

องค์ประกอบทางเคมีของใบจัน



นางสาวกนกวรรณ เบญจนาค

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จุฬาลงกรณ์มหาวิทยาลัย

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CHEMICAL CONSTITUENTS OF *MILLETTIA ERYTHROCALYX* LEAVES

Miss Kanokwan Benchanak

สถาบันวิทยบริการ  
จุฬาลงกรณ์มหาวิทยาลัย

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จากการศึกษาองค์ประกอบทางเคมีของใบจั่น สามารถแยกสารในกลุ่ม flavonoid ได้ 7 ชนิด การพิสูจน์โครงสร้างของสารทั้งหมดที่แยกได้อาศัยการวิเคราะห์เชิงสเปกตรัมของ UV, IR, MS และ NMR ร่วมกับการเปรียบเทียบข้อมูลกับสารที่ทราบโครงสร้างแล้ว พบว่าสารที่แยกได้ประกอบด้วยสารใหม่ที่มีโครงสร้างในกลุ่ม furanoflavone คือ 3',5'-dimethoxy[2'',3'':7,8] furanoflavone และเป็นสารที่มีรายงานมาแล้ว ได้แก่ 3',4'-methylenedioxy-7-methoxy flavone, pongaglabrone, gamatin, milletenin C, millettocalyxin A และ millettocalyxin C

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ภาควิชา  
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เกสรชเวท  
ลายมือชื่ออาจารย์ที่ปรึกษา.....

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FLAVONE

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Chemical investigation of the constituents of *Millettia erythrocalyx* leaves led to the isolation of seven flavonoid compounds. The structure determinations of these compounds were accomplished by spectroscopic analyses (UV, IR, MS and NMR) and comparison with previously reported data of known compounds. These isolates were identified as a new furanoflavone, namely 3',5'-dimethoxy[2'',3'':7,8]furanoflavone, and the known compounds 3',4'-methylenedioxy-7-methoxyflavone, pongaglabrone, gamatin, milletenin C, millettocalyxin A and millettocalyxin C.

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**LIST OF ABBREVIATIONS AND SYMBOLS**

$\alpha$	=	Alpha
Acetone- $d_6$	=	Deuterated acetone
$\beta$	=	Beta
CA	=	Chemical Abstract
$CDCl_3$	=	Deuterated chloroform
$CHCl_3$	=	Chloroform
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)
DEPT	=	Distortionless Enhancement by Polarization Transfer
$\delta$	=	Chemical shift
ESIMS	=	Electrospray Ionization Mass Spectrometry
EtOAc	=	Ethyl acetate
EtOH	=	Ethanol
g	=	Gram
$^1H$ NMR	=	Proton Nuclear Magnetic Resonance
HMBC	=	$^1H$ -detected Heteronuclear Multiple Bond Coherence
HMQC	=	$^1H$ -detected Heteronuclear Multiple Quantum Coherence
HRESIMS	=	High Resolution Electrospray Ionization Mass Spectrometry
Hz	=	Hertz
IR	=	Infrared Spectrum
$J$	=	Coupling constant

**LIST OF ABBREVIATIONS AND SYMBOLS (continued)**

Kg	=	Kilogram
L	=	Liter
$\lambda_{\max}$	=	Wavelength at maximal absorption
$\epsilon$	=	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
NOE	=	Nuclear Overhauser Effect
NOESY	=	Nuclear Overhauser Effect Spectroscopy
<i>o</i>	=	Ortho
<i>p</i>	=	Para
pet. ether	=	Petroleum ether
ppm	=	Part per million
pyridine- $d_5$	=	Deuterated pyridine
$\nu_{\max}$	=	Wave number at maximal absorption
s	=	Singlet (for NMR spectra)
t	=	Triplet (for NMR spectra)



**LIST OF ABBREVIATIONS AND SYMBOLS (continued)**

TLC	=	Thin Layer Chromatography
UV	=	Ultraviolet
UV-VIS	=	Ultraviolet and Visible Spectrophotometry



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# CHAPTER I

## Introduction

The genus *Millettia* belongs to the subfamily Papilionoideae of the family Leguminosae, with more than 200 species distributed in tropical Africa, Asia and Australia. Plants in this genus are well known for elaborating flavonoids and isoflavonoids.

The plants in the genus *Millettia* are trees or large shrubs, usually climbers. Leaves odd-pinnate. Flowers showy, in axillary racemes, often fascicled, simple or paniculate and terminal. Calyx campanulate; teeth generally short or nearly obsolete. Corolla much exerted; petals with long claws; standard broad; keel not beaked. Stamens monadelphous or diadelphous, filaments filiform; anthers uniform. Ovary sessile, linear, few-ovuled; style filiform, incurved, glabrous, stigma capitate. Pod linear or oblong, 1- or few-seeded, flat or turgid, late in dehiscing or hardly dehiscent (Chopra, Budhwar and Ghosh, 1965)

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

- |                                     |   |
|-------------------------------------|---|
| <i>Millettia atropurpurea</i> Wall. | = <i>Collerya atropurpurea</i> (Wall.)Schott  |
| <i>M. brandisiana</i> Kurz          | กระพี้จัน Kra phi chan, จัน Chan, พี้จัน Phi Chan<br>(General); ปี้จัน Pi Chan (Northern).  |
| <i>M. caerulea</i> Baker.           | บัวเปาะเต๊ะ Pua-po-do (Karen Mae Hong<br>Son); ผักเยี่ยววัว Phak yiao wua<br>(Nakhonsawan, Northern); หางไหลแดง Hang<br>Lai daeng (Kanchanaburi). |
| <i>M. decipiens</i> Prain           | ปารี Pa ri (Malay-Narathiwat).  |
| <i>M. extensa</i> Benth.            | กำวเครือ Kao khrua, กวาวเครือ Kwao khrua<br>(Chiang Mai); ตานครบ Tan krop (Lampang).  |

<i>M. glaucescens</i> Kurz	ยะดา Ya-daa (Malay-Narathiwat); หยี่น้ำ Yi nam (Peninsular).
<i>M. kangensis</i> Craib	กระเจาะ Kra cho, ขะเจาะ Kha cho, ขะเจาะน้ำ Kha cho nam (Chiang Mai).
<i>M. kityana</i> Craib	เครือข้าวเย็น Khrua khao yen, ลางเย็น Lang yen, ฮางเย็น Hang yen (Northern).
<i>M. latifolia</i> Dunn	ขะเจาะ Kha cho (General).
<i>M. leucantha</i> Kurz	กะเขาะ Kaso (Central); กระเจาะ Kra cho,
var. <i>leucantha</i>	ขะเจาะ Kha cho (Northern); กระพีเขาควาง Kra phi khao khwai (Prachuap Khiri Khan); ขะแมบ Kha maep, คำแมบ Kham maep (Chiang Mai).
<i>M. leucanthe</i> Kurz	กระเจี๊าะ Kra cho, ขะเจี๊าะ Kha cho
var. <i>buteoides</i> (Gagnep.)P.K.Loc	(Lampang); กระท่อน Kra thon,
( <i>M. buteoides</i> Gagnep. var.	(Phetchabun <i>Phitsanulok</i> ); ไม้กระพวงน้ำผัก
<i>siamensis</i> Craib, <i>M. pendula</i>	Mai kra tong nam phak (Loei); สะท่อน
Benth.)	Sa thon (Saraburi); สาร Sa thon (Ubon Ratchathani).
<i>M. macrostachya</i> Collett & Hemsl.	ขะเจาะน้ำ Kha cho nam (Chiang Mai).
var. <i>macrostachya</i>	
<i>M. macrostachya</i> Collett & Hemsl.	ขะเจาะหลวง Kha cho luang, ขะเจาะใหญ่
var. <i>tecta</i> Craib	Kha cho yai (Narathiwat).
<i>M. pachycarpa</i> Benth.	เกดะ Ke-tha (Karen-Chiang Mai); เครือไหล Khrua lai (Chiang Mai).

<i>M. peguensis</i> Ali	ตอหิ To-hi (Karen-Kanchanaburi).
( <i>M. ovalifolia</i> Kurz)	
<i>M. pulcha</i> Benth. Kurz	จันพอ Chan pho (Northern).
<i>M. racemosa</i> (Roxb.)Benth.	= <i>Endosamara racemosa</i> (Roxb.)R. Geesink
<i>M. sericea</i> (Vent.)Benth.	จะไนโค๊ะ Cha-nai-kho, ปาตู Paa-tu (Malay-Narathiwat); นอเราะะ No-ro (Malay-Yala, Pattani); ยิมแมเก๊ะ Yim-mae-ko (Malay-Yala); อ้อยสามสวน Oi sam suan (Nong Khai).
<i>M. thorelii</i> Gagnep.	= <i>Derris thorelii</i> Craib
<i>M. utilis</i> Dunn	สะท้อนน้ำผัก Sathon nam phak (Loei).
<i>M. xylocarpa</i> Miq.	กะเงาะ Ka cho, ขะเงาะ Kha cho (General); คะแมด Kha maet (Chiang Mai); จักจัน Chakkachan (Loei); ฟี่ฟง Phi phong (Phrae); ยะดา Ya-da (Malay-Yala); ไยยี Yai-yi (Karen-Mae Hong Son); สาทร Sa thon, หยี่น้ำ Yi nam (Pattani-Yala).

Although *Millettia erythrocalyx* Gagnep. has not been recorded in Thai Plant Names (Smitinand, 2001), but the herbarium specimens of this species have been kept at the Royal Forest Department, Ministry of Agriculture and Co-operatives.

*Millettia erythrocalyx* Gagnep. has a local name as Jun. It is a medium-sized tree reaching 7-8 m. Bark: grayish. Branchlets: rusty strigose, become glabrescent, spotted lenticels prominent. Leaflets 7-11, ovate- or elliptic-lanceolate, papery, 3-6 x 1.5-2 cm., base narrowly cordate, apex caudate, glabrous and shining adaxially, scarlet strigose on midrib and margins abaxially. Pseudoracemes: axillary on the tip of branches, 6-7 cm., densely dark brown strigose; flower 8-9 mm., calyx 3 mm., deep red, sparsely hairy, teeth

truncate, ciliated; corolla lilac, vexillum glabrous, round tapering at base, with 2 minute callus; ovary villose, ovules 4-5. Pod: linear-oblong, 9-10 x 2 cm., flat, slightly curved, tapering to the base, brown tomentose when young, become glabrescent, valves woody, spirally twisted. Seeds: 2-3, chestnut brown, 13 x 10 mm., lens-shaped, smooth. It has been found in Thailand, Laos and Cambodia (Zhi, 2002).

The isolations of several phenolic compounds from the root and the stem bark of *Millettia erythrocalyx* Gagnep. have been earlier described (Sritularak, 2002a and 2002b). The compounds from the stem bark of *M. erythrocalyx* are millettocalyxins A-C, pongol methyl ether, 2'-hydroxy-3,4-methylenedioxy-4'- $\gamma,\gamma$ -dimethylallyloxychalcone, derricidin, 7- $\gamma,\gamma$ -dimethylallyloxyflavanone, ponganone I, karanjin, milletenone, ovalifolin, milletenin C, 3',4'-methylenedioxy-7-methoxyflavone, pongaglabrone, prunetin, vicenin II, isovitexin, lupeol and dihydrophaseic acid-4'-*O*- $\beta$ -D-glucopyranoside. The compounds found in the root of *M. erythrocalyx* are 6-methoxy-[2'',3'':7,8]-furanoflavanone, 2,5-dimethoxy-4-hydroxy-[2'',3'':7,8]-furanoflavan, 3,4-methylenedioxy-2',4'-dimethoxychalcone, 1-(4-hydroxy-5-benzofuranyl)-3-phenyl-2-propen-1-one, derricidin, purpurenone, pongaglabol, ponganone I, pongamol, ovalitenone, milletenone, ponganoneV and lanceolatin B. So far, the constituents of the leaves of this plant have not been reported. Therefore, in this investigation the following objectives are put forward:

1. to isolate and purify chemical constituents of the leaves of *M. erythrocalyx*
2. to determine the chemical structure of each isolated compound



**Figure 1** *Millettia erythrocalyx* Gagnep.

## CHAPTER II

### HISTORICAL

#### 1. Chemical Constituents of *Millettia* species

Chemical investigations of several *Millettia* species have shown them to be a good source of flavonoids. In addition, other classes of natural compounds such as rotenoids, nitrogenous compounds, terpenoids and miscellaneous substances have been found (Tables 1 and 2).

**Table 1** Distribution of flavonoids in *Millettia* species.

Plant and compound	Category	Plant part	Reference
<i>Millettia auriculata</i>			
Auricularin [1]	Isoflavone	Root	Rao, Prasad and Ganapaty, 1992
Auriculasin [2]	Isoflavone	Leaf	Minhaj <i>et al.</i> , 1976
Auriculatin [3]	Isoflavone	Root	Shabbir <i>et al.</i> , 1968
Auriculin [4]	Isoflavone	Root	Shabbir and Zaman, 1970
Aurmillone [5]	Isoflavone	Seed	Raju and Srimannarayana, 1978
2'-Deoxyisoauriculatin [6]	Isoflavone	Root	Rao, Prasad and Ganapaty, 1992
Isoauriculasin [7]	Isoflavone	Leaf	Minhaj <i>et al.</i> , 1976
Isoauriculatin [8]	Isoflavone	Root	Shabbir and Zaman, 1970
Isoaurmillone [9]	Isoflavone	Pod	Gupta <i>et al.</i> , 1983
2'-O-Methylisoauriculatin [10]	Isoflavone	Root	Rao, Prasad and Ganapaty, 1992
Millettin [11]	Isoflavone	Seed	Raju <i>et al.</i> , 1981
Scandenone [12]	Isoflavone	Root	Rao, Prasad and Ganapaty, 1992
<i>M. conraui</i>			
Conrauinone A [13]	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998a
Conrauinone B [14]	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998a
Conrauinone C [15]	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998b

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
Conrauinone D [16]	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998b
7-Hydroxy-6-methoxy-3'-4'-methylenedioxyisoflavone [17]	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998b
5-Methoxydurmillone [18]	Isoflavone	Stem bark	Fuendjiep <i>et al.</i> , 1998a
<i>M. dura</i>			
Calopogonium isoflavone A [19]	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1996
6-Demethyldurallone [20]	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1996
7,2'-Dimethoxy-4',5'-methylenedioxyisoflavone [21]	Isoflavone	Stem bark Root bark	Dagne, Mammo and Bekele, 1991
Durallone [22]	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1996
Durlettone [23]	Isoflavone	Seed	Ollis, Rhodes and Sutherland, 1967
Durmillone [24]	Isoflavone	Seed Stem bark	Ollis, Rhodes and Sutherland, 1967 Yenesew, Midiwo and Waterman, 1996
Ferrugone [25]	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1997
Formononetin [26]	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1997
4-Hydroxyderricin [27]	Chalcone	Stem bark Root bark	Dagne, Mammo and Bekele, 1991
4-Hydroxylonchocarpin [28]	Chalcone	Stem bark Root bark	Dagne, Mammo and Bekele, 1991
Isoerythrin-A-4'-(3-methylbut-2-enyl)ether [29]	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1996
Jamaicin [30]	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1997



**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
Maximaisoflavone B [31]	Isoflavone	Stem bark Root bark	Dagne, Mammo and Bekele, 1991
Maximaisoflavone D [32]	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1996
Maximaisoflavone H [33]	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1996
6-Methoxycalopogonium isoflavone A [34]	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1997
Milldurone [35]	Isoflavone	Seed	Ollis, Rhodes and Sutherland, 1967
Predurallone [36]	Isoflavone	Seed pod	Yenesew, Midiwo and Waterman, 1996
<i>M. erythrocalyx</i>			
Derricidin [37]	Chalcone	Stem bark	Sritularak <i>et al.</i> , 2002a
2,5-Dimethoxy-4-hydroxy-[2'',3'':7,8]-furanoflavan [38]	Flavan	Root	Sritularak <i>et al.</i> , 2002b
7- $\gamma,\gamma$ -Dimethylallyloxyflavanone [39]	Flavanone	Stem bark	Sritularak <i>et al.</i> , 2002a
1-(4-Hydroxy-5-benzofuranyl)-3-phenyl-2-propen-1-one [40]	Chalcone	Root	Sritularak <i>et al.</i> , 2002b
2'-Hydroxy-3,4-methylenedioxy 4'- $\gamma,\gamma$ -dimethylallyloxychalcone [41]	Chalcone	Stem bark	Sritularak <i>et al.</i> , 2002a
Lanceolatin B [42]	Flavone	Root	Sritularak <i>et al.</i> , 2002b
6-Methoxy-[2'',3'':7,8]-furanoflavanone [43]	Flavanone	Root	Sritularak <i>et al.</i> , 2002b
3,4-Methylenedioxy-2',4'-dimethoxy chalcone [44]	Chalcone	Root	Sritularak <i>et al.</i> , 2002b
3',4'-Methylenedioxy-6,7-dimethoxy flavone (Milletenin C) [45]	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a
3',4'-Methylenedioxy-7-methoxy flavone [46]	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a
Milletenone [47]	Chalcone	Stem bark	Sritularak <i>et al.</i> , 2002a

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
Millettocalyxin A [48]	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a
Millettocalyxin B [49]	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a
Millettocalyxin C [50]	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a
Ovalifolin [51]	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a
Ovalitenone [52]	Dibenzoyl- methane	Root	Sritularak <i>et al.</i> , 2002b
Pongaglabol [53]	Flavone	Root	Sritularak <i>et al.</i> , 2002b
Pongaglabrone [54]	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a
Pongamol [55]	Chalcone	Root	Sritularak <i>et al.</i> , 2002b
Ponganone I [56]	Chalcone	Stem bark	Sritularak <i>et al.</i> , 2002a
Ponganone V [57]	Flavanone	Root	Sritularak <i>et al.</i> , 2002b
Pongal methyl ether [58]	Flavone	Stem bark	Sritularak <i>et al.</i> , 2002a
Prunetin [59]	Isoflavone	Stem bark	Sritularak <i>et al.</i> , 2002a
Purpurenone [60]	Chalcone	Root	Sritularak <i>et al.</i> , 2002b
<i>M. ferruginea</i> subsp. <i>darassana</i>			
Barbigerone [61]	Isoflavone	Seed	Dagne and Bekele, 1990
Calopogonium isoflavone A[19]	Isoflavone	Seed	Dagne and Bekele, 1990
Durmillone [24]	Isoflavone	Seed pod	Dagne, Bekele and Waterman, 1989
Ferrugone [25]	Isoflavone	Seed	Dagne and Bekele, 1990
		Stem bark	Dagne, Bekele and Waterman, 1989
Flemichapparin B [62]	Pterocarpene	Seed	Dagne and Bekele, 1990
		Stem bark	Dagne, Bekele and Waterman, 1989
7-Hydroxy-5,6-dimethoxy-3',4'- methylenedioxyisoflavone [63]	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
Ichthynone [64]	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989
Jamaicin [30]	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989
5-Methoxydurmillone [18]	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989
Predurmillone [65]	Isoflavone	Seed	Dagne and Bekele, 1990
Prefurrugone [66]	Isoflavone	Seed	Dagne and Bekele, 1990
<i>M. ferruginea</i> subsp. <i>ferruginea</i>			
Barbigerone [61]	Isoflavone	Seed	Dagne and Bekele, 1990
Calopogonium isoflavone A[19]	Isoflavone	Seed	Dagne and Bekele, 1990
Calopogonium isoflavone B[67]	Isoflavone	Stem bark	Dagne and Bekele, 1990
Durmillone [24]	Isoflavone	Seed	Dagne and Bekele, 1990
Ferrugone [25]	Isoflavone	Seed	Dagne and Bekele, 1990
		Root bark	Dagne <i>et al.</i> , 1990
7- <i>O</i> -Geranylformononetin [68]	Isoflavone	Root bark	Dagne <i>et al.</i> , 1990
4'- <i>O</i> -Geranylisoliquiritigenin [69]	Chalcone	Root bark	Dagne <i>et al.</i> , 1990
4'-Hydroxyisolonchocarpin [70]	Flavanone	Stem bark	Dagne, Bekele and Waterman, 1989
4-Hydroxy lonchocarpin [28]	Chalcone	Stem bark	Dagne, Bekele and Waterman, 1989
Isojamaicin [71]	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989
Jamaicin [30]	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989
		Root bark	Dagne <i>et al.</i> , 1990
5-Methoxydurmillone [18]	Isoflavone	Stem bark	Dagne, Bekele and Waterman, 1989
		Root bark	Dagne <i>et al.</i> , 1990
Nordurlettone [72]	Isoflavone	Seed	Dagne <i>et al.</i> , 1990

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
Prebarbigerone [73]	Isoflavone	Seed	Dagne and Bekele, 1990
Pre-5-methoxydurmillone [74]	Isoflavone	Root bark	Dagne and Bekele, 1990
<i>M. griffoniana</i>			
Calopogonium isoflavone B [67]	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997
3',4'-Dihydroxy-7-O-[(E)-3,7-dimethyl-2,6-octadienyl]isoflavone [75]	Isoflavone	Root bark	Yankep <i>et al.</i> , 1998
7,2'-Dimethoxy-4',5'-methylene dioxyisoflavone [21]	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997
Durmillone [24]	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997
7-O-Geranylformononetin [68]	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997
4'-O-Geranylisoliquiritigenin [69]	Chalcone	Root bark	Yankep, Fomum and Dagne, 1997
7-O-Geranylpseudobaptigenin [76]	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997
Griffonianone B [77]	Isoflavone	Root bark	Yankep <i>et al.</i> , 2001
Griffonianone C [78]	Isoflavone	Root bark	Yankep <i>et al.</i> , 2001
7-Hydroxy-6-methoxy-3',4'-methylenedioxyisoflavone [17]	Isoflavone	Root bark	Yankep <i>et al.</i> , 2001
Jamaicin [30]	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997
Maximaisoflavone G [79]	Isoflavone	Root bark	Yankep <i>et al.</i> , 2001
4'-Methoxy-7-O-[(E)-3-methyl-7-hydroxymethyl-2,6-octadienyl]isoflavone [80]	Isoflavone	Root bark	Yankep <i>et al.</i> , 1998
Odorantin [81]	Isoflavone	Root bark	Yankep, Fomum and Dagne, 1997

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
<i>M. hemsleyana</i>			
Dihydroisomilletinone methyl ether [82]	Chalcone	Stem bark	Mahmoud and Waterman, 1985
Dihydromilletinone methyl ether [83]	Chalcone	Stem bark	Mahmoud and Waterman, 1985
Lanceolatin B [42]	Flavone	Stem bark	Mahmoud and Waterman, 1985
3',4'-Methylenedioxy-7-methoxy flavone [46]	Flavone	Stem bark	Mahmoud and Waterman, 1985
Milletinone [47]	Chalcone	Stem bark	Mahmoud and Waterman, 1985
Pongaflavone [84]	Flavone	Stem bark	Mahmoud and Waterman, 1985
<i>M. ichthyochtona</i>			
3,6-Dimethoxyfurano[4'',5'':8,7] flavone [85]	Flavone	Leaf	Kamperdick <i>et al.</i> ,1998
Jamaicin [30]	Isoflavone	Leaf	Kamperdick <i>et al.</i> ,1998
2',4',5'-Trimethoxy-2'',2''-dimethyl pyrano[5'',6'':6,7]isoflavone [86]	Isoflavone	Leaf	Kamperdick <i>et al.</i> ,1998
<i>M. laurentii</i>			
Calycosin [87]	Isoflavone	Wood	Kamnaing <i>et al.</i> , 1999
Glyricidin [88]	Isoflavone	Wood	Kamnaing <i>et al.</i> , 1999
Laurentinol [89]	Flavonol	Wood	Kamnaing <i>et al.</i> , 1999
Laurentiquinone [90]	Isoflavan	Wood	Kamnaing <i>et al.</i> , 1999
<i>M. leucantha</i>			
Desmethoxykanugin [91]	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
Dihydromilletinone methyl ether [83]	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
2',4'-Dimethoxy-3,4-methylene-dioxychalcone [92]	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
2'-Hydroxy-3,4,4',6'-tetramethoxy chalcone [93]	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
Karanjin [94]	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
Lanceolatin B [42]	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
3',4'-methylenedioxy-7-methoxy-flavone [46]	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
3',4'-methylenedioxy-5,7-methoxy-flavone [95]	Flavone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
2,4,6, $\beta$ -tetramethoxy-3',4'-methylenedioxychalcone [96]	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
2',4',6'-trimethoxy-3,4-methylene-dioxychalcone [97]	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
2',4',6'-trimethoxy-3,4-methylene-dioxydihydrochalcone [98]	Chalcone	Stem bark	Phrutivorapongkul <i>et al.</i> , 2003
<i>M. ovalifolia</i>			
1-(4-Hydroxy-5-benzofuranyl)-3-phenyl-2-propen-1-one [40]	Chalcone	Root	Saxena <i>et al.</i> , 1987
7-Hydroxy-6,8-di-C-prenyl flavanone (Ovaliflavanone A) [99]	Flavanone	Seed	Gupta and Krishnamurti, 1976a
7-Hydroxy-8-C-prenylflavanone (Ovaliflavanone B) [100]	Flavanone	Seed	Gupta and Krishnamurti, 1976a
Kanjone [101]	Flavone	Seed	Gupta and Krishnamurti, 1976a
Karanjin [94]	Flavone	Seed	Gupta and Krishnamurti, 1976a
Lanceolatin B [42]	Flavone	Seed	Gupta and Krishnamurti, 1976a
Milletein A [102]	Flavanone	Leaf	Khan and Zaman, 1974

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
Milleteenin B [103]	Flavanone	Leaf	Khan and Zaman, 1974
Milleteenin C [45]	Flavone	Leaf	Khan and Zaman, 1974
Milleteenone [47]	Chalcone	Leaf	Khan and Zaman, 1974
Ovalichalcone [104]	Chalcone	Seed	Gupta and Krishnamurti, 1977a
Ovalichromene [105]	Flavanone	Seed	Gupta and Krishnamurti, 1976b
Ovalichromene A [106]	Flavanone	Seed	Gupta and Krishnamurti, 1976c
Ovalichromene B [107]	Flavanone	Seed	Gupta and Krishnamurti, 1976c
Ovaliflavanone C [108]	Flavanone	Seed	Islam, Gupta and Krishnamurti, 1980
Ovaliflavanone D [109]	Flavanone	Seed	Islam, Gupta and Krishnamurti, 1980
Ovalifolin [51]	Flavone	Leaf	Khan and Zaman, 1974
Ovalitenin A [110]	Chalcone	Seed	Gupta and Krishnamurti, 1977b
Ovalitenin B [111]	Chalcone	Seed	Gupta and Krishnamurti, 1976c
Ovalitenin C [112]	Chalcone	Seed	Islam, Gupta and Krishnamurti, 1980
Ovalitenone [52]	Dibenzoyl-methane	Seed	Gupta and Krishnamurti, 1977b
Pongachalcone [113]	Chalcone	Seed	Gupta and Krishnamurti, 1976c
Pongaglabrone [54]	Flavone	Seed	Gupta and Krishnamurti, 1976a
Pongamol [55]	Chalcone	Seed	Gupta and Krishnamurti, 1976b
Pongapin [114]	Flavone	Seed	Gupta and Krishnamurti, 1976b
<i>M. pachycarpa</i>			
Chalcone [115]	Chalcone	Seed	Singhal <i>et al.</i> , 1983
5-Hydroxy-4'-methoxy-6",6"-dimethylpyrano(2",3":7,8) isoflavone [116]	Isoflavone	Seed	Singhal <i>et al.</i> , 1983
Isoflavone [117]	Isoflavone	Leaf	Singhal <i>et al.</i> , 1981
Isoflavone [118]	Isoflavone	Leaf	Singhal <i>et al.</i> , 1981
Isoflavone [119]	Isoflavone	Leaf	Singhal <i>et al.</i> , 1982
Isoflavone [120]	Isoflavone	Leaf	Singhal <i>et al.</i> , 1982

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
Isoflavone [121]	Isoflavone	Seed	Singhal <i>et al.</i> , 1983
Isolonchocarpin [122]	Flavanone	Root	Shao <i>et al.</i> , 2001a
Lupinifolol [123]	Flavanonol	Aerial part	Singhal <i>et al.</i> , 1980
5-Methoxykaranjin [124]	Flavone	Root	Lu <i>et al.</i> , 1999
Pachycarin A [125]	Flavone	Root	Chen <i>et al.</i> , 1999
Pachycarin B [126]	Flavone	Root	Chen <i>et al.</i> , 1999
Pachycarin C [127]	Flavone	Root	Shao <i>et al.</i> , 2001b
Pachycarin D [128]	Flavone	Root	Shao <i>et al.</i> , 2001b
Pachycarin E [129]	Flavone	Root	Shao <i>et al.</i> , 2001b
Pinnatin [130]	Flavone	Root	Shao <i>et al.</i> , 2001a
Pomiferin [131]	Isoflavone	Seed	Singhal <i>et al.</i> , 1983
Pongaflavone [84]	Flavone	Root	Shao <i>et al.</i> , 2001a
5,7,3',4'-Tetrahydroxy-6,8-diprenyl isoflavone [132]	Isoflavone	Aerial part Seed	Singhal <i>et al.</i> , 1980 Singhal <i>et al.</i> , 1983
5,7,4'-Trihydroxy-6,8-diprenyl isoflavone [133]	Isoflavone	Aerial part Seed	Singhal <i>et al.</i> , 1980 Singhal <i>et al.</i> , 1983
5,7,4'-Trihydroxy-6,3'-diprenyl isoflavone [134]	Isoflavone	Aerial part	Singhal <i>et al.</i> , 1980
<i>M. peguensis</i>			
Kanjone [101]	Flavone	Stem bark	Ganapaty <i>et al.</i> , 1998
Lanceolatin B [42]	Flavone	Stem bark	Ganapaty <i>et al.</i> , 1998
Milletenone [47]	Chalcone	Stem bark	Ganapaty <i>et al.</i> , 1998
Ovaliflavanone A [99]	Flavanone	Stem bark	Ganapaty <i>et al.</i> , 1998
Ovalitenone [52]	Dibenzoyl-methane	Stem bark	Ganapaty <i>et al.</i> , 1998
Pongaglabol [53]	Flavone	Leaf	Ganapaty <i>et al.</i> , 1998
Pongamol [55]	Chalcone	Leaf	Ganapaty <i>et al.</i> , 1998



**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
<i>M. pendura</i>			
Clausequinone [135]	Isoflavan	Heart wood	Hayashi <i>et al.</i> , 1978
Equol [136]	Isoflavan	Heart wood	Hayashi <i>et al.</i> , 1978
(-)-Maackiain [137]	Pterocarpan	Heart wood	Hayashi <i>et al.</i> , 1978
Pendulone [138]	Isoflavan	Heart wood	Hayashi <i>et al.</i> , 1978
<i>M. pervilleana</i>			
3'- <i>O</i> -Demethylpervilleanone [139]	Isoflavone	Root bark	Galeffi <i>et al.</i> , 1997
Emoroidocarpan [140]	Pterocarpan	Root bark	Palazzino <i>et al.</i> , 2003
Pervilleanone [141]	Isoflavanone	Root bark	Galeffi <i>et al.</i> , 1997
Pervilline [142]	Pterocarpan	Root bark	Palazzino <i>et al.</i> , 2003
Pervillinine [143]	Pterocarpan	Root bark	Palazzino <i>et al.</i> , 2003
<i>M. pulchra</i>			
7,4'-Dihydroxy-8,3',5'-triprenyl dihydroflavanol [144]	Flavanonol	Aerial part	Baruah <i>et al.</i> , 1984
(-)-Maackiain [137]	Pterocarpan	Aerial part	Baruah <i>et al.</i> , 1984
6 $\alpha$ -Methoxyhomopterocarpan [145]	Pterocarpan	Aerial part	Baruah <i>et al.</i> , 1984
6 $\alpha$ -Methoxypterocarpan [146]	Pterocarpan	Aerial part	Baruah <i>et al.</i> , 1984
5,7,2',4'-Tetrahydroxy-6,3'-diprenylisoflavone [147]	Isoflavone	Aerial part	Baruah <i>et al.</i> , 1984
5,7,4'-Trihydroxy-2'-methoxy-6,3'-diprenylisoflavone [148]	Isoflavone	Aerial part	Baruah <i>et al.</i> , 1984
5,7,4'-Trihydroxy-8,3',5'-triprenyl flavanone [149]	Flavanone	Aerial part	Baruah <i>et al.</i> , 1984
(-)-Pterocarpan [150]	Pterocarpan	Aerial part	Baruah <i>et al.</i> , 1984
(-)-Sophoranone [151]	Flavanone	Aerial part	Baruah <i>et al.</i> , 1984
<i>M. racemosa</i>			
(+)-Cyclomillinol [152]	Isoflavan	Stem	Kumar, Krupadanam and Srimannarayana, 1989
Demethylvestitol [153]	Isoflavan	Stem	Rao, Prashant and Krupadanam, 1996

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
(-)-Isomillinol B [154]	Isoflavan	Stem	Rao and Krupadanam, 1994
Laxifloran [155]	Isoflavan	Stem	Rao and Krupadanam, 1994
(+)-Millinol [156]	Isoflavan	Stem	Kumar, Krupadanam and Srimannarayana, 1989
(+)-Millinol B [157]	Isoflavan	Stem	Kumar, Krupadanam and Srimannarayana, 1989
Millinolol [158]	Isoflavan	Stem	Rao, Prashant and Krupadanam, 1996
Neomillinol [159]	Isoflavan	Stem	Rao, Prashant and Krupadanam, 1996
Neovestitol [160]	Isoflavan	Stem	Rao, Prashant and Krupadanam, 1996
Vestitol [161]	Isoflavan	Stem	Rao and Krupadanam, 1994
<i>M. rubiginosa</i> Durmillone [24]	Isoflavone	Root	Desai <i>et al.</i> , 1977
Ichthynone [64]	Isoflavone	Root	Desai <i>et al.</i> , 1977
<i>M. sanagana</i> Kanjone [101]	Flavone	Root bark	Mbafor <i>et al.</i> , 1995
Lanceolatin B [42]	Flavone	Root bark	Mbafor <i>et al.</i> , 1995
5-Methoxyfurano[7,8:4",5"]flavone [124]	Flavone	Root bark	Mbafor <i>et al.</i> , 1995
Pongamol [55]	Chalcone	Root bark	Mbafor <i>et al.</i> , 1995
Sanaganone [162]	Flavone	Root bark	Mbafor <i>et al.</i> , 1995

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
<i>M. thonningii</i>			
Alpinumisoflavone [163]	Isoflavone	Seed	Olivares <i>et al.</i> , 1982
3',5-Dihydroxy-4'-methoxy-2'',2''-dimethylpyrano-(5'',6'':6,7) isoflavone [164]	Isoflavone	Seed	Olivares <i>et al.</i> , 1982
Dimethylalpinumisoflavone [165]	Isoflavone	Seed	Olivares <i>et al.</i> , 1982
		Root bark	Asomaning <i>et al.</i> , 1995
		Root, pod	Asomaning <i>et al.</i> , 1999
5- <i>O</i> -Methylalpinumisoflavone [166]	Isoflavone	Root	Asomaning <i>et al.</i> , 1999
4'-Methylalpinumisoflavone [167]	Isoflavone	Seed	Olivares <i>et al.</i> , 1982
5- <i>O</i> -Methyl-4'- <i>O</i> -(3-methyl-2-butenyl) –alpinumisoflavone [168]	Isoflavone	Root bark	Asomaning <i>et al.</i> , 1995
		Root	Asomaning <i>et al.</i> , 1999
Robustone [169]	Isoflavone	Seed	Khalid and Waterman, 1983
Thonninginisoflavone [170]	Isoflavone	Root bark	Asomaning <i>et al.</i> , 1995
<i>M. uraramensis</i> subsp. <i>usaramensis</i>			
Barbigerone [61]	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1998
4'- <i>O</i> -Geranylisoliquiritigenin [69]	Chalcone	Stem bark	Yenesew, Midiwo and Waterman, 1998
Isoliquiritigenin [171]	Chalcone	Stem bark	Yenesew, Midiwo and Waterman, 1998
Jamaicin [30]	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1998
Maximaisoflavone G [79]	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1998
Norisojamaicin [172]	Isoflavone	Stem bark	Yenesew, Midiwo and Waterman, 1998
$\alpha$ ,4,2''-Trihydroxy-4- <i>O</i> -geranyl dihydrochalcone [173]	Chalcone	Stem bark	Yenesew, Midiwo and Waterman, 1998

**Table 1 (continued)**

Plant and compound	Category	Plant part	Reference
<i>M. zechiana</i>			
Cyanidin-3,5-diglucoside [174]	Glycoside	Flower	Parvez and Ogbeide, 1990
3-Hydroxy-4'-methoxyflavone [175]	Flavonol	Flower	Parvez and Ogbeide, 1990
8-Hydroxyquercetin 7-glucoside [176]	Glycoside	Flower	Parvez and Ogbeide, 1990
Kaempferol 3-glucoside [177]	Glycoside	Flower	Parvez and Ogbeide, 1990
Kaempferol 3-rhamnoside [178]	Glycoside	Flower	Parvez and Ogbeide, 1990
Malvidin 3,5-diglucoside [179]	Glycoside	Flower	Parvez and Ogbeide, 1990
Pelargonidin 3-rhamnoside [180]	Glycoside	Flower	Parvez and Ogbeide, 1990
Quercetin 3-glucoside [181]	Glycoside	Flower	Parvez and Ogbeide, 1990

**Table 2 Distribution of miscellaneous compounds in *Millettia* species.**

Plant and compound	Category	Plant part	Reference
<i>M. auriculata</i> (-)-Sumatrol [182]	Rotenoid	Root Seed	Shabbir <i>et al.</i> , 1968 Rao, Prasad and Ganapaty, 1992
<i>M. dura</i> 6a, 12a-Dehydrodeguelin [183]	Rotenoid	Seed	Ollis, Rhodes and Sutherland, 1967
Deguelin [184]	Rotenoid	Seed	Dagne, Mammo and Bekele, 1991
(-)-Millettone [185]	Rotenoid	Seed Seed pod	Ollis, Rhodes and Sutherland, 1967 Yenesew, Midiwo and Waterman, 1997
(-)-Millettosin [186]	Rotenoid	Seed	Ollis, Rhodes and Sutherland, 1967
(-)-Rotenone [187]	Rotenoid	Seed	Ollis, Rhodes and Sutherland, 1967
(-)-Tephrosin [188]	Rotenoid	Seed Seed pod	Ollis, Rhodes and Sutherland, 1967 Yenesew, Midiwo and Waterman, 1997
<i>M. ferruginea</i> subsp. <i>darassana</i> (-)-12a-Hydroxyrotenone [189]	Rotenoid	Seed	Dagne and Bekele, 1990
(-)-Tephrosin [188]	Rotenoid	Seed	Dagne and Bekele, 1990
<i>M. ferruginea</i> subsp. <i>Ferruginea</i> (-)-Rotenone [187]	Rotenoid	Seed	Dagne and Bekele, 1990

**Table 2 (continued)**

Plant and compound	Category	Plant part	Reference
<i>M. griffoniana</i> Griffonianone A [190]	Rotenoid	Root	Yankep <i>et al.</i> , 2001
4-Hydroxy-5,6,7-trimethoxy-3-(3',4'-methylenedioxy)phenyl coumarin [191]	Coumarin	Root bark	Yankep <i>et al.</i> , 1998
<i>M. laurentii</i> O-Acetylmillaurine [192]	Alkaloid	Seed	Ngamga <i>et al.</i> , 1993
5a,9a-Dihydro-5a-hydroxy millaurine [193]	Alkaloid	Seed	Ngamga, Free and Fomum, 1994
Millettonine [194]	Alkaloid	Stem bark	Kamnaing <i>et al.</i> , 1994
Millaurine [195]	Alkaloid	Seed	Ngamga <i>et al.</i> , 1993
<i>M. ovalifolia</i> Azulene [196]	Monoterpenoid	Leaf	Nigam <i>et al.</i> , 1982
$\alpha$ -Boneol [197]	Monoterpenoid	Leaf	Nigam <i>et al.</i> , 1982
1,8-Cineol [198]	Monoterpenoid	Leaf	Nigam <i>et al.</i> , 1982
3,4-Dimethoxycinnamic acid [199]	Phenylpropanoid	Seed	Krishnamurti and Islam, 1987
Heptacosanol [200]	Alkane	Seed	Krishnamurti and Islam, 1987
Linelyl acetate [201]	Monoterpenoid	Leaf	Nigam <i>et al.</i> , 1982
Methyl chavicol [202]	Phenylpropanoid	Leaf	Nigam <i>et al.</i> , 1982
Ovalin [203]	Alkaloid	Seed	Gupta and Krishnamurti, 1979
19-Oxo-5 $\alpha$ -carda-14,20(22)-dienolide-3-O- $\beta$ -D-glucopyrano- side [204]	Cardenolide	Root	Bose and Chakraborty, 2000
Pi-cymene [205]	Monoterpenoid	Leaf	Nigam <i>et al.</i> , 1982
$\alpha$ -Pinene [206]	Monoterpenoid	Leaf	Nigam <i>et al.</i> , 1982
$\beta$ -Pinene [207]	Monoterpenoid	Leaf	Nigam <i>et al.</i> , 1982

**Table 2 (continued)**

Plant and compound	Category	Plant part	Reference
$\beta$ -Sitosterol [208]	Steroid	Seed	Gupta and Krishnamurti, 1976b
$\alpha$ -Terpinolene [209]	Monoterpenoid	Leaf	Nigam <i>et al.</i> , 1982
$\alpha$ -Thujene [210]	Monoterpenoid	Leaf	Nigam <i>et al.</i> , 1982
<i>M. pachycarpa</i>			
(-)-12a-Hydroxyrotenone [189]	Rotenoid	Root	Singhal <i>et al.</i> , 1982
(+)-12a-Hydroxyrot-2'-enonic acid [211]	Rotenoid	Root	Singhal <i>et al.</i> , 1982
(-)-Rotenone [187]	Rotenoid	Root	Singhal <i>et al.</i> , 1982
Rot-2'-enonic acid [212]	Rotenoid	Root	Singhal <i>et al.</i> , 1982
<i>M. pendura</i>			
$\beta$ -Amyrin [213]	Triterpenoid	Stem bark	Rathore, Nagar and Gupta, 1983
Daucosterol [214]	Steroid	Seed	Rathore, Nagar and Gupta, 1983
Ellagic acid [215]	Coumarin	Stem bark	Rathore, Nagar and Gupta, 1983
Galactose [216]	Carbohydrate	Stem bark	Rathore, Nagar and Gupta, 1983
Gallic acid [217]	Benzenoid	Stem bark	Rathore, Nagar and Gupta, 1983
$\beta$ -Methylgalactoside [218]	Carbohydrate	Stem bark	Rathore, Nagar and Gupta, 1983
Octacosan-1-ol [219]	Alkane	Stem bark	Rathore, Nagar and Gupta, 1983
Rhamnose [220]	Carbohydrate	Stem bark	Rathore, Nagar and Gupta, 1983
Stigmasterol [221]	Steroid	Stem bark	Rathore, Nagar and Gupta, 1983
$\beta$ -Sitosterol [208]	Steroid	Stem bark	Rathore, Nagar and Gupta, 1983

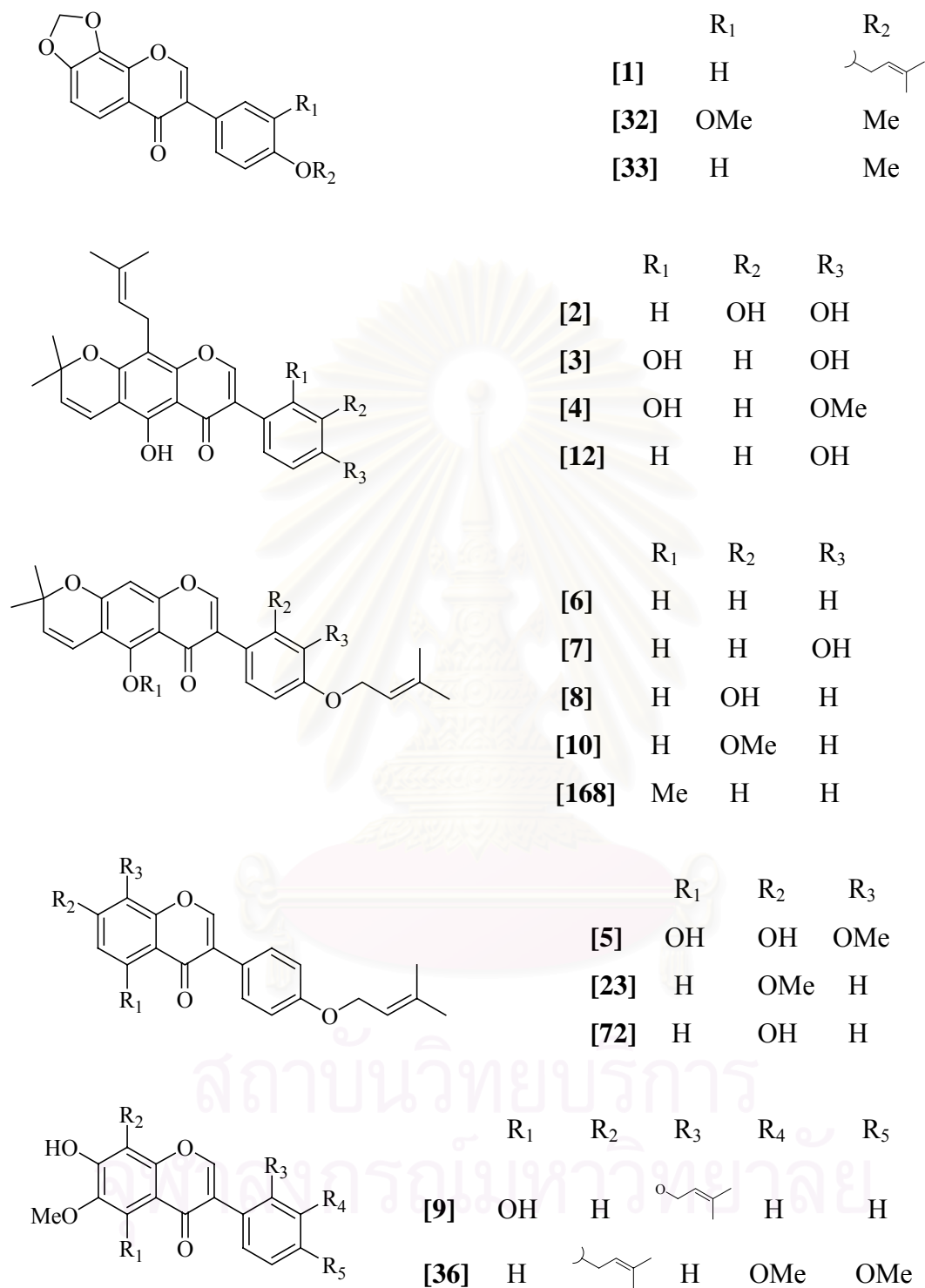
**Table 2 (continued)**

Plant and compound	Category	Plant part	Reference
<i>M. pervilleana</i> 3 $\alpha$ -Hydroxyrotenone [222] Pervilleanine [223] (-)-Rotenone [187]	Rotenoid 3-Arylcoumarin Rotenoid	Root bark Root bark Root bark	Palazzino <i>et al.</i> , 2003 Palazzino <i>et al.</i> , 2003 Palazzino <i>et al.</i> , 2003
<i>M. racemosa</i> $\beta$ -Amyrin [213] Behenic acid [224] $\beta$ -Sitosterol [208] Stigmasterol [221]	Triterpenoid Lipid Steroid Steroid	Stem Stem Stem Root Root	Rao and Krupadanam, 1994 Rao and Krupadanam, 1994 Rao and Krupadanam, 1994 Desai <i>et al.</i> , 1977
<i>M. reticulate</i> 1,8-Cineol [198] Limonene [225] $\alpha$ -Pinene [206] $\beta$ -Pinene [207] $\delta$ -Pinene [226]	Monoterpenoid Monoterpenoid Monoterpenoid Monoterpenoid Monoterpenoid	Flower Flower Flower Flower Flower	Gong and Wu, 1998 Gong and Wu, 1998 Gong and Wu, 1998 Gong and Wu, 1998 Gong and Wu, 1998
<i>M. thonningii</i> $\beta$ -Amyrin [213] Robustic acid [227] Thonningine-A [228] Thonningine-B [229] Thonningine-C [230]	Triterpenoid 3-Aryl coumarin Coumarin Coumarin Coumarin	Root bark Seed Seed Seed Seed	Asomaning <i>et al.</i> , 1995 Olivares <i>et al.</i> , 1982 Khalid and Waterman, 1983 Khalid and Waterman, 1983 Saxena <i>et al.</i> , 1987

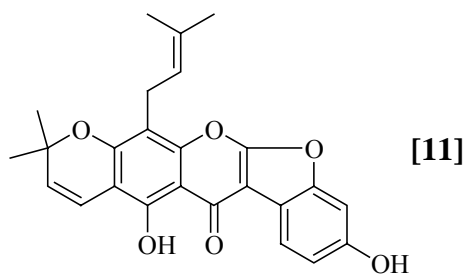


**Table 2 (continued)**

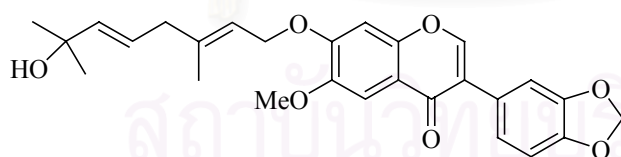
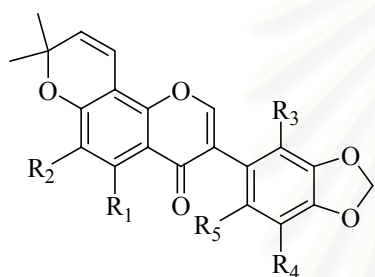
Plant and compound	Category	Plant part	Reference
<i>M. usaramensis</i> subsp. <i>usaramensis</i> (+)-12a-Epimillettosin [231]	Rotenoid	Stem bark	Yenesew, Miwido and Waterman, 1998
(+)-12 $\alpha$ -Hydroxy-12-dihydro usararotenoid A [232]	Rotenoid	Stem bark	Yenesew, Miwido and Waterman, 1998
(+)-Usararotenoid A [233]	Rotenoid	Stem bark	Yenesew, Miwido and Waterman, 1998
(+)-Usararotenoid B [234]	Rotenoid	Stem bark	Yenesew, Miwido and Waterman, 1998
4- <i>O</i> -Geranylcinnamyl acetate [235]	Phenylpropanoid	Stem bark	Yenesew, Miwido and Waterman, 1998



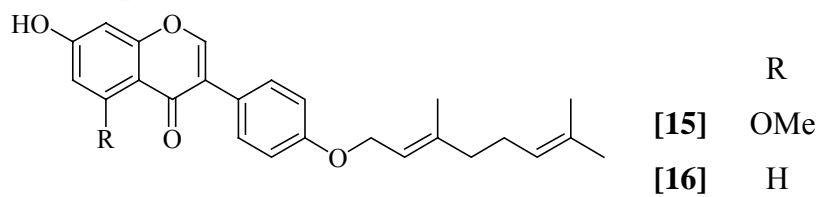
**Figure 2** Structures of isoflavones isolated from *Millettia* species.



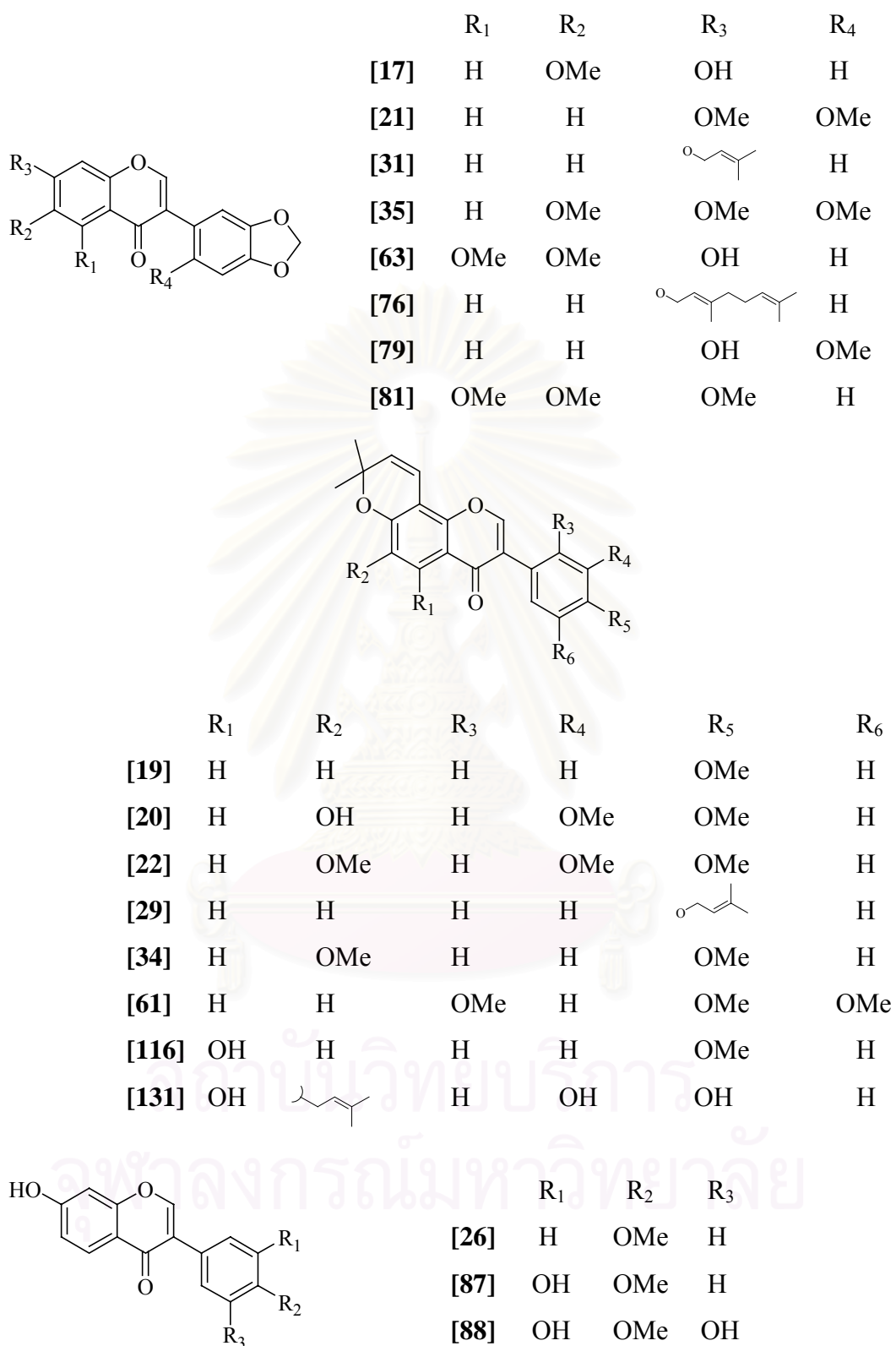
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
[13]	OMe	OMe	H	H	OMe
[18]	OMe	OMe	H	H	H
[24]	H	OMe	H	H	H
[25]	H	H	OMe	OMe	H
[30]	H	H	H	H	OMe
[64]	H	OMe	H	H	OMe
[67]	H	H	H	H	H
[71]	H	H	OMe	OMe	H
[77]	OMe	OH	H	H	H
[172]	H	H	H	OH	H



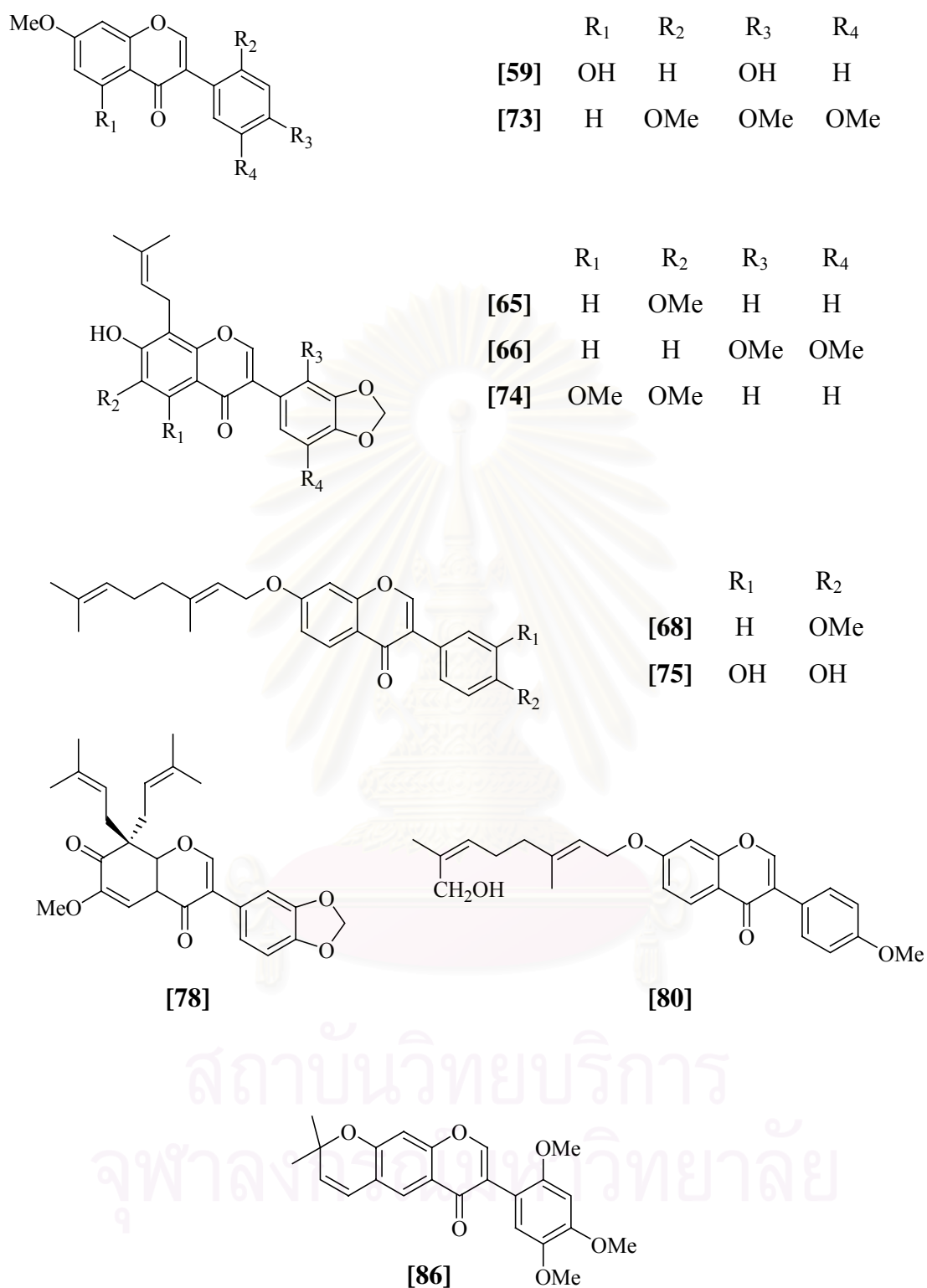
[14]



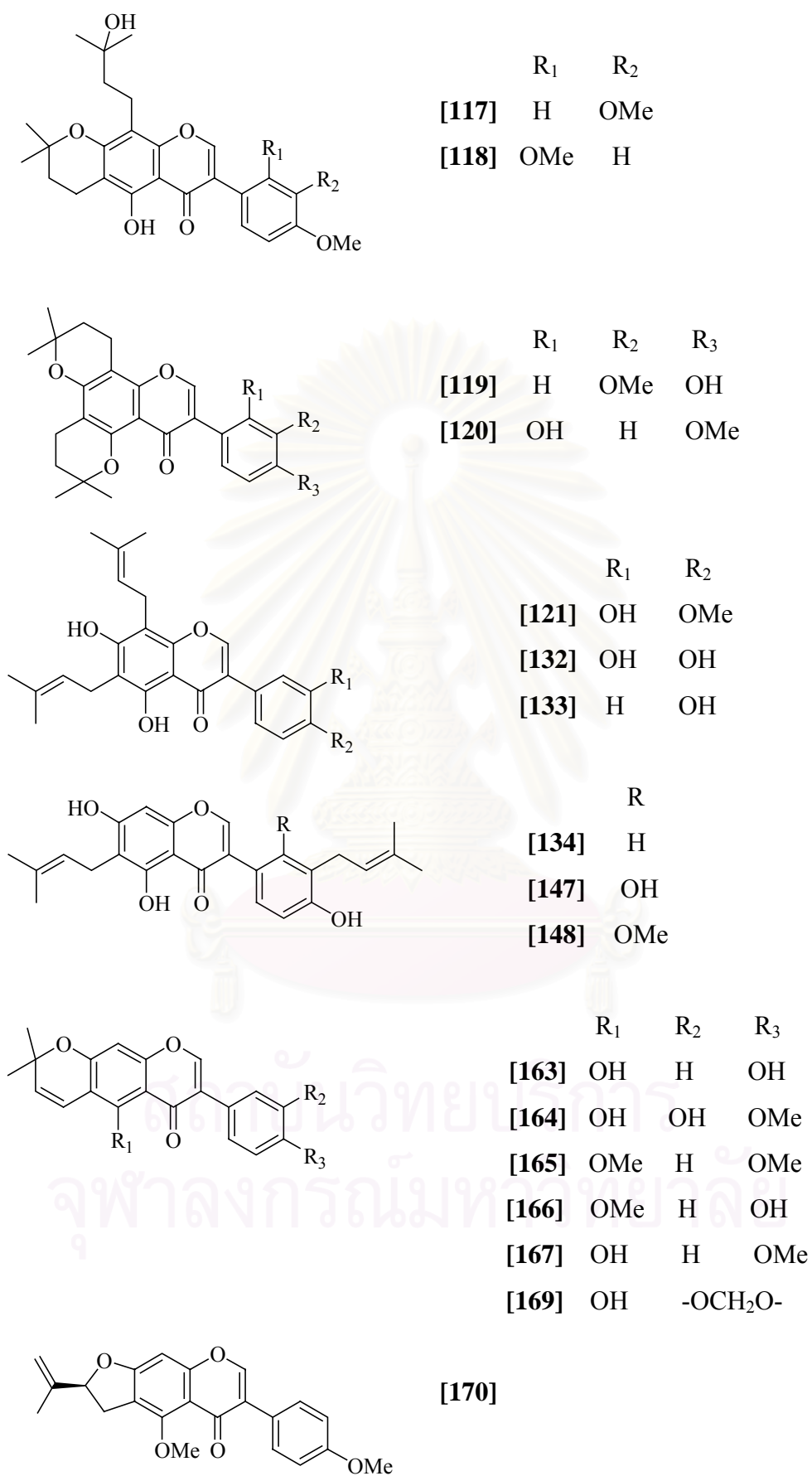
**Figure 2** Structures of isoflavones isolated from *Millettia* species (continued).



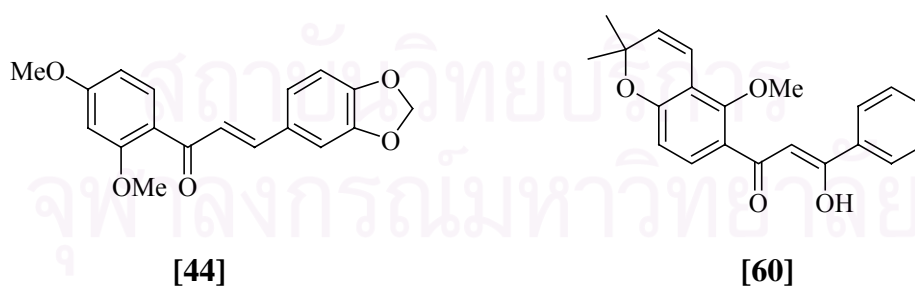
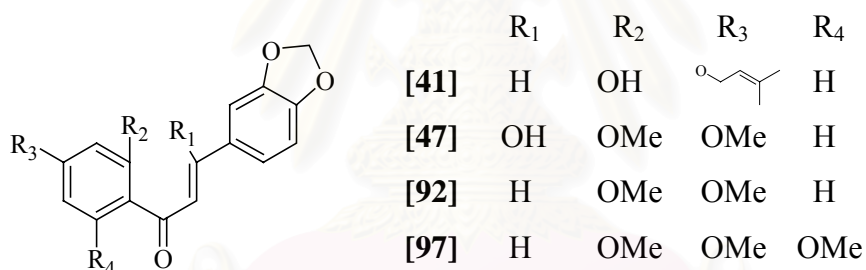
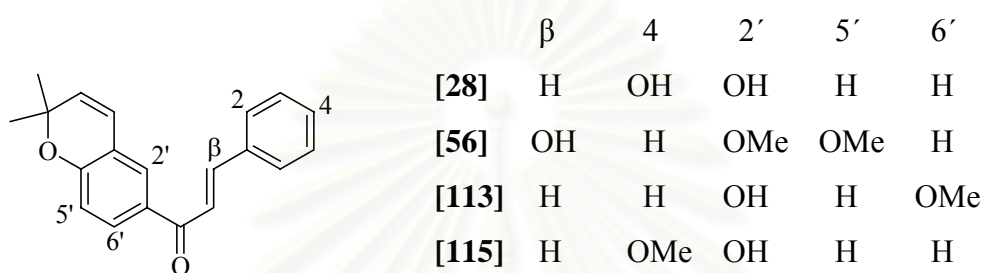
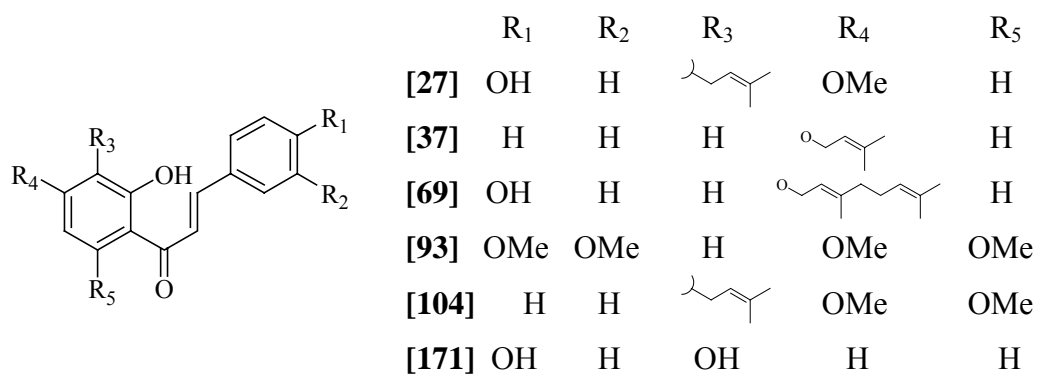
**Figure 2** Structures of isoflavones isolated from *Millettia* species (continued).



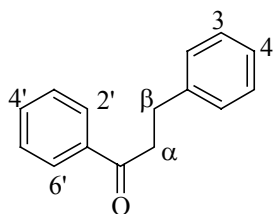
**Figure 2** Structures of isoflavones isolated from *Millettia* species (continued).



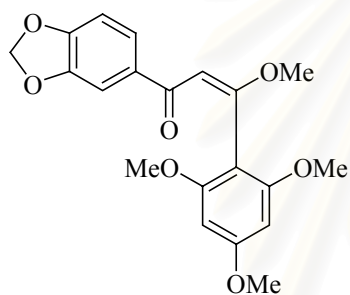
**Figure 2** Structures of isoflavones isolated from *Millettia* species (continued).



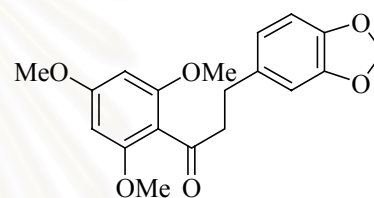
**Figure 3** Structures of chalcones isolated from *Millettia* species.



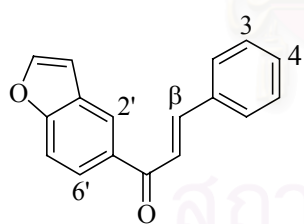
	$\alpha$	$\beta$	2	3	4	2'	3'	4'
[82]	H	OMe	OMe	H	OMe	H	-OCH <sub>2</sub> O-	
[83]	H	OMe	H	-OCH <sub>2</sub> O-		OMe	H	OMe
[173]	OH	H	H	H	OH	OH	H	



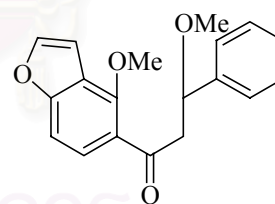
[96]



[98]



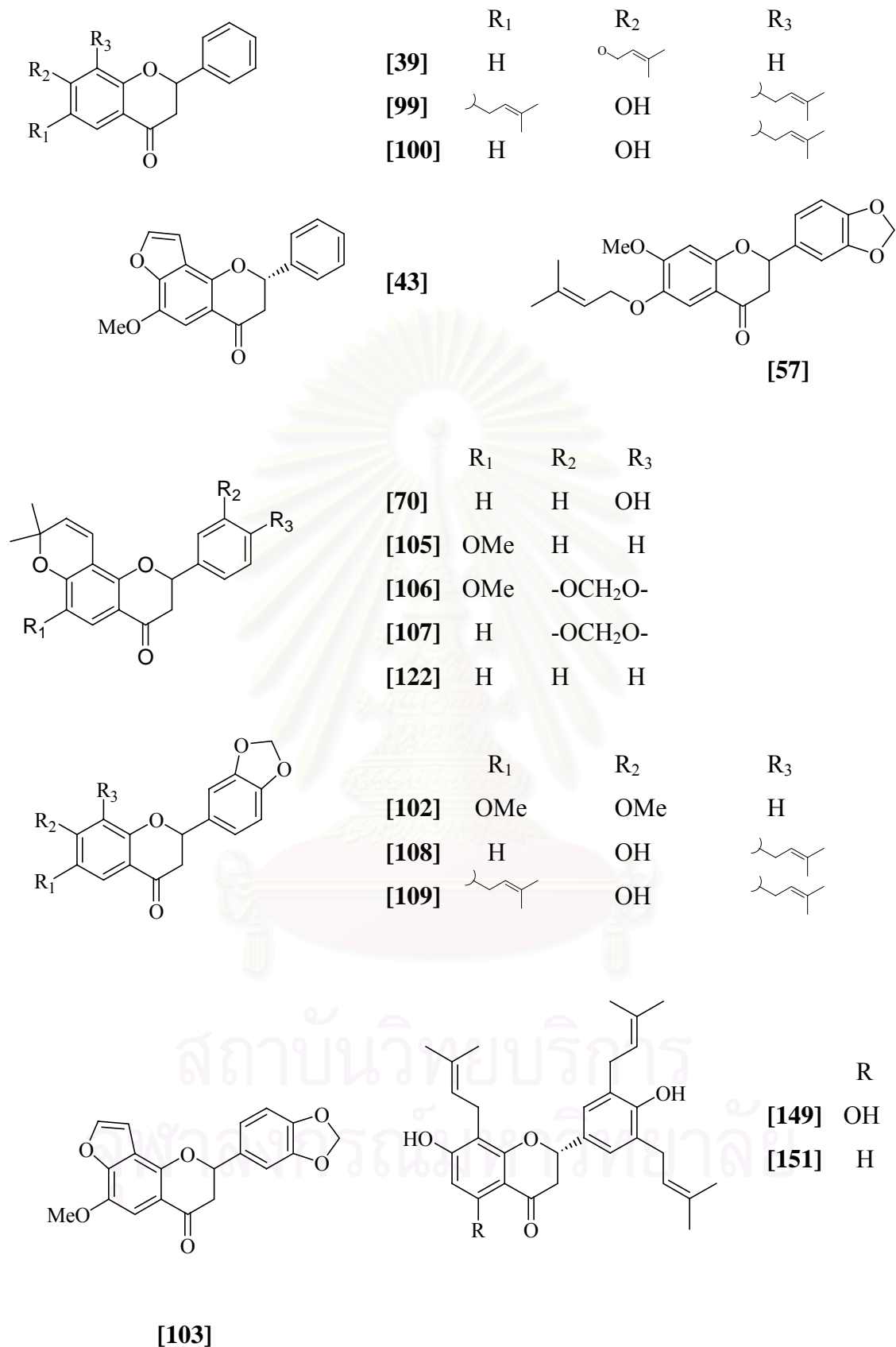
	$\beta$	3	4	2'
[40]	H	H	H	OH
[55]	OH	H	H	OMe
[110]	H	H	H	OMe
[112]	H	-OCH <sub>2</sub> O-		OMe



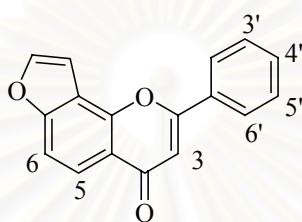
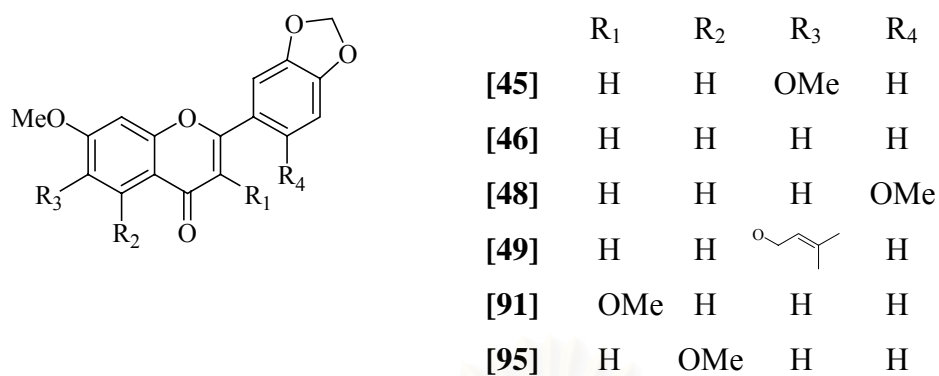
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
**Figure 3** Structures of chalcones isolated from *Millettia* species (continued).

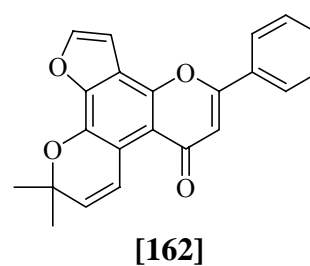
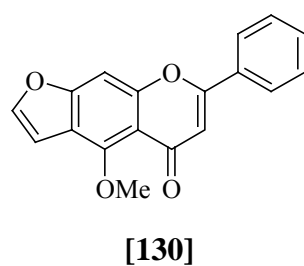
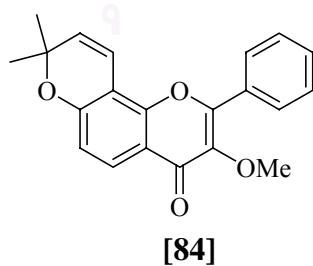




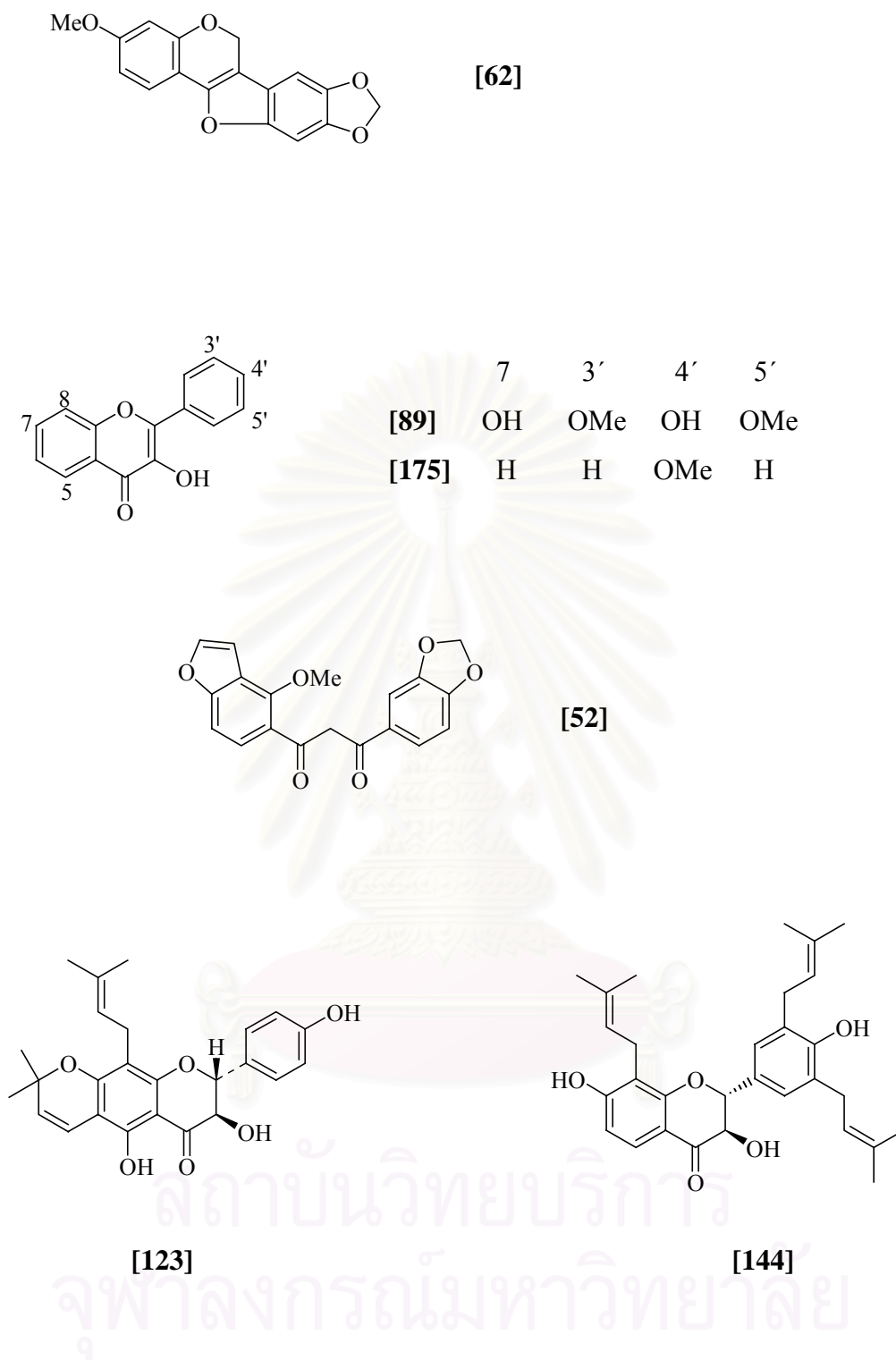
**Figure 4** Structures of flavanones isolated from *Millettia* species



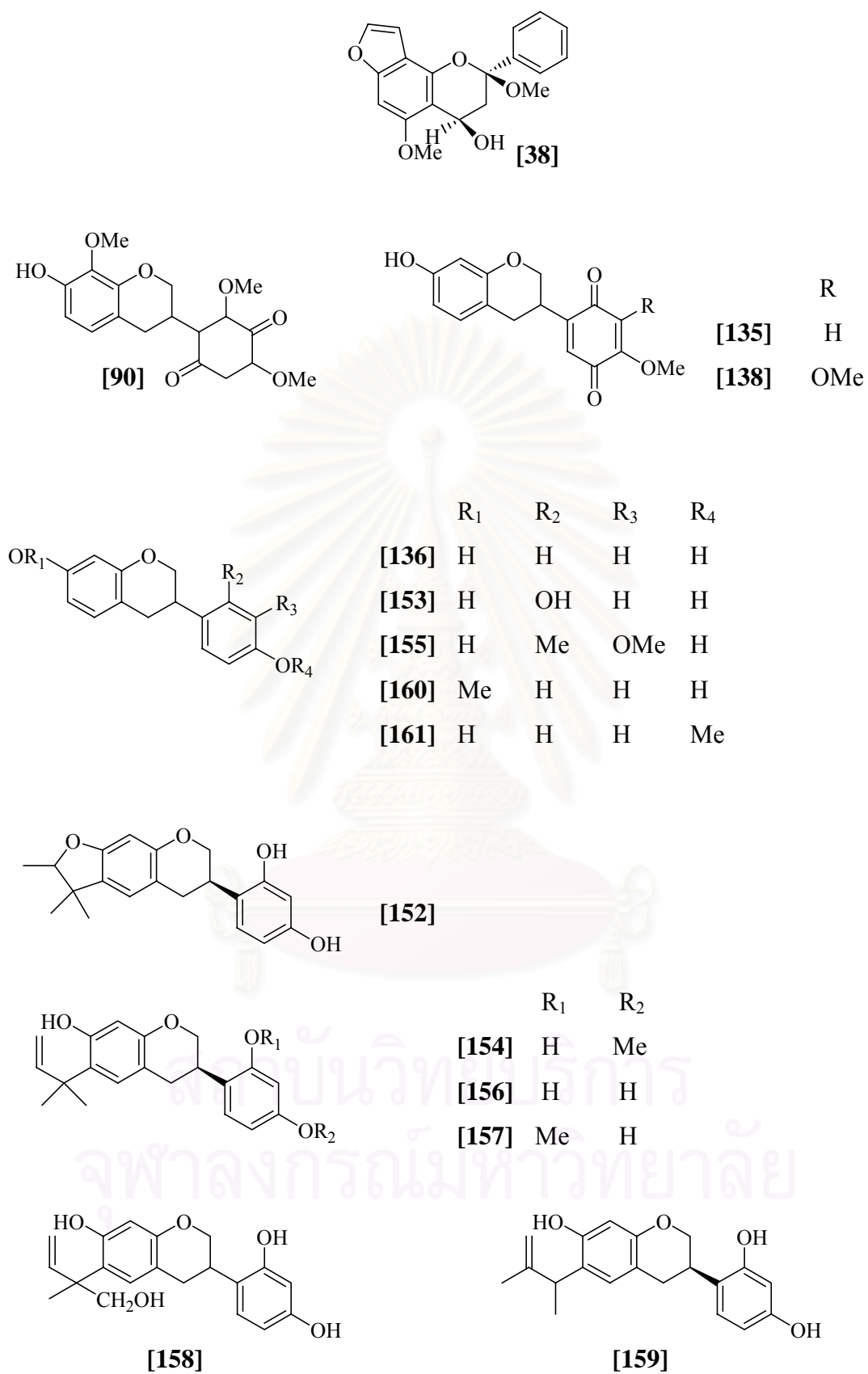
	3	5	6	2'	3'	4'	5'	6'
[42]	H	H	H	H	H	H	H	H
[50]	H	H	H	OMe	H	H	OMe	H
[51]	H	H		H	H	H	H	H
[53]	H	OH	H	H	H	H	H	H
[54]	H	H	H	H	-OCH <sub>2</sub> O-		H	H
[58]	H	H	H	H	OMe	H	H	H
[85]	OMe	H	OMe	H	H	H	H	H
[94]	OMe	H	H	H	H	H	H	H
[101]	H	H	OMe	H	H	H	H	H
[114]	OMe	H	H	H	-OCH <sub>2</sub> O-		H	H
[124]	H	OMe	H	H	H	H	H	H
[125]	OMe	H	H	H	OMe	OMe	H	H
[126]	OMe	H	H	OMe	H	H	H	OMe



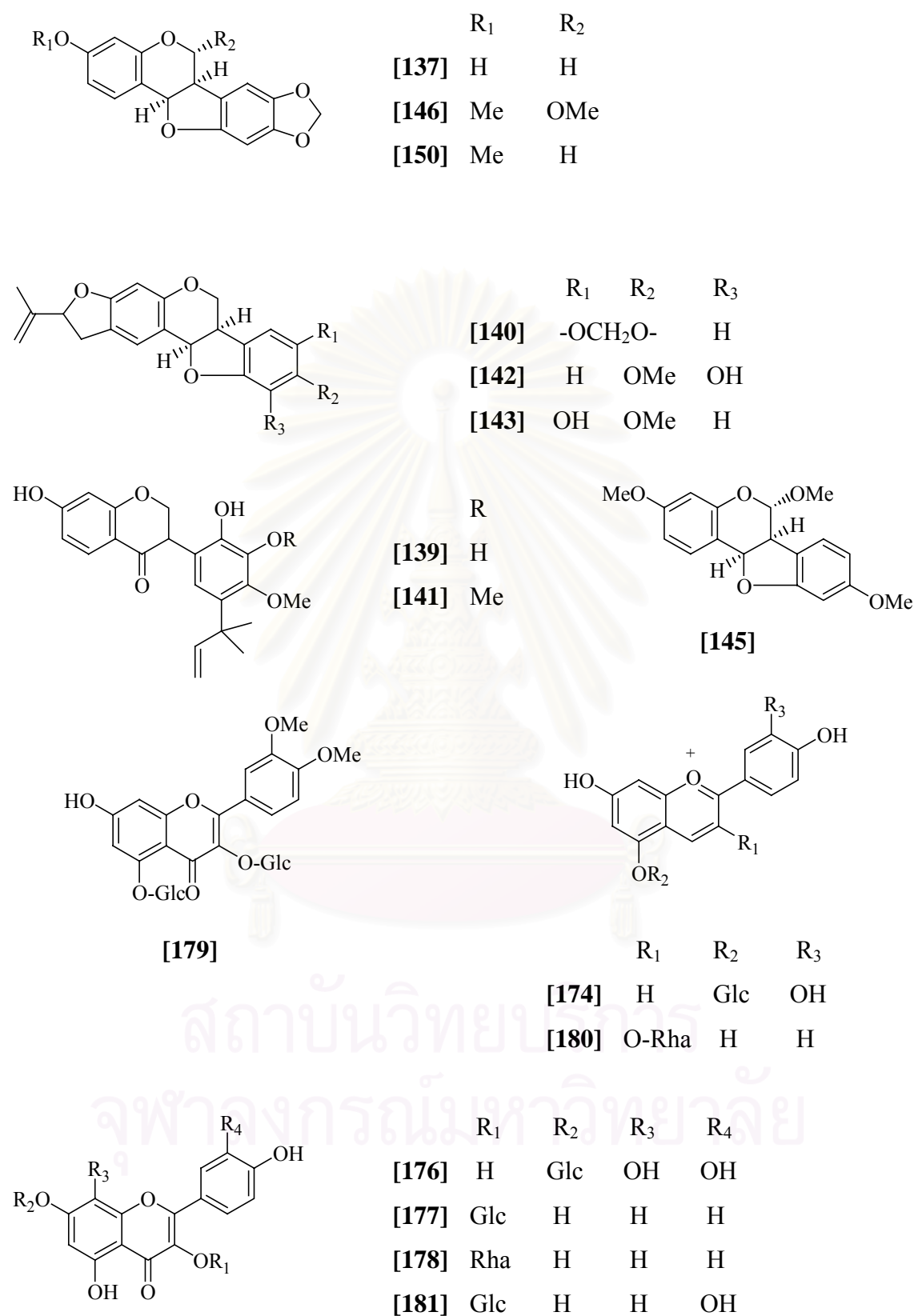
**Figure 5** Structures of flavones isolated from *Millettia* species.



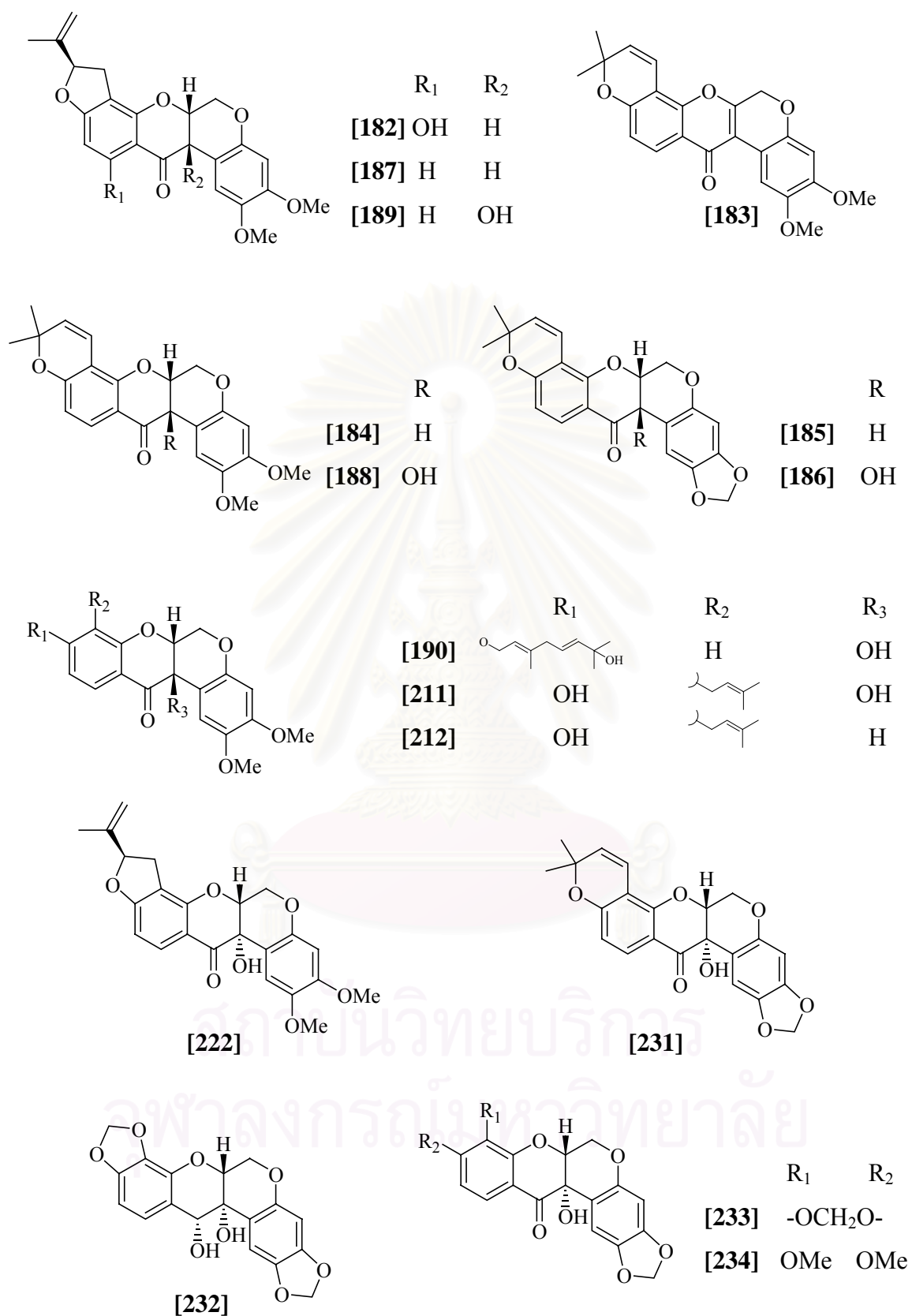
**Figure 6** Structures of pterocarpene (**62**), flavonols (**89**, **175**), dibenzoylmethane (**52**) and flavanonols (**123**, **144**) isolated from *Millettia* species.



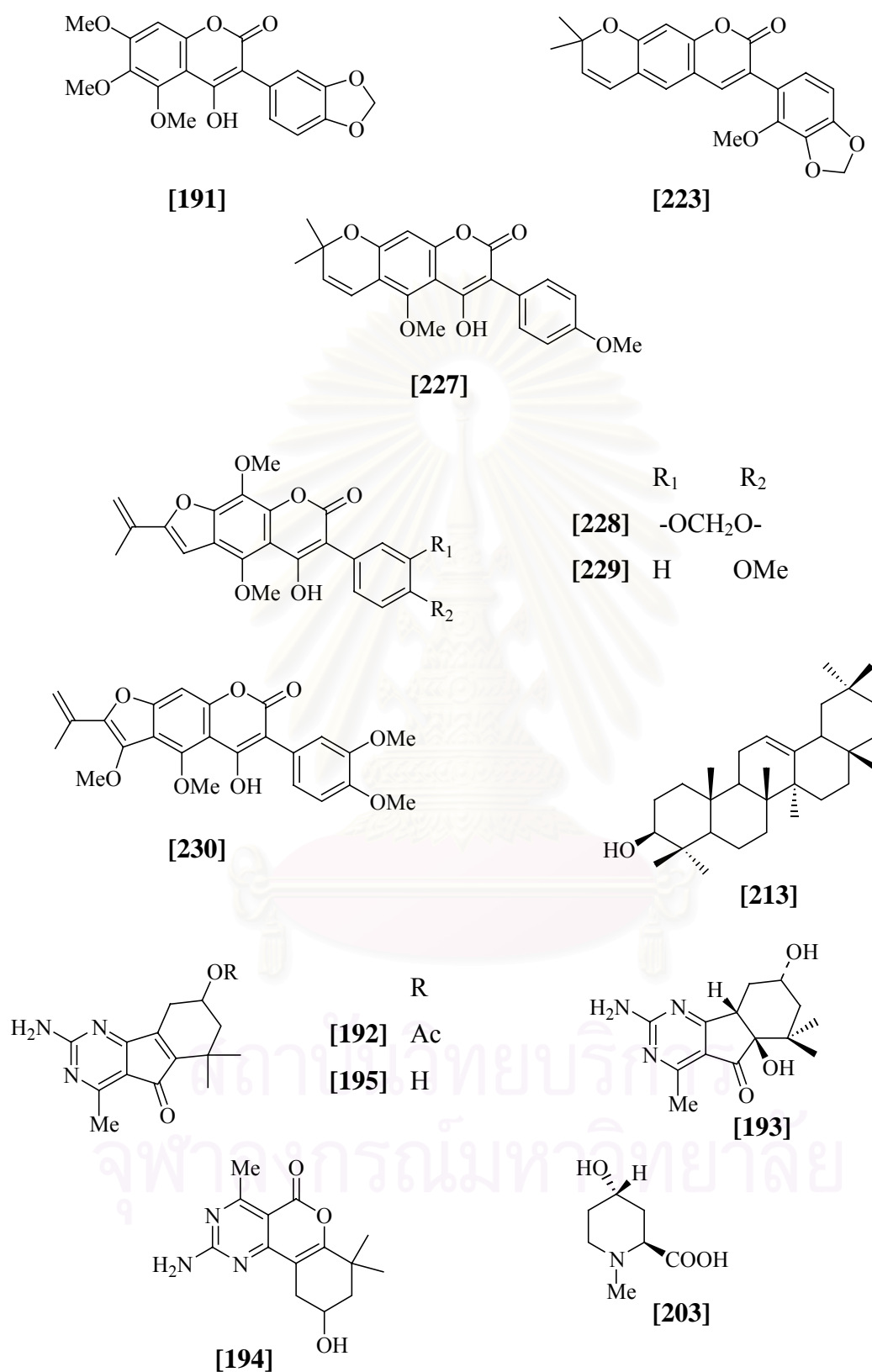
**Figure 7** Structures of flavan (**38**) and isoflavans (**90**, **135-6**, **138**, **152-161**) isolated from *Millettia* species.



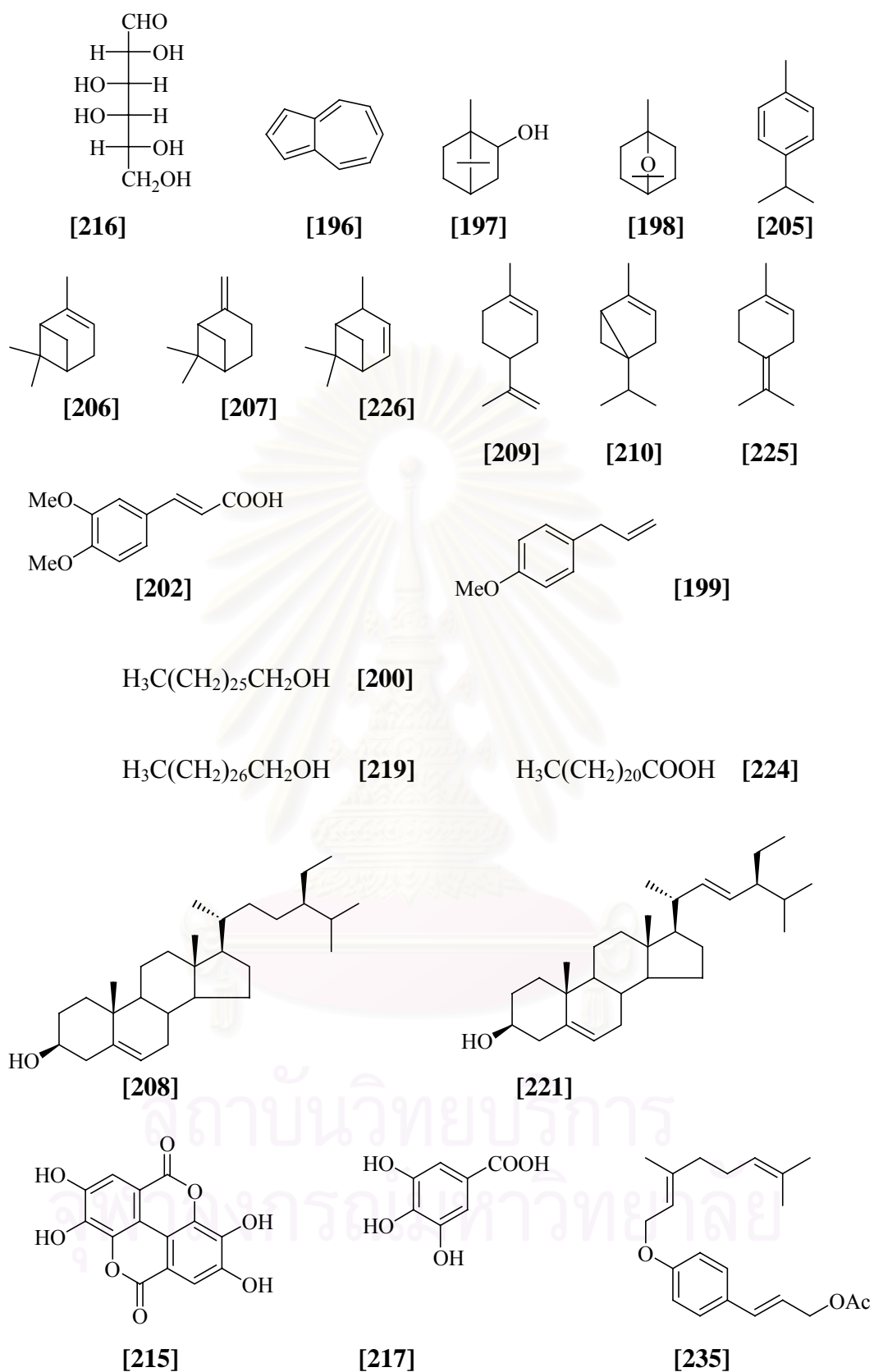
**Figure 8** Structures of pterocarpans (137, 140-3, 145-6, 150), isoflavanones (139, 141) and glycosides (174, 176-181) isolated from *Millettia* species.



**Figure 9** Structures of rotenoids isolated from *Millettia* species



**Figure 10** Structures of 3-arylcoumarins (**191**, **223**, **227-230**), triterpenoid (**213**) and alkaloids (**192-5**, **203**) isolated from *Millettia* species.



**Figure 11** Structures of miscellaneous compounds isolated from *Millettia* species.



## CHAPTER III

### EXPERIMENTAL

#### 1. Sources of Plant Materials

The leaves of *Millettia erythrocalyx* Gagnep. were collected from Tayang district, Petchaburi Province, Thailand, in April 1999. Authentication was performed by comparison with herbarium specimens (BKF No. 130496) at the National Park Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, and voucher specimens (KL-032542) are on deposit at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

#### 2. General Techniques

##### 2.1 Analytical Thin-Layer Chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F <sub>254</sub> (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	6 cm
Temperature	:	Laboratory temperature (30-35°C)
Detection	:	1. Ultraviolet light (254 and 365 nm) 2. Anisaldehyde and heating at 105°C for 10 min.

##### 2.2 Column Chromatography

###### 2.2.1 Vacuum Liquid Column Chromatography

Adsorbent	:	Silica gel 60 (No. 7734) particle size 0.063-0.200 mm (70-230 mesh ASTM) (E. Merck)
Packing method	:	Dry packing
Sample loading	:	The sample was dissolved with an organic solvent, mixed with the adsorbent, triturated, dried and then placed gently on top of the column.
Detection	:	Fractions were examined by TLC observing under UV light (254 and 365 nm).

### 2.2.2 Flash Column Chromatography

Adsorbent	:	Silica gel 60 (No. 9385) particle size 0.400-0.063 nm (230-400 mesh ASTM) (E. Merck)
Packing method	:	Wet packing
Sample loading	:	The sample was dissolved with the eluent and then applied gently on top of the column.
Detection	:	Fractions were examined in the same manner as described in section 2.3.1.

## 2.3 Spectroscopy

### 2.3.1 Ultraviolet (UV) Absorption Spectra

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

### 2.3.2 Infrared (IR) Absorption Spectra

IR film spectra were recorded on a Perkin Elmer FT-IR 1760X spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

### 2.3.3 Mass Spectra

Electrospray ionization mass spectra (ESIMS) and high resolution electrospray ionization mass spectra (HRESIMS) were measured on a mass spectrometer LCT (LC/MS) Micromass (Biotech Central Research Unit, National Center for Genetic Engineering and Biotechnology (NSTDA), Science Park, Pathumthani, Thailand).

### 2.3.4 Proton and Carbon-13 Nuclear Magnetic resonance ( $^1\text{H}$ and $^{13}\text{C}$ -NMR) Spectra

$^1\text{H}$  NMR (300 MHz) and  $^{13}\text{C}$  NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer (Faculty of Pharmaceutical Sciences, Chulalongkorn University)

$^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) spectra were obtained with a Bruker Avance DRX-500 spectrometer (Biotech Central Research Unit, National Center

for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA))

Solvents for NMR spectra were deuterated chloroform (chloroform-*d*) and deuterated acetone (acetone-*d*<sub>6</sub>). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

## **2.4 Physical property**

### **2.4.1 Melting Points**

Melting points were obtained on a Gallenkamp Melting Point Apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

## **2.5 Solvents**

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

## **3. Extraction and Isolation**

### **3.1 Extraction and Isolation of Compounds from the leaves of *Millettia erythrocalyx***

#### **3.1.1 Extraction**

The dried leaves of *Millettia erythrocalyx* (3.5 kg) were macerated with hexane (3 x 20 L), ethyl acetate (3 x 20 L) and ethanol (3 x 23 L) to give a hexane extract (78 g), an ethyl acetate extract (43 g) and an ethanol extract (283 g) after evaporation of the organic solvent.

#### **3.1.2 Isolation**

##### **3.1.2.1 Isolation of Compounds from Hexane Extract**

The hexane extract (78 g) was separated by vacuum liquid column chromatography using a sintered glass filter column of silica gel 60 (No. 7734, 400 g). The eluates were collected 300 ml per fraction. Elution was performed in a polarity gradient manner with mixtures of petroleum ether and ethyl acetate (100:0 to 0:100). Seventy-five fractions were collected. Fractions with similar TLC pattern (silica gel, EtOAc-Pet. Ether 4:1) were combined to give 14 fractions, as shown in Table 3.

**Table 3 Chromatographic fractions of the hexane extract of *M. erythrocalyx* leaves**

Fraction	Combined fractions	Weight (g)
HQ-1	1	1.5
HQ-2	2	0.5
HQ-3	3	1.6
HQ-4	4	0.3
HQ-5	5	0.9
HQ-6	6	6.1
HQ-7	7-8	10.0
HQ-8	9-12	10.3
HQ-9	13-22	12.2
HQ-10	23-30	9.7
HQ-11	31-33	2.6
HQ-12	34-57	7.8
HQ-13	58-66	0.3
HQ-14	67-75	1.2

### 3.1.2.2 Isolation of Compound MEL1 (3',4'-Methylenedioxy-7-methoxyflavone)

Fraction HQ-12 (7.8 g) was separated by vacuum liquid column chromatography using a sinter glass filter column of silica gel 60 (No. 9385, 100 g). Elution was performed in a polarity gradient manner with petroleum ether and ethyl acetate as the eluents (100:0 to 0:100). Fractions with similar TLC patterns (silica gel, Pet. Ether-EtOAc 1: 4) were combined to yield 10 fractions: fractions A (464 mg), B (679 mg), C (1.7 g), D (1.2 g), E (739 mg), F (375 mg), G (1.5 g), H (541 mg), I (160 mg) and J (439 mg).

Fraction D (1.2 g) was subjected to column chromatography with petroleum ether-ethyl acetate gradient elution. Fractions with similar TLC pattern were combined to afford 15 fractions: fractions D1 (18 mg), D2 (27 mg), D3 (29 mg), D4 (179 mg), D5 (292 mg), D6 (71 mg), D7 (27 mg), D8 (23 mg), D9 (16 mg), D10 (16 mg), D11 (21 mg), D12 (21 mg), D13 (95 mg), D14 (2 mg), D15 (11 mg). Fraction D8 (23 mg) was recrystallized from petroleum ether-chloroform to give white needles of compound MEL1 (2 mg,  $R_f$  0.33, silica gel, pet. ether-EtOAc 1:1). This compound was identified as 3',4'-methylenedioxy-7-methoxyflavone.

### 3.1.2.3 Isolation of Compound MEL2 (3',5'-dimethoxy[2'',3'':7,8] furanoflavone)

Fraction D4 (179 mg) was separated by column chromatography (silica gel 60 No. 9385) using the mixture of petroleum ether-EtOAc (4:1) as eluent. Fractions were combined according to their chromatographic pattern (pet. ether-EtOAc 1:1) to give D4-1 to D4-5. Fraction D4-2 gave compound MEL2 (2 mg,  $R_f$  0.4, silica gel, pet. ether-EtOAc 1:1). Compound MEL2 (3 mg) was also obtained by separating fraction D4-5 (7 mg) on a silica gel column (gradient elution, pet. ether-EtOAc 83.5:16.5 to 50:50). This compound was later identified as a new furanoflavone, 3',5'-dimethoxy[2'',3'':7,8] furanoflavone.

### 3.1.2.4 Isolation of Compound MEL3 (Pongaglabrone)

Fraction D5 (292 mg) was subjected to silica gel 60 column chromatography, eluted with gradient mixtures of ethyl acetate and pet. ether (20:80 to 50:50). Six combined fractions (D5-1 to D5-6) were collected from this column. Fraction D5-4 (232 mg) was continuously separated on a silica gel 60 column with isocratic elution of pet. ether-EtOAc (6:1) until giving 6 fractions (D5-4-1 to D5-4-6). Compound MEL3 (4 mg,  $R_f$  0.38, silica gel, pet. ether-EtOAc 1:1) was obtained as a white powder from fraction D5-4-2. This compound was identified as pongaglabrone.

Fraction D5-4-6 (88 mg) was purified by silica gel 60 column chromatography using gradient elution of pet. ether-EtOAc (83.5:16.5 to 50:50). The eluates were collected and combined according to their chromatographic pattern (pet. ether-EtOAc 1:1) to give 4 fractions (D5-4-6A to D5-4-6D). Compound MEL1 (5.6 mg,  $R_f$  0.33, silica gel, pet. ether-EtOAc 1:1) was obtained by recrystallization of fraction D5-4-6C (13 mg) from  $\text{CHCl}_3$ -pet. ether.

### 3.1.2.5 Isolation of Compound MEL4 (Gamatin) and MEL5 (Milletenin C)

Fraction H (541 mg) was separated on a silica gel 60 column (gradient elution, pet. ether-EtOAc 80:20 to 50:50). Fractions with similar chromatographic pattern were combined to give 9 fractions (H1 to H9).

Fraction H6 (140 mg) was separated over a silica gel 60 column with mixtures of pet. ether-EtOAc (80:20 to 50:50) as eluent. Similar fractions were combined after examination by TLC (pet. ether-EtOAc 1:1) to give 3 fractions (H6-1 to H6-3).

Fraction H6-2 (42 mg) was further separated on a silica gel 60 column, eluted with pet. ether-EtOAc (1:2). Sixteen fractions were collected, examined by TLC (silica gel, pet. ether-EtOAc 1:2) and combined to give 4 major fractions (H6-2-1 to H6-2-4). Fraction H6-2-3 (31 mg) was subjected to silica gel 60 column chromatography, eluted with 0.5% MeOH in CHCl<sub>3</sub> to give 21 fractions. Fractions with similar TLC patterns (silica gel, pet. ether-EtOAc 1:2) were combined to give 3 fractions (H6-2-3A to H6-2-3C). Fraction H6-2-3C, after removal of solvent, gave compound MEL4 (4 mg, *R<sub>f</sub>* 0.20, silica gel, pet. ether-EtOAc 1:2) as colorless needles, which was later identified as gamatin. Fraction H6-2-3A, after removal of solvent, gave compound MEL5 (13 mg, *R<sub>f</sub>* 0.24, silica gel, pet. ether-EtOAc 1:2) as a white powder. This compound was identified as milletenin C.

Fraction H6-2-3B (14 mg) was further purified on a silica gel 60 column, eluted with 0.5% MeOH in CHCl<sub>3</sub> to afford 4 mg of compound MEL5 (milletenin C, *R<sub>f</sub>* 0.24, silica gel, pet. ether-EtOAc 1:2) and compound MEL4 (gamatin, 7 mg, *R<sub>f</sub>* 0.20, silica gel, pet. ether-EtOAc 1:2).

#### 3.1.2.6 Isolation of Compound MEL6 (Milletocalyxin A)

Fraction G (1.5 g) was subjected to silica gel 60 column chromatography (gradient elution, pet. ether-EtOAc 85.7:14.3 to 33.3:66.7) to give 38 fractions. Similar fractions were combined after examination by TLC (pet. ether-EtOAc 1:1) to give 5 fractions (G1-G5).

Fraction G4 (915 mg) was further separated on a silica gel 60 column. Elution was performed with pet. ether-EtOAc gradient (85.7:14.3 to 50:50) to give 27 fractions. The eluates were combined on the basis of their TLC composition (silica gel, pet. ether-EtOAc 1:1) to yield 6 fractions (G4-1 to G4-6).

Fraction G4-4 (541 mg) was further separated by silica gel 60 column chromatography, eluted with gradient mixtures of pet. ether-EtOAc (85.7:14.3 to 50:50). Fractions (37 fractions) with similar chromatographic patterns were combined (TLC, silica gel, pet. ether-EtOAc 1:1) to give 5 fractions (G4-4-1 to G4-4-5).

Fraction G4-4-4 (179 mg) was purified on a silica gel 60 column with 0.5% MeOH in CHCl<sub>3</sub> as eluent. Fractions 5-10 from this column, after removal of the solvent, gave compound MEL6 (14 mg, *R<sub>f</sub>* 0.33, silica gel, pet. ether-EtOAc 1:2) as a white powder. It was identified as millettocalyxin A.

### 3.1.2.7 Isolation of Compound MEL7 (Millettocalyxin C)

Fraction G2 (15 mg) was subjected to silica gel 60 column chromatography, eluted with pet. ether-EtOAc (5:1) to give 11 fractions. Similar fractions were combined after examination by TLC (pet. ether-EtOAc 1:2) to give 3 fractions (G2-1 to G2-3). Fraction G2-2 (3 mg) was recrystallized from  $\text{CHCl}_3$  –pet. ether to give a yellow powder of compound MEL7 (1 mg,  $R_f$  0.45, silica gel, pet. ether-EtOAc 1:2). This compound was identified as millettocalyxin C.

Fraction G3 (135 mg) was separated on a silica gel 60 (No. 9385) column. Elution was performed with pet. ether-EtOAc (5:1) to give 29 fractions. Fractions with similar TLC pattern (silica gel, pet. ether-EtOAc 1:2) were combined to give 4 fractions (G3-1 to G3-4). Fraction G3-2 (2 mg) gave compound MEL7 (millettocalyxin C,  $R_f$  0.45, silica gel, pet. ether-EtOAc 1:2) as a yellow powder.

Repeated column chromatography of fraction G3-3 (45 mg, silica gel 60, pet. ether-EtOAc 5:1) gave 5 mg of compound MEL1 (3',4'-methylenedioxy-7-methoxyflavone,  $R_f$  0.45, silica gel, pet. ether-EtOAc 1:2).

Fraction D5-4-6A-3 (2 mg) was purified on a silica gel 60 column (gradient elution, pet. ether-EtOAc 83.5:16.5 to 66.7:33.3) to afford 1 mg of compound MEL7 as a yellow powder (millettocalyxin C,  $R_f$  0.45, silica gel, pet. ether-EtOAc 1:2).

Fraction D5-4-5 (3 mg) was further separated on a silica gel 60 column (gradient elution, pet. ether-EtOAc 85.7:14.3 to 80:20). Fractions (10 fractions) showing similar TLC pattern were combined (silica gel, pet. ether-EtOAc 1:2) to give 2 fractions (D5-4-5A and D5-4-5B). Repeated column chromatography of fraction D5-4-5B (2 mg) (silica gel, pet. ether-EtOAc 5:1) gave 1 mg of compound MEL7 (millettocalyxin C,  $R_f$  0.45, silica gel, pet. ether-EtOAc 1:2).

## 4. Physical and Spectral data of Isolated Compounds

### 4.1 Compound MEL1

Compound MEL1 was obtained as a white powder (13 mg), soluble in  $\text{CHCl}_3$ .

Melting Point : 201-202°C

ESIMS :  $[\text{M}+\text{H}]^+$   $m/z$  297.11(positive ion mode); **Figure 12**

UV :  $\lambda_{\text{max}}$  nm (log  $\epsilon$ ), in methanol; **Figure 13**  
236 (4.13), 334 (4.06)

- IR :  $\nu_{\max}$   $\text{cm}^{-1}$ , Film; **Figure 14**  
 2916, 1643, 1609, 1589, 1502, 1449, 1240, 1202, 1165, 1134, 1032
- $^1\text{H}$  NMR :  $\delta$  ppm, 300 MHz, in acetone- $d_6$ , **Table 4; Figure 15**
- $^{13}\text{C}$  NMR :  $\delta$  ppm, 75 MHz, in acetone- $d_6$ , **Table 4; Figure 16**

#### 4.2 Compound MEL2

Compound MEL2 was obtained as colorless needles (5 mg), soluble in  $\text{CHCl}_3$ .

Melting Point : 178-180°C

HRESIMS :  $[\text{M}+\text{H}]^+$   $m/z$  323.0916 (calcd. for  $\text{C}_{19}\text{H}_{14}\text{O}_5 = 323.0920$ ); **Figure 18**

ESIMS:  $[\text{M}+\text{H}]^+$   $m/z$  323.13 (positive ion mode); **Figure 17**

UV :  $\lambda_{\max}$  nm (log  $\epsilon$ ), in methanol; **Figure 19**  
 215 (3.87), 240 (3.55), 263 (3.44), 303 (3.49)

IR :  $\nu_{\max}$   $\text{cm}^{-1}$ , Film; **Figure 20**  
 1630, 1607, 1583, 1426, 1210, 1160, 1140, 1060

$^1\text{H}$  NMR :  $\delta$  ppm, 300 MHz, in  $\text{CDCl}_3$ , **Table 5; Figure 21**

$^{13}\text{C}$  NMR :  $\delta$  ppm, 75 MHz, in  $\text{CDCl}_3$ , **Table 5; Figure 22**

#### 4.3 Compound MEL3

Compound MEL3 was obtained as a white powder (4 mg), soluble in  $\text{CHCl}_3$ .

Melting Point : 220-222°C

ESIMS :  $[\text{M}+\text{H}]^+$   $m/z$  307.11 (positive ion mode); **Figure 30**

UV :  $\lambda_{\max}$  nm (log  $\epsilon$ ), in methanol; **Figure 31**  
 215 (4.50), 240 (4.35), 326 (4.24)

IR :  $\nu_{\max}$   $\text{cm}^{-1}$ , Film; **Figure 32**  
 2917, 1641, 1596, 1505, 1447, 1405, 1347, 1256, 746

$^1\text{H}$  NMR :  $\delta$  ppm, 300 MHz, in  $\text{CDCl}_3$ , **Table 6; Figure 33**

$^{13}\text{C}$  NMR :  $\delta$  ppm, 75 MHz, in  $\text{CDCl}_3$ , **Table 6; Figure 34**

#### 4.4 Compound MEL4

Compound MEL4 was obtained as colorless needles (11 mg), soluble in  $\text{CHCl}_3$ .

Melting Point : 227-228°C

ESIMS :  $[\text{M}+\text{H}]^+$   $m/z$  337.13 (positive ion mode); **Figure 36**

UV :  $\lambda_{\max}$  nm (log  $\epsilon$ ), in methanol; **Figure 37**  
 250 (4.38), 333 (4.24)

IR :  $\nu_{\max}$   $\text{cm}^{-1}$ , Film; **Figure 38**  
 2920, 1646, 1583, 1507, 1452, 1329, 1261, 1160, 1072, 756



$^1\text{H}$  NMR :  $\delta$  ppm, 300 MHz, in  $\text{CDCl}_3$ , **Table 7; Figure 39**

$^{13}\text{C}$  NMR :  $\delta$  ppm, 75 MHz, in  $\text{CDCl}_3$ , **Table 7; Figure 40**

#### 4.5 Compound MEL5

Compound MEL5 was obtained as a white powder (17 mg), soluble in  $\text{CHCl}_3$ .

Melting Point : 250-252°C

ESIMS :  $[\text{M}+\text{H}]^+$   $m/z$  327.16 (positive ion mode); **Figure 48**

UV :  $\lambda_{\text{max}}$  nm (log  $\epsilon$ ), in methanol; **Figure 49**  
211 (4.71), 240 (4.47), 333 (4.50)

IR :  $\nu_{\text{max}}$   $\text{cm}^{-1}$ , Film; **Figure 50**  
2918, 1639, 1591, 1508, 1478, 1453, 1335, 1265, 1085, 752

$^1\text{H}$  NMR :  $\delta$  ppm, 300 MHz, in  $\text{CDCl}_3$ , **Table 8; Figure 51**

$^{13}\text{C}$  NMR :  $\delta$  ppm, 75 MHz, in  $\text{CDCl}_3$ , **Table 8; Figure 52**

#### 4.6 Compound MEL6

Compound MEL6 was obtained as a white powder (14 mg), soluble in  $\text{CHCl}_3$ .

Melting Point : 210-212°C

ESIMS :  $[\text{M}+\text{Na}]^+$   $m/z$  350.06 (positive ion mode)  
 $[\text{M}+\text{H}]^+$   $m/z$  327.12 (positive ion mode); **Figure 53**

UV :  $\lambda_{\text{max}}$  nm (log  $\epsilon$ ), in methanol; **Figure 54**  
247 (4.31), 300 (4.14), 360 (4.27)

IR :  $\nu_{\text{max}}$   $\text{cm}^{-1}$ , Film; **Figure 55**  
1648, 1620, 1569, 1502, 1262, 1195, 1170, 1023, 826

$^1\text{H}$  NMR :  $\delta$  ppm, 300 MHz, in acetone- $d_6$ , **Table 9; Figure 56**

$^{13}\text{C}$  NMR :  $\delta$  ppm, 75 MHz, in acetone- $d_6$ , **Table 9; Figure 57**

#### 4.7 Compound MEL7

Compound MEL7 was obtained as a yellow powder (5 mg), soluble in  $\text{CHCl}_3$ .

Melting Point : 166-168°C

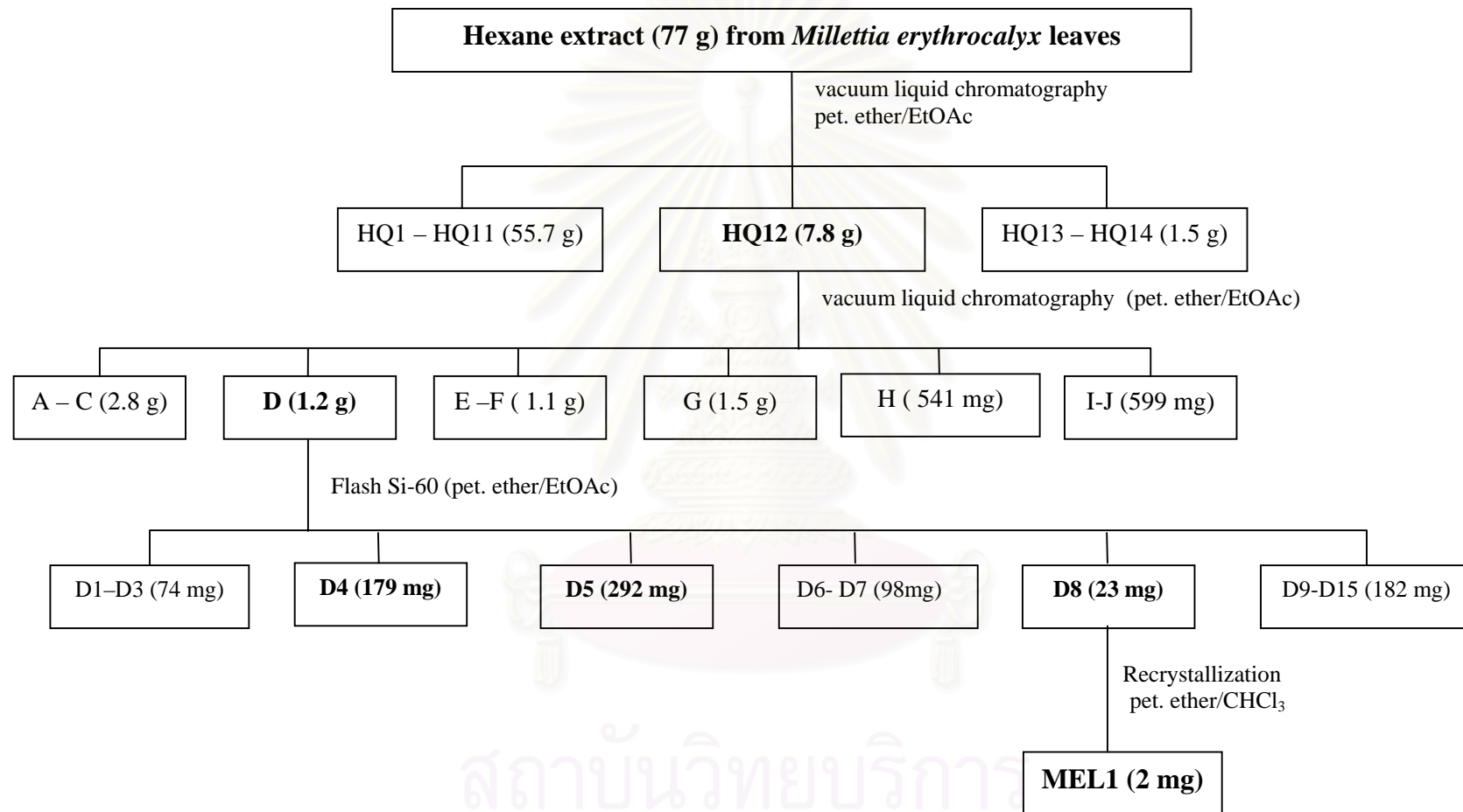
ESIMS :  $[\text{M}+\text{H}]^+$   $m/z$  323.14 (positive ion mode); **Figure 60**

UV :  $\lambda_{\text{max}}$  nm (log  $\epsilon$ ), in methanol; **Figure 61**  
248 (4.25), 293 (3.95)

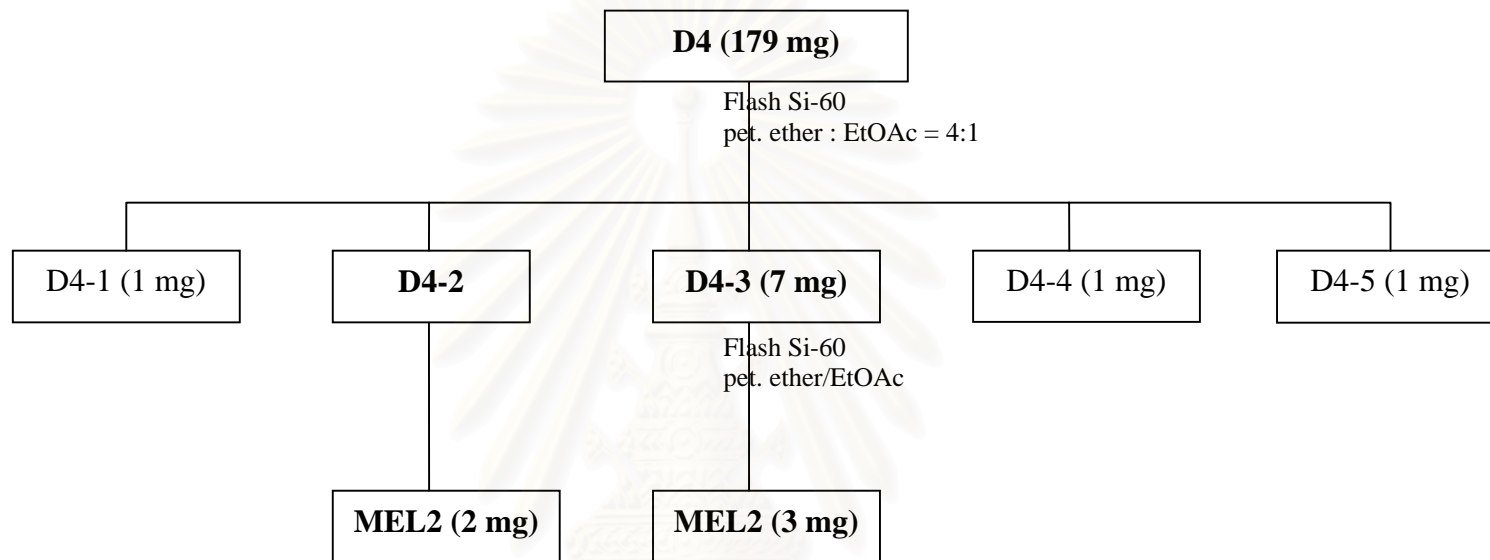
IR :  $\nu_{\text{max}}$   $\text{cm}^{-1}$ , Film; **Figure 62**  
1626, 1588, 1466, 1411, 1362, 1239, 1186, 1074, 753

$^1\text{H}$  NMR :  $\delta$  ppm, 300 MHz, in  $\text{CDCl}_3$ , **Table 10; Figure 63**

$^{13}\text{C}$  NMR :  $\delta$  ppm, 75 MHz, in  $\text{CDCl}_3$ , **Table 10; Figure 64**

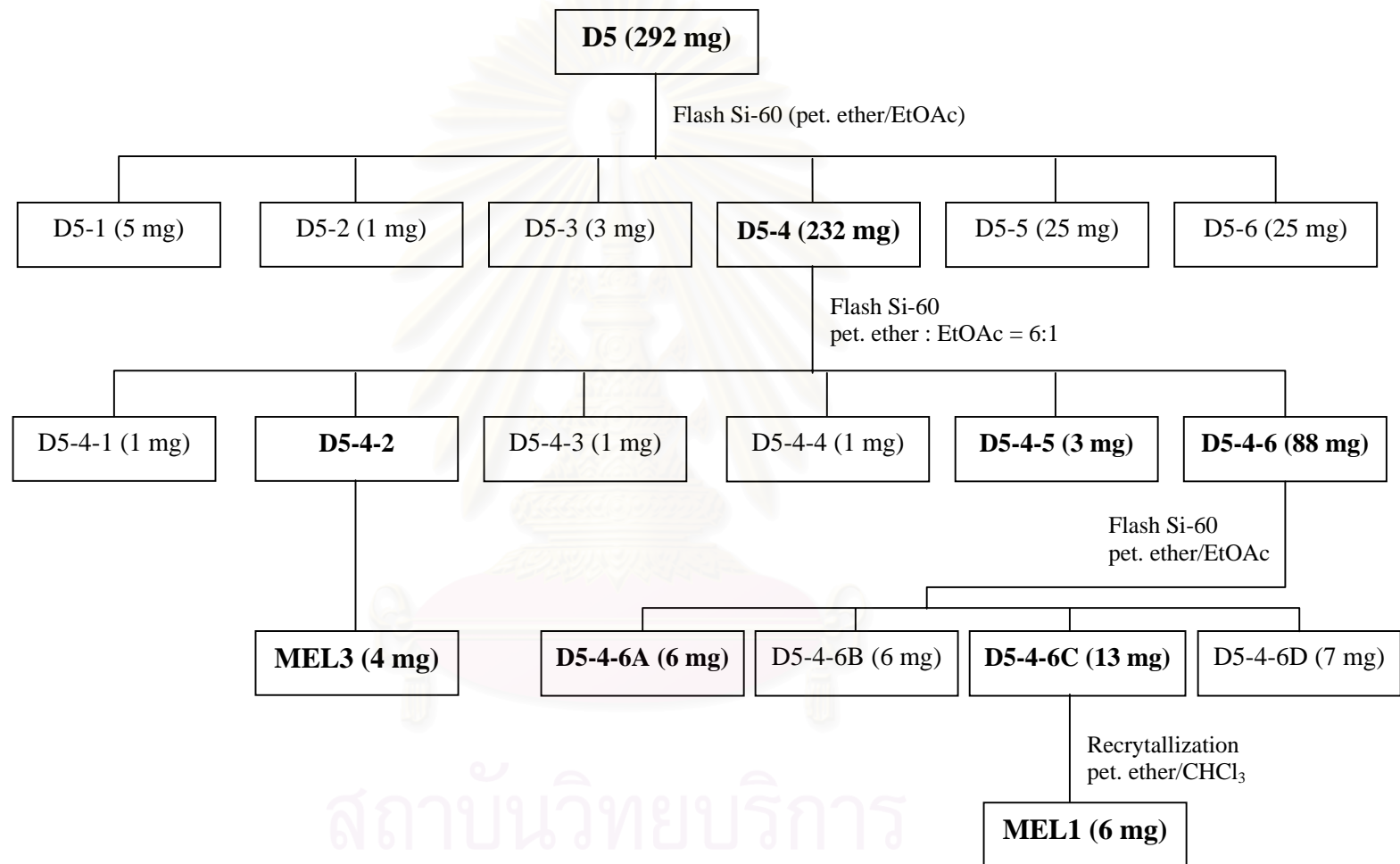


**Scheme 1** Separation of the hexane extract of *Millettia erythrocalyx* leaves.

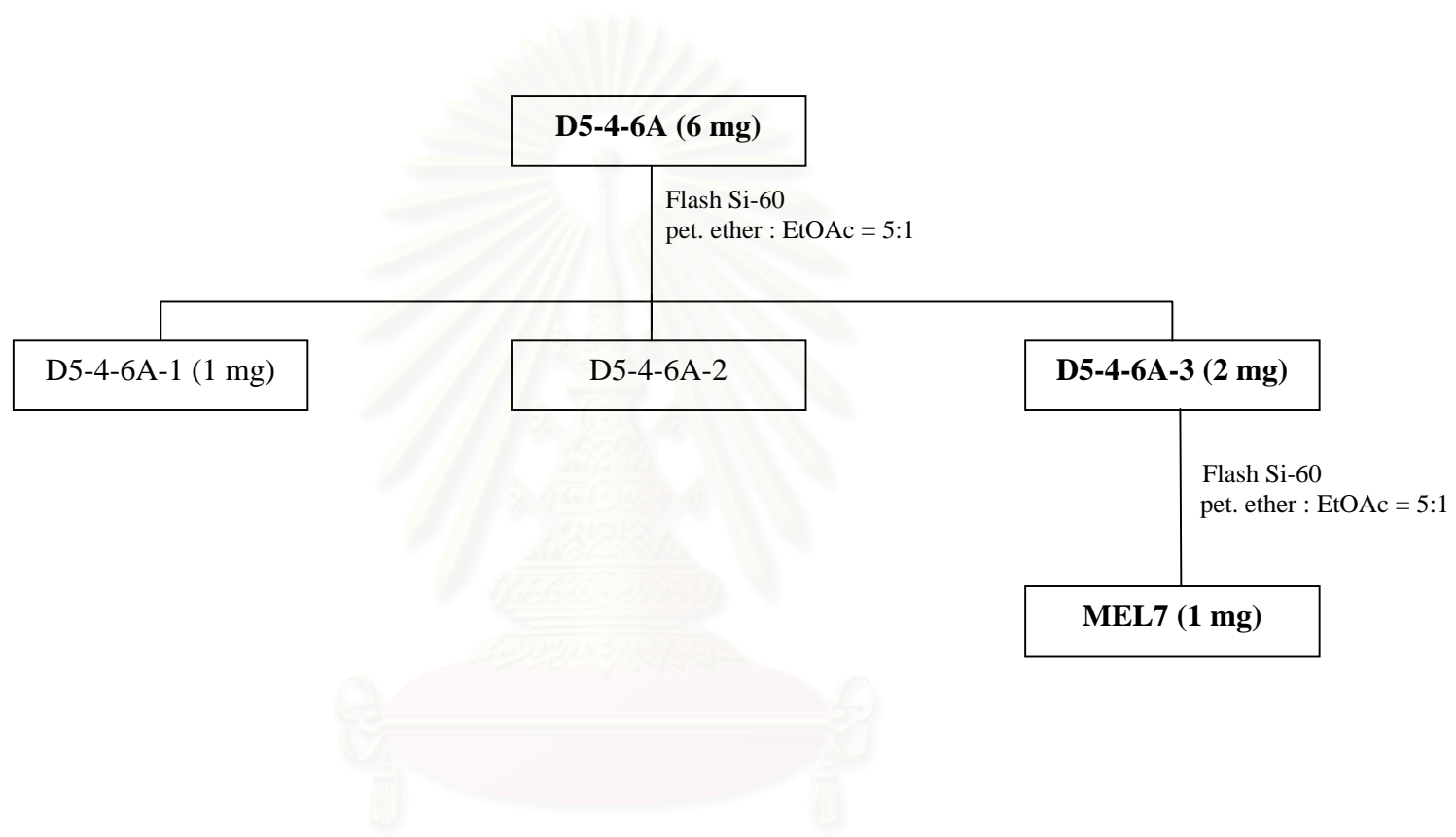


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**Scheme 2** Separation of fraction D4 from the hexane extract of *M. erythrocalyx* leaves.

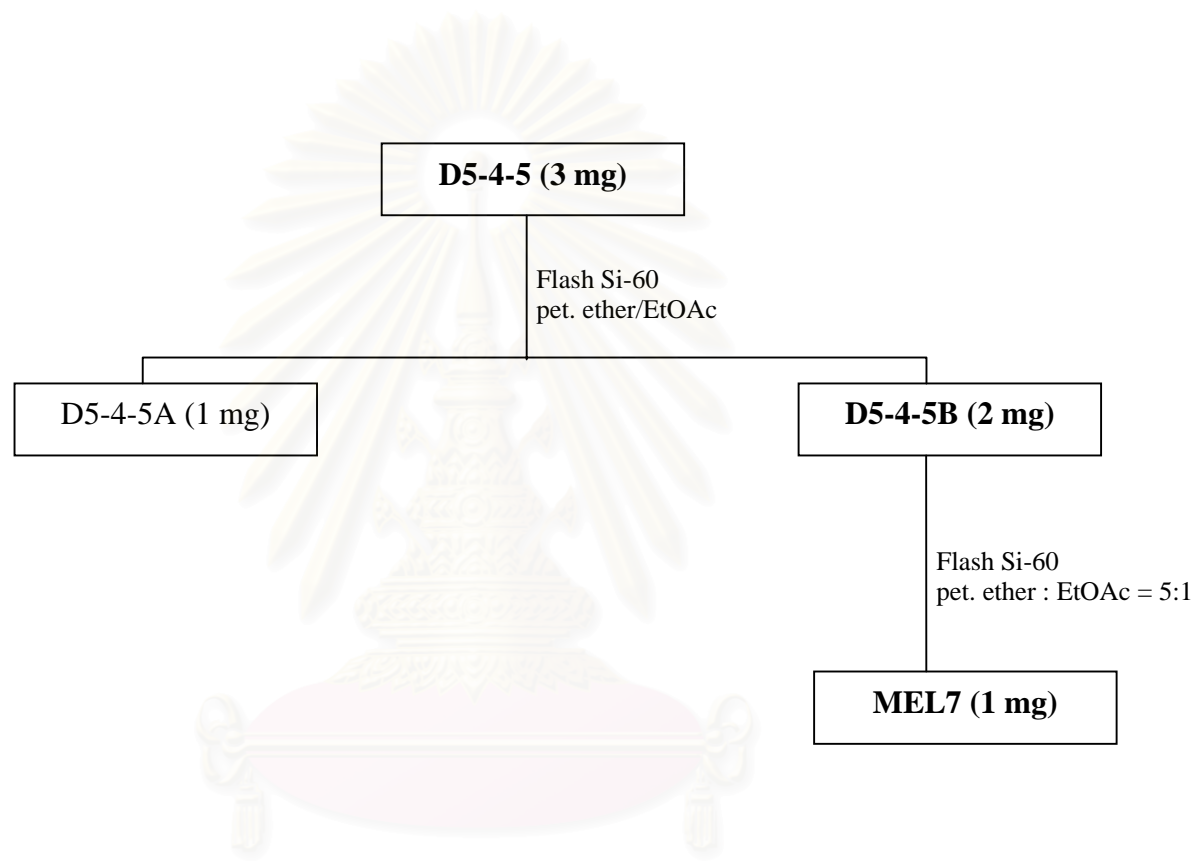


**Scheme 3** Separation of fraction D5 from the hexane extract of *M. erythrocalyx* leaves.



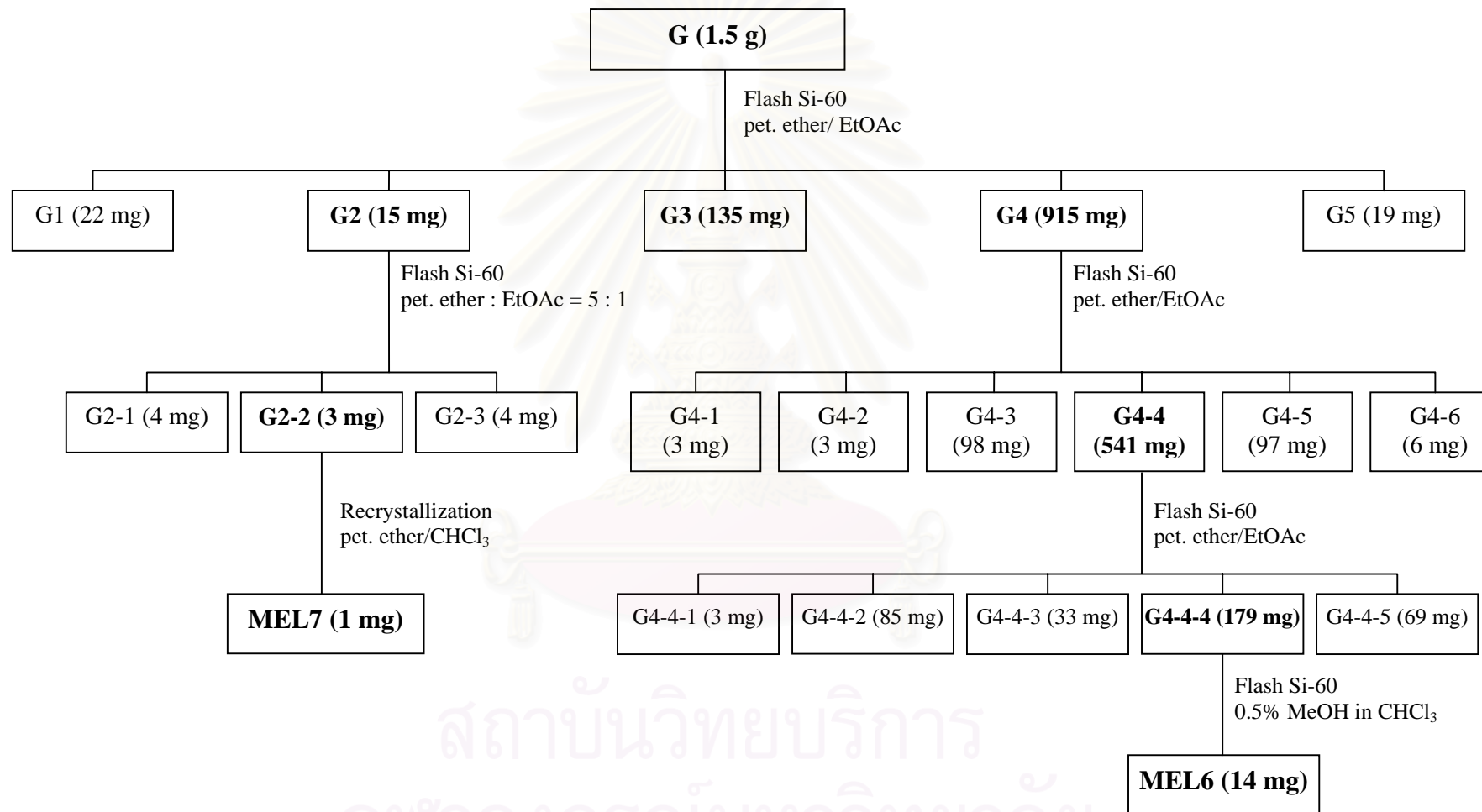
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**Scheme 4** Separation of fraction D5-46A from the hexane extract of *M. erythrocalyx* leaves.

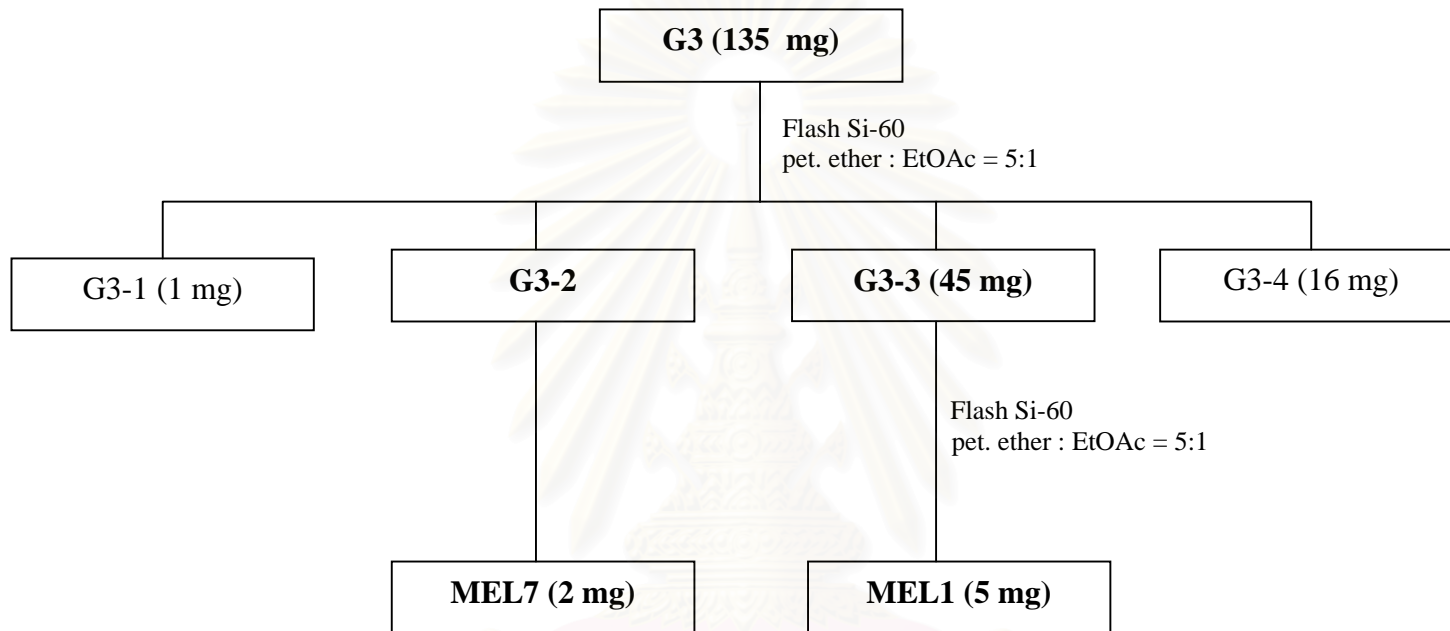


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**Scheme 5** Separation of fraction D5-45 from the hexane extract of *M. erythrocalyx* leaves.

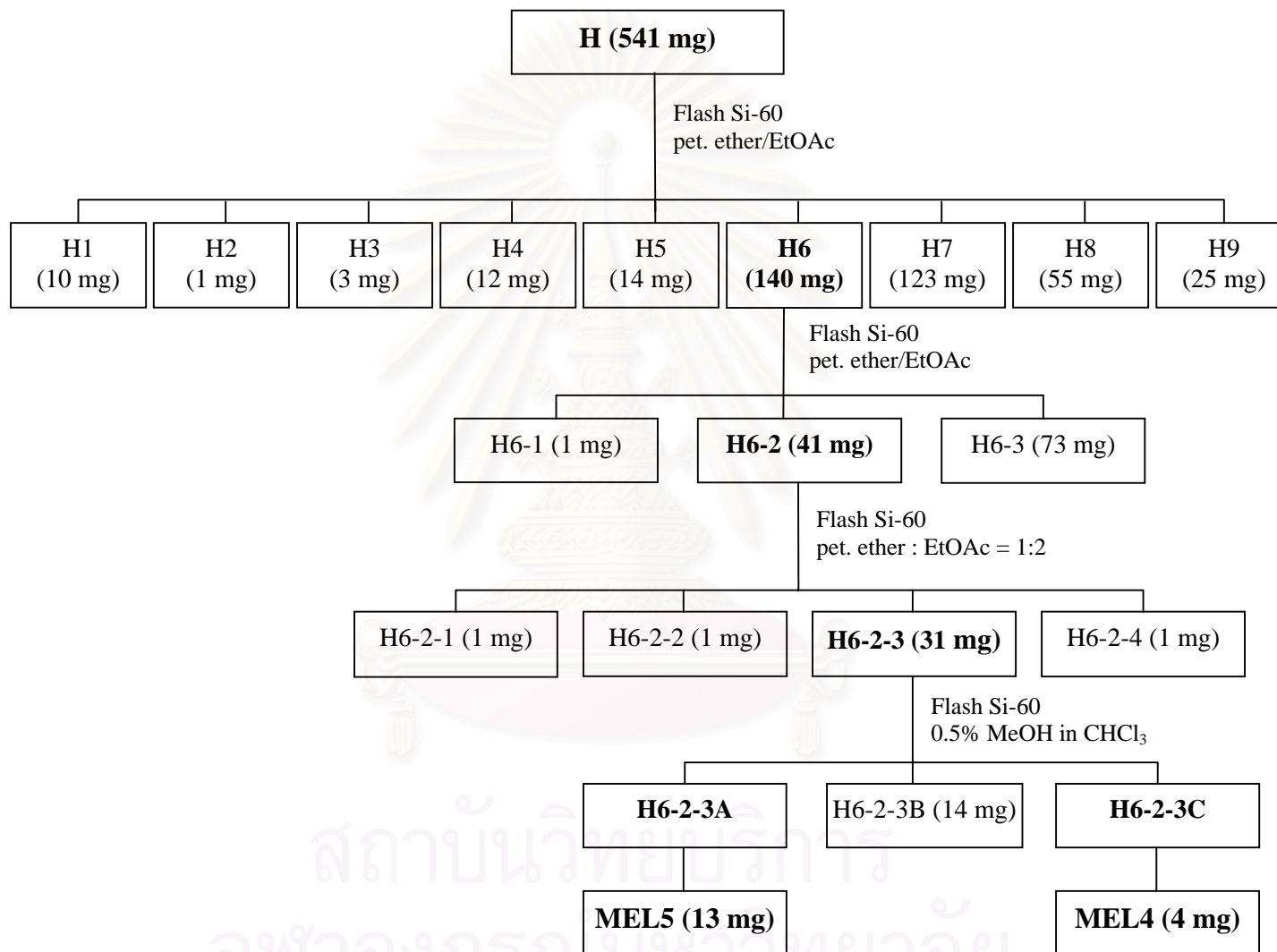


**Scheme 6** Separation of fraction G from the hexane extract of *M. erythrocalyx* leaves.

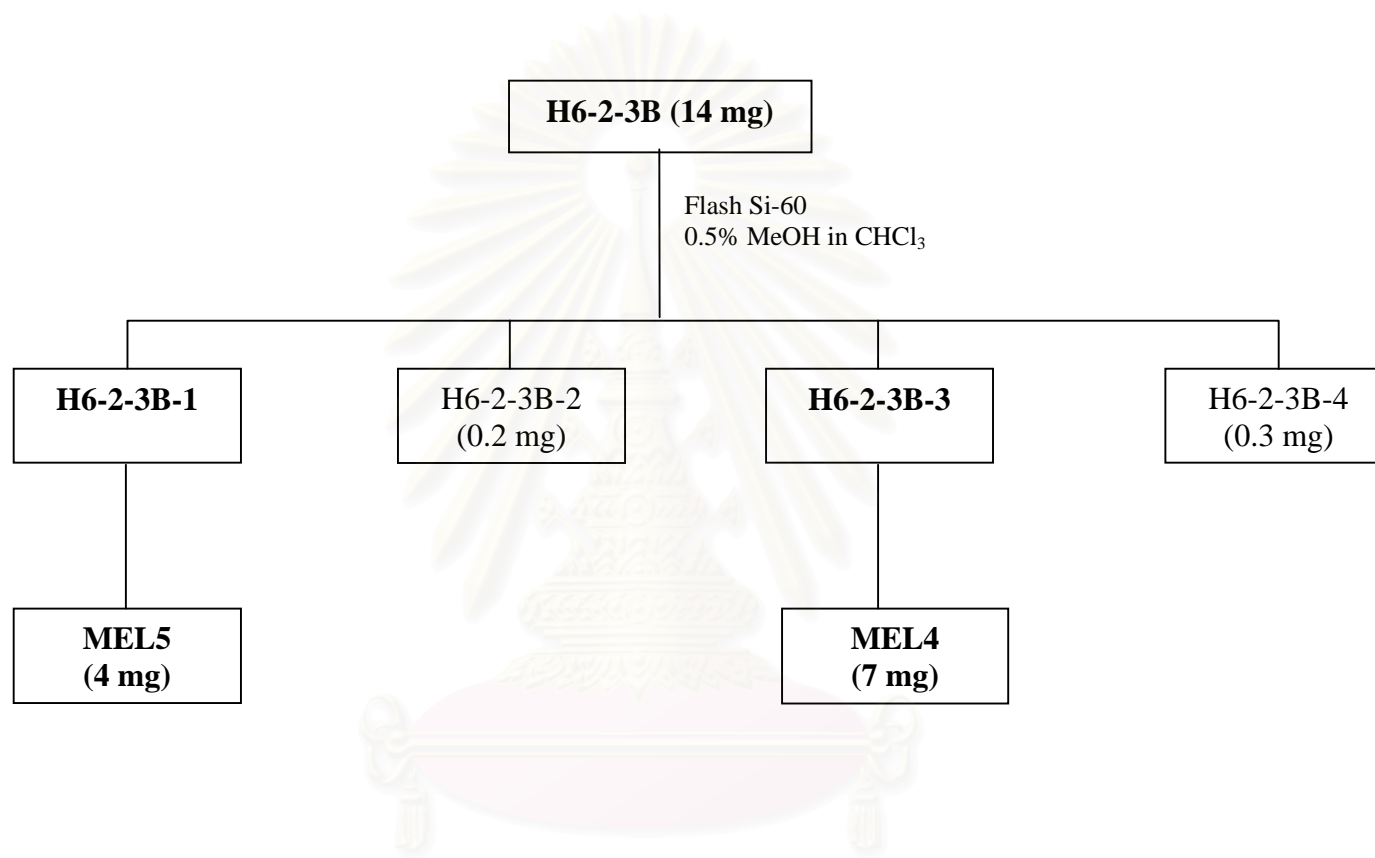


**Scheme 7** Separation of fraction G3 from the hexane extract of *M. eythrocalyx* leaves.





**Scheme 8** Separation of fraction H from the hexane extract of *M. erythrocalyx* leaves.



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**Scheme 9** Separation of fraction H6-21C from the hexane extract of *M. erythrocalyx* leaves.

## CHAPTER IV

### RESULTS AND DISCUSSION

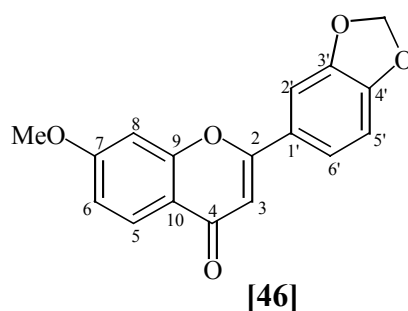
The leaves of *Millettia erythrocalyx* Gagnep. (3.5 kg) were extracted with hexane, ethyl acetate and ethanol, successively, to give a hexane extract (78 g), an ethyl acetate extract (43 g) and an ethanol extract (283 g), respectively. The hexane extract was further separated using several chromatographic techniques to yield seven pure compounds (MEL1 to MEL7).

The structures of all of the isolates were determined by interpretation of their UV, IR, NMR and MS data, and further confirmed by comparison of these spectral properties with literature values.

#### 1. Structure Determination of Isolated Compounds

##### 1.1 Structure Determination of Compound MEL1

Compound MEL1 was obtained as a white powder. It has a molecular formula of  $C_{17}H_{12}O_5$ , as indicated by the molecular ion peak at  $m/z$  297.1 in the ESIMS (**Figure 12**). The IR spectrum (**Figure 14**) showed absorption bands at 1643 (conjugated carbonyl), 1589 (conjugated C=C) and 1449 ( $CH_2$  bending) $cm^{-1}$ , and the UV absorptions at 236 and 334 nm (**Figure 13**) were indicative of a flavone nucleus (Markham, 1982). The  $^1H$  NMR spectrum (**Table 4, Figure 15**) exhibited signals for an aromatic singlet signal (H-3) at  $\delta$  6.67 (1H, s), a methoxyl at  $\delta$  3.97 (1H, s) and a methylenedioxy at  $\delta$  6.14 (2H, s). The presence of two doublets at  $\delta$  7.23 ( $J = 2.1$  Hz, H-8) and  $\delta$  7.98 ( $J = 8.7$  Hz, H-5) and a double doublet at  $\delta$  7.03 ( $J = 8.7, 2.1$  Hz, H-6) placed the methoxyl group at C-7. The ABM splitting system at  $\delta$  7.55 (d,  $J = 1.5$  Hz, H-2'), 7.02 (d,  $J = 8.4$  Hz, H-5') and 7.65 (dd,  $J = 8.4, 1.5$  Hz, H-6'), along with the  $^3J$  correlation peak of H-2' with C-2 ( $\delta$  163.14), indicated the location of the methylenedioxy group at C-3' and C-4'. These  $^1H$  and  $^{13}C$  NMR data were in good agreement with those reported for 3',4'-methylenedioxy-7-methoxyflavone [**46**] (Mahmoud and Waterman, 1985).

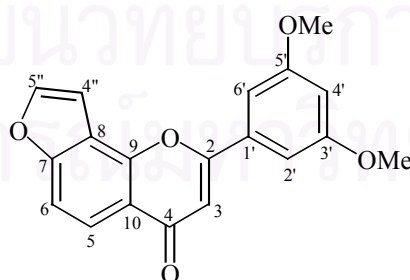


**Table 4 NMR Spectral data of compound MEL1 (acetone- $d_6$ ) and 3',4'-methylenedioxy-7-methoxyflavone (pyridine- $d_5$ )**

Position	Compound MEL1		3',4'-methylenedioxy-7-methoxyflavone
	$^1\text{H}$ (mult., $J$ in Hz)	$^{13}\text{C}$	$^1\text{H}$ (mult., $J$ in Hz)
2	-	163.1	-
3	6.67 (s)	106.8	7.08 (s)
4	-	177.1	-
5	7.98 (d, 8.7)	127.2	8.35 (d, 9.0)
6	7.03 (d, 8.7, 2.1)	115.1	7.05 (dd, 9.0, 2.0)
7	-	165.1	-
8	7.23 (d, 2.1)	101.5	7.15 (d, 2.0)
9	-	158.7	-
10	-	118.5	-
1'	-	126.7	-
2'	7.55 (d, 1.5)	106.9	7.60 (d, 2.0)
3'	-	149.5	-
4'	-	151.5	-
5'	7.02 (d, 8.4)	109.4	6.99 (d, 8.0)
6'	7.65 (dd, 8.4, 1.5)	122.1	7.54 (dd, 8.0, 2.0)
MeO-7	3.97 (s)	56.4	3.80 (s)
-OCH <sub>2</sub> O-	6.14 (s)	103.0	6.08 (s)

## 1.2 Structure Determination of Compound MEL2

Compound MEL2, colorless needles, exhibited a molecular ion  $[M+H]^+$  peak at  $m/z$  323.1 in the ESIMS, indicating a molecular formula of  $C_{19}H_{14}O_5$ . The IR spectrum displayed absorption bands at 1630 (conjugated C=O stretching), 1583 (conjugated C=C) and 1210 and 1060 (C-O stretching)  $cm^{-1}$  (**Figure 20**), and the UV absorptions at 240, 263 and 303 nm (**Figure 19**) were indicative of a furanoflavone (Mbafor *et al.*, 1995). This was supported by the  $^1H$  and  $^{13}C$  NMR signals (**Table 5, Figures 21 and 22**) for H-3 at  $\delta$  6.84 (1H, s) and C-3 at  $\delta$  106.8, for a furan ring at  $\delta$  7.18 (d,  $J = 2.1$ , H-4'')/  $\delta$  104.2 (C-4'') and  $\delta$  7.76 (d,  $J = 2.1$ , H-5'')/  $\delta$  145.8 (C-5''). The furan ring could be fused in an angular position at C-7 and C-8, as supported by the presence of two doublets at  $\delta$  8.15 (d,  $J = 9.0$ , H-5) and  $\delta$  7.55 (d,  $J = 9.0$ , H-6) and the HMBC correlations of H-5 with C-4 ( $\delta$  178.2) and H-6 with C-10 ( $\delta$  119.4) (**Figures 25-27**). Furthermore, the HMBC correlations of C-7 ( $\delta$  158.4) with H-4'' and H-5'' and C-8 ( $\delta$  117.2) with H-4'' and H-5'' were also observed. The presence of two methoxyl groups was revealed by the proton resonance at  $\delta$  3.88 (6H, s) and the carbon signal at  $\delta$  55.6. To determine the location of the two methoxyls on ring B, a NOESY experiment (**Figures 28 and 29**) was carried out. The NOE interactions of the methoxyl protons with the proton at  $\delta$  7.07 (d,  $J = 2.1$ , H-2') and with the proton at  $\delta$  6.63 (t,  $J = 2.1$ , H-4') placed the methoxyls at C-3' and C-5'. Interactions through  $^3J$  coupling of C-2 with H-2' and H-6', and C-4' ( $\delta$  103.3) with H-2' and H-6', were also observed in the HMBC spectrum. On the basis of the above spectroscopic data, compound MEL2 was identified as a new compound, 3',5'-dimethoxy [2'',3'':7,8]furanoflavone [**236**].



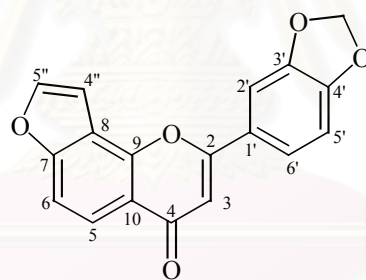
[236]

Table 5 NMR Spectral data of compound MEL2 (CDCl<sub>3</sub>)

Position	Compound MEL2		HMBC
	<sup>1</sup> H (mult., <i>J</i> in Hz)	<sup>13</sup> C	
2	-	162.5	H-3, H-2', H-6'
3	6.84 (s)	108.5	-
4	-	178.2	H-3, H-5
5	8.15 (d, 9.0)	121.8	-
6	7.55 (d, 9.0)	110.2	-
7	-	158.4	H-6, H-5, H-4'', H-5''
8	-	117.2	H-6, H-4'', H-5''
9	-	150.8	H-5
10	-	119.4	H-3, H-6
1'	-	133.7	H-3, H-2', H-6'
2'	7.07 (d, 2.1)	104.5	H-4'
3'	-	161.3	H-2', H-4', MeO-3'
4'	6.63 (t, 2.1)	103.3	H-2', H-6'
5'	-	161.3	H-4', H-6', MeO-5'
6'	7.07 (d, 2.1)	104.5	H-4'
4''	7.18 (d, 2.1)	104.2	H-5''
5''	7.76 (d, 2.1)	145.8	H-4''
MeO-3'	3.88 (s)	55.6	-
MeO-5'	3.88 (s)	55.6	-

### 1.3 Structure Determination of Compound MEL3

Compound MEL3 was obtained as a white powder. The molecular formula was determined as  $C_{18}H_{10}O_5$  by ESIMS of its  $[M+H]^+$  ion at  $m/z$  307.1. Its IR spectrum (**Figure 32**) exhibited absorption bands at  $1641\text{ cm}^{-1}$  (conjugated C=O),  $1596\text{ cm}^{-1}$  (conjugated C=C) and  $1447\text{ cm}^{-1}$  ( $CH_2$  bending). The UV spectrum maximum absorptions at 240 and 326 nm (**Figure 31**) and the  $^1H$  NMR signal at  $\delta$  6.74 (1H, s, H-3) were suggestive of a furanoflavone nucleus (**Table 6, Figure 33**). The  $^1H$  and  $^{13}C$  NMR signals for a furan ring at  $\delta$  7.18 (1H, d,  $J = 1.8$ , H-4'')/  $\delta$  104.1 (C-4'') and  $\delta$  7.75 (1H, d,  $J = 1.8$ , H-5'')/  $\delta$  145.8 (C-5'') were observed. In the  $^1H$  NMR spectrum, the signal for 3', 4'-methylenedioxy substitution on ring B appeared at  $\delta$  6.08 (2H, s), and three protons signals at  $\delta$  7.38 (1H, d,  $J = 1.8$  Hz), 6.95 (1H, d,  $J = 8.1$  Hz) and 7.51 (1H, dd,  $J = 8.1, 1.8$  Hz) were assigned to H-2', H-5' and H-6', respectively. Compound MEL3 was identified as pongaglabrone **[54]** based on the above spectral data. Its  $^1H$  NMR properties are in agreement with previously published values (Garcez *et al.*, 1988).



**[54]**

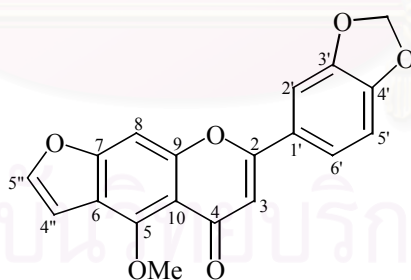
**Table 6 NMR Spectrum data of compound MEL3 (CDCl<sub>3</sub>) and pongaglabrone (CDCl<sub>3</sub>)**

Position	Compound MEL3		Pongaglabrone
	<sup>1</sup> H (mult., <i>J</i> in Hz)	<sup>13</sup> C	<sup>1</sup> H (mult., <i>J</i> in Hz)
2	-	162.3	-
3	6.74 (s)	107.1	6.80 (s)
4	-	178.1	-
5	8.14 (d, 9.0)	121.8	8.22 (d, 8.0)
6	7.54 (d, 9.0)	110.1	7.59 (d, 8.0)
7	-	158.3	-
8	-	117.1	-
9	-	150.7	-
10	-	119.3	-
1'	-	125.8	-
2'	7.38 (d, 1.8)	106.2	7.44 (d, 2.0)
3'	-	148.5	-
4'	-	150.6	-
5'	6.95 (d, 8.1)	108.9	7.00 (d, 8.0)
6'	7.51 (dd, 8.1, 1.8)	121.3	7.59 (dd, 8.0, 2.0)
4''	7.18 (d, 1.8)	104.1	7.20 (d, 2.0)
5''	7.75 (d, 1.8)	145.8	7.82 (d, 2.0)
-OCH <sub>2</sub> O-	6.08 (s)	101.9	6.14 (s)



#### 1.4 Structure Determination of compound MEL4

Compound MEL4 was obtained as colorless needles. The ESI mass spectrum (**Figure 36**) displayed the quasi-molecular ion peak  $[M+H]^+$  at  $m/z$  337.13, analysed for  $C_{19}H_{12}O_6$ . The UV spectrum (**Figure 37**) exhibited absorption maxima at 250, 333 nm. The IR spectrum (**Figure 38**) showed  $\nu_{max}$  at 1646 (conjugated C=O), 1583 (conjugated C=C) and 1452 ( $CH_2$  bending)  $cm^{-1}$ . The  $^1H$  NMR signal at  $\delta$  6.55 (1H, s, H-3) was characteristic of a furanoflavone nucleus. The  $^1H$  NMR signals (**Table 7, Figure 39**) at  $\delta$  7.03 (1H, d,  $J = 2.1$  Hz) and  $\delta$  7.58 (1H, d,  $J = 2.1$  Hz) were assigned as H-4'' and H-5'' for the furan ring. The methoxyl signal appeared at  $\delta$  4.18 ppm. The HMBC correlations (**Figures 45-47**) of H-8 with C-6 and C-10 indicated the location of the furan ring on C-6 and C-7, and the correlations of C-5 with protons of the methoxyl group suggested the attachment of the methoxyl group at C-5. This was supported by the NOESY correlation between H-4'' and the methoxyl group at C-5 (**Figures 43 and 44**). The characteristic signal for the methylenedioxy substituted B-ring was found at  $\delta$  6.05 (2H, s). The presence of an ABX spin system at  $\delta$  7.32 (1H, d,  $J = 1.5$  Hz, H-2'),  $\delta$  6.90 (1H, d,  $J = 8.1$  Hz, H-5') and  $\delta$  7.46 (1H, dd,  $J = 8.1, 1.5$  Hz, H-6') in the  $^1H$  NMR spectrum placed the methylenedioxy group at C-3' and C-4' positions. Based on the above spectral evidence, compound MEL4 was identified as gamatin [237] (Pathak and Khanna, 1981).



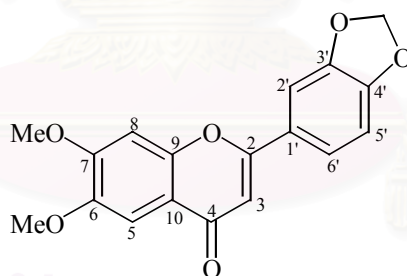
[237]

Table 7 NMR Spectral data of compound MEL4 (CDCl<sub>3</sub>) and gamatin (CDCl<sub>3</sub>)

Position	Compound MEL4		Gamatin	HMBC
	<sup>1</sup> H (mult., <i>J</i> in Hz)	<sup>13</sup> C	<sup>1</sup> H (mult., <i>J</i> in Hz)	
2	-	161.0	-	H-3, H-2', H-6'
3	6.55 (s)	106.9	6.43 (s)	-
4	-	178.4	-	H-3
5	-	153.6	-	MeO-5
6	-	117.3	-	H-8, H-4'', H-5''
7	-	158.0	-	H-4'', H-5''
8	7.34 (s)	106.2	6.75 (s)	-
9	-	155.7	-	H-8
10	-	112.8	-	H-3, H-8
1'	-	125.6	-	H-3, H-2', H-5'
2'	7.32 (d, 1.5)	95.4	7.19 (d, 9.0)	-
3'	-	148.4	-	H-5', -OCH <sub>2</sub> O-
4'	-	150.4	-	H-6', -OCH <sub>2</sub> O-
5'	6.90 (d, 8.1)	108.7	6.89 (d, 8.5)	-
6'	7.46 (dd, 8.1, 1.5)	121.2	7.19 (d, 9.0)	H-2'
4''	7.03 (d, 2.1)	105.4	7.30 (d, 2.0)	H-5''
5''	7.58 (d, 2.1)	145.2	7.47 (d, 2.5)	H-4''
MeO-5	4.18 (s)	61.9	4.12 (s)	-
-	6.05 (s)	101.9	5.94 (s)	-
OCH <sub>2</sub> O-				

### 1.5 Structure Determination of compound MEL5

Compound MEL5, a white powder, showed its quasi-molecular ion  $[M+H]^+$  at  $m/z$  327.2 in the ESIMS (**Figure 48**), indicating a molecular of  $C_{18}H_{14}O_6$ . The UV spectral data displayed maximum absorptions at 211, 240 and 333 nm (**Figure 49**). The IR spectrum showed  $\nu_{max}$  at 1639 (conjugated C=O), 1591 (conjugated C=C) and 1453 ( $CH_2$  bending)  $cm^{-1}$  (**Figure 50**). The  $^1H$  NMR spectrum exhibited a singlet signal of H-3 at  $\delta$  6.65 (**Table 8, Figure 51**). These data were indicative of a flavone nucleus. Two proton singlet signals at  $\delta$  7.54 and 6.95 were assigned to the aromatic protons H-5 and H-8 of ring A. The methylenedioxy group on ring B was observed at  $\delta$  6.05 (2H, s) and  $\delta_C$  101.9 ppm. Furthermore, the  $^1H$  NMR spectrum revealed the presence of two methoxyl groups at  $\delta$  3.97 and 3.99 ppm. The NOESY correlation of the methoxyl groups with H-5 and H-8 suggested the attachment of the methoxyls group at C-6 and C-7 (**Figure 54**). By analyses of the  $^1H$  and  $^{13}C$  NMR data and comparison with previously reported data (Parma, Gupta and Sharma, 1989), compound MEL5 was identified as milletenin C [45].



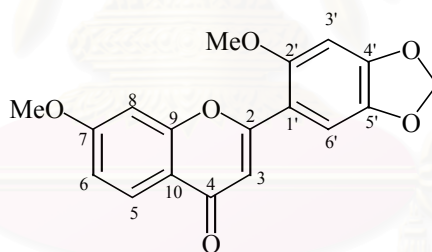
[45]

**Table 8 NMR Spectral data of compound MEL5 (CDCl<sub>3</sub>) and milletenin C (CDCl<sub>3</sub>)**

Position	Compound MEL5		Milletenin C	
	<sup>1</sup> H (mult., <i>J</i> in Hz)	<sup>13</sup> C	<sup>1</sup> H (mult., <i>J</i> in Hz)	<sup>13</sup> C
2	-	162.5	-	162.4
3	6.65 (s)	106.2	6.62 (s)	101.8
4	-	177.6	-	177.4
5	7.54 (s)	104.5	7.60 (s)	121.1
6	-	147.7	-	126.1
7	-	154.4	-	162.4
8	6.95 (s)	99.7	6.96 (s)	99.8
9	-	152.1	-	147.8
10	-	117.3	-	104.7
1'	-	126.0	-	121.1
2'	7.33 (d, 1.5)	106.2	7.34 (d, 1.5)	106.2
3'	-	148.5	-	147.8
4'	-	150.4	-	147.8
5'	6.91 (d, 8.4)	108.7	6.93 (d, 8.0)	106.3
6'	7.45 (dd, 8.4, 1.5)	121.1	7.44 (dd, 8.0, 1.5)	108.7
MeO-6	3.97 (s)	56.4	3.98 (s)	56.4
MeO-7	3.99 (s)	56.5	4.00 (s)	56.4
-OCH <sub>2</sub> O-	6.05 (s)	101.9	6.05 (s)	101.8

### 1.6 Structure Determination of compound MEL6

Compound MEL6 was obtained as a white powder. It showed a quasi-molecular  $[M+H]^+$  ion peak at  $m/z$  327.1 in the ESIMS (**Figure 55**), suggesting a molecular formula of  $C_{18}H_{14}O_6$ . The UV spectrum showed maximum absorptions at 247, 300, 360 nm (**Figure 56**). The IR spectrum displayed  $\nu_{max}$  at 1648 (conjugated C=O), 1620 and 1569  $cm^{-1}$  (conjugated C=C) (**Figure 57**). The  $^1H$  NMR signal at  $\delta$  6.88 (1H, s, H-3) was indicative of a flavone skeleton. The  $^1H$  and  $^{13}C$  NMR spectra (**Table 9, Figures 58 and 59**) revealed the presence of a methylenedioxy group at  $\delta_H$  6.10 (2H, s) and  $\delta_C$  102.8 ppm and two methoxyl groups at  $\delta_H$  3.96 (s)/  $\delta_C$  56.1 and  $\delta_H$  3.96 (s)/ $\delta_C$  56.8 ppm. For ring A, the two doublets at  $\delta$  7.98 (d,  $J = 9.0$  Hz, H-5) and  $\delta$  7.21 (d,  $J = 2.4$  Hz, H-8) and the double doublet at  $\delta$  7.01 (dd,  $J = 9.0, 2.4$  Hz, H-6), placed one of the methoxyls at C-7. The two proton signals at  $\delta$  6.90 (1H, s) and  $\delta$  7.47 (1H, s) suggested their *para*-relation on ring B (H-3' and H-6'). Thus, the other methoxyl group should be located at C-2'. The  $^1H$  and  $^{13}C$  NMR data were in good agreement with those of millettocalyxin A **[48]**, a flavone earlier isolated from the stem bark of this plant (Sritularak *et al.*, 2002a).



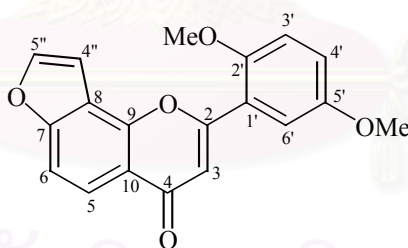
**[48]**

**Table 9 NMR Spectral data of compound MEL6 (acetone-*d*<sub>6</sub>) and millettocalyxin A (acetone-*d*<sub>6</sub>)**

Position	Compound MEL6		Millettocalyxin A	
	<sup>1</sup> H (mult., <i>J</i> in Hz)	<sup>13</sup> C	<sup>1</sup> H (mult., <i>J</i> in Hz)	<sup>13</sup> C
2	-	160.5	-	160.8
3	6.88 (s)	111.5	6.94 (s)	111.7
4	-	177.2	-	177.6
5	7.98 (d, 9.0)	126.9	8.03 (d, 8.8)	127.1
6	7.01 (dd, 9.0, 2.4)	114.6	7.06 (dd, 8.8, 2.4)	114.9
7	-	164.7	-	165.0
8	7.21 (d, 2.4)	101.1	7.26 (d, 2.4)	101.4
9	-	158.6	-	158.9
10	-	118.1	-	118.4
1'	-	113.1	-	113.5
2'	-	155.8	-	156.1
3'	6.90 (s)	95.9	6.95 (s)	96.1
4'	-	151.7	-	151.9
5'	-	142.4	-	142.7
6'	7.47 (s)	108.0	7.52 (s)	108.2
-OCH <sub>2</sub> O-	6.10 (s)	102.8	6.15 (s)	103.1
MeO-7	3.96 (s)	56.1	4.02 (s)	56.4
MeO-2'	3.96 (s)	56.8	4.02 (s)	57.1

### 1.7 Structure Determination of compound MEL7

Compound MEL7, a yellow powder, exhibited a quasi-molecular ion  $[M+H]^+$  peak at  $m/z$  323.1 in the ESIMS (**Figure 62**), analyzed for  $C_{19}H_{14}O_5$ . It showed UV absorptions at 248 and 293 nm (**Figure 63**). The IR spectrum displayed absorption bands at 1626 (conjugated C=O), 1588 (conjugated C=C), 1186 and 1074 (C-O stretching)  $cm^{-1}$  (**Figure 64**). The  $^1H$  NMR signal at 7.20 (1H, s, H-3) was suggestive of a furanoflavone nucleus (**Table 10, Figure 65**) (Mbafor *et al.*, 1995). The  $^1H$  NMR spectrum provided signals for a furan ring at  $\delta$  7.15 (1H, d,  $J = 1.5$  Hz, H-4'') and 7.73 ppm (1H, d,  $J = 1.8$  Hz, H-5''). This spectrum also exhibited the presence of two methoxyls at  $\delta$  3.86 (3H, s) and 3.89 ppm (3H, s). The *ortho*-coupled protons at  $\delta$  8.15 (1H, d,  $J = 8.7$  Hz) and 7.54 ppm (1H, d,  $J = 8.7$  Hz) were assigned to H-5 and H-6, respectively. The three coupled aromatic protons at  $\delta$  6.98 (1H, d,  $J = 9.0$  Hz), 7.04 (1H, dd,  $J = 9.0, 3.0$  Hz) and 7.49 (1H, d,  $J = 3.0$  Hz) were assigned to H-3', H-4' and H-6', respectively. By comparing the above spectral information with reported the  $^1H$  and  $^{13}C$  NMR data, compound MEL7 was identified as millettocalyxin C [**50**], which was previously isolated from the stem bark of this plant (Sritularak *et al.*, 2002a).



[50]

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**Table 10 NMR Spectral data of compound MEL7 (CDCl<sub>3</sub>) and millettocalyxin C (CDCl<sub>3</sub>)**

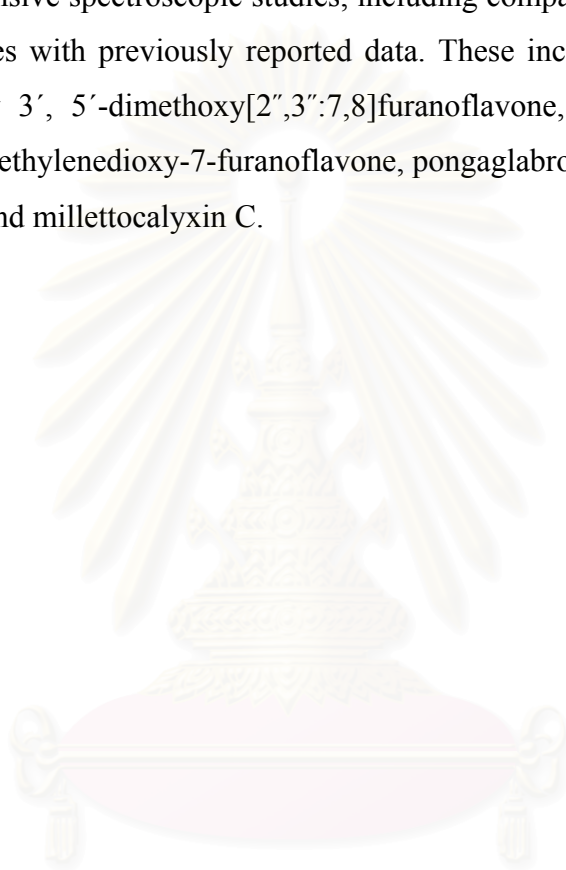
Position	Compound MEL7		Millettocalyxin C	
	<sup>1</sup> H (mult., <i>J</i> in Hz)	<sup>13</sup> C	<sup>1</sup> H (mult., <i>J</i> in Hz)	<sup>13</sup> C
2	-	159.8	-	159.8
3	7.20 (s)	113.2	7.23 (s)	113.2
4	-	178.6	-	178.7
5	8.15 (d, 8.7)	121.8	8.16 (d, 9.0)	121.8
6	7.54 (d, 8.7)	110.0	7.54 (d, 9.0)	110.0
7	-	158.3	-	158.3
8	-	117.1	-	117.2
9	-	151.0	-	151.0
10	-	119.2	-	119.3
1'	-	121.4	-	121.4
2'	-	152.4	-	152.5
3'	6.98 (d, 9.0)	113.1	6.99 (d, 9.0)	113.2
4'	7.04 (dd, 9.0, 3.0)	117.2	7.04 (dd, 9.0, 3.0)	117.3
5'	-	153.5	-	153.6
6'	7.49 (d, 3.0)	114.7	7.50 (d, 3.0)	114.7
4''	7.15 (d, 1.8)	104.2	7.16 (d, 2.0)	104.3
5''	7.73 (d, 1.8)	145.6	7.75 (d, 2.0)	145.7
MeO-2'	3.89 (s)	56.2	3.95 (s)	56.2
MeO-5'	3.86 (s)	55.9	3.91 (s)	56.0



## CHAPTER V

### CONCLUSION

Phytochemical investigation of the leaves of *Millettia erythrocalyx* Gagnep. led to the isolation of seven flavonoid compounds. The structure of all of these isolates were determined by extensive spectroscopic studies, including comparison of their UV, IR, MS and NMR properties with previously reported data. These include a new furanoflavone compound, namely 3', 5'-dimethoxy[2'',3'':7,8]furanoflavone, and the known flavone compounds 3',4'-methylenedioxy-7-furanoflavone, pongaglabrone, gamatin, milletenin C, millettocalyxin A and millettocalyxin C.



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**APPENDICES**

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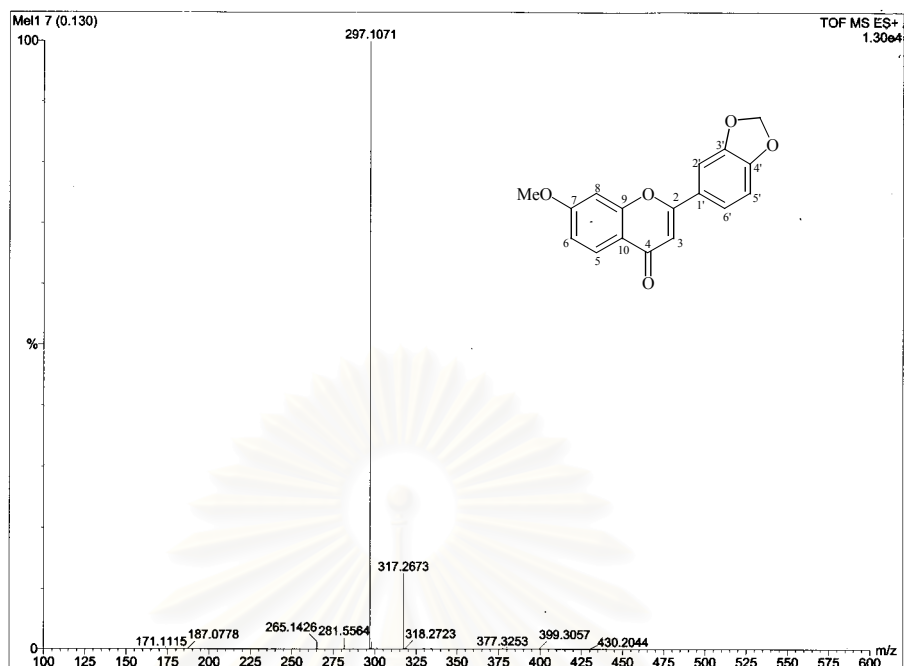


Figure 12 ESI Mass spectrum of compound MEL1

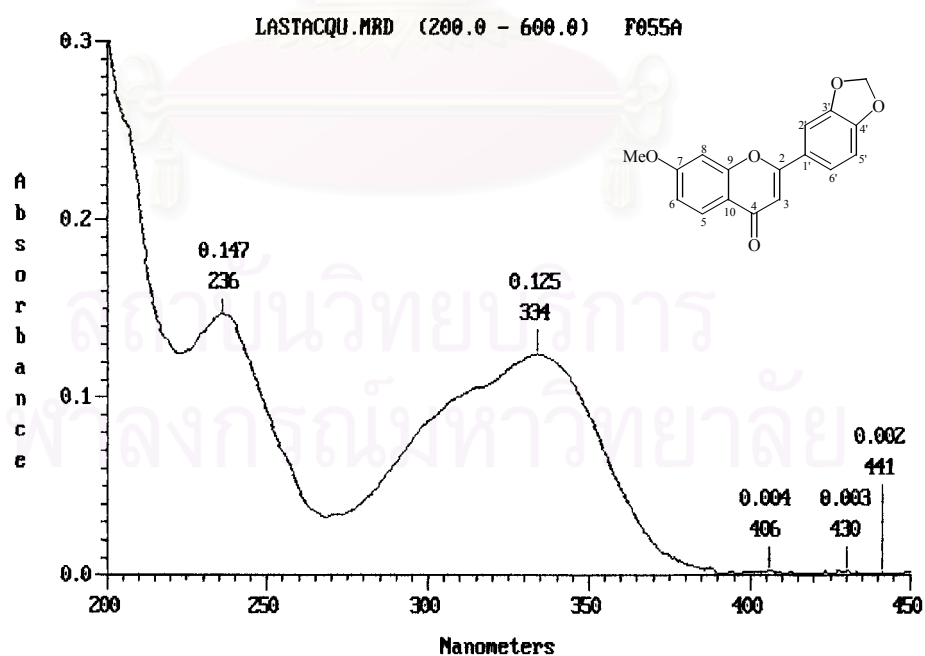


Figure 13 UV Spectrum of compound MEL1 (MeOH)

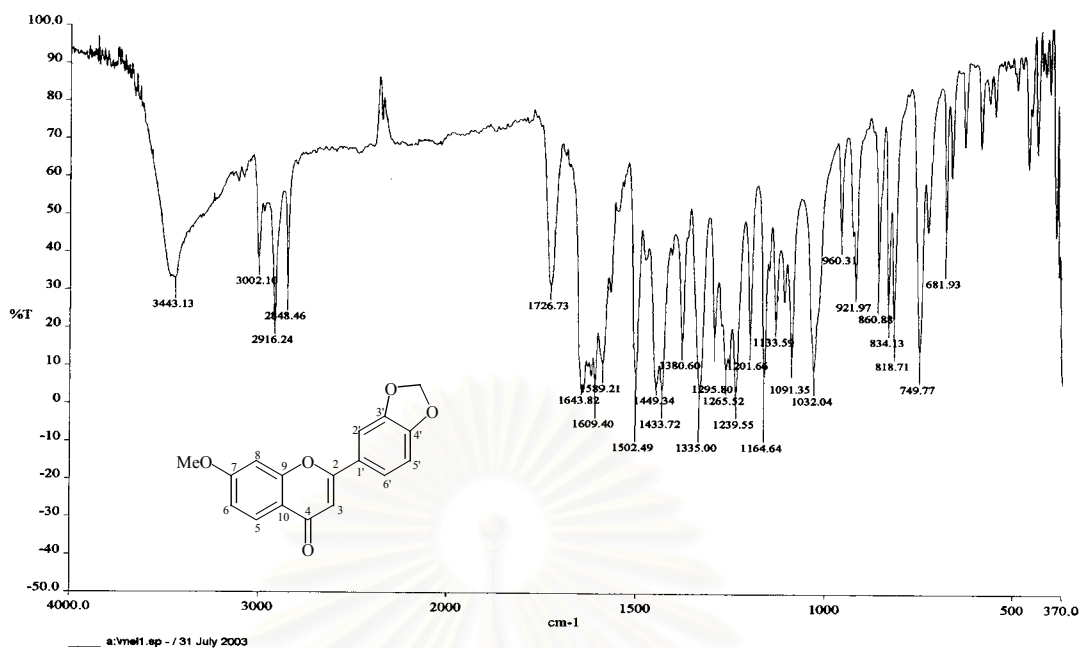


Figure 14 IR Spectrum of compound MEL1 (film)

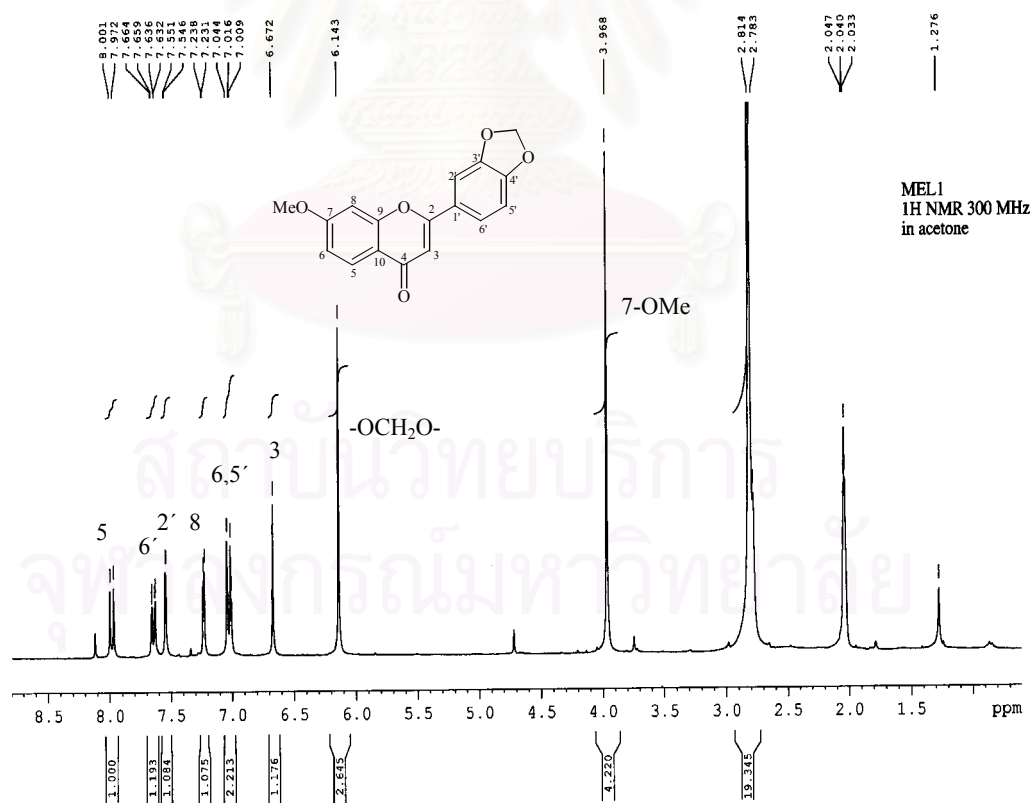


Figure 15 <sup>1</sup>H-NMR (300 MHz) Spectrum of compound MEL1 (acetone-d<sub>6</sub>)

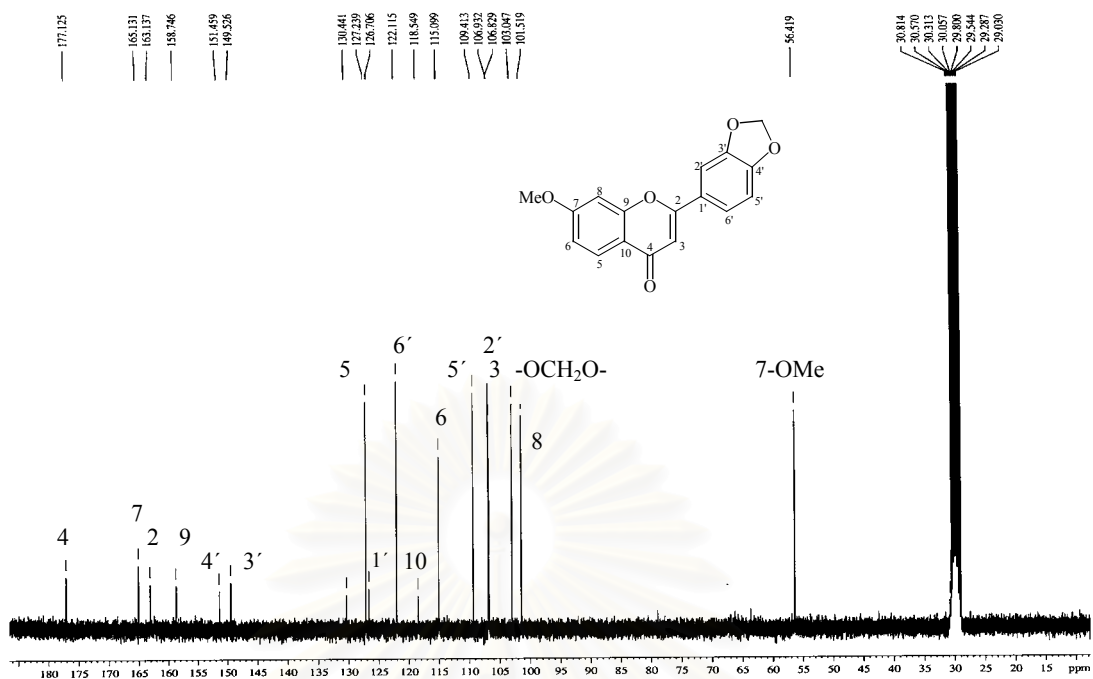


Figure 16  $^{13}\text{C-NMR}$  (75 MHz) Spectrum of compound MEL1 (acetone- $d_6$ )

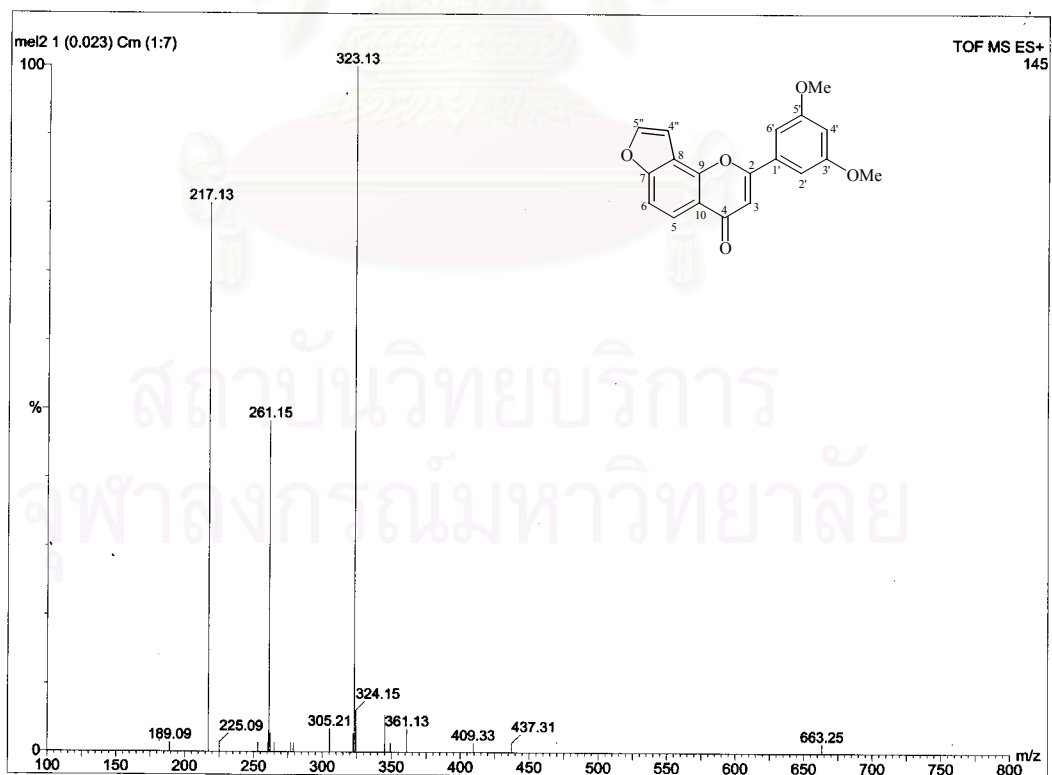


Figure 17 ESI Mass spectrum of compound MEL2

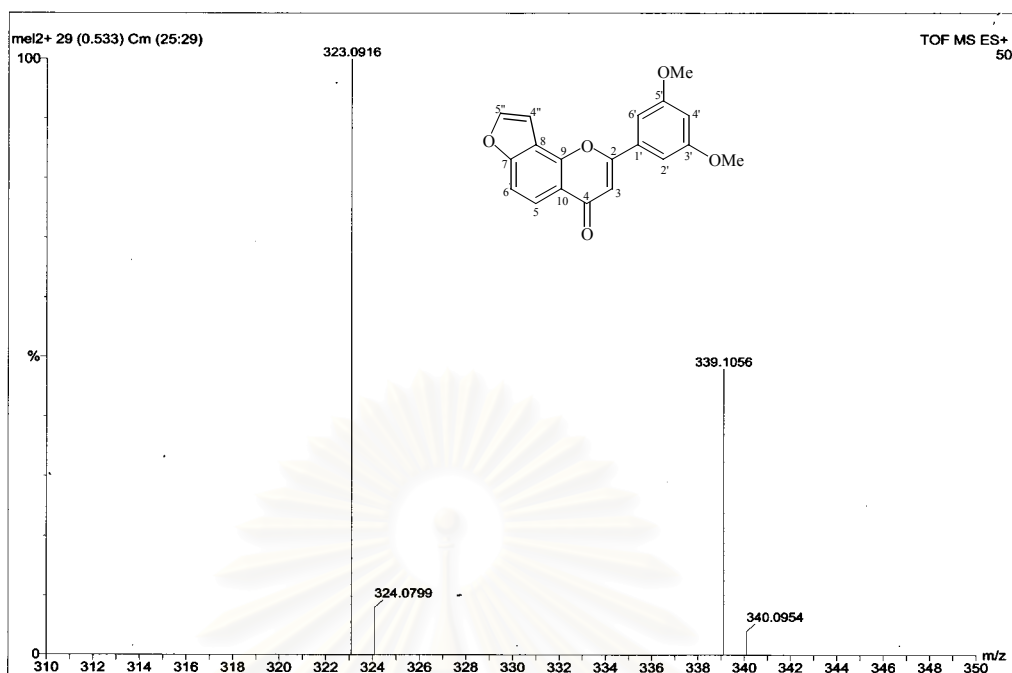


Figure 18 HRESI Mass Spectrum of compound MEL2

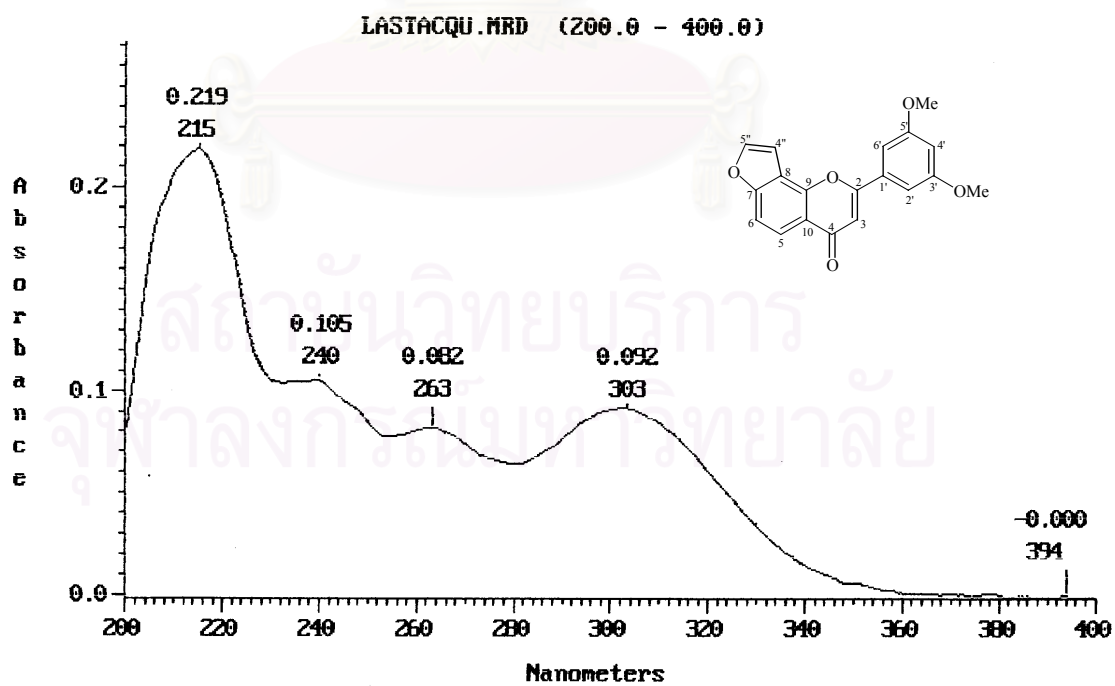
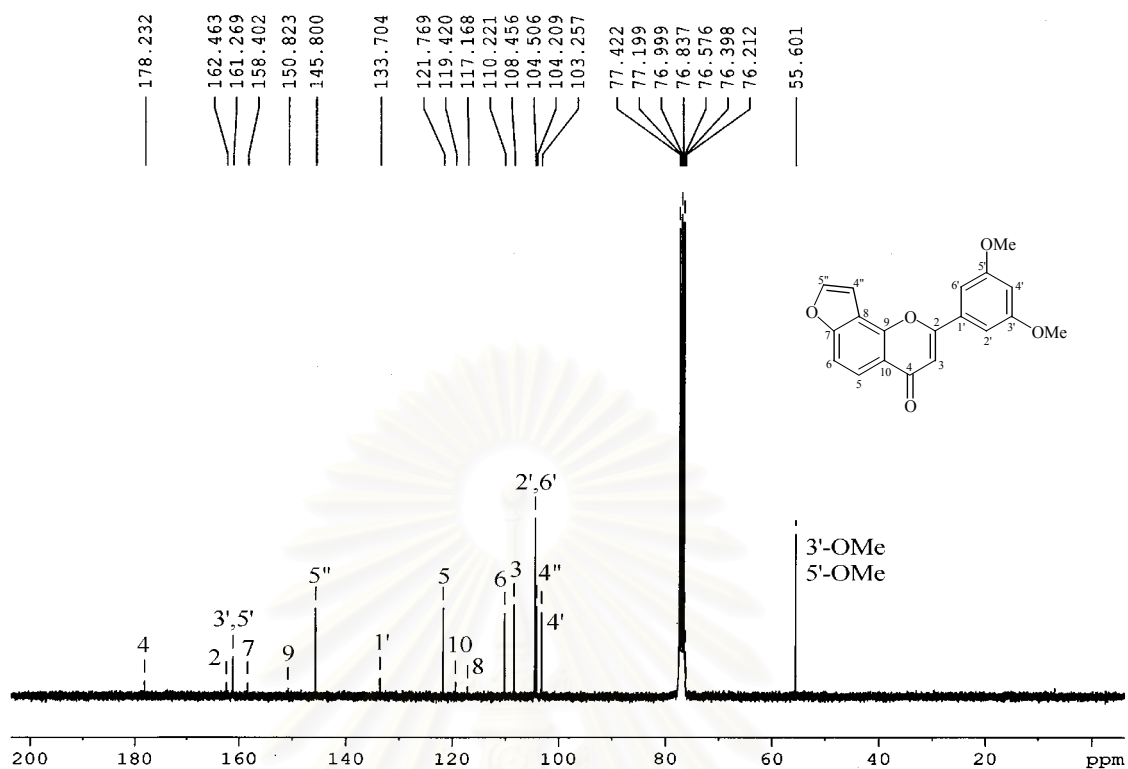
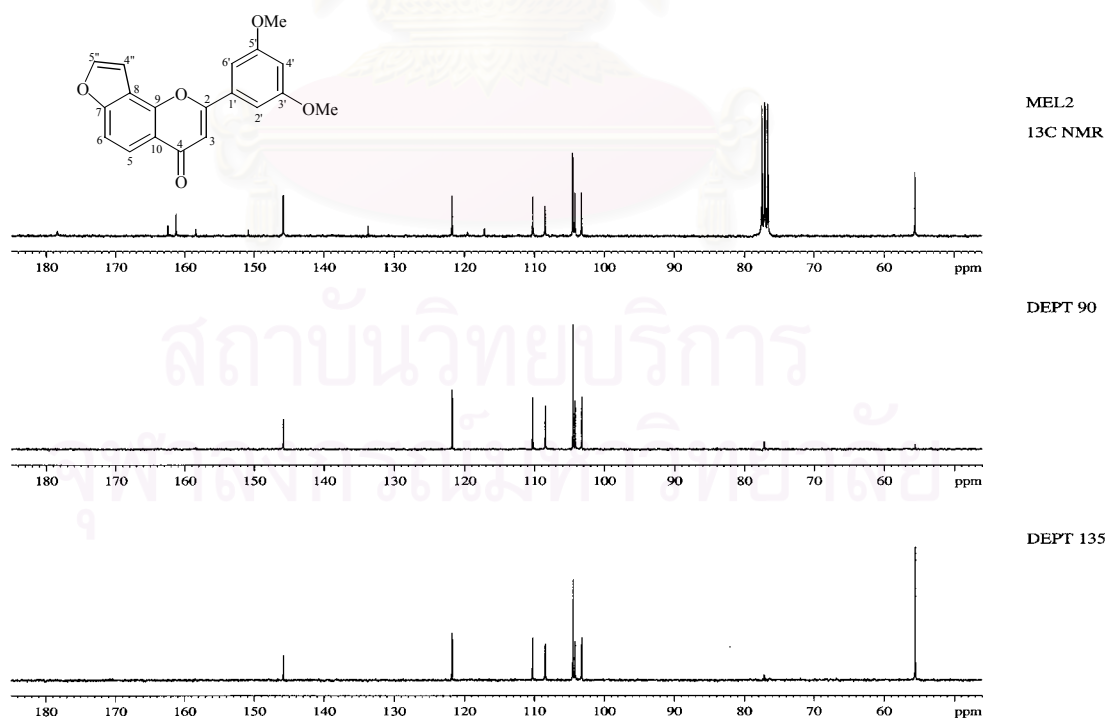


Figure 19 UV Spectrum of compound MEL2 (MeOH)





**Figure 22**  $^{13}\text{C}$ -NMR Spectrum (75 MHz) of compound MEL2 ( $\text{CDCl}_3$ )



**Figure 23**  $^{13}\text{C}$ -NMR, DEPT 90 and DEPT 135 Spectra (75 MHz) of compound MEL2 (in  $\text{CDCl}_3$ )

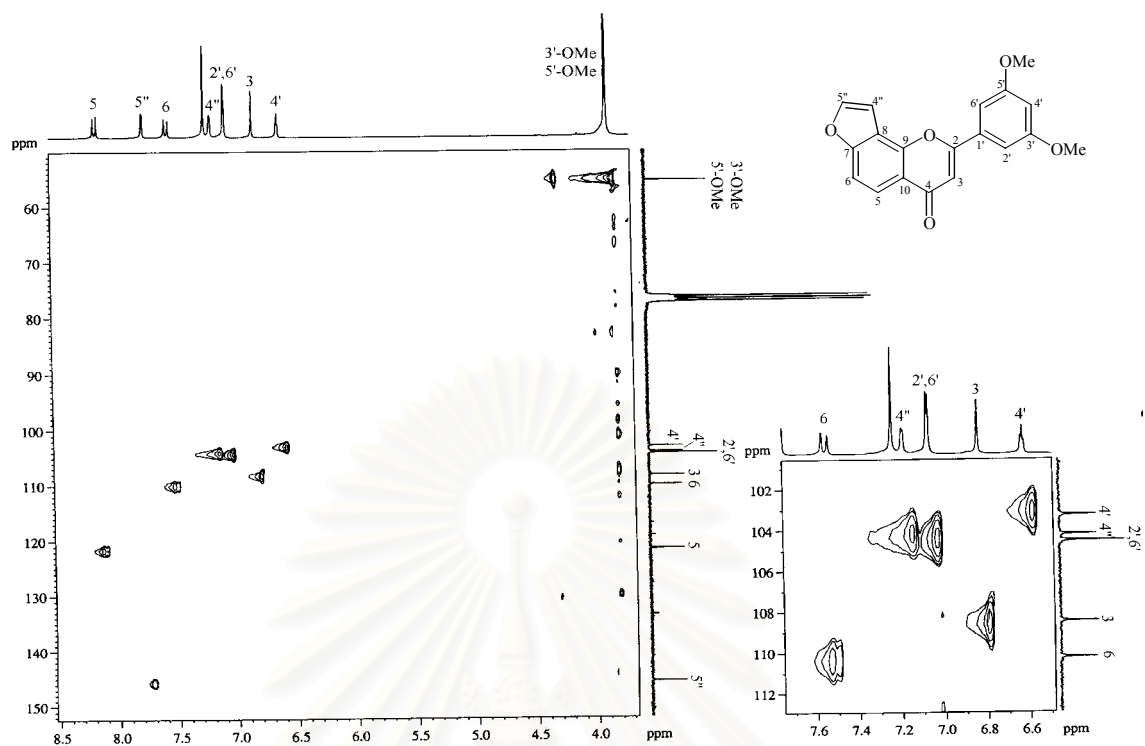


Figure 24 HMQC Spectrum of compound MEL2 (CDCl<sub>3</sub>)

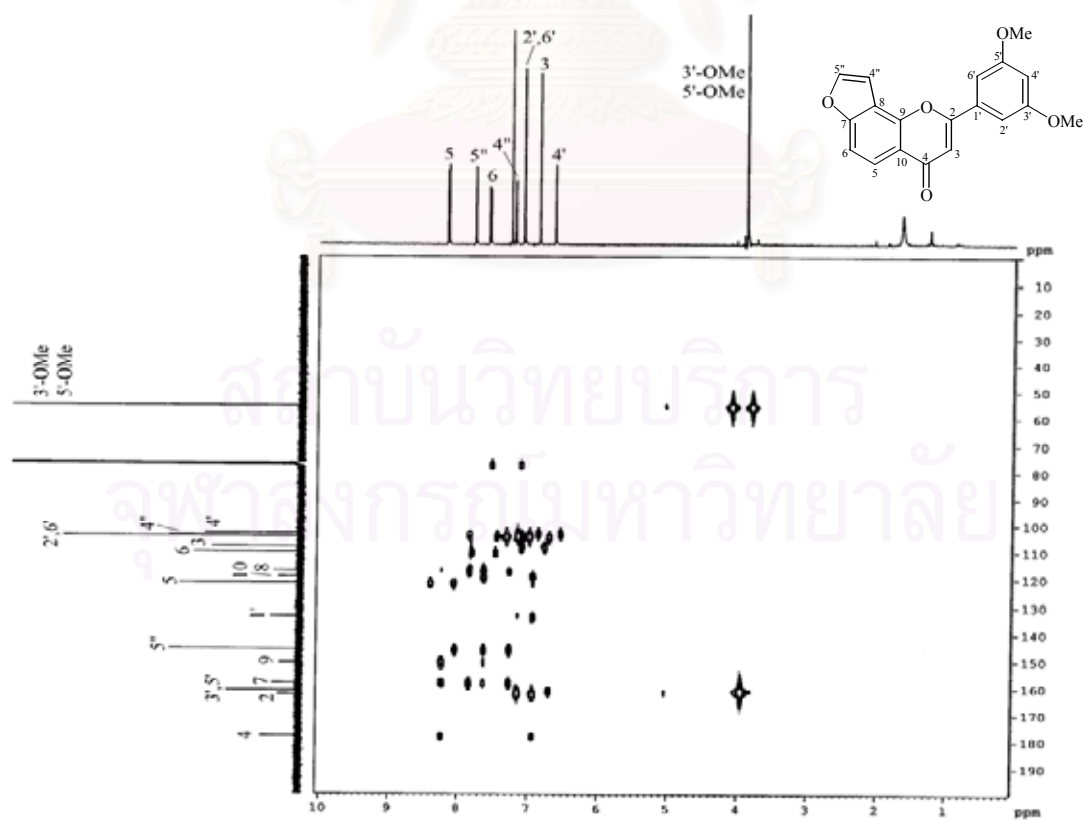
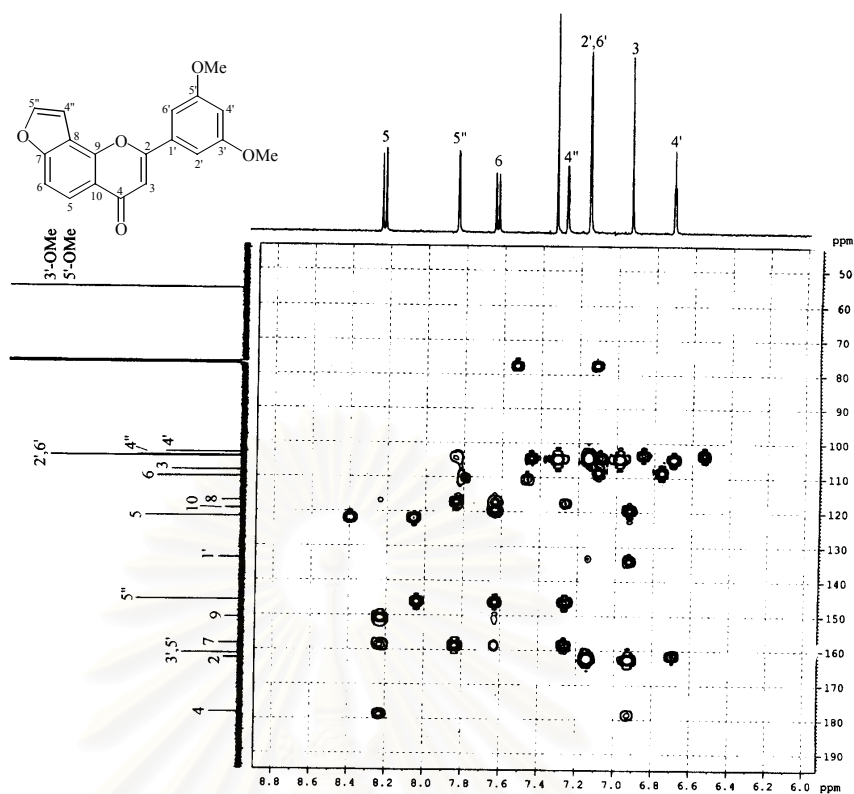
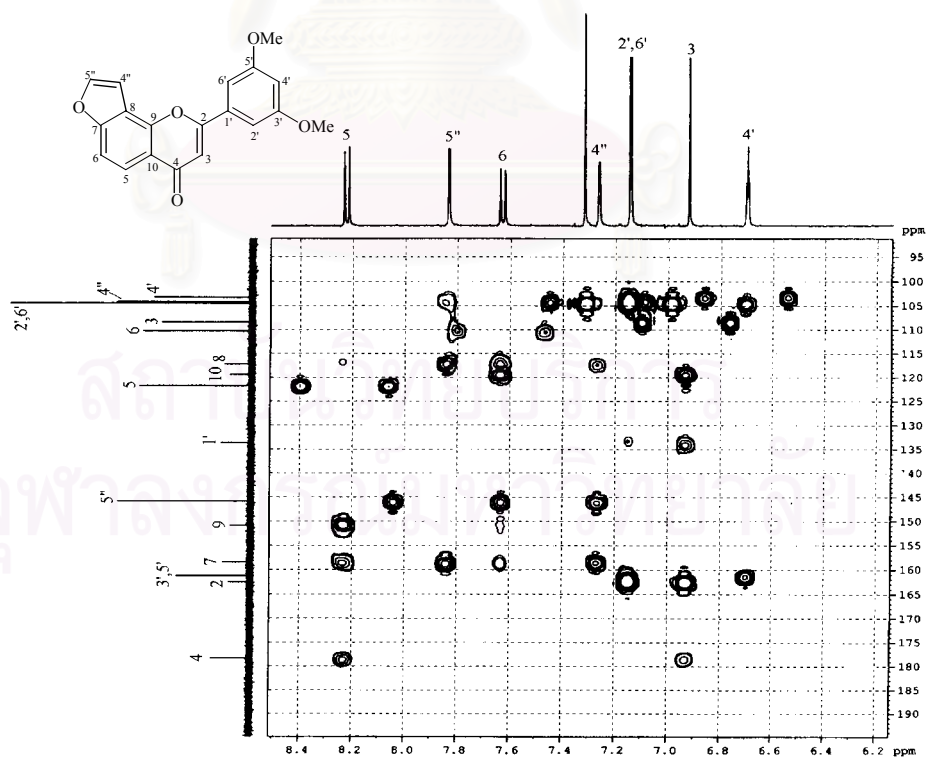


Figure 25 HMBC Spectrum of MEL2 (CDCl<sub>3</sub>)

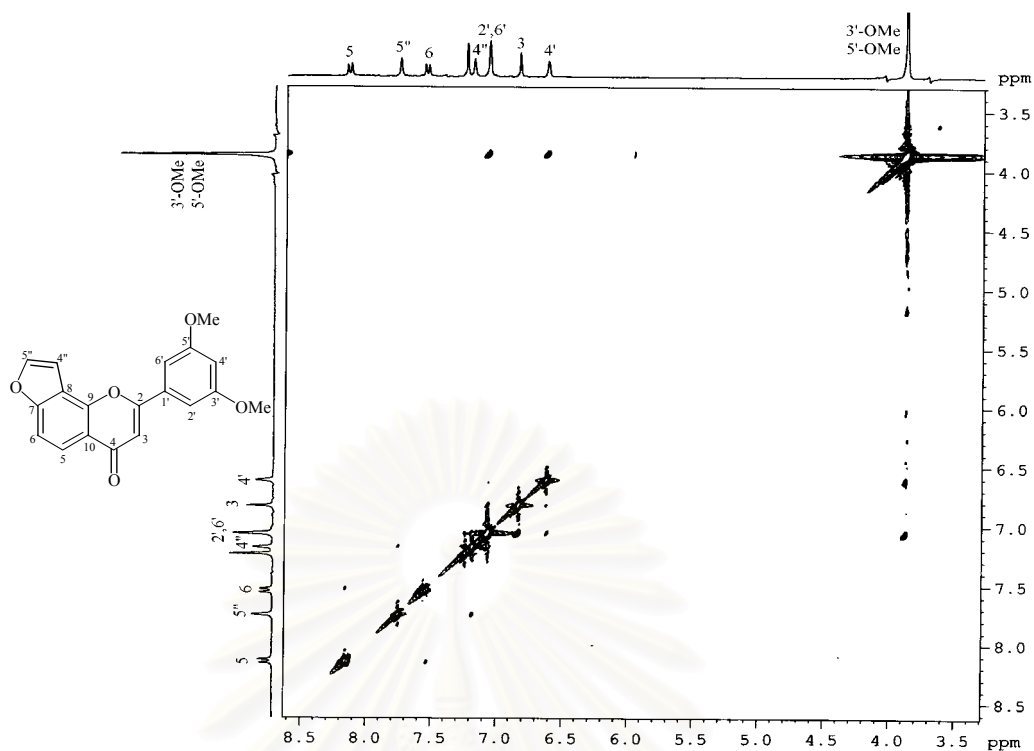


**Figure 26** HMBC Spectrum of compound MEL2 (CDCl<sub>3</sub>)  
(expanded,  $\delta_{\text{H}}$  6.0-8.8 ppm,  $\delta_{\text{C}}$  42-194 ppm)

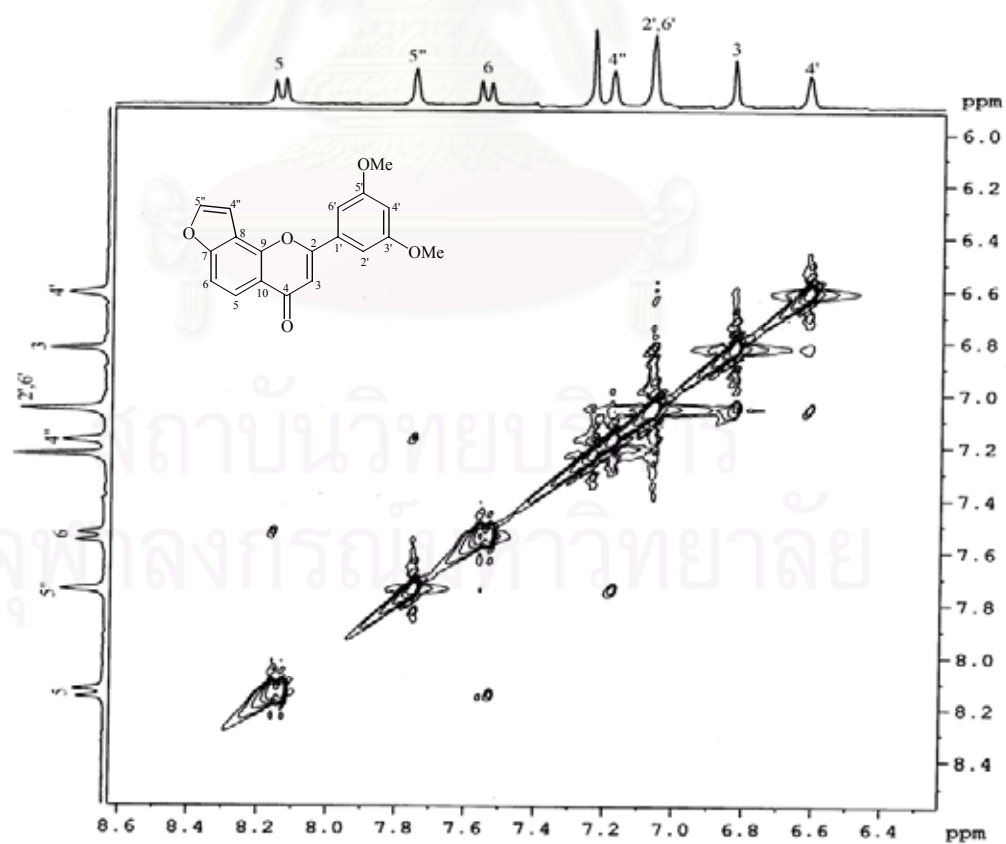


**Figure 27** HMBC Spectrum of compound MEL2 (CDCl<sub>3</sub>)  
(expanded,  $\delta_{\text{H}}$  6.2-8.5 ppm,  $\delta_{\text{C}}$  91-195 ppm)





**Figure 28** NOESY Spectrum of compound MEL2 (CDCl<sub>3</sub>)



**Figure 29** NOESY Spectrum of compound MEL2 (CDCl<sub>3</sub>)  
(expanded,  $\delta_{\text{H}}$  6.0-8.6 ppm)

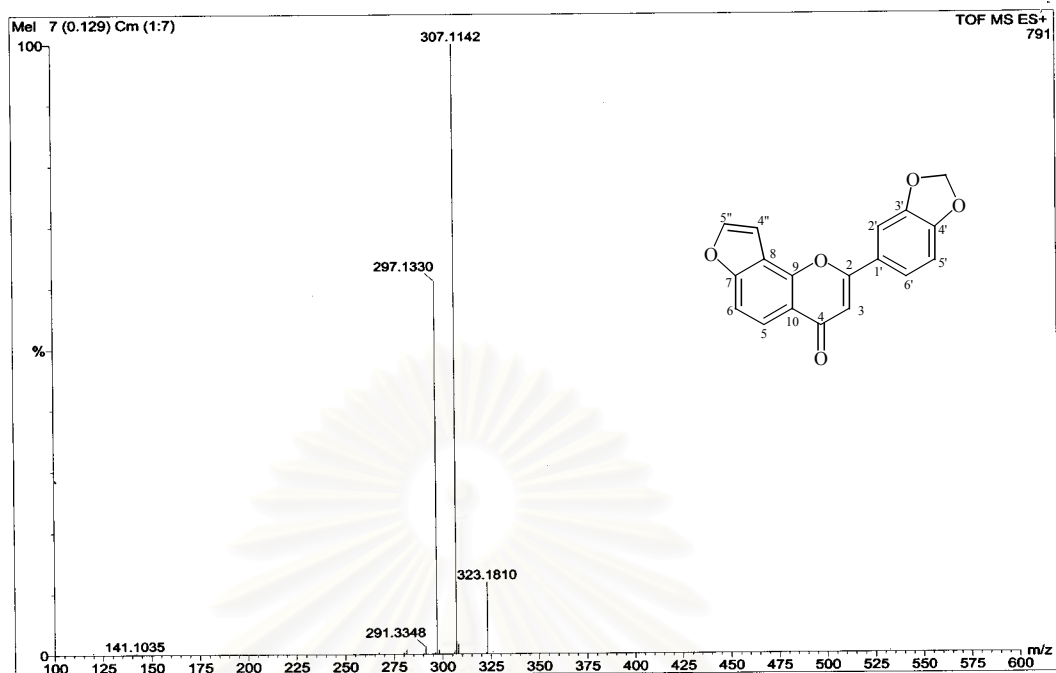


Figure 30 ESI Mass spectrum of compound MEL3 (CDCl<sub>3</sub>)

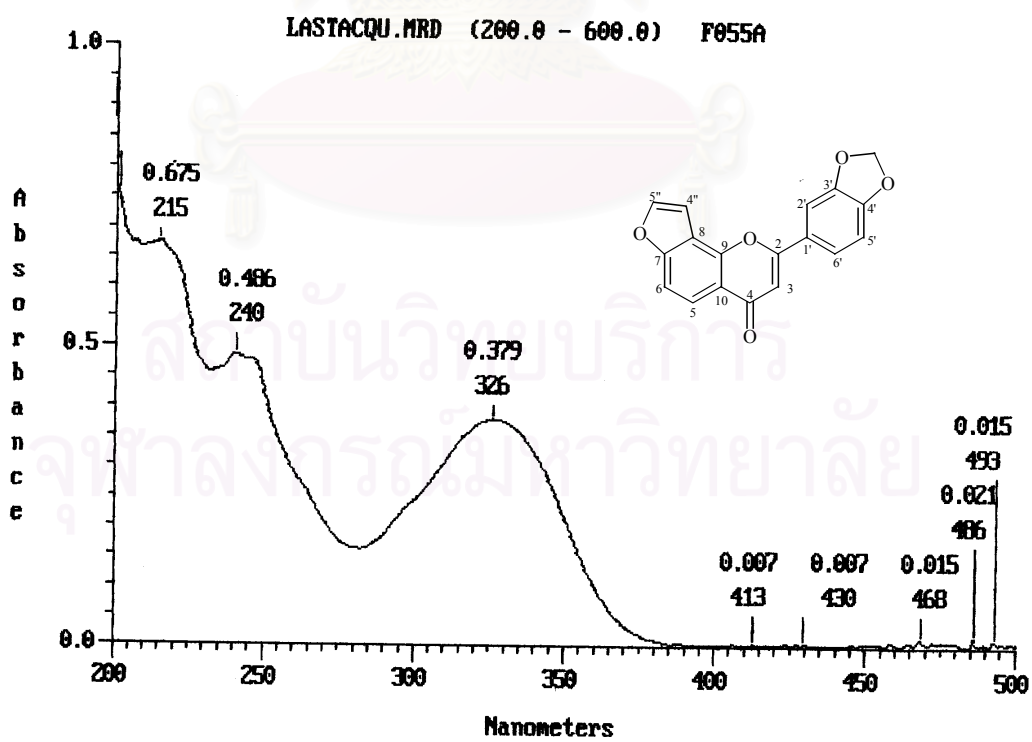


Figure 31 UV Spectrum of compound MEL3 (MeOH)

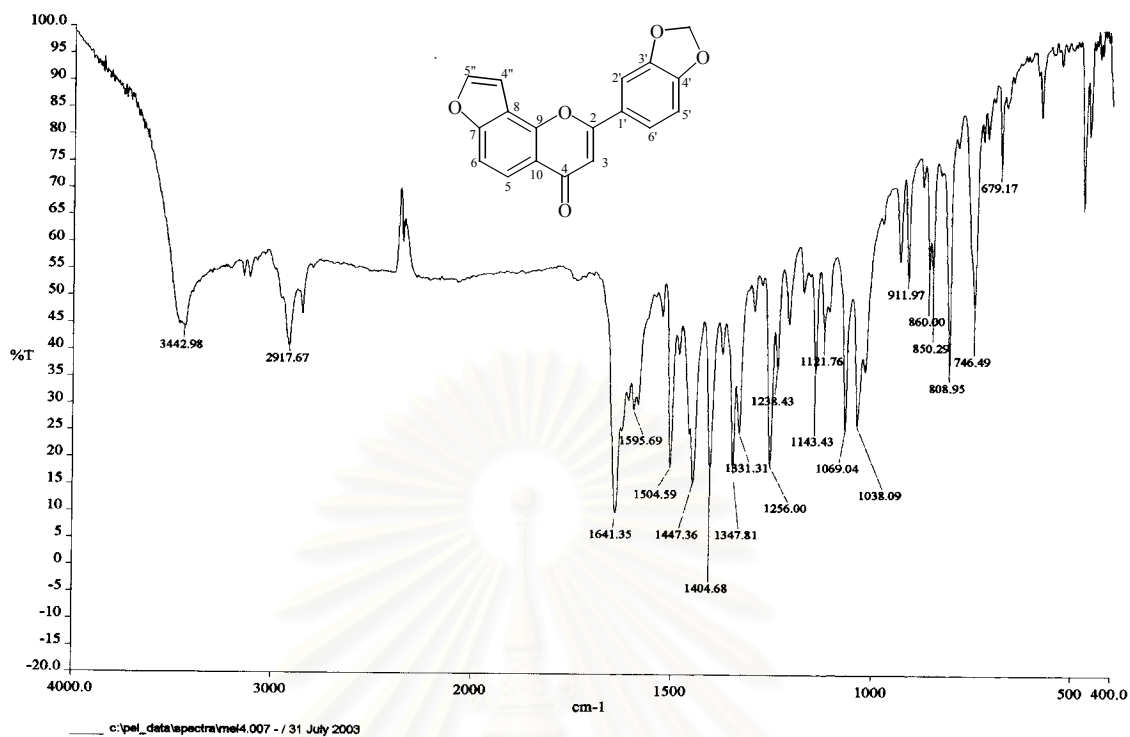


Figure 32 IR Spectrum of compound MEL3 (film)

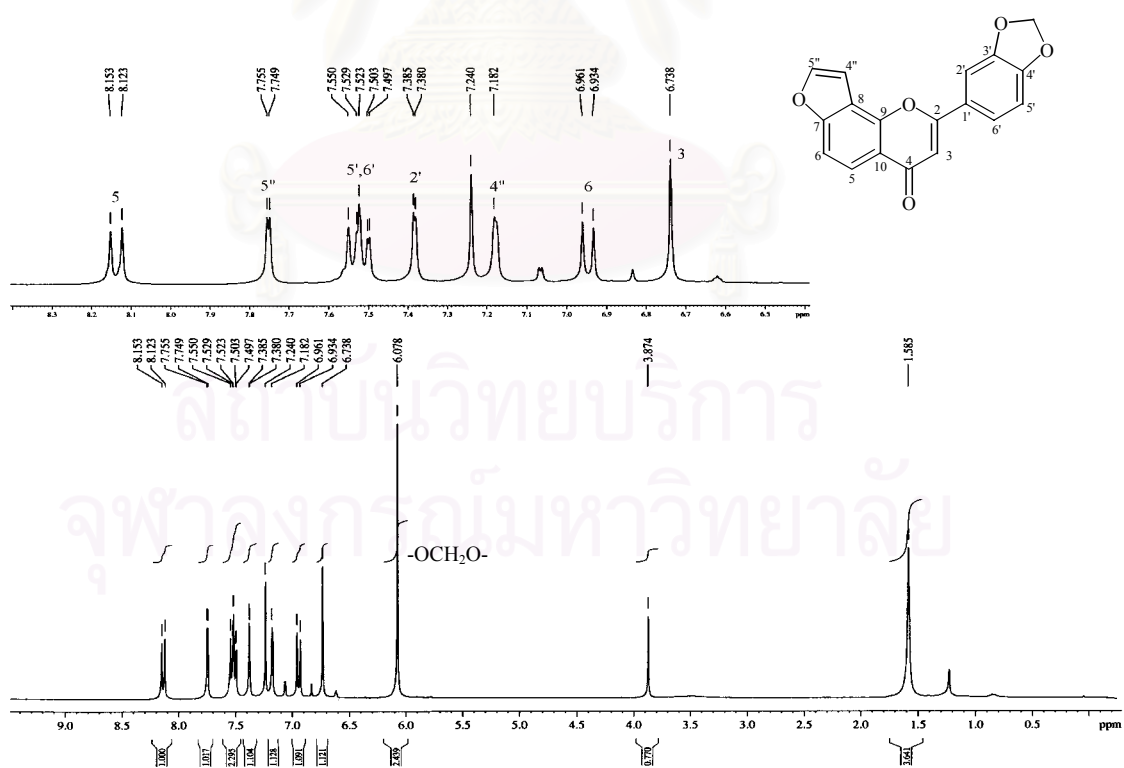
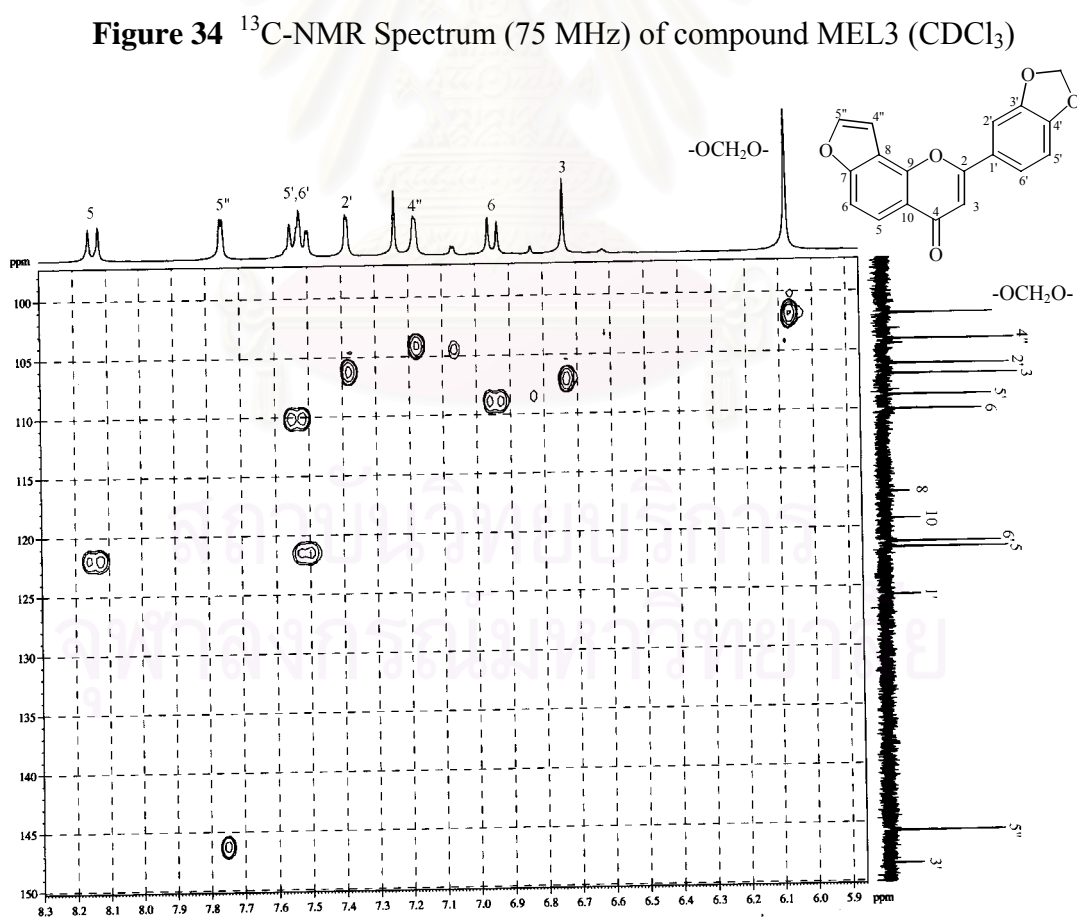
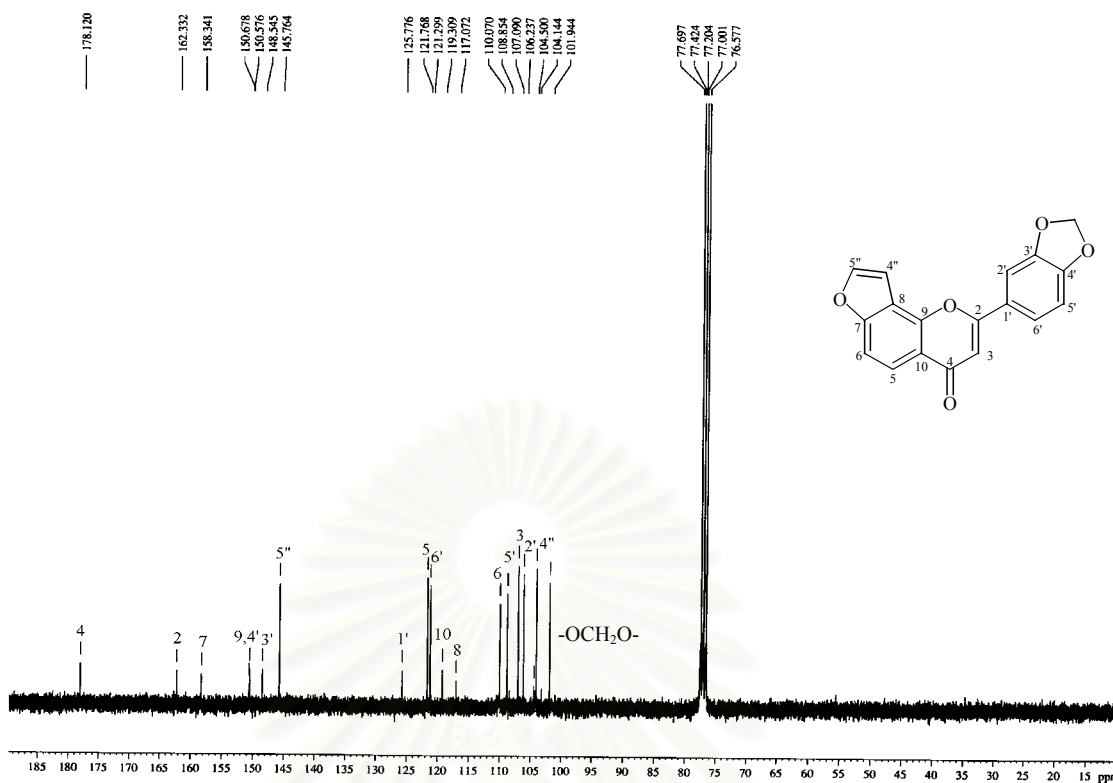


Figure 33 <sup>1</sup>H-NMR Spectrum (300 MHz) of compound MEL3 (CDCl<sub>3</sub>)



**Figure 35** HMBC Spectrum of compound MEL3 ( $\text{CDCl}_3$ ) (expanded,  $\delta_{\text{H}}$  5.9-8.3 ppm,  $\delta_{\text{C}}$  97-150 ppm)

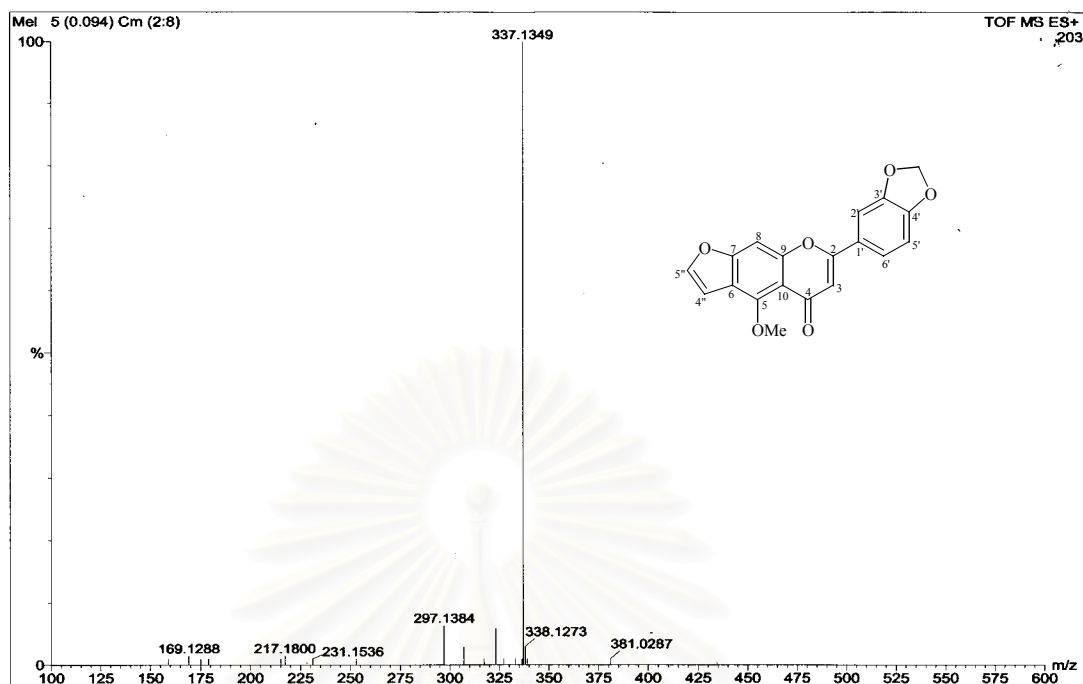


Figure 36 ESI Mass spectrum of compound MEL4

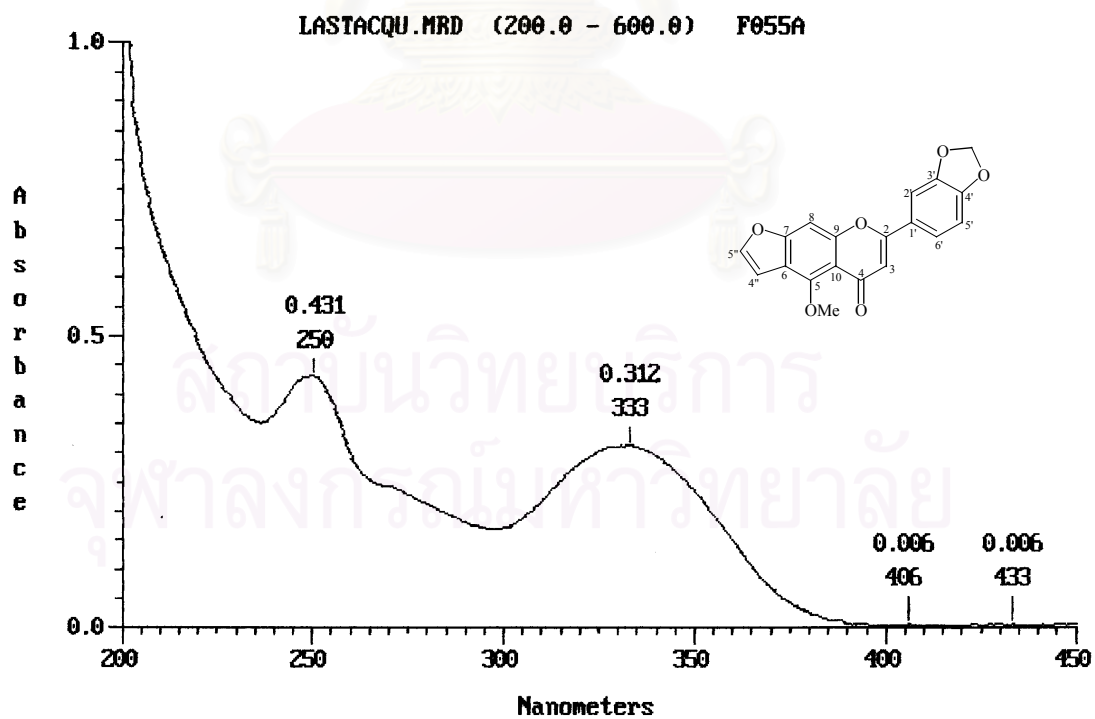


Figure 37 UV Spectrum of compound MEL4 (MeOH)

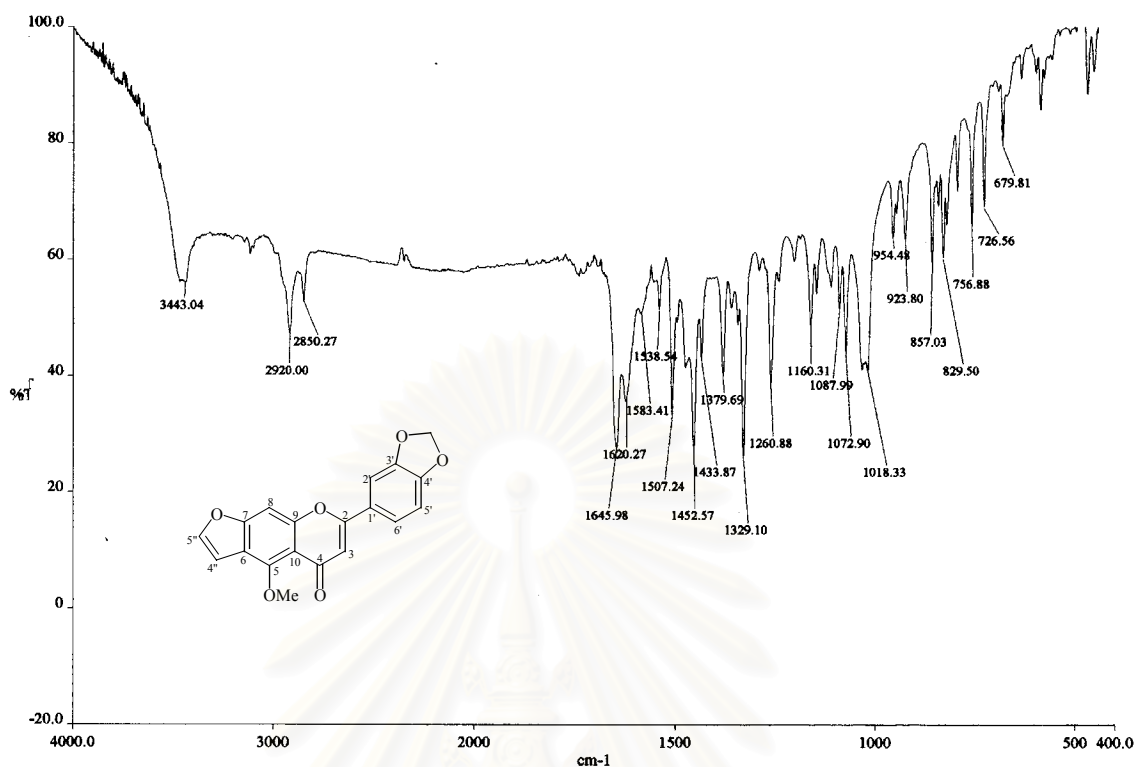


Figure 38 IR Spectrum of compound MEL4 (film)

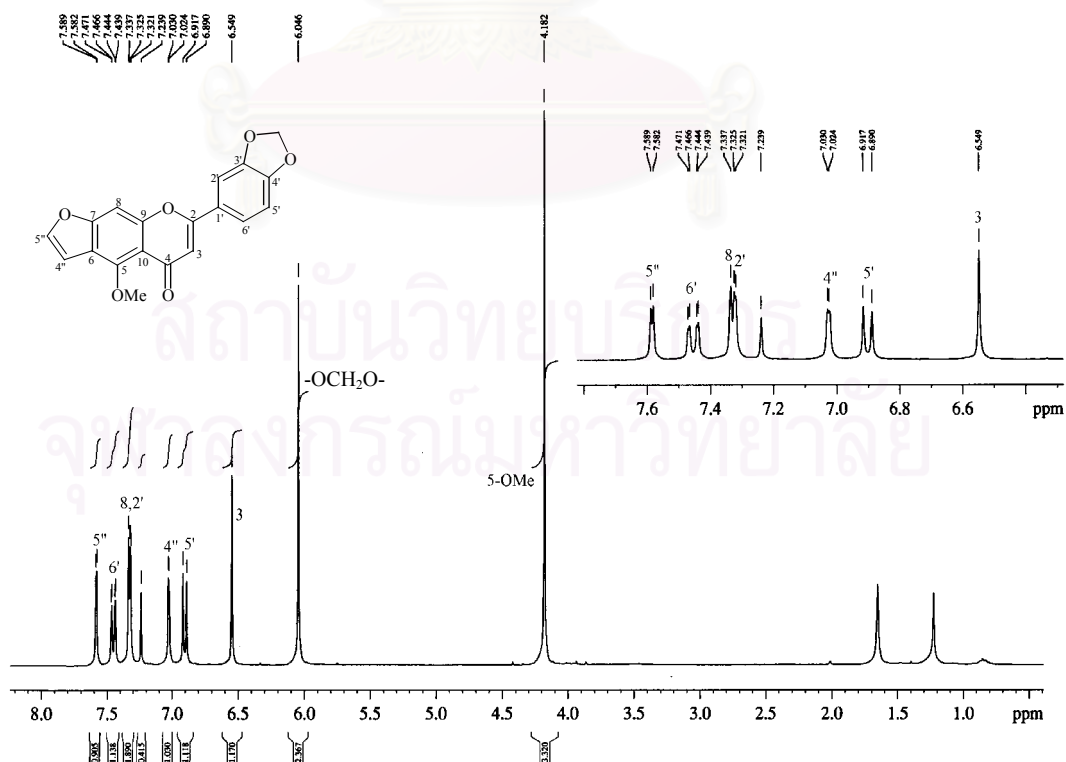


Figure 39 <sup>1</sup>H-NMR Spectrum (300 MHz) of compound MEL4 (CDCl<sub>3</sub>)

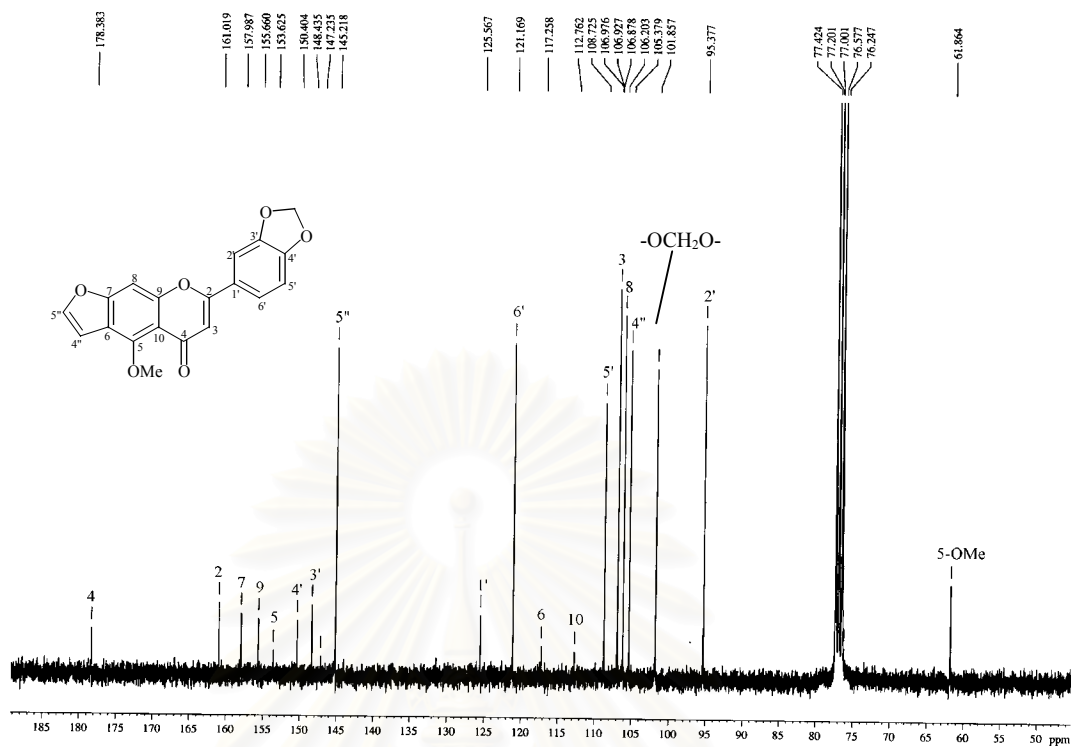


Figure 40  $^{13}\text{C}$ -NMR Spectrum (75 MHz) of compound MEL4 ( $\text{CDCl}_3$ )

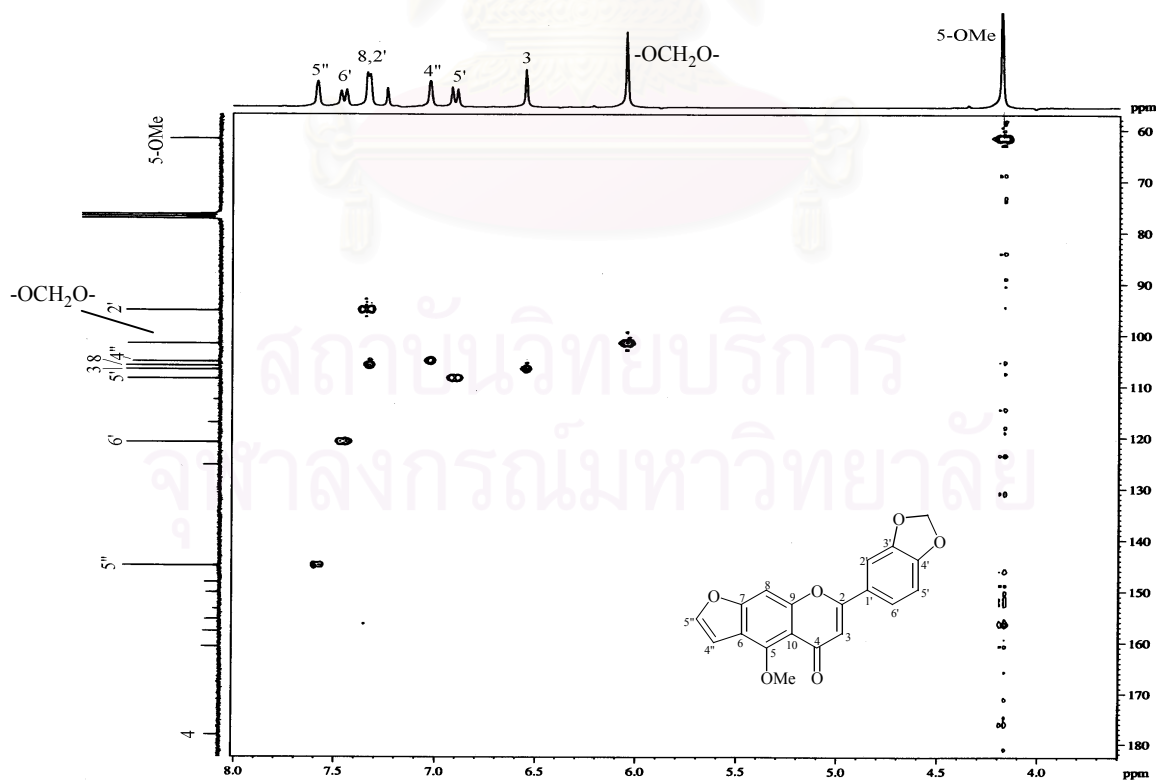
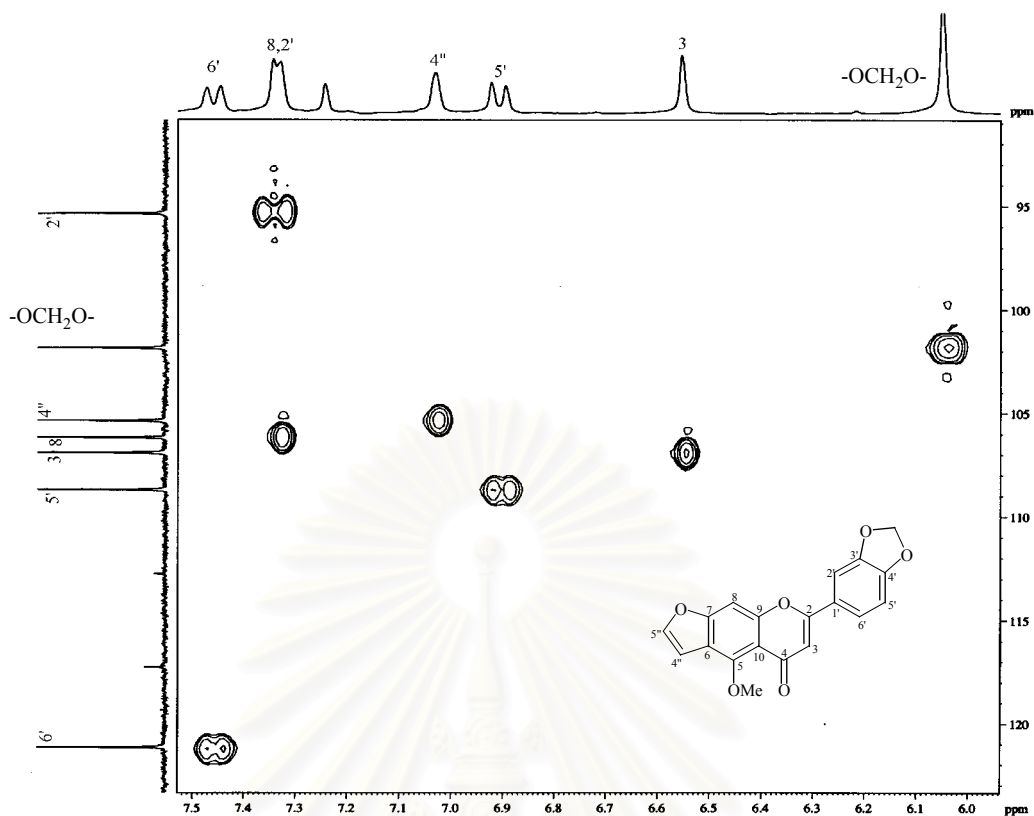
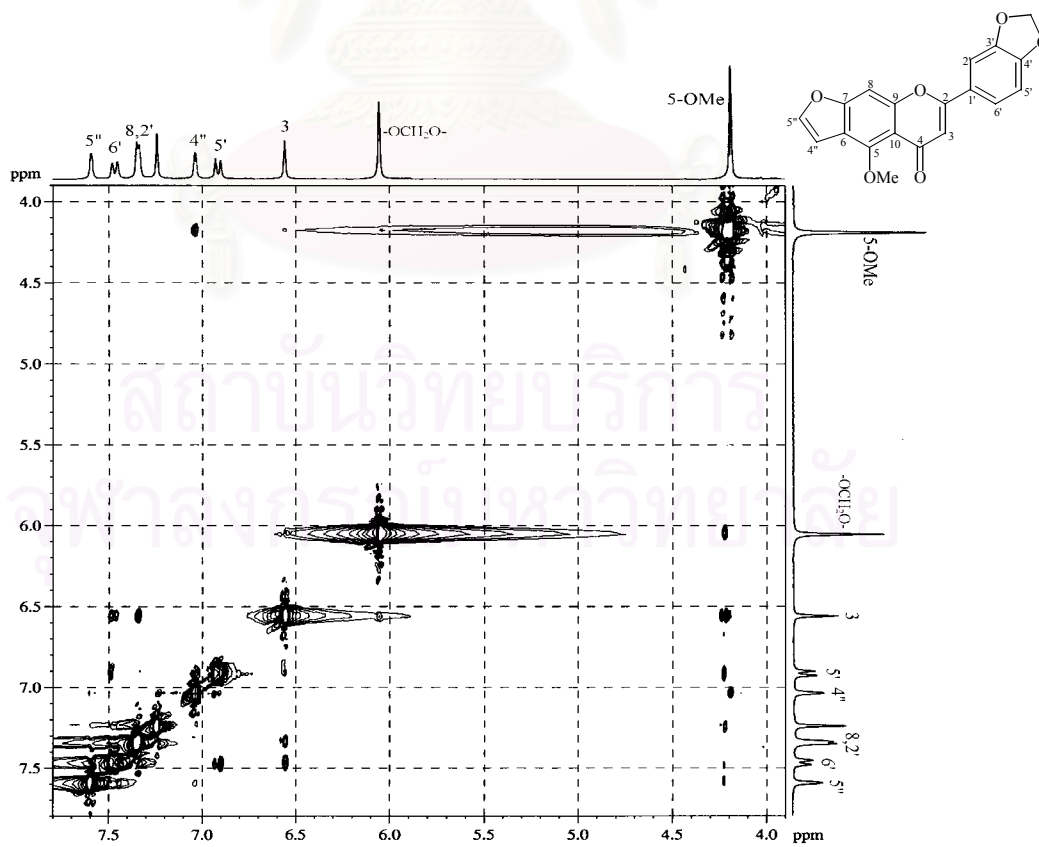


Figure 41 HMBC Spectrum of compound MEL4 ( $\text{CDCl}_3$ )

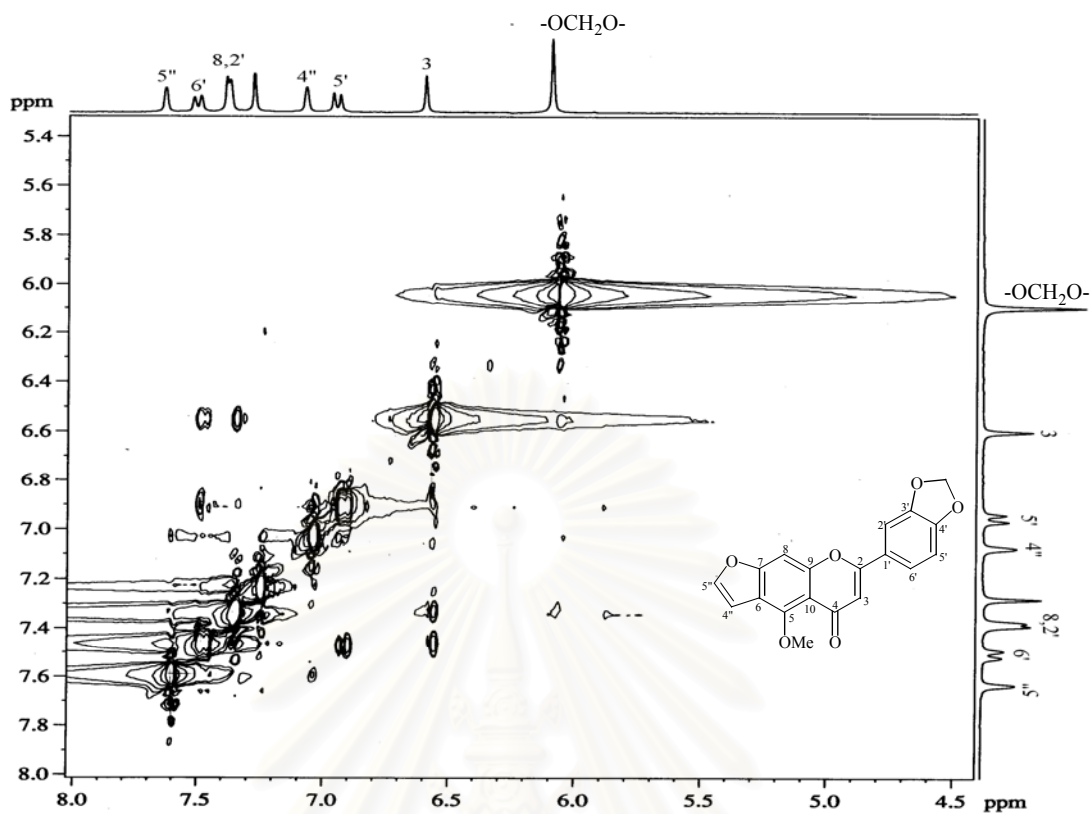


**Figure 42** HMQC Spectrum of compound MEL4 ( $CDCl_3$ )  
(expanded,  $\delta_H$  5.4-7.8 ppm,  $\delta_C$  91-123 ppm)

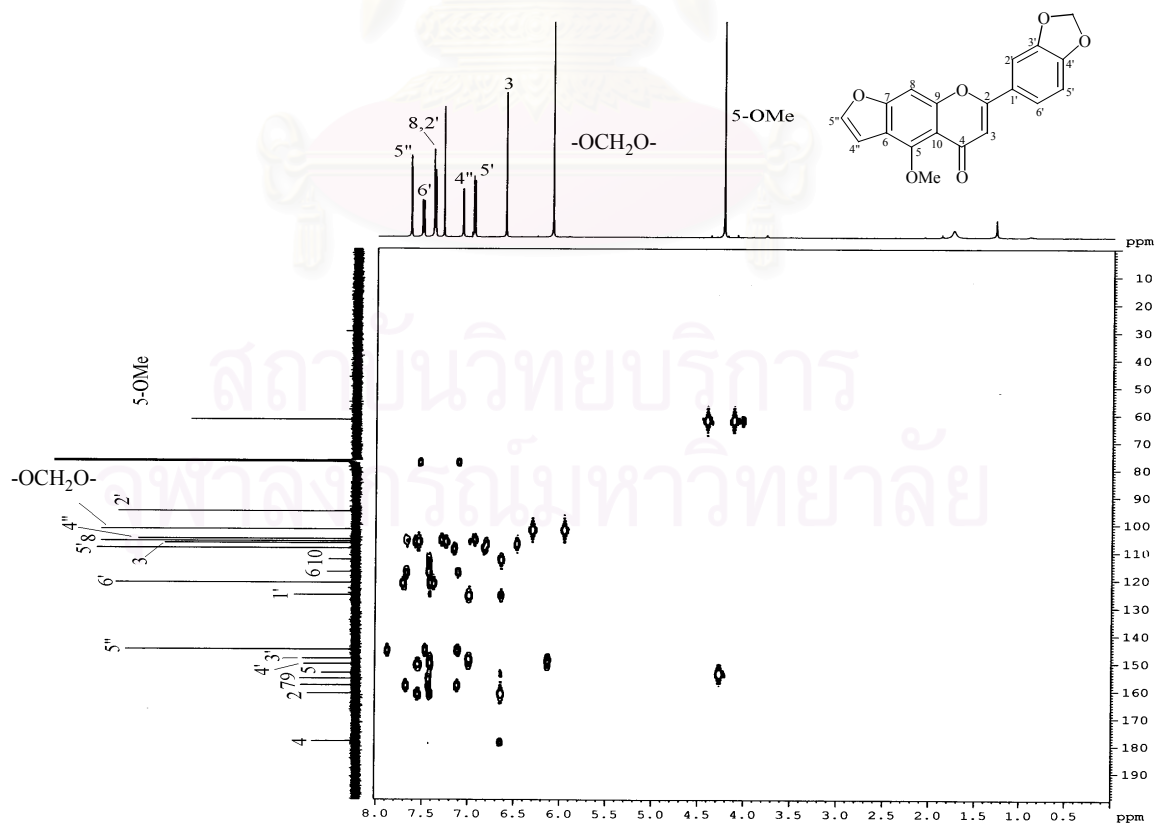


**Figure 43** NOESY Spectrum of compound MEL4

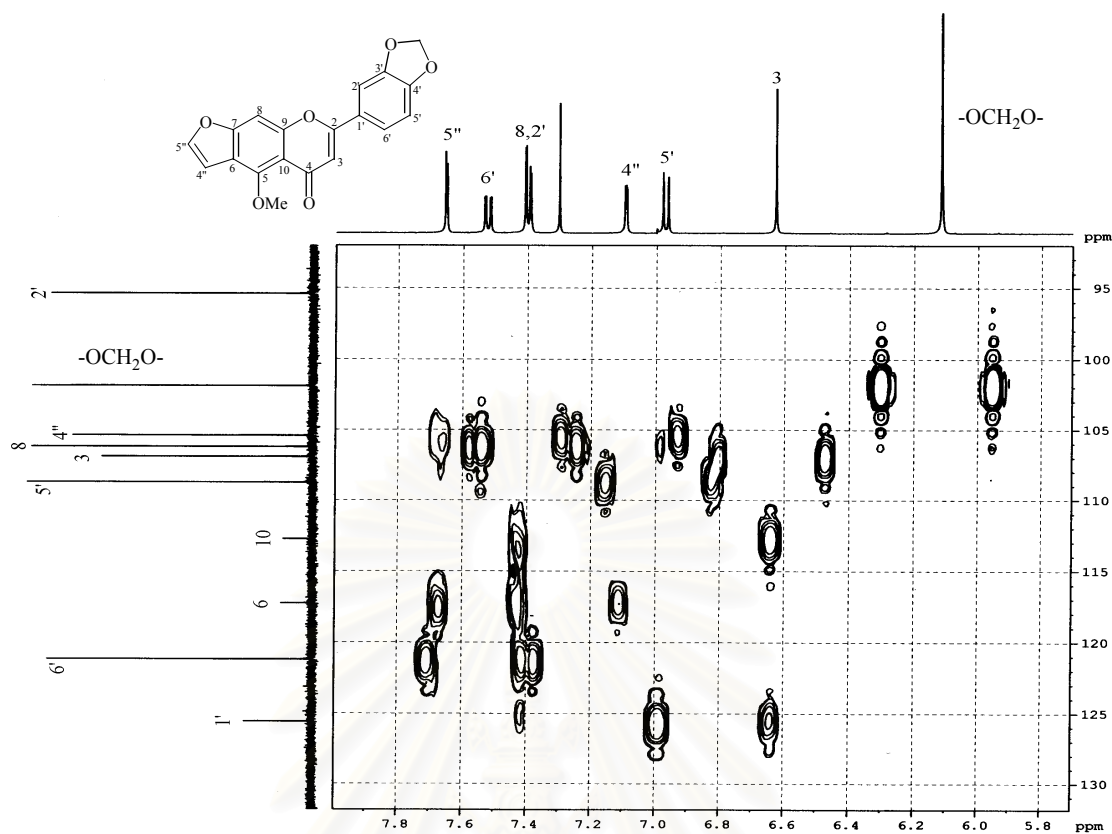




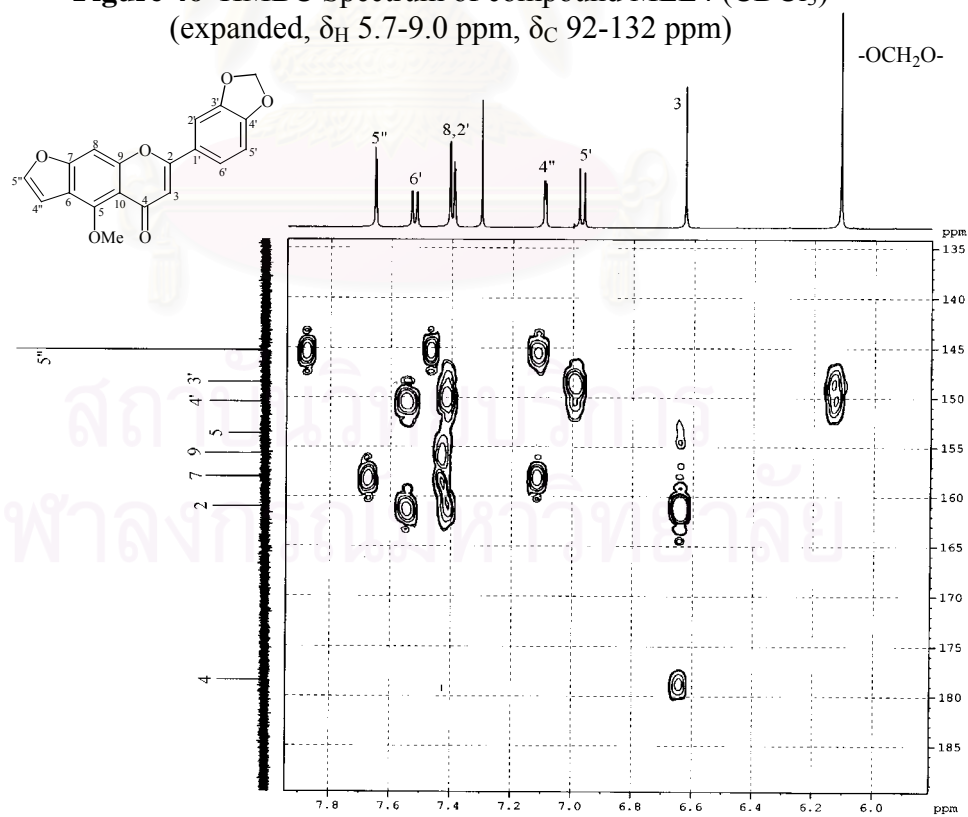
**Figure 44** NOESY Spectrum of compound MEL4  
(expanded,  $\delta_{\text{H}}$  4.5-8.0)



**Figure 45** HMBC Spectrum of compound MEL4 (CDCl<sub>3</sub>)



**Figure 46** HMBC Spectrum of compound MEL4 (CDCl<sub>3</sub>)  
(expanded,  $\delta_H$  5.7-9.0 ppm,  $\delta_C$  92-132 ppm)



**Figure 47** HMBC Spectrum of compound MEL4 (CDCl<sub>3</sub>)  
(expanded,  $\delta_H$  5.8-7.9 ppm,  $\delta_C$  85-190 ppm)

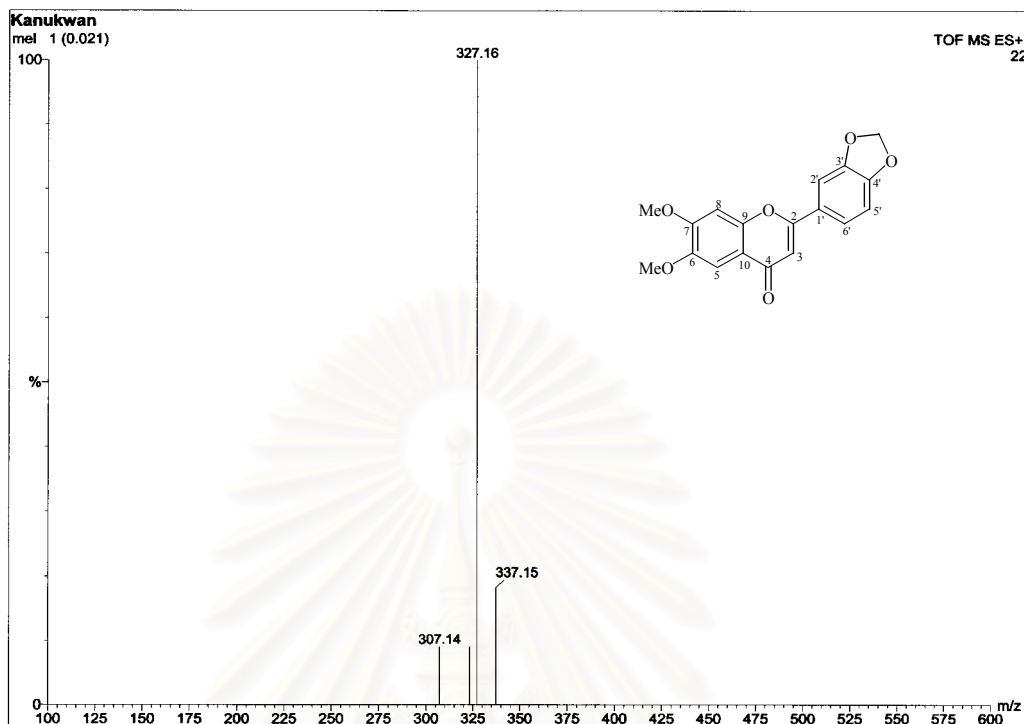


Figure 48 ESI Mass spectrum of compound MEL5 ( $\text{CDCl}_3$ )

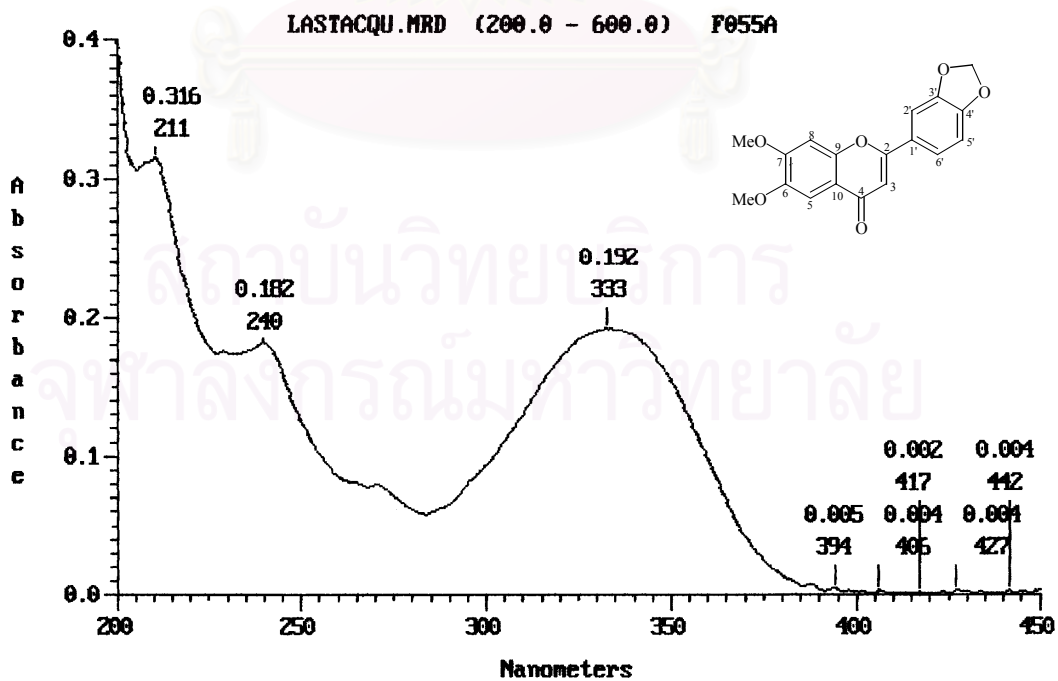
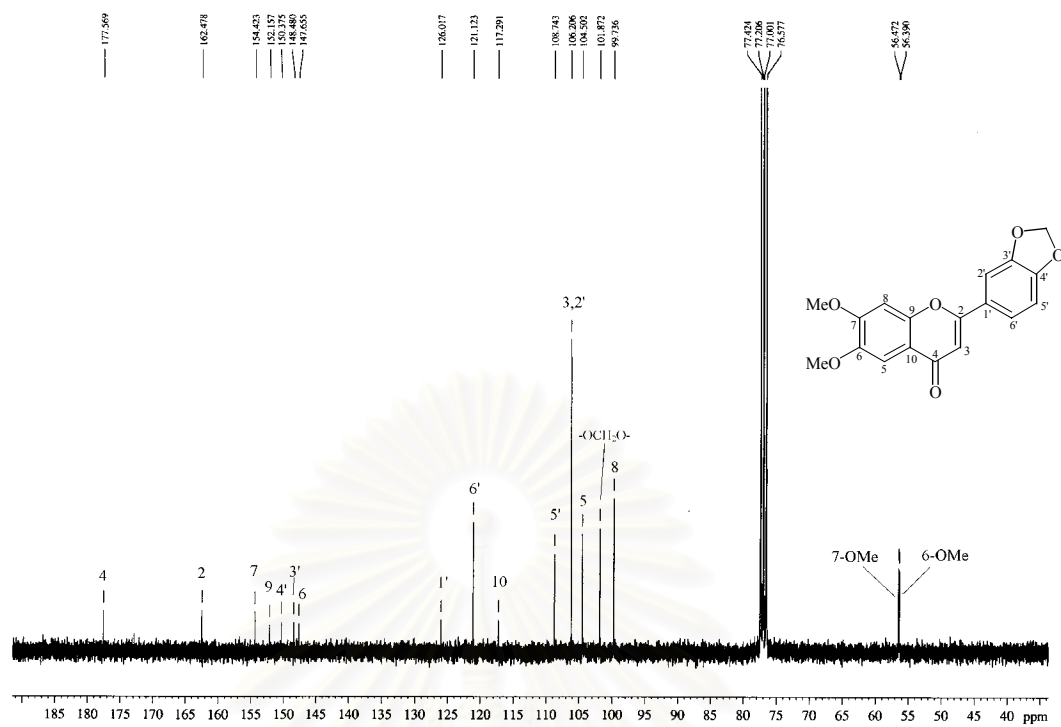
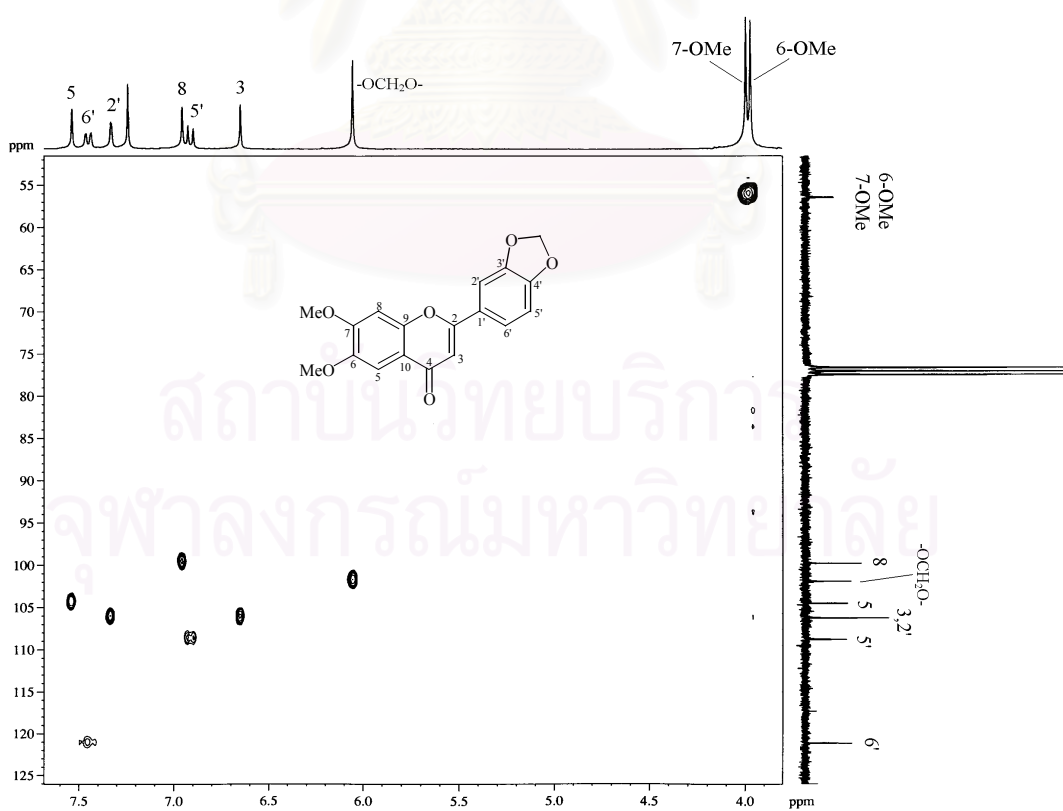


Figure 49 UV Spectrum of compound MEL5 (MeOH)

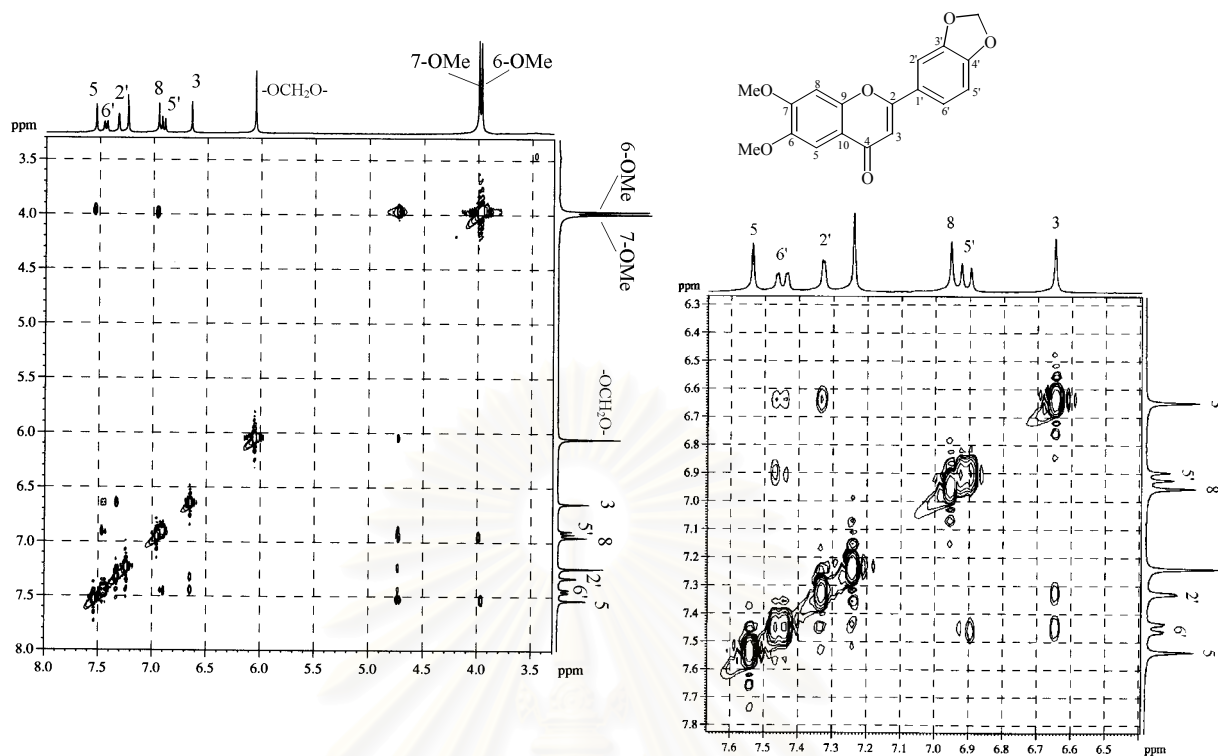




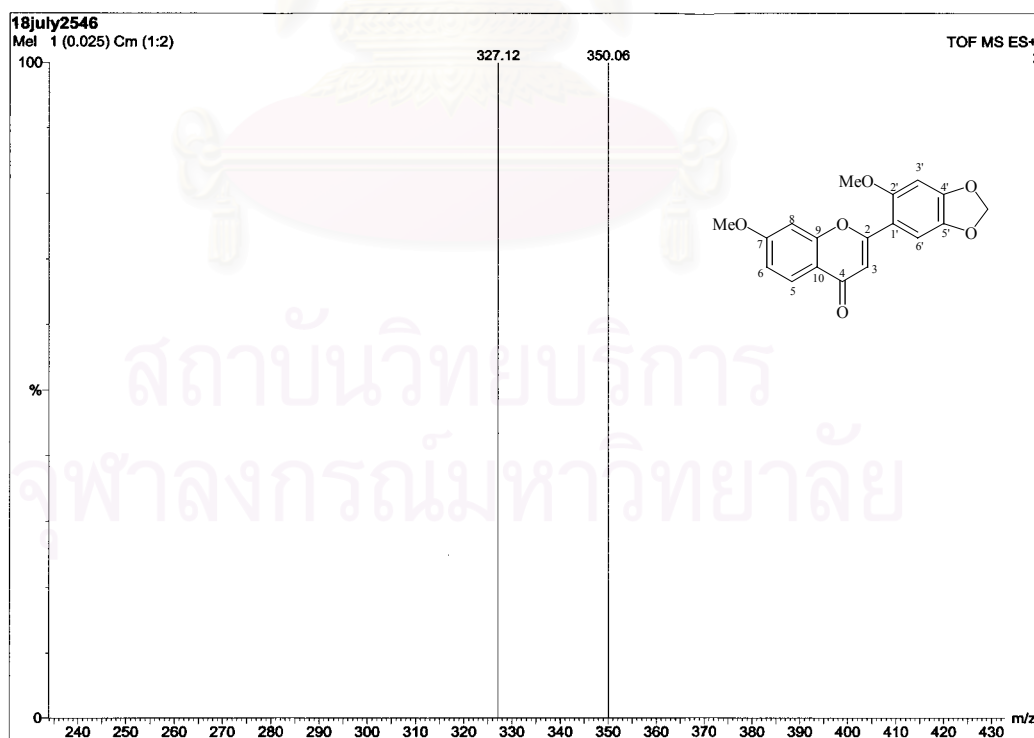
**Figure 52** <sup>13</sup>C-NMR Spectrum (75 MHz) of compound MEL5 (CDCl<sub>3</sub>)



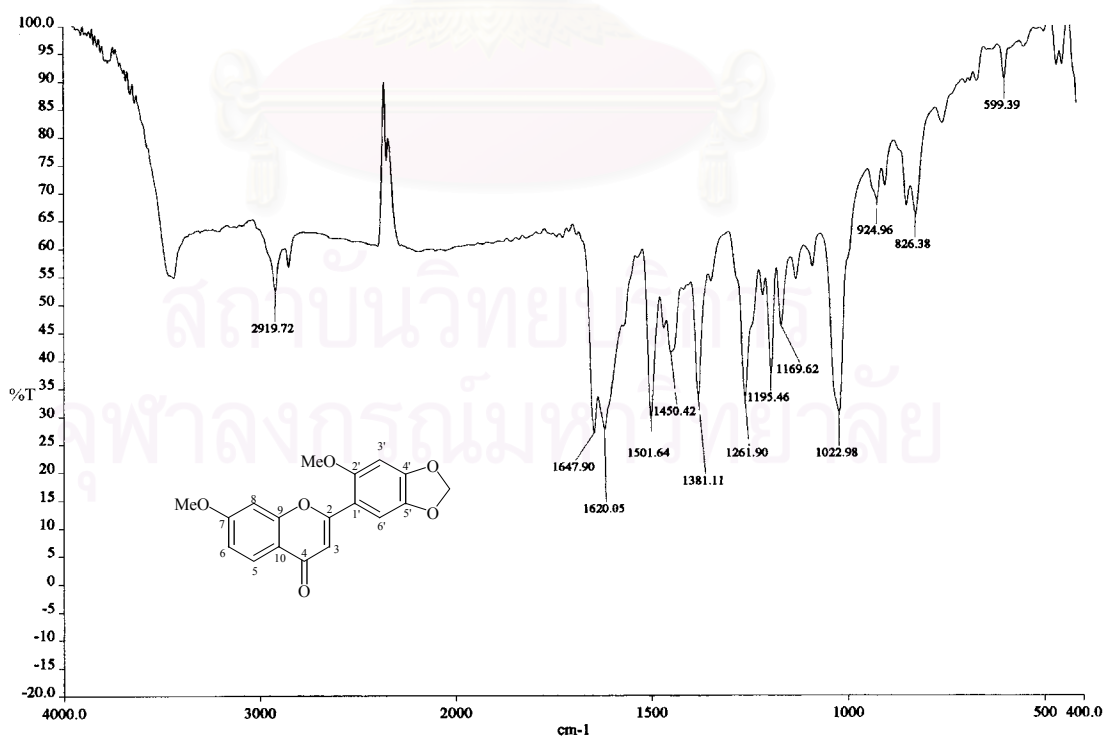
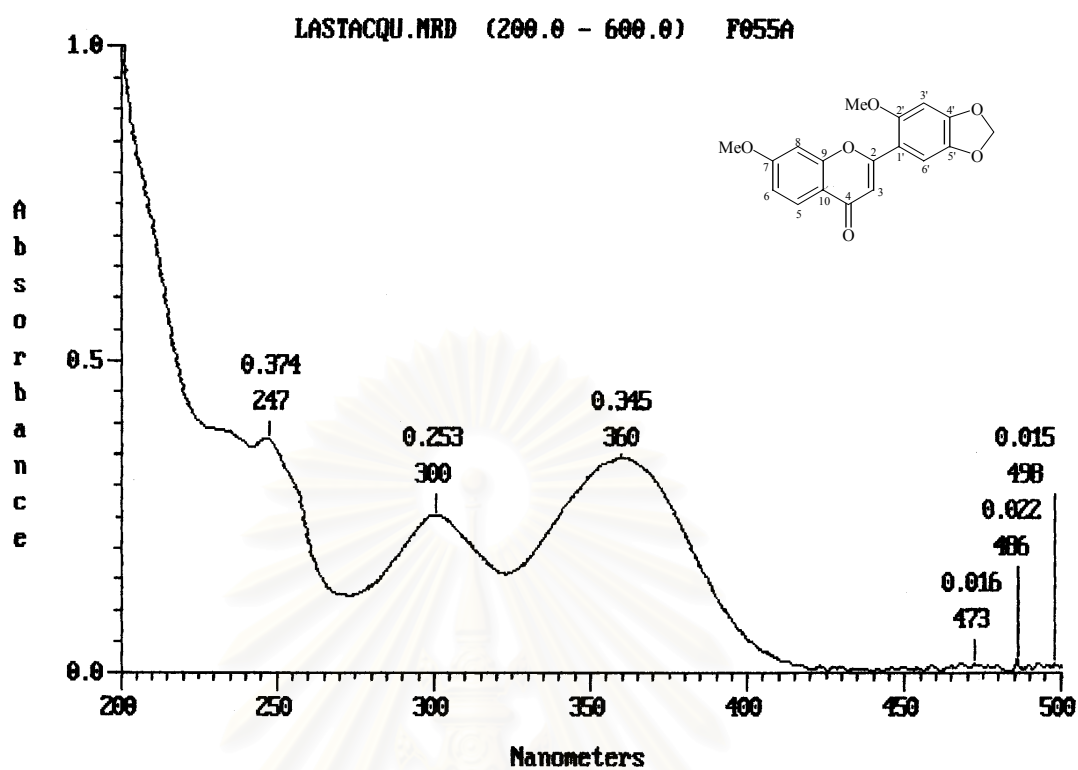
**Figure 53** HMQC Spectrum of compound MEL5 (CDCl<sub>3</sub>)



**Figure 54** NOESY Spectrum of compound MEL5 (CDCl<sub>3</sub>)



**Figure 55** ESI Mass spectrum of compound MEL6



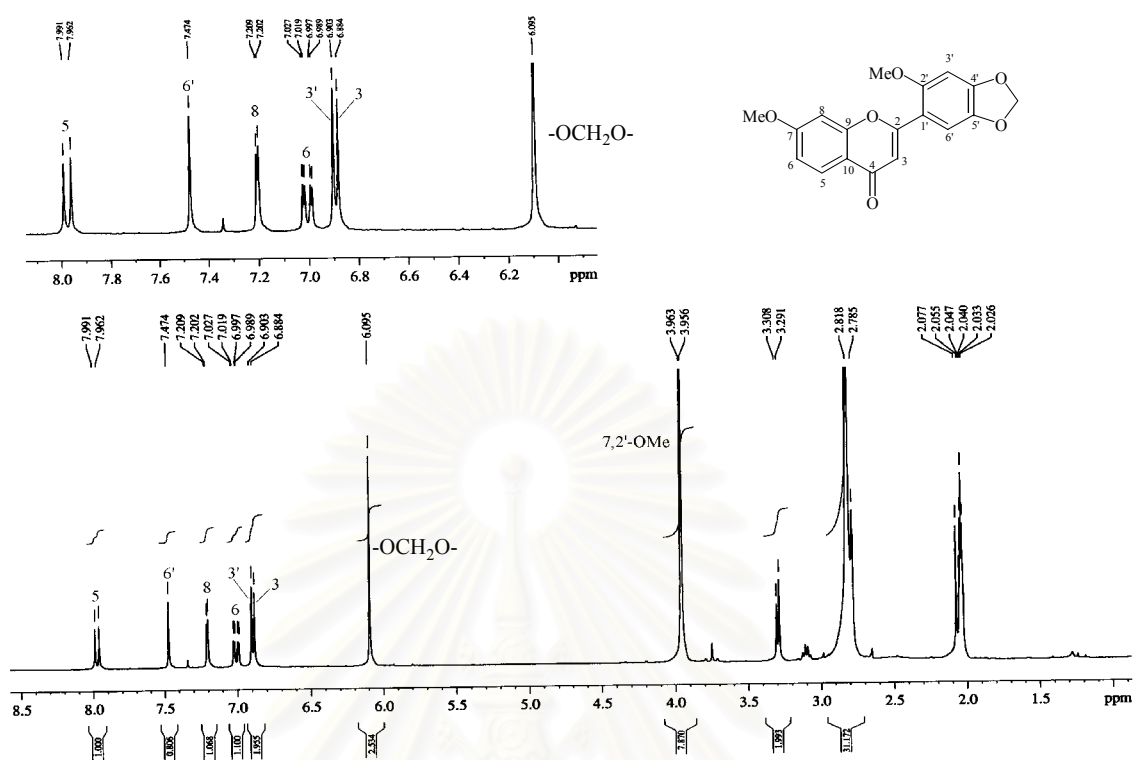


Figure 58  $^1\text{H-NMR}$  Spectrum (300 MHz) of compound MEL6 (acetone- $d_6$ )

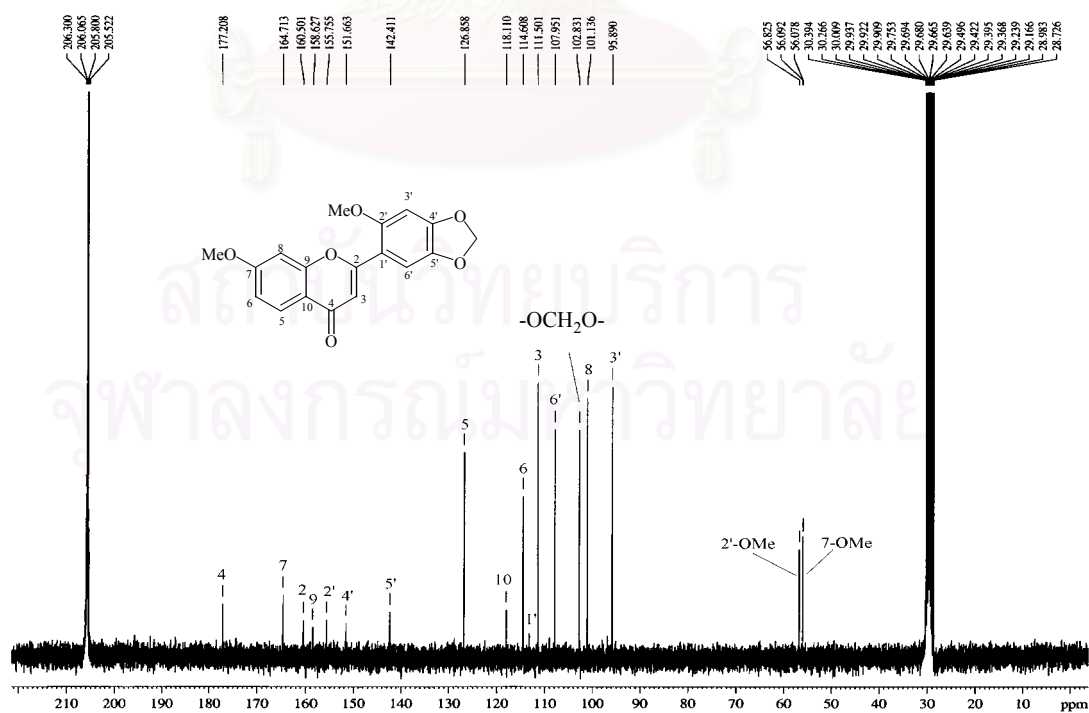


Figure 59  $^{13}\text{C-NMR}$  Spectrum (75 MHz) of compound MEL6 (acetone- $d_6$ )



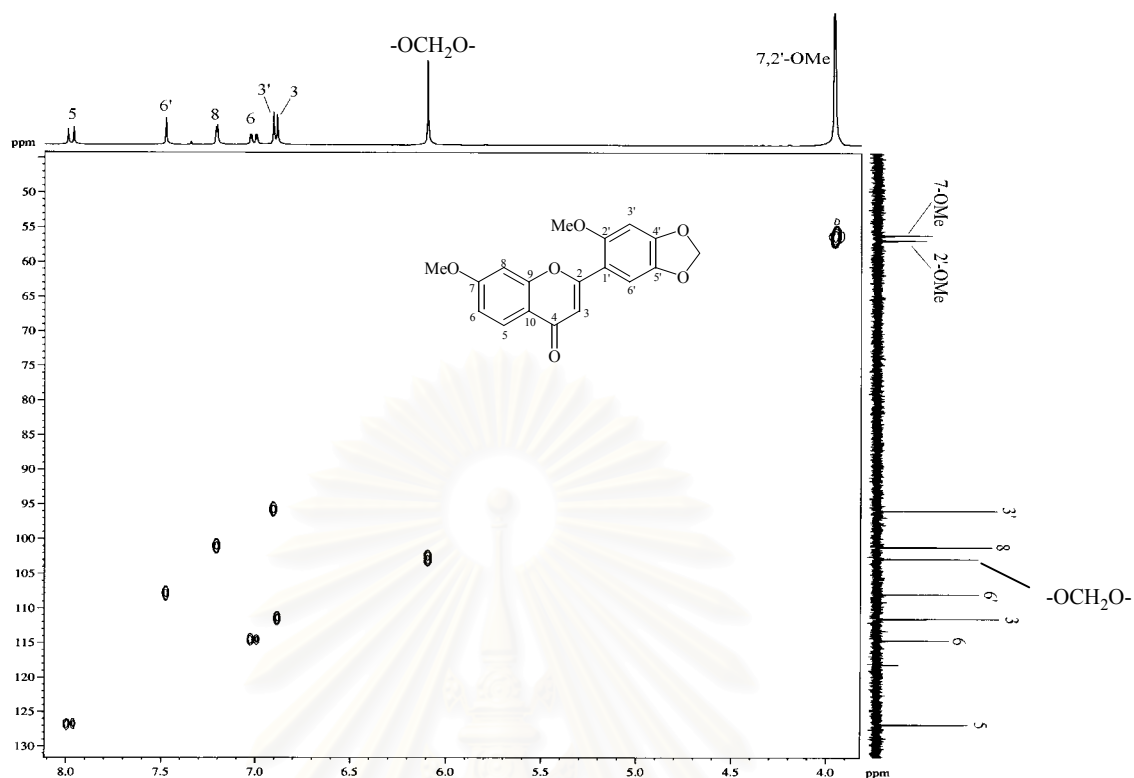


Figure 60 HMQC Spectrum of compound MEL6 (acetone- $d_6$ )

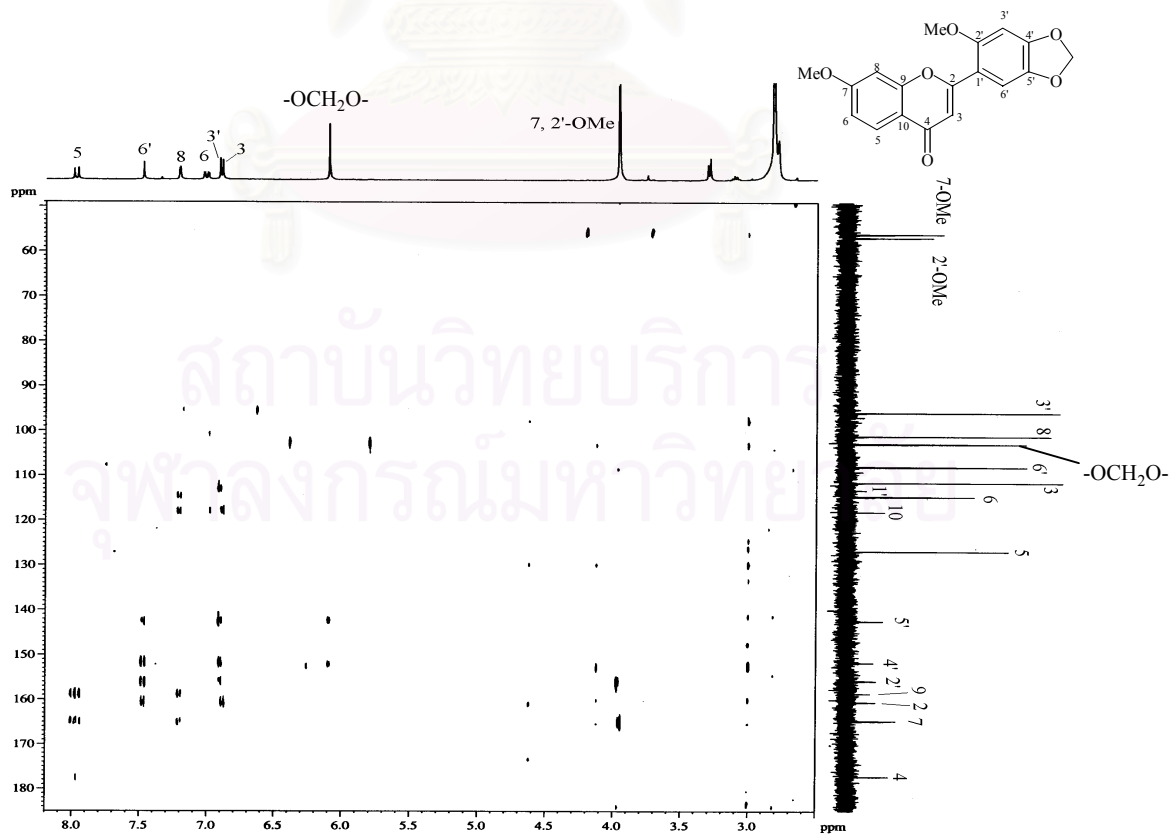


Figure 61 HMBC Spectrum of compound MEL6 (acetone- $d_6$ )

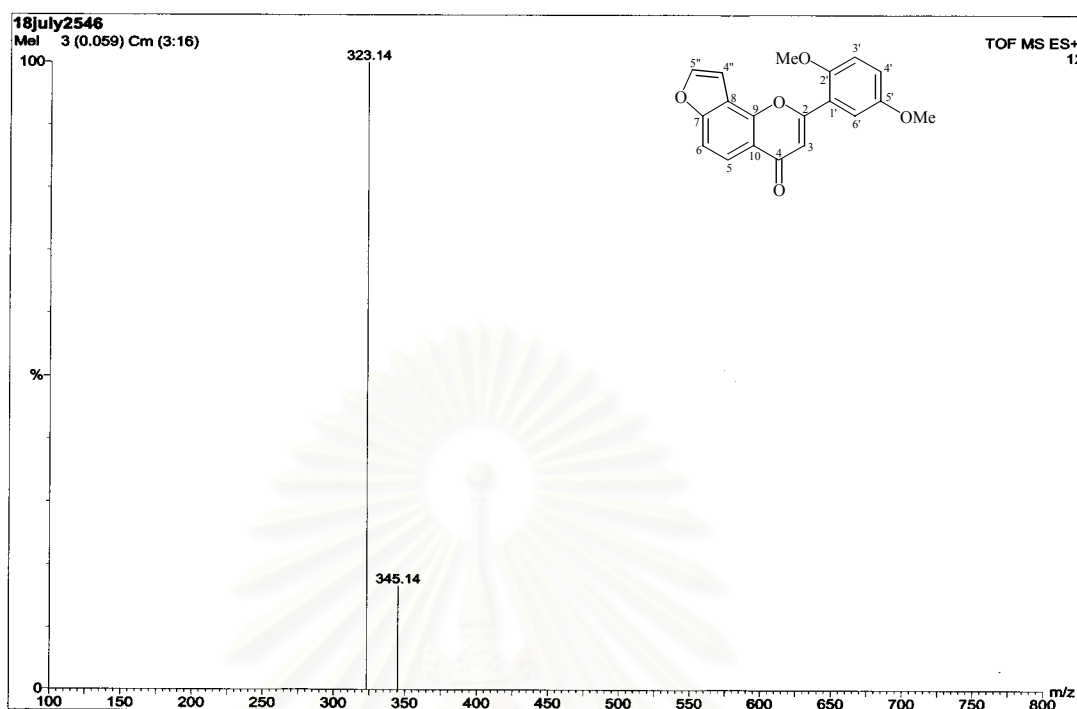


Figure 62 ESI Mass spectrum of compound MEL7

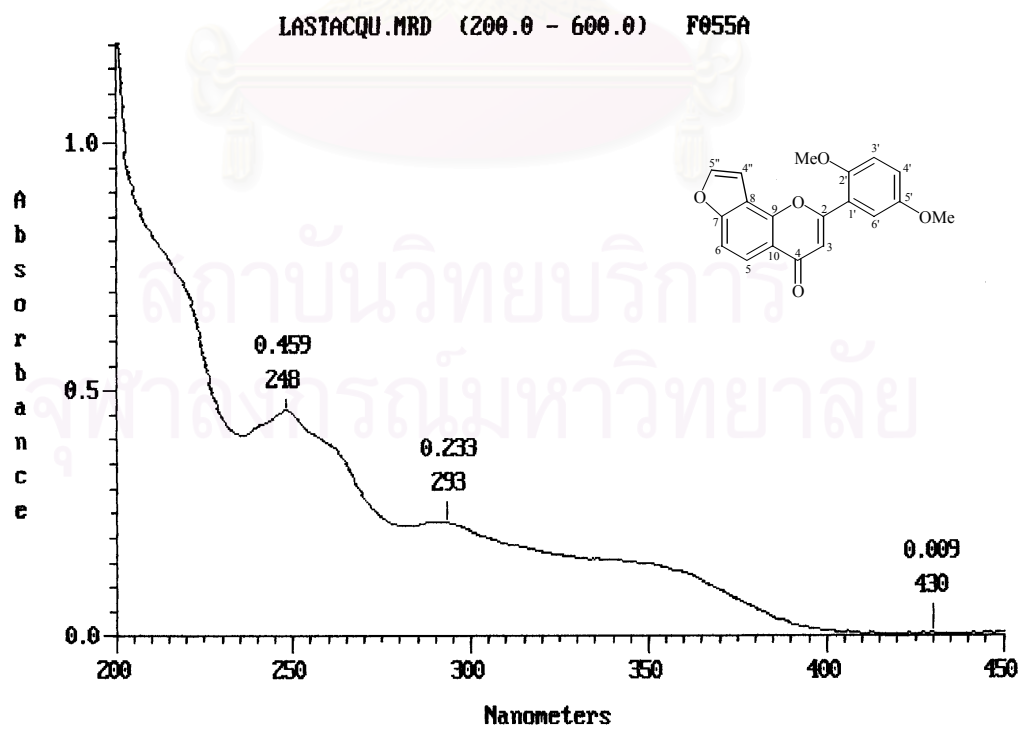


Figure 63 UV Spectrum of compound MEL7 (MeOH)

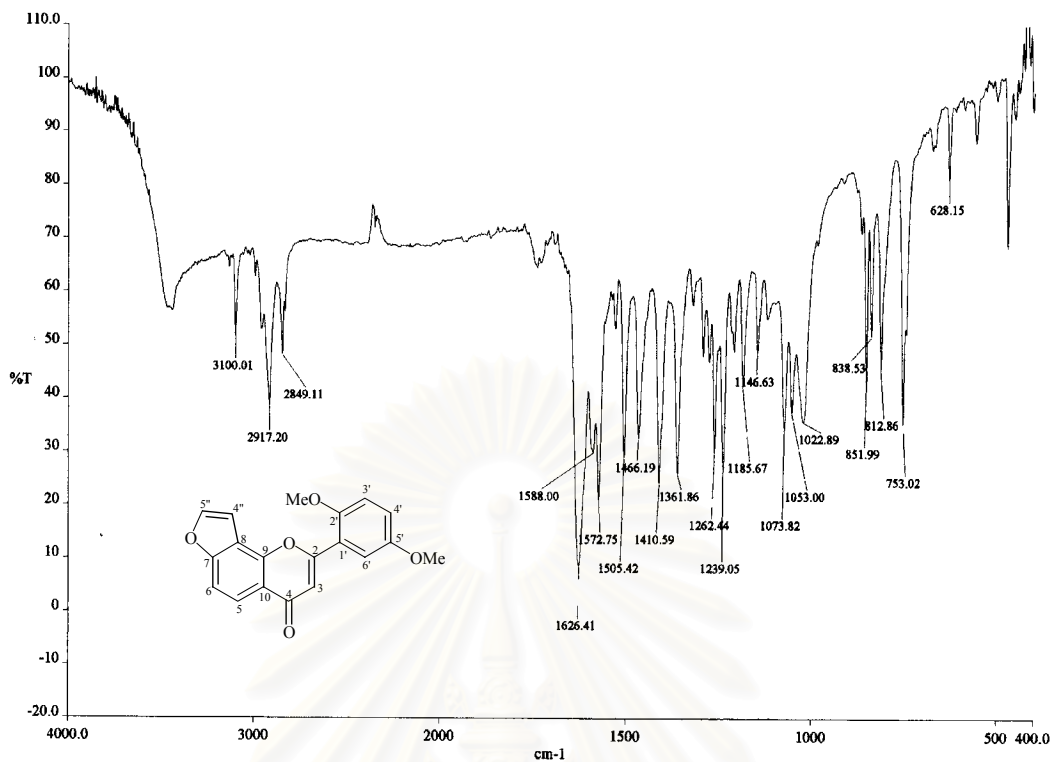


Figure 64 IR Spectrum of compound MEL7 (film)

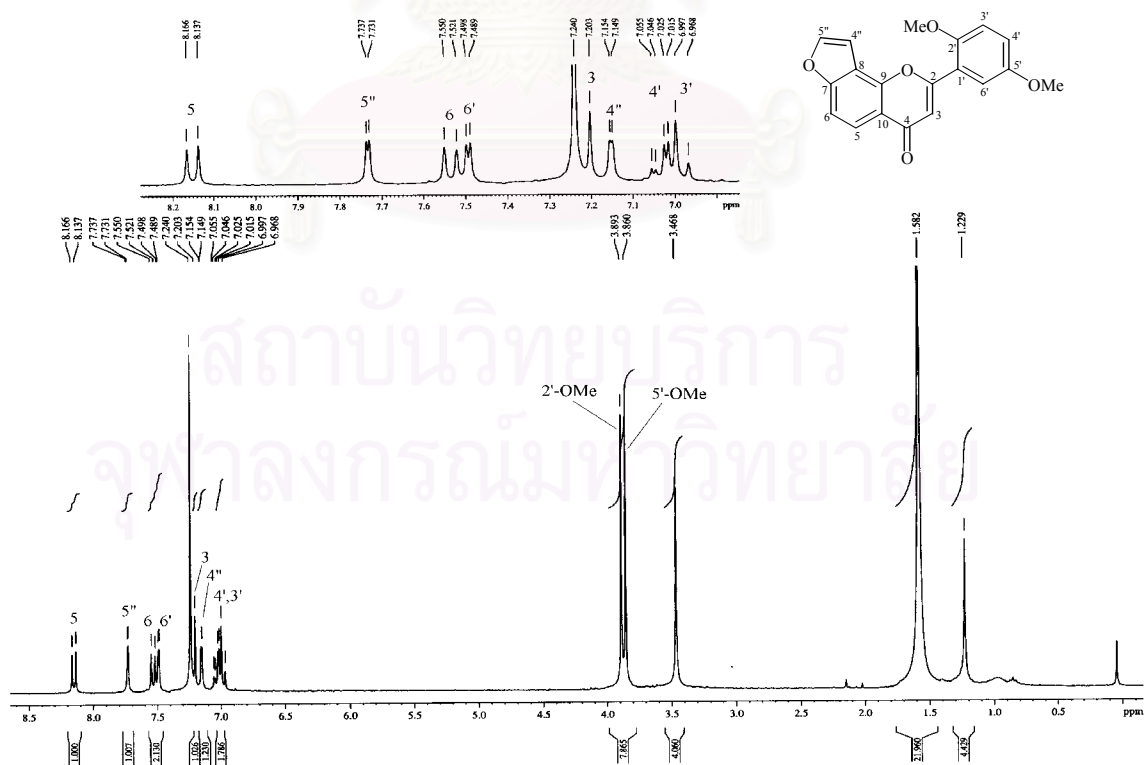
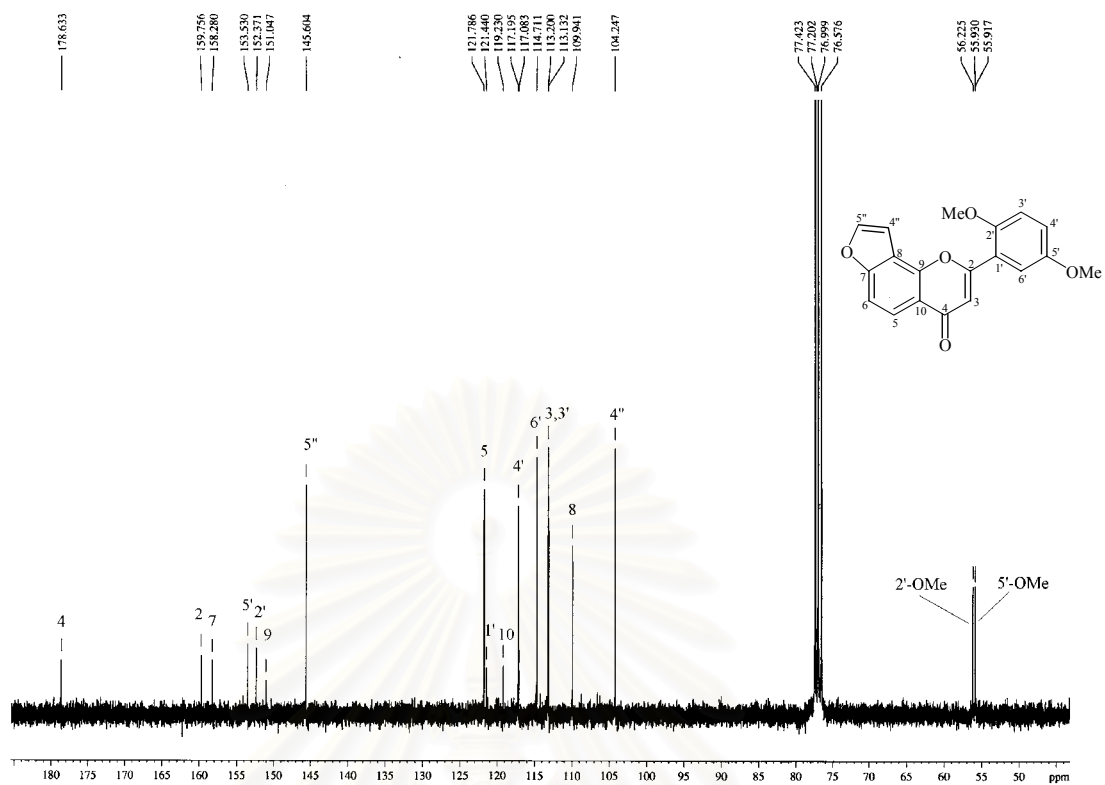


Figure 65 <sup>1</sup>H-NMR Spectrum (300 MHz) of compound MEL7 (CDCl<sub>3</sub>)



**Figure 66**  $^{13}\text{C}$ -NMR Spectrum (75 MHz) of compound MEL7 ( $\text{CDCl}_3$ )

สถาบันวิทยบริการ  
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## VITA

Miss Kanokwan Benchanak was born on June 14, 1976 in Nakhonratchasima, Thailand. She received her Bachelor's degree of Science in Pharmacy in 1998 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University. She currently works at Buayai Hospital, Nakhonratchasima.



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