CHEMICAL CONSTITUENTS FROM LEAVES AND PODS OF *MILLETTIA BRANDISIANA*

By

Anan Athipornchai

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree
MASTER OF SCIENCE
Department of Chemistry
Graduate School
SILPAKORN UNIVERSITY
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การศึกษาองค์ประกอบทางเคมีจากใบและฝักกระพี้จั่น

โดย

นายอนันต์ อธิพรชัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเคมีอินทรีย์

ภาควิชาเคมี

บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

ปีการศึกษา 2551

The graduate school, Silpakorn University has approved and accredited the thesis title of “Chemical Constituents from Leaves and Pods of *Millettia brandisiana*” submitted by Mr. Anan Athipornchai as a partial fulfillment of the requirements for the degree of Master of Science, program of Organic Chemistry.

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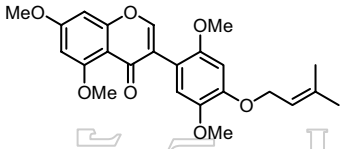
49302205 : สาขาวิชาเคมีอินทรีย์
คำสำคัญ : *Millettia brandisiana*/ isoflavones/ rotenoids/ anti-inflammatory activity
อนันต์ อธิพรชัย : การศึกษาองค์ประกอบทางเคมีจากใบและฝักกระพี้จั่น. อาจารย์ที่ปรึกษาวิทยานิพนธ์ : ผศ. ดร. อรษา ปานเจริญ. 152 หน้า.

การศึกษาองค์ประกอบทางเคมีจากใบและฝักกระพี้จั่น (*Millettia brandisiana*) ด้วยวิธีทางโครมาโทรกราฟี่ พบไอโซฟลาโวนชนิดใหม่ 7 สาร (**MB1 MB21-MB23 MB29** และ **MB31-MB32**) ชาลโคนชนิดใหม่ 2 สาร (**MB16** และ **MB17**) และสารที่ยังไม่มีรายงานการสกัดได้จากธรรมชาติแต่ได้มีการสังเคราะห์แล้ว 2 สาร (**MB2** และ **MB24**) นอกจากนี้ยังพบสารที่มีรายงานแล้ว 24 สาร (**MB3-MB15 MB18-MB20 MB25-MB28 MB30** และ **MB33-MB35**) การทดสอบฤทธิ์ต้านการอักเสบพบว่า **MB11** แสดงฤทธิ์ต้านการอักเสบดีกว่า phenylbutazone



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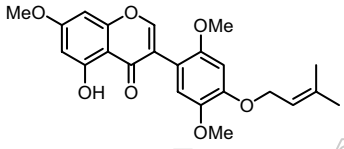

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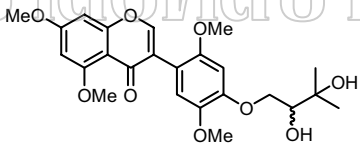

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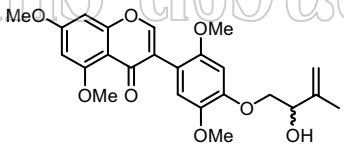

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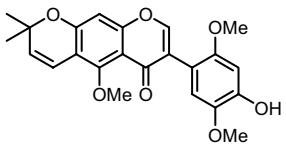

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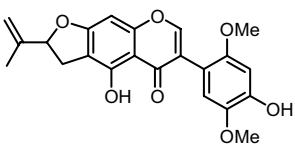

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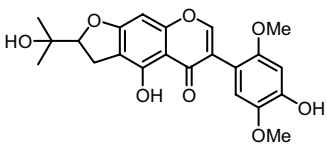

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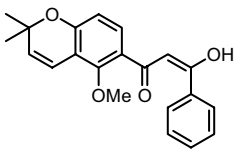

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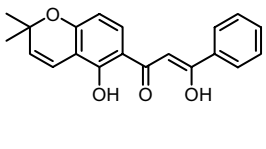

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49302205 : MAJOR : ORGANIC CHEMISTRY
KEY WORDS : *MILLETTIA BRANDISIANA*/ ISOFLAVONES/ ROTENOIDS/
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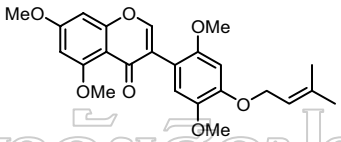
ANAN ATHIPORNCHAI : CHEMICAL CONSTITUENTS FROM LEAVES AND PODS OF *MILLETTIA BRANDISIANA*. THESIS ADVISOR : ASSIST. PROF. ORASA PANCHAROEN, Ph.D. 152 pp.

Chemical investigation of the leaves and pods of *Millettia brandisiana* resulted in the isolation of seven new isoflavones (**MB1**, **MB21-MB23**, **MB29** and **MB31-MB32**), two new chalcones (**MB16** and **MB17**) and two synthetically known isoflavones (**MB2** and **MB24**) together with twenty-four known compounds (**MB3-MB15**, **MB18-MB20**, **MB25**-**MB28 MB30** and **MB33-MB35**). The structures of these compounds were elucidated on the basis of spectroscopic analysis. In addition, **MB11** showed higher anti-inflammatory activity than that of phenylbutazone.



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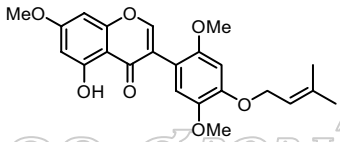

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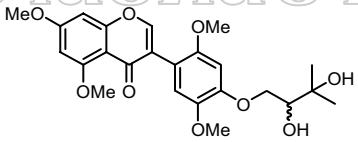

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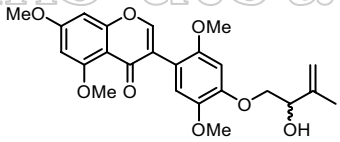

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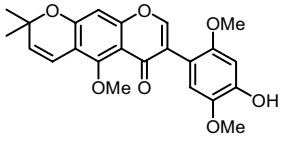

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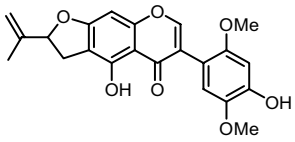

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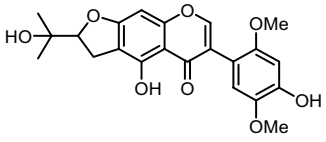

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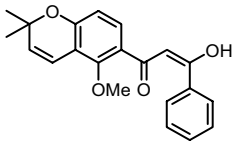

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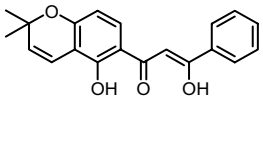

MB17

Department of Chemistry Graduate School, Silpakorn University Academic Year 2008

Student's signature.....

Thesis advisor's signature.....

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CHAPTER 1

INTRODUCTION

1.1 Introduction

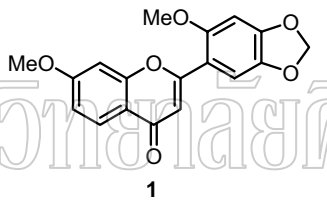
The genus *Millettia* belongs to the subfamily Papilionoideae of the family Leguminosae, with more than 200 species distributed in tropical Africa, Asia and Australia (Thulin, 1983). Previous phytochemical studies on some *Millettia* species revealed the presence of chalcones, isoflavones, rotenoids (Degne, Yenesew and Waterman, 1989; Yenesew, Midiwo and Waterman, 1998), isoflavans (Khalid and Waterman, 1983), flavanones, isocoumarins (Baruah *et al*., 1984) and pterocapans (Sritularak *et al*., 2002a). Many leguminous plants, especially the genera *Derris*, *Lonchocarpus*, *Millettia*, *Mundulea* and *Tephrosia* have been used as fish poisons and insecticides (Thasana, Chuankamnerdkarm and Ruchirawat, 2001; Kumar, Krupadanam and Srimannarayana, 1989).

Millettia brandisiana Kurz, a member of the Leguminosae, is widely distributed in the northern, central and northeastern regions of Thailand. It is a medium-sized tree, 8-20 m tall and known as “Kra-pee-jan” (Veesommai and Kaewduengtian, 2004). Trunk is a simple straight. Bark is gray-yellow or gray-brown, smooth or finely straight. Twigs are yellow-brown or green when young, smooth straight. Leaves are deep green coriaceous ovate-oblong, shortly acuminate, base rounded, nerves 10 pairs. Flowers are in clusters in the axils of fallen leaves, pale pink or purple, unisexual or bisexual. Fruits are linear-oblong, flat, slightly curved tapering to the base, brown tomentose when young. Seeds are chesturt brown, lens-shaped, smooth. Normally it flowers in March to May.

Figure 1. *Millettia brandisiana* Kurz

1.2 Review of literatures

In 2002, Sritularak *et al*. investigated the chemical constituents of the stem bark of *Millettia erythrocalyx*. Three new flavones, millettocalyxin A-C (**1-3**) and a new natural product pongol methyl ether (**4**) were isolated, along with ten known compounds, 7-*γ,γ*-dimethylallyloxyflavanone (**5**), 2'-hydroxy-3,4-methylenedioxy-4'-*γ,γ*-dimethylallyloxychalcone (**6**), derricidin (**7**), 5-hydroxyprunetin (**8**), pongaglabrone (**9**), 3',4'-methylenedioxy-7-methoxyflavone (**10**), 3',4'-methylene dioxy-6,7-dimethoxyflavone (**11**), ponganone I (**12**), milletenone (**13**) and ovalifolin (**14**). The structures of the new isolates were elucidated on the basis of spectroscopic data interpretation (Sritularak *et al*. 2002a).



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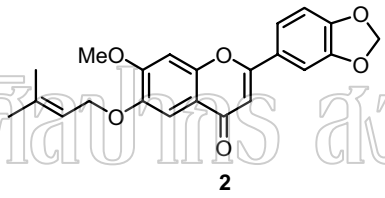

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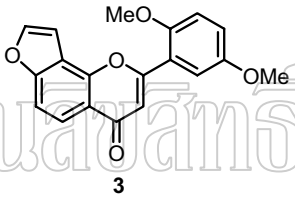

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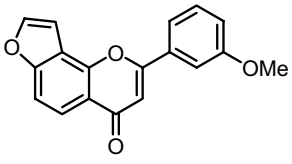

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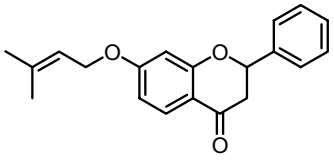

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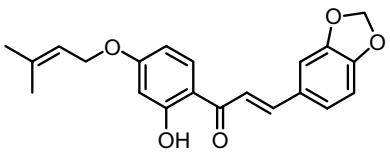

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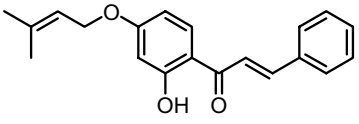

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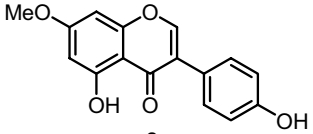

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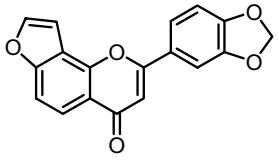

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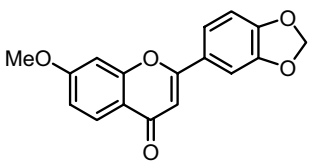

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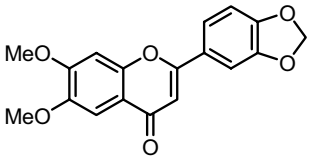

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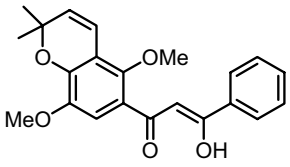

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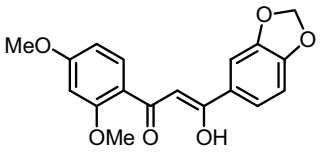

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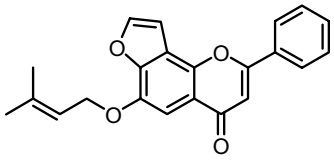

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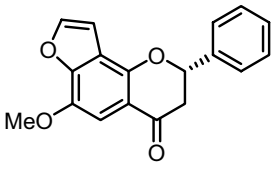
In 2002, Sritularak *et al*. also reported the isolation of two new flavonoids, 6-methoxy-[2",3" : 7,8]-furanoflavanone (**15**) and 2,5-dimethoxy-4-hydroxy-[2",3" : 7,8]-furanoflavan (**16**) and a new natural compound, 3,4-methylenedioxy-2',4'-dimethoxychalcone (**17**) from the roots of *Millettia erythrocalyx* together with ten known compounds, derricidin (**7**), ponganone I (**12**), milletenone (**13**), 1-(4-hydroxy-5-benzofuranyl)-3-phynyl-2-propen-1-one (**18**), purpurenone (**19**), pongaglabol (**20**), ovalitenone (**21**), pongamol (**22**), ponganone V (**23**) and lanceolatin (**24**). Their structures were elucidated on the basis of spectroscopic analysis (Sritularak *et al*. 2002b).



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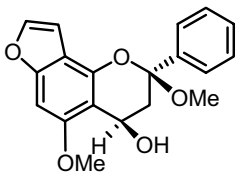

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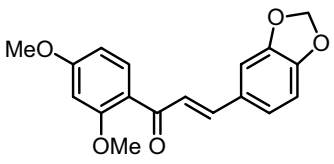

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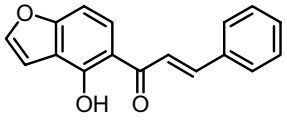

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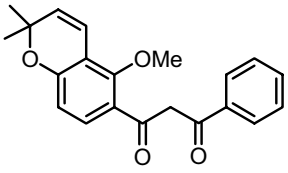

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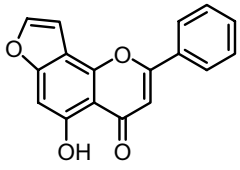

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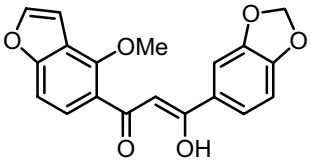

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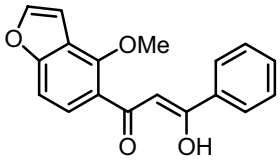

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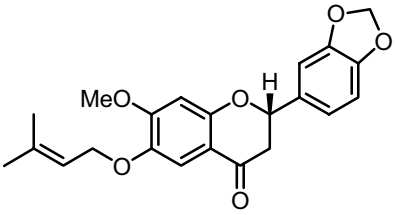

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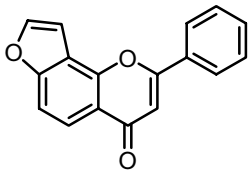
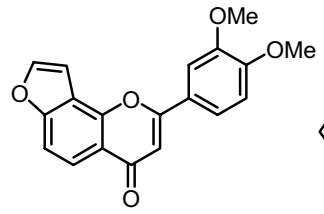


24

In 2005, Likhitwitayawuid *et al*. isolated a new flavone, 3',5'-dimethoxy-[2",3" : 7,8]-furanoflavone (**25**) and three known compounds, milletocalyxin A (**1**), pongol methyl ether (**4**) and ovalifolin (**14**) from the leaves of *Millettia erythrocalyx*. The structures were elucidated by analysis of spectroscopic data. In addition, the compounds isolated from the leaves were evaluated for anti-Herpes Simplex Virus activity (HSV-1 and HSV-2). It was found that flavones milletocalyxin A (**1**), pongol methyl ether (**4**) and ovalifolin (**14**) possessed moderate activity against both types of HSV (Likhitwitayawuid *et al*., 2005).

In 2006, Sritularak and Likhitwitayawuid studied the chemical constituents of the pods of *Millettia erythrocalyx*. Five new flavonoids, 2'-hydroxy-3,4-dimethoxy-[2",3" : 4',3']-furanochalcone (**26**), 2',3-dihydroxy-4-methoxy-4'-*γ,γ*-dimethylallyloxy chalcone (**27**), (-)-(2*S*)-6,3',4'-trimethoxy-[2",3" : 7,8]-furanoflavanone (**28**), 3',4'-methylenedioxy-[2",3" : 7,8]-furanoflavonol (**29**) and 6,3'-dimethoxy-[2",3" : 7,8]-furanoflavone (**30**) were isolated together with six known compounds, milletocalyxin C (**3**), pongol methyl ether (**4**), 2'-hydroxy-3,4-methylenedioxy-4'-*γ,γ*-dimethyl allyloxychalcone (**6**), derricidin (**7**), 3',4'-methylenedioxy-7-methoxyflavone (**10**) and ovalifolin (**14**). Their structures were elucidated through analysis of their spectroscopic data (Sritularak and Likhitwitayawuid, 2006).



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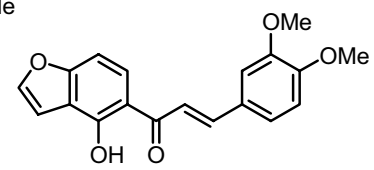

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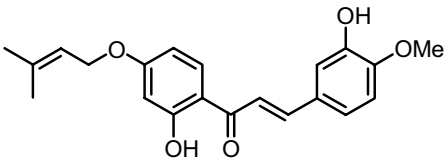

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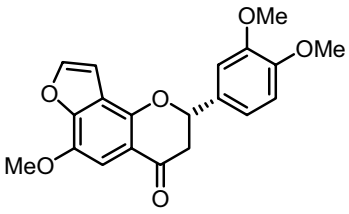

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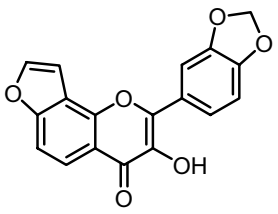

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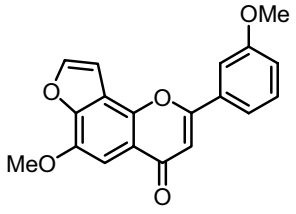
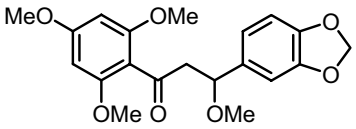


30

In 2003, Pattanaprateep, Ruangrungsi and Cordell reported the isolation of two new chalcones, 3,4-methylenedioxy-2',4',6',β-tetramethoxydihydrochalcone (**31**) and 2,4-dimethoxy-3',4'-methlenendioxydihydrochalcone (**32**) from the stem bark of *Millettia decipiens* along with five known flavonoids, dihydromilletenone methyl ether (**33**), 2,4,β-trimethoxy-3',4'-methylenendioxydihydrochalcone (**34**), β-hydroxy-2,4-dimethoxy-3',4'-methylenendioxychalcone (**35**), 2,4,6,β-tetramethoxy-3',4'-methylene dioxychalcone (**36**) and desmethoxykanugin (**37**). The structure identification was achieved on the basis of spectroscopic analysis (Pattanaprateep, Ruangrungsi and Cordell, 2003).



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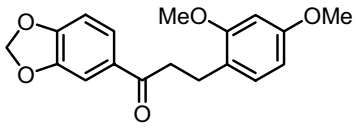

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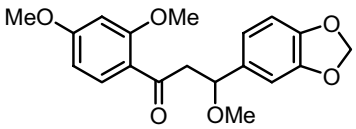

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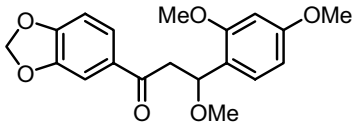

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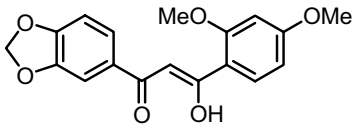

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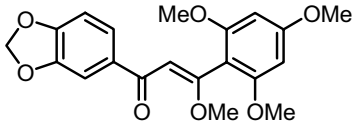

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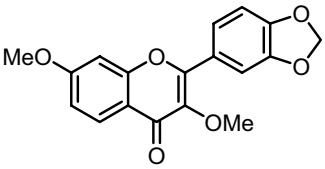
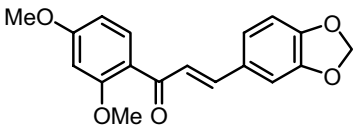


37

In 2003, Phrutivorapongkul *et al*. isolated four new chalcones, 2,4,6,*β*-tetramethoxy-3',4'-methylenendioxychalcone (**36**), 2',4'-dimethoxy-3,4-methylenedi oxychalcone (**38**), 2',4',6'-trimethoxy-3,4-methylenedioxydihydrochalcone (**39**) and 2',4',6'-trimethoxy-3,4-methylenedioxychalcone (**40**) along with two known chalcones, dihydromilletenone methyl ether (**33**) and 2'-hydroxy-3,4,4',6'-tetramethoxychalcone (**41**) and five known flavones, 3',4'-methylenedioxy-7-methoxyflavone (**10**), desmethoxykanugin (**37**), karanjin (**42**), lanceolatin B (**43**), and 3',4'-methylenedioxy-5,7-dimethoxyflavone (**44**) from the stem bark of *Millettia leucantha*. The structure elucidation of the isolates was achieved with the aid of extensive 1D and 2D NMR studies. Moderate cytotoxic activity was observed in chalcones (**38**, **40**), whereas dihydrochalcones (**33**, **39**) showed moderate anti-Herpes Simplex Virus (HSV) activity. Interestingly, flavone **37** showed significant anti-inflammatory effects inhibiting both cyclooxygenase (COX)-1 and -2 (Phrutivorapongkul *et al*., 2003).



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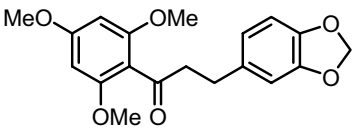

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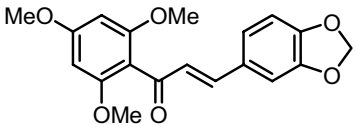

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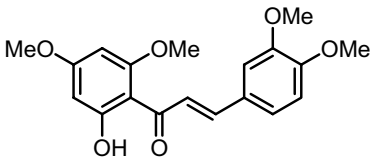

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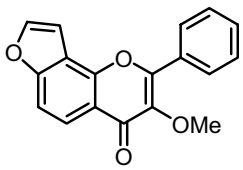

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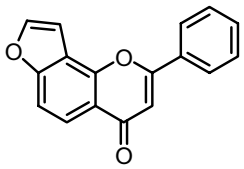

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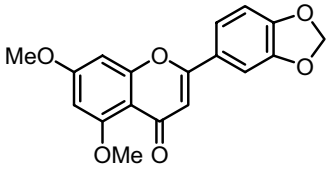
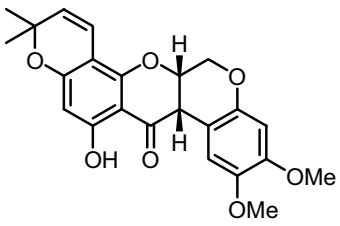


44

In 2007, Pancharoen, Petveroj and Phongpaichit isolated four known rotenoids, α-toxicarol (**45**), 12a-hydroxy-α-toxicarol (**46**), 6-deoxyclitoriacetal (**47**) and 6a,12a-dehydro-α-toxicarol (**48**) from the hexane extract of the flowers of *Millettia brandisiana*. Their structures were determined by spectroscopic methods. In addition, rotenoids **46** and **47** were evaluated for antimicrobial activity and found to be inactive at 128 μg/mL (Pancharoen, Petveroj and Phongpaichit, 2007).



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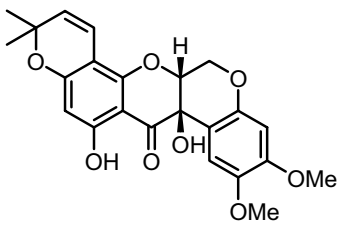

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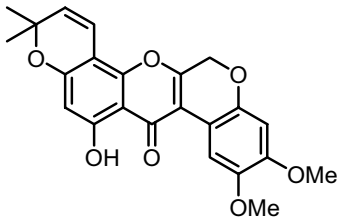

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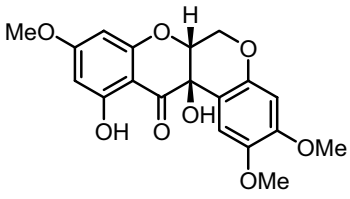
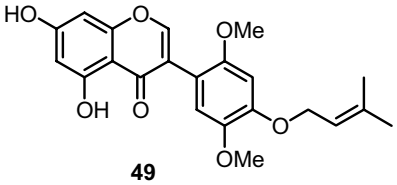


47

In 2007, Kikuchi *et al*. studied a screening program for natural products with tumor-selective apoptosis-inducing properties. Six new isoflavonoids, brandisianin A-F (**49-54**) were isolated from the methanol extract of the leaves of *Millettia brandisiana* together with five known compounds, 4'-demethyltoxicarol isoflavone (**55**), viridiflorin (**56**), naringenin (**57**), olibergin (**58**) and (-)-epicatechin (**59**). Among these compounds brandisianin D (**52**) exhibited death-receptor 5 (DR5) expression enhancement activity in a luciferase assay based in DLD-1/*Sac*I cells. The results suggested that brandisianin D (**52**) might overcome TRAIL-resistance by an increase in DR5 expression (Kikuchi *et al*., 2007).



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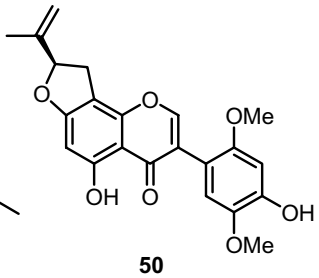

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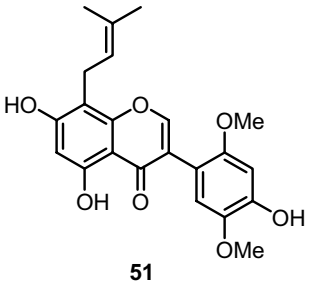

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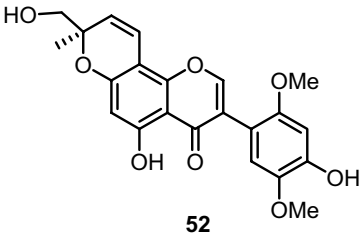

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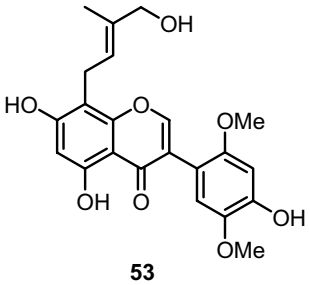

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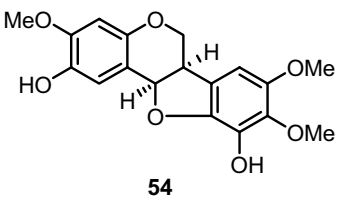

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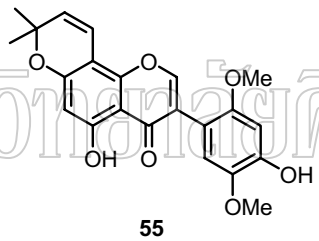

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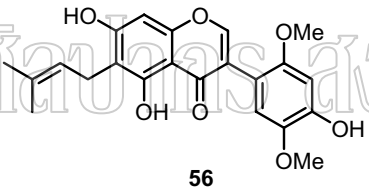

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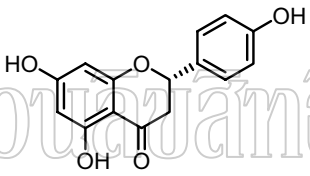

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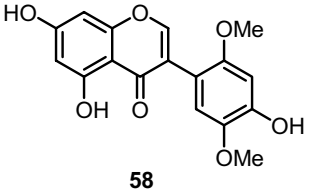

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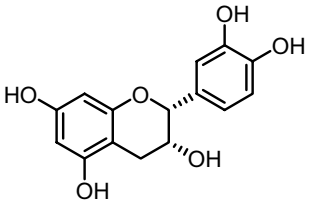

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1.3 The Objective

To dates, a few species of *Millettia* in Thailand have been studied. *Millettia brandisiana* Kurz is a plant growing throughout Thailand, with no previous record of chemical examination. It was therefore of interest to investigate chemical constituents from different parts of this plant. This work involved the isolation, purification and structural determination of compounds from the leaves and pods of *M. brandisiana* (collected in Nakorn Pathom). While our project was in progress, another group of researchers reported the isolation of six new flavonoids from the leaves of this plant (collected in Khon Kaen) (Kikuchi *et al*., 2007).

CHAPTER 2

EXPERIMENTAL

2.1 Instruments and chemicals

Melting points were determined by a Kofler hot stage apparatus and uncorrected. Optical rotations were measured in chloroform solutions on a JASCO P-1010 polarimeter. Ultraviolet spectra (UV) were obtained on a HP-8453 UV-Vis spectrophotometer. Principle bands (λ_{max}) were recorded as wavelengths (nm) in methanol or ethanol solutions. Infrared spectra (IR) were recorded on a Perkin Elmer GX-FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on a Bruker AVANCE 300 (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) spectrometer. Chemical shifts are in δ (ppm) with tetramethylsilane (TMS) as an internal standard. Inverse-detected heteronuclear correlations were measured using HMQC and HMBC pulse sequences with a pulse field gradient. EIMS were obtained using a Hewlett Packard 5989B. Vacuum liquid chromatography (VLC) and column chromatography (CC) were carried out using Scharlau silica gel 60 (0.06-0.23 mm). Gel filtration (size exclusion chromatography) was carried out using sephadex LH-20 (Merck). Pre-coated thin layer chromatography (TLC) aluminum sheets of silica gel 60 F_{254} (20 x 20 cm, layer thickness 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized under ultraviolet light or sprayed with 1% $CeSO_4$ in 10% aq.H_2SO_4 following by heating. Preparative thin layer chromatography (PLC) was carried out on glass plates using silica gel 60 F_{254} (20 x 20 cm, layer thickness 0.25, 0.5 and 1.0 mm, Merck). Organic solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except solvents for UV, IR and optical rotation were analytical grade reagents.

2.2 Plant material

The leaves and pods of *Millettia brandisiana* were collected at Silpakorn University, Sanamchandra Palace campus, Nakorn Pathom, Thailand in June 2003. A voucher specimen (SUMB603) was identified by Assoc. Prof. Nijisiri Ruangrungsi and deposited at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

2.3 Chemical investigation of the leaves

2.3.1 Extraction and isolation

The dried leaves of *Millettia brandisiana* (1.27 kg) were extracted with ethanol (5L x 3) at room temperature. The combined extract was concentrated to dryness to give 186.7 g of residue. The ethanol extract was sequentially partitioned between organic solvents and water to yield ethyl acetate extract (79.4 g), *n*-butanol extract (30.6 g) and water extract (76.5 g). The ethyl acetate extract was further dissolved in hexane to afford a hexane-soluble fraction (22.3 g) and a hexane-insoluble fraction (57.1 g).

2.3.2 Chemical investigation of hexane-soluble fraction

Separation of the hexane-soluble fraction (22.3 g) was carried out by VLC using hexane and EtOAc in a polarity gradient manner. On the basis of their TLC characteristics, similar fractions were combined to give 12 fractions (A1-A12, Table 1).

Table 1. Fractions obtained from hexane-soluble fraction.

Fraction	Weight (g)	Physical characteristic
A1	0.71	yellow solid
A2	3.85	yellow solid
A3*	2.39	deep yellow viscous liquid
A4*	2.67	deep yellow viscous liquid
A5*	1.19	deep green viscous liquid
A6*	0.60	deep green viscous liquid
A7*	1.70	deep green viscous liquid
A8*	1.54	deep green viscous liquid
A9*	0.93	brown viscous liquid
A10*	0.93	brown viscous liquid
A11	2.40	brown viscous liquid
A12	1.11	brown viscous liquid

* TLC characteristics of these fractions showed major spots under UV and their ^{1}H NMR spectra showed aromatic proton signals.

The combined A3 and A4 (5.06 g) was further purified by CC using a step gradient of hexane and acetone to give 11 fractions (B1-B11, Table 2).

Table 2. Fractions obtained from the combined A3 and A4.

Fraction	Weight (mg)	Physical characteristic
B1	2173.4	yellow solid
B2	1335.2	yellow solid
B3	254.4	yellow viscous liquid
B4*	284.5	green viscous liquid
B5*	177.1	green viscous liquid
B6*	143.4	green viscous liquid
B7*	4.3	green viscous liquid
B8*	20.3	green viscous liquid
B9*	22.0	brown viscous liquid
B10	51.9	brown viscous liquid
B11	150.6	brown viscous liquid

* Fractions were further investigated.

Fraction B4 (284.5 mg) was separated by PLC using hexane-CH_2Cl_2 (1:9) as the developing solvent to give **MB11**, crystallization from acetone yielded yellow needles (117.1 mg).

Fraction B5 (177.1 mg) was purified by PLC using hexane-CH_2Cl_2-EtOAc (4:1:1, 8 runs) as the mobile phase to yield **MB13**, crystallization from acetone gave deep yellow plates (10.1 mg).

Fraction B6 (143.4 mg) was separated by PLC using CH_2Cl_2-MeOH (30:1) as the developing solvent to give **MB7**, crystallization from acetone yielded pale yellow needles (34.3 mg).

The combined fraction B7, B8 and B9 (46.6 mg) was purified by PLC using CH_2Cl_2-MeOH (30:1) as the mobile phase to yield **MB9**, crystallization from acetone afforded yellow needles (3.9 mg).

The combined fraction A5 and A6 (1.79 g) was separated by CC and the column was eluted by hexane with a step gradient of acetone to afford 8 fractions (C1-C8, Table 3).

Table 3. Fractions obtained from the combined A5 and A6.

Fraction	Weight (mg)	Physical characteristic
C1	14.7	yellow viscous liquid
C2	291.6	green viscous liquid
C3	291.7	green viscous liquid
C4	226.7	green viscous liquid
C5*	319.8	green viscous liquid
C6*	308.0	green viscous liquid
C7	112.5	brown viscous liquid
C8	17.0	brown viscous liquid

* Fractions were further investigated.

Fraction C5 (319.8 mg) was separated by PLC using hexane-acetone (3:1, 3 runs) as the developing solvent to give **MB10**, crystallization from acetone gave yellow plates (21.0 mg).

Fraction C6 (308.0 mg) was further purified by CC and the column was eluted with gradients of hexane and acetone to yield 4 fractions (D1-C4, Table 4). Fraction D2 was identified as compound **MB11**, crystallization from acetone yielded yellow needles (178.8 mg).

Table 4. Fractions obtained from C6.

Fraction	Weight (mg)	Physical characteristic
D1	26.9	green viscous liquid
D2*	178.8	yellow solid
D3	45.0	green viscous liquid
D4	73.2	green viscous liquid

* Fractions were further investigated.

Fraction A7 (1.70 g) was purified by CC and the column was eluted with a step gradient hexane and acetone to afford 9 fractions (E1-E9, Table 5).

Table 5. Fractions obtained from A7.

Fraction	Weight (mg)	Physical characteristic
E1	36.8	green viscous liquid
E2	122.7	green viscous liquid
E3	95.5	green viscous liquid
E4	44.3	green viscous liquid
E5	51.7	green viscous liquid
E6	101.9	green viscous liquid
E7	31.5	green viscous liquid
E8*	532.7	green viscous liquid
E9	94.7	brown viscous liquid

* Fractions were further investigated.

Fraction E8 (532.7 mg) was purified by CC and the column was eluted with CH_2Cl_2 to give **MB7**, crystallization from acetone afforded pale yellow needles (373.6 mg).

Fraction A8 (1.54 g) was separated by PLC using hexane-CH_2Cl_2-EtOAc (10:6:1, 4 runs) as the mobile phase to yield F1 as a yellow viscous liquid (50.3 mg) and F2 as a green viscous liquid (794.0 mg). The fractions F1 (50.3 mg) and F2 (794.0 mg) were purified by PLC using hexane-acetone (3:1, 4 runs) as the developing solvent to afford **MB4**, crystallization from CH_2Cl_2 gave yellow plates (2.6 mg), **MB3**, crystallization from CH_2Cl_2 yielded yellow needles (7.2 mg), **MB11**, crystallization from acetone afforded yellow needles (309.2 mg) and **MB7**, crystallization from acetone gave pale yellow needles (186.8 mg).

The combined fraction A9 and A10 (1.86 g) was subjected to CC and the column was eluted by CH_2Cl_2 with a step gradient of MeOH to give 11 fractions (G1-G11, Table 6).

Table 6. Fractions obtained from the combined A9 and A10.

Fraction	Weight (mg)	Physical characteristic
G1	27.0	green viscous liquid
G2*	86.9	green viscous liquid
G3*	60.0	green viscous liquid
G4*	86.1	deep green viscous liquid
G5*	303.5	deep green viscous liquid
G6	84.6	deep green viscous liquid
G7	202.5	deep green viscous liquid
G8	318.6	brown viscous liquid
G9	192.1	brown viscous liquid
G10	182.4	brown viscous liquid
G11	107.1	brown viscous liquid

* Fractions were further investigated.

Fraction G2 (86.9 mg) and G3 (60.0 mg) were purified by PLC using hexane-acetone (3:1, 4 runs) as the mobile phase to yield **MB11**, crystallization from acetone afforded yellow needles (26.0 mg) and **MB7**, crystallization from acetone yielded pale yellow needles (63.6 mg).

Fraction G4 (86.1 mg) was separated by PLC using hexane-acetone-MeOH (3:1:0.4, 4 runs) as the developing solvent to afford **MB9**, crystallization from acetone gave yellow needles (3.4 mg).

Fraction G5 (303.5 mg) was purified by PLC using hexane-acetone-MeOH (3:1:0.8, 3 runs) as the mobile phase to give **MB1**, crystallization from acetone afforded white needles (7.0 mg).

2.3.3 Chemical investigation of hexane-insoluble fraction

Separation of the hexane-insoluble fraction (57.1 g) was performed by VLC using hexane and EtOAc in a polarity gradient manner. On the basis of their TLC characteristic, similar fractions were combined to yield 17 fractions (H1-H17, Table 7).

Table 7. Fractions obtained from hexane-insoluble fraction.

Fraction	Weight (g)	Physical characteristic
H1*	0.66	yellow viscous liquid
H2*	0.34	yellow viscous liquid
H3*	0.96	green viscous liquid
H4*	2.19	deep green viscous liquid
H5*	2.99	deep green viscous liquid
H6*	10.09	deep green viscous liquid
H7*	5.73	deep green viscous liquid
H8	5.95	deep green viscous liquid
H9	1.60	deep green viscous liquid
H10	1.43	deep green viscous liquid
H11*	3.23	brown viscous liquid
H12*	0.50	brown viscous liquid
H13	1.18	brown viscous liquid
H14	0.87	brown viscous liquid
H15	1.68	black viscous liquid
H16	1.44	black viscous liquid
H17	6.30	black viscous liquid

* Fractions were further investigated.

The combined fraction H1, H2 and H3 (1.96 g) was subjected to CC and the column was eluted with gradients of hexane and EtOAc to afford 11 fractions (I1-I11, Table 8).

Table 8. Fractions obtained from the combined fractions H1, H2 and H3.

Fraction	Weight (mg)	Physical characteristic
I1	109.1	orange solid
I2	665.8	orange solid
I3	82.1	yellow viscous liquid
I4	74.4	yellow viscous liquid
I5	100.4	green viscous liquid
I6	150.1	green viscous liquid
I7*	88.6	green viscous liquid
I8*	99.0	green viscous liquid
I9*	68.3	green viscous liquid
I10*	226.3	green viscous liquid
I11*	54.0	green viscous liquid

* Fractions were further investigated.

Fraction I7 (88.6 mg) and I8 (99.0 mg) were purified by PLC using hexane-acetone (3:1) as the developing solvent to give **MB12**, crystallization from acetone yielded deep yellow plates (18.3 mg) and a mixture of **MB12** and **MB10** as yellow solid (15.3 mg).

Fraction I9 (68.3 mg) was separated by PLC using hexane-acetone (3:1, 2 runs) as the mobile phase to afford **MB10**, crystallization from acetone gave yellow plates (7.2 mg) and a mixture of **MB3** and **MB10** as yellow solid (7.6 mg).

Fractions I10 (226.3 mg) and I11 (54.0 mg) were purified by PLC using hexane-acetone (3:1, 4 runs) as the developing solvent to yield **MB11**, crystallization from acetone afforded yellow needles (63.7 mg), **MB7**, crystallization from acetone gave pale yellow needles (41.8 mg) and **MB13**, crystallization from acetone yielded deep yellow plates (8.8 mg).

The combined fraction H4, H5 and H7 (10.9 g) was separated by CC and the column was eluted with a step gradient of hexane and acetone to afford 10 fractions (J1-J10, Table 9).

Table 9. Fractions obtained from the combined fractions H4, H5 and H7.

Fraction	Weight (mg)	Physical characteristic
J1*	590.5	yellow viscous liquid
J2*	292.5	yellow viscous liquid
J3*	741.3	yellow viscous liquid
J4	103.4	green viscous liquid
J5*	345.3	green viscous liquid
J6*	1684.4	green viscous liquid
J7*	555.6	green viscous liquid
J8*	1968.7	brown viscous liquid
J9	406.9	brown viscous liquid
J10	196.6	brown viscous liquid

* Fractions were further investigated.

Fraction J1 (590.5 mg) was purified by PLC using hexane-acetone (3:1, 3 runs) as the mobile phase to give **MB2**, crystallization from acetone afforded white plates (17.4 mg).

Fraction J2 (292.5 mg) was purified by CC and the column was eluted with CH_2Cl_2 to afford **MB12**, crystallization from acetone gave deep yellow plates (49.2 mg).

Fraction J3 (741.3 mg) was purified by PLC using CH_2Cl_2 (4 runs) as the developing solvent to yield **MB6**, crystallization from acetone afforded pale yellow plates (2.3 mg), **MB8**, crystallization from acetone gave deep yellow plates (23.7mg) and **MB9**, crystallization from acetone yielded yellow needles (3.0 mg).

Purification of J5 (345.3 mg) by CC with CH_2Cl_2 afforded a mixture that yielded **MB6** as pale yellow plates (41.6 mg) and **MB11** as yellow needles (273.0 mg) after crystallization from acetone.

The combined fraction J6 and J7 (2.24 g) was purified by CC using CH_2Cl_2 to afford **MB11**, crystallization from acetone yielded yellow needles (1.92 g).

Fraction J8 (1.97 g) was purified by CC and the column was eluted with CH_2Cl_2 to yield **MB7**, crystallization from acetone gave pale yellow needles (1.94 g).

Fraction H6 (10.09 g) was separated by CC and the column was eluted with a step gradient of hexane and EtOAc to give 11 fractions (K1-K11, Table 10).

Table 10. Fractions obtained from H6.

Fraction	Weight (mg)	Physical characteristic
K1	77.4	green viscous liquid
K2	218.4	green viscous liquid
K3*	311.3	green viscous liquid
K4	327.1	green viscous liquid
K5	194.8	deep green viscous liquid
K6*	3363.1	deep green viscous liquid
K7	2823.5	deep green viscous liquid
K8	396.8	deep green viscous liquid
K9*	1154.9	brown viscous liquid
K10*	672.2	brown viscous liquid
K11	201.6	brown viscous liquid

* Fractions were further investigated.

Fraction K3 (311.3 mg) was purified by CC using CH_2Cl_2 to yield a green solid (120.4 mg) which was recrystallized from acetone to afford **MB14**, crystallization from acetone yielded colorless needles (50.2 mg).

Fraction K6 (3.39 g) was purified by CC and the column was eluted with gradients of hexane and EtOAc to give 6 fractions (L1-L6, Table 11).

Table 11. Fractions obtained from K6.

Fraction	Weight (mg)	Physical characteristic
L1	137.9	green viscous liquid
L2	279.3	green viscous liquid
L3*	873.9	deep green viscous liquid
L4*	411.0	deep green viscous liquid
L5	1343.5	brown viscous liquid
L6	144.8	brown viscous liquid

* Fractions were further investigated.

Fraction L3 (873.9 mg) was purified by PLC using hexane-CH_2Cl_2-EtOAc (6:1:1, 2 runs) as the developing solvent to afford **MB10**, crystallization from acetone gave yellow plates (31.5 mg).

Fraction L4 (411.0 mg) was separated by PLC using hexane-CH_2Cl_2-EtOAc (6:1:1, 3 runs) as the mobile phase to give a yellow viscous liquid (72.3 mg) which was purified by PLC using hexane-acetone (3:1, 3 runs) as the developing solvent to yield **MB6**, crystallization from acetone afforded pale yellow plates (2.7 mg).

Fraction K9 (1.15 g) was purified by CC using CH_2Cl_2 to give **MB7**, crystallization from acetone yielded pale yellow needles (890.4 mg).

Fraction K10 (672.2 mg) was purified by CC and the column was eluted with CH_2Cl_2 to yield **MB11**, crystallization from acetone afforded yellow needles (49.1 mg), **MB7**, crystallization from acetone yielded pale yellow needles (251.6mg) and **MB5**, crystallization from acetone gave colorless needles (10.0 mg).

The combined fraction H11 and H12 (3.74 g) was separated by CC and the column was eluted with a step gradient of hexane and acetone to afford 9 fractions (M1-M9, Table 12).

Table 12. Fractions obtained from the combined fractions H11 and H12.

Fraction	Weight (mg)	Physical characteristic
M1	37.3	green viscous liquid
M2*	107.6	green viscous liquid
M3	475.7	green viscous liquid
M4*	406.7	green viscous liquid
M5	301.9	green viscous liquid
M6	307.5	deep green viscous liquid
M7	388.2	deep green viscous liquid
M8	360.7	deep green viscous liquid
M9	382.2	brown viscous liquid

* Fractions were further investigated.

Addition of acetone to fraction M2 (107.6 mg) gave **MB12** (77.5 mg) as a deep yellow plate.

Fraction M4 (406.7 mg) was purified by CC and the column was eluted with CH_2Cl_2 to yield a yellow solid (322.1 mg) which was crystallized from acetone to afford **MB1** as white needles (119.0 mg).

2.4 Chemical investigation of the pods

2.4.1 Extraction and isolation

The dried pods of *M. brandisiana* (1.33 kg) were immersed in hexane, ethyl acetate and methanol at room temperature. After evaporation, hexane extract (4.14 g), ethyl acetate extract (2.41 g) and methanol extract (20.8 g) were obtained.

2.4.2 Chemical investigation of hexane and ethyl acetate extract

The hexane and ethyl acetate extracts showed similar TLC behavior, they were combined to give a dark green thick liquid (6.55 g). The residue was separated by CC using hexane and acetone in a polarity gradient manner to afford 16 fractions (N1-N16, Table 13).

Table 13. Fractions obtained from the combined hexane and ethyl acetate extract.

Fraction	Weight (mg)	Physical characteristic
N1*	948.0	yellow viscous liquid
N2*	906.2	yellow viscous liquid
N3*	56.4	yellow viscous liquid
N4*	91.3	green viscous liquid
N5*	552.3	green viscous liquid
N6*	294.4	green viscous liquid
N7*	216.2	green viscous liquid
N8*	108.6	green viscous liquid
N9*	178.0	deep green viscous liquid
N10*	167.4	deep green viscous liquid
N11*	666.4	deep green viscous liquid
N12*	75.2	deep green viscous liquid

Table 13. (continued)

Fraction	Weight (mg)	Physical characteristic
N13*	127.5	deep green viscous liquid
N14*	246.3	brown viscous liquid
N15	148.7	brown viscous liquid
N16	134.6	brown viscous liquid

* Fractions were further investigated.

Fraction N1 (948.0 mg) was purified by PLC using hexane-acetone (95:5, 2 runs) as the developing solvent to give 8 fractions (O1-O8, Table 14).

Table 14. Fractions obtained from N1.

Fraction	Weight (mg)	Physical characteristic
O1	62.7	yellow viscous liquid
O2	296.0	yellow viscous liquid
O3*	209.4	yellow viscous liquid
O4*	40.7	yellow viscous liquid
O5*	101.1	yellow viscous liquid
O6*	43.1	green viscous liquid
O7	7.8	green viscous liquid
O8	6.1	green viscous liquid

* Fractions were further investigated.

Fraction O3 (209.4 mg) was purified by PLC using hexane-acetone (98:2, 3 runs) as the mobile phase to yield **MB17**, crystallization from acetone afforded yellow needles (63.2 mg).

Fraction O4 (40.7 mg) was separated by PLC using hexane (5 runs) as the developing solvent to afford **MB17**, crystallization from acetone yielded yellow needles (7.8 mg), **MB15**, crystallization from acetone gave yellow plates (3.6 mg) and a yellow viscous liquid (7.4 mg). The liquid was purified by PLC using hexane-acetone (99:1, 3 runs) as the mobile phase to give **MB15**, crystallization from acetone afforded yellow plates (1.3 mg) and **MB20**, crystallization from acetone yielded yellow needles (2.2mg).

Fraction O5 (101.1 mg) was purified by PLC using hexane-acetone (95:5, 4 runs) as the developing solvent to yield **MB19**, crystallization from acetone afforded pale yellow needles (50.6 mg) and **MB33**, crystallization from acetone gave colorless needles (23.9 mg).

Crystallization of O6 (43.1 mg) from hexane afforded **MB35** as white solid (6.2 mg).

Fraction N2 (906.2 mg) was separated on by PLC using hexane-acetone (95:5, 4 runs) as the developing solvent to yield 6 fractions (P1-P6, Table 15).

Table 15. Fractions obtained from N2.

Fraction	Weight (mg)	Physical characteristic
P1	27.1	yellow viscous liquid
P2*	129.7	yellow viscous liquid
P3*	178.8	yellow viscous liquid
P4*	29.9	yellow viscous liquid
P5	47.2	yellow viscous liquid
P6	36.7	yellow viscous liquid

* Fractions were further investigated.

Fraction P2 (129.7 mg) was purified by PLC using hexane (5 runs) as the mobile phase to give **MB17**, crystallization from acetone afforded yellow needles (30.5 mg).

Fraction P3 (178.8 mg) was purified by PLC using hexane-acetone (98:2, 5 runs) as the developing solvent to yield **MB17**, crystallization from acetone gave yellow needles (3.8 mg), **MB15**, crystallization from acetone afforded yellow plates (2.7 mg) and a yellow viscous liquid (20.9 mg). The liquid was purified by PLC using hexane-CH_2Cl_2 (9:1, 5 runs) as the mobile phase to afford **MB19**, crystallization from acetone yielded pale yellow needles (1.6 mg).

Fraction P4 (29.9 mg) was purified by PLC using hexane-acetone (95:5, 2 runs) as the developing solvent to give **MB19**, crystallization from acetone afforded pale yellow needles (13.9 mg).

Fraction N3 (56.4 mg) was separated by PLC using hexane-acetone (9:1) as the mobile phase to yield **MB17**, crystallization from acetone gave yellow needles (2.0 mg), **MB19**, crystallization from acetone afforded pale yellow needles (2.6 mg) and **MB14**, crystallization from acetone yielded colorless needles (3.5 mg).

Fraction N4 (91.3 mg) was purified by PLC using hexane-acetone (9:1) as the developing solvent to afford **MB17**, crystallization from acetone gave yellow needles (2.3 mg) and **MB14**, crystallization from acetone yielded colorless needles (23.0 mg).

Crystallization of N5 (552.3 mg) from acetone gave **MB14** as colorless needles (2.4 mg) and a yellow viscous liquid (469.8 mg). Repetitive PLC of the liquid using hexane-acetone (9:1, 2 runs) as the mobile phase afforded **MB15**, crystallization from acetone gave yellow plates (3.9 mg), **MB16**, crystallization from acetone yielded yellow plates (3.1 mg), **MB14**, crystallization from acetone afforded colorless needles (120.3 mg) and **MB18**, crystallization from acetone gave pale yellow needles (2.0 mg).

Fraction N6 (294.4 mg) was subjected to CC and the column was eluted with CH_2Cl_2 to afford 4 fractions (Q1-Q4, Table 16).

Table 16. Fractions obtained from N6.

Fraction	Weight (mg)	Physical characteristic
Q1	7.8	yellow viscous liquid
Q2*	93.7	yellow viscous liquid
Q3*	33.5	yellow viscous liquid
Q4*	145.0	yellow viscous liquid

* Fractions were further investigated.

Purification of the fraction Q2 (93.7 mg) by repeated PLC using hexane-acetone (4:1, 3 runs) as the developing solvent yielded **MB21**, crystallization from acetone afforded pale yellow needles (3.4 mg).

Repeated PLC of the combined fraction Q3 and Q4 (178.5 mg) using hexane-acetone (9:1, 5 runs) as the mobile phase gave **MB18**, crystallization from acetone yielded pale yellow needles (49.7 mg).

Fraction N7 (216.2 mg) was separated by PLC using hexane-acetone (4:1, 4 runs) as the developing solvent to yield 8 fractions (R1-R8, Table 17).

Table 17. Fractions obtained from N7.

Fraction	Weight (mg)	Physical characteristic
R1	1.2	yellow viscous liquid
R2	2.1	yellow viscous liquid
R3*	48.2	yellow viscous liquid
R4*	34.4	yellow viscous liquid
R5	8.2	yellow viscous liquid
R6*	13.2	yellow solid
R7*	6.2	yellow solid
R8	5.0	yellow viscous liquid

* Fractions were further investigated.

Purification of the fraction R3 (48.2 mg) by repeated PLC using hexane-acetone (4:1) and CH_2Cl_2 as the mobile phase afforded **MB21**, crystallization from acetone gave pale yellow needles (4.7 mg) and **MB18**, crystallization from acetone yielded pale yellow needles (11.9 mg).

Fraction R4 (34.4 mg) was purified by PLC using CH_2Cl_2 (3 runs) as the developing solvent to give **MB10**, crystallization from acetone afforded yellow plates (10.0 mg), **MB4**, crystallization from CH_2Cl_2 yielded yellow plates (2.5 mg), **MB11**, crystallization from acetone gave yellow needles (2.4 mg) and a mixture of **MB18** and **MB35** as a yellow viscous liquid (2.7 mg).

The ^{1}H NMR spectra of R6 (13.2 mg) and R7 (6.2 mg) demonstrated that they are **MB11**, crystallization from acetone afforded yellow needles (13.2 mg) and **MB21**, crystallization from acetone yielded pale yellow needles (6.2 mg), respectively.

Repetitive PLC of the fraction N8 (108.6 mg) using hexane-acetone (3:1) and CH_2Cl_2 as the mobile phase yielded **MB10**, crystallization from acetone afforded yellow plates (7.5 mg), **MB6**, crystallization from acetone gave pale yellow plates (2.8 mg) and **MB28**, crystallization from acetone yielded yellow plates (3.6 mg).

Repeated PLC of the fraction N9 (178.0 mg) using hexane-acetone (3:1, 5 runs) and CH_2Cl_2 as the developing solvent afforded **MB28**, crystallization from acetone gave yellow plates (17.7 mg), **MB6**, crystallization from acetone yielded pale yellow plates (4.4 mg), **MB11**, crystallization from acetone afforded yellow needles (12.7 mg), **MB25**, crystallization from CH_2Cl_2 gave yellow solid (2.0 mg), **MB7**, crystallization from acetone yielded pale yellow needles (1.7 mg) and the mixture of **MB6** and **MB11** as yellow solid (12.1 mg).

Purification of the fraction N10 (167.4 mg) by repeated PLC using hexane-acetone (3:1, 3 runs) and hexane-EtOAc (3:2, 3 runs) as the mobile phase gave the mixture of **MB11** and **MB25** as yellow solid (5.0 mg), **MB7**, crystallization from acetone afforded pale yellow needles (8.4 mg) and **MB29**, crystallization from acetone yielded yellow plates (4.6 mg).

Crystallization of N11 (666.4 mg) from acetone afforded **MB1** as white needles (227.8 mg) and a yellow viscous liquid (438.6 mg). Further crystallization of the liquid from acetone yielded S1 as a yellow viscous liquid (43.4 mg) and S2 as a yellow viscous liquid (395.2 mg).

Fraction S1 (43.4 mg) was purified by PLC using CH_2Cl_2-MeOH (30:1, 2 runs) as the developing solvent to give **MB1**, crystallization from acetone afforded white needles (0.7 mg), **MB24**, crystallization from acetone yielded yellow plates (1.0 mg) and **MB23**, crystallization from acetone gave yellow plates (2.5 mg).

Repetitive PLC of S2 (395.2 mg) using hexane-acetone-MeOH (3:1:0.2, 4 runs), hexane-EtOAc (3:2, 4 runs), CH_2Cl_2-MeOH (30:1, 2 runs) and CH_2Cl_2 as the mobile phase gave **MB7**, crystallization from acetone afforded pale yellow needles (1.4 mg), **MB24**, crystallization from acetone yielded yellow plates (7.8 mg), **MB1**, crystallization from acetone gave white needles (100.6 mg), **MB5**, crystallization from acetone afforded colorless needles (4.2 mg), **MB27** as yellow solid (3.3 mg), **MB30**, crystallization from CH_2Cl_2 yielded yellow plates (1.8 mg), **MB31** as white powder (3.4 mg), **MB25** as yellow solid (1.6 mg), **MB32**, crystallization from CH_2Cl_2

afforded yellow plates (2.7 mg), **MB26**, crystallization from CH_2Cl_2 gave yellow plates (1.8 mg) and **MB23**, crystallization from acetone yielded yellow plates (2.9 mg).

Repeated PLC of the fraction N12 (75.2 mg) using CH_2Cl_2-MeOH (30:1, 4 runs) and hexane-EtOAc-MeOH (3:2:0.2, 3 runs) as the developing solvent yielded **MB1**, crystallization from acetone afforded white needles (1.2 mg), **MB31** as white powder (0.8 mg) and **MB24**, crystallization from acetone gave yellow plates (0.7 mg).

Purification of the fraction N13 (127.5 mg) by repeated PLC using CH_2Cl_2-MeOH (30:1, 4 runs) and CH_2Cl_2 as the mobile phase gave **MB24**, crystallization from acetone afforded yellow plates (41.6 mg) and **MB23**, crystallization from acetone yielded yellow plates (4.3 mg).

Further crystallization of N14 (246.3 mg) from acetone afforded **MB34** as white plates (18.1 mg) and a yellow viscous liquid (228.2 mg). Repetitive PLC of the liquid (228.2 mg) using CH_2Cl_2-MeOH (30:1, 4 runs) and CH_2Cl_2-MeOH (20:1, 2 runs) yielded **MB24**, crystallization from acetone afforded yellow plates (3.6 mg), **MB23**, crystallization from acetone gave yellow plates (2.5 mg) and **MB22**, crystallization from acetone yielded yellow plates (2.2mg).

Acetylation of MB34

MB34 (10.2 mg) was acetylated with acetic anhydride (2 mL) in pyridine (1 mL) at room temperature overnight. The reaction mixture was poured into ice water and then extracted with CH_2Cl_2. The lower layer was separated and washed with 10% hydrochloric acid and then water. The organic fraction was dried over anhydrous sodium sulfate and evaporated. The residue (14.3 mg) was further purified by PLC using hexane-acetone-MeOH (3:2:0.1, 2 runs). The acetylated product (**MB34OAc**) was obtained as white plates (10.5 mg) after crystallization from acetone.

Anti-inflammatory assay

Animals

Male Sprague-Dawley rats weighing 40-60 g purchased from the National Laboratory Center, Nakorn Pathom, Thailand, were used. The experiment was conducted according to the Ethical Principles and Guidelines for the Use of Animals prepared by the National Research Council of Thailand. The young rats were used in this study as described by Brattsand *et al*. (Brattsand *et al*., 1982) because of the thin skin of the ear and being easy to induce edema by irritant.

Ethyl Phenylpropiolate (EPP)-Induced Ear Edima in Rats

Topical anti-inflammatory activity of compounds **MB7** and **MB11** was assessed by the method described by Brattsand *et al*. Male rats of 40-60 g body weight were used.

The inflammogen EPP was dissolved in acetone and ear edema was induced by topical application of EPP at a dose of 1 mg/20 μL/ear to the inner and outer surfaces of both ears using an automatic microliter pipette. Test substances also dissolved in acetone (20 μL/ear) were administered topically just before the inflammogen. The thickness of the ear was measured with vernier calipers before and at 15, 30, 60 and 120 min after edema induction. The effect of the test substances on the ear edema was compared with that of the vehicle-control group and the present inhibition was calculated.

Statistical Analysis

The data from the experiment were expressed as mean ± S.E.M. Statistical comparison between groups was analyzed by oneway analysis of nariance (ANOVA) and *post hoc* least-significant difference (LSD) test. p values, <0.05 were considered significant.

MB1: 4'-*γ,γ*-dimethylallyloxy-5,7,2',5'-tetramethoxyisoflavone : white needles; m.p. 152-154 °C. UV λ_{max} (EtOH) nm (log ε): 226(5.00), 256(5.50), 290(4.85). IR (acetone film) cm^{-1}: 2936, 2852, 1651, 1571, 1511, 1463, 1285, 1214, 1154, 1042. EIMS *m/z* (%): 426$[M]^+$ (3), 358 (29), 343 (9), 327 (23), 181 (8), 149 (11), 69 (100). HR-ESI-MS *m/z*: 427.1818 $[M+H]^+$ (Calcd. for $C_{24}H_{26}O_7$ + H 427.1757). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 18.

MB2: 7,4'-*bis*-(*γ,γ*-dimethylallyloxy)-5-hydroxyisoflavone (7,4'-di-*O*-prenyl genistein) : white plates; m.p. 112-115 °C. UV λ_{max} (EtOH) nm (log ε): 205 (4.63), 263 (4.40), 238 (4.13). IR (acetone film) cm^{-1}: 3521, 2926, 1714, 1651, 1580, 1510, 1171, 1053. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table19.

MB3: 5-hydroxy-7,2',4',5'-tetramethoxyisoflavone (robustigenin) : yellow needles; m.p. 160-162 °C [(Chibber and Sharma, 1979), 174-175 °C]. UV λ_{max} (MeOH) nm (log ε): 205 (5.02), 260 (4.61), 292 (4.39). IR (acetone film) cm^{-1}: 3509, 2923, 2851, 1652, 1511, 1440, 1301, 1157, 1032. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 20.

MB4: 5-hydroxy-2',4',5'-trimethoxy-6",6"-dimethylchromeno-(7,8,2",3")-isoflavone (toxicarol isoflavone) : yellow plates; m.p. 195-196 °C [(Dagne, Mammo and Sterner, 1992), 213-214 °C]. UV λ_{max} (MeOH) nm (log ε): 277 (3.33). IR (acetone film) cm^{-1}: 3459, 2926, 2856, 1647, 1430. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 20.

MB5: 6-*γ,γ*-dimethylallyl-5,7,4'-trihydroxy-2',5'-dimethoxyisoflavone (viridiflorin) : colorless needles; m.p. 226-228 °C [(Gomez *et al.*, 1985), 220-222 °C]. UV λ_{max} (EtOH) nm (log ε): 207 (4.74), 264 (4.31), 297 (4.11), 282 (4.02), 244 (4.09). IR (acetone film) cm^{-1}: 3520, 2955, 2919, 1711, 1650, 1513, 1061, 903. EIMS *m/z* (%): 398$[M]^+$ (58), 355 (100), 343 (72), 313 (18), 256 (13). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 21.

MB6: (6a*S*,12a*S*)-6,6a-dihydro-11-hydroxy-2,3,9-trimethoxy-[1]benzopyrano [3,4-*b*][1]benzopyran-12(12aH)-one (sermundone) : pale yellow plates; m.p. 210-212 °C [(Nakatani, Ohta and Matsui, 1972), 236-237 °C]. $[\alpha]_D^{28}$ +216.1° (*c* = 0.03, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 203 (4.49), 292 (4.17). IR (acetone film) cm^{-1}: 3436, 2939, 2853, 1645, 1580, 1515, 1461, 1437, 1368, 1307, 1273, 1218, 1159, 1101, 917, 829. EIMS *m/z* (%): 358$[M]^+$ (28), 192 (100), 177 (22), 95 (15), 69 (11). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 22.

MB7: (6a*R*,12a*R*)-6,6a-dihydro-11,12a-dihydroxy-2,3,9-trimethoxy-[1]benzo pyrano[3,4-*b*][1]benzopyran-12(12aH)-one (6-deoxyclitoriacetal): pale yellow needles; m.p. 86-90 °C [(Lin *et al*., 1992), 130-131 °C]. $[\alpha]_D^{28}$ +222.5° (*c* = 0.6, $CHCl_3$), [(Lin *et al*., 1992), +233° (*c* = 0.1, $CHCl_3$)]. UV λ_{max} (MeOH) nm (log ε): 293 (4.31); IR (acetone film) cm^{-1}: 3428, 2922, 2853, 1644, 1510, 1450, 1337, 1157, 1104. EIMS *m/z* (%): 374$[M]^+$ (25), 356 (2), 208 (100), 193 (9), 165 (16), 138 (6). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 23.

MB8: 11-hydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(6H)-one (6a,12a-dehydrosermundone) : deep yellow plates; m.p. 203-204 °C [(Nakatani, Ohta and Matsui, 1972), 208-210 °C]. UV λ_{max} (EtOH) nm (log ε): 209 (5.28), 276 (5.26), 325 (4.94). IR (acetone film) cm^{-1}: 3400, 2917, 2848, 1655, 1582, 1506, 1196, 1048. EIMS *m/z* (%): 356$[M]^+$ (64), 341 (13), 95 (19), 76 (18), 69 (100), 55 (34). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 24.

MB9: 6,11-dihydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(6H)-one (stemonal) : yellow needles; m.p. 190-192 °C [(Shiengthong *et al.*, 1974), 215-216 °C]. $[\alpha]_D^{28}$ +22.4° (*c* = 0.1, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε): 204 (4.06), 274 (4.07). IR (acetone film) cm^{-1}: 3399, 1658, 1578, 1505, 1165, 1028. EIMS *m/z* (%): 372$[M]^+$ (59), 343 (100), 327 (25), 299 (5), 284 (4), 257 (2), 217 (2), 167 (5), 138 (3), 122 (5), 95 (5), 69 (27). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 25.

MB10: (7a*S*,13a*S*)-13,13a-dihydro-6-hydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(7aH)-one (α-toxicarol): yellow plates; m.p. 88-90 °C [(Jang *et al.*, 2003), 102-104 °C]. $[\alpha]_D^{28}$ +43.5° (*c* = 0.2, $CHCl_3$), [(Jang *et al.*, 2003), +34.4° (*c* = 0.2, $CHCl_3$)]. UV λ_{max} (MeOH) nm (log ε): 228 (3.93), 273 (4.16), 295 (3.78), 310 (3.70), 363 (3.15). IR (acetone film) cm^{-1}: 3300, 2920, 1638, 1587, 1513, 1454, 1276, 1198, 1120. EIMS *m/z* (%): 410$[M]^+$ (52), 395 (15), 208 (38), 192 (100), 179 (15). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 26.

MB11: (7a*R*,13a*R*)-13,13a-dihydro-6,7a-dihydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(7aH)-one (12a-hydroxy-α-toxicarol): yellow needles; m.p. 107-110 °C [(Andrei *et al*., 1997), 226 °C]. $[\alpha]_D^{28}$ +21.2° (*c* = 0.8, $CHCl_3$), [(Andrei *et al*., 1997), -1.7° (*c* = 0.5, $CHCl_3$)]. UV λ_{max} (MeOH) nm (log ε): 230 (4.26), 274 (4.51), 310 (4.05), 366 (3.52). IR (acetone film) cm^{-1}: 3422, 2920, 1693, 1585, 1509, 1162, 1119. EIMS *m/z* (%): 426$[M]^+$ (15), 393 (1), 219 (12), 208 (100), 181 (5), 165 (13). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 27.

MB12: 6-hydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(13H)-one (6a,12a-dehydro-α-toxicarol): deep yellow plates; m.p. 232-234 °C [(Lin and Kuo, 1993), 261-263 °C]. UV λ_{max} (EtOH) nm (log ε): 203 (4.14), 221 (4.15), 273 (4.19), 330 (3.72). IR (acetone film) cm^{-1}: 3400, 2933, 1658, 1580, 1510, 1290, 1044, 872, 814, 789. EIMS *m/z* (%): 408$[M]^+$ (63), 393 (100), 365 (6), 349 (3), 307 (3), 204 (3), 152 (3), 77 (3), 69 (15), 55 (8). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 28.

MB13: 6,13-dihydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano [3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(13H)-one (6-hydroxy-6a,12a-dehydro-α-toxicarol): deep yellow plates; m.p. 165 °C (dec.), [(Somleva and Ognyanov, 1985), 170 °C (dec.)]. $[\alpha]_D^{28}$ -16.5° (*c* = 0.1, $CHCl_3$), [(Somleva and Ognyanov, 1985), -11.3° (*c* = 0.2, $CHCl_3$)]. UV λ_{max} (EtOH) nm (log ε): 221 (4.18), 274 (4.37), 327 (3.82). IR (acetone film) cm^{-1}: 3350, 2920, 2850, 1656, 1583, 1513, 1278, 1041. EIMS *m/z* (%): 424$[M]^+$ (12), 328 (14), 152 (12), 91 (100), 77 (38), 65 (10), 55 (40). ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 29.

MB14: stigmast-5-en-3*β*-ol (*β*-sitosterol): colorless needles; m.p. 138-140 °C, [(Moghaddam *et al*., 2007), 141 °C]. $[\alpha]_D^{28}$ -51.2° (*c* = 0.2, CH_2Cl_2). IR (Nujol) cm^{-1}: 3425, 2924, 2854, 2725, 1304, 1253, 1193, 1168, 1154, 1054, 970. ^1H NMR ($CDCl_3$) δ_H 5.35 (1H, *br d*, *J* = 5.2 Hz, H-6), 3.53 (1H, *t*, *J* = 4.2, 11.7 Hz, H-3), 1.03 (3H, *s*, Me-19), 0.92 (3H, *d*, *J* = 6.6 Hz, Me-21), 0.85 (3H, *t*, *J* = 6.3 Hz, Me-29), 0.84 (3H, *d*, *J* = 6.3 Hz, Me-26), 0.81 (3H, *d*, *J* = 6.6 Hz, Me-27), 0.68 (3H, *s*, Me-18).

MB15: (*Z*)-2'-methoxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxy chalcone (purpurenone): yellow plates; m.p. 79-80 °C, [(Rao and Raju, 1984 and Magalhaes *et al.*, 1996), 75.9 °C]. UV λ_{max} (MeOH) nm (log ε): 360 (4.39), 256 (4.35). IR (CH_2Cl_2 film) cm^{-1}: 3423, 2925, 2851, 1634, 1592, 1575, 1459, 1370, 1311, 1279, 1217, 1114, 1077, 986, 776, 693. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 30.

MB16: (*E*)-2'-methoxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxy chalcone: yellow plates; m.p. 97-98 °C. UV λ_{max} (MeOH) nm (log ε): 201 (4.02), 255 (3.66), 225 (3.66), 341 (3.12). IR (CH_2Cl_2 film) cm^{-1}: 3425, 2925, 2854, 1740, 1700, 1593, 1464, 1372, 1281, 1259, 1215, 1163, 1114, 1078, 1022, 983, 806, 739, 695. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 31.

MB17: (*Z*)-2'-hydroxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxy chalcone: yellow needles; m.p. 85-87 °C. UV λ_{max} (MeOH) nm (log ε): 270 (4.82). IR (CH_2Cl_2 film) cm^{-1}: 3430, 3060, 2977, 2917, 2849, 1688, 1602, 1574, 1487, 1455, 1430, 1375, 1336, 1270, 1242, 1129, 1112, 1076, 897, 764, 714, 688, 626. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 32.

MB18: 6",6"-dimethylchromeno-(7,8,2",3")-flavone: pale yellow needles; m.p. 116-118 °C, [(Magalhaes *et al.*, 1996), 137.0 °C]. UV λ_{max} (MeOH) nm (log ε): 225 (4.50), 270 (4.49), 324 (4.15). IR (CH_2Cl_2 film) cm^{-1}: 2975, 2932, 2845, 1635, 1592, 1577, 1450, 1438, 1369, 1381, 1363, 1284, 1214, 1189, 1131, 1114, 1082, 1033, 895, 825, 770, 742, 688. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 33.

MB19: 6",6"-dimethylchromeno-(7,8,2",3")-flavanone (isolonchocarpin): pale yellow needles; m.p. 115-116 °C, [(Rao and Raju, 1979), 108-110 °C]. $[\alpha]_D^{28}$ -63.7° (*c* = 0.05, CH_2Cl_2), [(Rao and Raju, 1979), -93.0° ($CHCl_3$)]. UV λ_{max} (MeOH) nm (log ε): 267 (4.42), 308 (3.97). IR (CH_2Cl_2 film) cm^{-1}: 2928, 2855, 1742, 1686, 1638, 1596, 1578, 1441, 1393, 1377, 1336, 1275, 1213, 1164, 1113, 1100, 1069, 899, 817, 757, 728, 698. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 34.

MB20: 5-hydroxy-6",6"-dimethylchromeno-(7,8,2",3")-flavanone (obovatin): yellow needles; m.p. 118-120 °C, [(Chen *et al.*, 1978), 123-124 °C]. $[\alpha]_D^{28}$ -123.1° (*c* = 0.04, $CHCl_3$), [(Chen *et al*., 1978), -93.8° ($CHCl_3$)]. UV λ_{max} (MeOH) nm (log ε): 202 (4.43), 271 (4.38), 221 (4.35). IR (CH_2Cl_2 film) cm^{-1}: 3255, 2929, 2872, 1727, 1642, 1591, 1480, 1441, 1407, 1373, 1338, 1304, 1276, 1247, 1213, 1197, 1162, 917, 881, 932, 766, 697. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 35.

MB21: 4'-*γ,γ*-dimethylallyloxy-5-hydroxy-7,2',5'-trimethoxyisoflavone: pale yellow needles; m.p. 124-126 °C. UV λ_{max} (MeOH) nm (log ε): 202 (3.96), 260 (3.62), 291 (3.36). IR (CH_2Cl_2 film) cm^{-1}: 3452, 2933, 2854, 1655, 1619, 1585, 1509, 1450, 1397, 1358, 1303, 1272, 1212, 1197, 1156, 1040, 1004, 828, 769, 710, 644. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 36.

MB22: 4'-(2,3-dihydroxy-3-methylbutyloxy)-5,7,2',5'-tetramethoxyisoflavone: yellow plates; m.p. 79-80 °C. $[\alpha]_D^{28}$ +50.6° (*c* = 0.01, CH_2Cl_2). UV λ_{max} (MeOH) nm (log ε): 205 (4.42), 255 (4.10), 288 (3.90). IR (CH_2Cl_2 film) cm^{-1}: 3437, 2919, 2844, 1732, 1645, 1608, 1568, 1510, 1463, 1420, 1362, 1278, 1216, 1148, 1079, 1021, 821, 753. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 37.

MB23: 4'-(2-hydroxy-3-methyl-3-butenyloxy)-5,7,2',5'-tetramethoxyisoflavone : yellow plates; m.p. 128-130 °C. $[\alpha]_D^{28}$ -28.7° (*c* = 0.02, CH_2Cl_2). UV λ_{max} (MeOH) nm (log ε): 201 (2.75), 255 (2.32), 290 (2.10). IR (CH_2Cl_2 film) cm^{-1}: 3442, 2924, 2851, 1645, 1614, 1570, 1512, 1456, 1422, 1362, 1288, 1216, 1155, 1083, 1040, 904, 824, 767, 734. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 38.

MB24: 4'-hydroxy-5,7,2',5'-tetramethoxyisoflavone: yellow plates; m.p. 168-170 °C [(Tsukayama *et al.*, 1980), 191-193 °C]. UV λ_{max} (MeOH) nm (log ε): 276 (4.55), 290 (4.58). IR (CH_2Cl_2 film) cm^{-1}: 3429, 2935, 2827, 1645, 1570, 1515, 1456, 1423, 1290, 1217, 1155, 1084, 1063, 1042, 988, 824. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 39.

MB25: 5,4'-dihydroxy-7,2',5'-trimethoxyisoflavone (derrugenin): yellow solid; m.p. 178-180 °C [(Tsukayama *et al.*, 1980), 212-213 °C]. UV λ_{max} (MeOH) nm (log ε): 204 (3.72), 260 (3.28). IR (CH_2Cl_2 film) cm^{-1}: 3430, 2923, 2851, 1732, 1652, 1617, 1512, 1461, 1456, 1373, 1290, 1197, 1155, 1041, 825, 765. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 40.

MB26: 6-hydroxy-7,4'-dimethoxyisoflavone (alfalone): yellow plates; m.p. 224-226 °C [(Kobayashi *et al.*, 1988), 246-247 °C]. UV λ_{max} (MeOH) nm (log ε): 202 (4.00), 265 (3.59). IR (CH_2Cl_2 film) cm^{-1}: 3444, 2923, 2852, 1735, 1628, 1456, 1369, 1275, 1243, 1174, 1033, 831, 795, 748. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 41.

MB27: 7-hydroxy-6,4'-dimethoxyisoflavone (afrormosin): yellow solid; m.p. 198-200 °C. UV λ_{max} (MeOH) nm (log ε): 201 (4.44), 262 (4.09). IR (CH_2Cl_2 film) cm^{-1}: 3437, 2923, 2851, 1725, 1635, 1540, 1515, 1474, 1442, 1369, 1281, 1232, 1177, 1112, 1025, 816, 797, 736. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 42.

MB28: 5,4'-dihydroxy-2',5'-dimethoxy-6",6"-dimethylchromeno-(6,7,2",3")-isoflavone (elongatin): yellow plates; m.p. 85-86 °C [(Smalberger, Vleggaar and Weber, 1975), 181-182 °C]. UV λ_{max} (MeOH) nm (log ε): 202 (4.39), 279 (4.22). IR (CH_2Cl_2 film) cm^{-1}: 3426, 2971, 2937, 2842, 1652, 1627, 1599, 1581, 1515, 1464, 1422, 1361, 1296, 1197, 1148, 1131, 1059, 1039, 827, 773, 698. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 43.

MB29: 4'-hydroxy-5,2',5'-trimethoxy-6",6"-dimethylchromeno-(6,7,2",3")-isoflavone: yellow plates; m.p. 90-92 °C. UV λ_{max} (MeOH) nm (log ε): 266 (4.09), 224 (4.08). IR (CH_2Cl_2 film) cm^{-1}: 3445, 2924, 2851, 1732, 1645, 1605, 1513, 1464, 1365, 1288, 1207, 1149, 1127, 1063, 1039, 959, 836, 734. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 44.

MB30: 5,4'-dihydroxy-5"-isopropenyl-4",5"-dihydrofurano-(6,7,2",3")-isoflavone (licoagroisoflavone): yellow plates; m.p. 185-186 °C [(Li *et al.*, 2001), 196-198 °C]. $[\alpha]_D^{28}$ -69.6° (*c* = 0.01, CH_2Cl_2), [(Li *et al.*, 2001), -68.1° (*c* = 0.81, MeOH)]. UV λ_{max} (MeOH) nm (log ε): 204 (4.35), 267 (4.23). IR (CH_2Cl_2 film) cm^{-1}: 3412, 2921, 2850, 1717, 1645, 1581, 1515, 1463, 1369, 1306, 1263, 1220, 1176, 1111, 1075, 902, 837, 818. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 45.

MB31: 5,4'-dihydroxy-2',5'-dimethoxy-5"-isopropenyl-4",5"-dihydrofurano-(6,7,2",3")-isoflavone: white powder; m.p. 252-253 °C. $[\alpha]_D^{28}$ -517.4° (*c* = 0.01, CH_2Cl_2). UV λ_{max} (MeOH) nm (log ε): 204 (4.07), 264 (3.70), 295 (3.54). IR (CH_2Cl_2 film) cm^{-1}: 3425, 2916, 2828, 2447, 1735, 1621, 1542, 1510, 1456, 1364, 1287, 1210, 1151, 1064, 1038, 976, 855, 815, 753. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 46.

MB32: 5,4'-dihydroxy-2',5'-dimethoxy-5''-(2-hydroxyisopropyl)-4'',5''-dihydro furano-(6,7,2'',3'')-isoflavone: yellow plates; m.p. 169-170 °C. $[\alpha]_D^{28}$ -83.4° (*c* = 0.02, CH_2Cl_2). UV λ_{max} (MeOH) nm (log ε): 204 (4.55), 263 (4.18), 297 (4.05). IR (CH_2Cl_2 film) cm^{-1}: 3429, 2935, 2852, 1732, 1662, 1628, 1513, 1456, 1417, 1369, 1294, 1204, 1165, 1110, 1061, 1035, 960, 821, 733. ^1H and ^{13}C NMR ($CDCl_3$) data: see Table 47.

MB33: stigmast-5-en-3-one (*β*-sitosterone): colorless needles; m.p. 78-80 °C, [(Cambie *et al.,* 1991), 84-90 °C]. $[\alpha]_D^{24}$ +32.6° (*c* = 0.3, CH_2Cl_2). IR (CH_2Cl_2 film) cm^{-1}: 2935, 2870, 1735, 1674, 1613, 1463, 1376, 1266, 1229, 1184, 972, 865, 738. ^1H NMR ($CDCl_3$) δ_H 5.72 (1H, *br s*, H-6), 1.18 (3H, *s*, Me-19), 0.92 (3H, *d*, *J* = 6.6 Hz, Me-21), 0.85 (3H, *t*, *J* = 6.3 Hz, Me-29), 0.84 (3H, *d*, *J* = 6.3 Hz, Me-26), 0.81 (3H, *d*, *J* = 6.6 Hz, Me-27), 0.71 (3H, *s*, Me-18).

MB34: sitosterol-3-*O*-*β*-D-glucopyranoside: white plates; m.p. 260 °C (decompose), [(Mbafor, Ndom and Fomum, 1997), 259 °C (decompose)]. $[\alpha]_D^{24}$ -64.5° (*c* = 0.03, MeOH). IR (Nujol) cm^{-1}: 3373, 2924, 2854, 1307, 1168, 1074, 1021, 722. ^1H NMR ($CDCl_3$ + CD_3OD) δ_H 5.35 (1H, *br d*, *J* = 4.8 Hz, H-6), 4.40 (1H, *d*, *J* = 7.8 Hz, H-1'), 3.84 (1H, *dd*, *J* = 3.3, 12.0 Hz, H-6'β), 3.76 (1H, *dd*, *J* = 4.5, 12.0 Hz, H-6'α), 3.56 (1H, *tt*, *J* = 4.8, 10.2 Hz, H-3), 3.51-3.24 (4H, *m*, H-2',3',4',5'), 0.99 (3H, *s*, Me-19), 0.90 (3H, *d*, *J* = 6.3 Hz, Me-21), 0.84 (3H, *t*, *J* = 6.9 Hz, Me-29), 0.82 (3H, *d*, *J* = 6.6 Hz, Me-26), 0.80 (3H, *d*, *J* = 6.3 Hz, Me-27), 0.66 (3H, *s*, Me-18).

MB34OAc: sitosterol-3-*O*-*β*-D-glucopyranoside tetraacetate: white plates; m.p. 144-146 °C. $[\alpha]_D^{24}$ -258.9° (*c* = 0.1, CH_2Cl_2). IR (Nujol) cm^{-1}: 2923, 2853, 1751, 1737, 1216, 1155, 1038, 722. ^1H NMR ($CDCl_3$) δ_H 5.37 (1H, *br d*, *J* = 5.1 Hz, H-6), 5.22 (1H, *t*, *J* = 9.6 Hz, H-3'), 5.09 (1H, *t*, *J* = 9.6 Hz, H-4'), 4.97 (1H, *dd*, *J* = 8.1, 9.6 Hz, H-2'), 4.61 (1H, *d*, *J* = 8.1 Hz, H-1'), 4.27 (1H, *dd*, *J* = 4.8, 12.0 Hz, H-6'β), 4.12 (1H, *dd*, *J* = 2.4, 12.0 Hz, H-6'α), 3.96 (1H, *ddd*, *J* = 2.4, 4.8, 9.6 Hz, H-5'), 3.50 (1H, *tt*, *J* = 4.5, 10.8 Hz, H-3), 2.09, 20.6, 20.4, 20.2 (3H, *s*, each, 4xOAc), 1.00 (3H, *s*, Me-19), 0.93 (3H, *d*, *J* = 6.3 Hz, Me-21), 0.86 (3H, *t*, *J* = 6.3 Hz, Me-29), 0.85 (3H, *d*, *J* = 6.3 Hz, Me-26), 0.83 (3H, *d*, *J* = 6.6 Hz, Me-27), 0.69 (3H, *s*, Me-18).

MB35: lup-20(29)-en-3β-ol (lupeol): white solid; m.p. 220-223 °C, [(Rosa, Giulio and Tommonaro, 1997), 215-216 °C]. $[\alpha]_D^{24}$ +26.2° (*c* = 0.2, CH_2Cl_2), [(Rosa, Giulio and Tommonaro, 1997), +26.0° (*c* = 0.01, $CHCl_3$)]. IR (CH_2Cl_2 film) cm^{-1}: 3418, 2918, 2850, 1455, 1397, 1297, 1188, 1108, 1040, 1012, 984, 882, 852, 766, 746. ^1H NMR ($CDCl_3$) δ_H 4.71 (1H, *d*, *J* = 2.1 Hz, H-29a), 4.59 (1H, *d*, *J* = 2.1 Hz, H-29b), 3.21 (1H, *dd*, *J* = 5.4, 10.8 Hz, H-3), 2.38 (1H, *ddd*, *J* = 6.0, 11.1, 16.8 Hz, H-19), 1.70 (3H, *s*, Me-30), 1.05 (3H, *s*, H-26), 0.99 (3H, *s*, Me-23), 0.96 (3H, *s*, Me-27), 0.85 (3H, *s*, Me-25), 0.81 (3H, *s*, Me-28), 0.78 (3H, *s*, Me-24).

Table 18. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB1**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.79 (*s*)	152.3	3	4, 8a, 1'
3	-	122.6		
4	-	175.3		
4a	-	110.1		
5	-	163.7		
6	6.38 (*d*, 2.1)	96.1	5	8, 4a
7	-	161.4		
8	6.47 (*d*, 2.1)	92.6	8a	6, 4a
8a	-	159.9		
1'	-	112.6		
2'	-	151.7		
3'	6.62 (*s*)	100.1	2', 4'	1', 5'
4'	-	148.8		
5'	-	143.4		
6'	6.95 (*s*)	115.9	1', 5'	3, 2', 4'
1"	4.64 (*d*, 6.6)	66.1	2"	4', 3"
2"	5.54 (*t*, 6.6)	120.1		
3"	-	137.6		
4"	1.80 (*s*)*	25.9*	3"	2", 5"
5"	1.78 (*s*)*	18.3*	3"	2", 4"
5-OMe	3.90 (*s*)	55.7		5
7-OMe	3.94 (*s*)	56.4		7
2'-OMe	3.74 (*s*)	56.9		2'
5'-OMe	3.84 (*s*)	56.6		5'

* Interchangeable values

Table 19. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB2**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.88 (*s*)	152.6	3	4, 8a
3	-	123.7		
4	-	180.9		
4a	-	106.2		
5	-	162.7		
6	6.41 (*d*, 2.1)	98.7	5	8, 4a
7	-	164.8		
8	6.43 (*d*, 2.1)	93.1	7, 8a	6, 4a
8a	-	157.9		
1'	-	122.9		
2'	7.46 (*dd*, 1.8, 8.7)	130.1		3, 4', 6'
3'	7.00 (*dd*, 1.8, 8.7)	114.8	4'	1', 5'
4'	-	159.1		
5'	7.00 (*dd*, 1.8, 8.7)	114.8	4'	1', 3'
6'	7.46 (*dd*, 1.8, 8.7)	130.1		3, 4', 2'
1"	4.57 (*d*, 7.5)	64.9	2"	4', 3"
2"	5.54 (*t*, 7.5)	119.5		
3"	-	138.6		
4"	1.78 (*s*)	18.3	3"	2"
5"	1.79 (*s*)	18.2	3"	2"
1"'	4.59 (*d*, 7.5)	65.5	2"'	7, 3"'
2"'	5.51 (*t*, 7.5)	118.6		
3"'	-	139.3		
4"'	1.82 (*s*)	25.8	3"'	2"'
5"'	1.83 (*s*)	25.8	3"'	2"'
5-OH	12.88 (*s*)	-		

Table 20. ^{1}H and ^{13}C NMR ($CDCl_3$) spectral data of **MB3** and **MB4**

Position	**MB3** δ_H (*multiplicity, J_{Hz}*)	**MB3** δ_C	**MB4** δ_H (*multiplicity, J_{Hz}*)	**MB4** δ_C
2	7.90 (*s*)	154.8	7.93 (*s*)	154.6
3	-	121.4	-	122.0
4	-	182.0	-	187.2
4a	-	110.3	-	108.8
5	-	159.7	-	157.3
6	6.40 (*d*, 2.4)	98.1	6.31 (*s*)	94.9
7	-	166.5	-	160.0
8	6.43 (*d*, 2.4)	92.4	-	101.6
8a	-	158.1	-	162.8
1'	-	111.3	-	111.4
2'	-	151.3	-	149.5
3'	6.65 (*s*)	98.2	6.65 (*s*)	98.3
4'	-	149.3	-	145.5
5'	-	141.5	-	143.9
6'	6.90 (*s*)	115.2	6.90 (*s*)	115.6
4"	-	-	6.71 (*d*, 10.2)	128.1
5"	-	-	5.60 (*d*, 10.2)	115.2
6"	-	-	-	77.2
7"	-	-	1.49 (*s*)	28.3
8'	-	-	1.49 (*s*)	28.3
5-OH	12.98 (*s*)	-	12.97 (*s*)	-
7-OMe	3.90 (*s*)*	55.8*	-	-
2'-OMe	3.82 (*s*)*	56.2*	3.81 (*s*)*	56.2*
4'-OMe	3.88 (*s*)*	56.2*	3.88 (*s*)*	56.6*
5'-OMe	3.95 (*s*)*	56.6*	3.95 (*s*)*	56.9*

* Interchangeable values

Table 21. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB5**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.79 (*s*)	154.5	3	4, 8a
3	-	120.3		
4	-	180.9		
4a	-	105.6		
5	-	159.5		
6	-	111.4		
7	-	161.5		
8	6.53 (*s*)	93.3	7, 8a	6, 4a
8a	-	156.0		
1'	-	110.3		
2'	-	152.3		
3'	6.62 (*s*)	100.3	2', 4'	1', 5'
4'	-	146.9		
5'	-	140.6		
6'	6.82 (*s*)	114.8	5'	3, 2', 4'
1"	3.37 (*d*, 7.2)	21.4	6, 2"	5, 7, 3"
2"	5.52 (*t*, 7.2)	121.8		
3"	-	135.0		
4"	1.68 (*s*)	25.7	3"	2", 5"
5"	1.79 (*s*)	17.8	3"	2", 4"
5-OH	13.03 (*s*)	-		
2'-OMe	3.70 (*s*)	56.4		2'
5'-OMe	3.83 (*s*)	56.7		5'

Table 22. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB6**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	6.86 (*s*)	110.3	2	3, 4a, 12a
1a	-	104.4		
2	-	144.0		
3	-	149.7		
4	6.49 (*s*)	101.1	3, 4a	2, 1a
4a	-	147.3		
6eq	4.18 (*d*, 12.0)	66.0	6a	
6ax	4.61 (*dd,* 3.3, 12.0)	-	6a	
6a	4.90 (*dd,* 3.3, 4.2)	71.8		
7a	-	162.0		
8	6.02 (*d*, 2.4)	94.2	7a	10
9	-	168.4		
10	6.06 (*d,* 2.4)	95.4	11	11a
11	-	164.9		
11a	-	101.4		
12	-	194.4		
12a	3.86 (*d*, 4.2)	43.8	1a	
11-OH	12.10 (*s*)	-	11	10, 11a
2-OMe	3.81 (*s*)	56.3		2
3-OMe	3.83 (*s*)	55.9		3
9-OMe	3.79 (*s*)	55.7		9

Table 23. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB7**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	6.70 (*s*)	109.2	2, 1a	3, 4a, 12a
1a	-	108.3		
2	-	144.1		
3	-	151.4		
4	6.50 (*s*)	101.2	3, 4a	2, 1a
4a	-	148.4		
6eq	4.47 (*d*, 11.7)	63.7	6a	12a
6ax	4.60 (*dd,* 2.4, 11.7)	-	6a	12a
6a	4.57 (*d,* 2.4)	75.6	6a, 12a	12, 1a
7a	-	161.6		
8	6.00 (*d*, 2.4)	94.6	9, 7a	10, 11a
9	-	169.1		
10	6.06 (*d,* 2.4)	95.6	9, 11	8, 11a
11	-	164.3		
11a	-	100.2		
12	-	195.0		
12a	-	67.0		
11-OH	11.54 (*s*)	-	11	10, 11a
12-OH	4.21 (*s*)	-	12a	12, 6a
2-OMe	3.76 (*s*)	56.4		2
3-OMe	3.83 (*s*)	55.8		3
9-OMe	3.78 (*s*)	55.9		9

Table 24. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB8**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	8.27 (*s*)	109.9	2	3, 4a, 12a
1a	-	109.7		
2	-	144.1		
3	-	149.2		
4	6.56 (*s*)	100.5	3, 4a	2, 1a
4a	-	146.3		
6	4.99 (*s*)	64.8	6a	4a, 12a
6a	-	157.3		
7a	-	156.6		
8	6.38 (*d*, 2.4)	98.4*	9, 7a	10
9	-	165.3		
10	6.38 (*d*, 2.4)	92.6*	9, 11	8, 11a
11	-	162.8		
11a	-	106.2		
12	-	179.2		
12a	-	110.9		
11-OH	12.94 (*s*)	-	11	10, 11a
2-OMe	3.96 (*s*)	56.4		2
3-OMe	3.88 (*s*)	55.9		3
9-OMe	3.87 (*s*)	55.8		9

* Interchangeable values

Table 25. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB9**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	6.45(*s*)	109.6	2, 1a	3, 4a
1a	-	108.2		
2	-	143.3		
3	-	149.5		
4	6.70 (*s*)	101.7	3, 4a	2, 1a
4a	-	144.4		
6	6.20 (*s*)	88.9	6a	12a
6a	-	155.6		
7a	-	157.5		
8	6.47 (*d*, 2.4)	92.7	9, 7a	10, 11a
9	-	165.5		
10	6.42 (*d*, 2.4)	98.2	11	8, 11a
11	-	162.4		
11a	-	106.4		
12	-	180.3		
12a	-	142.8		
6-OH	3.86 (*s*)	-		
11-OH	12.83 (*s*)	-		
2-OMe	3.98 (*s*)	56.3		2
3-OMe	3.91 (*s*)	55.9		3
9-OMe	3.90 (*s*)	55.8		9

Table 26. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB10**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	6.88 (*s*)	110.3	2, 1a	3, 4a, 12a
1a	-	104.4		
2	-	144.0		
3	-	149.7		
4	6.47 (*s*)	101.1	3, 4a	2, 1a
4a	-	147.3		
6eq	4.18 (*d*, 12.0)	66.0	6a	4a, 12a
6ax	4.63 (*dd,* 3.0, 12.0)	-	6a	4a, 12a
6a	4.89 (*dd,* 3.0, 4.2)	71.9		
7a	-	155.9		
8	-	101.8		
9	-	162.8		
10	5.97 (*s*)	97.8	11	8, 11a
11	-	164.5		
11a	-	101.2		
12	-	194.2		
12a	3.86 (*d*, 4.2)	43.5	12, 1a	
4'	6.57 (*d*, 10.2)	115.4		9, 7a, 6'
5'	5.48 (*d*, 10.2)	126.4	6'	8, 7', 8'
6'	-	78.3		
7'	1.45 (*s*)	28.6	6'	5', 8'
8'	1.38 (*s*)	28.3	6'	5', 7'
11-OH	12.21 (*s*)	-	11	10, 11a
2-OMe	3.81(*s*)	55.9		2
3-OMe	3.86 (*s*)	55.3		3

Table 27. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB11**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	6.73 (*s*)	109.3	2	3, 4a, 12a
1a	-	108.4		
2	-	144.0		
3	-	151.3		
4	6.50 (*s*)	101.1	3, 4a	2, 1a
4a	-	148.3		
6eq	4.48 (*dd*, 0.9, 12.0)	63.6	6a	12a
6ax	4.62 (*dd,* 2.4, 12.0)	-	6a	12a
6a	4.55 (*dd,* 0.9, 2.4)	75.7	12a	
7a	-	155.5		
8	-	102.0		
9	-	164.0		
10	6.00 (*s*)	98.0	11	8, 11a
11	-	163.6		
11a	-	99.0		
12	-	194.8		
12a	-	66.8		
4'	6.53 (*d*, 10.2)	115.1		9, 7a, 6'
5'	5.48 (*d*, 10.2)	126.6	6'	8
6'	-	78.6		
7'	1.33 (*s*)	28.6	6'	5', 8'
8'	1.45 (*s*)	28.4	6'	5', 7'
11-OH	11.65 (*s*)	-	11	10, 11a
12-OH	4.19 (*s*)	-	12a	12, 6a
2-OMe	3.77 (*s*)	56.4		2
3-OMe	3.84 (*s*)	55.9		3

Table 28. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB12**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	8.26 (*s*)	109.7	2	3, 4a, 12a
1a	-	109.9		
2	-	144.2		
3	-	149.2		
4	6.57 (*s*)	100.6	3, 4a	2, 1a
4a	-	146.3		
6	5.01 (*s*)	64.8	6a	4a, 12a
6a	-	156.8		
7a	-	150.9		
8	-	101.1		
9	-	159.3		
10	6.30 (*s*)	100.7	9, 11	8, 11a
11	-	162.4		
11a	-	106.0		
12	-	179.3		
12a	-	110.9		
4'	6.62 (*d*, 10.0)	114.4		9, 7a, 6'
5'	5.61 (*d*, 10.0)	127.8	6'	8, 7', 8'
6'	-	78.1		
7'	1.50 (*s*)	28.2	6'	5', 8'
8'	1.50 (*s*)	28.2	6'	5', 7'
11-OH	13.02 (*s*)	-		
2-OMe	3.95 (*s*)	56.4		2
3-OMe	3.90 (*s*)	55.9		3

Table 29. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB13**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	8.38 (*s*)	109.2	2	3, 4a, 12a
1a	-	107.5		
2	-	144.5		
3	-	149.6		
4	6.65 (*s*)	101.4	3, 4a	2, 1a
4a	-	142.5		
6	6.20 (*s*)	89.1	6a	4a
6a	-	154.1		
7a	-	151.6		
8	-	101.3		
9	-	159.8		
10	6.30 (*s*)	100.6	9, 11	8, 11a
11	-	162.2		
11a	-	105.9		
12	-	180.4		
12a	-	110.7		
4'	6.71 (*d*, 10.2)	114.4		9, 6'
5'	5.63 (*d*, 10.2)	127.7	6'	8
6'	-	78.3		
7'	1.49 (*s*)	28.3	6'	8'
8'	1.51 (*s*)	29.7	6'	7'
6-OH	-	-		
11-OH	12.81 (*s*)	-	11	10, 11a
2-OMe	3.94 (*s*)	56.3		2
3-OMe	3.88 (*s*)	55.8		3

Table 30. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB15**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	-	135.7		
2	7.99 (*d*, 6.9)	127.1		4, 6, β
3	7.49 (*t*, 6.9)	128.6		1, 5
4	7.54 (*t*, 6.9)	132.1		2, 6
5	7.49 (*t*, 6.9)	128.6		1, 3
6	7.99 (*d*, 6.9)	127.1		2, 4, β
α	7.18 (*s*)	96.7	β, β'	
β	-	184.5		
β'	-	185.2		
1'	-	121.8		
2'	-	156.2		
3'	-	115.1		
4'	-	157.6		
5'	6.70 (*d*, 8.7)	113.0		1', 3'
6'	7.72 (*d*, 8.7)	130.8		2'
4"	6.68 (*d*, 9.9)	116.5		4', 6"
5"	5.72 (*d*, 9.9)	130.7	6"	3'
6"	-	77.2		
7"	1.49 (*s*)	28.1	6"	5"
8"	1.49 (*s*)	28.1	6"	5"
2'-OMe	3.83 (*s*)	62.7		2'
β-OH	16.92 (*br s*)	-		

Table 31. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB16**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	-	134.9		
2	7.96 (*dd*, 1.2, 7.2)	128.9		4, 6, β
3	7.48 (*dd,* 7.2, 7.8)	128.7		1, 5
4	7.60 (*dt*, 1.2, 7.8)	133.7		2, 6
5	7.48 (*dd*, 7.2, 7.8)	128.7		1, 3
6	7.96 (*dd*, 1.2, 7.2)	128.9		2, 4, β
α	7.15 (*s*)	78.3	β, β'	
β	-	190.3		
β'	-	191.6		
1'	-	121.8		
2'	-	157.1		
3'	-	114.2		
4'	-	159.4		
5'	6.63 (*d*, 8.7)	113.2	4'	1', 3'
6'	7.71 (*d*, 8.7)	131.7		2', 4', β'
4"	6.46 (*d*, 9.9)	116.6	3'	2', 4', 6"
5"	5.64 (*d*, 9.9)	130.2	6"	3'
6"	-	77.2		
7"	1.44 (*s*)	28.0	6"	5"
8"	1.44 (*s*)	28.0	6"	5"
2'-OMe	3.71 (*s*)	63.2		2'
β-OH	3.49 (*br s*)	-		

Table 32. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB17**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
1	-	133.8		
2	7.93 (*d*, 6.9)	126.6		4, 6, β
3	7.49 (*t*, 6.9)	128.7		1, 5
4	7.53 (*t*, 6.9)	132.0		2, 6
5	7.49 (*t*, 6.9)	128.7		1, 3
6	7.93 (*d*, 6.9)	126.6		2, 4, β
α	6.72 (*s*)	92.0	β, β'	1
β	-	175.7		
β'	-	194.6		
1'	-	112.6		
2'	-	159.7		
3'	-	109.7		
4'	-	159.5		
5'	6.40 (*d*, 9.0)	108.6		1', 3'
6'	7.60 (*d*, 9.0)	129.5		2', β'
4"	6.77 (*d*, 9.9)	116.0		2', 4', 6"
5"	5.62 (*d*, 9.9)	128.2	6"	3'
6"	-	78.0		
7"	1.49 (*s*)	28.3	6"	5"
8"	1.49 (*s*)	28.3	6"	5"
2'-OH	12.82 (*s*)	-	2'	1', 3'
β-OH	15.34 (*s*)	-		

Table 33. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB18**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	-	162.6		
3	6.78 (*s*)	107.4	2, 4	4a, 1'
4	-	178.0		
4a	-	117.8		
5	8.00 (*d*, 8.7)	126.1		4, 7, 8a
6	6.87 (*d*, 8.7)	115.1	7	8, 4a
7	-	157.6		
8	-	109.5		
8a	-	152.3		
1'	-	132.0		
2'	7.90 (*dd*, 2.1, 7.5)	126.0		2, 4'
3'	7.55-7.53 (*m*)	129.1	2'	1', 5'
4'	7.55-7.53 (*m*)	131.4		
5'	7.55-7.53 (*m*)	129.1	6'	1', 3'
6'	7.90 (*dd*, 2.1, 7.5)	126.0		2, 4'
4"	6.94 (*d*, 9.9)	115.2		7, 8a, 6"
5"	5.77 (*d*, 9.9)	130.5	6"	8
6"	-	77.7		
7"	1.51 (*s*)	28.2	6"	5"
8"	1.51 (*s*)	28.2	6"	5"

Table 34. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB19**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	5.48 (*dd*, 3.0, 12.9)	79.8		
3β	3.01 (*dd*, 12.9, 16.8)	44.4	2, 4	1'
3α	2.84 (*dd*, 3.0, 16.8)	-	2, 4	
4	-	190.6		
4a	-	114.8		
5	7.75 (*d*, 8.7)	127.9		4, 7, 8a
6	6.51 (*d*, 8.7)	111.2		8, 4a
7	-	159.7		
8	-	109.5		
8a	-	157.7		
1'	-	139.0		
2'	7.48 (*dd,* 1.8, 7.8)	126.0	3'	6'
3'	7.44 (*dd,* 7.8, 8.1)	128.8		5'
4'	7.41 (*dt,* 1.8, 8.1)	128.6	3', 5'	
5'	7.44 (*dd,* 7.8, 8.1)	128.8		3'
6'	7.48 (*dd,* 1.8, 7.8)	126.0	5'	2'
4"	6.66 (*d*, 9.9)	115.9		7, 8a, 6"
5"	5.58 (*d*, 9.9)	128.9	6"	8
6"	-	77.6		
7"	1.47 (*s*)	28.1	6"	5"
8"	1.45 (*s*)	28.4	6"	5"

Table 35. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB20**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	5.44 (*dd*, 3.3, 12.9)	79.1		
3α	2.83 (*dd*, 3.3, 17.1)	43.3	4	
3β	3.06 (*dd*, 12.9, 17.1)	-	2, 4	1'
4	-	195.7		
4a	-	102.9		
5	-	163.8		
6	6.01 (*s*)	97.7	5, 7	4a, 8
7	-	162.3		
8	-	102.0		
8a	-	156.8		
1'	-	138.5		
2'	7.46 (*dd,* 1.8, 7.8)	126.0	3'	2, 4'
3'	7.44 (*dd,* 7.2, 7.8)	128.8	2', 4'	1'
4'	7.41 (*dt,* 1.8, 7.2)	128.7	3', 5'	2', 6'
5'	7.44 (*dd,* 7.2, 7.8)	128.8	4', 6'	1'
6'	7.46 (*dd,* 1.8, 7.8)	126.0	5'	2, 4'
4"	6.55 (*d*, 9.9)	115.6	8	7, 8a, 6"
5"	5.47 (*d*, 9.9)	126.5	6"	8
6"	-	78.2		
7"	1.45 (*s*)	28.5	6"	8"
8"	1.43 (*s*)	28.3	6"	7"
5-OH	12.09 (*s*)	-	5	4a, 6

Table 36. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB21**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.89 (*s*)	154.8	3	4, 8a, 1'
3	-	120.7		
4	-	180.8		
4a	-	106.4		
5	-	162.6		
6	6.40 (*d*, 2.1)	98.1	5	8
7	-	165.4		
8	6.43 (*d*, 2.1)	92.4	8a	6
8a	-	158.0		
1'	-	110.9		
2'	-	151.8		
3'	6.66 (*s*)	100.0	2', 4'	1', 5'
4'	-	149.4		
5'	-	143.6		
6'	6.89 (*s*)	115.4	5'	3, 2', 4'
1"	4.66 (*d*, 6.6)	66.2	2"	4', 3"
2"	5.55 (*br t*, 6.6)	119.9		
3"	-	137.9		
4"	1.81 (*s*)	25.9	3"	2", 5"
5"	1.79 (*s*)	18.3	3"	2", 4"
5-OH	12.90 (*s*)	-		
7-OMe	3.89 (*s*)	55.8		7
2'-OMe	3.78 (*s*)	56.8		2'
5'-OMe	3.86 (*s*)	56.7		5'

Table 37. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB22**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.80 (*s*)	152.5	3	4, 8a, 1'
3	-	122.4		
4	-	175.3		
4a	-	110.1		
5	-	163.9		
6	6.39 (*d*, 2.1)	96.2	5	4a
7	-	161.4		
8	6.48 (*d*, 2.1)	92.7	8a	4a
8a	-	160.0		
1'	-	114.0		
2'	-	151.9		
3'	6.64 (*s*)	101.0	2', 4'	1', 5'
4'	-	148.3		
5'	-	143.5		
6'	6.98 (*s*)	116.1	5'	3, 2', 4'
1"α	4.13 (*dd*, 6.0, 9.9)	72.4		
1"β	4.33 (*dd*, 2.4, 9.9)	-		
2"	3.75 (*dd*, 2.4, 6.0)	75.1		
3"	-	71.9		
4"	1.35 (*s*)	26.8	3"	2", 5"
5"	1.30 (*s*)	25.6	3"	2", 4"
5-OMe	3.92 (*s*)	55.8		5
7-OMe	3.94 (*s*)	56.5		7
2'-OMe	3.74 (*s*)	56.9		2'
5'-OMe	3.84 (*s*)	56.6		5'

Table 38. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB23**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.80 (*s*)	152.4	3	4, 8a, 1'
3	-	122.4		
4	-	175.4		
4a	-	109.9		
5	-	163.8		
6	6.39 (*d*, 2.1)	96.2	5, 7	8, 4a
7	-	161.4		
8	6.48 (*d*, 2.1)	92.6	8a	6, 4a
8a	-	159.9		
1'	-	114.5		
2'	-	151.9		
3'	6.66 (*s*)	102.4	2', 4'	1', 5'
4'	-	148.4		
5'	-	144.0		
6'	6.99 (*s*)	116.6	1', 5'	3, 2', 4'
1"α	3.98 (*t*, 9.9)	74.8		
1"β	4.18 (*dd*, 2.7, 9.9)	-	2"	4', 3"
2"	4.49 (*br d*, 9.9)	73.4		
3"	-	143.2		
4"a	5.17 (*br s*)	112.6		2"
4"b	5.01 (*br s*)	-		
5"	1.84 (*s*)	19.0	3"	2", 4"
5-OMe	3.91 (*s*)	55.7		5
7-OMe	3.94 (*s*)	56.4		7
2'-OMe	3.74 (*s*)	56.8		2'
5'-OMe	3.85 (*s*)	56.7		5'

Table 39. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB24**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.81 (*s*)	152.5	3	4, 8a, 1'
3	-	122.3		
4	-	175.5		
4a	-	110.1		
5	-	163.8		
6	6.38 (*d*, 2.1)	96.1	5, 7	8, 4a
7	-	161.4		
8	6.47 (*d*, 2.1)	92.6	8a	6, 4a
8a	-	159.9		
1'	-	111.5		
2'	-	152.2		
3'	6.64 (*s*)	99.8	2', 4'	1', 5'
4'	-	146.3		
5'	-	140.2		
6'	6.97 (*s*)	115.2	1', 5'	3, 2', 4'
4'-OH	3.92 (*br s*)	-	4'	
5-OMe	3.91 (*s*)	55.7		5
7-OMe	3.94 (*s*)	56.4		7
2'-OMe	3.71 (*s*)	56.5		2'
5'-OMe	3.86 (*s*)	56.7		5'

Table 40. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB25**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.89 (*s*)	155.0	3	4, 8a
3	-	121.1		
4	-	180.8		
4a	-	109.7		
5	-	159.4		
6	6.38 (*d*, 2.4)	98.1		
7	-	165.4		
8	6.41 (*d*, 2.4)	92.4		
8a	-	157.4		
1'	-	109.9		
2'	-	152.3		
3'	6.67 (*s*)	100.0	2', 4'	1'
4'	-	146.8		
5'	-	140.7		
6'	6.89 (*s*)	114.4	5'	3, 4'
5-OH	12.89 (*s*)	-	5	
4'-OH	5.77 (*br s*)	-		
7-OMe	3.86 (*s*)	55.8		7
2'-OMe	3.74 (*s*)	56.4		2'
5'-OMe	3.86 (*s*)	56.7		5'

Table 41. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB26**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.95 (*s*)	151.9	3	4, 8a, 1'
3	-	123.7		
4	-	176.2		
4a	-	119.7		
5	7.76 (*s*)	108.8	6	4, 7, 8a
6	-	142.8		
7	-	152.3		
8	6.90 (*s*)	99.0	7, 8a	6, 4a
8a	-	152.6		
1'	-	123.8		
2'	7.53 (*d*, 9.0)	130.2	1'	3, 4'
3'	6.99 (*d*, 9.0)	114.0	4'	1'
4'	-	159.5		
5'	6.99 (*d*, 9.0)	114.0	4'	1'
6'	7.53 (*d*, 9.0)	130.2	1'	3, 4'
6-OH	3.76 (*br s*)	-		7
7-OMe	4.04 (*s*)	55.4		7
4'-OMe	3.86 (*s*)	56.5		4'

Table 42. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB27**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.95 (*s*)	152.0	3	4, 1'
3	-	124.2		
4	-	175.7		
4a	-	119.5		
5	7.67 (*s*)	104.8		4, 7, 8a
6	-	146.4		
7	-	151.3		
8	7.00 (*s*)	102.6	7, 8a	4a
8a	-	153.4		
1'	-	124.4		
2'	7.52 (*d*, 8.7)	130.2	1'	3, 4'
3'	6.99 (*d*, 8.7)	114.0	4'	1'
4'	-	159.5		
5'	6.99 (*d*, 8.7)	114.0	4'	1'
6'	7.52 (*d*, 8.7)	130.2	1'	3, 4'
7-OH	-	-		
6-OMe	4.04 (*s*)	56.5		6
4'-OMe	3.87 (*s*)	55.3		4'

Table 43. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB28**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.83 (*s*)	154.8	3	4, 8a, 1'
3	-	120.3		
4	-	180.9		
4a	-	106.2		
5	-	156.8		
6	-	105.5		
7	-	159.3		
8	6.33 (*s*)	94.9	7, 8a	6, 4a
8a	-	157.3		
1'	-	110.0		
2'	-	152.3		
3'	6.66 (*s*)	100.0	2', 4'	1', 5'
4'	-	146.8		
5'	-	140.3		
6'	6.87 (*s*)	114.4	1', 5'	3, 2', 4'
4"	6.72 (*d*, 9.9)	115.6	6	5, 7, 6"
5"	5.62 (*d*, 9.9)	128.1	6"	6
6"	-	77.9		
7"	1.47 (*s*)	28.3	6"	5"
8"	1.47 (*s*)	28.3	6"	5"
5-OH	13.19 (*s*)	-		
4'-OH	5.78 (*br s*)	-		
2'-OMe	3.73 (*s*)	56.4		2'
5'-OMe	3.87 (*s*)	56.6		5'

Table 44. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB29**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.80 (*s*)	152.7	3	4, 8a, 1'
3	-	122.2		
4	-	175.1		
4a	-	104.1		
5	-	155.9		
6	-	113.3		
7	-	157.9		
8	6.60 (*s*)	100.7	7, 8a	6
8a	-	158.7		
1'	-	111.4		
2'	-	152.2		
3'	6.63 (*s*)	99.9	2', 4'	1', 5'
4'	-	146.5		
5'	-	140.2		
6'	6.93 (*s*)	114.8	1', 5'	3, 2', 4'
4"	6.76 (*d*, 10.2)	116.2		5, 7, 6"
5"	5.74 (*d*, 10.2)	130.7	6"	6
6"	-	77.7		
7"	1.49 (*s*)	28.3	6"	5"
8"	1.49 (*s*)	28.3	6"	5"
4'-OH	3.94 (*br s*)	-		
5-OMe	3.90 (*s*)	62.8		5
2'-OMe	3.74 (*s*)	56.4		2'
5'-OMe	3.88 (*s*)	56.7		5'

Table 45. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB30**

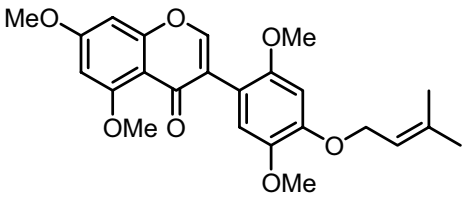
Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.85 (*s*)	152.6	3	4, 8a, 1'
3	-	124.0		
4	-	180.9		
4a	-	105.6		
5	-	160.3		
6	-	109.1		
7	-	163.1		
8	6.49 (*s*)	95.2	8a	6, 4a
8a	-	156.7		
1'	-	123.4		
2'	7.41 (*d*, 8.7)	130.4		4', 6'
3'	6.92 (*d*, 8.7)	115.5	4'	1'
4'	-	154.8		
5'	6.92 (*d*, 8.7)	115.5	4'	1'
6'	7.41 (*d*, 8.7)	130.4		2', 4'
4"a	2.95 (*dd*, 7.8, 15.0)	28.2	6	
4"b	3.20 (*dd*, 2.1, 15.0)	-	6, 5"	5, 7
5"	4.45 (*br d*, 7.8)	77.2		
6"	-	146.6		
7"a	5.01 (*br s*)	110.5		5", 8"
7"b	4.90 (*br s*)	-		5", 8"
8"	1.89 (*s*)	18.6	6"	5", 7"
5-OH	13.27 (*s*)	-	5	6, 4a
4'-OH	-	-		

Table 46. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB31**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.86 (*s*)	154.9	3	4, 8a, 1'
3	-	122.3		
4	-	182.7		
4a	-	106.3		
5	-	159.9		
6	-	109.6		
7	-	163.0		
8	6.49 (*s*)	94.7	8a	4a
8a	-	156.7		
1'	-	110.4		
2'	-	152.5		
3'	6.68 (*s*)	100.6	2', 4'	1', 5'
4'	-	147.3		
5'	-	141.0		
6'	6.88 (*s*)	115.2	5'	3, 2', 4'
4"α	2.94 (*dd*, 8.1, 15.0)	28.9	6, 5"	5, 7, 6"
4"β	3.20 (*dd*, 2.1, 15.0)	-	6	5, 7, 6"
5"	4.44 (*br d*, 7.5)	76.2		
6"	-	147.1		
7"a	5.01 (*br s*)	110.2		5", 8"
7"b	4.90 (*br s*)	-		5", 8"
8"	1.87 (*s*)	18.4	6"	5", 7"
5-OH	13.31 (*s*)	-		
4'-OH	5.77 (*br s*)	-	4'	
2'-OMe	3.76 (*s*)	56.5		2'
5'-OMe	3.88 (*s*)	56.8		5'

Table 47. ^{1}H, ^{13}C NMR and HMBC ($CDCl_3$) spectral data of **MB32**

Position	δ_H (*multiplicity, J_{Hz}*)	δ_C	HMBC	
			^{2}J	^{3}J
2	7.88 (*s*)	154.8	3	4, 8a
3	-	120.3		
4	-	181.0		
4a	-	106.7		
5	-	157.1		
6	-	108.9		
7	-	165.9		
8	6.39 (*s*)	88.9	7, 8a	6, 4a
8a	-	158.2		
1'	-	110.0		
2'	-	152.3		
3'	6.68 (*s*)	100.0	2', 4'	1', 5'
4'	-	146.8		
5'	-	140.3		
6'	6.89 (*s*)	114.4	5'	3, 2', 4'
4"α	3.13 (*dd*, 8.1, 15.6)	26.9	6	
4"β	3.23 (*dd*, 9.3, 15.6)	-		
5"	4.81 (*dd*, 8.1, 9.3)	91.8		
6"	-	72.0		
7"	1.38 (*s*)	25.9	6"	5", 8"
8"	1.26 (*s*)	23.9	6"	5", 7"
5-OH	13.15 (*s*)	-		
4'-OH	5.77 (*br s*)	-		
6"-OH	-	-		
2'-OMe	3.76 (*s*)	56.5		2'
5'-OMe	3.89 (*s*)	56.7		5'



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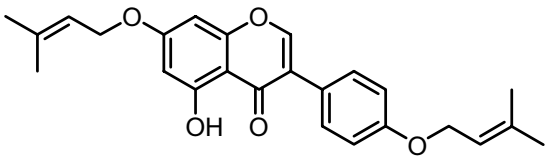

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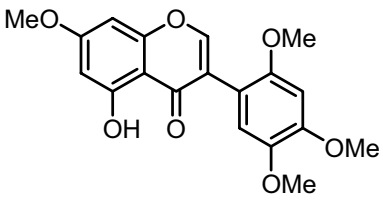

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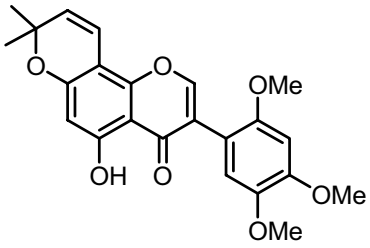

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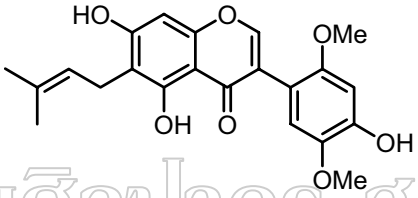

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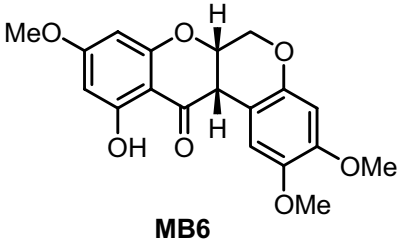
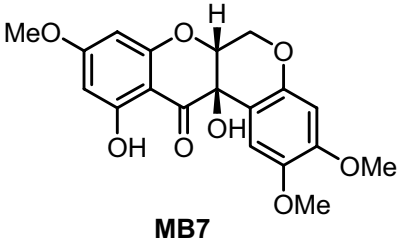


MB6



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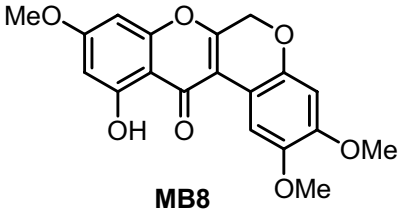

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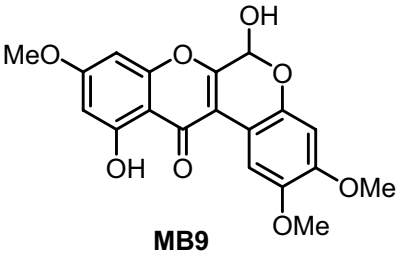

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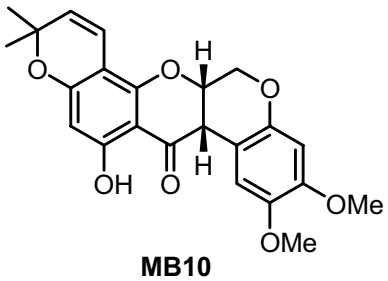

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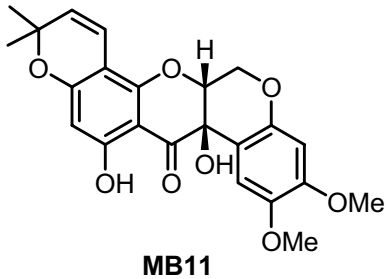

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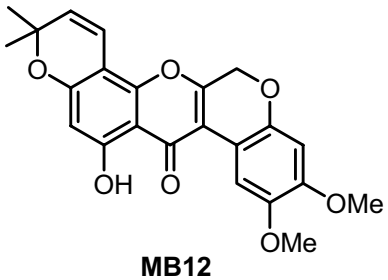

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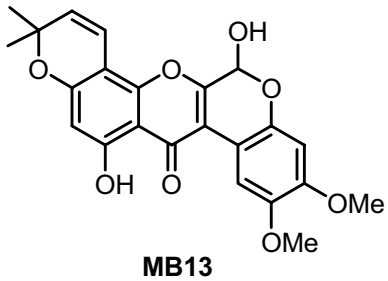

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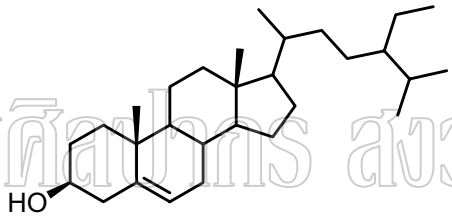

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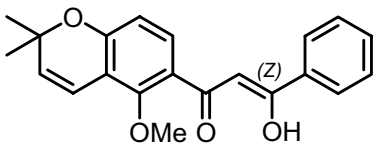

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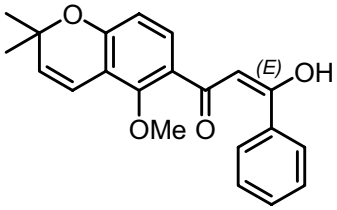

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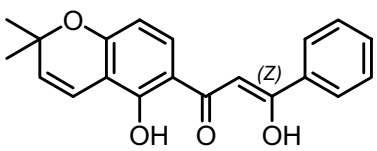

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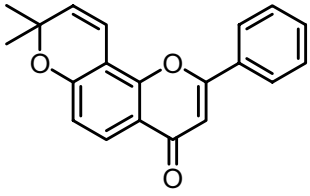

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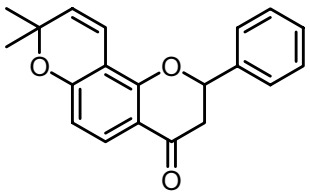

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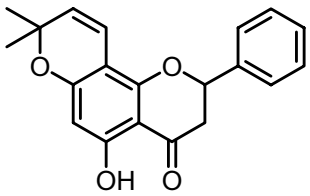

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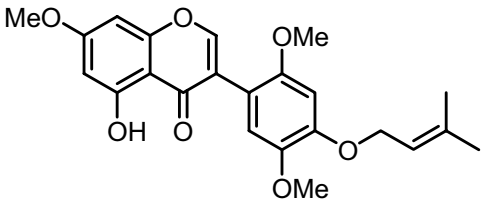

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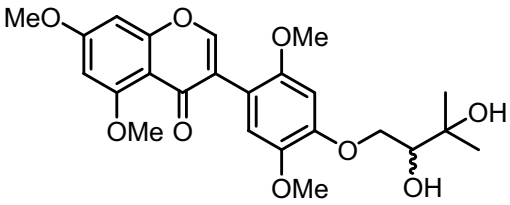

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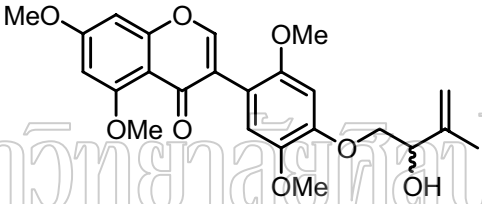

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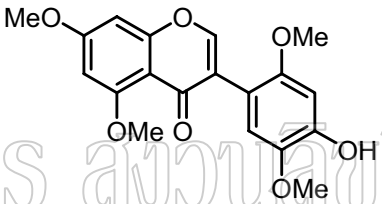

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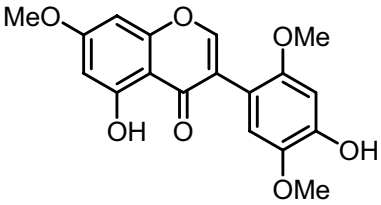

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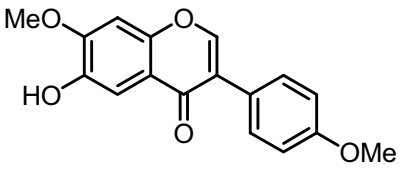

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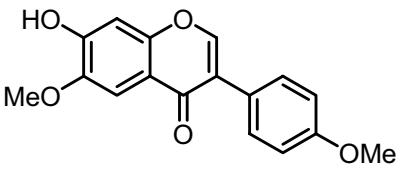

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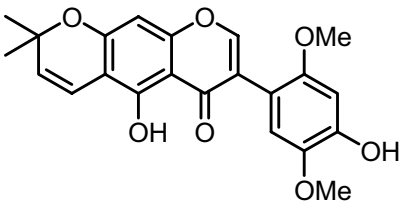

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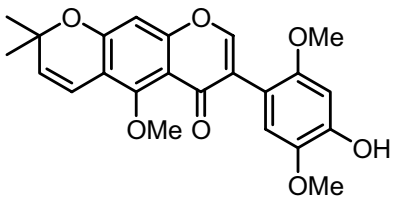

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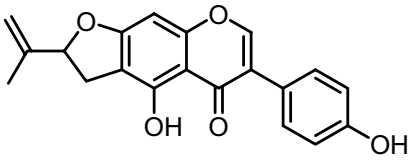

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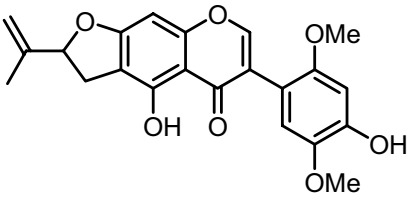

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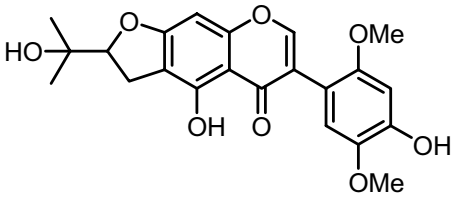

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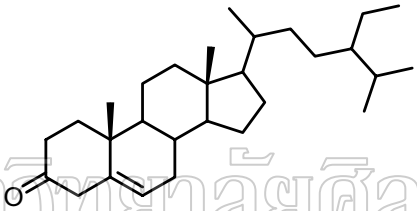

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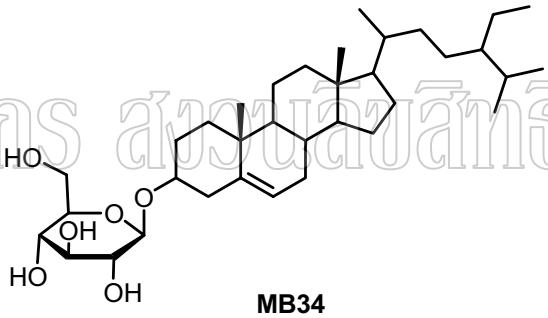

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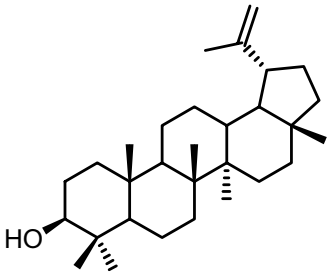


MB35

CHAPTER 3

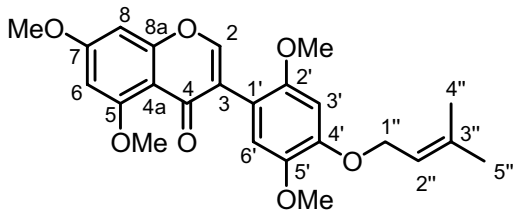
RESULTS AND DISCUSSION

3.1 Structural determination of compounds isolated the leaves and pods of *Millettia brandisiana*

The hexane and ethyl acetate extracts from the leaves and pods of *M. brandisiana* were separated by chromatographic methods to afford eight new compounds, two synthetically known isoflavones and twenty-five known compounds. Purification of the ethyl acetate extract from the leaves gave a new isoflavone, 4'-*γ,γ*-dimethylallyloxy-5,7,2',5'-tetramethoxyisoflavone (**MB1**), a synthetically known isoflavone, 7,4'-*bis*-(*γ,γ*-dimethylallyloxy)-5-hydroxyisoflavone (**MB2**), three known isoflavones, robustigenin (**MB3**), toxicarol isoflavone (**MB4**) and viridiflorin (**MB5**), eight known rotenoids, sermundone (**MB6**), 6-deoxyclitoriacetal (**MB7**), 6a,12a-dehydrosermundone (**MB8**), stemonal (**MB9**), α-toxicarol (**MB10**), 12a-hydroxy-α-toxicarol (**MB11**), 6a,12a-dehydro-α-toxicarol (**MB12**) and 6-hydroxy-6a,12a-dehydro-α-toxicarol (**MB13**) and *β*-sitosterol (**MB14**). Investegration of the haxnae and ethyl acetate extracts from the pods afforded two new *β*-hydroxylchalcones, (*E*)-2'-methoxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone (**MB16**) and (*Z*)-2'-hydroxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone (**MB17**), a known *β*-hydroxylchalcone, (*Z*)-2'-methoxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone (**MB15**), a known flavone, 6",6"-dimethylchromeno-(7,8,2",3")-flavone (**MB18**), two known flavanones, isolonchocarpin (**MB19**) and obovatin (**MB20**), seven new isoflavones, **MB1**, 4'-*γ,γ*-dimethylallyloxy-5-hydroxy-7,2',5'-trimethoxyisoflavone (**MB21**), 4'-(2,3-dihydroxy-3-methylbutyloxy)-5,7,2',5'-tetra methoxyisoflavone (**MB22**), 4'-(2-hydroxy-3-methyl-3-butenyloxy)-5,7,2',5'-tetra methoxyisoflavone (**MB23**), 4'-hydroxy-5,2',5'-trimethoxy-6",6"-dimethylchromeno-(6,7,2",3")-isoflavone (**MB29**), 5,4'-dihydroxy-2',5'-dimethoxy-5"-isopropenyl-4",5"-

dihydrofurano-(6,7,2",3")-isoflavone (**MB31**) and 5,4'-dihydroxy-2',5'-dimethoxy-5"-(2-hydroxyisopropyl)-4",5"-dihydrofurano-(6,7,2",3")-isoflavone (**MB32**), a syntheticcally known isoflavone, 4'-hydroxy-5,7,2',5'-tetramethoxyisoflavone (**MB24**), seven known isoflavones, **MB4**, **MB5**, derrugenin (**MB25**), alfalone (**MB26**), afrormosin (**MB27**), elongatin (**MB28**) and licoagroisoflavone (**MB30**), four known rotenoids, **MB6**, **MB7**, **MB10** and **MB11**, three sterols, **MB14**, *β*-sitosterone (**MB33**) and sitosterol-3-*O*-*β*-D-glucopyranoside (**MB34**) and lupeol (**MB35**). The structures of all compounds were elucidated by spectroscopic analysis including 2D NMR techniques and comparison spectral data with those previously reported in the literatures.

MB1 : 4'-γ,γ-dimethylallyloxy-5,7,2',5'-tetramethoxyisoflavone



MB1 is white needles, m.p. 152-154 °C. Its molecular formula was determined to be $C_{24}H_{26}O_7$ by HR-ESI-MS ($[M+H]^+$ *m/z* 427.1818, calcd. 427.1757). The UV spectrum showed maxima absorptions at λ 290 and 256 nm. The IR spectrum showed a strong absorption band corresponding to the carbonyl group at 1651 cm^{-1}. The ^1H NMR spectrum (Table 18) exhibited a characteristic signal of an isoflavone proton at δ_H 7.79 (*s*, H-2). The ^{13}C, DEPT and HMQC spectra showed 24 carbon signals, corresponding to four methoxyls, two methyls, one methylene, six methines and eleven quaternary carbons. Two methoxyls were attached to ring A, as indicated by signals at δ_H 3.90 and 3.94 (3H each, *s*) which were confirmed by ^3J correlations between 5-OMe (δ_H 3.90) to C-5 (δ_C 163.7) and 7-OMe (δ_H 3.94) to C-7 (δ_C 161.4) in the HMBC spectrum (Table 18). This was supported by an AM spin system at δ_H 6.38 (*d*, *J* = 2.1 Hz, H-6) and δ_H 6.47 (*d*, *J* = 2.1 Hz, H-8). In addition, the signals for a γ,γ-dimethylallyloxy group [δ_H 1.78, 1.80 (3H each, *s*, 2xMe), δ_H 4.64 (*d*, *J* = 6.6 Hz, H_2-1") and δ_H 5.54 (*t*, *J* = 6.6 Hz, H-2")], as well as two methoxyls at δ_H 3.84 and 3.74 (3H each, *s*) were observed. For ring B, the appearance of two aromatic proton singlets at δ_H 6.62 and 6.95 indicated that the *para*-correlation of two protons was deduced. Its HMBC spectrum exhibited ^3J correlations of the singlets at δ_H 6.62 to C-1' (δ_C 112.6) and δ_H 6.95 to C-3 (δ_C 122.6) suggesting that the two protons were H-3' and H-6', respectively. From the NOE difference experiment, irradiation of H-6' (δ_H 6.95) enhanced the signal of the methoxyl at δ_H 3.84 (2.6%) indicating that this methoxyl group was located at C-5' which was confirmed by the HMBC correlation of the signal at δ_H 3.84 to C-5' (δ_C 143.4). Irradiation of H-3' (δ_H 6.62) enhanced the signal of the

methoxyl at δ_H 3.74 (3.8%) and the methylene (H_2-1") at δ_H 4.64 (5.8%), while irradiation of 5'-OMe (δ_H 3.84) enhanced the signal of H-6' (11.6%) and irradiation of H_2-1" (δ_H 4.64) enhanced the signal of H-3' (14.4%). Irradiation of the methoxyl at δ_H 3.74 enhanced the signal of H-3' (12.1%) but no NOE effect to the signal of 5'-OMe was observed. The placement of the second methoxyl group at C-4' was unlikely; the γ,γ-dimethylallyloxy unit was therefore placed at C-4'. This was supported ^3J correlation of H_2-1" (δ_H 4.64) to C-4' (δ_C 148.8) and 2'-OMe (δ_H 3.74) to C-2' (δ_C 151.7) in the HMBC spectrum. **MB1** was then identified as 4'-γ,γ-dimethylallyloxy-5,7,2',5'-tetramethoxyisoflavone. It was a new isoflavone derivative.

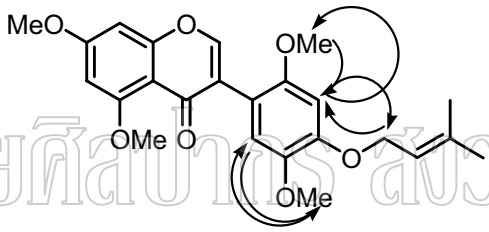
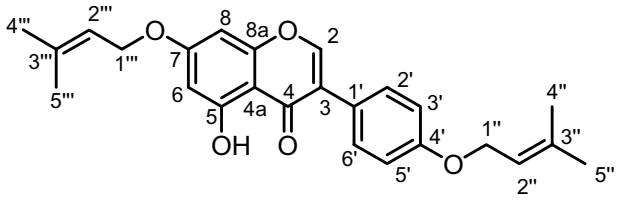


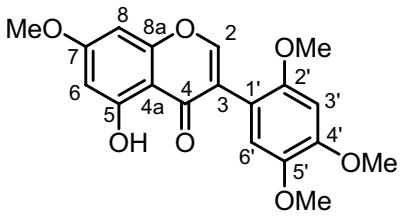
Figure 2. Selected NOE difference correlations of **MB1**

MB2 : 7,4'-*bis*-(*γ,γ*-dimethylallyloxy)-5-hydroxyisoflavone
(7,4'-di-*O*-prenylgenistein)



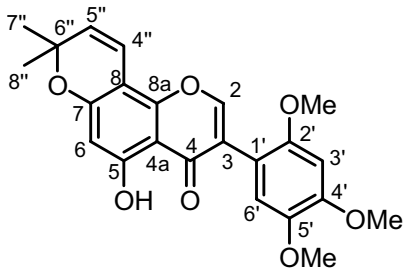
MB2 is white plates, m.p. 112-115 °C. Its molecular formula was $C_{25}H_{26}O_5$ as indicated by the mass spectrum ($[M]^+$ *m/z* 388). The UV spectrum showed maxima absorptions at λ 263, 238 and 205 nm. IR absorption bands at 3511 and 1651 cm^{-1} suggested the presence of hydroxyl group and carbonyl group, respectively. The ^1H NMR spectrum (Table 19) exhibited a characteristic signal of an isoflavone proton at δ_H 7.88 (*s*, H-2) and a singlet of a chelated hydroxyl group (5-OH) at δ_H 12.88. The ^{13}C, DEPT and HMQC spectra showed 25 carbon signals, corresponding to four methyls, two methylenes, nine methines and ten quaternary carbons. For ring A, the ^1H NMR spectrum of **MB2** displayed an AM spin system at δ_H 6.41 (*d*, *J* = 2.1 Hz, H-6) and δ_H 6.43 (*d*, *J* = 2.1 Hz, H-8). The 4'-oxysubstituted pattern of B-ring was readily deduced from proton signals forming an A_2B_2 system at δ_H 7.46 (*dd*, *J* = 8.7, 1.8 Hz, H-2', H-6') and δ_H 7.00 (*dd*, *J* = 8.7, 1.8 Hz, H-3', H-5'). In addition, the signals for two *γ,γ*-dimethylallyloxy groups {[δ_H 1.78, 1.79 (3H each, 2xMe), δ_H 4.57 (2H, *d*, *J* = 7.5 Hz) and δ_H 5.54 (1H, *t*, *J* = 7.5 Hz)] and [δ_H 1.82, 1.83 (3H each, 2xMe), δ_H 4.59 (2H, *d*, *J* = 7.5 Hz) and δ_H 5.51 (1H, *t*, *J* = 7.5 Hz)]}were observed. The HMBC spectrum exhibited ^3J correlations of the methylene protons at δ_H 4.57 to C-4' (δ_C 159.1) and δ_H 4.59 to C-7 (δ_C 164.8) suggesting that the two *γ,γ*-dimethylallyloxy units were therefore placed at C-4' and C-7, respectively. **MB2** was thus concluded to be 7,4'-*bis*-(*γ,γ*-dimethylallyloxy)-5-hydroxyisoflavone (7,4'-di-*O*-prenylgenistein). This compound was a synthetically known but naturally new isoflavone (Jain and Sharma, 1974).

MB3 : 5-hydroxy-7,2',4',5'-tetramethoxyisoflavone (robustigenin)



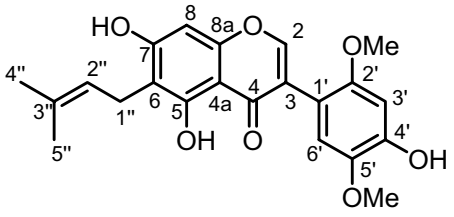
MB3 is yellow needles, m.p. 160-162 °C. The UV spectrum showed maxima absorptions at λ 292, 260 and 205 nm. The IR spectrum exhibited the absorption bands of O-H stretching (3459 cm^{-1}) and C=O stretching (1647 cm^{-1}). The ^{1}H NMR spectrum (Table 20) showed the characteristic resonances of an isoflavone proton at δ_H 7.93 (*s*, H-2) and a hydrogen-bonded hydroxyl proton at δ_H 12.97 (*s*, 5-OH). The ^{13}C, DEPT and HMQC spectra showed 19 carbon signals, corresponding to four methoxyls, five methines and ten quaternary carbons. In addition, four singlets for methoxyl group (δ_H 3.95, 3.90, 3.88 and 3.82) were observed. A methoxyl group (δ_H 3.90, 3H, *s*, 7-OMe) was attached to ring A due to the presence of an AM spin system at δ_H 6.43 (*d*, *J* = 2.4 Hz, H-6) and δ_H 6.40 (*d*, *J* = 2.4 Hz, H-8). For ring B, the appearance of two singlets of aromatic protons at δ_H 6.65 and 6.90 indicated that the *para*-correlation of two protons was deduced. Two singlets of aromatic protons at δ_H 6.65 and 6.90 were then assigned to H-3' and H-6', respectively. The three methoxyl groups at δ_H 3.95, 3.82 and 3.88 (3H each, *s*) were therefore placed at C-4', C-2' and C-5', according to the HMBC correlations. Comparison of the ^{1}H NMR data with those of 5-hydroxy-7,2',4',5'-tetramethoxyisoflavone, **MB3** was thus determined to be 5-hydroxy-7,2',4',5'-tetramethoxyisoflavone (robustigenin) (Chibber and Sharma, 1979).

MB4 : 5-hydroxy-2',4',5'-trimethoxy-6",6"-dimethylchromeno-(7,8,2",3")-isoflavone (toxicarol isoflavone)



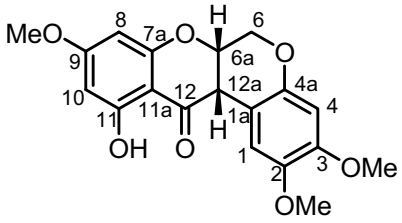
MB4 is yellow plates, m.p. 195-196 °C. The UV spectrum showed maxima absorptions at λ 277 nm. The IR spectrum showed absorption bands corresponding to the hydroxyl and carbonyl group at 3509 and 1652 cm^{-1}, respectively. The ^{1}H NMR spectrum (Table 20) was similar to that of **MB3** (robustigenin) except the presence of signals of a 6",6"-dimethylchromene unit [δ_H 6.71 (*d*, *J* = 10.2 Hz, H-4"), δ_H 5.60 (*d*, *J* = 10.2 Hz, H-5") and δ_H 1.49 (6H, *s*, 2xMe)] in **MB4** instead of the signals for one aromatic proton and one methoxyl group. The ^{13}C, DEPT and HMQC spectra showed 23 carbon signals, corresponding to three methoxyls, two methyls, six methines and twelve quaternary carbons. For ring A, the singlet at δ_H 6.31 (1H) was attributed to H-6. The 6",6"-dimethylchromene unit [δ_H 6.71 (*d*, *J* = 10.2 Hz, H-4"), δ_H 5.60 (*d*, *J* = 10.2 Hz, H-5") and δ_H 1.49 (6H, *s*, 2xMe)] was therefore placed at C-7 and C-8 of isoflavone skeleton. For ring B, two singlets of aromatic protons at δ_H 6.65 and 6.90 were assigned to H-3' and H-6', respectively. The three methoxyl groups at δ_H 3.95, 3.81 and 3.88 (3H each, *s*) were therefore placed at C-4', C-2' and C-5' as a result from the HMBC spectrum. Comparison of the ^{1}H NMR data with those of 5-hydroxy-2',4',5'-trimethoxy-6",6"-dimethylchromeno-(7,8,2",3")-isoflavone, **MB4** was thus identified as 5-hydroxy-2',4',5'-trimethoxy-6",6"-dimethylchromeno-(7,8,2",3")-isoflavone (toxicarol isoflavone) (Dagne, Mammo and Sterner, 1992).

MB5 : 6-*γ,γ*-dimethylallyl-5,7,4'-trihydroxy-2',5'-dimethoxyisoflavone (viridiflorin)



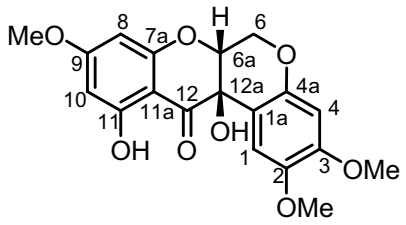
MB5 is colorless needles, m.p. 226-228 °C. Its molecular formula was $C_{22}H_{22}O_7$ as indicated by mass spectrum ($[M]^+$ *m/z* 398). This compound exhibited UV absorption bands at λ 297, 282, 264, 244 and 207 nm and IR spectral bands at 3520 (hydroxyl group), 1650 (carbonyl group) cm^{-1}. The ^1H NMR spectrum (Table 21) showed the characteristic resonance of an isoflavone proton at δ_H 7.79 (*s*, H-2) and a singlet of a chelated hydroxyl group at δ_H 13.03 (5-OH). The ^{13}C, DEPT and HMQC spectra showed 22 carbon signals, corresponding to two methoxyls, two methyls, one methylene, five methines and twelve quaternary carbons. The signal for a *γ,γ*-dimethylallyl group [δ_H 1.68, 1.79 (3H each, 2xMe), δ_H 3.37 (*d*, *J* = 7.2 Hz, H_2-1") and δ_H 5.52 (*t*, *J* = 7.2 Hz, H-2")] and one aromatic proton (δ_H 6.53, 1H, *s*) was observed. The HMBC spectrum of **MB5** exhibited ^3J correlations of δ_H 3.37 (H_2-1") to C-5 (δ_C 159.5) and C-7 (δ_C 161.5). The placement of the aromatic proton at C-6 was unlikely; the *γ,γ*-dimethylallyl unit was therefore placed at C-6. This was supported by a ^3J correlation of the hydroxyl group (δ_H 13.03, *s*, 5-OH) to C-6 (δ_C 111.4) in the HMBC spectrum. For ring B, the appearance of two aromatic proton singlets at δ_H 6.62 and 6.82 indicated the *para*-correlation of two protons. The HMBC spectrum of **MB5** exhibited ^3J correlations of the singlets at δ_H 6.62 to C-1' (δ_C 110.3) and δ_H 6.82 to C-3 (δ_C 120.3) suggesting that the two protons were H-3' and H-6', respectively. In addition, two methoxyl groups at δ_H 3.70 (3H, *s*) and 3.83 (3H, *s*) were therefore located at C-2' (δ_C 152.3) and C-5' (δ_C 140.6), respectively. This was supported by comparison of their spectral data with that **MB1**. Accordingly, the structure of **MB5** was proposed to be 6-*γ,γ*-dimethylallyl-5,7,4'-trihydroxy-2',5'-dimethoxyisoflavone or known as viridiflorin (Gomez *et al*., 1985).

MB6 : (6a*S*,12a*S*)-6,6a-dihydro-11-hydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(12aH)-one (sermundone)



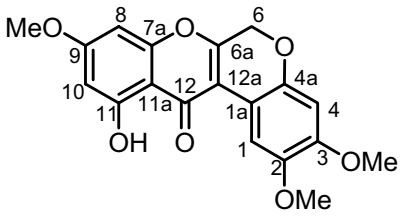
MB6 is pale yellow plates, m.p. 210-212 °C. The molecular formula was $C_{19}H_{18}O_7$ as indicated by mass spectrum ($[M]^+$ *m/z* 358). The UV spectrum of M**B6** showed maxima absorptions at λ 292 and 203 nm. The IR spectrum exhibited absorption bands at 3436 (O-H), 1645 (C=O), 1580 and 1515 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 19 carbon signals, corresponding to three methoxyls, one methylene, six methines and nine quaternary carbons. The ^1H NMR (Table 22) signals at δ_H 4.18 (*d*, *J* = 12.0 Hz, H-6eq), 4.61 (*dd*, *J* = 3.3, 12.0 Hz, H-6ax), 4.90 (*dd*, *J* = 3.3, 4.2 Hz, H-6a) and 3.86 (*d*, *J* = 4.2 Hz, H-12a) and the signals at ^{13}C NMR δ_C 43.8 (C-12a), 66.0 (C-6) and 71.8 (C-6a) implied that **MB6** is a rotenoid. The ^1H NMR spectrum displayed, in addition to signals for three methoxyl groups, two doublets (*J* = 2.4 Hz) at δ_H 6.02 and 6.06 for H-8 and H-10, respectively. Two singlets at δ_H 6.86 and 6.49 integrating for a single proton each were assigned to H-1 and H-4, respectively. Based on the H-1 chemical shift value (δ_H 6.86) the B/C ring junction in **MB6** was determined to be *cis*- (Crombie and Lown, 1962). The three methoxyl groups at δ_H 3.81, 3.83 and 3.79 (3H each, *s*) were therefore placed at C-2, C-3 and C-9, respectively. The unambiguous assignment of quaternary carbons and the locations of the methoxyls and hydroxyl group were established by 2D NMR correlations and comparison of the data with those of (6a*S*,12a*S*)-6,6a-dihydro-11-hydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(12aH)-one. The structure of **MB6** was thus determined to be (6a*S*,12a*S*)-6,6a-dihydro-11-hydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(12aH)-one (sermundone) (Nakatani, Ohta and Matsui, 1972).

MB7 : (6a*R*,12a*R*)-6,6a-dihydro-11,12a-dihydroxy-2,3,9-trimethoxy-[1]benzopyrano [3,4-*b*][1]benzopyran-12(12aH)-one (6-deoxyclitoriacetal)



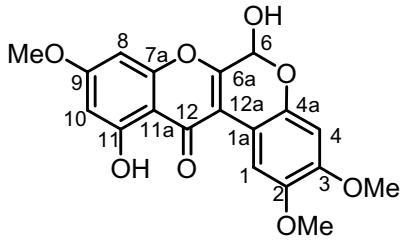
MB7 is pale yellow needles, m.p. 86-90 °C. Its molecular formula was $C_{19}H_{18}O_8$ as indicated by mass spectrum ($[M]^+$ *m/z* 374). The UV spectrum showed maxima absorption at λ 293 nm. The IR spectrum showed strong absorption bands at 3428 (O-H) and 1644 (C=O) cm^{-1}. The ^1H NMR spectrum (Table 23) was similar to that of **MB6** (sermundone) except the presence of a signal due to a hydroxyl group (δ_H 4.21, *br s*) and the signal of H-12a (δ_H 3.86, *d*, *J* = 4.2 Hz). The ^{13}C, DEPT and HMQC spectra showed 19 carbon signals, corresponding to three methoxyls, one methylene, five methines and ten quaternary carbons. A doublet of doublet at δ_H 4.60 (*J* = 2.4, 11.7 Hz) was attributed to H-6ax and two doublets at δ_H 4.57 (*J* = 2.4 Hz) and 4.47 (*J* = 11.7 Hz) were assigned to H-6a and H-6eq, respectively. The placement of the hydroxyl group (δ_H 4.21, *br s*) was then at C-12a. **MB7** was deduced to be a 12a-hydroxyrotenoid on the basis of both the NMR and mass spectral data. In addition, the signals for four aromatic protons were observed at δ_H 6.70 (*s*, H-1), 6.50 (*s*, H-4), 6.00 (*d*, *J* = 2.4, H-8) and 6.06 (*d*, *J* = 2.4, H-10). A singlet at δ_H 11.54 (1H) was assigned to a chelated phenolic proton (11-OH). The three methoxyl groups at δ_H 3.76, 3.83 and 3.78 (3H each, *s*) were therefore placed at C-2, C-3 and C-9, respectively. Comparison of the ^1H NMR data with those of (6a*R*,12a*R*)-6,6a-dihydro-11,12a-dihydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*] [1]benzopyran-12(12aH)-one, the structure of **MB7** was thus determined to be (6a*R*,12a*R*)-6,6a-dihydro-11,12a-dihydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*] [1]benzopyran-12(12aH)-one (6-deoxyclitoriacetal) (Lin *et al*., 1992).

MB8 : 11-hydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(6H)-one (6a,12a-dehydrosermundone)



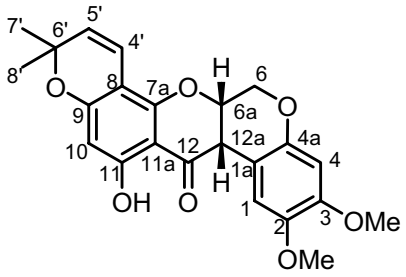
MB8 was obtained as deep yellow plates, m.p. 203-204 °C. The UV spectrum showed maxima absorptions at λ 325, 276 and 209 nm. The mass spectrum gave the molecular formula of $C_{19}H_{16}O_7$ and showed fragmentation peaks at 356 (M^+, 64%), 341 (13%), 95 (19%), 76 (18%) and 69 (100%). The IR spectrum showed absorption bands at 3400 (O-H), 1655 (C=O), 1614, 1582 and 1506 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 19 carbon signals, corresponding to three methoxyls, one methylene, four methines and eleven quaternary carbons. The ^1H NMR spectrum (Table 24) was similar to that of **MB6** (sermundone) except that **MB8** exhibited a deshielded signal of a characteristic dehydrorotenoid proton at δ_H 8.27 (*s*, H-1). The signal of four aromatic protons were observed at δ_H 8.27 (*s*, H-1), 6.56 (*s*, H-4) and 6.38 (*s*, H-8 and H-10). Two singlets at δ_H 4.99 (2H) and 12.94 (1H) were assigned to H_2-6 and a chelated phenolic proton (11-OH), respectively. The three methoxyl groups at δ_H 3.96, 3.88 and 3.87 (3H each, *s*) were therefore placed at C-2, C-3 and C-9, respectively. By comparison of the ^1H NMR data with those of 11-hydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(6H)-one, **MB8** can be assigned as 11-hydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(6H)-one (6a,12a-dehydrosermundone) (Bai *et al.*, 2004 and Baran-Marzak, Massicot and Molho, 1971).

MB9 : 6,11-dihydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(6H)-one (stemonal)



MB9 is yellow needles, m.p. 190-192 °C. Its molecular formula was $C_{19}H_{16}O_8$ as indicated by the mass spectrum ($[M]^+$ *m/z* 372). The UV spectrum showed maxima absorptions at λ 274 and 204 nm. The IR spectrum exhibited bands at 3399 (O-H), 1658 (C=O), 1615, 1578 and 1505 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 19 carbon signals, corresponding to three methoxyls, five methines and eleven quaternary carbons. The ^1H NMR spectrum (Table 25) was similar to that of **MB8** (sermundone) except the signal integrating for one proton (H-6) of **MB9** appeared downfield at δ_H 6.20 (1H, *s*) compared to that of **MB8** (δ_H 4.99, 2H, *s*). The substitution of one proton of H_2-6 by a hydroxyl group could be expected. **MB9** was deduced to be a 6-hydroxy-6a,12a-dehydrorotenoid on the basis of the NMR and mass spectral data. A broad singlet at δ_H 3.86 (1H) which disappeared after D_2O exchange confirmed the presence of a 6-OH. A singlet of a chelated hydroxyl group (11-OH) at δ_H 12.83 was also observed. Two singlets at δ_H 8.45 and 6.70 integrating for a single proton each were assigned to H-1 and H-4. The two doublets (J = 2.4 Hz) at δ_H 6.42 and 6.47 were attributed to H-8 and H-10, respectively. The three methoxyl groups at δ_H 3.98, 3.91 and 3.90 (3H each, *s*) were therefore placed at C-2, C-3 and C-9, respectively. By comparison of the ^1H NMR data with those of 6,11-dihydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(6H)-one, **MB9** was determined to be 6,11-dihydroxy-2,3,9-trimethoxy-[1]benzopyrano[3,4-*b*][1]benzopyran-12(6H)-one (stemonal) (Shiengthong, 1974).

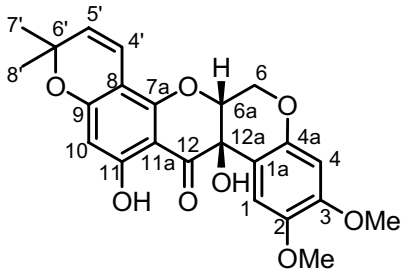
MB10 : (7a*S*,13a*S*)-13,13a-dihydro-6-hydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(7aH)-one (α-toxicarol)



MB10 is yellow plates, m.p. 88-90 °C. The molecular formula was $C_{23}H_{22}O_7$ as indicated by the mass spectrum ($[M]^+$ *m/z* 410). The UV spectrum showed maxima absorptions at λ 363, 310, 295, 273 and 228 nm. The IR spectrum showed absorption bands corresponding to the hydroxyl (3300 cm^{-1}), aromatic (1587, 1513 and 1454 cm^{-1}) and carbonyl group (1639 cm^{-1}). The ^{13}C, DEPT and HMQC spectra showed 23 carbon signals, corresponding to two methoxyls, two methyls, one methylene, seven methines and eleven quaternary carbons. The ^1H NMR spectrum (Table 26) was similar to that of **MB6** (sermundone) except that **MB10** had the signals of a 6',6'-dimethylchromene ring [δ_H 6.57 and 5.48 (1H each, *d*, *J* = 10.0 Hz), 1.45 and 1.38 (3H each, *s*)] instead of the signals of one aromatic proton and one methoxyl group. The 6',6'-dimethylchromene ring was therefore placed at C-8 and C-9 of rotenoid skeleton. This was confirmed by ^3J correlations of H-4' (δ_H 6.57) to C-7a (δ_C 155.9) and C-9 (δ_C 162.8) and H-5' (δ_H 5.48) to C-8 (δ_C 101.8) in the HMBC spectrum. The two doublets of doublets at δ_H 4.63 (*J* = 2.4, 11.7 Hz) and 4.89 (*J* = 2.4, 3.0 Hz) and two doublets at δ_H 4.18 (*J* = 12.0 Hz) and 3.86 (*J* = 4.2 Hz) were assigned to H-6ax, H-6a, H-6eq and H-12a, respectively. The singlet of a chelated hydroxyl group (11-OH) at δ_H 12.21 was observed. The signals of three aromatic protons were observed at δ_H 6.88 (*s*, H-1), 6.47 (*s*, H-4) and 5.97 (*s*, H-10). The two methoxyl groups at δ_H 3.76 and 3.78 (3H each, *s*) were therefore placed at C-2 and C-3, respectively. By comparison of the ^1H NMR data with those of (7a*S*,13a*S*)-13,13a-dihydro-6-hydroxy-9,10-dimethoxy-3,3-

dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(7aH)-one, **MB10** was thus identified as (7a*S*,13a*S*)-13,13a-dihydro-6-hydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(7aH)-one (α-toxicarol) (Jang *et al*., 2003).

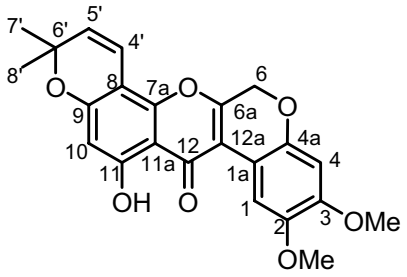
MB11 : (7a*R*,13a*R*)-13,13a-dihydro-6,7a-dihydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(7aH)-one (12a-hydroxy-α-toxicarol)



MB11 is yellow needles, m.p. 107-110 °C. The mass spectrum gave the molecular formula of $C_{23}H_{22}O_8$ and showed the fragmentation peaks at 426 (M^+, 15%), 393 (M^+, 1%), 219 (M^+, 12%), 208 (M^+, 100%), 181 (M^+, 15%) and 165 (M^+, 13%). The UV spectrum showed maxima absorptions at λ 366, 310, 274 and 230 nm. The IR spectrum showed absorption bands at 3422 (O-H), 1693 (C=O), 1588 and 1509 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 23 carbon signals, corresponding to two methoxyls, two methyls, one methylene, six methines and twelve quaternary carbons. The ^1H NMR spectrum (Table 27) was similar to that of **MB10**, except that **MB11** had a signal of a hydroxyl group at δ_H 4.19 (1H, *br s*) instead of the signal of H-12a at δ_H 3.86 (1H, *d*, *J* = 4.2 Hz). Thus, the hydroxyl group (δ_H 4.19) was located at C-12a (δ_C 66.8). This was supported by ^3J correlations of the 12a-OH (δ_H 4.19) to C-12 (δ_C 194.8) and C-6a (δ_C 75.7) in the HMBC spectrum. The three doublets of doublets at δ_H 4.48 (*J* = 0.9, 12.0 Hz) and 4.62 (*J* = 2.4, 12.0 Hz) and 4.55 (*J* = 0.9, 2.4 Hz) were assigned to H-6eq, H-6ax and H-6a, respectively. The singlet of a chelated hydroxyl group (11-OH) at δ_H 11.65 was observed. The signals of three aromatic protons appeared at δ_H 6.73 (*s*, H-1), 6.50 (*s*, H-4) and 6.00 (*s*, H-10). The two methoxyl groups at δ_H 3.77 and 3.84 (3H each, *s*) were therefore placed at C-2 and C-3, respectively. The 6',6'-dimethylchromene ring [δ_H 6.53 and 5.48 (*d*, *J* = 10.2 Hz, H-4' and H-5'), 1.45 and 1.33 (*s*, H_3-7' and H_3-8')] was located at C-8 and C-9. This

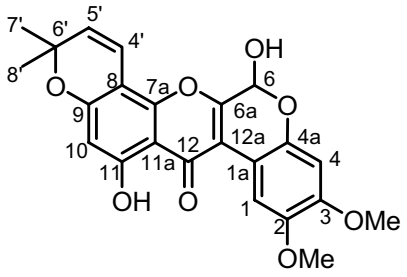
was confirmed by ^{3}J correlations of H-4' (δ_H 6.53) to C-9 (δ_C 164.0) and C-7a (δ_C 155.5) in the HMBC spectrum. By comparison of the ^{1}H NMR data with those of (7a*R*,13a*R*)-13,13a-dihydro-6,7a-dihydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1] benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(7aH)-one, **MB11** was elucidated as (7a*R*,13a*R*)-13,13a-dihydro-6,7a-dihydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1] benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(7aH)-one (12a-hydroxy-α-toxicarol) (Andrei *et al*., 1997).

MB12 : 6-hydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano [2,3-*h*][1]benzopyran-7(13H)-one (6a,12a-dehydro-α-toxicarol)



MB12 is deep yellow plates, m.p. 232-234 °C. The molecular formula was $C_{23}H_{20}O_7$ as indicated by the mass spectrum ($[M]^+$ *m/z* 408). This compound exhibited UV absorption bands at λ 330, 273, 221 and 203 nm and IR absorption bands at 3400 (hydroxyl group), 1658 (carbonyl group), 1580 and 1510 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 23 carbon signals, corresponding to two methoxyls, two methyls, one methylene, five methines and thirteen quaternary carbons. The ^1H NMR spectrum (Table 28) was similar to that of **MB11**, except that **MB12** showed a deshielded signal of a characteristic dehydrorotenoid proton at δ_H 8.26 (*s*, H-1). The signals of three aromatic protons were observed at δ_H 8.26 (*s*, H-1), 6.57 (*s*, H-4) and 6.30 (*s*, H-10). Two singlets at δ_H 5.01 (2H) and 13.02 (1H) were assigned to H_2-6 and a chelated phenolic proton (11-OH), respectively. The two methoxyl groups at δ_H 3.95 and 3.90 (3H each, *s*) were therefore placed at C-2 and C-3, respectively. The 6',6'-dimethylchromene ring [δ_H 6.62 and 5.61 (*d*, *J* = 10.0 Hz, H-4' and H-5'), 1.50 (*s*, H_3-7' and H_3-8')] was located at C-8 and C-9 of 6a,12a-dehydrorotenoid skeleton. This was confirmed by ^3J correlations of H-4' (δ_H 6.62) to C-9 (δ_C 159.3) and C-7a (δ_C 150.9) in the HMBC spectrum. By comparison of the ^1H NMR data with those of 6-hydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1] benzopyran-7(13H)-one, **MB12** was determined to be 6-hydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(13H)-one (6a,12a-dehydro-α-toxicarol) (Lin and Kuo, 1993).

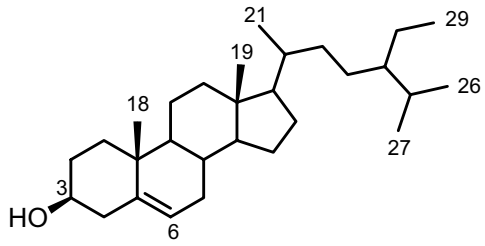
MB13 : 6,13-dihydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*] pyrano[2,3-*h*][1]benzopyran-7(13H)-one
(6-hydroxy-6a,12a-dehydro-α-toxicarol)



MB13 is deep yellow plates, m.p. 165 °C (decompose). The molecular formula was $C_{23}H_{20}O_8$ as indicated by the mass spectrum ($[M]^+$ *m/z* 424). The UV spectrum showed maxima absorption bands at λ 327, 274 and 221 nm. The IR spectrum showed absorption bands at 3350 (O-H), 1656 (C=O), 1583 and 1513 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 23 carbon signals, corresponding to two methoxyls, two methyls, six methines and thirteen quaternary carbons. The ^1H NMR spectrum (Table 29) was similar to that of **MB12**, except that the signal of H-6 of **MB13** appeared downfield at δ_H 6.20 (1H, *s*) compared to that of **MB12** (δ_H 5.01, 2H, *s*). Thus, the substitution of one proton of H_2-6 by a hydroxyl group could be expected. **MB13** was deduced to be a 6-hydroxy-6a,12a-dehydrorotenoid on the basis of the NMR and mass spectral data. In addition, the singlet of a chelated hydroxyl group (11-OH) at δ_H 12.81 was observed. The signals of three aromatic protons were observed at δ_H 8.38 (*s*, H-1), 6.65 (*s*, H-4) and 6.30 (*s*, H-10). The two methoxyl groups at δ_H 3.94 and 3.88 (3H each, *s*) were therefore placed at C-2 and C-3, respectively. The 6',6'-dimethylchromene ring [δ_H 6.71 and 5.63 (*d*, *J* = 10.2 Hz, H-4' and H-5'), 1.49 and 1.51 (*s*, H_3-7' and H_3-8')] was then assigned at C-8 and C-9. This was confirmed by a ^3J correlation of H-4' (δ_H 6.71) to C-9 (δ_C 159.8) in the HMBC spectrum. By comparison of the ^1H NMR data with those of 6,13-dihydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(13H)-one, **MB13**

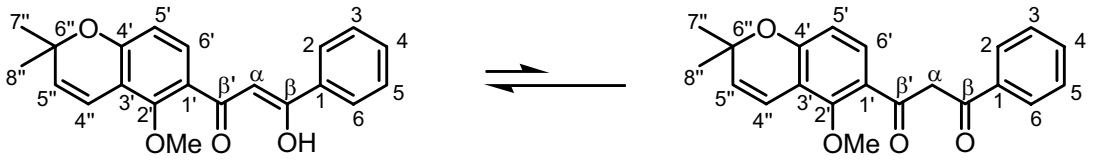
was thus determined to be 6,13-dihydroxy-9,10-dimethoxy-3,3-dimethyl-(3H)-[1]benzo pyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-7(13H)-one (6-hydroxy-6a,12a-dehydro-α-toxicarol) (Somleva and Ognyanov, 1985).

MB14 : stigmast-5-en-3β-ol (β-sitosterol)



MB14 was obtained as colorless needles, m.p. 138-140 °C, $[\alpha]_D^{28}$ -51.2°, (*c* = 0.2, CH_2Cl_2). The IR spectrum showed absorption bands at 3425 (O-H), 2924, 2854 (CH_2, CH_3), 2725 (C=C), 1168, 1154 and 1054 (C-O) cm^{-1}. The ^1H NMR spectrum showed the resonances of an oxymethine proton at δ_H 3.53 (1H, *tt*, *J* = 4.2, 11.7 Hz, H-3) and an olefinic proton at δ_H 5.35 (1H, *br d*, *J* = 5.2 Hz, H-6). The signals for six methyls at δ_H 1.03 (3H, *s*, Me-19), 0.92 (3H, *d*, *J* = 6.6 Hz, Me-21), 0.85 (3H, *t*, *J* = 6.3 Hz, Me-29), 0.84 (3H, *d*, *J* = 6.3 Hz, Me-26), 0.81 (3H, *d*, *J* = 6.6 Hz, Me-27) and 0.68 (3H, *s*, Me-18) were observed. By comparison of the ^1H NMR data with those of stigmast-5-en-3β-ol, **MB14** was identified as stigmast-5-en-3β-ol (β-sitosterol) (Moghaddam *et al.*, 2007).

MB15 : (*Z*)-2'-methoxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone



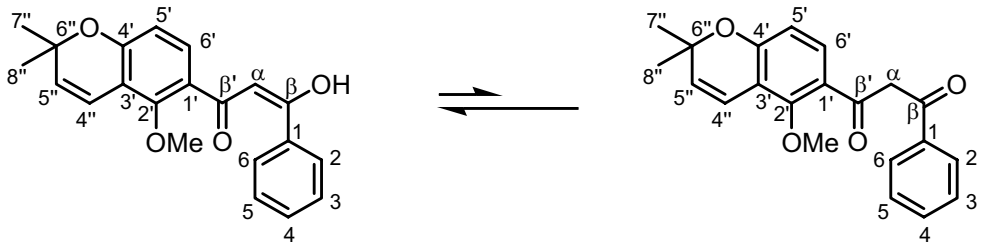
MB15 **MB15a**

MB15 is yellow plates, m.p. 79-80 °C. The UV spectrum showed maxima absorptions at λ 360 and 256 nm. The IR spectrum showed absorption bands at 3423 (O-H), 1634 and 1592 (C=O) and 1575 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 21 carbon signals, corresponding to a methoxyl, two methyls, ten methines, six quaternary and two carbonyl carbons. The ^{1}H NMR spectrum (Table 30) showed a singlet of a deshielded hydroxyl group at δ_H 16.92 and a singlet of an olefinic proton at δ_H 7.18 which correlated with a carbon at δ_C 96.7 in the HMQC spectrum. These features and the similarity to known compounds implied the presence of a partial structure such as -COCH=C(OH)- in **MB15**, which suggested that **MB15** was a *β*-hydroxychalcone derivative in a (*Z*)-configuration (Kikuchi, Chen and Tsuda, 1990). This result was confirmed by ^{2}J correlations of the sharp singlet at δ_H 7.18 (H-α) to the signals of two carbonyl groups at δ_C 184.5 (C=O) and δ_C 185.2 (C=O) in the HMBC spectrum. The ^{1}H NMR spectrum showed the presence of an unsubstituted benzene ring attributable to ring B [δ_H 7.99 (*d*, *J* = 6.9 Hz, H-2 and H-6), 7.54 (*t*, *J* = 6.9 Hz, H-4) and 7.49 (*t*, *J* = 6.9 Hz, H-3 and H-5)]. The HMBC spectrum displayed a ^{3}J correlation of a singlet at δ_H 3.83 to the methoxyl carbon (δ_C 62.7), the methoxyl group was then located at C-2'. The signals for two *ortho*-coupled aromatic protons [δ_H 6.70 and 7.72 (*d*, *J* = 8.7 Hz, H-5' and H-6'] and a 6",6"-dimethylchromene ring [δ_H 1.49 (*s*, 2xMe), 5.72 (*d*, *J* = 9.9 Hz, H-5") and 6.68 (*d*, *J* = 9.9 Hz, H-4")] were observed. The 6",6"-dimethylchromene unit was therefore placed at C-3' and C-4' of the chalcone skeleton which was confirmed by ^{3}J correlations of H-4" (δ_H 6.68) to C-4' (δ_C 157.6) and H-5" (δ_H 5.72) to C-3' (δ_C 115.1) in the HMBC spectrum. These results

were supported by the NOE difference experiment, irradiation of 2'-OMe (δ_H 3.83) enhanced the signals of H-4" (5.69%) and H-α (5.47%) while irradiation of H-6' (δ_H 7.72) enhanced the signals of H-5' (23.39%) and H-α (2.91%). Irradiation of H-2 and H-6 (δ_H 7.99) enhanced the signal of H-α (30.63%). Irradiation of H-α (δ_H 7.18) enhanced the signals of 2'-OMe (1.47%), H-2 and H-6 (16.13%) and H-6' (2.16%) but no NOE effect to the signal of *β*-hydroxyl was observed. By comparison of the ^{1}H NMR data with those of (*Z*)-2'-methoxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone (purpurenone), **MB15** was thus determined to be (*Z*)-2'-methoxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone (purpurenone) (Rao and Raju, 1984 and Magalhaes *et al.*, 1996).

Figure 3. Selected NOE difference correlations of **MB15**

MB16 : (*E*)-2'-methoxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone



MB16 **MB16a**

MB16 was obtained as yellow plates, m.p. 97-98 °C. The UV spectrum showed maxima absorptions at λ 341, 255, 225 and 201 nm. The IR spectrum showed absorption bands at 3425 (O-H), 1740 and 1700 (C=O), 1593 and 1570 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 21 carbon signals, corresponding to a methoxyl, two methyls, ten methines, six quaternary and two carbonyl carbons. The ^{1}H NMR spectrum (Table 31) was almost identical to that of **MB15**. The presence of a 6",6"-dimethylchromene unit in ring A was deduced by the appearance of signals at δ_H 1.44 (*s*, 2xMe), 5.64 (*d*, *J* = 9.9 Hz, H-5") and 6.46 (*d*, *J* = 9.9 Hz, H-4"). The HMBC spectrum of **MB16** exhibited ^{3}J correlations of H-4" (δ_H 6.46) to C-2' (δ_C 157.1) and C-4' (δ_C 159.4) and H-5" (δ_H 5.64) to C-3' (δ_C 114.2). The methoxyl group was placed at C-2' as indicated by a ^{3}J correlation of the singlet at δ_H 3.71 to C-2' (δ_C 157.1) in the HMBC spectrum. The two *ortho*-coupled protons [δ_H 6.63 and 7.71 (*d*, *J* = 8.7 Hz)] were thus assigned at C-5' and C-6', respectively. The singlet for an olefinic proton at δ_H 7.15 (H-α) was observed. The broad singlet of a hydroxyl group at δ_H 3.49 which was exchangeable with D_2O was assigned to *β*-OH. This signal appeared at a higher field compared to that of **MB15**. For ring B, the resonance at δ_H 7.96 (*dd*, *J* = 1.2, 7.2 Hz) was attributed to H-2 and H-6. The signals for H-3, H-5 and H-4 were separated into 2 sets compared to those of **MB15**. The signals for H-3 and H-5 were resonated at δ_H 7.48 (*dd*, *J* = 7.2, 7.8 Hz); while that of H-4 appeared at δ_H 7.60 (*dt*, *J* = 1.2, 7.8 Hz). These results indicated that **MB16** could be an (*E*)-isomer of **MB15**. This was confirmed by the NOE difference experiment, irradiation of 2'-OMe (δ_H 3.71)

enhanced the signals of H-4" (1.08%) and H-α (0.93%) while irradiation of H-α (δ_H 7.15) enhanced the signals of H-6' (0.62%), 2'-OMe (0.29%) and the *β*-hydroxyl (2.60%) but no NOE effect to the signal of H-2 and H-6 (δ_H 7.96) was observed. On the basis of the above evidences, **MB16** was characterized as (*E*)-2'-methoxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone, which was a new *β*-hydroxy chalcone derivative.

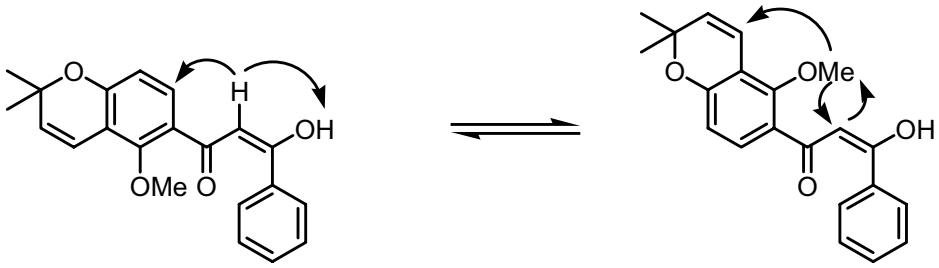
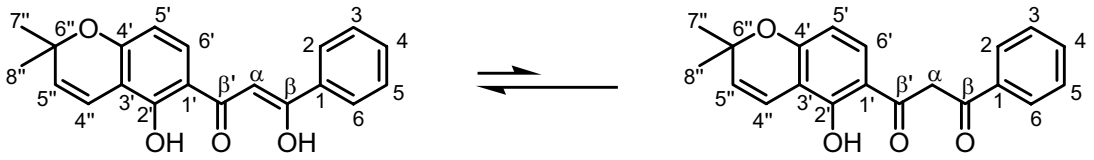


Figure 4. Selected NOE difference correlations of **MB16**

MB17 : (*Z*)-2'-hydroxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone



MB17 **MB17a**

MB17 was obtained as yellow needles, m.p. 85-87 °C. The UV spectrum showed maxima absorption at λ 270 nm. Its IR spectrum showed absorption bands corresponding to the hydroxyl group at 3430 cm^{-1} and carbonyl group at 1688 and 1602 cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 20 carbon signals, corresponding to two methyls, ten methines, six quaternary and two carbonyl carbons. The ^{1}H NMR spectrum (Table 32) was similar to that of **MB15**, except the presence of a strongly chelated hydroxyl group at δ_H 12.82 (1H, *s*) in **MB17** instead of the methoxyl group at δ_H 3.83 (*s*) in **MB15**. In addition, the ^{1}H NMR spectrum showed a singlet of a deshielded hydroxyl group at δ_H 15.34 (*β*-OH) and a singlet of an olefinic proton at δ_H 7.18 (s, H-α). The presence of an unsubstituted ring B was deduced by the appearance of the signals at δ_H 7.93 (*d*, *J* = 6.9 Hz, H-2 and H-6), 7.53 (*t*, *J* = 6.9 Hz, H-4) and 7.49 (*t*, *J* = 6.9 Hz, H-3 and H-5). The resonances of two aromatic protons at δ_H 6.40 and 7.60 (1H each, *d*, *J* = 8.7 Hz) indicated that the *ortho*-correlation of two protons was deduced. The HMBC spectrum of **MB17** exhibited ^{3}J correlations of the doublets at δ_H 6.40 to C-1' (δ_C 112.6) and C-3' (δ_C 109.7) and δ_H 7.60 to C-β' (δ_C 194.6) and C-2' (δ_C 159.7) suggesting that the two protons were H-5' and H-6', respectively. The signals for a 6",6"-dimethylchromene ring [δ_H 1.49 (*s*, 2xMe), 5.62 (*d*, *J* = 9.9 Hz, H-5") and 6.77 (*d*, *J* = 9.9 Hz, H-4")] were observed. This unit was located at C-3' and C-4' of the chalcone skeleton which was confirmed by ^{3}J correlations of H-4" (δ_H 6.77) to C-2' (δ_C 159.7) and H-5" (δ_H 5.62) to C-3' (δ_C 109.7) in the HMBC spectrum. These results indicated that **MB17** could be a 2'-hydroxy-*β*-hydroxychalcone derivative in a (*Z*)-configulation on the basis of the NMR data. This

result was confirmed by the NOE difference experiment, irradiation of H-2 and H-6 (δ_H 7.93) enhanced the signal of H-α (8.49%) while irradiation of H-6' (δ_H 7.60) enhanced the signal of H-α (13.76%). Irradiation of H-α (δ_H 6.72) enhanced the signal of H-2 and H-6 (5.11%) and H-6' (11.37%) but no NOE effect to the signal of the *β*-hydroxyl (δ_H 15.34) was observed. The structure of **MB17** was thus concluded to be (*Z*)-2'-hydroxy-6",6"-dimethylchromeno-(3',4',2",3")-*β*-hydroxychalcone. **MB17** was a new *β*-hydroxychalcone derivative.

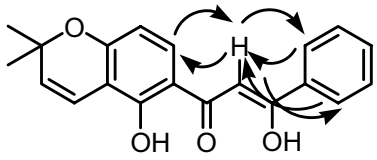
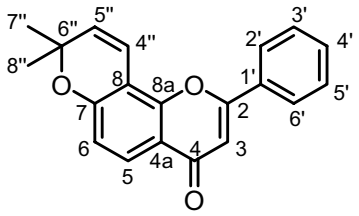


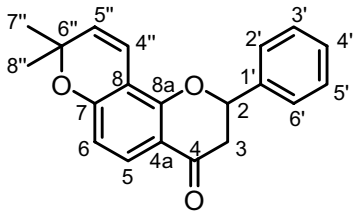
Figure 5. Selected NOE difference correlations of **MB17**

MB18 : 6",6"-dimethylchromeno-(7,8,2",3")-flavone



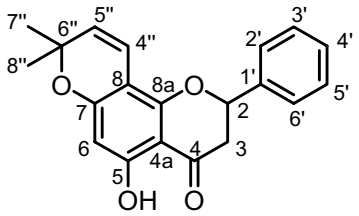
MB18 is pale yellow needles, m.p. 116-118 °C. The UV spectrum showed maxima absorptions at λ 324, 270 and 225 nm. The IR spectrum exhibited bands at 1635 (C=O), 1592 and 1577 (aromatic) cm^{-1}. The ^{1}H NMR spectrum (Table 33) exhibited a characteristic signal of a flavone proton at δ_H 6.78 (1H, *s*, H-3). The ^{13}C, DEPT and HMQC spectra showed 20 carbon signals, corresponding to two methyls, ten methines and eight quaternary carbons. A doublet of doublet (J = 2.1, 7.5 Hz) at δ_H 7.90 (H-2' and H-6') and a multiplet at δ_H 7.55-7.53 (H-3', H-4' and H-5') indicated that the B-ring was unsubstituted. The appearance of a singlet at δ_H 1.51 assignable to a gem-dimethyl group (H_3-7" and H_3-8") and two doublets (J = 9.9 Hz) at δ_H 6.94 and 5.77 integrating for one proton each, corresponding to vinyllic protons (H-4" and H-5", suggested the presence of a 6",6"-dimethylchromene residue in ring A. In addition, the two aromatic proton doublets at δ_H 8.00 and 6.87 (1H each, *d*, J = 8.7 Hz) indicated that the *ortho*-correlation of two protons was deduced. The HMBC spectrum of **MB18** exhibited ^{3}J correlations of the doublets at δ_H 8.00 to C-4 (δ_C 178.0) and C-8a (δ_C 152.3) and δ_H 6.87 to C-4a (δ_C 117.8) and C-8 (δ_C 109.5) suggested that the two protons were H-5 and H-6, respectively. Thus, the 6',6'-dimethylchromene unit was located at C-7 and C-8 of the flavone skeleton. This was confirmed by ^{3}J correlations of H-4" (δ_H 6.94) to C-7 (δ_C 157.6) and C-8a (δ_C 152.3) and H-5" (δ_H 5.77) to C-8 (δ_C 109.5) in the HMBC spectrum. By comparison of the ^{1}H NMR data with those of 6",6"-dimethylchromeno-(7,8,2",3")-flavone, **MB18** was elucidated as 6",6"-dimethyl chromeno-(7,8,2",3")-flavone (Magalhaes *et al.*, 1996).

MB19 : 6",6"-dimethylchromeno-(7,8,2",3")-flavanone (isolonchocarpin)



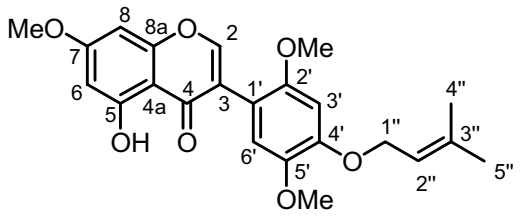
MB19 is pale yellow needles, m.p. 115-116 °C. The UV spectrum showed maxima absorptions at λ 308 and 267 nm. The IR spectrum exhibited absorption bands at 1742 (C=O), 1638, 1596 and 1578 (aromatic) cm^{-1}. The ^{1}H NMR spectrum (Table 34) exhibited a characteristic signal of a flavanone protons at δ_H 5.48 (1H, *dd*, *J* = 3.0, 12.9 Hz, H-2), 3.01 (1H, *dd*, *J* = 12.9, 16.8 Hz, H-3α) and 2.84 (1H, *dd*, *J* = 3.0, 16.8 Hz, H-3β). The ^{13}C, DEPT and HMQC spectra showed 20 carbon signals, corresponding to two methyls, one methylene, ten methines and seven quaternary carbons. The ^{1}H NMR spectrum showed a similar substitution pattern compared to that of **MB18**. It showed the presence of an unsubstituted ring B [δ_H 7.48 (*dd*, *J* = 1.8, 7.8 Hz, H-2' and H-6'), 7.44 (*dd*, *J* = 7.8, 8.1 Hz, H-3' and H-5') and 7.41 (*dt*, *J* = 1.8, 8.1 Hz, H-4')]. For ring A, the *ortho*-correlation of two protons was deduced, as indicated by two doublets at δ_H 7.75 and 6.51 (1H each, *d*, *J* = 8.7 Hz) which was confirmed by ^{3}J correlations in the HMBC spectrum of the doublets at δ_H 7.75 to C-4 (δ_C 190.6) and C-8a (δ_C 157.7) and δ_H 6.51 to C-4a (δ_C 114.8) and C-8 (δ_C 109.5). The signals for a 6",6"-dimethylchromene ring [δ_H 1.45 and 1.47 (3H each, *s*, 2xMe), 5.58 (*d*, *J* = 9.9 Hz, H-5") and 6.66 (*d*, *J* = 9.9 Hz, H-4")] were observed. This unit was therefore located at C-7 and C-8 of the flavanone skeleton. This was confirmed by ^{3}J correlations of H-4" (δ_H 6.66) to C-7 (δ_C 157.7) and C-8a (δ_C 157.7) and H-5" (δ_H 5.58) to C-8 (δ_C 109.5) in the HMBC spectrum. By comparison of the ^{1}H NMR data with those of 6",6"-dimethylchromeno-(7,8,2",3")-flavanone, **MB19** was identified as 6",6"-dimethylchromeno-(7,8,2",3")-flavanone (isolonchocarpin) (Rao and Raju, 1979).

MB20 : 5-hydroxy-6",6"-dimethylchromeno-(7,8,2",3")-flavanone (obovatin)



MB20 is yellow plates, m.p. 118-120 °C. The UV spectrum showed maxima absorptions at λ 271, 221 and 202 nm. The IR spectrum showed absorption bands at 3255 (O-H), 1642 (C=O), 1591 and 1480 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 20 carbon signals, corresponding to two methyls, one methylene, nine methines and eight quaternary carbons. The ^{1}H NMR spectrum (Table 35) was similar to that of **MB19**, except that **MB20** had the signal of a phenolic hydroxyl group at δ_H 12.09 (1H, *s*, 5-OH). **MB20** was deduced to be a 5-hydroxyflavanone on the basis of the NMR spectral data. Two doublets of doublets at δ_H 7.46 (*dd*, *J* = 1.8, 7.8 Hz, H-2' and H-6') and 7.44 (*dd*, *J* =7.2, 7.8 Hz, H-3' and H-5'), and a doublet of triplet δ_H 7.41 (*J* = 1.8, 7.2 Hz, H-4') indicated the unsubstituted B-ring. For ring A, a singlet at δ_H 6.01 (1H) was then assigned to H-6. The signals appeared at δ_H 1.43 and 1.45 (3H each, *s*, H-7" and H-8"), 5.47 (*d*, *J* = 9.9 Hz, H-5") and 6.55 (*d*, *J* = 9.9 Hz, H-4") were due to the protons of a 6",6"-dimethylchromene ring. This result was supported by ^{3}J correlations in the HMBC spectrum of H-4" (δ_H 6.55) to C-7 (δ_C 162.3) and C-8a (δ_C 156.8) and H-5" (δ_H 5.47) to C-8 (δ_C 102.0). By comparison of the ^{1}H NMR data with those of 5-hydroxy-6",6"-dimethylchromeno-(7,8,2",3")-flavanone, **MB20** can be assigned as 5-hydroxy-6",6"-dimethylchromeno-(7,8,2",3")-flavanone (obovatin) (Chen *et al*., 1978 and Andrei *et al.*, 2000).

MB21 : 4'-γ,γ-dimethylallyloxy-5-hydroxy-7,2',5'-trimethoxyisoflavone



MB21 is pale yellow needles, m.p. 124-126 °C. This compound exhibited the UV absorption bands at λ 291, 260 and 202 nm. The IR spectrum showed absorption bands at 3452 (O-H), 1655 (C=O), 1618, 1585 and 1509 (aromatic) cm^{-1}. The ^{1}H NMR spectrum (Table 36) was similar to that of **MB1** except that one of the methoxyl signals was missing. The appearance of singlets at δ_H 12.90 (5-OH) and δ_H 7.89 (H-2) suggested that MB-21 was a 5-hydroxyisoflavone. The ^{13}C, DEPT and HMQC spectra showed 23 carbon signals, corresponding to three methoxyls, two methyls, one methylene, six methines and eleven quaternary carbons. One of the methoxyl groups was attached to ring A, as indicated by signal at δ_H 3.89 (3H, *s*, 7-OMe) which was confirmed by a ^{3}J correlation of 7-OMe (δ_H 3.89) to C-7 (δ_C 165.4) in the HMBC spectrum. This was supported by an AM spin system at δ_H 6.40 (*d*, *J* = 2.1 Hz, H-6) and δ_H 6.43 (*d*, *J* = 2.1 Hz, H-8). The signal for a γ,γ-dimethylallyloxy group [δ_H 1.79, 1.80 (3H each, 2xMe), 4.66 (*d*, *J* = 6.6 Hz, H_2-1") and 5.55 (*br t*, *J* = 6.6 Hz, H-2")] as well as two methoxyls at δ_H 3.78 and 3.86 (3H each, *s*) were observed. The appearance of two aromatic proton singlets at δ_H 6.66 and 6.89 indicated that the *para*-correlation of two protons was deduced in ring B. The HMBC spectrum of **MB21** exhibited ^{3}J correlations of the singlets at δ_H 6.66 to C-1' (δ_C 110.9) and δ_H 6.89 to C-3 (δ_C 120.7) suggesting that the two protons were H-3' and H-6', respectively. From the NOE difference experiment, irradiation of H-6' (δ_H 6.89) enhanced the signal of the meythoxyl at δ_H 3.86 (3.51%) indicating that this methoxyl group was located at C-5' (δ_C 143.6) which was confirmed by the HMBC correlation of 5'-OMe (δ_H 3.86) to C-5' (δ_C 143.6). Irradiation of H-3' (δ_H 6.66) enhanced the signals of the methoxyl group at

δ_H 3.78 (3.05%) and the methylene (H_2-1") at δ_H 4.66 (5.20%); while irradiation of 5'-OMe (δ_H 3.86) enhanced the signal of H-6' (13.05%). Irradiation of the methoxyl at δ_H 3.78 enhanced the signal of H-3' (9.55%) but no NOE effect to the signal of 5'-OMe was observed. Irradiation of H_2-1" (δ_H 4.66) enhanced the signal of H-3' (11.28%) but no NOE effect to the signal of H-2 (δ_H 7.89) was observed. The placement of the second methoxyl group at C-4' was unlikely, the γ,γ-dimethylallyloxy unit was therefore placed at C-4'. This was supported by ^3J correlations of H_2-1" (δ_H 4.66) to C-4' (δ_C 149.4) and 2'-OMe (δ_H 3.78) to C-2' (δ_C 151.8) in the HMBC spectrum. The substitution pattern of ring B was similar to that of **MB1**. **MB21** was thus determined to be 4'-γ,γ-dimethylallyloxy-5-hydroxy-7,2',5'-trimethoxyisoflavone which was a new isoflavone derivative.

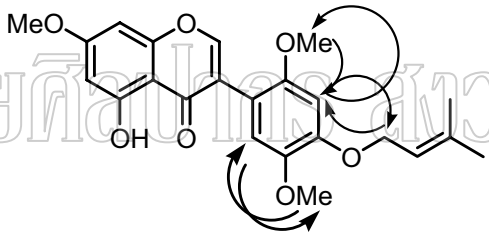
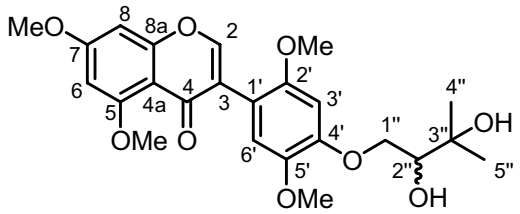


Figure 6. Selected NOE difference correlations of **MB21**

MB22 : 4'-(2,3-dihydroxy-3-methylbutyloxy)-5,7,2',5'-tetramethoxyisoflavone



MB22 was isolated as yellow plates, m.p. 79-80 °C. The UV spectrum showed maxima absorptions at λ 288, 255 and 205 nm. Its IR spectrum showed absorption bands at 3437 (O-H), 1645 (C=O), 1608, 1568 and 1510 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 24 carbon signals, corresponding to four methoxyls, two methyls, one methylene, six methines and eleven quaternary carbons. The ^{1}H NMR spectrum (Table 37) exhibited a characteristic signal of an isoflavone proton at δ_H 7.80 (*s*, H-2). The proton signals of **MB22** were almost identical with those of **MB1**, except in regard to the signals of the substituent at C-4'. For ring A, the two methoxyl groups at δ_H 3.92 and 3.94 (3H each, *s*) were assigned to 5-OMe and 7-OMe which were confirmed by ^{3}J correlations of 5-OMe (δ_H 3.92) to C-5 (δ_C 163.9) and 7-OMe (δ_H 3.94) to C-7 (δ_C 161.4) in the HMBC spectrum. This was supported by the doublets for two aromatic protons (J = 2.1 Hz) at δ_H 6.39 (H-6) and δ_H 6.48 (H-8). For ring B, the two singlets δ_H 6.64 and 6.98 (1H each) were assigned to H-3' and H-6' while those two at δ_H 3.74 and 3.84 (3H each) were attributed to 2'-OMe and 5'-OMe. This was supported by ^{3}J correlations of H-3' (δ_H 6.64) to C-1' (δ_C 114.0), H-6' (δ_H 6.98) to C-3 (δ_C 122.4), 2'-OMe (δ_H 3.74) to C-2' (δ_C 151.9) and 5'-OMe (δ_H 3.84) to C-5' (δ_C 143.5) in the HMBC spectrum. The presence of a 2,3-dihydroxy-3-methylbutyloxy unit in **MB22** was deduced from its NMR spectra which showed singlets for two methyls [(δ_H 1.30, H_3-5") and (δ_H 1.35, H_3-4")] and an ABX system [δ_H 3.75 (*dd*, J = 2.4, 6.0 Hz, H-2"), δ_H 4.13 (*dd*, J = 6.0, 9.0 Hz, H-1"α) and δ_H 4.33 (*dd*, J = 2.4, 9.0 Hz, H-1"β)]. The ^{13}C signals at δ_C 75.1 and 71.9 were assigned to C-2" (oxymethine carbon) and C-3" (oxygen bearing quarternary carbon) which were

supported by ^{3}J correlations of the singlets at δ_H 1.30 and 1.35 to C-2" (δ_C 75.1) and ^{2}J correlations of those singlets to C-3" (δ_C 71.9) in the HMBC spectrum. This unit was located at C-4' which was confirmed by NOE difference experiment, irradiation of H-3' (δ_H 6.64) enhanced the signal of H-1"a (1.12%) and 2'-OMe (0.32%) and irradiation of H-1"a (δ_H 4.33) enhanced the signal of H-3' (0.68%). On the basis of the above evidences, **MB22** was characterized as 4'-(2,3-dihydroxy-3-methylbutyloxy)-5,7,2',5'-tetramethoxyisoflavone. It was a new isoflavone derivative.

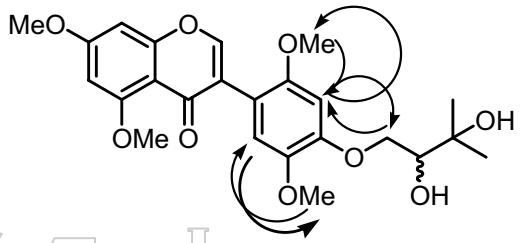
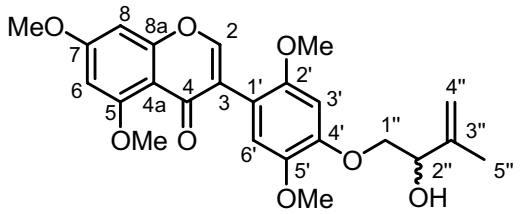


Figure 7. Selected NOE difference correlations of **MB22**

MB23 : 4'-(2-hydroxy-3-methyl-3-butenyloxy)-5,7,2',5'-tetramethoxyisoflavone



MB23 was obtained as yellow plates, m.p. 128-130 °C. The UV spectrum showed maxima absorptions at λ 290, 255 and 201 nm. It showed the strong IR absorption bands at 3442 (O-H), 1645 (C=O), 1613, 1570 and 1512 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 24 carbon signals, corresponding to four methoxyls, one methyl, two methylenes, six methines and eleven quaternary carbons. The ^{1}H NMR spectrum (Table 38) showed a characteristic resonance of a isoflavone proton at δ_H 7.80 (*s*, H-2) and a substitution pattern similar to that of **MB22**, except in regard to the signals of the substituent at C-4'. Two of the methoxyl groups were attached to ring A, as indicated by signals at δ_H 3.91 and 3.94 (3H each, *s*) which was assigned to 5-OMe and 7-OMe which were confirmed by ^{3}J correlations of 5-OMe (δ_H 3.91) to C-5 (δ_C 163.8) and 7-OMe (δ_H 3.94) to C-7 (δ_C 161.4) in the HMBC spectrum. This was supported by the appearance of an AM spin pattern at δ_H 6.39 (*d*, *J* = 2.1 Hz, H-6) and δ_H 6.48 (*d*, *J* = 2.1 Hz, H-8). For ring B, two singlets at δ_H 6.66 and 6.99 (1H each) were assigned to H-3' and H-6' while the two methoxyls at δ_H 3.74 and 3.85 were placed at C-2' and C-5', the pattern similar to that of **MB22**. This was supported by ^{3}J correlations of H-3' (δ_H 6.66) to C-1' (δ_C 114.5), H-6' (δ_H 6.99) to C-3 (δ_C 122.4), 2'-OMe (δ_H 3.74) to C-2' (δ_C 151.9) and 5'-OMe (δ_H 3.85) to C-5' (δ_C 144.0) in the HMBC spectrum. In addition, the presence of a 2-hydroxy-3-methyl-3-butenyloxy unit was indicated by a singlet at δ_H 1.84 (H_3-5"), two broad singlets at δ_H 5.01 and 5.17 (1H each, H_2-4") and an ABX system at δ_H 3.98 (*t*, *J* = 9.9 Hz, H-1"α), δ_H 4.18 (*dd*, *J* = 2.7, 9.9 Hz, H-1"β) and δ_H 4.49 (*br d*, *J* = 9.9 Hz, H-2"). The ^{13}C signals at δ_C 73.4, 112.6 and 143.2 were assigned to C-2" (oxymethine carbon), C-4" (methylene carbon)

and C-3" (sp^2quarternary carbon) which were supported by ^3J correlations of the singlet at δ_H 1.84 to C-2" (δ_C 73.4) and C-4" (δ_C 112.6), the broad singlet at δ_H 5.17 to C-2" (δ_C 73.4) and ^2J correlation of the singlet at δ_H 1.84 to C-3" (δ_C 143.2) in the HMBC spectrum. From the NOE difference experiment, irradiation of H-3' (δ_H 6.66) enhanced the signals of H-1"β (2.25%) and 2'-OMe (2.73%); while irradiation of H-1"β (δ_H 4.18) enhanced the signal of H-3' (5.51%). This unit was therefore located at C-4' which was confirmed by a ^3J correlation of H-1"β (δ_H 4.18) to C-4' (δ_C 148.4) in the HMBC spectrum. On the basis of the above spectroscopic studies, **MB23** was thus identified as 4'-(2-hydroxy-3-methyl-3-butenyloxy)-5,7,2',5'-tetramethoxyisoflavone. This compound appears to be novel.

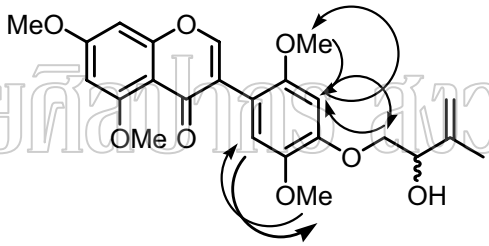
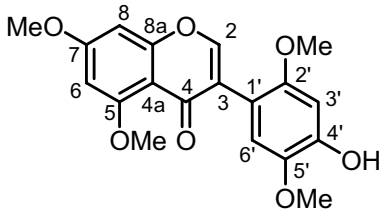


Figure 8. Selected NOE difference correlations of **MB23**

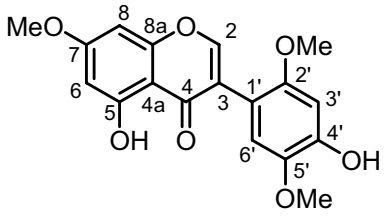
MB24 : 4'-hydroxy-5,7,2',5'-tetramethoxyisoflavone



MB24 was isolated as yellow plates, m.p. 168-170 °C. Its UV spectrum showed maxima absorptions at λ 290 and 276 nm. The IR spectrum showed the strong absorption bands at 3509 (O-H), 1768 (C=O), 1627 (aromatic) cm^{-1}. The ^{1}H NMR spectrum (Table 39) exhibited a characteristic signal of an isoflavone proton at δ_H 7.81 (*s*, H-2) and a substitution pattern similar to that of **MB1**, except that the signals of a *γ,γ*-dimethylallyloxy unit were missing. The ^{13}C, DEPT and HMQC spectra showed 19 carbon signals, corresponding to four methoxyls, five methines and ten quaternary carbons. Five substituents were attached to the isoflavone nucleus, as indicated by the signals for four methoxyls at δ_H 3.94, 3.91, 3.86 and 3.71 (3H each, *s*) and a hydroxyl at δ_H 3.92 (1H, *br s*, exchangeable with D_2O) in the NMR spectrum. Two methoxyls were attached to ring A, as indicated by the signals at δ_H 3.91 and 3.94 which were confirmed by ^{3}J correlations between 5-OMe (δ_H 3.91) to C-5 (δ_C 163.8) and 7-OMe (δ_H 3.94) to C-7 (δ_C 161.4) in the HMBC spectrum. This was supported by an AM spin system at δ_H 6.38 (*d*, *J* = 2.1 Hz, H-6) and δ_H 6.47 (*d*, *J* = 2.1 Hz, H-8). For ring B, the appearance of two aromatic proton singlets at δ_H 6.64 and 6.97 suggested that the *para*-correlation of two protons was deduced. The HMBC spectrum of **MB24** exhibited ^{3}J correlations of the singlets at δ_H 6.64 to C-1' (δ_C 111.5) and δ_H 6.97 to C-3 (δ_C 122.3) suggesting that the two protons were H-3' and H-6', respectively. The two methoxyls at δ_H 3.71 and 3.86 and a hydroxyl at δ_H 3.92 in ring B were then assigned to 2'-OMe, 5'-OMe and 4'-OH, respectively. This was confirmed by ^{3}J correlations of 2'-OMe (δ_H 3.71) to C-2' (δ_C 140.2) and 5'-OMe (δ_H 3.86) to C-5' (δ_C 152.2) and a ^{2}J correlation of 4'-OH (δ_H 3.92) to C-4' (δ_C 146.3) in the HMBC spectrum. Thus, **MB24**

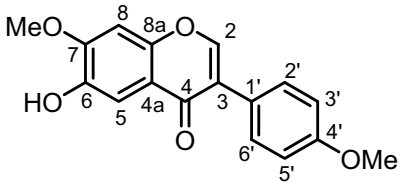
was identified as 4'-hydroxy-5,7,2',5'-tetramethoxyisoflavone (Tsukayama *et al.*, 1980). Although **MB24** was earlier synthesized, this is the first time it has been found as a naturally occurring compound.

MB25 : 5,4'-dihydroxy-7,2',5'-trimethoxyisoflavone (derrugenin)



MB25 is yellow solid, m.p. 178-180 °C. The UV spectrum showed maxima absorptions at λ 260 and 204 nm. The IR spectrum showed absorption bands at 3430 (O-H), 1652 (C=O), 1617, 1512 and 1461 (aromatic) cm^{-1}. The ^{1}H NMR spectrum (Table 40) exhibited a characteristic signal of an isoflavone proton at δ_H 7.89 (*s*, H-2). The ^{13}C, DEPT and HMQC spectra showed 18 carbon signals, corresponding to three methoxyls, five methines and ten quaternary carbons. These features and the similarity to **MB24** implied that **MB25** was a 5-hydroxyisoflavone as indicated by the signal at δ_H 12.89 (*s*, 5-OH). The resonances of *meta*-coupling (H-6 and H-8) were observed at δ_H 6.38 (1H, *d*, *J* = 2.4 Hz) and δ_H 6.41 (1H, *d*, *J* = 2.4 Hz). For ring B, the appearance of two aromatic proton singlets at δ_H 6.67 and 6.89 indicated that the *para*-correlation of two protons was deduced. The HMBC spectrum of **MB25** showed ^{3}J correlations of the singlets at δ_H 6.67 to C-1' (δ_C 109.9) and δ_H 6.89 to C-3 (δ_C 121.1) suggesting that the two protons were H-3' and H-6', respectively. A broad singlet at δ_H 5.77 (1H) was assigned to a non chelated hydroxyl group (4'-OH). A singlet for six hydrogens at δ_H 3.86 and a singlet for three hydrogens at δ_H 3.74 were assigned to three methoxyl groups of 7-OMe, 5'-OMe and 2'-OMe, respectively. These results were confirmed by ^{3}J correlations of 7-OMe (δ_H 3.86) to C-7 (δ_C 165.4), 5'-OMe (δ_H 3.86) to C-5' (δ_C 140.7) and 2'-OMe (δ_H 3.74) to C-2' (δ_C 152.3) in the HMBC spectrum. On the basis of the above evidences, **MB25** was characterized as 5,4'-dihydroxy-7,2',5'-trimethoxyisoflavone. It was known as derrugenin (Tsukayama *et al.*, 1980).

MB26 : 6-hydroxy-7,4'-dimethoxyisoflavone (alfalone)



MB26 was obtained as yellow plates, m.p. 224-226 °C. The UV spectrum showed maxima absorptions at λ 265 and 202 nm. The IR spectrum showed absorption bands at 3444 (O-H), 1735 (C=O), 1628 and 1509 (aromatic) cm^{-1}. The ^{1}H NMR spectrum (Table 41) showed a characteristic resonance of an isoflavone proton at δ_H 7.95 (*s*, H-2). The ^{13}C, DEPT and HMQC spectra showed 17 carbon signals, corresponding to two methoxyls, seven methines and eight quaternary carbons. Three substituents were attached to the isoflavone nucleus, as indicated by singlets for two methoxyls at δ_H 3.86 and 4.04 (3H each, *s*) and singlet for a hydroxyl at δ_H 3.76 (1H, *br s*, exchangeable with D_2O) in the ^{1}H NMR spectrum. For ring A, the appearance of two aromatic proton singlets at δ_H 7.76 and 6.90 indicated that the *para*-correlation of two protons was deduced. The HMBC spectrum of **MB26** exhibited ^{3}J correlations of the singlets at δ_H 7.76 to C-4 (δ_C 176.2) and C-8a (δ_C 152.6) and δ_H 6.90 to C-4a (δ_C 119.7) suggesting that the two protons were H-5 and H-8, respectively. A methoxyl (δ_H 4.04, *s*) and a hydroxyl (δ_H 3.76, *br s*) were attached to ring A which were confirmed by ^{3}J correlations of 7-OMe (δ_H 4.04) to C-7 (δ_C 152.3) and 6-OH (δ_H 3.76) to C-7 (δ_C 152.3) in the HMBC spectrum. This was supported by the NOE difference experiment, irradiation of 7-OMe (δ_H 4.04) enhanced the signal of the aromatic proton (H-8) at δ_H 6.90 (10.13%). For ring B, the two doublets (J = 9.0 Hz) integrating for two protons each at δ_H 7.53 (H-2' and H-6') and δ_H 6.90 (H-3' and H-5') suggested the presence of a 1,4-disubstituted benzene nucleus. The second methoxyl at δ_H 3.86 (3H, *s*) was therefore placed at C-4' which was confirmed by a ^{3}J correlation of 4'-OMe (δ_H 3.86) to C-4' (δ_C 159.5) in the HMBC spectrum. This was supported by the NOE

difference experiment, irradiation of 4'-OMe (δ_H 3.86) enhanced the signal of the aromatic protons (H-3' and H-5') at δ_H 6.99 (3.47%). By comparison of the ^{1}H NMR data with those of 6-hydroxy-7,4'-dimethoxyisoflavone, **MB26** was identified as 6-hydroxy-7,4'-dimethoxyisoflavone (alfalone) (Kobayashi *et al*., 1988).

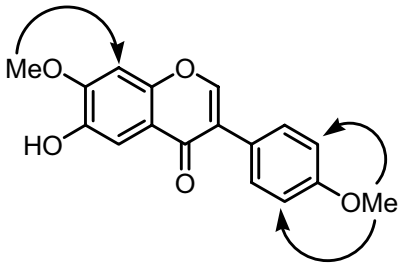
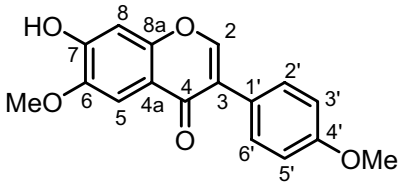


Figure 9. Selected NOE difference correlations of **MB26**

MB27 : 7-hydroxy-6,4'-dimethoxyisoflavone (afrormosin)



MB27 was isolated as yellow solid, m.p. 198-200 °C. The UV spectrum showed maxima absorptions at λ 262 and 201 nm. The IR spectrum showed absorption bands of a carbonyl group at 1635 cm^{-1} and a hydroxyl group at 3437 cm^{-1}. The ^{1}H NMR spectrum (Table 42) was similar to that of **MB26**, except that the signal of H-8 of **MB27** appeared at lower field (δ_H 7.00, *s*) compared to that of **MB26** (δ_H 6.90, *s*). The ^{13}C, DEPT and HMQC spectra showed 17 carbon signals, corresponding to two methoxyls, seven methines and eight quaternary carbons. These data indicated that **MB27** was an isomer of **MB26**. For ring A, the two protons in the aromatic region at δ_H 7.67 (1H, *s*) and δ_H 7.00 (1H, *s*) were proposed for H-5 and H-8, respectively. This was confirmed by ^{3}J correlations of H-5 (δ_H 7.67) to C-4 (δ_C 175.7) and C-8a (δ_C 153.4) and H-8 (δ_H 7.00) to C-4a (δ_C 119.5) in the HMBC spectrum. The signals for two methoxyl groups at δ_H 4.04 and 3.87 (3H each, *s*) were observed. From the NOE difference experiment, irradiation of the methoxyl at δ_H 4.04 enhanced the signal of H-5 (4.81%) but no NOE effect to the signal of the methoxyl at δ_H 3.87 was observed. The methoxyl group at δ_H 4.04 was therefore located at C-6 which was confirmed by a ^{3}J correlation 6-OMe (δ_H 4.04) to C-6 (δ_C 146.4) in the HMBC spectrum. In addition, the 4'-oxysubstituted pattern of B-ring was readily deduced from the proton signals forming an AA'BB' system at δ_H 7.52 (*d*, J = 8.7 Hz, H-2' and H-6') and δ_H 6.99 (*d*, J = 8.7 Hz, H-3' and H-5'). Thus, the placement of the second methoxyl (δ_H 3.87) was assigned at C-4' which was confirmed by a ^{3}J correlation 4'-OMe (δ_H 3.87) to C-4' (δ_C 159.5) in the HMBC spectrum. This was supported by the NOE difference experiment, irradiation of 4'-OMe (δ_H 3.87) enhanced the signal of the aromatic protons H-3' and

H-5' (1.55%). By comparison of the ^{1}H NMR data with those of 7-hydroxy-6,4'-dimethoxyisoflavone, **MB27** was thus concluded to be 7-hydroxy-6,4'-dimethoxy isoflavone (afrormosin) (Caballero and Smith, 1986).

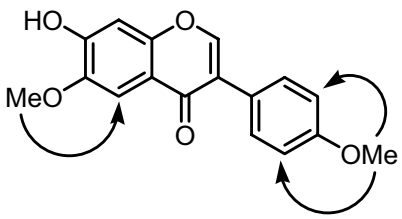
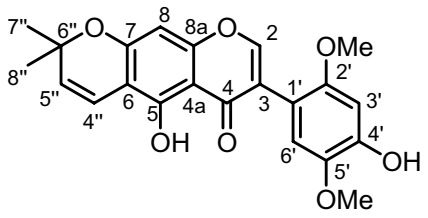


Figure 10. Selected NOE difference correlations of **MB27**

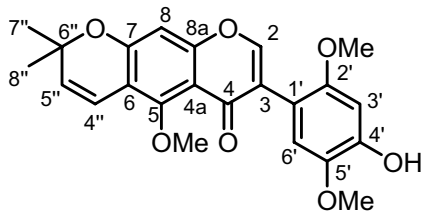
MB28 : 5,4'-dihydroxy-2',5'-dimethoxy-6",6"-dimethylchromeno-(6,7,2",3")-isoflavone (elongatin)



MB28 is yellow plates, m.p. 85-86 °C. The UV spectrum showed maxima absorptions at λ 279 and 202 nm. The IR spectrum showed absorption bands at 3426 (O-H), 1652 (C=O), 1599, 1581 and 1515 (aromatic) cm^{-1}. The ^{1}H NMR spectrum (Table 43) exhibited a characteristic signal of an isoflavone proton at δ_H 7.83 (*s*, H-2) and a signal of a chelated hydroxyl group at δ_H 13.19 (*s*, 5-OH). The ^{13}C, DEPT and HMQC spectra showed 22 carbon signals, corresponding to two methoxyls, two methyls, six methines and twelve quaternary carbons. The signals for a 6",6"-dimethylchromene ring [δ_H 1.47 (6H, *s*, 2xMe), δ_H 5.62 (*d*, *J* = 9.9 Hz, H-5") and δ_H 6.72 (*d*, *J* = 9.9 Hz, H-4")] and an aromatic proton at δ_H 6.33 (1H, *s*) were observed in ring A. The HMBC spectrum of **MB28** exhibited ^{3}J correlations of H-4" (δ_H 6.72) to C-5 (δ_C 156.8) and C-7 (δ_C 159.3) and H-5" (δ_H 5.62) to C-6 (δ_C 105.5) suggesting that the 6",6"-dimethylchromene unit was located at C-6 and C-7 of ring A. The aromatic proton singlet (δ_H 6.33) was then assigned at C-8 which was confirmed by ^{2}J correlations of H-8 (δ_H 6.33) to C-7 (δ_C 159.3) and C-8a (δ_C 157.3) and a ^{3}J correlation of H-2 (δ_H 7.83) to C-8a (δ_C 157.3) in the HMBC spectrum. For ring B, the two aromatic proton singlets at δ_H 6.66 and 6.87 indicated that the *para*-correlation of two protons was deduced. The HMBC spectrum of **MB28** showed ^{3}J correlations of the singlets at δ_H 6.66 to C-1' (δ_C 110.0) and δ_H 6.87 to C-3 (δ_C 120.3) suggesting that the two protons were H-3' and H-6', respectively. Two sharp singlets with three protons each at δ_H 3.73 and 3.87 and a broad singlet with one proton at δ_H 5.78 were assigned to two methoxyl groups (2'-OMe and 5'-OMe) and a hydroxyl group (4'-OH),

respectively. This was supported by ^{3}J correlations of 2'-OMe (δ_H 3.73) to C-2' (δ_C 152.3) and 5'-OMe (δ_H 3.87) to C-5' (δ_C 140.3) in the HMBC spectrum. By comparison of the ^{1}H NMR data with those of 5,4'-dihydroxy-2',5'-dimethoxy-6",6"-dimethyl chromeno-(6,7,2",3")-isoflavone, **MB28** was identified as 5,4'-dihydroxy-2',5'-dimethoxy-6",6"-dimethylchromeno-(6,7,2",3")-isoflavone (elongatin) (Smalberger, Vleggaar and Weber, 1975).

MB29 : 4'-hydroxy-5,2',5'-trimethoxy-6",6"-dimethylchromeno-(6,7,2",3")-isoflavone



MB29 was obtained as yellow plates, m.p. 90-92 °C. The UV spectrum showed maxima absorptions at λ 266 and 224 nm. The IR spectrum exhibited absorption bands at 3445 (O-H), 1732 (C=O), 1645, 1605 and 1513 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 23 carbon signals, corresponding to three methoxyls, two methyls, six methines and twelve quaternary carbons. The ^{1}H NMR spectrum (Table 44) was similar to that of **MB28**, except that **MB29** had an additional signal of a methoxyl group at δ_H 3.90 (3H, *s*) whereas the signal of a chelated hydroxyl group was missing. The methoxyl group at δ_H 3.90 was located at C-5 which was confirmed by a ^{3}J correlation of 5-OMe (δ_H 3.90) to C-5 (δ_C 155.9) in the HMBC spectrum. A singlet of *gem*-dimethyl protons at δ_H 1.49 (*s*, 2xMe) and two doublets (J = 10.2 Hz) of *cis*-olefinic protons at δ_H 6.72 (H-4") and 5.74 (H-5") were attributed to a 6",6"-dimethylchromene ring at C-6 and C-7 in the isoflavone skeleton which was supported by the NOE experiment, irradiation of H-4" (δ_H 6.72) enhanced the signal of 5-OMe (5.78%). This was confirmed by ^{3}J correlations of H-4" (δ_H 6.72) to C-5 (δ_C 155.9) and C-7 (δ_C 157.9), H-5" (δ_H 5.74) to C-6 (δ_C 113.3) and H-8 (δ_H 6.60) to C-6 (δ_C 113.3) in the HMBC spectrum. For ring B, two sharp singlets at δ_H 6.63 and 6.93 were assigned to the *para* oriented H-3' and H-6' which were confirmed by ^{3}J correlations of H-3' (δ_H 6.63) to C-1' (δ_C 111.4) and H-6' (δ_H 6.93) to C-3 (δ_C 122.2) in the HMBC spectrum. From the NOE difference experiment, irradiation of H-6' (δ_H 6.93) enhanced the signal of the methoxyl at δ_H 3.88 (4.92%) indicating that this methoxyl group was located at C-5' which was confirmed by the HMBC correlation of the signal at δ_H 3.88 to C-5' (δ_C 140.2). Irradiation of H-3' (δ_H 6.63) enhanced the

signals of the methoxyl at δ_H 3.74 (6.72%) and the hydroxyl group at δ_H 3.94 (7.16%); while irradiation of 5'-OMe (δ_H 3.88) enhanced the signals of H-6' (20.86%) and irradiation of the hydroxyl group (δ_H 3.94) enhanced the signal of H-3' (5.84%). Irradiation of the methoxyl at δ_H 3.74 enhanced the signal of H-3' (27.62%) but no NOE effect to the signal of 5'-OMe was observed. The hydroxyl group (δ_H 3.94) was thus located at C-4'. This was supported by a ^3J correlation of 2'-OMe to C-2' (δ_C 152.2) in the HMBC spectrum. Based on the above spectral evidences, **MB29** was identified as 4'-hydroxy-5,2',5'-trimethoxy-6",6"-dimethylchromeno-(6,7,2",3")-isoflavone which was a new isoflavone derivative.

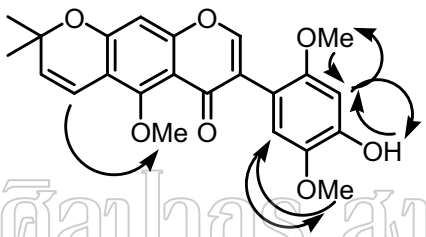
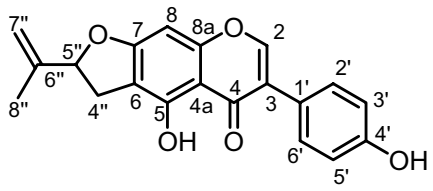


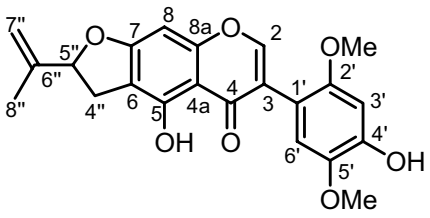
Figure 11. Selected NOE difference correlations of **MB29**

MB30 : 5,4'-dihydroxy-5"-isopropenyl-4",5"-dihydrofurano-(6,7,2",3")-isoflavone (licoagroisoflavone)



MB30 is yellow plates, m.p. 185-186 °C, $[\alpha]_D^{28}$ -69.6°, (*c* = 0.01, CH_2Cl_2). The UV spectrum showed maxima absorptions at λ 267 and 204 nm. The IR spectrum exhibited absorption bands at 3412 (O-H), 1645 (C=O), 1581 and 1515 (aromatic) cm^{-1}. The ^1H NMR spectrum (Table 45) showed a characteristic signal of an isoflavone proton at δ_H 7.85 (*s*, H-2). The ^{13}C, DEPT and HMQC spectra showed 20 carbon signals, corresponding to one methyl, two methylenes, seven methines and ten quaternary carbons. The presence of a 5"-isopropenyl-4",5"-dihydrofuran unit was indicated by the signals at δ_H 1.89 (*s*, H_3-8"), 3.20 (*dd*, *J* = 2.1, 15.0 Hz, H-4"α), 2.95 (*dd*, *J* = 7.8, 15.0 Hz, H-4"β), 4.45 (*br d*, *J* = 7.5 Hz, H-5"), 4.90 (*br s*, H-7"a) and 5.01(*br s*, H-7"b). This unit was assigned at C-6 and C-7 of the isoflavone skeleton which was supported by ^3J correlations of H-8 (δ_H 6.49) to C-4a (δ_C 105.6) and C-6 (δ_C 109.1), H-4"β (δ_H 2.95) to C-5 (δ_C 160.3) and C-7 (δ_C 163.1) and 5-OH (δ_H 13.27) to C-4a (δ_C 105.6) and C-6 (δ_C 109.1) in the HMBC spectrum. From the NOE difference experiment, irradiation of H_2-4" showed no NOE effect to the signal of the chelated hydroxyl (5-OH). The 4'-oxysubstituted pattern of B-ring was readily deduced from the proton signals forming an AA'BB' system at δ_H 7.41 (*d*, *J* = 8.7 Hz, H-2' and H-6') and 6.92 (*d*, *J* = 8.7 Hz, H-3' and H-5'). By comparison of the ^1H NMR data and physical properties with those of 5,4'-dihydroxy-5"-isopropenyl-4",5"-dihydrofurano-(6,7,2",3")-isoflavone**,** **MB32** was assigned as 5,4'-dihydroxy-5"-isopropenyl-4",5"-dihydrofurano-(6,7,2",3")-isoflavone (licoagroisoflavone) (Li *et al.*, 2001).

MB31 : 5,4'-dihydroxy-2',5'-dimethoxy-5"-isopropenyl-4",5"-dihydrofurano-dihydrofurano-(6,7,2",3")-isoflavone



MB31 is white powder, m.p. 252-253 °C, $[\alpha]_D^{28}$ -517.4°, (*c* = 0.01, CH_2Cl_2). The UV spectrum exhibited maxima absorptions at λ 295, 264 and 204 nm. The IR spectrum showed absorption bands at 3425 (O-H), 1735 (C=O), 1621, 1542 and 1510 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 22 carbon signals, corresponding to two methoxyls, one methyl, two methylenes, five methines and twelve quaternary carbons. The ^1H NMR spectrum (Table 46) exhibited a characteristic resonance of isoflavone proton at δ_H 7.86 (*s*, H-2) and a signal of a chelated hydroxyl proton at δ_H 13.31 (*s*, 5-OH). These data were similar to those of **MB30** except that **MB31** had additional two methoxyl groups at δ_H 3.76 and 3.88 (3H each). The presence of a 5"-isopropenyl-4",5"-dihydrofuran unit was indicated by the signals at δ_H 1.87 (*s*, H_3-8"), 3.20 (*dd*, *J* = 2.1, 15.0 Hz, H-4"α), 2.94 (*dd*, *J* = 8.1, 15.0 Hz, H-4"β), 4.44 (*br d*, *J* = 7.5 Hz, H-5"), 4.90 (*br s*, H-7"b) and 5.01(*br s*, H-7"a). This unit was assigned at C-6 and C-7 of the isoflavone skeleton which was confirmed by ^3J correlations of H-8 (δ_H 6.49) to C-4a (δ_C 106.3) and H-4"β (δ_H 2.94) to C-5 (δ_C 159.9) and C-7 (δ_C 163.0) in the HMBC spectrum. From the NOE difference experiment, irradiation of H_2-4" showed no NOE effect to the signal of the chelated hydroxyl (5-OH). For ring B, the two singlets at δ_H 6.63 and 6.93 (1H each) were assigned for two aromatic protons H-3' and H-6', respectively. This was supported by ^3J correlations of H-3' (δ_H 6.63) to C-1' (δ_C 110.4) and H-6' (δ_H 6.93) to C-3 (δ_C 122.3) in the HMBC spectrum. In addition, two singlets for two methoxyls at δ_H 3.76 and 3.88 (3H each) and a broad singlet for a hydroxyl at δ_H 5.77 (1H) were observed. From

the NOE difference experiment, irradiation of H-3' (δ_H 6.63) enhanced the signal of the methoxyl at δ_H 3.76 (7.73%) and irradiation of the methoxyl at δ_H 3.76 enhanced the signal of H-3' (10.12%). Irradiation of H-6' (δ_H 6.93) enhanced the signal of the methoxyl at δ_H 3.88 (8.45%) while irradiation of the methoxyl at δ_H 3.88 enhanced the signal of H-6' (12.54%) but no NOE effect to the signal of the methoxyl at δ_H 3.76 was observed. Two methoxyl groups at δ_H 3.76 and 3.88 were then located at C-2' and C-5', while the hydroxyl group at δ_H 5.77 was placed at C-4'. This was confirmed by ^3J correlations between 2'-OMe (δ_H 3.76) to C-2' (δ_C 152.5) and 5'-OMe (δ_H 3.88) to C-5' (δ_C 141.0) in the HMBC spectrum. By comparison of the spectral data and physical properties to those of brandisianin B (Kikuchi *et al*., 2007), **MB31** was thus determined to be 5,4'-dihydroxy-2',5'-dimethoxy-5''-isopropenyl-4'',5''-dihydrofurano-(6,7,2'',3'')-isoflavone. It was a new isoflavone derivative with the linear 5''-isopropenyl-4'',5''-dihydrofuran; while brandisianin B was the angular isomer.

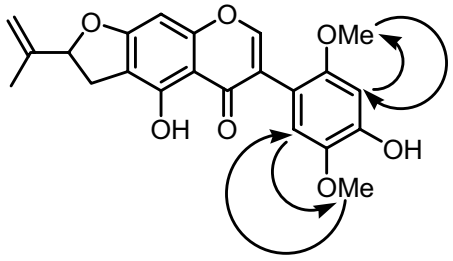
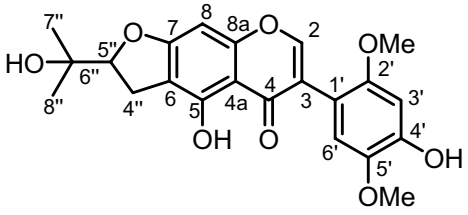


Figure 12. Selected NOE difference correlations of **MB31**

MB32 : 5,4'-dihydroxy-2',5'-dimethoxy-5"-(2-hydroxyisopropyl)-4",5"-dihydro furano-(6,7,2",3")-isoflavone



MB32 was obtained as yellow plates, m.p. 169-170 °C $[\alpha]_D^{28}$ -83.4°, (*c* = 0.02, CH_2Cl_2). The UV spectrum showed maxima absorptions at λ 297, 263 and 204 nm. The IR spectrum showed absorption bands at 3429 (O-H), 1732 (C=O), 1628 and 1513 (aromatic) cm^{-1}. The ^{13}C, DEPT and HMQC spectra showed 22 carbon signals, corresponding to two methoxyls, two methyls, one methylene, five methines and twelve quaternary carbons. The ^{1}H NMR spectrum (Table 46) were almost identical to that of **MB31**, except in regard to the signals of a dihydrofuran unit in ring A. The signals appeared at δ_H 1.26 and 1.38 (3H each, *s*, 2xMe), 3.13 (*dd*, *J* = 8.1, 15.6 Hz, H-4"α), 3.23 (*dd*, *J* = 9.3, 15.6 Hz, H-4"β) and 4.81 (*dd*, *J* = 8.1, 9.3 Hz, H-5") were due to the protons of a 5"-(2-hydroxyisopropyl)-4",5"-dihydrofuran unit which was assigned at C-6 and C-7 of the isoflavone skeleton. This was supported by ^{3}J correlations of H-8 (δ_H 6.39) to C-4a (δ_C 106.7) and C-6 (δ_C 108.9) in the HMBC spectrum. In addition, the singlets at δ_H 6.68 and 6.89 (1H each, *s*) were assigned to H-3' and H-6' which were confirmed by ^{3}J correlations of H-3' (δ_H 6.68) to C-1' (δ_C 110.0) and H-6' (δ_H 6.89) to C-3 (δ_C 120.3) in the HMBC spectrum. The singlets at δ_H 3.76 and 3.89 (3H each) and a broad singlet signal at δ_H 5.77 (1H) were assigned for two methoxyl groups (2'-OMe and 5'-OMe) and a hydroxyl group (4'-OH). This was confirmed by ^{3}J correlations of 2'-OMe (δ_H 3.76) to C-2' (δ_C 152.3) and 5'-OMe (δ_H 3.89) to C-5' (δ_C 140.3) in the HMBC spectrum. These results were supported by the NOE difference experiment, irradiation of H-3' (δ_H 6.68) enhanced the signals of 2'-OMe (1.84%) and 4'-OH (1.33%) while irradiation of H-6' (δ_H 6.89) enhanced the

signal of 5'-OMe (1.40%). Irradiation of 5'-OMe (δ_H 3.89) enhanced the signal of H-6' (4.96%). Irradiation of 4'-OH (δ_H 5.77) enhanced the signal of 5'-OMe (0.25%); while irradiation of 2'-OMe (δ_H 3.76) enhanced the signal of H-3' (6.67%) but no NOE effect of the signal of 5'-OMe was observed. The substitution pattern of ring B was similar to those of **MB5**, **MB24**, **MB25**, **MB28**, **MB29** and **MB30**. Based on the above spectral evidences, **MB32** was identified as 5,4'-dihydroxy-2',5'-dimethoxy-5''-(2-hydroxyisopropyl)-4'',5''-dihydrofurano-(6,7,2'',3'')-isoflavone which was a new isoflavone derivative.

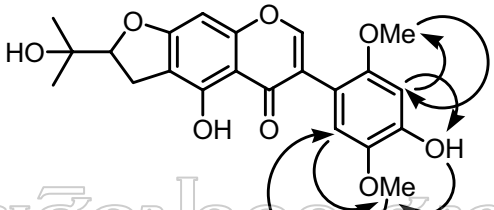
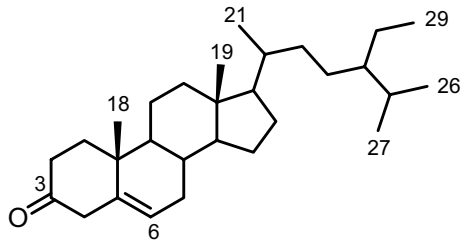


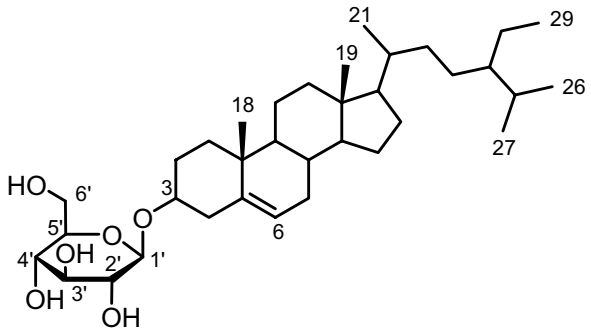
Figure 13. Selected NOE difference correlations of **MB32**

MB33 : stigmast-5-en-3-one (*β*-sitosterone)



MB33 was obtained as colorless needles, m.p. 78-80 °C, $[\alpha]_D^{28}$ +32.6°, (*c* = 0.3, CH_2Cl_2). The IR spectrum showed absorption bands at 2935, 2867 (C-H), 1674 (C=O), 1266, 1229 and 1184 (C-O) cm^{-1}. The ^1H NMR spectrum showed the resonance of an olefinic proton at δ_H 5.72 (1H, *br s*, H-6). The signals for six methyls at δ_H 1.18 (3H, *s*, Me-19), 0.92 (3H, *d*, *J* = 6.6 Hz, Me-21), 0.85 (3H, *t*, *J* = 6.3 Hz, Me-29), 0.84 (3H, *d*, *J* = 6.3 Hz, Me-26), 0.81 (3H, *d*, *J* = 6.6 Hz, Me-27) and 0.71 (3H, *s*, Me-18) were observed. By comparison of the ^1H NMR data and the physical properties with those of stigmast-5-en-3-one, **MB33** was identified as stigmast-5-en-3-one (*β*-sitosterone) (Cambie *et al.,* 1991).

MB34 : sitosterol-3-*O*-*β*-D-glucopyranoside

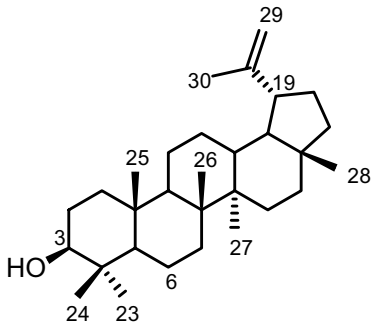


MB34 is white plates, m.p. 260 °C (decompose), $[\alpha]_D^{28}$ -64.5°, (*c* = 0.03, MeOH). The IR spectrum showed absorption bands at 3373 (O-H), 2924, 2854 (C-H), 1168, 1074 and 1021 (C-O) cm^{-1}. The ^{1}H NMR spectrum showed the resonances of an oxymethine proton at δ_H 3.56 (1H, *tt*, *J* = 4.8, 10.2 Hz, H-3) and an olefinic proton at δ_H 5.35 (1H, *br d*, *J* = 4.8 Hz, H-6). The signals for six methyls at δ_H 0.99 (3H, *s*, Me-19), 0.90 (3H, *d*, *J* = 6.3 Hz, Me-21), 0.84 (3H, *t*, *J* = 6.9 Hz, Me-29), 0.82 (3H, *d*, *J* = 6.6 Hz, Me-26), 0.80 (3H, *d*, *J* = 6.3 Hz, Me-27) and 0.66 (3H, *s*, Me-18) were observed. A doublet at δ_H 4.40 which showed a large coupling constant (*J* = 7.8 Hz) was assigned to the anomeric proton H-1' of *β*-D-glucose. The other proton signals of glucose appeared at δ_H 3.84 (1H, *dd*, *J* = 3.3, 12.0 Hz, H-6'β), 3.76 (1H, *dd*, *J* = 4.5, 12.0 Hz, H-6'α) and 3.51-3.24 (4H, *m*, H-2',3',4',5'). In addition, the presence of glucose was acetylation of **MB34** to yield the tetraacetate derivative (**MB34OAc**).

The ^{1}H NMR spectrum of **MB34OAc** showed signals for four acetate methyls at δ_H 2.09, 2.06, 2.04 and 2.02 (3H each, *s*). The downfield shifts of the proton signals of glucose appeared at δ_H 4.61 (1H, *d*, *J* = 8.1 Hz, H-1'), 4.97 (1H, *dd*, *J* = 8.1, 9.6 Hz, H-2'), 5.22 (1H, *t*, *J* = 9.6 Hz, H-3'), 5.09 (1H, *t*, *J* = 9.6 Hz, H-4'), 3.96 (1H, *ddd*, *J* = 2.4, 4.8, 9.6 Hz, H-5'), 4.12 (1H, *dd*, *J* = 2.4, 12.0 Hz, H-6'α) and 4.27 (1H, *dd*, *J* = 4.8, 12.0 Hz, H-6'β).

By comparison of the ^{1}H NMR data and physical properties with those of sitosterol-3-*O*-*β*-D-glucopyranoside, **MB34** was identified as sitosterol-3-*O*-*β*-D-glucopyranoside (Mbafor, Ndom and Fomum, 1997).

MB35 : lup-20(29)-en-3β-ol (lupeol)



MB35 was obtained as white solid, m.p. 220-223 °C, $[\alpha]_D^{28}$ +26.2°, (*c* = 0.2, CH_2Cl_2). The IR spectrum showed absorption bands at 3418 (O-H), 2918, 2850 (C-H), 1188, 1108, 1040 and 1012 (C-O) cm^{-1}. The ^1H NMR spectrum showed the resonances of an oxymethine proton at δ_H 3.21 (1H, *dd*, *J* = 5.4, 10.8 Hz, H-3), a methine proton at δ_H 2.38 (1H, *ddd*, *J* = 6.0, 11.1, 16.8 Hz, H-19) and terminal olefinic methylene protons at δ_H 4.71 (1H, *d*, *J* = 2.1 Hz, H-29a) and 4.59 (1H, *d*, *J* = 2.1 Hz, H-29b). The signals for seven methyls at δ_H 1.70 (3H, *s*, Me-30), 1.05 (3H, *s*, H-26), 0.99 (3H, *s*, Me-23), 0.96 (3H, *s*, Me-27), 0.85 (3H, *s*, Me-25), 0.81 (3H, *s*, Me-28), 0.78 (3H, *s*, Me-24) were observed. By comparison of the ^1H NMR data and physical properties with those of lup-20(29)-en-3β-ol (lupeol), **MB35** was identified as lup-20(29)-en-3β-ol (lupeol) (Rosa, Giulio and Tommonaro, 1997).

3.2 Evaluation of biological activities

Anti-inflammatory activity

6-Deoxyclitoriacetal (**MB7**) and 12a-hydroxy-α-toxicarol (**MB11**) were tested for anti-inflammatory activity using ethyl phenylpropiolate (EPP)-induced ear edema in rats; the results are shown in Table 48. **MB11** showed higher anti-inflammatory activity than that of phenylbutazone.

Table 48. Inhibitory effect of compounds **MB7**, **MB11** and phenylbutazone on ethyl phenylpropiolate (EPP)-induced ear edema in rats

Group	Dose mg/ear	Time after topical application of EPP							
		15 min		30 min		1 h		2 h	
		ED (μm)	ED (%)	ED (μm)	ED (%)	ED (μm)	ED (%)	ED (μm)	ED (%)
Control acetone	-	80±29.4	-	205±9.6	-	285±9.6	-	225±9.6	-
Phenylbutazone	1	15±5.0	81.3	65±15.0**	65.9	135±20.7*	52.6	135±20.6*	47.1
MB7	1	10±5.8	87.5	95±5.0**	53.7	125±9.6**	56.1	135±5.0**	47.1
MB11	1	10±5.8	87.5	75±5.0**	63.4	95±5.1**	66.7	80±8.2**	68.6

Values are expressed as mean ± S.E.M. (N=4). Statistically significant from control group: *P<0.05, **P<0.001

CHAPTER 4

CONCLUSIONS

The genus *Millettia* of the family Leguminosae has been known to be rich in furanoflavanoids and pyranoflavonoids, with more than 20 structures being identified from six plant species (Maurya and Yadav, 2005). *Millettia* furanoflavonoids and pyranoflavonoids could be classified according to their basic skeleton as chalcones, flavones, flavanols, flavanones and flavans. Almost all of the members in the last four categories have the furan and pyran structure located on ring A in an angular position.

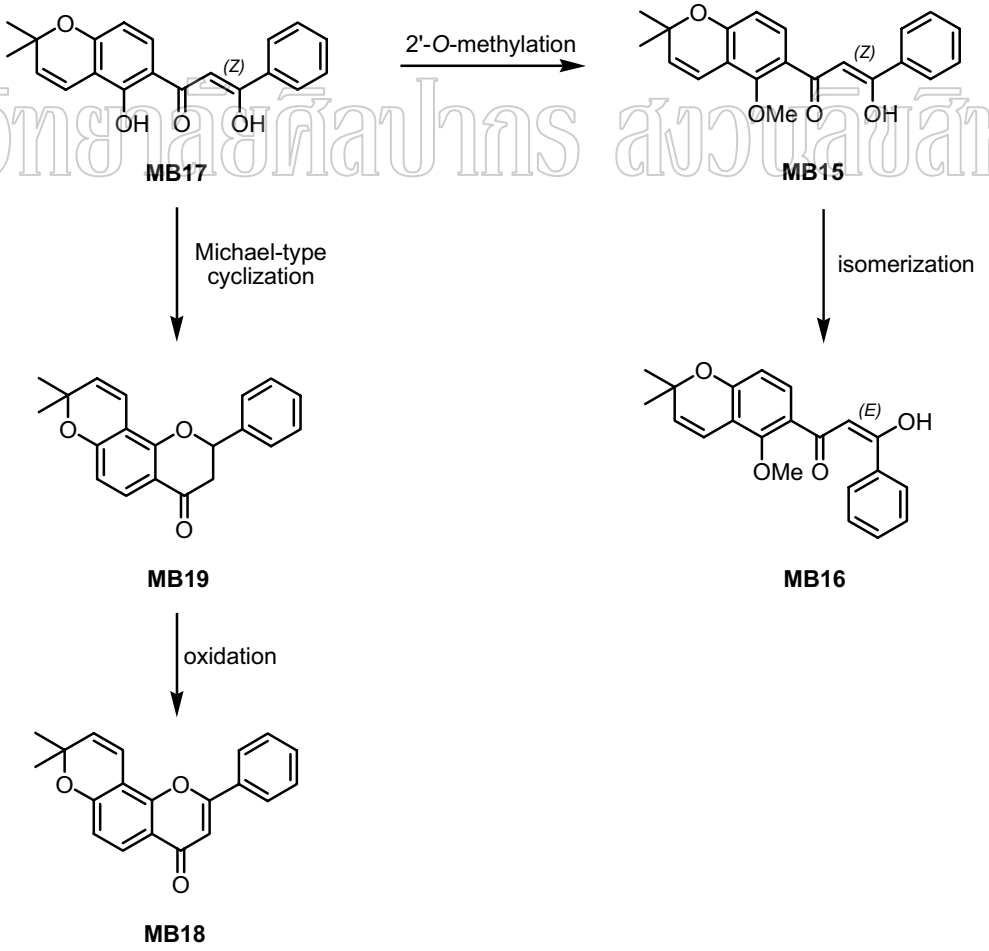
In the present report, the study of the chemical constituents from the leaves and pods of *Millettia brandisiana* Kurz resulted in the isolation of thirty-five compounds: seven new isoflavones (**MB1**, **MB21-MB23**, **MB29** and **MB31-MB32**), two new chalcones (**MB16** and **MB17**) and two synthetically known isoflavones (**MB2** and **MB24**) together with twenty-four known compounds (**MB3-MB14**, **MB15**, **MB18-MB20**, **MB25**, **MB26-MB28**, **MB30** and **MB33-MB35**).

While our project was in progress, Kikuchi *et al*. reported six new isoflavonoids isolated from the methanol extract of the leaves of the same plant (collected in Khon Kaen), some of which showed tumor-selective apoptosis-inducing properties (Kikuchi *et al*., 2007). We later described the isolation of isoflavones and rotenoids from the leaves (collected in Nakorn Pathom). A new isoflavone and a synthetically known isoflavone together with twelve known compounds were obtained from the leaves of *Millettia brandisiana*; a major isolated rotenoid (**MB11**) showed higher anti-inflammatory activity than phenylbutazone using EPP induced ear edema in rat (Pancharoen *et al*., 2008).

Relationship of compounds from *Millettia brandisiana*

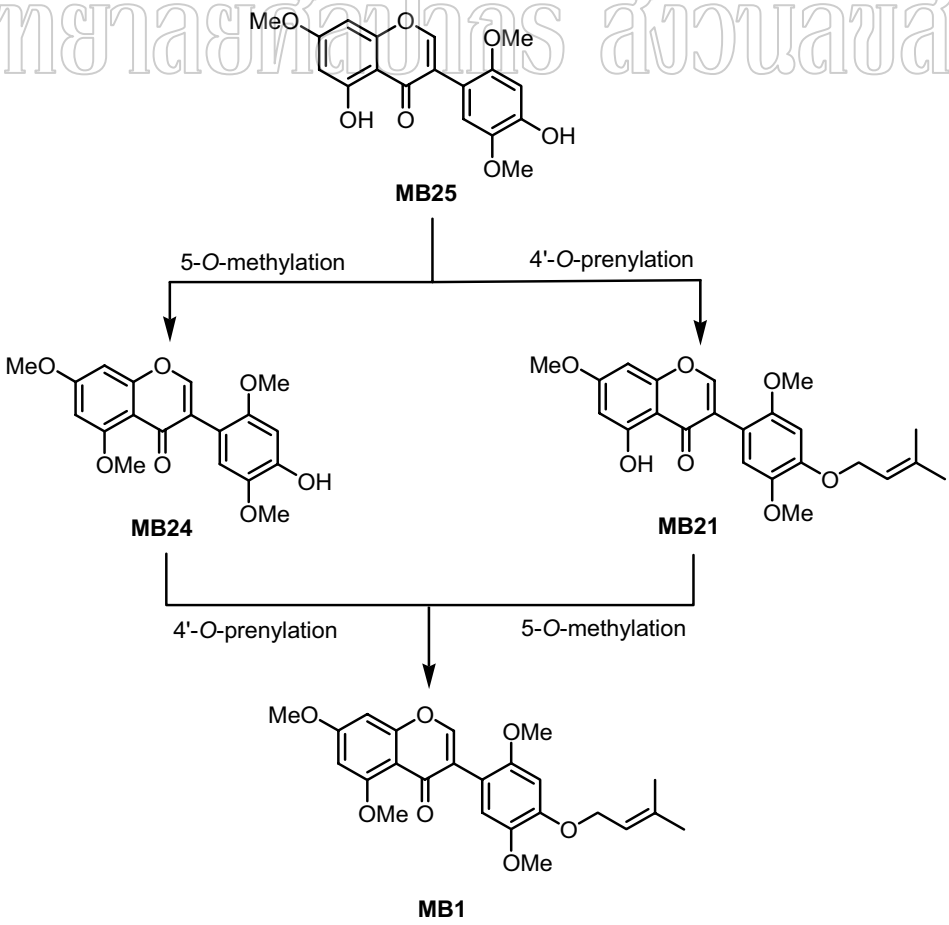
Chromatographic separation of the extracts from the leaves and pods of *Millettia brandisiana* has revealed that isoflavones and rotenoids were two main components in this plant. The chalcones, flavones, flavanones, sterols and triterpene were also present. Chalcones act as precursors for a vast range of flavonoid derivatives found throughout the plant kingdom.

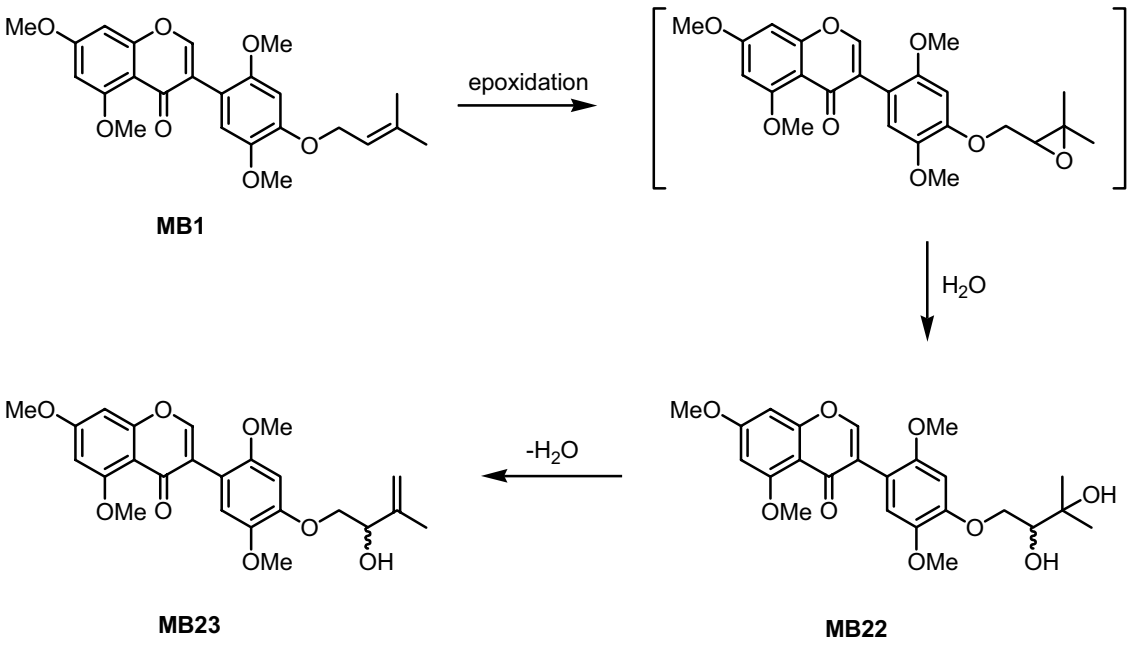
Michael-type cyclization of **MB17** gave isolonchocarpin (**MB19**). 2'-*O*-Methylation of **MB17** yielded **MB15** and isomerization of **MB15** afforded **MB16**. Oxidation of isolonchocapin (**MB19**) gave 6",6"-dimethylchromeno-(7,8,2",3")-flavone (**MB18**).



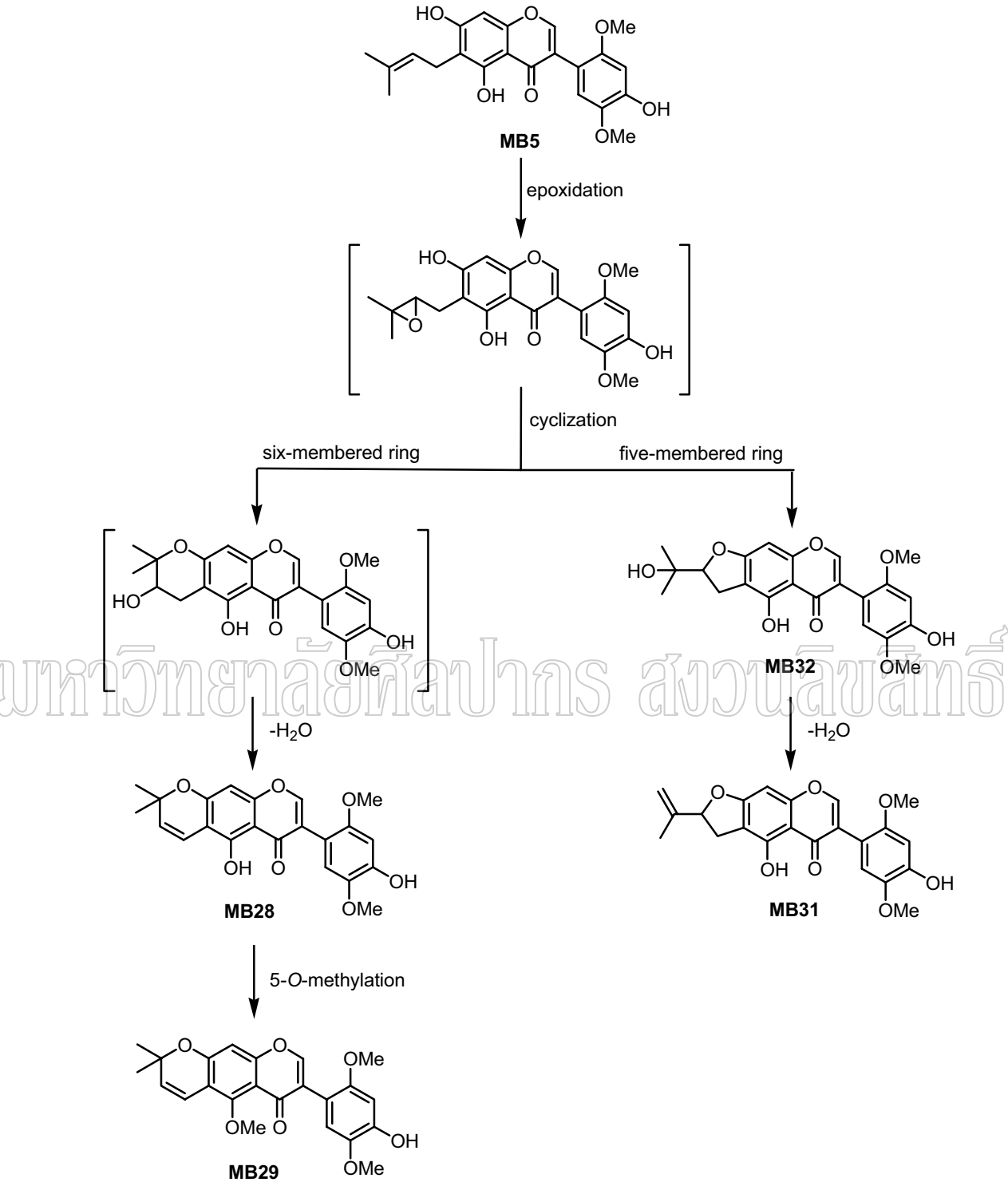
The isoflavonoids form a quite distinct subclass of flavonoids, being structural variants in which the shikimate-derived aromatic ring has migrated to the adjacent carbon of the heterocycle. Nevertheless, hundreds of different isoflavonoids have been identified and structural complexity is brought about by hydroxylation and alkylation reactions, varying the oxidation level of the heterocyclic ring, or forming additional heterocyclic rings.

Derrugenin (**MB25**) which was isolated in a small amount from the pods of *Millettia brandisiana*, was the precursor of some isoflavones in this plant. 5-*O*-Methylation of **MB25** gave **MB24**; while 4'-*O*-prenylation afforded **MB21**. **MB-1,** obtained from **MB21** and **MB24**, was a precursor of **MB22** and **MB23**. It is possible to speculate the biosynthesis of these isoflavones (**MB22** and **MB23**) from **MB1** which is in fact present in this plant. Accordingly, the epoxide intermediate arising from **MB-1**, could undergo ring opening reaction by H_2O to afford **MB22** which upon dehydration yielded **MB23**.



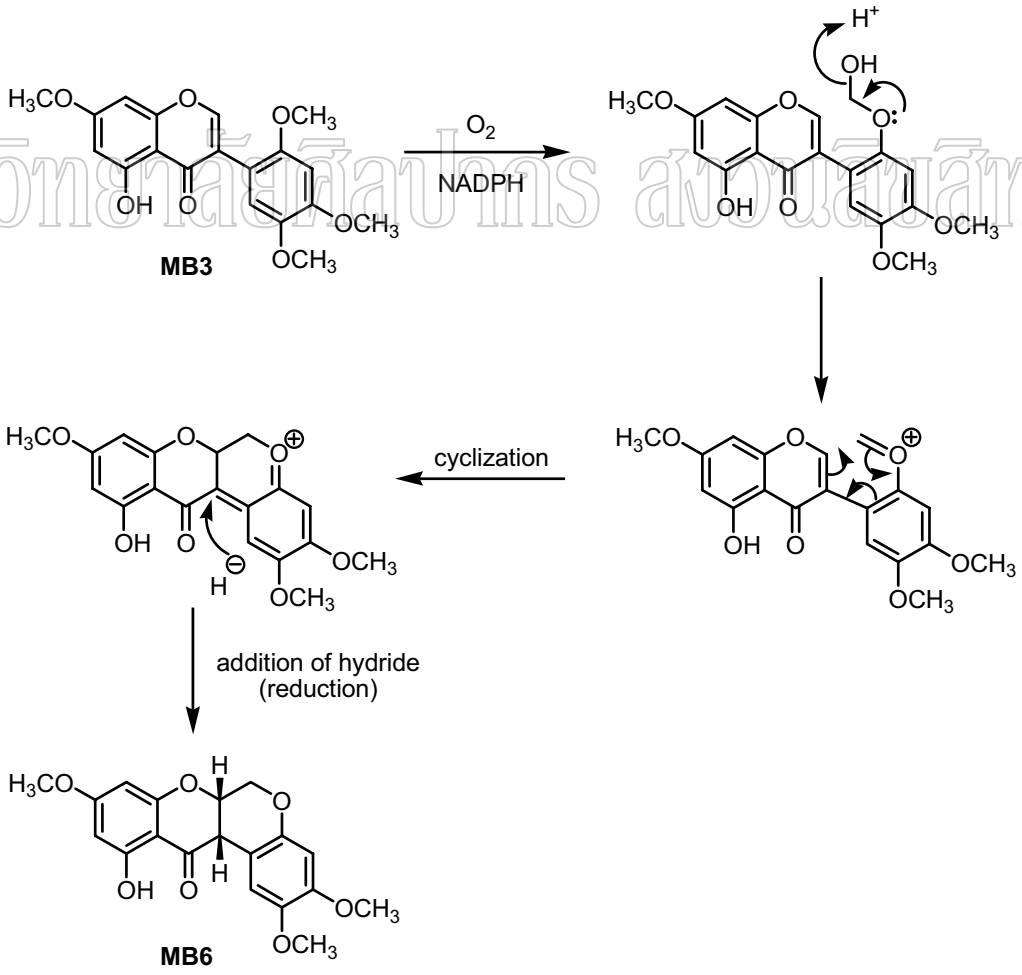


Viridiflorin (**MB5)** was a precursor of four isoflavones (**MB28-MB29** and **MB31-MB32)**. The cyclization had been postulated to involve an intermediate epoxide, so that nucleophilic attack of the phenol on to the epoxide group might lead to formation of either five-membered furan or six-membered pyran heterocycles as commonly encountered in natural products. Cyclization by opening of the epoxide ring and dehydration gave elongatin (**MB28**), further 5-*O*-methylation yielded **MB29**. Alternatively, cyclization to a five-membered ring gave **MB32**, which upon dehydration afforded **MB31**.

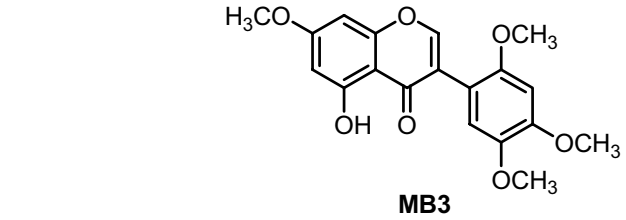


The presence of both isoflavones and rotenoids in the *Millettia brandisiana* is in good agreement with the biosynthesis. The rotenoids may thus be characterized in derivation as biosynthetically advanced isoflavonoids and this agree with the circumstantial evidence of their occurrence.

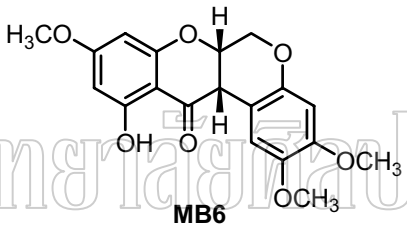
Although two isoflavones, robustigenin (**MB3**) and toxicarol isoflavone (**MB4**), were isolated in small amounts from the leaves, a series of corresponding rotenoids and dehydrorotenoids were obtained with 6-deoxyclitoriacetal (**MB7**) and 12a-hydroxy-α-toxicarol (**MB11**) as the main components. The rotenoids were formed by ring cyclization of 2'-methoxyisoflavone.



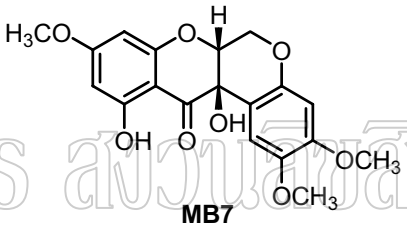
Four rotenoids (**MB6-MB9**) were obtained from robustigenin (**MB3**), while rotenoids with an angular chromene ring (**MB10-MB13**) were obtained from toxicarol isoflavone (**MB4**).



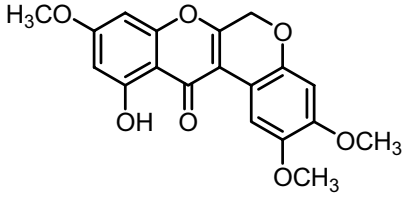
MB3



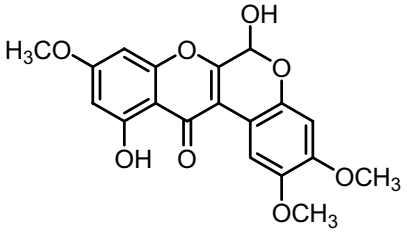
MB6



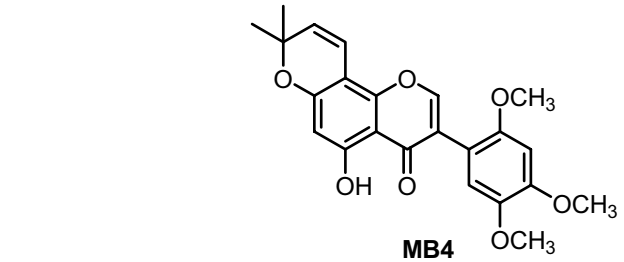
MB7



MB8

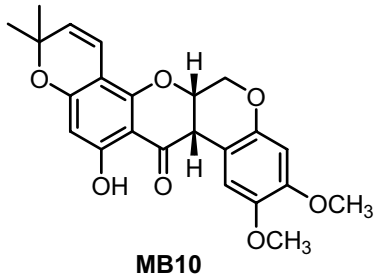


MB9

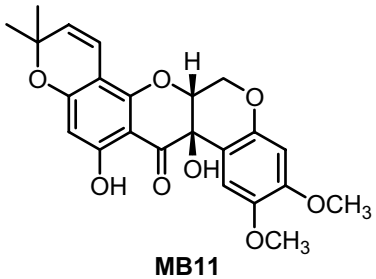


MB4

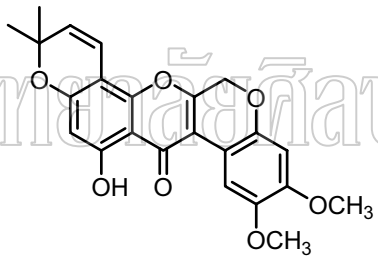
cyclization



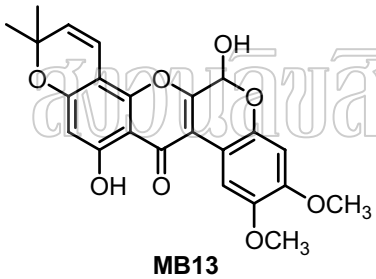
MB10



MB11



MB12



MB13

In conclusion, isoflavones and rotenoids have been isolated from the leaves of *M. brandisiana* (collected in Nakorn Pathom) whereas the leaves collected in Khon Kaen yielded isoflavones, no rotenoids were isolated (Kikuchi *et al*., 2007). Only an isoflavone, viridiflorin (**MB5**), were obtained from both plant samples. There is biogeographic variation in the flavonoids isolated from *M. brandisiana* collected in different parts of Thailand. It is interesting to note that *M. brandisiana* is the first Thai *Millettia* species to produce isoflavones and rotenoids; other species of *Millettia* in Thailand have yielded only flavones and chalcones (Phrutivorapongkul, *et al*., 2003 and Srituralak and Likhitwitayawuid, 2006).

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APPENDIX

ABBREVIATIONS AND SYMBOLS

s	=	singlet
br s	=	broad singlet
d	=	doublet
dd	=	doublet of doublet
dt	=	doublet of triplet
m	=	multiplet
δ	=	chemical shift relative to tetramethylsilane (TMS)
J	=	coupling constant
ppm	=	part per million
$[\alpha]_D$	=	specific rotation
ε	=	molar absorptivity coefficient
λ_{max}	=	maximum wavelength
ν_{max}	=	absorption frequencies
cm^{-1}	=	reciprocal centimeter (wave number)
m.p.	=	melting point
°C	=	degree celcius
m/z	=	a value of mass divided by change
%	=	percent
c	=	concentation
g	=	gram
mg	=	milligram
µg	=	microgram
mL	=	milliliter
µm	=	micrometer
nm	=	nanometer
h	=	hour
min	=	minute
Hz	=	Hertz
MHz	=	Megahertz

MS	=	Mass Spectroscopy
EI-MS	=	Electron-Ionization Mass Spectrometry
UV	=	Ultraviolet-Visible
IR	=	Infrared
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimentional Nuclear Magnetic Resonance
COSY	=	Correlation Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
NOE	=	Nuclear Overhauser Effect Spectroscopy
HPLC	=	High Performance Liguid Chromatography
CC	=	Column Chromatography
PLC	=	Preparative Thin Layer Chromatography
VLC	=	Vacuum Liquid Chromatography
CH_2Cl_2	=	dichloromethane
EtOAc	=	ethyl acetate
MeOH	=	methanol
$CDCl_3$	=	deuterochloroform
CD_3OD	=	deuteromethanol
H_2SO_4	=	sulfuric acid
$CeSO_4$	=	cerium sulfate

PUBLICATIONS

PUBLICATIONS

Pancharoen, O., Athipornchai, A., Panthong, A. and Taylor, W. C. (2008) "Isoflavones and rotenoids from the leaves of *Millettia brandisiana*", *Chem. Pharm. Bull.*, **56**, 835-838.

PRESENTATIONS

1. Anan Athipornchai, Orasa Pancharoen, Channarong Choowong, Lampa Yossombat and Ampai Panthong (2006) "Rotenoids from Leaves of *Millettia brandisiana*", *Poster Presentation*, The 32nd Congress on Science and Technology of Thailand (STT.32) on 10-12 October 2006, Queen Sirikit National Convention Center (QSNCC), Bangkok, Thailand.

2. Anan Athipornchai and Orasa Pancharoen (2007) "Chemical Constituents from the pods of *Millettia brandisiana*" *Poster* presentation, The Sixth Princess Chulabhorn International Science Congress on 25-29 November 2007 Bangkok, Thailand.

3. Anan Athipornchai, Suteera Bunkum and Orasa Pancharoen (2008) "Chemical Constituents from the bark of *Millettia brandisiana*" *Poster* presentation, The 34th Congress on Science and Technology of Thailand (STT.34) on 31 October - 2 November 2008, Queen Sirikit National Convention Center (QSNCC), Bangkok, Thailand.

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