

CHAPTER 2

Experimental

2.1 Plant Materials

The plants used in this research were listed in Table 2.1

Table 2.1 Plant materials used in the research

Scientific Name	Local Name	Place	Year
<i>V. scandens</i>	Gu-si-pah-doh (Lua language)	Haw Mai (Lua) village, Bahng Hin Fohn Subdistrict, Mae Jam, Chiang Mai province	March 2008
<i>C. nervosum</i> var. <i>paniala</i>	Makiang	Chiang Mai Horticulture Research Center, Office of agricultural research and development region 1, Lampang province	July-August 2009

2.2 Chromatography techniques

2.2.1 Flash Column Chromatography (FCC)

Flash column chromatography was carried out using the adaptation of classical column chromatography by applying the pressure from the air pump to the head of column.

Adsorbent: Silica gel 60 PF₂₅₄ and sephadexTM LH-20

Packing method: Slurry packing

Sample loading: The sample was dissolved in a small amount of suitable organic solvent, mixed with a small quantity of silica gel, air dried and added gently onto the top of column.

Elution: After loading of the sample, the column was eluted with suitable organic solvent system using isocratic or gradient technique. The eluents is optimized in small scale pretests, often using TLC with the same stationary phase.

2.2.2 Thin Layer Chromatography (TLC)

Techniques: One way, as ascending.
Adsorbent: Silica gel 60 PF₂₅₄ pre-coated on aluminum plate 3×4 cm and 1×4 cm.

2.2.3 Preparative Thin Layer Chromatography (PTLC)

Techniques: One way, as ascending.
Adsorbent: Silica gel 60 PF₂₅₄ by spreading aqueous slurry on to plate size 20×20 cm. left standing to dry at room temperature and subsequently activated for 4 hours at 120 °C in an oven.

2.2.4 Detection on chromatography plate

Ultraviolet light (UV₂₅₄): The compound which contains unsaturated bonds especially conjugated system is visible as quenching spot under UV light at 254 nm.

Anisaldehyde reagent: Developing this reagent by prepared with *p*-anisaldehyde : EtOH : H₂SO₄ : CH₃COOH (2.03 : 93.11 : 3.06 : 1.80). The spot of organic compounds appears specific colors with this reagent after heating at 90–110 °C for 2–4 minutes.

2.3 Chemicals and Apparatus and Instruments

2.3.1 Chemicals

Chemicals used in this research were listed in Table 2.2

Table 2.2 Chemicals and solvents used in the research

Chemical	Molecular	Molecular weight	Grade	Supplier
Acetic acid	C ₂ H ₄ O ₂	60.08	99.8%	Merck
Acetone ^{*,**}	C ₃ H ₆ O	58.08	commercial	-
Aluminum plate	-	-	-	Merck
Ammonium chloride	NH ₄ Cl	53.49	≥ 99.0%	Scharlau
<i>p</i> -Anisaldehyde	C ₈ H ₈ O ₂	136.15	99%	ACRCS
Chloroform ^{**}	CHCl ₃	119.38	≥99.8%	Carlo Erbr
Chloroform- <i>d</i> ₁	CDCl ₃	120.38	≥ 99.8%	Wilmad
<i>m</i> -Chloroperbenzoic acid	C ₇ H ₅ ClO ₃	172.57	-	Fluka
Dichloromethane [*]	CH ₂ Cl ₂	84.93	commercial	-
Ethanol	C ₂ H ₆ O	46.07	commercial	-
Ethyl acetate [*]	C ₄ H ₈ O ₂	88.11	commercial	-
Hexane [*]	C ₆ H ₁₄	86.18	commercial	-
Hydrogen peroxide	H ₂ O ₂	34.01	50%	NCG
Methanol ^{*,***}	CH ₄ O	32.04	commercial	-
Methyl iodide	CH ₃ I	141.94	≥ 99.5%	Fluka
Phosphorus pentaoxide	P ₂ O ₅	141.94	≥ 97%	Fluka
Potassium carbonate ^{****}	K ₂ CO ₃	138.21	99%	Unilab
Sephadex TM LH-20	-	-	-	Amersham Biosciences
Silica gel 60 PF ₂₅₄	-	-	-	Merck
Sodium bicarbonate	NaHCO ₃	84.01	99–101%	BHD

Table 2.2 (Continued)

Chemical	Molecular	Molecular weight	Grade	Supplier
Sodium borohydride	NaBH ₄	37.83	97%	Labchem
Sodium hydroxide	NaOH	40.00	≥ 99%	Merck
Sodium metal	Na	22.99	-	May & Baker Ltd
Sulfuric acid	H ₂ SO ₄	98.08	96%	CarloErba
Tetrahydrofuran****	C ₄ H ₈ O	72.11	≥ 99.5%	Merck

Note *	Simple distillation
**	Refluxed over P ₂ O ₅ for 1 h followed by simple distillation
***	Refluxed over CaH ₂ for 1 h followed by simple distillation
****	Vacuumed and dried by hot gun
*****	Distilled from sodium/benzophenone under nitrogen atmosphere

2.3.2 Apparatus and Instruments

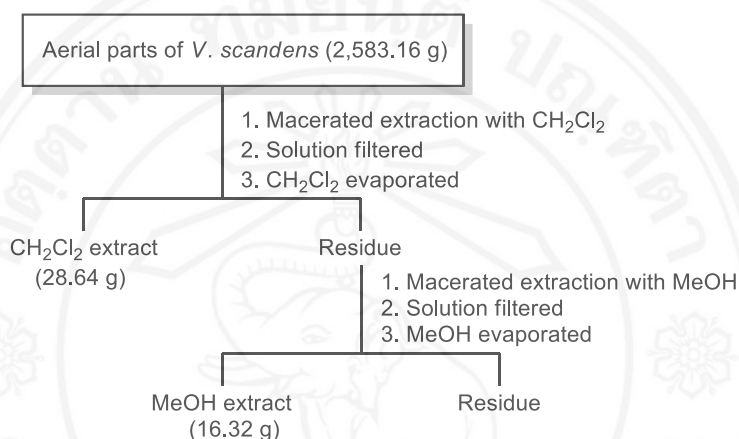
The apparatus and instruments were listed in Table 2.3

Table 2.3 Apparatus and instruments used in this research

Apparatus and instruments	Company	Model
High vacuum pump	Edwards	Edwards 18
Infrared spectrometer (FT-IR)	Bruker	TENSOR 27
Mass spectrometer (HRMS)	Waters	Micromass Q-TOF-2™
Melting point apparatus	SANYO	Gallenkamp
Nuclear Magnetic Resonance Spectrometer (NMR)	Bruker	-
Optical rotations	Atago	AP-300
Rotary evaporator	Büchi	R-200
UV-lamp 254	-	-
Weighting balance (2 and 4 positions)	Mettler Toledo	PG802-S and AB204-S

2.4 Extraction and Isolation of *V. scandens* Aerial Parts

The air-dried aerial parts of *V. scandens* (2,583.16 g) were successively extracted with dichloromethane and methanol twice (3 days each) at room temperature. The solvents evaporated under reduced pressure at temperature 40–45 °C, to obtain CH₂Cl₂ extract (green crude, 28.64 g, 1.12% yield) and the methanol extract (dark green gum, 16.32 g, 0.63% yield), respectively. The extraction sequence was shown in Scheme 2.1.

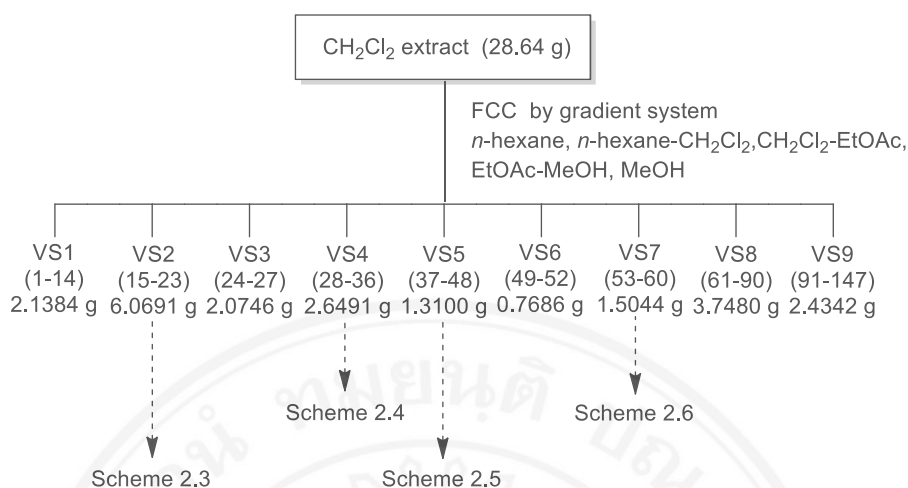


Scheme 2.1 Extraction of the aerial parts of *V. scandens*

2.4.1. Dichloromethane Extract (CH₂Cl₂ Extract)

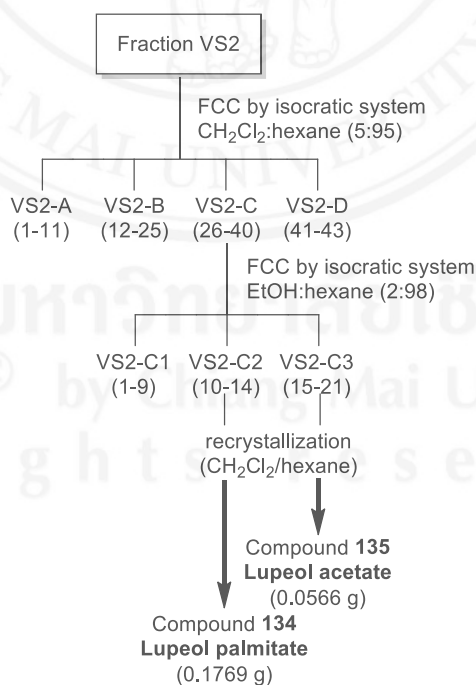
The CH₂Cl₂ extract (28.64 g) was fractionated by flash column chromatography (FCC, silica gel) and eluted with gradient mixtures of *n*-hexane, *n*-hexane-CH₂Cl₂, CH₂Cl₂-EtOAc, EtOAc-MeOH and MeOH with increasing polarity as solvent. All eluates were examined by TLC and anisaldehyde reagent and then combined to obtain 9 fractions (VS1-9). This isolation was shown in Scheme 2.2.

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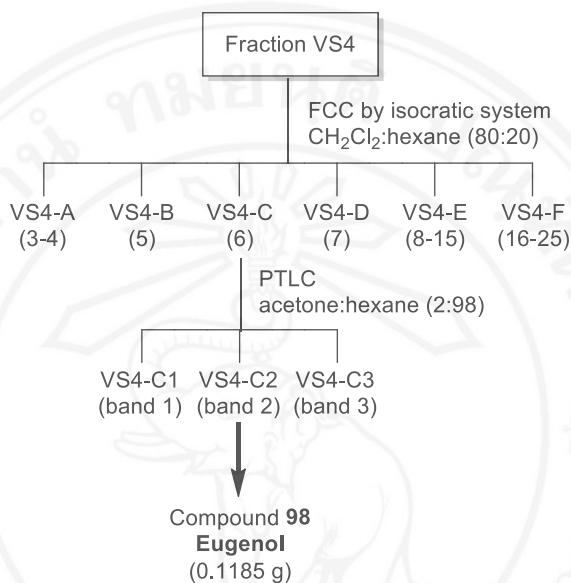
Scheme 2.2 Fractionation of the CH_2Cl_2 extract of the aerial parts of *V. scandens*

Fraction VS2; this combined fractions was rechromatographed over silica gel eluted with CH_2Cl_2 :hexane (5:95) to give 4 subfractions (VS2-A-D). Subfraction VS2-C (26-40) was subjected to column chromatography eluted with 2% EtOH in hexane to give 3 subfractions (VS2-C1-C3). The precipitate of subfractions VS2-C2 and VS2-C3 were recrystallized from CH_2Cl_2 /hexane to afford compounds **134** and **135** as white solid (0.1769 and 0.0566 g respectively) (Scheme 2.3).



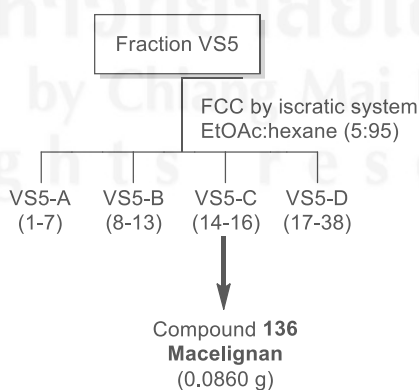
Scheme 2.3 Fractionation of fraction VS2 of the CH_2Cl_2 extract

Fraction VS4; this combined fractions was rechromatographed over silica gel eluted with CH₂Cl₂:hexane (80:20) to give 6 subfractions (VS4-A-F). The subfraction VS4-C was further purified by preparative thin layer chromatographed using 2% acetone in hexane as mobile phase to afford compound **98** as yellow oils (0.1185 g), as shown in Scheme 2.4.



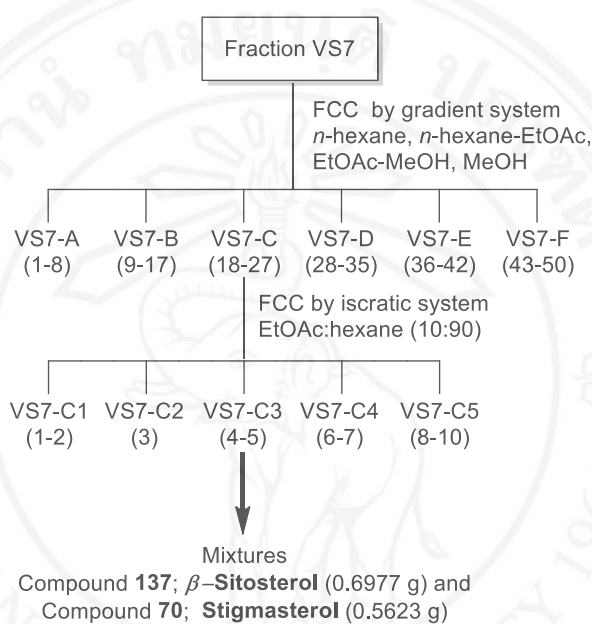
Scheme 2.4 Fractionation of fraction VS4 of the CH₂Cl₂ extract

Fraction VS5; this combined fractions was rechromatographed over silica gel eluted with EtOAc:hexane (5:95) to give 4 subfractions (VS5-A-D). A white solid of compound **136** (0.0860 g) was received from isolation of fraction VS5-C as shown in Scheme 2.5.



Scheme 2.5 Fractionation of fraction VS5 of the CH₂Cl₂ extract

Fraction VS7 was rechromatographed over silica gel and eluted with *n*-hexane, *n*-hexane-EtOAc, EtOAc-MeOH and MeOH with increasing amount of the more polar solvent to obtain 6 subfractions (VS7-A-F). Subfraction VS7-C was subjected to FCC and eluted with EtOAc:hexane (10:90) to give 5 subfractions. A mixture of compounds **137** (0.6977 g) and **70** (0.5623 g) were received from isolation of fraction VS7-C3 as a white solid (Scheme 2.6).

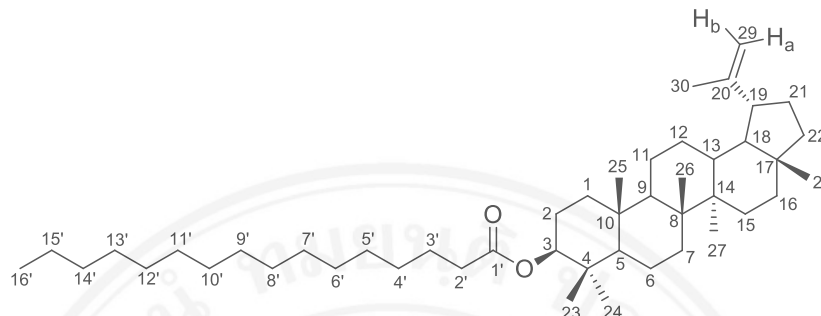


Scheme 2.6 Fractionation of fraction VS7 of the CH₂Cl₂ extract

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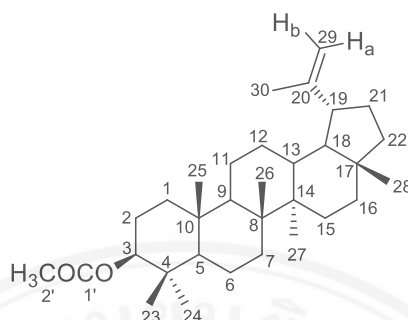
2.5 Structural elucidations of isolated compounds from *V. scandens*

2.5.1 Lupeol palmitate (134)



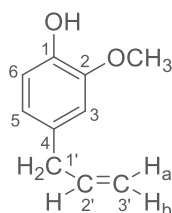
Molecular formula	$C_{46}H_{80}O_2$ (664 g/mol)
Physical characteristic	White solid 0.1769 g m.p. 83.5–84.0 °C (from CH_2Cl_2 /hexane) R_f 0.63 (EtOAc : hexane; 2 : 98)
FTIR (thin film) ν_{max}	3070 (C=C-H stretching), 2915 (C-H stretching of CH_2/CH_3), 1728 (C=O stretching of ester), 1641 (C=C stretching), 1470 (C-H bending of CH_2/CH_3), 1173 (C-O stretching), 716 (CH_2 rocking of C-(CH_2) _n -C) cm^{-1}
1H NMR ($CDCl_3$, 400 MHz)	δ : 0.78 (3H, <i>s</i> , H-28), 0.84 (6H, <i>s</i> , H-23, 25), 0.85 (3H, <i>s</i> , H-26), 0.88 (3H, <i>t</i> , H-16'), 0.94 (3H, <i>s</i> , H-27), 1.03 (3H, <i>s</i> , H-24), 1.68 (3H, <i>s</i> , H-30), 2.27 (2H, <i>t</i> , $J = 7.2$ Hz, H-2'), 2.38 (1H, <i>td</i> , $J = 11.1, 5.7$ Hz, H-19), 4.47 (1H, <i>m</i> , H-3), 4.57 (1H, <i>dd</i> , $J = 2.3, 1.3$, H-29a or 29b), 4.68 (1H, <i>d</i> , $J = 2.3$, H-29a or 29b)
^{13}C NMR ($CDCl_3$, 100 MHz)	δ : 14.1 (C-16'), 14.5 (C-27), 16.0 (C-24), 16.2 (C-26), 16.6 (C-25), 18.0 (C-28), 18.2 (C-6), 19.3 (C-30), 20.9 (C-11), 22.7 (C-15'), 23.7 (C-2), 25.1 (C-3'), 25.2 (C-12), 27.4 (C-15), 28.0 (C-23), 29.2–29.8 (C-4'–13'), 31.9 (C-14'), 34.2 (C-7), 34.8 (C-2'), 35.6 (C-16), 37.1 (C-10), 37.8 (C-4), 38.0 (C-13), 38.4 (C-1, 21), 40.0 (C-22), 40.8 (C-8), 42.8 (C-14), 43.0 (C-17), 48.0 (C-19), 48.3 (C-18), 50.3 (C-9), 55.4 (C-5), 80.6 (C-3), 109.4 (C-29), 150.9 (C-20), 173.6 (C-1')
EIMS m/z	664 [M^+], 649, 621, 445, 408, 189
HRMS (ESI) m/z	found 687.5640 for $C_{47}H_{83}O_2Na$ ($M+Na$) ⁺

2.5.2 Lupeol acetate (135)



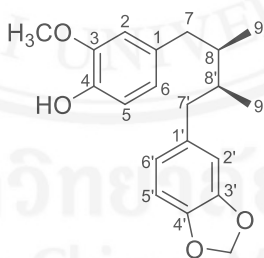
Molecular formula	$C_{32}H_{52}O_2$ (468 g/mol)
Physical characteristic	White solid 0.0566 g m.p. 210.0–212.0 °C (from EtOAc/hexane) R_f 0.60 (EtOAc : hexane; 2 : 98)
FTIR (thin film) ν_{max}	3076 (C=C-H stretching), 2946 (C-H stretching of CH_2/CH_3), 1731 (C=O stretching of ester), 1641 (C=C stretching), 1453 (C-H bending of CH_2/CH_3), 1249 (C-O stretching) cm^{-1}
1H NMR ($CDCl_3$, 400 MHz)	δ : 0.78 (1H, <i>m</i> , H-5), 0.83 (3H, <i>s</i> , H-28), 0.84 (9H, <i>s</i> , H-23, 24, 25), 0.93 (3H, <i>s</i> , H-27), 1.02 (3H, <i>s</i> , H-26), 1.68 (3H, <i>s</i> , H-30), 1.86-1.96 (2H, <i>m</i> , H-21), 2.04 (3H, <i>s</i> , H-2'), 2.38 (1H, <i>td</i> , $J = 11.1, 5.7$ Hz, H-19), 4.47 (1H, <i>dd</i> , $J = 10.4, 5.4$ Hz, H-3), 4.56 (1H, <i>dd</i> , $J = 2.3, 1.3$ Hz, H-29a or 29b), 4.68 (1H, <i>d</i> , $J = 2.3$ Hz, H-29a or 29b)
^{13}C NMR ($CDCl_3$, 100 MHz)	δ : 14.5 (C-27), 15.9 (C-24), 16.1 (C-25), 16.4 (C-26), 17.9 (C-28), 18.1 (C-6), 19.2 (C-30), 20.9 (C-11), 21.6 (C-2'), 23.7 (C-2), 25.0 (C-12), 27.4 (C-15), 27.9 (C-23), 29.8 (C-21), 34.2 (C-7), 35.5 (C-16), 37.0 (C-10), 37.7 (C-4), 38.0 (C-13), 38.3 (C-1), 39.9 (C-22), 40.8 (C-8), 42.8 (C-14,17), 48.0 (C-18), 48.2 (C-19), 50.3 (C-9), 55.4 (C-5), 80.9 (C-3), 109.3 (C-29), 150.9 (C-20), 171.0 (C-1')
EIMS m/z	468 [M^+], 453, 425, 408, 249, 189
HRMS (ESI) m/z	calcd for $C_{32}H_{52}O_2Na$ ($M+Na$) $^+$: 491.3865 found 491.3863

2.5.3 Eugenol (98)



Molecular formula	$C_{10}H_{12}O_2$ (164 g/mol)
Physical characteristic	Yellow oil 0.1185 g R_f 0.61 (acetone : hexane; 2 : 98)
FTIR (thin film) ν_{max}	3506 (O-H stretching), 3076 (C=C-H stretching), 2938 (C-H stretching of CH_2/CH_3), 1610 (C=C stretching), 1432 (C-H bending of CH_2/CH_3), 1268 (C-O stretching of ether) cm^{-1}
1H NMR ($CDCl_3$, 400 MHz)	δ : 3.32 (2H, <i>d</i> , $J = 6.7$ Hz, H-1'), 3.88 (3H, <i>s</i> , 2-OCH ₃), 5.07 (2H, <i>m</i> , H-3'), 5.50 (1H, <i>brs</i> , 1-OH), 5.96 (1H, <i>ddt</i> , $J = 16.9, 10.1, 6.7$, H-2'), 6.69 (1H, <i>m</i> , H-3,5), 6.85 (1H, <i>m</i> , H-6)
^{13}C NMR ($CDCl_3$, 100 MHz)	δ : 39.8 (C-1'), 55.8 (2-OCH ₃), 111.1 (C-3), 114.2 (C-6), 115.4 (C-3'), 121.1 (C-5), 131.8 (C-4), 137.8 (C-2'), 143.8 (C-1), 146.4 (C-2)
EIMS m/z	164 [M^+], 149, 137, 121
HRMS (ESI) m/z	calcd for $C_{10}H_{12}O_2Na$ ($M+Na$) ⁺ : 187.0735 found 187.0736

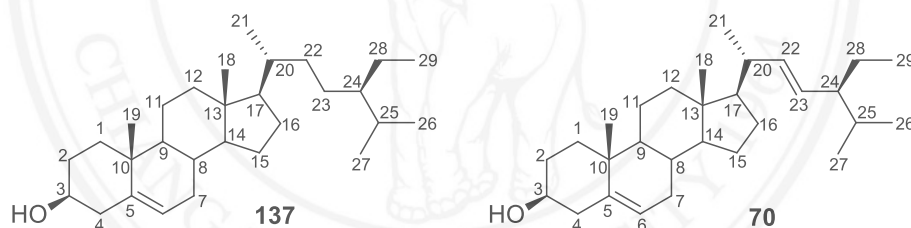
2.5.4 Macelignan (136)



Molecular formula	$C_{20}H_{24}O_4$ (328 g/mol)
Physical characteristic	Yellow gum 0.0860 g $[\alpha]_D^{27.5} = +7.8^\circ$ ($c = 0.32$, $CHCl_3$) R_f 0.43 (EtOAc : hexane; 5 : 95)
FTIR (thin film) ν_{max}	3538 (O-H stretching), 2960 (C-H stretching of CH_2/CH_3), 2776 (C-H stretching of OCH_2O), 1608 (C=C stretching), 1442 (C-H bending of CH_2/CH_3), 1038 (C-O stretching of ether), 933 (O- CH_2 -O stretching) cm^{-1}

¹ H NMR (CDCl ₃ , 400 MHz)	δ: 0.83 (3H, <i>d</i> , <i>J</i> = 6.6 Hz, H-9), 0.85 (3H, <i>d</i> , <i>J</i> = 6.6 Hz, H-9'), 1.74 (2H, <i>m</i> , H-8,8'), 2.25 (1H, <i>dd</i> , <i>J</i> = 13.6, 9.1 Hz, H-7'), 2.29 (1H, <i>dd</i> , <i>J</i> = 13.6, 5.0 Hz, H-7), 2.72 (2H, <i>dd</i> , <i>J</i> = 13.6, 5.0 Hz, H-7,7'), 3.86 (3H, <i>s</i> , 3-OCH ₃), 5.50 (1H, <i>brs</i> , 4-OH), 5.92 (2H, <i>dd</i> , 2.6, 1.4 Hz, OCH ₂ O), 6.61 (1H, <i>dd</i> , <i>J</i> = 8.2, 1.5 Hz, H-6), 6.62 (1H, <i>d</i> , <i>J</i> = 1.5 Hz, H-2'), 6.65 (1H, <i>dd</i> , <i>J</i> = 8.2, 1.6 Hz, H-6'), 6.66 (1H, <i>d</i> , <i>J</i> = 1.6 Hz, H-2), 6.73 (1H, <i>d</i> , <i>J</i> = 7.9 Hz, H-5), 6.83 (1H, <i>d</i> , <i>J</i> = 7.9 Hz, H-5')
¹³ C NMR (CDCl ₃ , 100 MHz)	δ: 16.1 (C-9), 16.2 (C-9'), 38.8 (C-7'), 39.0 (C-7), 39.2 (C-8'), 39.3 (C-8), 55.8 (3-OCH ₃), 100.7 (OCH ₂ O), 107.9 (C-5), 109.3 (C-2), 111.4 (C-2'), 114.0 (C-5'), 121.7 (C-6), 121.8 (C-6'), 133.7 (C-1'), 135.7 (C-1), 143.5 (C-4'), 145.4 (C-4), 146.3 (C-3'), 147.4 (C-3)
EIMS <i>m/z</i>	328 [M ⁺], 137, 135
HRMS (ESI) <i>m/z</i>	calcd for C ₂₀ H ₂₄ O ₄ Na (M+Na) ⁺ : 351.1573 found 351.1498

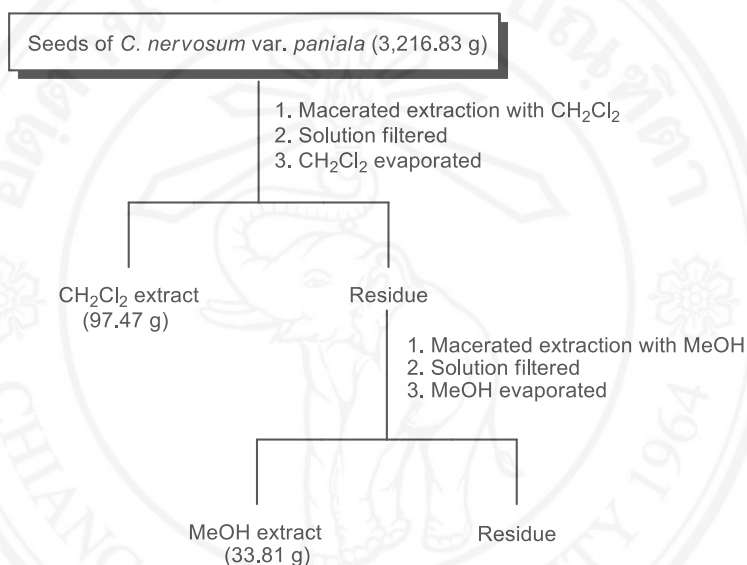
2.5.5 β-Sitosterol (137) and stigmasterol (70)



Molecular formular	C ₂₉ H ₅₀ O (414 g/mol) and C ₂₉ H ₄₈ O (412 g/mol)
Physical characteristic	White solid 1.2600 g m.p. 138.7–144.8 °C (from EtOAc/hexane) R _f 0.67 (EtOAc : hexane; 10 : 90)
FTIR (thin film) <i>v</i> _{max}	3396 (O-H stretching), 2937 (C-H stretching of CH ₂ /CH ₃), 1642 (C=C stretching), 1048 (C-O stretching) cm ⁻¹
¹ H NMR (CDCl ₃ , 400 MHz)	δ: 0.69 (3H, <i>s</i> , H-18), 0.79 (3H, <i>d</i> , <i>J</i> = 7.0 Hz, H-27), 0.81 (3H, <i>t</i> , H-29), 0.84 (3H, <i>d</i> , <i>J</i> = 6.5 Hz, H-26), 1.00 (3H, <i>s</i> , H-21), 1.02 (3H, <i>s</i> , H-19), 3.52 (1H, <i>m</i> , H-3), 5.01* (1H, <i>dd</i> , <i>J</i> = 15.2, 8.6 Hz, H-23), 5.14* (1H, <i>dd</i> , <i>J</i> = 15.2, 8.6 Hz, H-22), 5.43 (1H, <i>m</i> , H-6) * found that in stigmasterol
EIMS <i>m/z</i>	414 and 412 [M ⁺]

2.6 Extraction and Isolation of *C. nervosum* var. *paniala* seeds

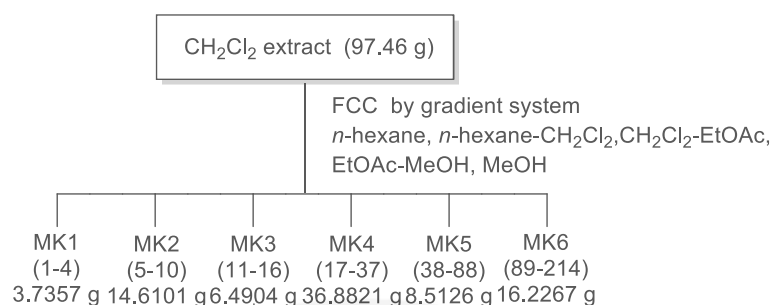
The air-dried seeds of *C. nervosum* var. *paniala* (3,216.83 g) were successively extracted with dichloromethane and methanol twice (3 days each) at room temperature. The extracts were concentrated under reduced pressure at 40–45 °C, to obtain the CH₂Cl₂ extract (brown sticky, 97.47 g, 3.03% yield) and the methanol extract (dark brown gum, 33.81 g, 1.05% yield), respectively. The extraction sequence was shown in Scheme 2.7.



Scheme 2.7 Extraction of the seeds of *C. nervosum* var. *paniala*

2.6.1 Dichloromethane Extract (CH₂Cl₂ Extract)

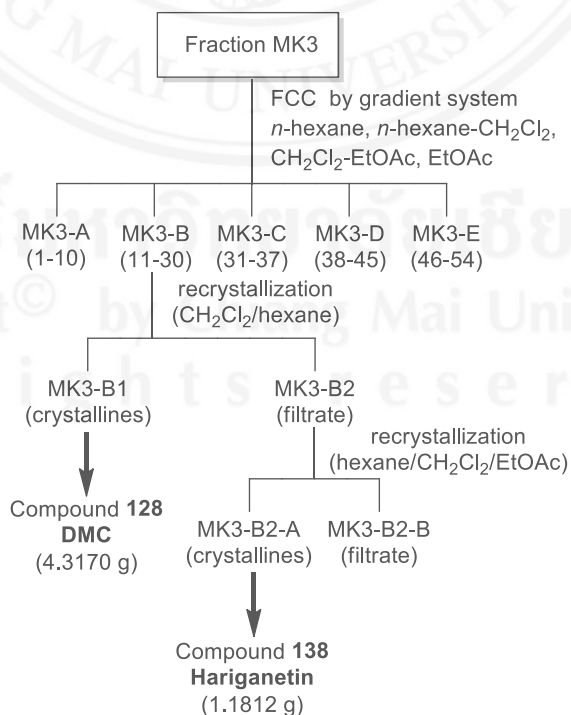
The CH₂Cl₂ extract (97.47 g) was fractionated by flash column chromatography (FCC, silica gel) and eluted with gradient mixtures of *n*-hexane, *n*-hexane-CH₂Cl₂, CH₂Cl₂-EtOAc, EtOAc-MeOH and MeOH with increasing polarity as solvent. All eluates were examined by TLC and anisaldehyde reagent and then combined to obtain 6 fractions (MK1-6). This isolation was shown in Scheme 2.8.



Scheme 2.10
 Scheme 2.9

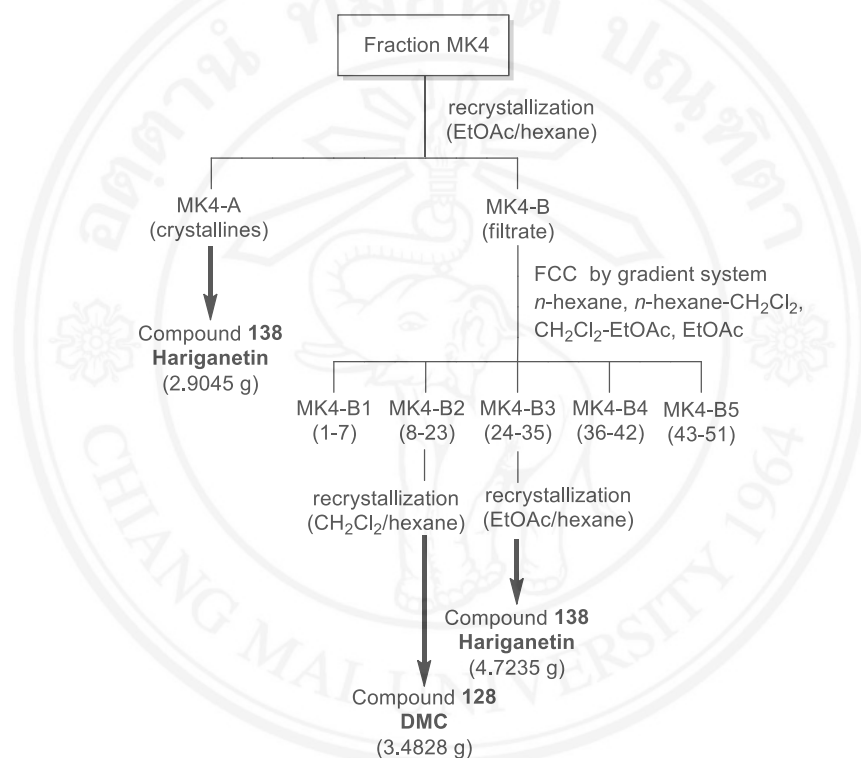
Scheme 2.8 Fractionation of the CH₂Cl₂ extract of the seeds of
C. nervosum var. *paniala*

Fraction MK3; this combined fractions was rechromatographed over silica gel eluted with gradient mixtures of *n*-hexane, *n*-hexane-CH₂Cl₂, CH₂Cl₂-EtOAc, and EtOAc with increasing polarity as solvent to give 5 subfractions (MK3-A-E). Compound **128** (4.3170 g) was received from recrystallized from CH₂Cl₂/hexane in subfractions MK3-B as orange solid. Subfraction MK3-B2 was recrystallized from hexane/CH₂Cl₂/EtOAc to afford compound **138** (1.1812 g) as orange solid as shown in Scheme 2.9



Scheme 2.9 Fractionation of fraction MK3 of the CH₂Cl₂ extract

Fraction MK4; the fraction was recrystallized from EtOAc/hexane to give 2 subfractions (MK4-A and B). Compound **138** (2.9045 g) was received from isolation of subfraction MK4-A. Subfraction MK4-B was rechromatographed over silica gel eluted with gradient mixtures of *n*-hexane, *n*-hexane-CH₂Cl₂, CH₂Cl₂-EtOAc, and EtOAc with increasing polarity to give 5 subfractions (MK4-B1-5). Compounds **128** (3.4828 g) and **138** (4.7235 g) were received from recrystallized from CH₂Cl₂/hexane and EtOAc/hexane of subfractions MK4-B2 and 3, respectively as shown in Scheme 2.10.

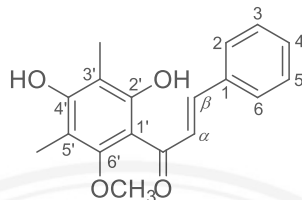


Scheme 2.10 Fractionation of fraction MK4 of the CH₂Cl₂ extract

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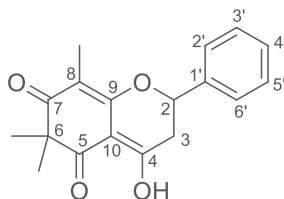
2.7 Structural elucidations of isolated compounds from *C. nervosum* var. *paniala*

2.7.1 2',4'-Dihydroxy-6'-methoxy-3',5'-dimethylchalcone (DMC) (128)



Molecular formula	$C_{18}H_{18}O_4$ (298 g/mol)
Physical characteristic	Orange solid 3.4650 g m.p. 120.8–122.3 °C (from CH_2Cl_2 /hexane) R_f 0.79 (EtOAc : hexane; 20 : 80)
FTIR (thin film) ν_{max}	3444 (O-H stretching), 2935 (C-H stretching of CH_3), 1628 (C=O stretching of ketone), 1554 (C=C stretching), 1450 (C-H bending of CH_3), 1165 (C-O stretching of ether), 1110 (C-O stretching of 2° alcohol), 972 (C-H bending of <i>trans</i> -alkene) cm^{-1}
1H NMR ($CDCl_3$, 400 MHz)	δ : 2.14 (3H, <i>s</i> , 5'- $\underline{CH_3}$), 2.16 (3H, <i>s</i> , 3'- $\underline{CH_3}$), 3.66 (3H, <i>s</i> , 6'- $\underline{OCH_3}$), 5.38 (1H, <i>s</i> , 4'- \underline{OH}), 7.41 (3H, <i>m</i> , H-3,4,5), 7.64 (2H, <i>m</i> , H-2,6), 7.84 (1H, <i>d</i> , $J = 15.7$ Hz, H_β), 7.99 (1H, <i>d</i> , $J = 15.7$ Hz, H_α), 13.69 (1H, <i>s</i> , 2'- \underline{OH})
^{13}C NMR ($CDCl_3$, 100 MHz)	δ : 7.6 (5'- $\underline{CH_3}$), 8.2 (3'- $\underline{CH_3}$), 62.3 (6'- $\underline{OCH_3}$), 106.6 (C-1'), 109.0 (C-3'), 109.0 (C-5'), 126.7 (C_α), 128.4 (C-5), 128.4 (C-3), 128.9 (C-6), 128.9 (C-2), 130.2 (C-4), 135.3 (C-1), 142.9 (C_β), 158.8 (C-6'), 159.3 (C-4'), 162.0 (C-2'), 193.4 (C=O)
EIMS m/z	298 [M^+], 221, 194, 166, 103, 77
HRMS (ESI) m/z	calcd for $C_{18}H_{18}O_4Na$ ($M+Na$) $^+$: 321.1103 found 321.1104

2.7.2 Hariganetin (138)

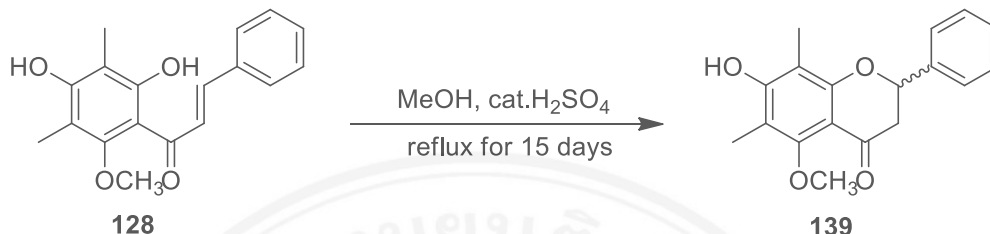


Molecular formula	$C_{18}H_{18}O_4$ (298 g/mol)
Physical characteristic	Orange solid 3.9056 g m.p. 140.4–141.5 °C (from EtOAc/hexane) R_f 0.43 (EtOAc : hexane; 20 : 80)
FTIR (thin film) ν_{max}	3450 (O-H stretching), 2974 (C-H stretching of CH_3), 1649 (C=O stretching of ketone), 1619 (C=O stretching of ketone), 1499 (C=C stretching), 1452 (C-H bending of CH_3), 1055 (C-O stretching of ether) cm^{-1}
1H NMR ($CDCl_3$, 400 MHz)	δ : 1.40 (3H, s, 6- \underline{CH}_3), 1.42 (3H, s, 6- \underline{CH}_3), 1.86 (3H, s, 8- \underline{CH}_3), 2.92 (1H, dd, $J = 17.9, 3.7$ Hz, H-3a), 3.03 (1H, dd, $J = 17.9, 10.8$ Hz, H-3b), 5.32 (1H, dd, $J = 10.8, 3.7$ Hz, H-2), 7.37-7.48 (5H, m, H-2',3',4',5',6'), 15.80 (1H, s, 4- \underline{OH})
^{13}C NMR ($CDCl_3$, 100 MHz)	δ : 7.9 (8- \underline{CH}_3), 23.1 (6- \underline{CH}_3), 25.5 (6- \underline{CH}_3), 38.2 (C-3), 52.3 (C-6), 76.0 (C-2), 101.4 (C-10), 107.3 (C-8), 125.8 (C-2',6'), 128.9 (C-3',4',5'), 138.0 (C-1'), 161.2 (C-9), 182.8 (C-4), 198.0 (C-7), 201.7 (C-5)
EIMS m/z	298 [M^+], 283, 255, 227
HRMS (ESI) m/z	calcd for $C_{18}H_{18}O_4Na$ ($M+Na$) $^+$: 321.1103 found 321.1102

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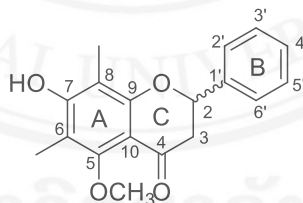
2.8 Derivatization of 2',4'-dihydroxy-6'-methoxy-3',5'-dimethyl-chalcone

2.8.1 7-Hydroxy-5-methoxy-6,8-dimethylflavanone (139)



A mixture of compound **128** (0.3852 g, 1.29 mmol) and conc. sulfuric acid (2 ml) in anhydrous methanol (40 ml) was heated under reflux for 15 days. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate solution at 0°C and the crude mixture was extracted several times with CH₂Cl₂. The combined organic extracts were washed with H₂O, dried over MgSO₄, filtered, and evaporated to dryness.

The crude product was purified by flash column chromatography on silica gel using EtOAc:hexane (3:7) as eluent to give flavones **139**, 7-hydroxy-5-methoxy-6,8-dimethylflavanone in 70% yield (0.2486 g) and 92% conversion from the starting material.

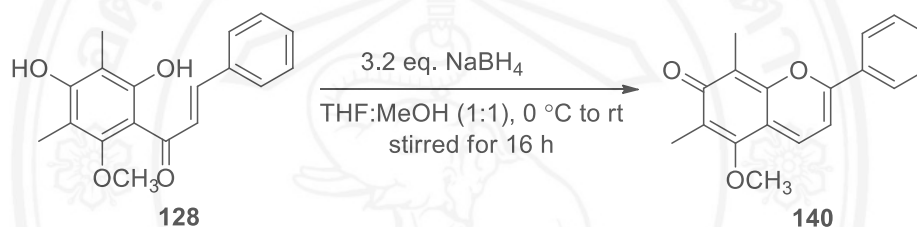


7-hydroxy-5-methoxy-6,8-dimethylflavanone (**139**)

Molecular formula	C ₁₈ H ₁₈ O ₄ (298 g/mol)
Physical characteristic	White solid 0.2486 g m.p. 210.0–211.2 °C (from EtOAc/hexane) R _f 0.38 (EtOAc : hexane; 20 : 80)
FTIR (thin film) ν _{max}	3395 (O-H stretching), 1650 (C=O stretching of ketone), 1580 (C=C stretching), 1453 (C-H bending of CH ₃), 1196 (C-O stretching of ether), 1108 (C-O stretching of 2° alcohol) cm ⁻¹

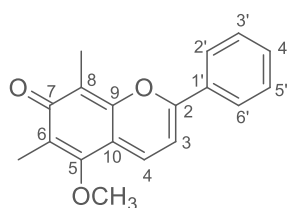
$^1\text{H NMR}$ (CDCl_3 , 400 MHz)	δ : 2.14 (6H, <i>s</i> , 6,8- CH_3), 2.83 (1H, <i>dd</i> , $J = 16.7, 3.1$ Hz, H-3a), 2.97 (1H, <i>dd</i> , $J = 16.7, 13.0$ Hz, H-3b), 3.81 (3H, <i>s</i> , 5- OCH_3), 5.41 (1H, <i>dd</i> , $J = 13.0, 3.0$ Hz, H-2), 5.46 (1H, <i>brs</i> , 7- OH), 7.35–7.50 (5H, <i>brm</i> , H-2',3',4',5',6')
$^{13}\text{C NMR}$ (CDCl_3 , 100 MHz)	δ : 7.8 (6- CH_3), 8.1 (8- CH_3), 45.7 (C-3), 61.2 (5- OCH_3), 78.6 (C-2), 107.0 (C-8), 109.1 (C-10), 111.3 (C-6), 125.8 (C-4'), 128.4 (C-3',5'), 128.7 (C-2',6'), 139.2 (C-1'), 157.7 (C-9), 158.9 (C-5), 159.6 (C-7), 189.7 (C-4)
HRMS (ESI) <i>m/z</i>	calcd for $\text{C}_{18}\text{H}_{18}\text{O}_4\text{Na}$ ($\text{M}+\text{Na}$) $^+$: 321.1103 found 321.1099

2.8.2 5-Methoxy-6,8-dimethyl-2-phenyl-7H-1-benzopyran-7-one (140)



Sodium borohydride (0.1480 g, 2.72 mmol) in a 100 ml round-bottomed flask equipped with a magnetic stirrer and fitted with a three-way stopcock with a septum cap and nitrogen inlet was added THF (15 ml). To a stirred 0°C solution, a solution of **128** (0.2540 g, 0.85 mmol) in THF (10 ml) was added and stirred for 15 min. The reaction mixture was stirred at room temperature for overnight. After that, dry MeOH (25 ml) was added to the reaction mixture and stirred for 16 h.

The reaction was neutralized with dilute HCl and extracted several times with CH_2Cl_2 . The combined organic extracts was washed with H_2O , dried over MgSO_4 , filtered, and evaporated to dryness. The crude product was purified by flash column chromatography on silica gel using EtOAc:hexane (3:7) as eluent to give compound **140**, 5-methoxy-6,8-dimethyl-2-phenyl-7H-1-benzopyran-7-one in 34% yield (0.0490 g) and 55% conversion from the starting material.

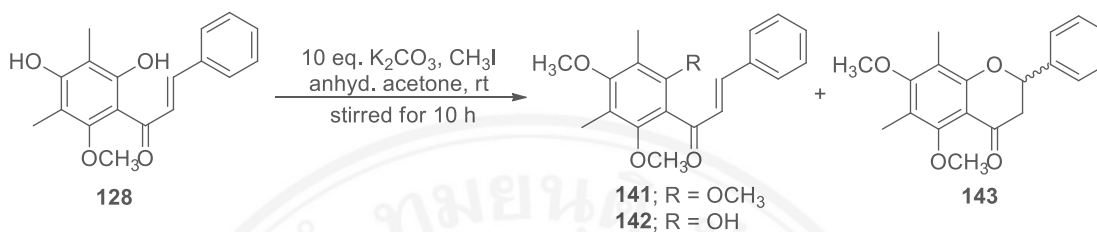


5-methoxy-6,8-dimethyl-2-phenyl-7H-1-benzopyran-7-one (140)

Molecular formular	$C_{18}H_{16}O_3$ (280 g/mol)
Physical characteristic	Red solid 0.0490 g m.p. 78.5–79.8 °C (from EtOAc/hexane) R_f 0.52 (EtOAc : hexane; 50 : 50)
FTIR (thin film) ν_{max}	2920 (C-H stretching of CH_3), 1655 (C=O stretching of ketone), 1509 (C=C stretching), 1453 (C-H bending of CH_3), 1131 (C-O stretching of ether) cm^{-1}
1H NMR ($CDCl_3$, 400 MHz)	δ : 2.17 (3H, <i>s</i> , 6- CH_3), 2.24 (3H, <i>s</i> , 8- CH_3), 3.85 (3H, <i>s</i> , 5- OCH_3), 6.86 (1H, <i>d</i> , $J = 7.40$ Hz, H-3), 7.52 (3H, <i>m</i> , H-3',4',5'), 7.67 (1H, <i>d</i> , $J = 7.40$ Hz, H-4), 7.92 (2H, <i>m</i> , H-2',6')
^{13}C NMR ($CDCl_3$, 100 MHz)	δ : 8.0 (6- CH_3), 10.1 (8- CH_3), 61.8 (5- OCH_3), 101.9 (C-3), 109.9 (C-6), 118.8 (C-10), 125.7 (C-3',5'), 127.4 (C-8), 129.2 (C-4'), 131.2 (C-2',6'), 131.3 (C-4), 131.5 (C-1'), 153.6 (C-9), 153.7 (C-5), 158.5 (C-2), 184.3 (C-7)
HRMS (ESI) m/z	calcd for $C_{18}H_{16}O_3H$ (M+H) ⁺ : 281.1178 found 281.1180

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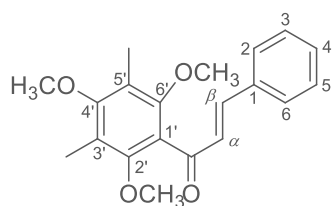
2.8.3 2',4',6'-Trimethoxy-3',5'-dimethylchalcone (141), 2'-hydroxy-4',6'-dimethoxy-3',5'-dimethylchalcone (142) and 5,7-dimethoxy-6,8-dimethylflavanone (143)



DMC **128** (0.2652 g, 0.89 mmol) in anhydrous acetone (20 ml) was stirred at room temperature and then potassium carbonate (1.2290 g, 8.88 mmol) was added to the reaction and left stirred for 15 min. After that the reaction mixture was added with MeI (0.60 ml, 0.89 mmol) at room temperature and stirred for 10 h, filtrated and extracted several times with CH_2Cl_2 . The combined organic extracts was washed with H_2O , dried over $MgSO_4$, filtered, and evaporated to dryness.

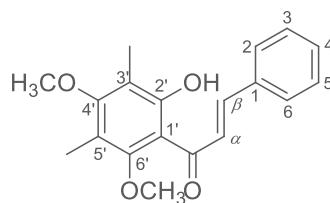
The crude product was purified by flash column chromatography on silica gel using gradient mixture solvent of *n*-hexane, *n*-hexane- CH_2Cl_2 , CH_2Cl_2 -EtOAc and EtOAc with increasing polarity as eluent to give two chalcones; 2',4',6'-trimethoxy-3',5'-dimethylchalcone (**141**) and 2'-hydroxy-4',6'-dimethoxy-3',5'-dimethylchalcone (**142**) and one flavanone; 5,7-dimethoxy-6,8-dimethylflavanone (**143**) in 69% yield (0.2018 g), 24% yield (0.0689 g) and 5% yield (0.0142 g), respectively and 100% conversion from the starting material.

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2',4',6'-trimethoxy-3',5'-dimethylchalcone (141)

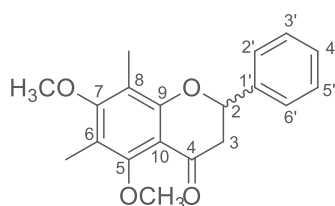
Molecular formular	$C_{20}H_{22}O_4$ (326 g/mol)
Physical characteristic	Brown oil 0.2018 g R_f 0.71 (EtOAc : hexane; 20 : 80)
FTIR (thin film) ν_{max}	2935 (C-H stretching of CH_3), 1683 (C=O stretching of ketone), 1633 (C=C stretching), 1450 (C-H bending of CH_3), 1110 (C-O stretching of ether), 960 (C-H bending of <i>trans</i> -alkene) cm^{-1}
1H NMR ($CDCl_3$, 400 MHz)	δ : 2.21 (6H, <i>s</i> , 3',5'- $\underline{CH_3}$), 3.68 (6H, <i>s</i> , 2',6'- $\underline{OCH_3}$), 3.73 (3H, <i>s</i> , 4'- $\underline{OCH_3}$), 7.04 (1H, <i>d</i> , $J = 16.1$ Hz, H_β), 7.32 (3H, <i>m</i> , H-3,4,5), 7.37 (1H, <i>d</i> , $J = 16.1$ Hz, H_α), 7.51 (2H, <i>m</i> , H-2,6)
^{13}C NMR ($CDCl_3$, 100 MHz)	δ : 9.2 (3',5'- $\underline{CH_3}$), 60.0 (4'- $\underline{OCH_3}$), 62.1 (2',6'- $\underline{OCH_3}$), 120.8 (C-3',5'), 124.8 (C-1'), 128.4 (C_α), 128.6 (C-2,6), 128.8 (C-3,5), 154.5 (C-2',6'), 195.1 (C=O), 130.5 (C-4), 134.6 (C-1), 145.6 (C_β), 159.2 (C-4')
HRMS (ESI) m/z	calcd for $C_{20}H_{22}O_4Na$ (M+Na) $^+$: 349.1416 found 349.1418



2'-hydroxy-4',6'-dimethoxy-3',5'-dimethylchalcone (142)

Molecular formular	$C_{19}H_{20}O_4$ (312 g/mol)
Physical characteristic	Yellow oil 0.0689 g R_f 0.58 (EtOAc : hexane; 20 : 80)
FTIR (thin film) ν_{max}	3481 (O-H stretching), 2926 (C-H stretching of CH_3), 1684 (C=O stretching of ketone), 1586 (C=C stretching), 1455 (C-H bending of CH_3), 1113 (C-O stretching of ether), 965 (C-H bending of <i>trans</i> -alkene) cm^{-1}
1H NMR ($CDCl_3$, 400 MHz)	δ : 2.17 (3H, <i>s</i> , 3'- $\underline{CH_3}$), 2.18 (3H, <i>s</i> , 5'- $\underline{CH_3}$), 3.66 (3H, <i>s</i> , 6'- $\underline{OCH_3}$), 3.75 (3H, <i>s</i> , 4'- $\underline{OCH_3}$), 7.41 (3H, <i>m</i> , H-3,4,5), 7.65 (2H, <i>m</i> , H-2,6), 7.86 (1H, <i>d</i> , $J = 15.7$ Hz, H_α), 7.97 (1H, <i>d</i> , $J = 15.7$ Hz, H_β), 13.09 (1H, <i>s</i> , 2'- \underline{OH})
^{13}C NMR ($CDCl_3$, 100 MHz)	δ : 8.7 (3'- $\underline{CH_3}$), 8.8 (5'- $\underline{CH_3}$), 60.1 (4'- $\underline{OCH_3}$), 62.3 (6'- $\underline{OCH_3}$), 111.9 (C-1'), 115.7 (C-3',5'), 125.8 (C_α), 128.5 (C-2,6), 129.0 (C-3,5), 130.4 (C-4), 135.2 (C-1), 143.4 (C_β), 158.7 (C-6'), 161.6 (C-2'), 163.6 (C-4'), 194.1 (C=O)
HRMS (ESI) m/z	calcd for $C_{19}H_{20}O_4Na$ ($M+Na$) $^+$: 335.1259 found 335.1259

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5,7-dimethoxy-6,8-dimethylflavanone (143)

Molecular formula	$C_{19}H_{20}O_4$ (312 g/mol)
Physical characteristic	White solid 0.0142 g M.p. 97.8–98.4 °C (from EtOAc/hexane) R_f 0.55 (EtOAc : hexane; 20 : 80)
FTIR (thin film) ν_{max}	2938 (C-H stretching of CH_2/CH_3), 1649 (C=O stretching of ketone), 1602 (C=C stretching), 1453 (C-H bending of CH_2/CH_3), 1108 (C-O stretching of ether) cm^{-1}
1H NMR ($CDCl_3$, 400 MHz)	δ : 2.16 (6H, <i>s</i> , 6,8- CH_3), 2.86 (1H, <i>dd</i> , $J = 16.6, 3.1$ Hz, H-3a), 2.98 (1H, <i>dd</i> , $J = 16.6, 13.1$ Hz, H-3b), 3.62 (3H, <i>s</i> , 7-O CH_3), 3.82 (3H, <i>s</i> , 5-O CH_3), 5.42 (1H, <i>dd</i> , $J = 13.1, 3.0$ Hz, H-2), 7.35–7.50 (5H, <i>brm</i> , H-2',3',4',5',6')
^{13}C NMR ($CDCl_3$, 100 MHz)	δ : 8.6 (6- CH_3), 9.1 (8- CH_3), 45.8 (C-3), 60.1 (7-O CH_3), 61.1 (5-O CH_3), 78.5 (C-2), 111.8 (C-8), 115.6 (C-10), 118.7 (C-6), 125.8 (C-3',5'), 128.4 (C-4'), 128.7 (C-2',6'), 139.1 (C-1'), 157.7 (C-9), 159.7 (C-5), 163.4 (C-7), 190.2 (C-4)
HRMS (ESI) m/z	calcd for $C_{19}H_{20}O_4Na$ (M+Na) $^+$: 335.1259 found 335.1262

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