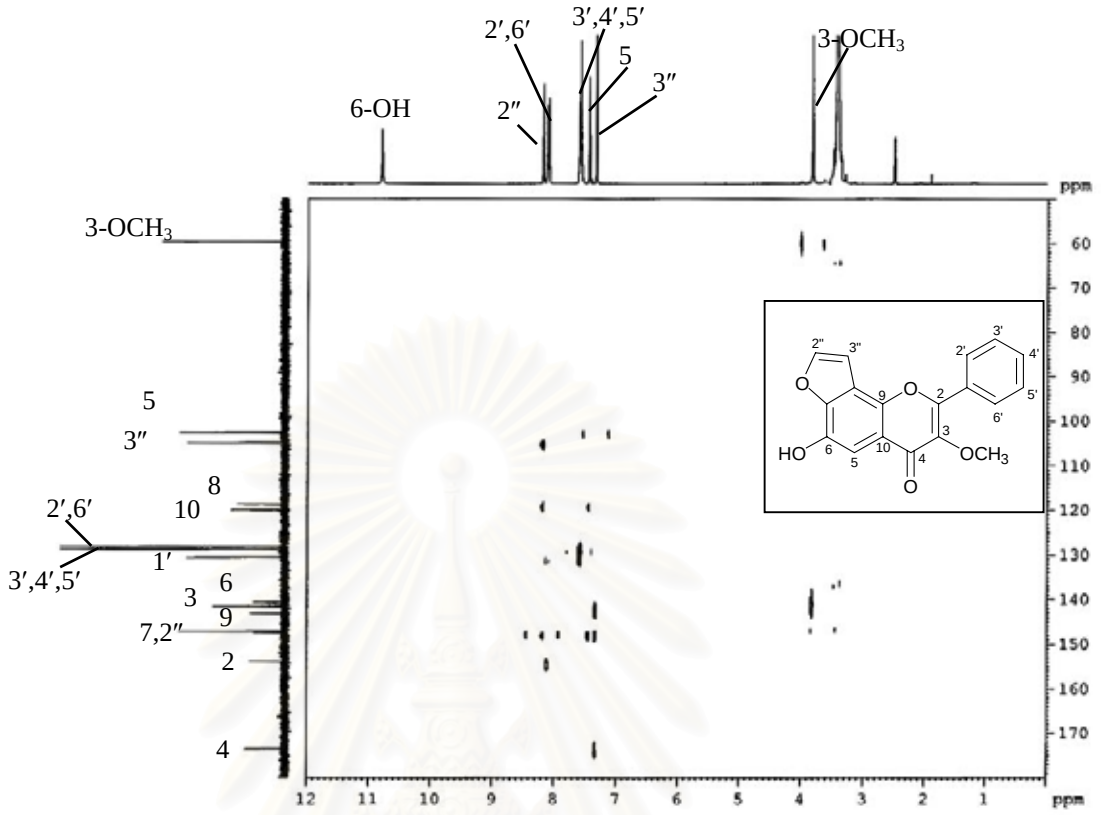


Figure 66 HMBC Spectrum (DMSO-d_6) of Compound MK 02

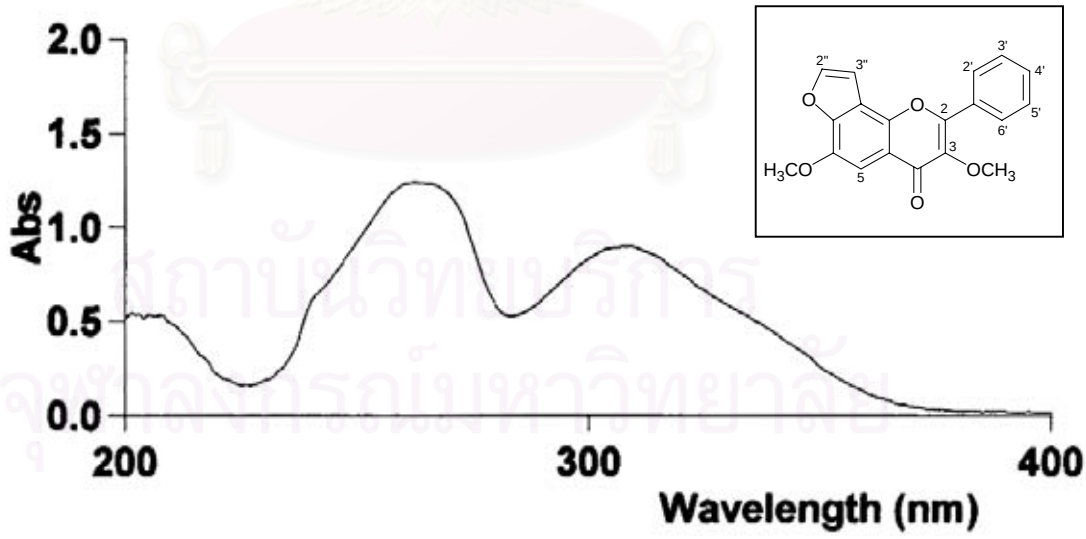


Figure 67 UV Spectrum of Compound MK 03 (methanol)

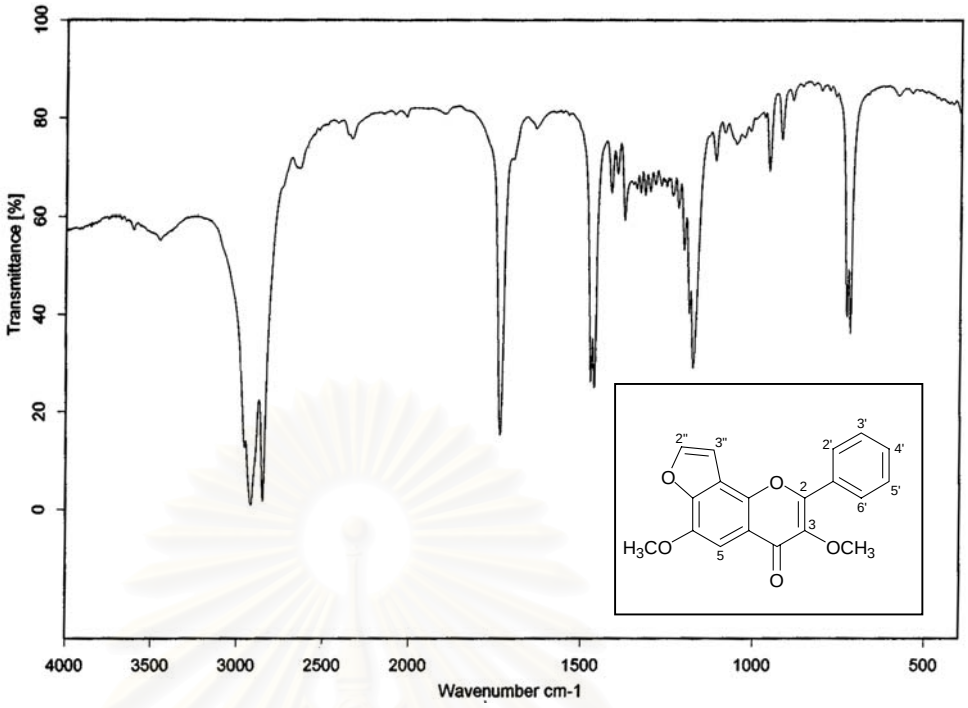


Figure 68 IR Spectrum of Compound MK 03 (film)

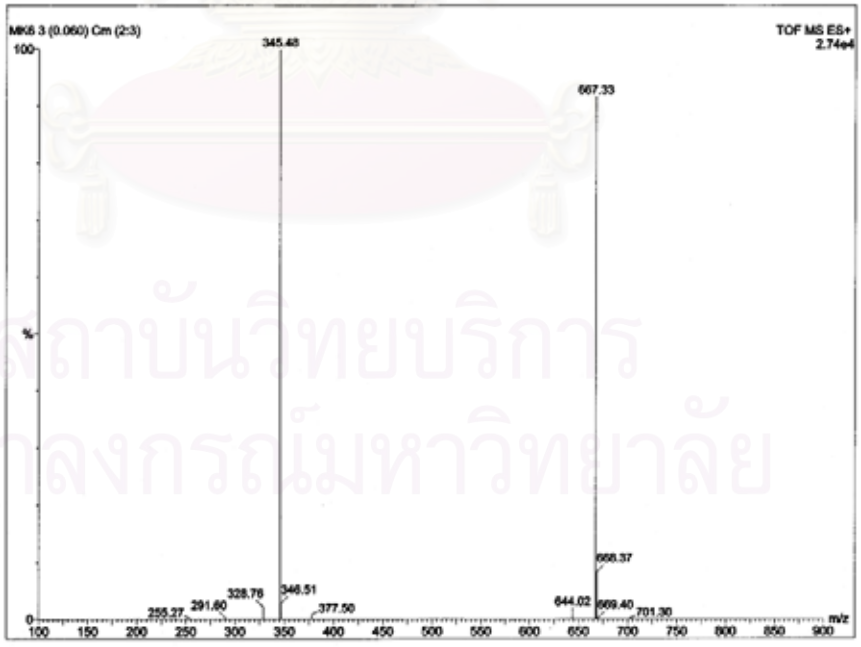


Figure 69 MS Spectrum of Compound MK 03

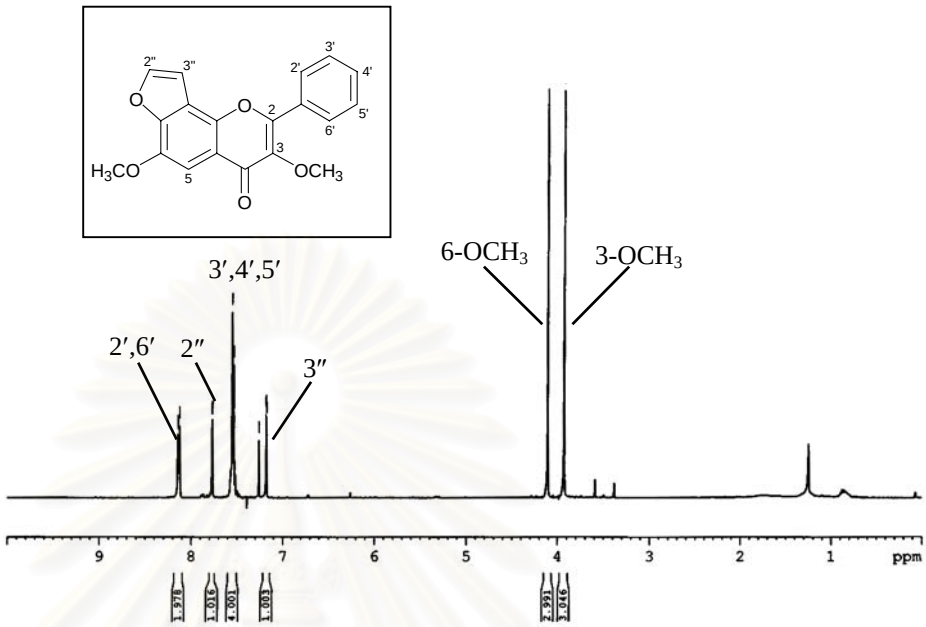


Figure 70 ^{1}H- NMR Spectrum ($CDCl_3$) of Compound MK 03

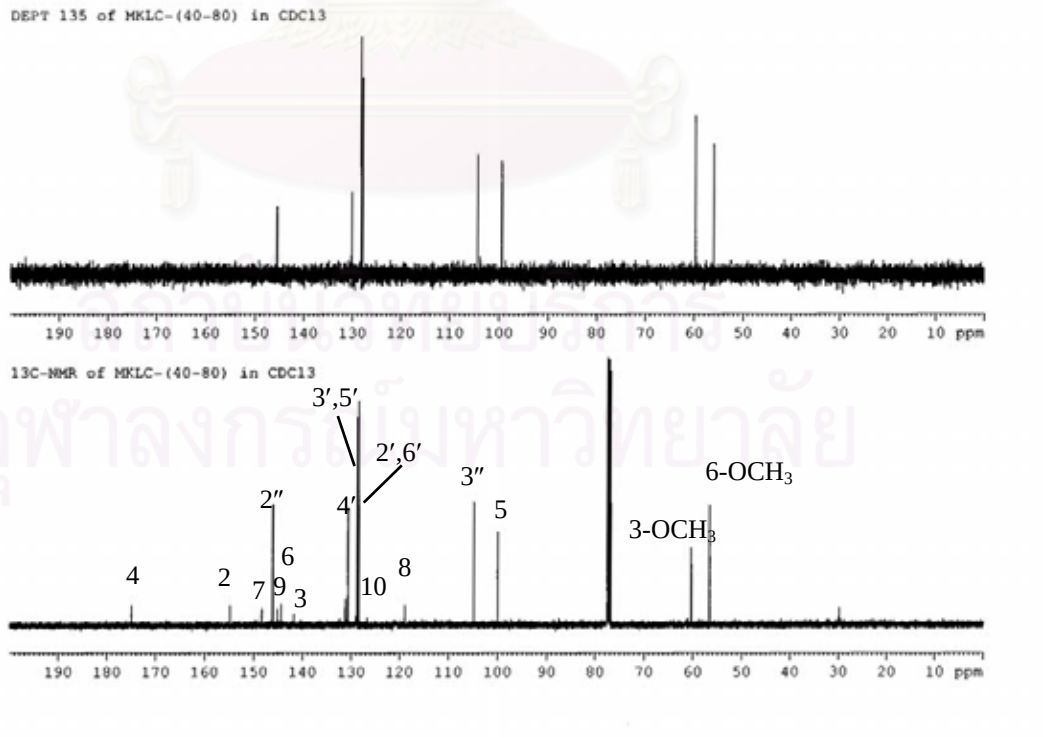


Figure 71 ^{13}C NMR and DEPT Spectra ($CDCl_3$) of Compound MK 03

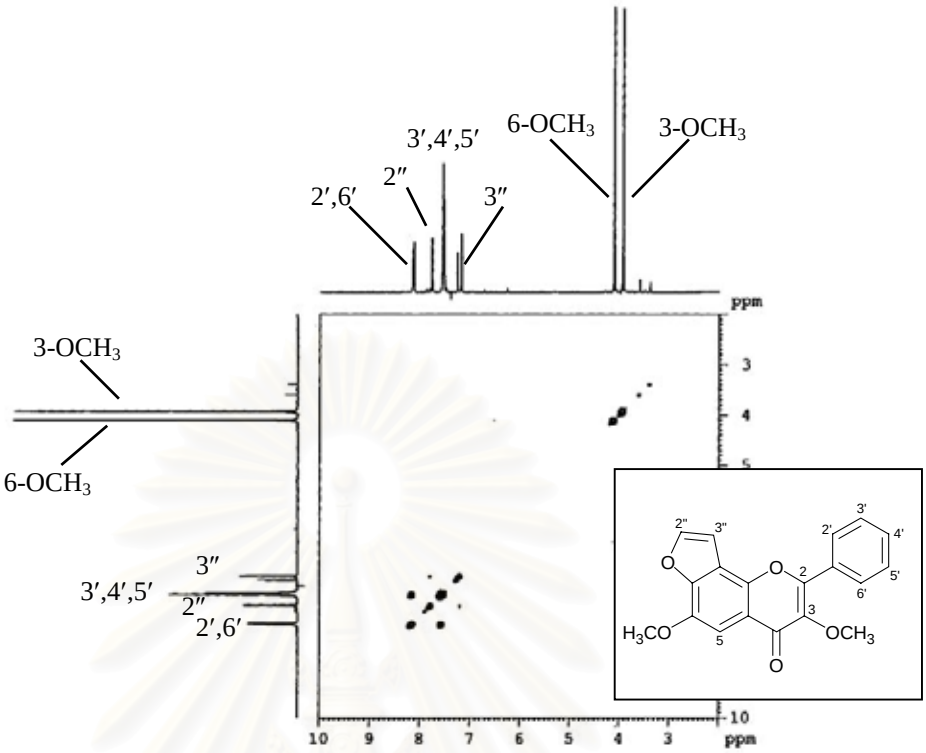


Figure 72 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Compound MK 03

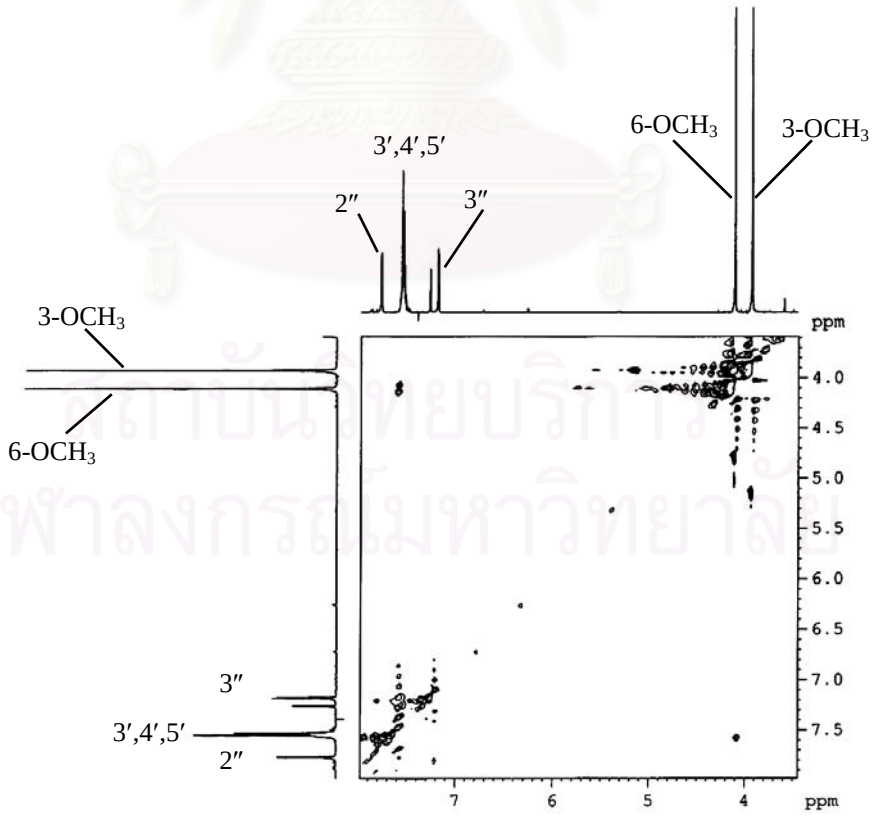


Figure 73 NOESY Spectrum ($CDCl_3$) of Compound MK 03

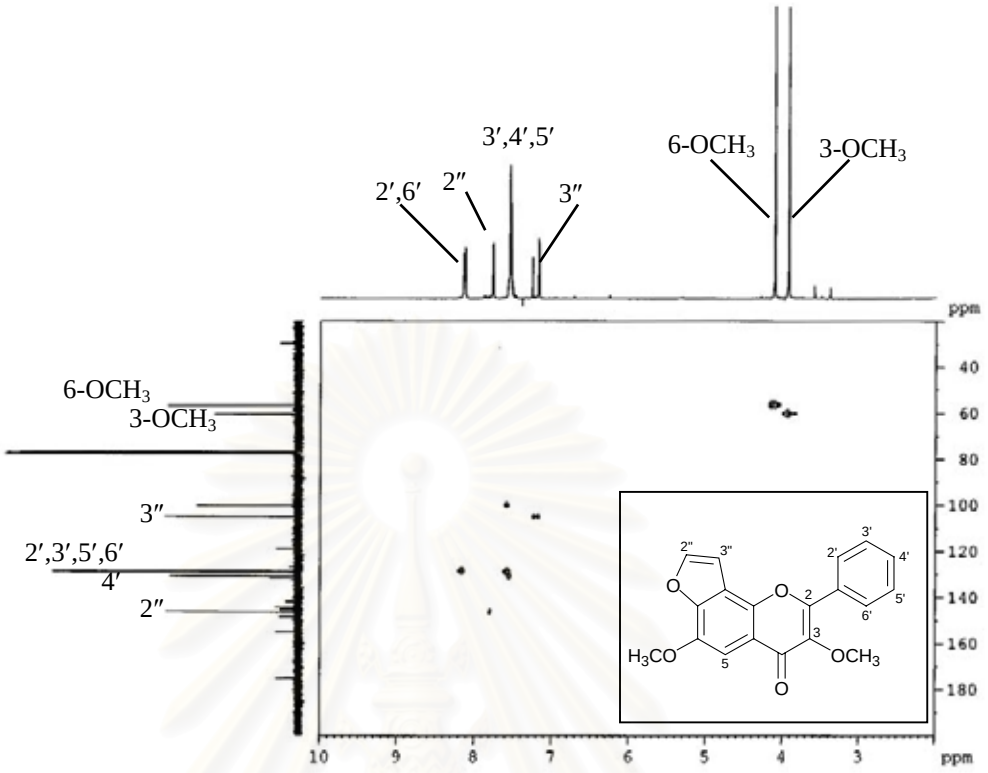


Figure 74 HMQC Spectrum ($CDCl_3$) of Compound MK 03

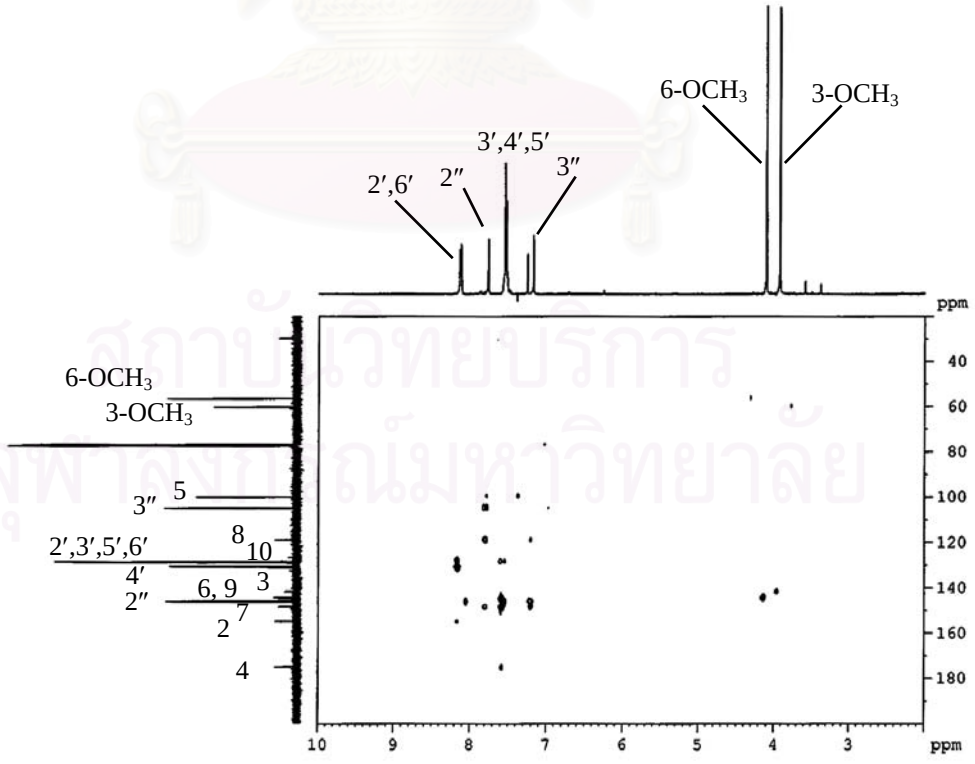


Figure 75 HMBC Spectrum ($CDCl_3$) of Compound MK 03

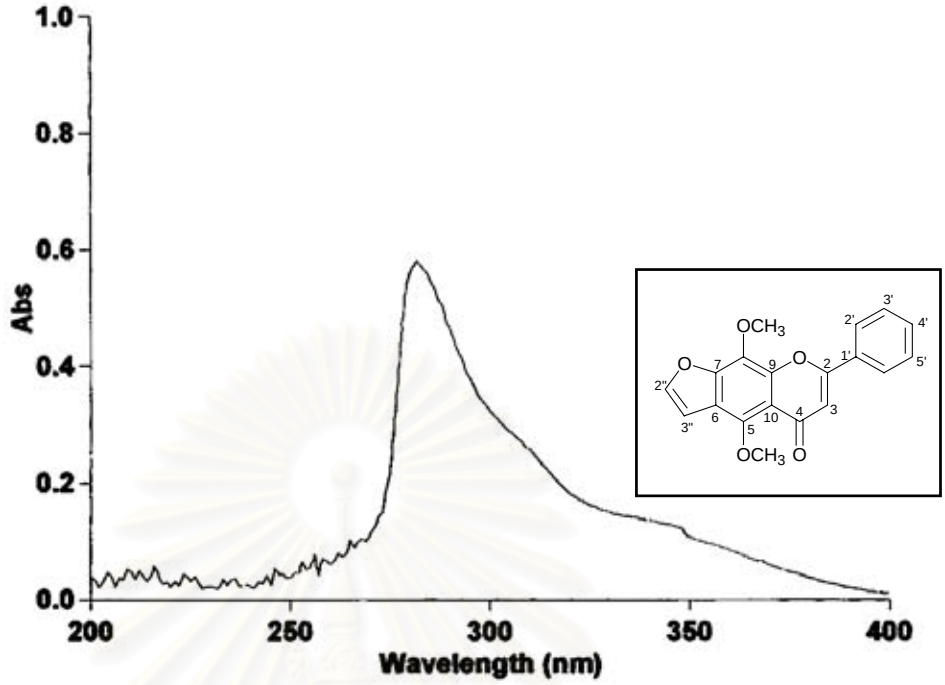


Figure 76 UV Spectrum of Compound MK 04 (chloroform)

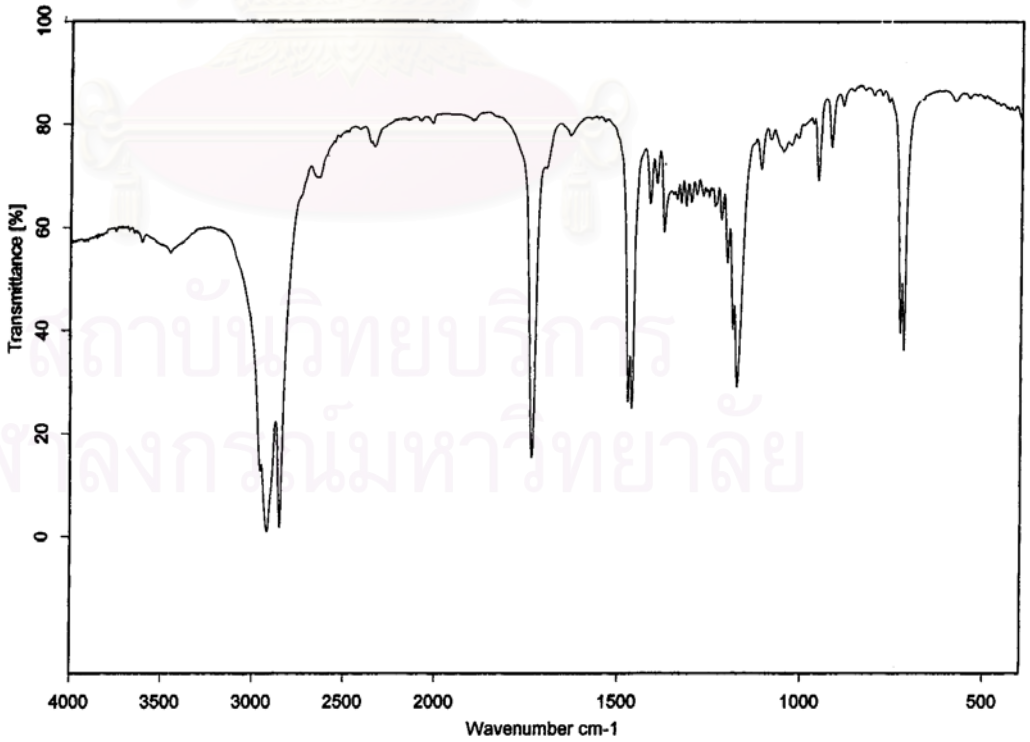


Figure 77 IR Spectrum of Compound MK 04 (film)

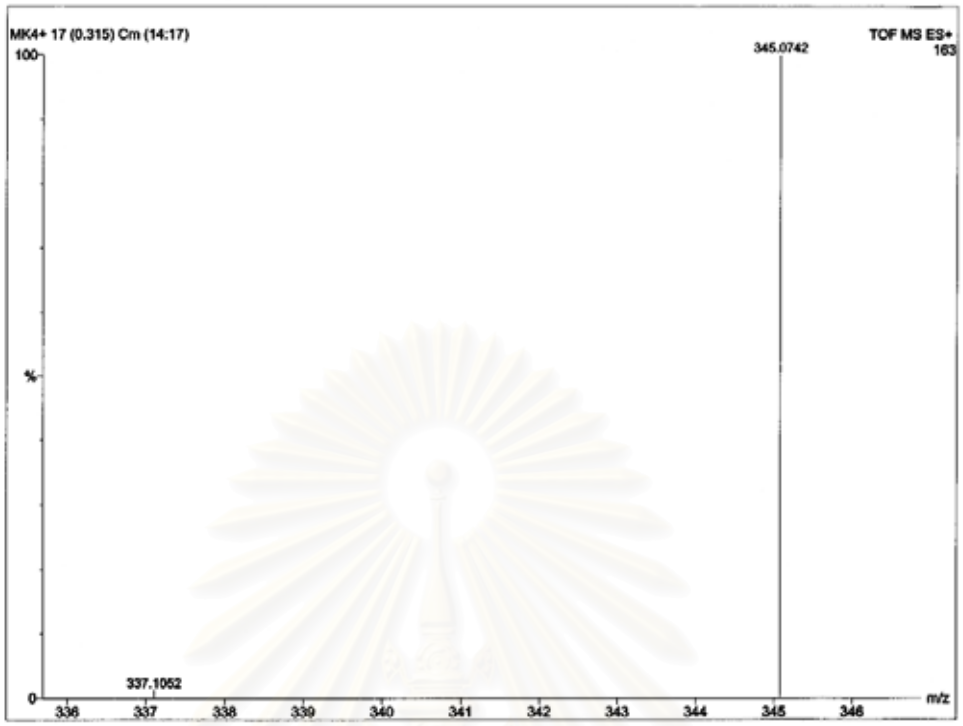


Figure 78 MS Spectrum of Compound MK 04

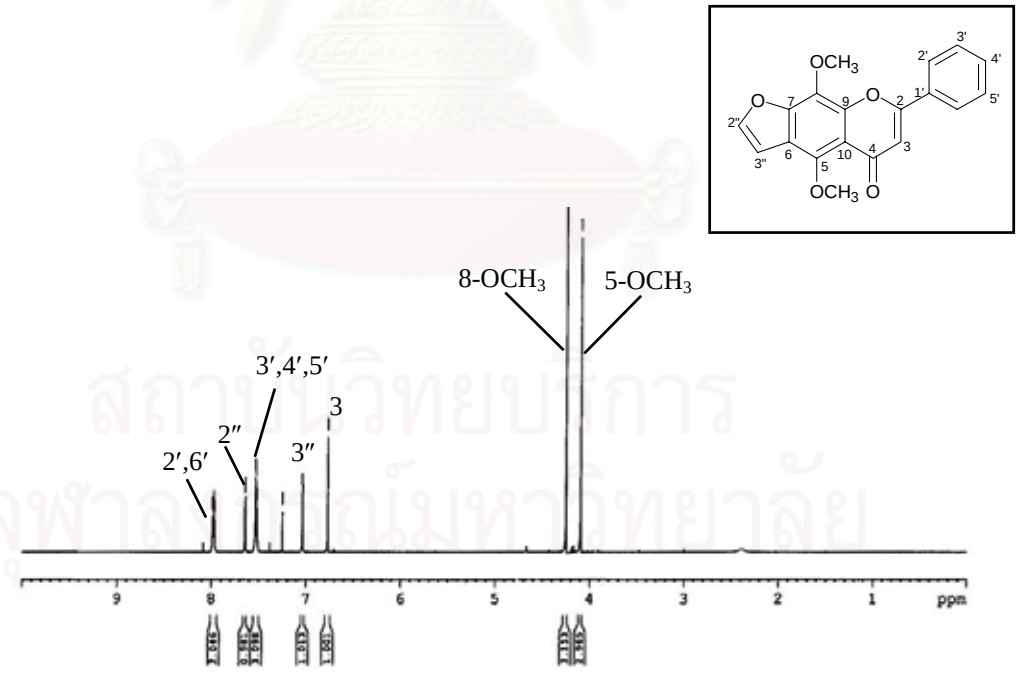


Figure 79 ^{1}H- NMR Spectrum ($CDCl_3$) of Compound MK 04

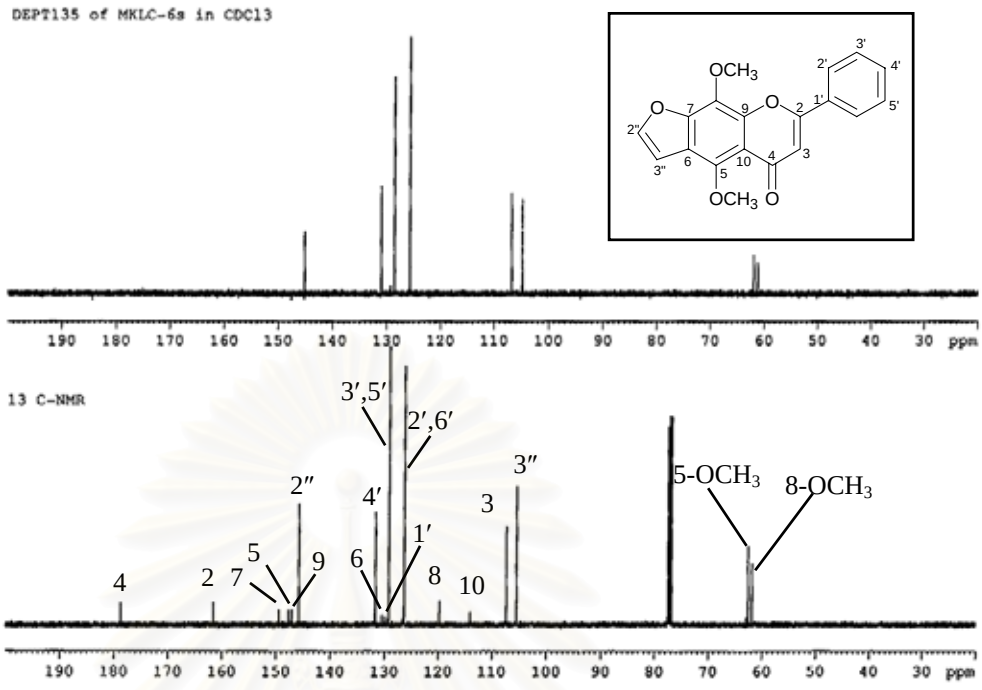


Figure 80 ^{13}C NMR and DEPT Spectra ($CDCl_3$) of Compound MK 04

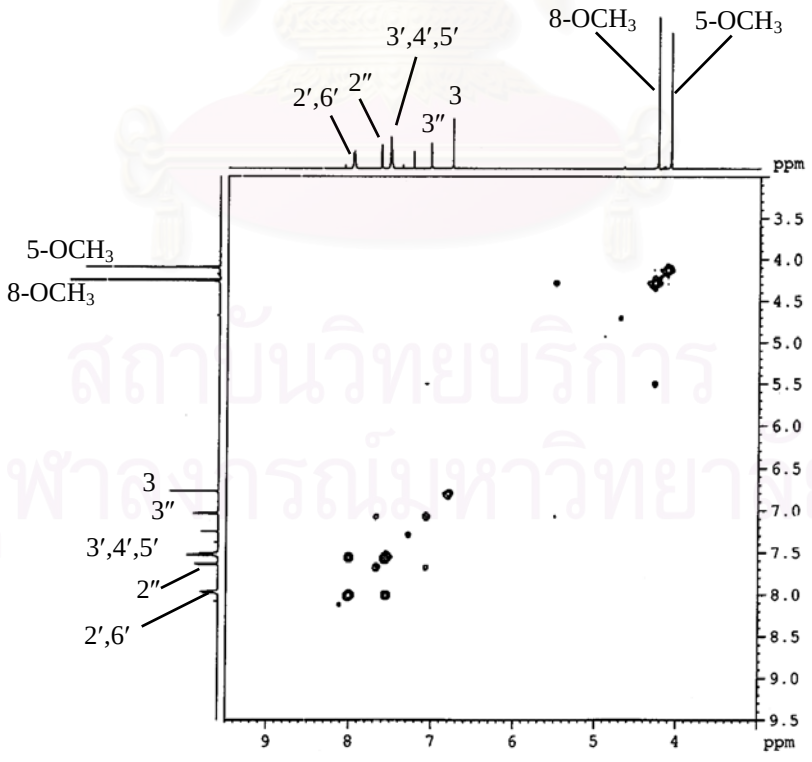


Figure 81 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Compound MK 04

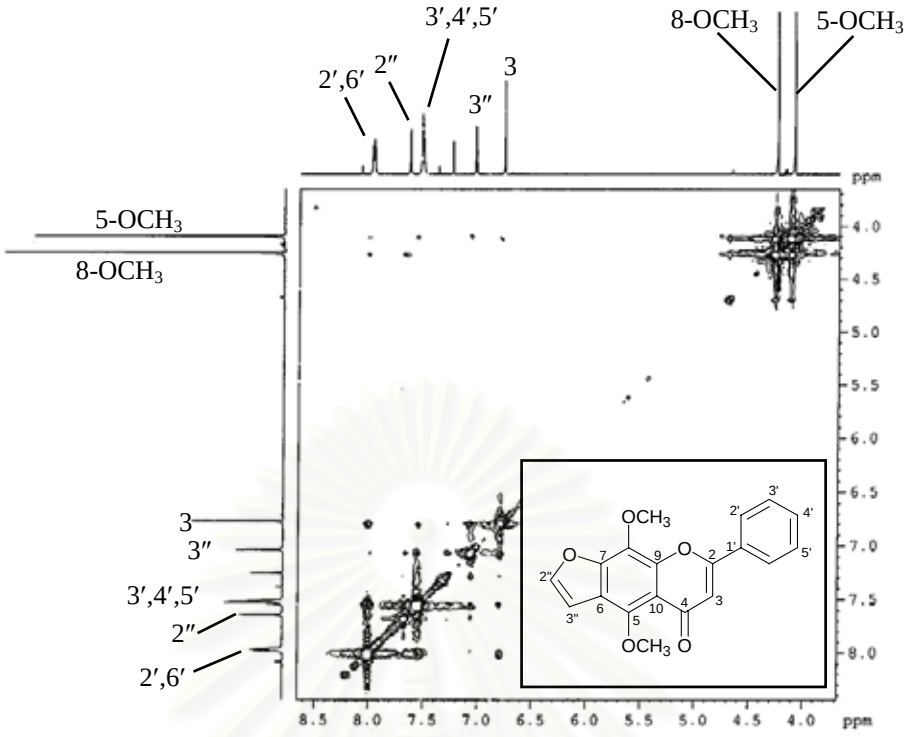


Figure 82 NOESY Spectrum ($CDCl_3$) of Compound MK 04

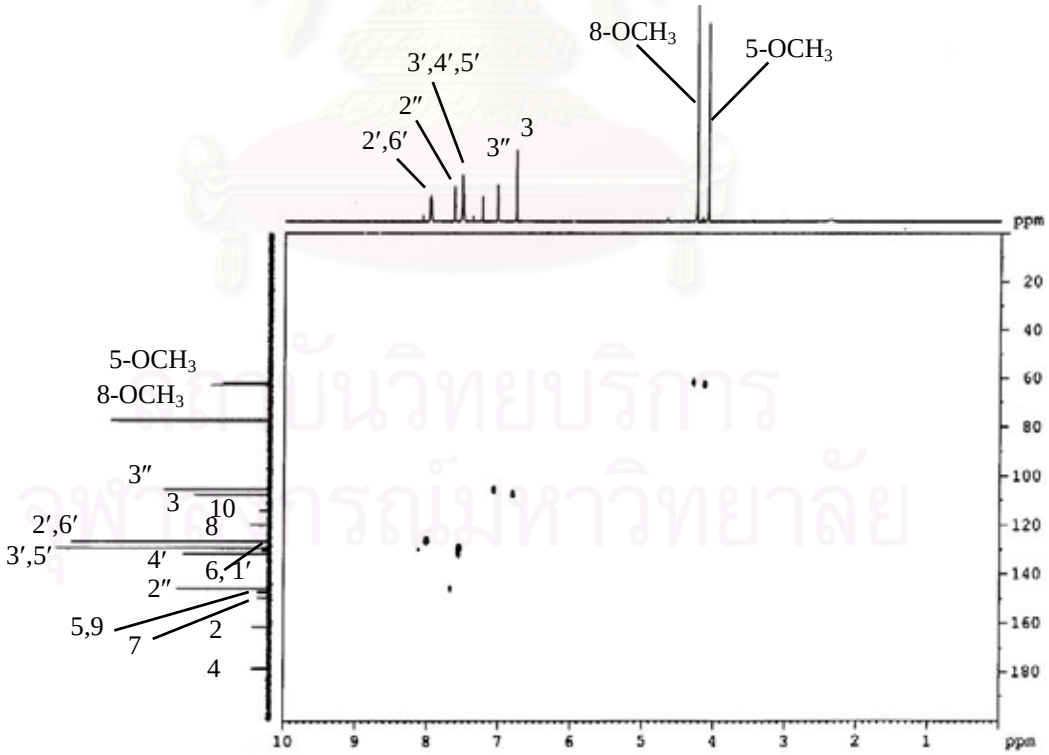


Figure 83 HMQC Spectrum ($CDCl_3$) of Compound MK 04

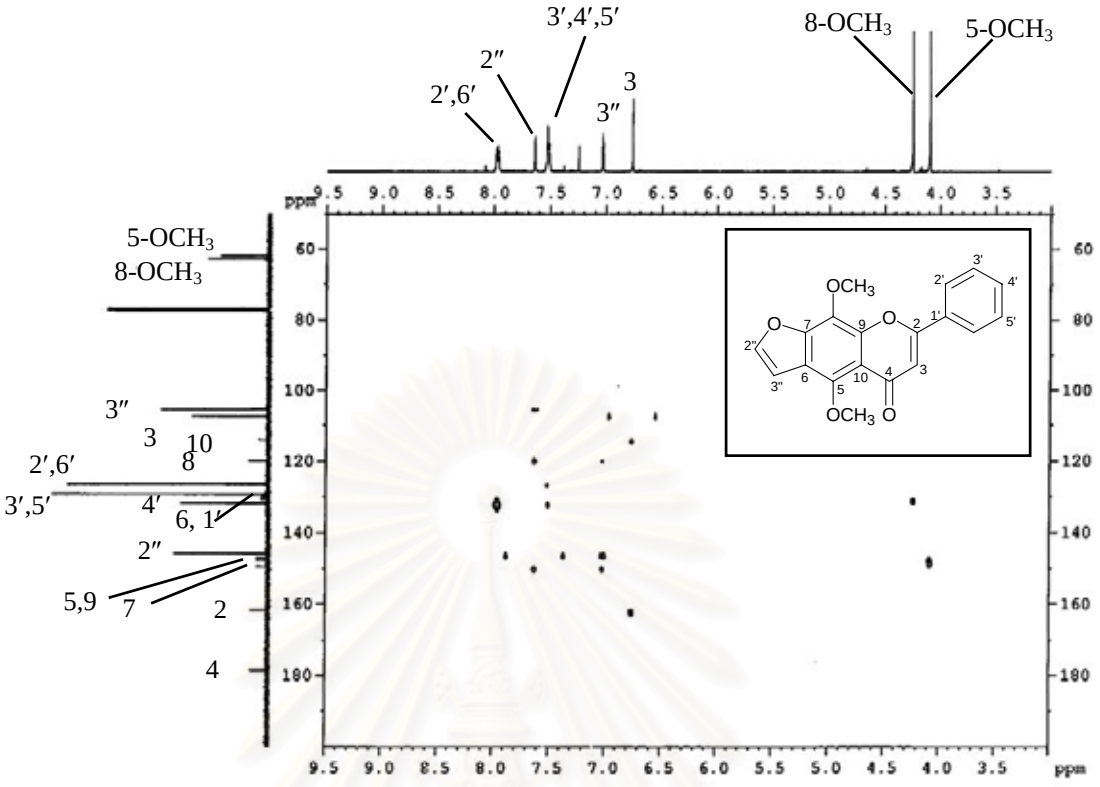


Figure 84 HMBC Spectrum ($CDCl_3$) of Compound MK 04

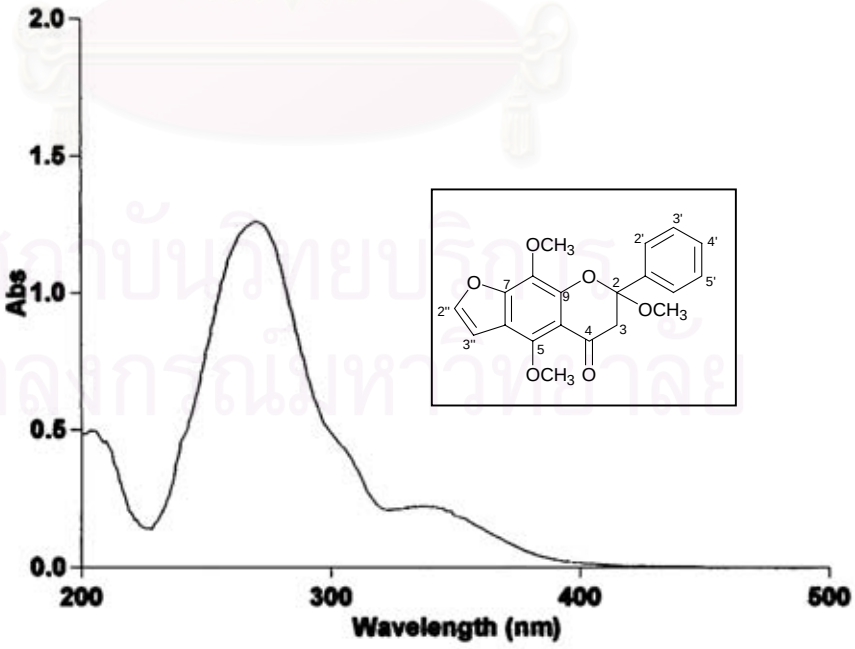


Figure 85 UV Spectrum of Compound MK 05 (chloroform)

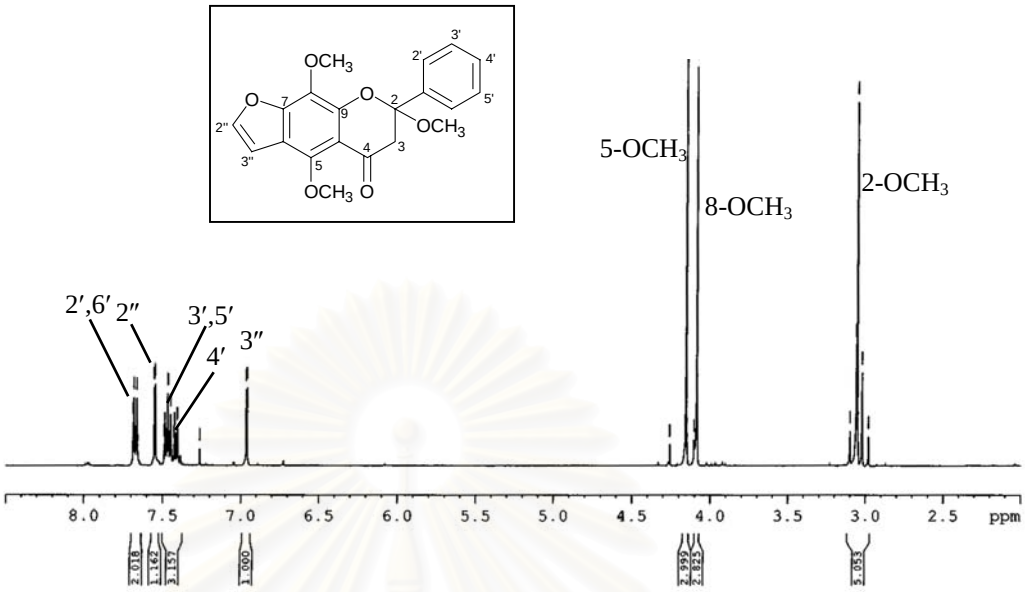


Figure 86 ^{1}H- NMR Spectrum ($CDCl_3$) of Compound MK 05

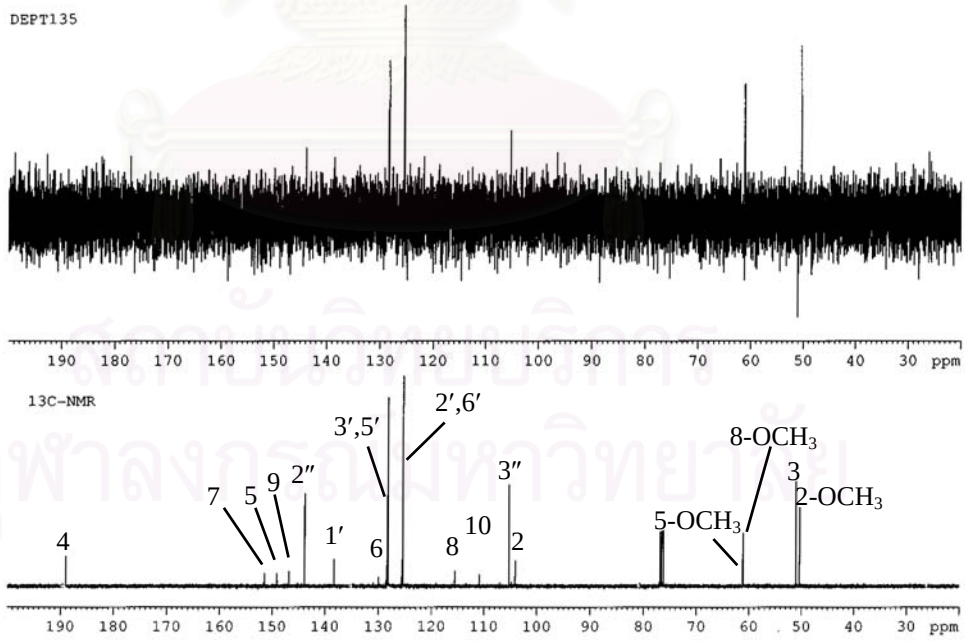


Figure 87 ^{13}C NMR and DEPT Spectra ($CDCl_3$) of Compound MK 05

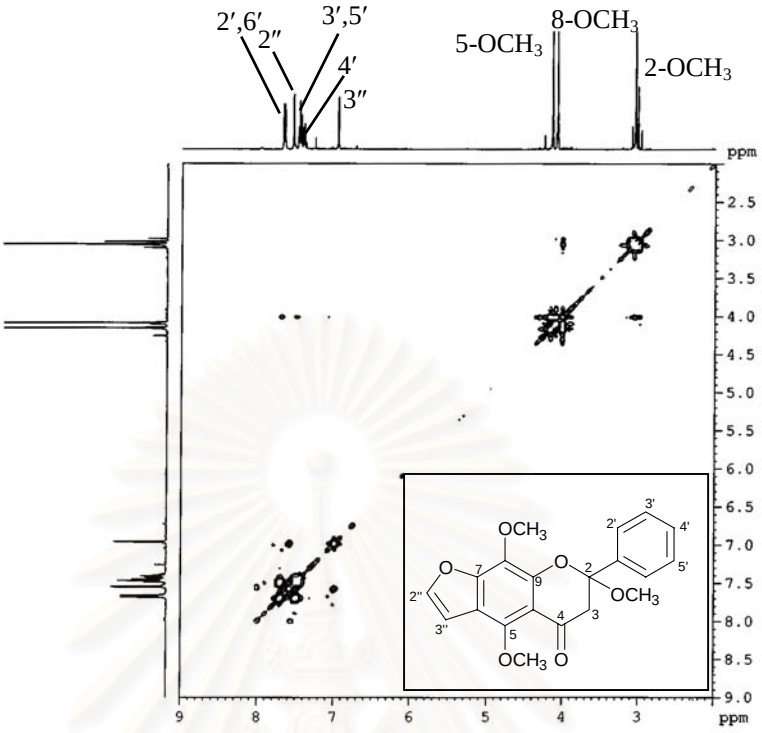


Figure 88 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Compound MK 05

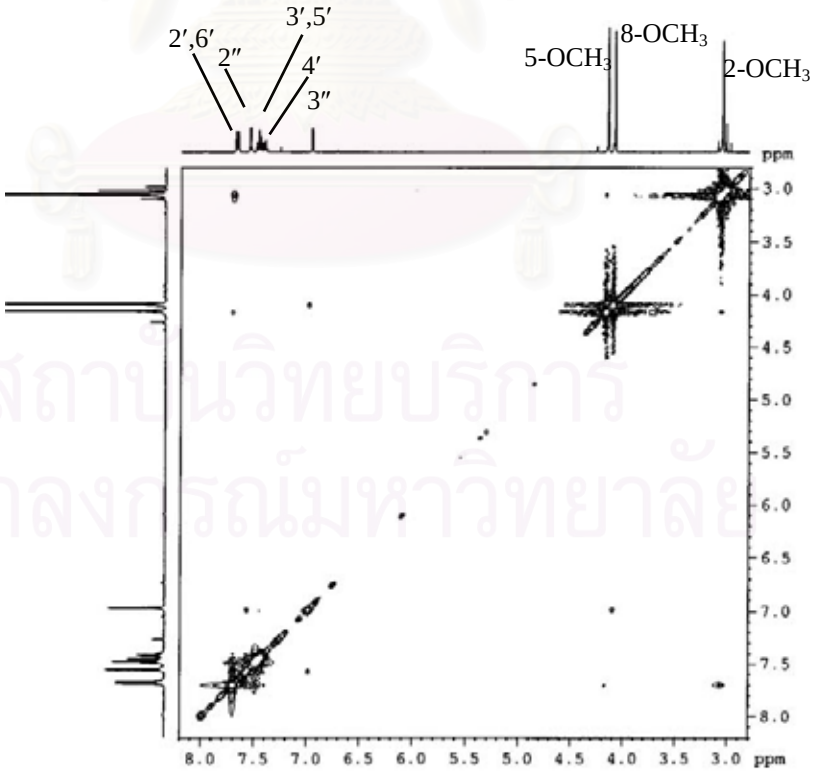


Figure 89 NOESY Spectrum ($CDCl_3$) of Compound MK 05

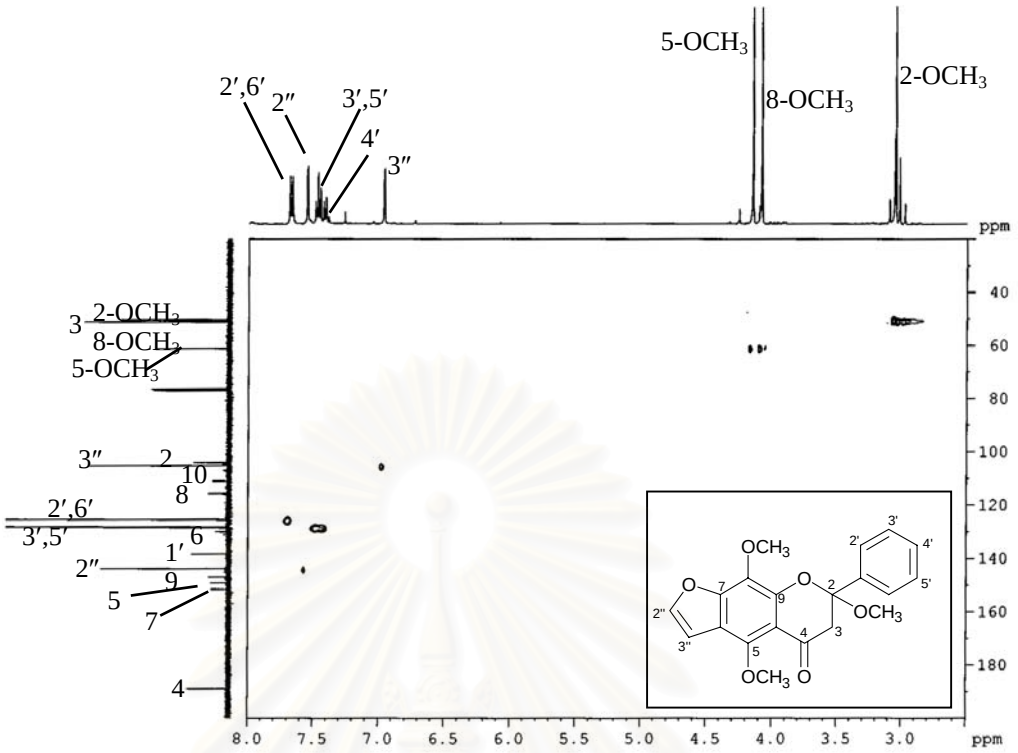


Figure 90 HMQC Spectrum ($CDCl_3$) of Compound MK 05

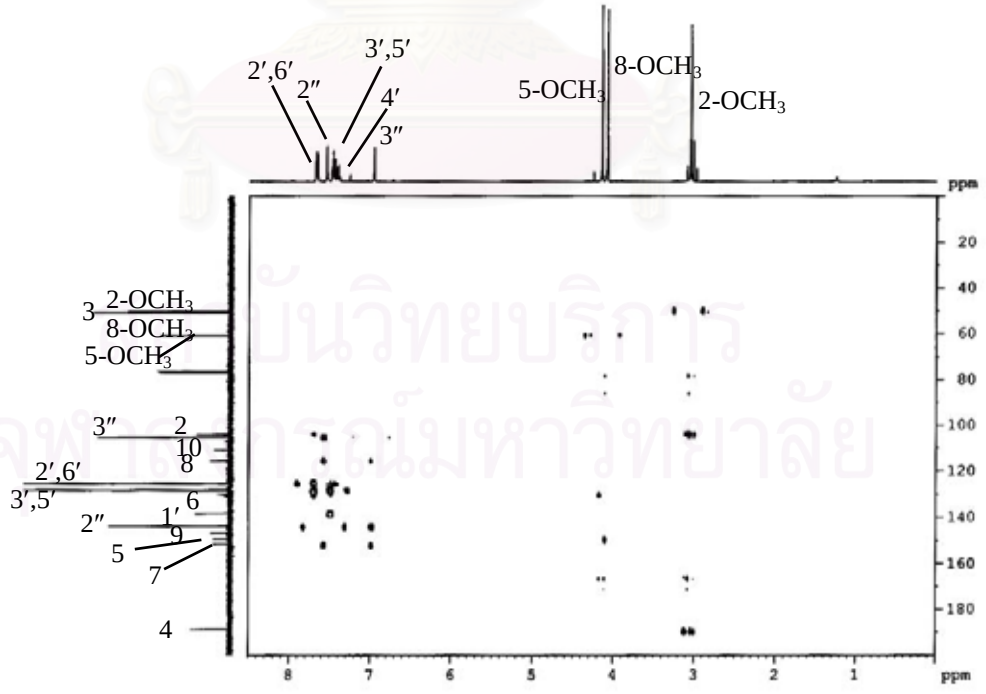


Figure 91 HMBC Spectrum ($CDCl_3$) of Compound MK 05

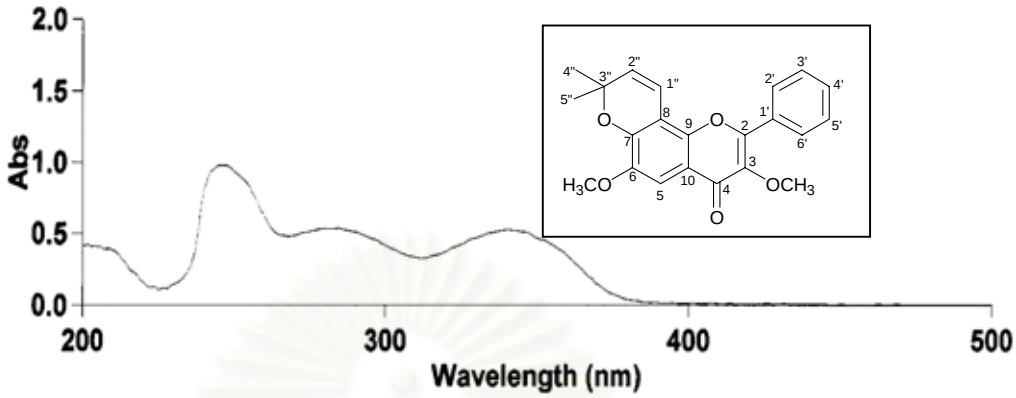


Figure 92 UV Spectrum of Compound MK 06 (chloroform)

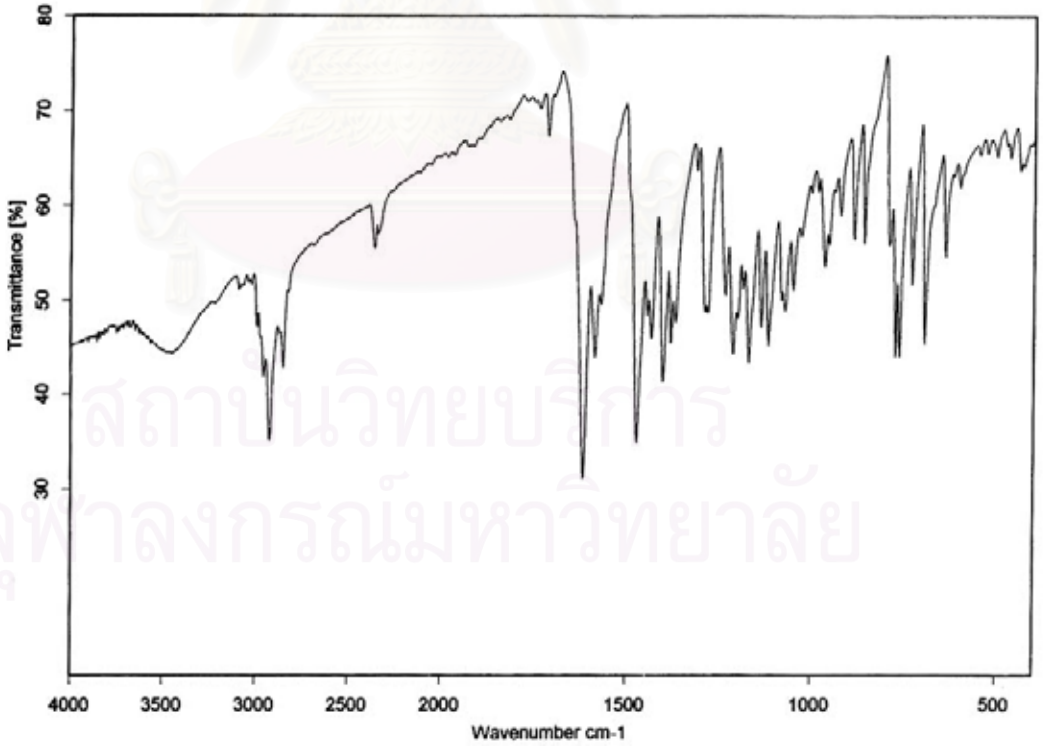


Figure 93 IR Spectrum of Compound MK 06 (film)

Figure 94 MS Spectrum of Compound MK 06

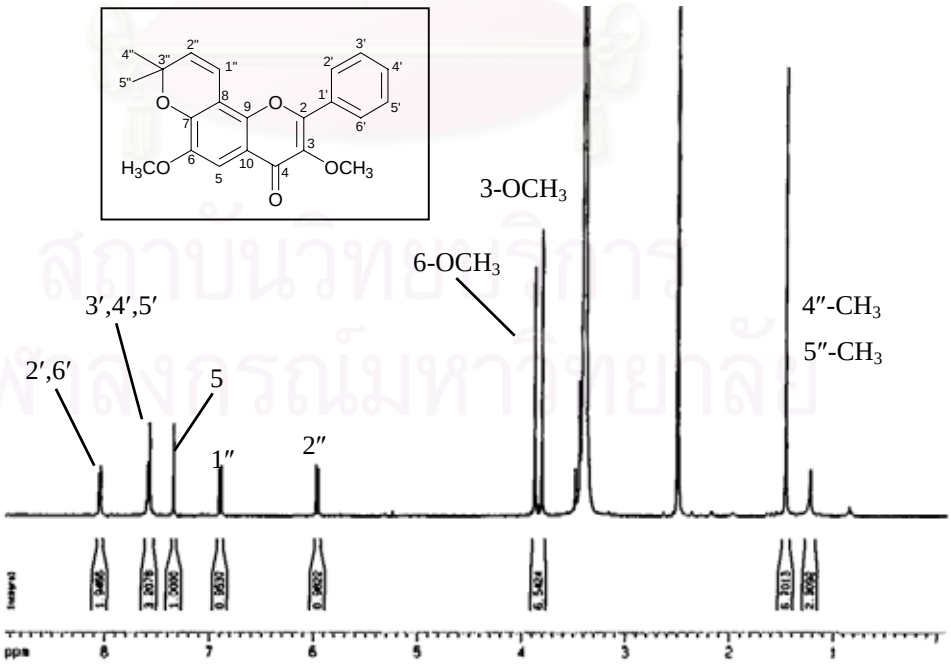


Figure 95 ^{1}H- NMR Spectrum (DMSO-d_6) of Compound MK 06

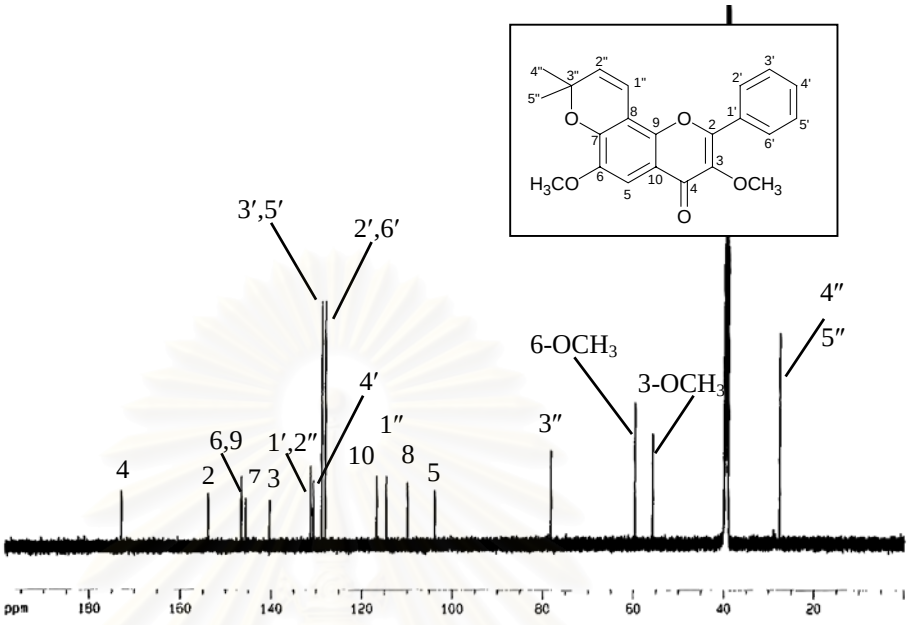


Figure 96 ^{13}C-NMR Spectrum (DMSO-d_6) of Compound MK 06

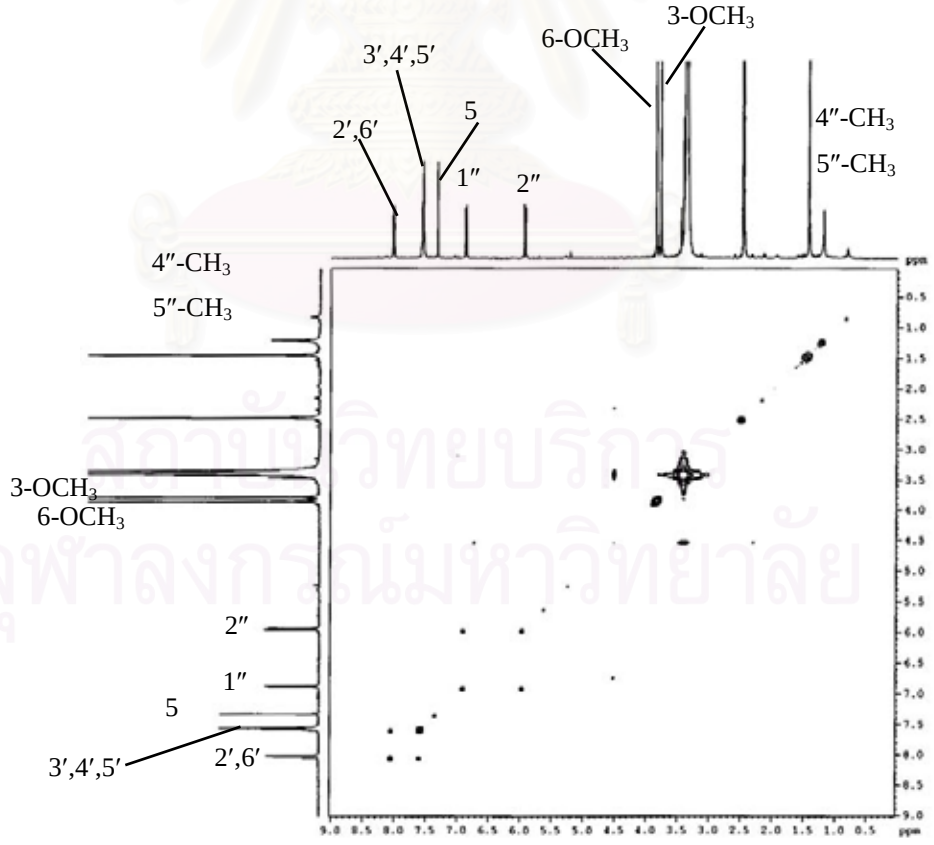


Figure 97 ^{1}H-^{1}H COSY Spectrum (DMSO-d_6) of Compound MK 06

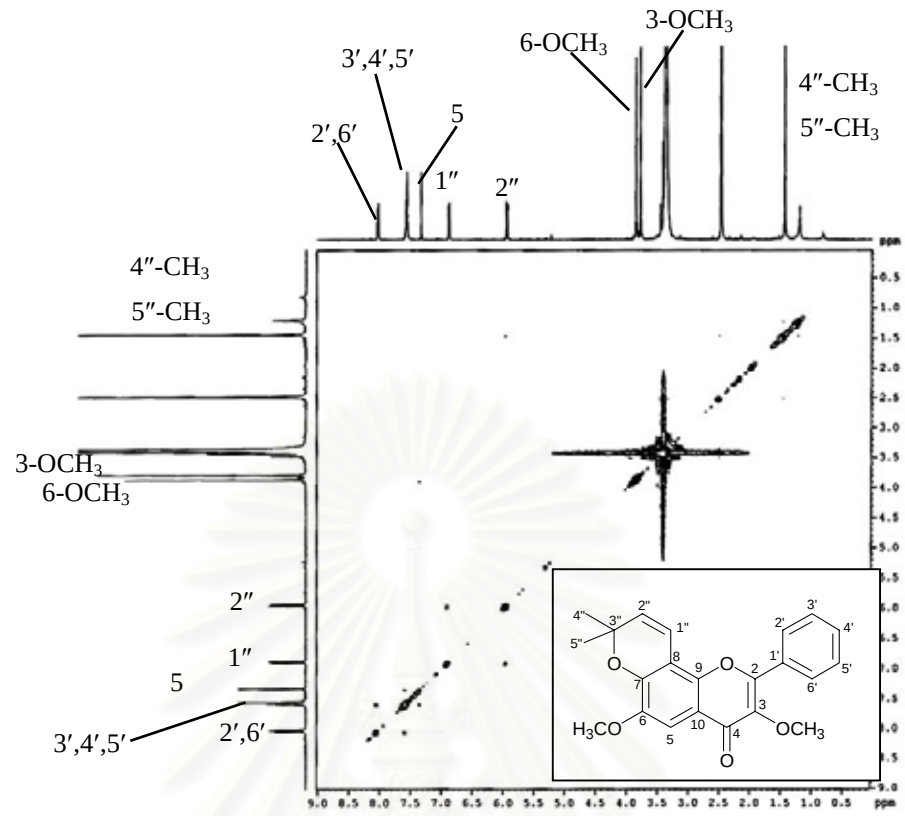


Figure 98 NOESY Spectrum (DMSO-d_6) of Compound MK 06

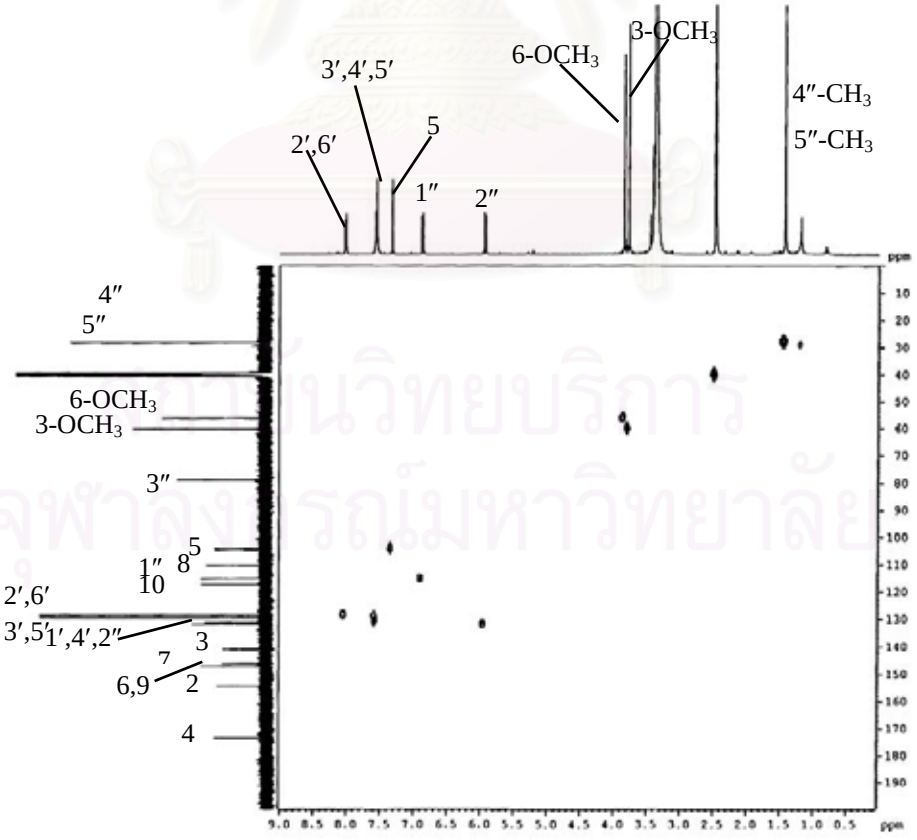


Figure 99 HMQC Spectrum (DMSO-d_6) of Compound MK 06

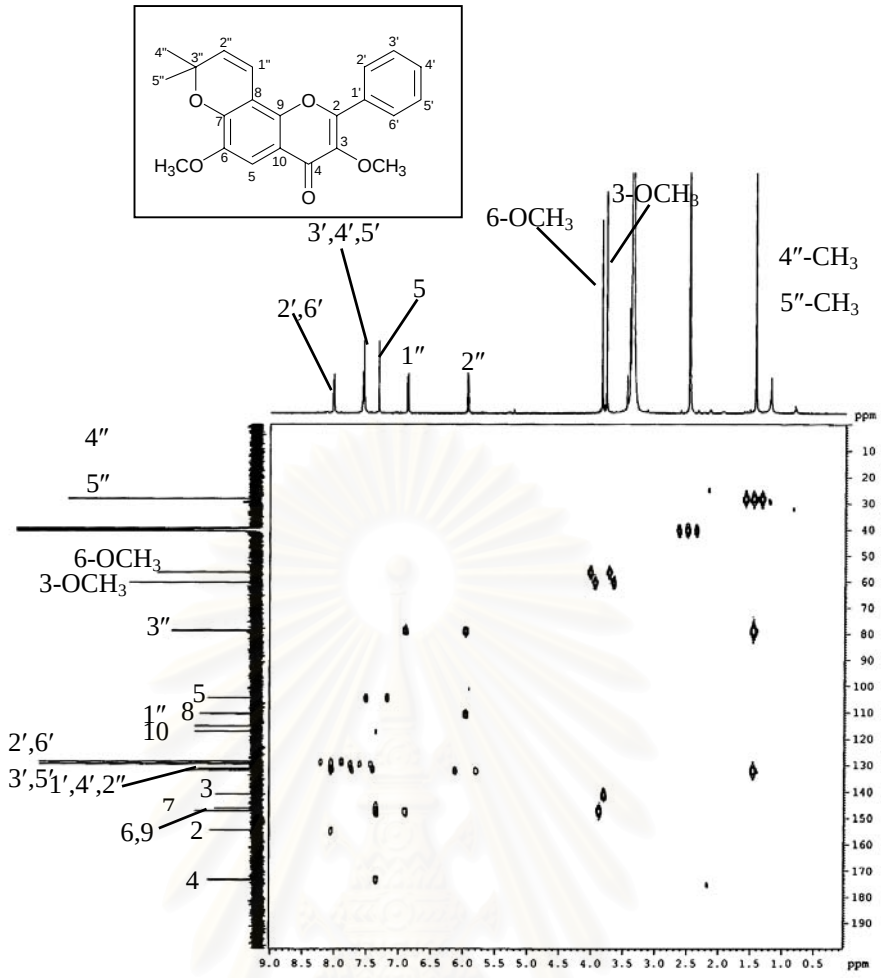


Figure 100 HMBC Spectrum (DMSO-d_6) of Compound MK 06

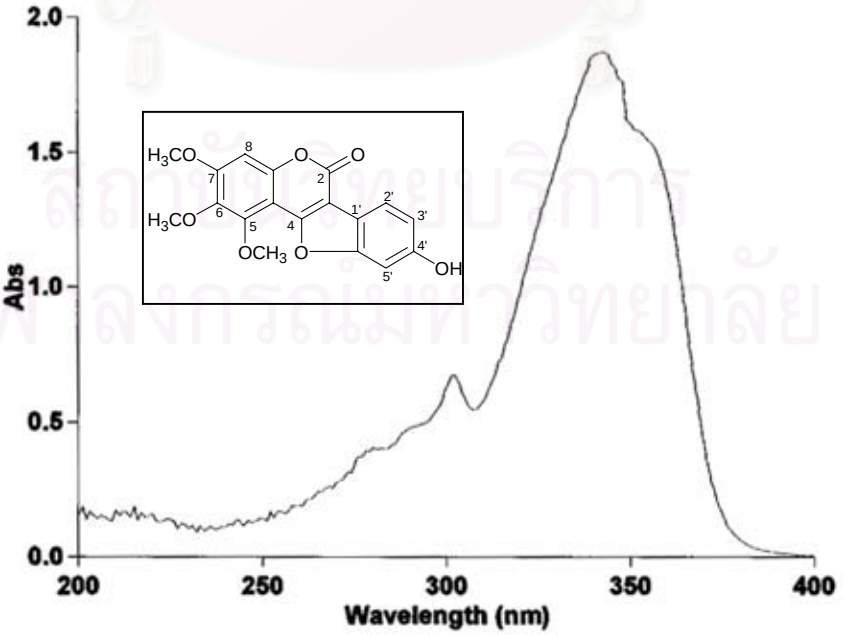


Figure 101 UV Spectrum of Compound MK 07 (chloroform)

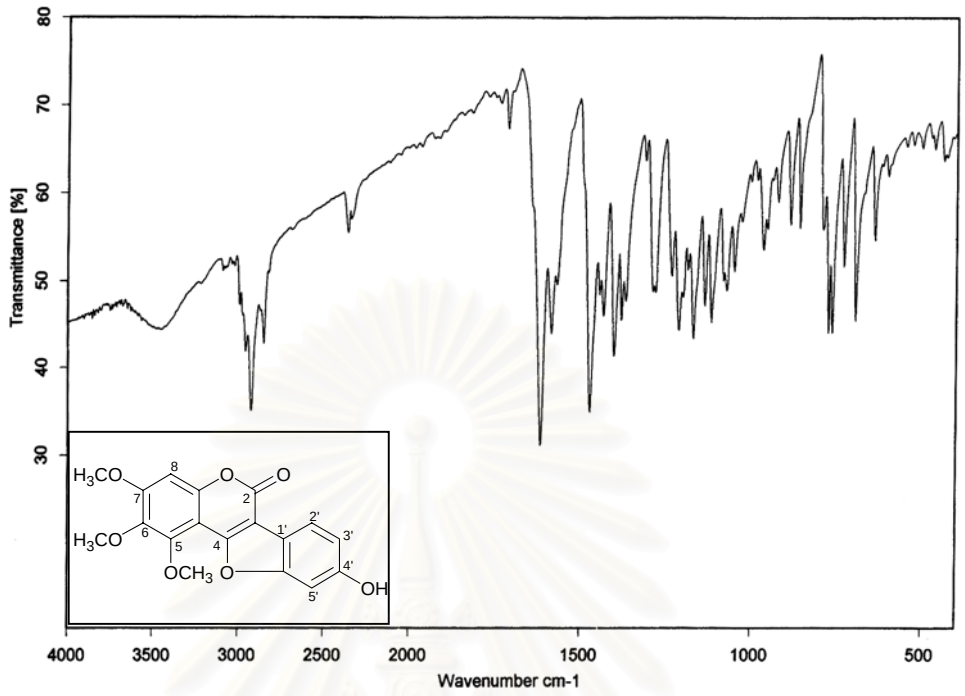


Figure 102 IR Spectrum of Compound MK 07 (film)

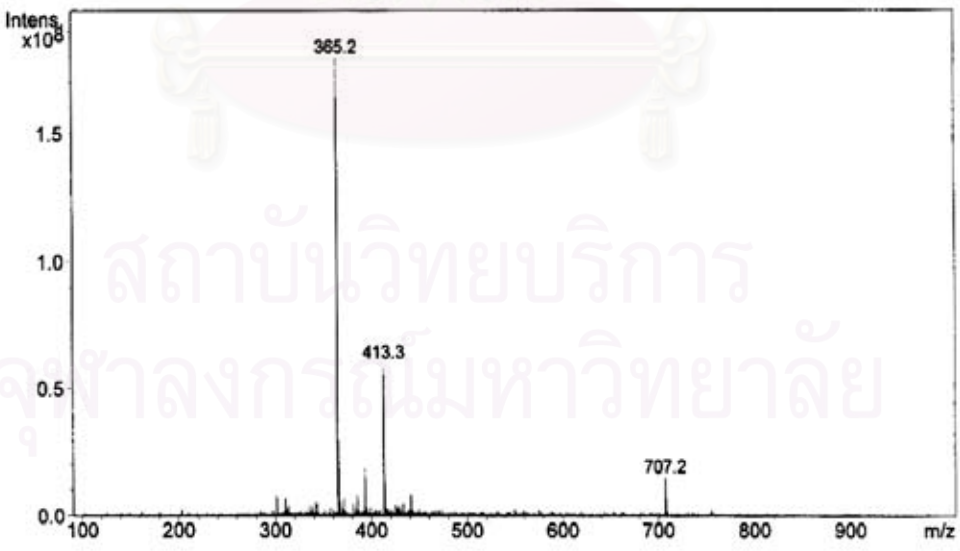


Figure 103 MS Spectrum of Compound MK 07

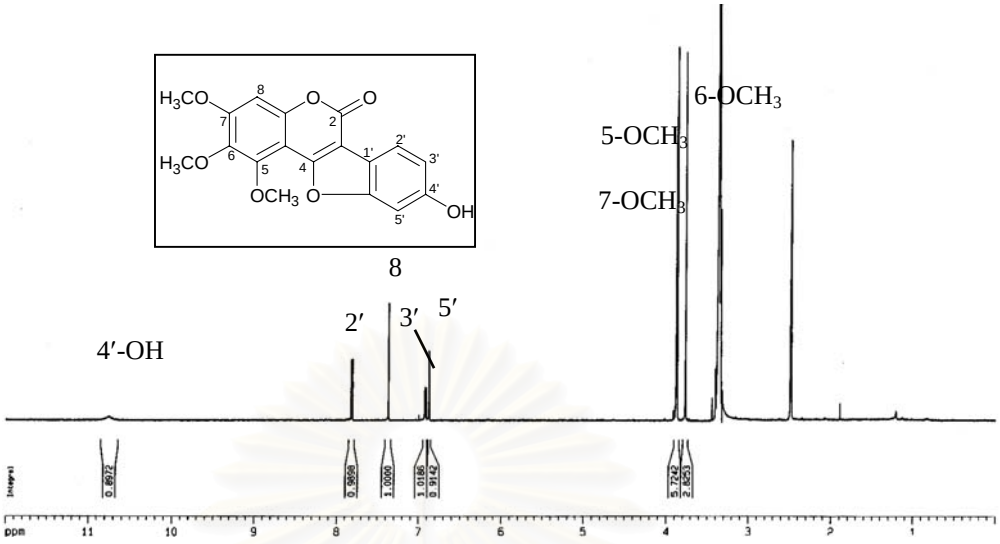


Figure 104 ^{1}H- NMR Spectrum (DMSO-d_6) of Compound MK 07

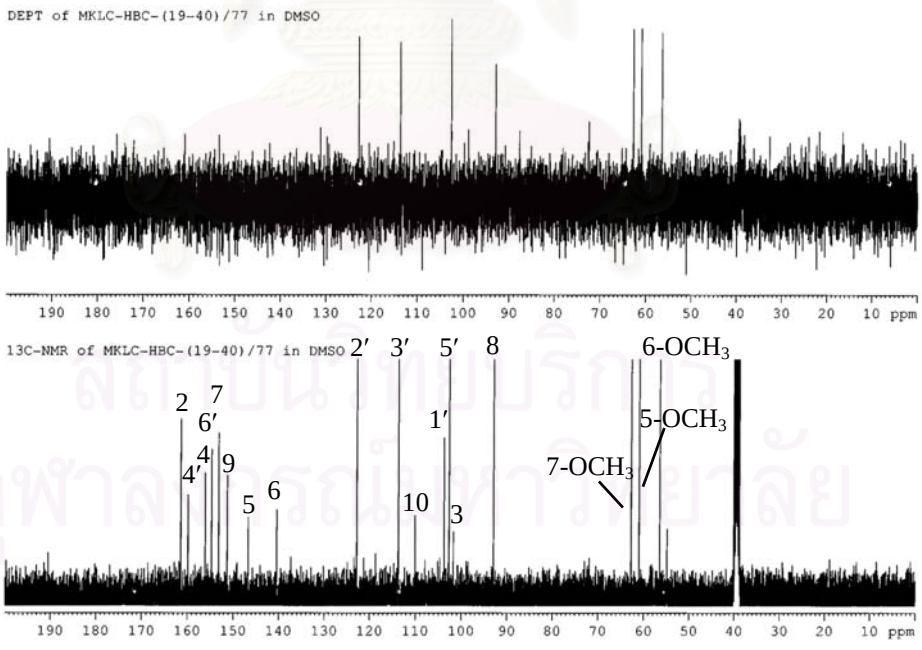


Figure 105 ^{13}C NMR and DEPT Spectra (DMSO-d_6) of Compound MK 07

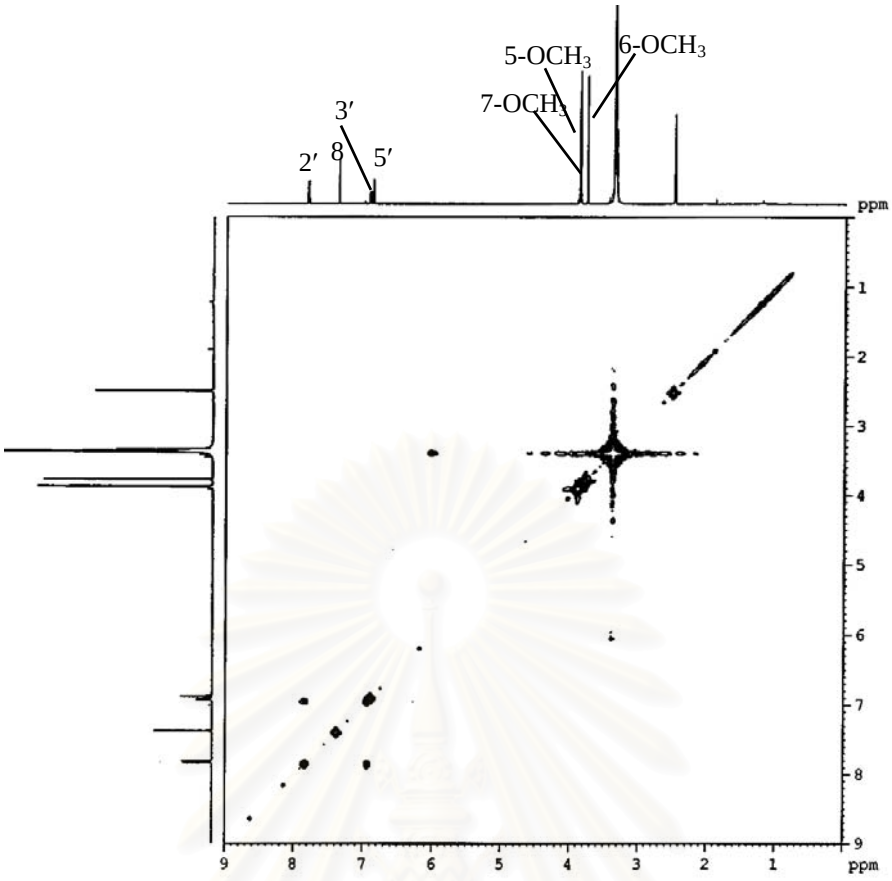


Figure 106 ^{1}H-^{1}H COSY Spectrum (DMSO-d_6) of Compound MK 07

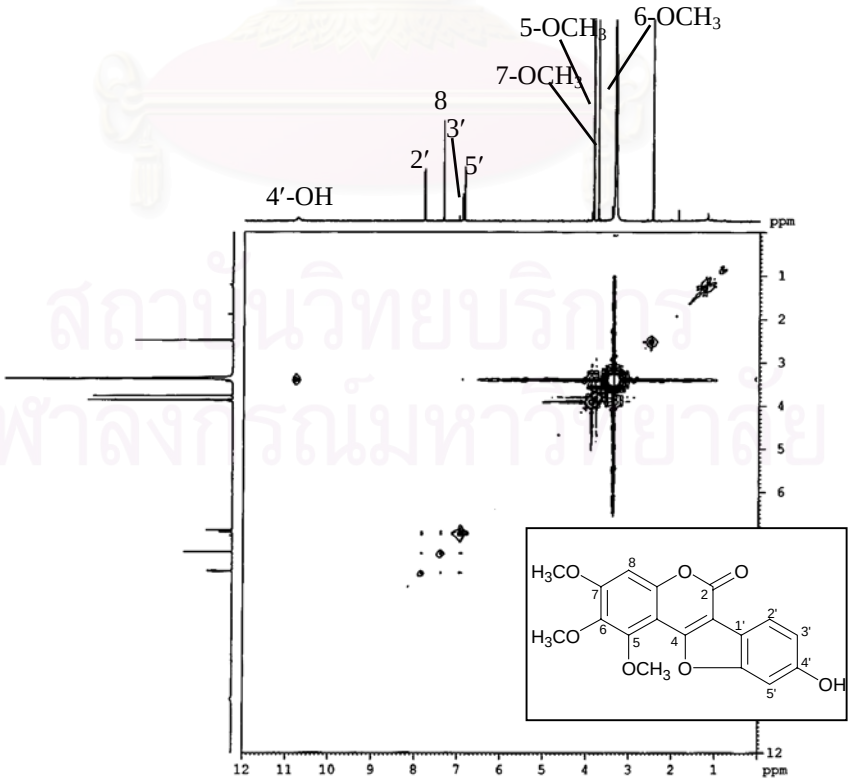


Figure 107 NOESY Spectrum (DMSO-d_6) of Compound MK 07

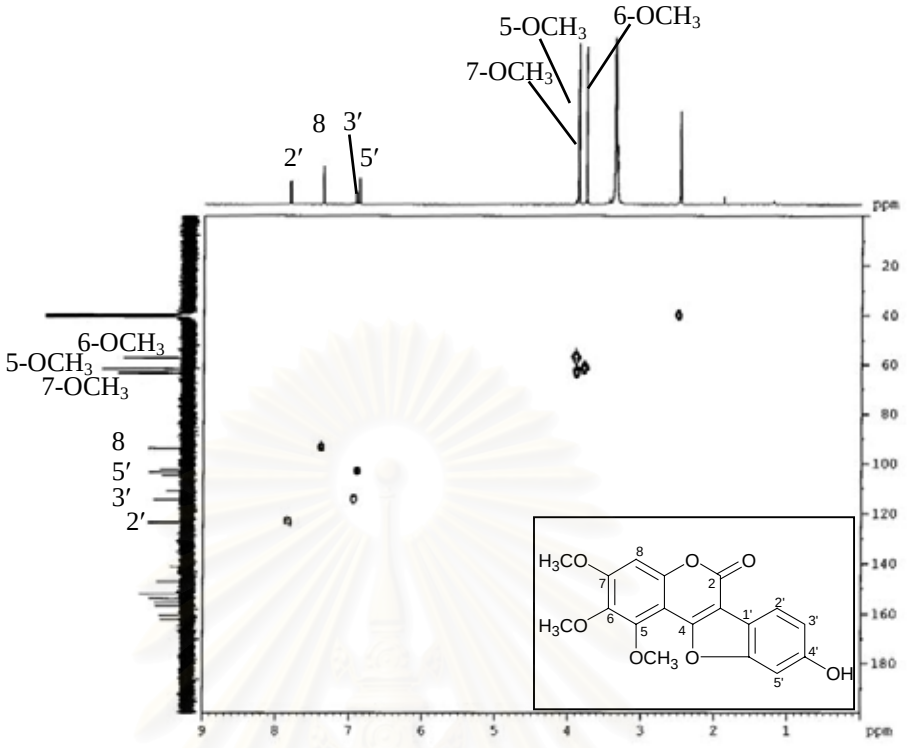


Figure 108 HMQC Spectrum (DMSO-d_6) of Compound MK 07

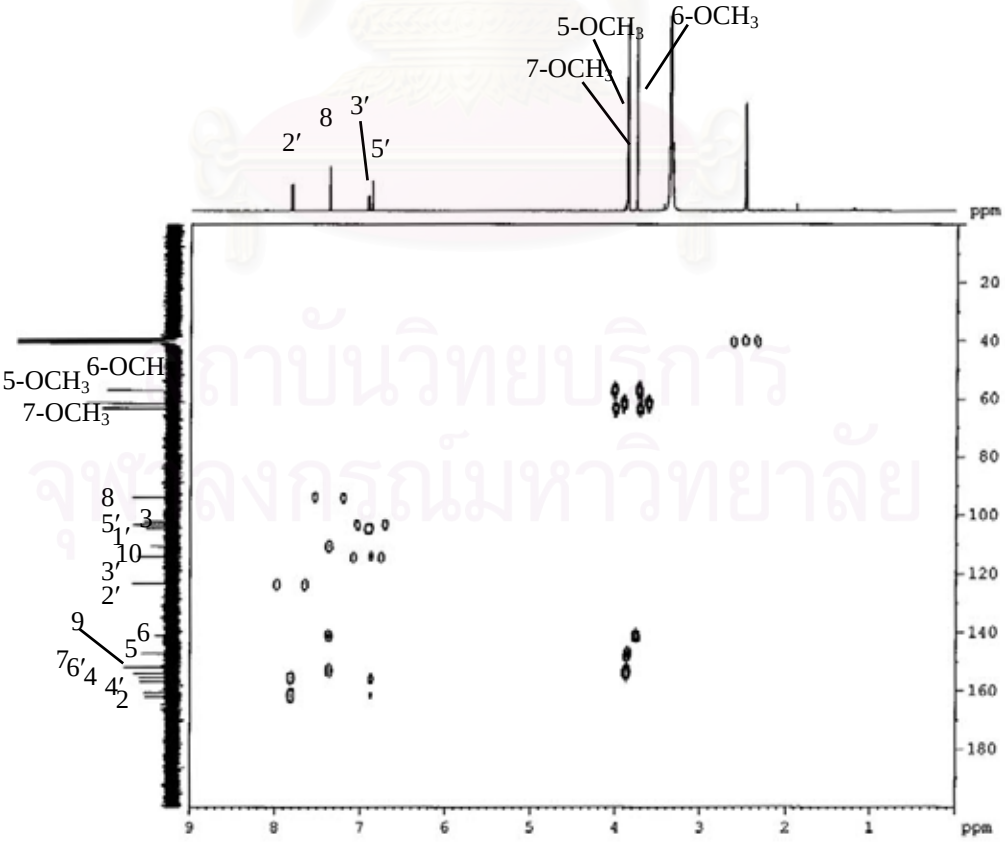


Figure 109 HMBC Spectrum (DMSO-d_6) of Compound MK 07

VITA

Miss Jiraporn Thongtan was born on December 3, 1965 in Bangkok, Thailand. She received her Bachelor's degree of Science in Chemistry from Ramkhamhaeng University in 1992, and Master's degree of Science in Applied Chemistry from Ramkhamhaeng University in 1997. She received a student grant from theThailand Graduated Institute of Science and Technology (TGIST).

Publication

Thongtan, J., Kittakoop, P., Ruangrugsi, N., Saenboonrueng, J., and Thebtaranonth, Y. 2003. New antimycobacterial and antimalarial 8,9-secokaurane diterpenes from *Croton kongensis*. **J. Nat. Prod**., 66(6): 868-870.

Poster Presentations

1. Thongtan, J., Kittakoop, P., Ruangrugsi, N., and Saenboonrueng, J. Antimycobacterial and antimalarial principle from *Croton kongensis*. 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. Thongtan, J., Kittakoop, P., Ruangrugsi, N., and Saenboonrueng, J. Antimycobacterial and antimalarial compounds from *Croton kongensis* and *Croton birmanicus*.The 20th Annual Research Meeting in Pharmaceutical Sciences, December 1, 2003, Bangkok, Thailand.