

CHAPTER III

EXPERIMENTAL

1. Source of Plant Materials

1.1 *Michelia rajaniana* Craib

The stem bark of *Michelia rajaniana* Craib was collected from Doi Suthep-Doi Pui National Park, ChiangMai Province, Thailand, in July, 1985 and authenticated by comparison with herbarium specimens at Royal Forest Department, Ministry of Agriculture and Cooperatives, Thailand. A voucher specimen of plant material has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

1.2 *Grangea maderaspatana* Poir.

The whole parts of *Grangea maderaspatana* Poir. were collected from Suphan Buri Province, Thailand, in May, 1986. Authentication was achieved by comparison with herbarium specimens at Botany Section, Technical Division, Ministry of Agriculture and Co-operatives, Thailand. A voucher specimen of plant material has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

2. General Techniques

2.1 Thin-layer Chromatography (TLC)

Analytical

Technique : one way, ascending

Absorbent : silica gel GF₂₅₄ (E. Merck) 30 gm/60 ml of distilled water

Plate size : 5 x 20 cm, 10 x 20 cm, and 20 x 20 cm

Layer thickness

: 250 μ

Activation : air dried for 15 minutes and then at 110 ° C for 1 hour.

Solvent systems

- a) benzene : acetone (1:1)
- b) benzene : acetone (4:1)
- c) benzene : ethyl acetate (4:1)
- d) benzene : ethyl acetate (1:2)
- e) chloroform : acetone (5:1)
- f) ethyl acetate : acetone (1:1)
- g) benzene : acetone (1:1)
- h) benzene : acetone (4:1)
- i) benzene : ethyl acetate (4:1)
- j) benzene : ethyl acetate (1:2)
- k) chloroform : acetone (5:1)

Distance : 15 cm

Temperature : 24-30 °C

Detection on chromatographic plate

: a) Ultraviolet light at wavelength 254 and 366 nm

b) Chromogenic spray reagents

- Dragendorff's spray reagent

Solution A : bismuth subnitrate (850 mg), distilled water (40 ml) and acetic acid (10 ml)

Solution B : potassium iodide (8 gm) and distilled water (20 ml)

Solution A and B, each of 5 ml, were mixed. Then 20 ml of glacial acetic acid and 70 ml of distilled water were added and used as spray reagent.

The alkaloids give orange spots as positive test.

- Mixture of 2 % resorcinol in methanol and 2 % sulphuric acid (1:1). Plate after spraying, was warmed in hot air oven. The colours developed are indicated the presence of various types of were sesquiterpene lactone.

2.2 Column Chromatography (CC)

Adsorbent : silica 0.040-0.063 mm (E. Merck)

Packing of column

: dry packing

Sample loading

: the portion of crude extract was dissolved in a small amount of volatile solvent, mixed with small a quantity of adsorbent, air dried, triturated and added onto the top of a dry column.

Examination of eluate

: fractions were examined by thin-layer chromatography using the chromogenic spray reagents and UV-light. Those fractions of similar pattern were combined.

2.3 Physical Constants

Optical Rotation

: Optical rotations were determined by Bendix-NPL automatic polarimeter.

Melting Points

: Melting points were determined by Gallenkamp melting point apparatus.

2.4 Spectroscopy

Ultraviolet-visible (UV) Absorption Spectra

Ultraviolet-visible absorption spectra were obtained on a Varian DMS 90 spectrophotometer.

Infrared (IR) Absorption spectra

IR absorption spectra were obtained on a Perkin-Elmer Model 1330 or 180 spectrophotometer.

Nuclear Magnetic Resonance (NMR) Spectra

Proton (^1H) and Carbon-13 (^{13}C) NMR spectra were taken on a Bruker WH 400 spectrometer with TMS (=0) as internal standard and with solvents as indicated.

Mass Spectra (MS)

Mass spectra were recorded on a Varian MAT CH 7 or VG Micromass 7070 F spectrometer.

2.5 Authentic Samples

Liriodenine, parthenolide and bisparthenolfdine obtained from *Paramichelia baillonii* HU were kindly supplied by Mr. Arthorn Rivepiboon, graduate student of Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3. Extraction and Purification

3.1 *Michelia rajaniana* Craib.

The fresh bark of *M. rajaniana* Craib. (3 kg) was with blended with 95 % ethanol, macerated twice for 3 day-periods (10 L and 8 L) and filtered by suction. The combined filtrate was evaporated under reduced pressure to dryness. The residue was suspended in water (1 L) and extracted with chloroform (8 x 500 ml). The combined chloroform fractions, after drying (Na_2SO_4 anhydrous) and evaporation, yielded 12.5 gm of syrupy mass (crude MR).

The crude MR was divided into 12 equal portion and each one was treated in the same manner. Each portion was chromatographed on a silica gel column (2.5 x 15 cm) using benzene : ethyl acetate (1:1) as eluent. Twenty five millilitre of each fraction was collected and compared by TLC. Those fractions of similar pattern were combined and evaporated to dryness. Fraction 1-13, 17-39 and 41-53 after evaporation, afforded residue A, B and C respectively.

a) Residue A was rechromatographed on silica gel (2.5 x 15 cm) column using benzene : acetone 4 : 1 as eluent to furnish 138 mg (0.0046 %) of MR-1 and 79 mg (0.00263 %) of MR-3.

b) Residue B was rechromatographed on silica gel (2.5 x 15 cm) column using chloroform : acetone 5 : 1 to afford 84 mg (0.0028 %) of MR-4 and 80 mg (0.00266 %) of MR-6

c) Residue C was rechromatographed on silica gel (2.5 x 15 cm) column using chloroform to give 344 (0.0115 %) of MR-7 and 150 mg (0.005 %) of MR-8

3.2 Granagea maderaspatana Poir.

The dried powdered plant material (2 kg) was macerated with 95 % ethanol (2 x 5 L) for 3 day-periods and filtered by suction. The combined filtrate was evaporated under reduced pressure until dryness to give syrupy mass (250 gm): The residue was suspended in water (500 ml), extracted with chloroform (6 x 300 ml). The combined chloroform fraction, after drying (Na_2SO_4 anhydrous) and evaporation, yielded 45 gm of syrupy mass (crude GM).

The crude GM was divided into 15 equal portions and each one was treated in the same manner. Each portion was chromatographed on a silica gel column (5 X 15 cm) using chloroform : acetone 5 : 1 as eluent. One hundred millilitre of each fraction was collected, evaporated and compared by TLC. Those fractions of similar pattern were combined and evaporated to dryness.

a) fractions 3-4 were designated as GM-1 (24 mg, 0.0012 %)

b) fractions 7-11 were designated as GM-2 (115 mg, 0.00775 %)

c) fractions 21-28 were designated as GM-3 (117 mg, 0.00585 %)

4. Identification of the Isolated Compounds

The isolated compounds were identified by comparison of hRf values, melting points, optical rotation, ultra-violet absorption spectra, infrared absorption spectra, nuclear magnetic resonance spectra and mass spectra with authentic samples and data published previously.

4.1 Identification of MR-1

MR-1 was crystallized from methanol as white needle crystals. It is soluble in benzene, chloroform, ethylacetate and acetone.

hRf Value

The hRf values given are obtained from the following systems :-

- a) benzene : acetone (1:1) = 85
- b) benzene : acetone (4:1) = 67
- c) benzene : ethyl acetate (4:1) = 62
- d) benzene : ethyl acetate (1:2) = 70
- e) chloroform : acetone (5:1) = 78

The thin-layer chromatograms of MR-1 are shown in Figures 3-7 (pp. 102-106)

Optical Rotation (in CHCl_3)

$$(\alpha)_D^{20} = -78^\circ$$

Melting Point

112-115° C

Molecular Weight

248

Infrared Absorption Spectrum (in CCl_4) (Figure 14, p. 113)

ν_{max} (cm^{-1})

3020, 2920, 1770, 1650, 1281, 1260, 1130 and 940

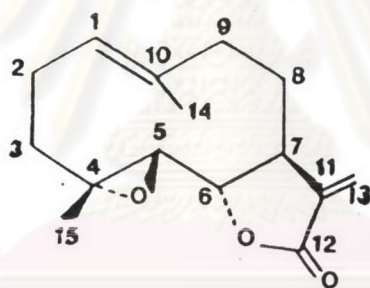
Proton NMR Spectrum (in CDCl_3 , 400 MHz) (Table 3, p. 67 and Figure 15, p. 114).

Mass Spectrum (EIMS)

m/z (% , relative intensity) (Figure 16, p. 115)

248 (M^+ , 2) , 230 (9), 191 (25), 190 (61), 177 (16.4),
175 (34.4), 155 (65), 119 (100), 91 (87.7), 67 (73.7),
43 (100) and 41 (77.2)

From the above data, MR-1 was in complete agreement with the structure of (-)-parthenolide (77). It is therefore concluded that MR-1 is (-)-parthenolide, the structure of which is shown below.



(-)- parthenolide

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4.2 Identification of MR-3

MR-3 was crystallized from ethyl acetate as white amorphous solid. It is soluble in benzene, chloroform, ethyl acetate and acetone.

hRf Value

The hRf values given obtained from the following systems :-

- a) benzene : acetone (1:1) = 76
- b) benzene : acetone (4:1) = 54
- c) benzene : ethyl acetate (4:1) = 51
- d) benzene : ethyl acetate (1:2) = 56
- e) chloroform : acetone (5:1) = 61

The thin-layer chromatograms of MR-3 are shown in Figure 3-7 (pp. 102-106)

Optical Rotation (in CHCl_3)

$$(\alpha)_D^{20} = -112^\circ$$

Melting Point

100-102° C

Molecular weight

513

Infrared Absorption Spectrum (in CCl_4) (Figure 17, p. 116)

ν_{max} (cm^{-1})

3365, 3020, 2920, 1770, 1450, 1215, 1175, 1000
and 940

Proton NMR Spectrum (in CDCl_3 , 400 MHz) (Table 3, p. 67 and Figure 18, p. 117)

Carbon-13 NMR Spectrum (in CDCl_3 , 100 MHz) (Table 4, p. 68 and Figure 19, p. 118)

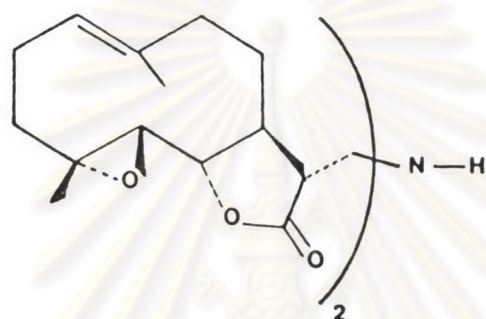
Mass Spectrum (EIMS)

m/z (%, relative intensity) (Figure 20, p. 119)
513 (M^+ , 1.74), 495 (5.9), 278 (100), 264 (13.61),
260 (15), 231 (12), 220 (19) and 43 (51)

HRMS (composition interpreted, calculated millimass)

513.3071 ($\text{C}_{30}\text{H}_{43}\text{NO}_6$, M^+ , 513.3090)
278.1752 ($\text{C}_{16}\text{H}_{24}\text{NO}_3$, $\text{M}-\text{C}_{14}\text{H}_{19}\text{O}_3$, 278.1756)
264.1600 ($\text{C}_{16}\text{H}_{22}\text{NO}_3$, $\text{M}-\text{C}_{15}\text{H}_{21}\text{O}_3$, 264.1594)

From the above data, MR-3 was in complete agreement with the structure of (-)-bisparthenolidine (77). It is therefore concluded the MR-3 is (-)-bisparthenolidine, the structure of which is shown below.



(-)-bisparthenolidine

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4.3 Identification of MR-4

MR-4 was crystallized from ethyl acetate as white needle crystals. It is soluble in chloroform, benzene and acetone.

hRf Value

The hRf values given are obtained with the following systems :-

- a) silica gel GF₂₅₄/benzene : acetone (1:1) = 73
- b) silica gel GF₂₅₄/benzene : acetone (4:1) = 45
- c) silica gel GF₂₅₄/benzene : ethyl acetate(4:1) = 40
- d) silica gel GF₂₅₄/benzene : ethyl acetate(1:2) = 51
- e) silica gel GF₂₅₄/chloroform : acetone (5:1) = 54

The thin-layer chromatograms of MR-4 shown in Figure 3-7 (pp. 102-106)

Molecular Weight

324

Infrared Absorption Spectrum (in CHCl₃)

ν_{\max} (cm⁻¹)

3620-3240 (br), 2932, 1777, 1734, 1262, 1099, 1069, 1020 and 1006.9

(Figure 21, p. 120)

Proton NMR Spectrum (in CDCl_3 , 400 MHz) (Figure 22, p. 121)

Proton	
1	5.30 (d, 10.2) ^a
2 _β	4.70 (ddd, 10.3, 6.1)
3 _α	1.23 (dd, 11.4)
3 _β	2.55-2.62 (dd)
5	2.73 (d, 8.8)
6	3.95 (dd, 8.8)
7	2.34-2.43 (ddd, 8.8, 12.1)
8 _β	4.92 (ddd, 8.2, 3.6)
9 _α , 9 _β	2.43-2.45 (m)
11	2.55-2.62 (dq, 6.7)
13	1.45 (d, 6.5)
14	1.87 (s)
15	1.30 (s)
2'	2.12 (s)

^aChemical shifts are in ppm from TMS, multiplicity and coupling constants are in parenthesis in Hertz.

Carbon-13 NMR Spectrum (in CDCl_3 , 100 MHz) (Table 4, p. 68, Figure 23, p. 122)

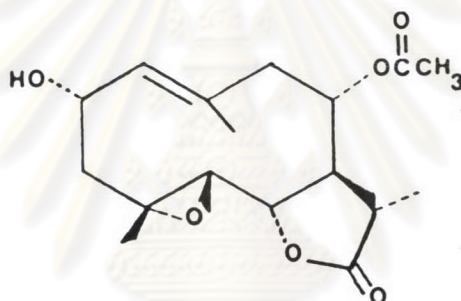
Mass Spectrum (EIMS)

m/z (% , relative intensity) (Figure 24, p. 123)

264 (6.2), 137 (12), 118 (17), 95 (50), 43 (100) and 29 (22)

From EIMS data, it does not show parent peak (324) but shows fragmentation peak ($324-60=264$) which loss hydroxyl and acetyl groups. These groups show in IR spectrum ^1H - and ^{13}C -NMR spectrum.

The results were concluded for the structure of MR-4 as a novel sesquiterpene lactone and the chemical structure was assigned as $\text{C}_{17}\text{H}_{23}\text{O}_7$, 8α -acetoxy- 2α -hydroxy-dihydroparthenolide and named paramicholide, the structure of which is shown below.



paramicholide

4.4 Identification of MR-6

MR-6 was crystallized from ethyl acetate as white needle crystals. It is soluble in chloroform, ethyl acetate and acetone.

hRf Value

The hRf values given are obtained from the following systems :-

- a) benzene : acetone (1:1) = 60
- b) benzene : acetone (4:1) = 30
- c) benzene : ethyl acetate (4:1) = 25

d) benzene : ethyl acetate (1:2) = 34.6

e) chloroform : acetone (5:1) = 33

The thin-layer chromatograms of MR-6 are shown in Figure 3-7 (pp. 102-106) .

Molecular Weight

307

Infrared Absorption Spectrum (in CHCl₃) (Figure 25, p. 124)

ν_{\max} (cm⁻¹)

3020, 2955.8, 2928.05, 2855.26, 1769.79, 1670.26,
908.55 and 864.17

Proton NMR Spectrum (in CDCl₃, 400 MHz) (Figure 26, p. 125)

Proton

1 5.17 (dd, br, 9.7, 1.5)

2_α 2.35-2.45 (m)

2_β 2.35-2.45 (m)

3_α 1.21 (ddd, 13.0, 6.0)

3_β 2.05-2.15 (m)

5 2.70 (d, 8.9)

6 3.87 (dd, 8.9)

7 2.4-2.45 (m)

8_α 2.35-2.45 (m)

8_β 2.05-2.15 (m)

9_{α}	2.05-2.15 (m)
9_{β}	2.2-2.3 (m)
11	2.4-2.45 (m)
13_a	3.64-3.69 (ddd)
13_b	3.50-3.56 (ddd)
14	1.69 (s)
15	1.30 (s)
2	2.02 (s)
N-H	6.31 (br.)

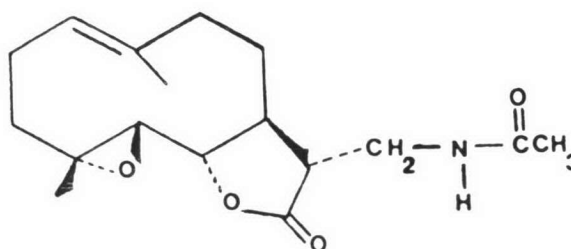
Carbon-13 NMR Spectrum (in CDCl_3 , 100 MHz) (Table 4, p. 68 ,
Figure 27 , p 126)

Mass Spectrum (EIMS)

m/z (% , relative intensity) (Figure 28, p.127)

307 (M^+ , 26), 235 (39), 230 (35), 218 (43)
217 (40.5), 208 (64.6), 205 (31), 191 (35)
190 (63), 117 (83), 131 (100), 95 (72)
72 (100), 43 (100)

The results were concluded for the structure of MR-6 as a novel sesquiterpene lactone and chemical structure was assigned as $\text{C}_{17}\text{H}_{25}\text{NO}_4$ and named N-acetylparthenolidine, the structure of which is shown below.



N-acetylparthenolidine

4.5 Identification of MR-7

MR-7 was crystallized from ethyl acetate as white needle crystals. It is soluble in acetone and alcohol.

hRf Value

The hRf values given are obtained from the following systems :-

- a) benzene : acetone (1:1) = 52
- b) benzene : acetone (4:1) = 26
- c) benzene : ethyl acetate (4:1) = 20
- d) benzene : ethyl acetate (1:2) = 28
- e) chloroform : acetone (5:1) = 30

The thin-layer chromatograms of MR-7 are shown in Figure 3-7 (pp. 102-106)

Molecular Weight

323

Infrared Absorption Spectrum (in CDCl_3) (Figure 29 p. 128)

ν_{max} (cm^{-1})

2932.47, 1768.28, 1656.14, 1079.48, 979.64,

and 908.47

Proton NMR Spectrum (in CDCl_3 , 400 MHz) (Figure 29, p. 129)

Proton	
1	5.2 (dd)
2 _α	2.2-2.4 (m)
2 _β	2.2-2.4 (m)
3 _α	1.25 (m)
3 _β	2.05-2.15 (m)
5	2.7 (d)
6	3.92 (dd)
7	2.65 (ddd)
8	4.1 (ddd)
9 _α	2.35-2.45 (m)
9 _β	2.35-2.45 (m)
11	3.57 (ddd)
13 _a	3.0 (ddd)
13 _b	3.77 (ddd)
14	1.75 (s)
15	1.3 (s)
2	2.1 (s)
N-M	6.95 (dd)
O-H	4.95 (d)

Carbon-13 NMR Spectrum (in CDCl_3 , 100 MHz) (Table 4, p. 68, Figure 31,

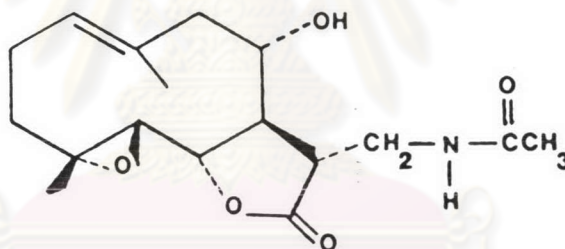
p.130)

Mass Spectrum (EIMS)

m/z (% , relative intensity) (Figure 32, p.131)

256 (100), 241 (19.5), 228 (16), 216 (12.3),
 214 (39), 212 (16.3), 196 (16), 188 (16.3),
 184 (31), 179 (37.8), 176 (13.5), 175 (13.5),
 156 (63.6), 68 (100), 43 (100)

The results were concluded for the structure of MR-7 as a novel sesquiterpene lactone and chemical structure was assigned as $C_{17}H_{25}NO_5$ and named N-acetyl-8 α -hydroxyparthenolidine, the structure of which is shown below.



N-acetyl-8 α -hydroxyparthenolidine

4.6 Identification of MR-8

MR-8 was crystallized from chloroform as yellow needle crystals. It is soluble in chloroform, acetone, ethyl acetate and alcohol. It gives orange colour with the dragendorff's reagent. This reaction is indicated that MR-8 might be alkaloid.

hRf Value

The hRf values given are obtained with the following systems :-

- a) benzene : acetone (1:1) = 43
 b) benzene : acetone (4:1) = 18
 c) benzene : ethyl acetate (4:1) = 14
 d) benzene : ethyl acetate (1:2) = 15
 e) chloroform : acetone (5:1) = 20

The thin layer chromatograms of MR-8 are shown in Figure 3-7 (pp.102-106)

Melting Point

278-281 °C

Ultraviolet-Visible Absorption Spectra (Figure 33 and 34, pp.132-133)

λ_{\max} (nm) (in 95 % ethanol)

250, 270, 310, 400 (SH) and 416 nm

λ_{\max} (nm) (in 0.1 N HCl in ethanol)

260, 282, 320, 396 and 452

Infrared Absorption Spectrum (in CH_2Cl_2) (Figure 35 p. 134)

ν_{\max} (cm^{-1})

3040, 2920, 1655, 1590, 1480, 1462, 1438, 1410, 1300,
 1220, 1200, 1050, 1010, 965, 890 and 865

Proton NMR Spectrum (Figure 36, p.135)

Proton	10 % DMSO-d ₆ :CDCl ₃	CDCl ₃
3	7.21 (s)	7.17 (s)
4	7.83 (br s)	7.74 (br s)
5	8.90 (br s)	8.80 (br s)
8	8.57 (d, <i>J</i> =8.0 Hz)	8.58 (d, <i>J</i> =8.1)
9	7.58 (t, <i>J</i> =8.0 Hz)	7.57 (t, <i>J</i> =8.0)
10	7.77 (t, <i>J</i> =8.0 Hz)	7.73 (t, <i>J</i> =8.0)
11	8.72 (d, <i>J</i> =8.0 Hz)	8.61 (d, <i>J</i> =8.1)
OCH ₂ O	6.41 (s)	6.37 (s)

Mass Spectrum (EIMS)

m/z (% , relative intensity) (Figure 36, p. 136)

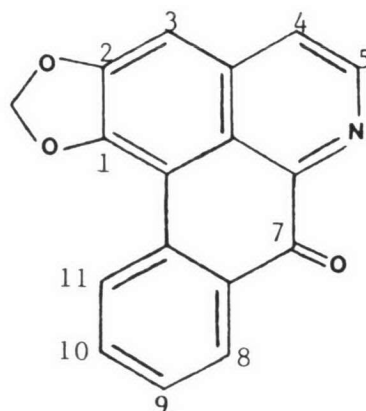
275 (M⁺, 80), 247 (14), 246 (10), 224 (62.9)

178 (19.5), 149 (36.8), 143 (66.5), 125 (20.6),

123 (22.8)

111 (30.9), 99 (100), 98 (70), 97 (48) and 49 (100)

This data are in agreement with the published values of liriiodenine (77). It is therefore concluded that MR-8 is liriiodenine, the structure of which is shown below.



liriiodenine

Table 3 $^1\text{H-NMR}$ Spectra of MR-1 and MR-3^a

Proton	MR-1 ^c	MR-3
1	5.21 (dd, br, 4.0, 12.2)	5.27 (dd, 2.2, 9.8)
2 _{α} ^b	2.38 (dd, 5.1, 13.1)	2.26 (dd, 6.0, 12.1)
2 _{β}	2.46 (ddd, 13.8, 12.2, 12.5)	2.40 (m)
3 _{α}	1.25 (m)	1.23 (dt, 5.9, 13.9)
3 _{β} ^b	2.17 (m)	1.88 (dd, 5.9, 14.6)
5	2.79 (d, 8.8)	2.74 (d, 8.8)
6	3.86 (dd, 8.8, 8.3)	3.86 (dd, 8.8)
7	2.78 (m)	2.40 (m)
8 _{α} ^b	1.72 (m)	2.18 (m)
8 _{β}	1.73 (m)	1.70 (m)
9 _{α}	2.09–2.24 (m)	2.10–2.18 (m)
9 _{β}	2.38 (m)	2.10–2.18 (m)
11 _{β}	-	2.40 (m)
13 _{a}	6.33 (d, 3.6)	3.15 (dd, 2.8, 13.1)
13 _{b}	5.62 (d, 3.1)	2.92 (dd, 2.8, 13.1)
14	1.72 (s)	1.67 (s)
15	1.31 (s)	1.30 (s)

^aChemical shifts are in ppm from TMS, multiplicity, coupling constants (Hz) are in parenthesis and the samples were dissolved in CDCl_3 .

^bpreviously assigned 200 MHz spectrum from Badesinsky *et al.*
 $\text{H}_{2\alpha}$, 2.09–2.24 (m); $\text{H}_{3\beta}$, 2.09–2.24 (m) and $\text{H}_{8\alpha}$, 2.09–2.24 (m) (78).

^cSpecific assignments possible at 400 MHz with 2D-COSY and decoupling experiments.

Table 4 ^{13}C -NMR Spectra of MR-1, MR-3, MR-4, MR-6 and MR-7 ^a

carbon	MR-1 ^c	MR-3	MR-4	MR-6	MR-7
1	125.3(-)	125.3(-)	131.0(-)	125.1 (+)	127.4(-)
2	24.2 ^b (+)	24.2(+) ^b	66.6(-)	24.1(+)	24.4(+)
3	36.2 ^b (+)	36.5(+) ^b	45.1(+)	63.3(+)	35.6(+)
4	61.5(+)	61.6(+)	60.6(+)	61.7(+)	61.9(+)
5	66.4(-)	66.1(-)	66.6(-)	62.2(-)	66.0(+)
6	82.5(-)	82.3(-)	78.6(-)	82.9(-)	78.9(-)
7	47.7(-)	49.0(-)	55.4(-)	48.4(-)	51.5(-)
8	41.2 ^b (+)	30.2(+) ^b	72.1(-)	29.8(+)	72.3(-)
9	30.2 ^b (+)	40.9(+)	49.3(+)	40.9(+)	39.6(+)
10	134.7(+)	134.3(+)	131.0(+)	134.6(+)	130.3(+)
11	139.5(-)	45.5(-)	39.6(-)	46.6(-)	46.8(-)
12	169.3(+)	176.7(+)	176.8(+)	176.6(+)	177.0(+)
13	121.0(+)	46.2(+)	18.3(-)	36.6(+)	52.4(+)
14	17.3(-)	17.2(-)	8.2(-)	17.2(-)	17.5(-)
15	17.0(-)	16.8(-)	17.6(-)	16.7(-)	17.3(-)
1'	-	-	169.8(+)	170.7(+)	173.2(+)
2'	-	-	21.0(-)	23.2(-)	22.9(-)

^a Chemical shifts are in ppm from TMS, solvent was CDCl_3 , (+) and(-) are signs from the attached proton test (APT).

^b Assignments may be interchanged.

^c Data taken from El-Ferally *et al.* (79).

4.7 Identification of GM-1

GM-1 was crystallized from n-hexane as rod crystals. It is soluble in chloroform, ethyl acetate and acetone.

hRf Value

The hRf values given are obtained from the following systems :-

- f) ethyl acetate : acetone (1:1) = 80
- g) benzene : acetone (1:1) = 70
- h) benzene : acetone (4:1) = 60
- i) benzene : ethyl acetate (4:1) = 52
- j) benzene : ethyl acetate (1:2) = 75
- k) chloroform : acetone (5:1) = 80

The thin layer chromatograms of GM-1 are shown in Figure 8-13, pp. 107-112.

Molecular Weight

232

Infrared Absorption Spectrum (in CCl_4) (Figure 38, p. 137)

ν_{max} (cm⁻¹)

3020, 2940, 1767, 1264, 1142.9 and 940

Proton NMR Spectrum (in CDCl_3 , 400 MHz) (Figure 39, p. 138)

Proton

1 _{α}	}	0.9-1.9 (m) ^a
1 _{β}		
2 _{α}		
2 _{β}		
3 _{α}		2.08
3 _{β}		2.1
6		5.26 (d, 5.9)
7		2.95 (m)
8 _{α}	}	0.9-1.9 (m) ^a
8 _{β}		
9 _{α}		
9 _{β}		
13 _a		6.14 (d, 1)
13 _b		5.57 (d, 1)
14		1.70 (s)
15		1.08 (s)

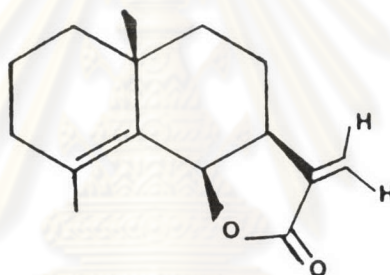
^aNo specific assignments with two dimension and decoupling experiments.

Carbon-13 NMR Spectrum (in CDCl_3 , 100 MHz) (Table 5, p. 77, Figure 40, p. 139)

Mass Spectrum (EIMS)

m/z (%, relative intensity) (Figure 41, p.140)
 232 (M^+ , 15.6), 218 (15.5), 217 (100), 161 (10.6)
 146 (16.6), 119 (9), 105 (14), 93 (12)
 91 (19), 81 (12), 79 (13), 55 (13) and 40 (18)

From the above data, GM-1 was in complete agreement with structure of frullanolide (43, 81, 82). It is therefore concluded that GM-1 is frullanolide, the structure of which is shown below.



frullanolide

4.8 Identification of GM-2

GM-2 was crystallized from benzene : ethyl acetate (4:1) as white needle crystals. It is soluble in chloroform, ethyl acetate and acetone.

hRf Value

The hRf values given are obtained from the following systems :-

f) ethyl acetate : acetone (1:1) = 68

g) benzene : acetone (1:1) = 60

h) benzene : acetone (4:1) = 42

1) benzene : ethyl acetate (4:1) = 39

j) benzene : ethyl acetate (1:2) = 61

k) chloroform : acetone (5:1) = 71

The thin layer chromatograms of GM-2 are shown in Figure 8-13. pp. 107-112

Melting Point

69.5-70.0

Molecular Weight

248

Infrared Absorption Spectrum (in CCl₄)

ν_{\max} (cm⁻¹)

3020, 3010, 2933, 1771.8, 1751.5, 1142.4 and 956

(Figure 42, p. 141)

Proton NMR Spectrum (in CDCl₃, 400 MHz) (Figure 43, p. 142)

Proton

1
α

1
β

2
α

2
β

3
α

3
β

6

1.35-2.2(m)^a

5.0 (s)

Proton	
8 _α	} 1.35-2.2 (m) ^a
8 _β	
9 _α	
9 _β	
13 _a	6.27 (d)
13 _b	5.81 (s)
14	1.01 (s)
15	1.78 (s)

^a No specific assignments with two dimension and decoupling experiments.

Carbon-13 NMR Spectrum (in acetone-d₆, 100 Hz) (Table 5, p.77, Figure 44, p. 143)

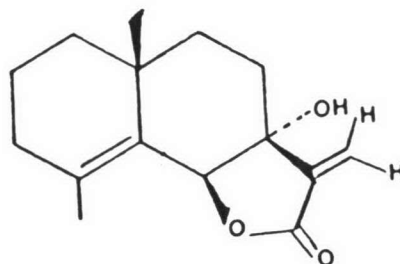
Mass Spectrum (EIMS)

m/z (% , relative intensity) (Figure 45, p.144)

248 (M⁺, 18), 233 (100), 215 (29.7), 187 (25)

178 (17) and 169 (30.5)

The results were concluded for the structure of GM-2 as a novel sesquiterpene lactone and the chemical structure was assigned as C₁₅H₂₀O₃ and named 7_α-hydroxyfrullanolide, the structure of which is shown below.



7 α -hydroxyfrullanolide

4.9 Identification of GM-3

GM-3 was crystallized from n-hexane : acetone (1:1) as needle crystals. It is soluble in chloroform, ethyl acetate and acetone,

hRf Value

The hRf values given are obtained from the following systems :
systems :-

- f) ethyl acetate : acetone (1:1) = 40
- g) benzene : acetone (1:1) = 36.6
- h) benzene : acetone (4:1) = 25
- i) benzene : ethyl acetate (4:1) = 20
- j) benzene : ethyl acetate (1:2) = 34
- k) chloroform : acetone (5:1) = 42

The thin layer chromatograms of GM-3 are shown in Figure 8-13, pp. 107-112.

Melting Point

135.0-139.0

Molecular Weight

266

Infrared Absorption Spectrum (in CHCl_3) (Figure 46, p. 145)

ν_{max} (cm⁻¹)

3600-3500, 2920, 1770.9, 1006.5 and 971

Proton NMR Spectrum (in CDCl_3 , 100 MHz) (Figure 47, p. 146)

Proton	
1_α	} 1.3-2.1 (m) ^a
1_β	
2_α	
2_β	
3	4.0 (dd)
6	4.89 (s)
8_α	} 1.3-2.1 (m) ^a
8_β	
9_α	
9_β	
11	2.8 (q, 7.2)
13	1.21 (d, 7.2)
14	1.04 (s)
15	1.96 (s)
O-H	2.6 (br)

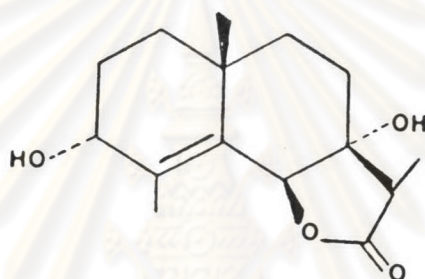
^a No specific assignments with two dimension and decoupling experiments.

Carbon-13 NMR Spectrum (in CDCl_3 , 100 MHz) (Table 5, p. 77, Figure 48, p. 147)

Mass Spectrum (EIMS)

m/z (%, relative intensity) (Figure 49, p. 148)
 266 (M^+ , 52), 251 (25), 248 (61.7), 215 (40.3),
 204 (36), 192 (33), 187 (47), 177 (37), 123 (100)

The results were concluded for the structure of GM-3 as a novel sesquiterpene lactone and the chemical structure was assigned as $C_{15}H_{22}O_4$ and named 7α , 3α -dihydroxy dihydrofrullanolide, the structure of which is shown below.



7α , 3α -dihydroxy dihydrofrullanolide

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Table 5 Carbon-13 Spectra of GM-1, GM-2 and GM-3

Carbon	GM-1	GM-2	GM-3
1	18.2 (+)	18.2 (+)	24.9 (+)
2	33.1 (+)	33.1 (+)	33.8 (+)
3	39.1 (+)	38.8 (+)	69.6 (-)
4	138.5 (+)	140.5 (+)	140.5 (+)
5	128.5 (+)	126.8 (+)	131.5 (+)
6	75.9 (-)	81.4 (-)	79.9 (-)
7	41.2 (-)	76.0 (+)	77.4 (+)
8	25.0 (+)	31.5 (+)	27.4 (+)
9	37.9 (+)	34.9 (+)	34.7 (+)
10	32.6 (+)	32.7 (+)	32.7 (+)
11	142.3 (+)	144.7 (+)	47.7 (-)
12	170.9 (+)	169.1 (+)	177.0 (+)
13	120.1 (+)	121 (+)	7.2 (-)
14	19.3 (-)	19.4 (-)	17.9 (-)
15	25.8 (-)	26.1 (-)	24.0 (-)

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