



CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The roots of *Murraya siamensis* Craib were obtained from Khao Luang, Sukhothai Province, Thailand, during October, 1988. The plant material was authenticated by comparison with herbaria at Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. The voucher specimen of plant material has been deposited at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

2. General Techniques

2.1 Thin layer chromatography (TLC)

2.1.1 Analytical TLC

Technique : One way, ascending

Adsorbent : Aluminium sheet silica gel 60 F₂₅₄ (E. Merck)
precoated plate

Plate size : 5 x 8 cm

Layer thickness : 0.2 mm

Solvent systems :

- a) Chloroform
- b) Chloroform : benzene (5:5)
- c) Chloroform : hexane (5:5)
- d) Chloroform : petroleum ether (5:5)
- e) Chloroform : petroleum ether (7:3)

Distance : 6 cm

Temperature : 25-30°C

Detections :

a) Ultraviolet light. The compounds which contain unsaturated bonds become visible as quenching spots (254 nm) and coumarins as fluorescent blue to green colored bands (365 nm) on TLC plate.

b) Chromogenic agents

i) Dragendorff's reagent. Alkaloids and other nitrogenous compounds exhibit orange color after spraying.

ii) Iodine vapour. The unsaturated organic compounds display yellow-brown spots.

iii) 10 % Sulfuric acid in methanol. Various organic compounds yielded characteristic colors after heating on a hot plate.

2.1.2 Preparative TLC

Technique : One way, ascending

Adsorbent : Silica gel 60 F₂₅₄ (E. Merck)

Plate size : 20 x 20 cm

Layer thickness : 2 mm

Solvent systems :

- a) Chloroform : hexane : ethyl acetate (5:3.5:1.5)
- b) Ethyl acetate : petroleum ether (8:2)

Distance : 15 cm

Temperature : 25-30°C

Detection :

Ultraviolet light. The compounds which contain unsaturated bonds become visible as quenching bands (254 nm) and coumarins as fluorescent blue to green colored bands (365 nm) on TLC plate.

2.2 Column chromatography

Column size : 2.5 x 15 cm, 4.0 x 7 cm,

4.0 x 15 cm, 5.0 x 15 cm

Adsorbent : Silica gel 0.040-0.063 mm (E. Merck)

Packing of column : Dry packing, then soaking with eluent.

Sample loading :

A portion of crude extract was dissolved in a small amount of organic solvent and then, added slowly onto the top of a dry column.

Solvents :

- a) Chloroform
- b) 10-80 % Chloroform in petroleum ether
- c) 95-98 % Chloroform in ethyl acetate
- d) Ethyl acetate : petroleum ether (1:1)

Temperature : 25-30°C

2.3 Physical constant

Melting point. Melting point of the isolated compounds were determined by Gallenkamp Melting Point Apparatus.

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) absorption spectra. The ultraviolet absorption spectra were obtained on a Perkin Elmer Lambda 3 UV/VIS spectrophotometry in 95 % ethanol with and without 0.1 M NaOH solution.

2.4.2 Infrared (IR) absorption spectra. The infrared absorption spectra were obtained on a Nicolet Model 20 SX/C FTIR spectrometer.

2.4.3 Nuclear magnetic resonance (NMR) spectra. The proton (^1H) and carbon-13 (^{13}C) NMR spectra were taken on a Bruker WH-400 Spectrometer with tetramethylsilane (TMS) and acetone- d_6 as internal standards respectively. The multiplicities for ^{13}C NMR spectra were determined by the attached proton test which produced positive (+) quaternary C and CH_2 signals and negative (-) CH and CH_3 signals.

2.4.4 Mass spectra. The mass spectra were obtained on a VG Micromass 7070 F or a ZAB-E Spectrometer.

3. Extraction and Isolation.

3.1 Extraction

The dried chipped roots of *Murraya siamensis* Craib (1 kilogram) were extracted thoroughly by maceration twice, for two-day period, with 95 % ethanol (6 and 5 liters). After combination, the extracts were evaporated under reduced pressure to yield 21.5 grams of syrupy mass. The residue was suspended in water (2 liters), followed by extraction with chloroform (4.5 liters). The combined extracts were dried with anhydrous sodium sulfate and evaporated under reduced pressure to give a crude chloroform extract (9.3 grams).

3.2 Isolation

The crude chloroform extract (9.3 grams) was divided into four equal portions. Each portion was purified by silica gel flash chromatography using chloroform as eluent. Twenty-five milliliters fractions were collected and examined by thin layer chromatography (TLC). Fractions containing homogeneous pattern were combined to furnish as following:-

Fractions 2-3 afforded a residue A

Fractions 5-7 afforded a residue B

Fractions 9 afforded a residue C

Fractions 11-23 afforded a residue D

Fractions 26-32 afforded a residue E

Residue A, B, C and E were purified by rechromatographed by using chloroform (A, B), 10-80 % chloroform in petroleum ether (C) and 95-98 % chloroform in ethyl acetate (E) as eluents respectively.

The purified chemical substances from four residues were designated as follows:-

Compound isolated from residue A was designated as MS-1 (230 mg)

Compound isolated from residue B was designated as MS-2 (120 mg)

Compound isolated from residue C was designated as MS-3 (24 mg)

Compound isolated from residue E was designated as MS-4 (16 mg)

Residue D was further purified by silica gel preparative TLC using chloroform : hexane : ethyl acetate (5:3.5:1.5) and ethyl acetate : petroleum ether (8:2) to yield 143 mg of MS-5, 28 mg of MS-6 and residue F. Purification of residue F by silica gel flash chromatography using ethyl acetate : petroleum ether (1:1) gave 22 mg of MS-7 and 21 mg of MS-8.

4. Characterization of the Isolated Compounds

The isolated compounds were characterized by the data of R_f values, melting points, ultraviolet absorption spectra, infrared absorption spectra, nuclear magnetic resonance spectra, mass spectra and compared with previously published data of known chemical compounds.

4.1 Characterization of MS-1

MS-1 was obtained as yellow needles

hRf value (figure 8-12 p. 160-164)

88 on TLC solvent system a
92 on TLC solvent system b
79 on TLC solvent system c
81 on TLC solvent system d
95 on TLC solvent system e

Melting point

169-171 °C

Molecular weight

279 (Mass spectrometry)

IR spectrum (in CCl₄) (figure 13 p. 165)

$\bar{\nu}$ max (cm⁻¹)

3468, 2966, 2926, 2849, 2731, 1637, 1606, 1496, 1340

^1H NMR spectrum (figure 14-15 p. 166-167)

Proton	Chemical shift (ppm)(δ)		
	Acetone- d_6	CDCl_3	Benzene- d_6
4	8.22 (s)	8.04	7.40
5	8.01 (d,7.6)	7.97	7.80
6	7.20 (t,7.6)	7.27	7.21
7	7.35 (t,7.6)	7.40	7.27
8	7.48 (d,7.6)	7.40	6.90
9	3.61 (d,6.8)	3.64	3.53
10	5.34 (br.t,6.8)	5.32	5.22
12	1.64 (d,1.0)	1.77	1.57
13	1.81 (s)	1.90	1.69
CHO	9.91 (s)	9.91	9.47
NH	10.62 (br.s)	8.20	7.48
OH	11.75 (s)	11.70	12.35

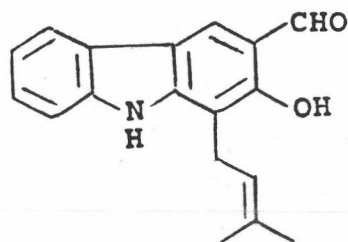
 ^{13}C NMR spectrum (in acetone- d_6) (figure 16 p. 168)

<u>Carbon</u>	<u>Chemical shift (ppm)(δ)</u>
1a	145.3 (+)
1	109.7 (+)
2	158.0 (+)
3	115.7 (+)
4	126.0 (-)
4a	117.7 (+)
5a	124.1 (+)
5	120.7 (-)
6	120.0 (-)
7	125.9 (-)
8	111.6 (-)
8a	141.5 (+)
3-CHO	196.3 (-)
9	23.0 (+)
10	121.7 (-)
11	132.7 (+)
12	17.6 (-)
13	25.3 (-)

Mass spectrum (EIMS) (figure 17 p. 169) m/z (% relative intensity)

279 (M^+ , 100), 264 (29.5), 248 (10.6), 236 (21.1),
 224 (97.2), 195 (9.6), 167 (18.3), 125 (4.6)

Therefore it is concluded that MS-1 is heptaphylline (26), the structure of which is shown below.



Heptaphylline
(MS-1)

4.2 Characterization of MS-2

MS-2 was obtained as yellow crystals.

hRf value (figure 8-12 p. 160-164)

79 on TLC solvent system a
85 on TLC solvent system b
71 on TLC solvent system c
71 on TLC solvent system d
89 on TLC solvent system e

Melting point

151-152°C

Molecular weight

309 (Mass spectrometry)

IR spectrum (in CCl₄) (figure 18 p. 170)

$\bar{\nu}$ max (cm⁻¹)

3420, 2958, 2925, 2848, 1641, 1618, 1316, 1154

^1H NMR spectrum (figure 19 p. 171)

Proton	Chemical shift (ppm)(δ)		
	Acetone- d_6	CDCl_3	Benzene- d_6
4	8.12 (s)	7.94	7.34
5	7.88 (d, 8.7)	7.84	7.66
6	6.82 (dd, 8.7, 2.2)	6.87	6.93
8	7.01 (d, 2.2)	6.91	6.44
9	3.59 (d, 6.9)	3.63	3.57
10	5.32 (br. t, 6.9)	5.32	5.26
12	1.64 (d, 1.0)	1.78	1.58
13	1.81 (s)	1.90	1.71
CHO	9.90 (s)	9.90	9.52
NH	10.51 (br. s)	8.10	7.39
OH	11.72 (s)	11.60	12.38
OCH_3	3.84 (s)	3.90	3.46

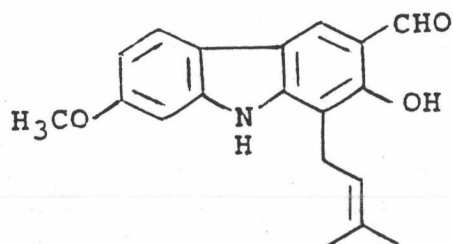
 ^{13}C NMR spectrum (in acetone- d_6) (figure 20 p. 172)

Carbon	Chemical shift (ppm)(δ)
1a	145.5 (+)
1	109.6 (+)
2	157.5 (+)
3	115.6 (+)
4	124.5 (-)
4a	117.9 (+)
5a	117.5 (+)
5	120.7 (-)
6	109.0 (-)
7	159.5 (+)
8	95.9 (-)
8a	142.9 (+)
3-CHO	196.3 (-)
7- OCH_3	55.3 (-)
9	22.9 (+)
10	121.8 (-)
11	132.6 (+)
12	17.6 (-)
13	25.3 (-)

Mass spectrum (EIMS) (figure 21 p. 173) m/z (% relative intensity)

309 (M^+ , 100), 294 (12.1), 266 (8.4), 254 (61),
238 (8.7), 210 (9.6)

Therefore it is concluded that MS-2 is 7-methoxyheptaphylline (28), the structure of which is shown below.



7-Methoxyheptaphylline

(MS-2)

4.3 Characterization of MS-3

MS-3 was obtained as yellow crystals.

hRf value (figure 8-12 p. 160-164)

24 on TLC solvent system a
 41 on TLC solvent system b
 27 on TLC solvent system c
 29 on TLC solvent system d
 67 on TLC solvent system e

Melting point

218-219°C

Molecular weight

241 (Mass spectrometry)

IR spectrum (in CCl₄) (figure 22 p. 174)

$\bar{\nu}$ max (cm⁻¹)

3381, 2925, 2850, 1769, 1690, 1647, 1616, 1316, 1154

¹H NMR spectrum (figure 23 p. 175)

Proton	Chemical shift (ppm) (δ)		
	Acetone-d ₆	CDCl ₃	Benzene-d ₆
1	6.85 (s)	6.84	6.6
4	8.29 (s)	8.05	7.36
5	7.92 (d, 8.8)	7.85	7.61
6	6.83 (dd, 8.8, 2.2)	6.88	6.86
8	7.02 (d, 2.2)	6.89	6.50
CHO	9.95 (s)	9.93	9.49
NH	10.72 (br.s)	8.12	6.31
OH	11.42 (s)	11.43	12.18
OCH ₃	3.85 (s)	3.90	3.47

¹³C NMR spectrum (in acetone-d₆) (figure 24 p. 176)

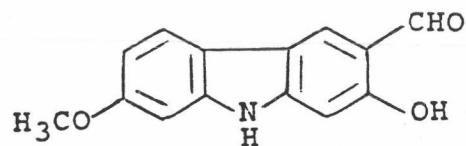
<u>Carbon</u>	<u>Chemical shift (ppm) (δ)</u>
1a	147.0 (+)
1	97.0 (-)
2	160.9 (+)
3	115.8 (+)
4	126.7 (-)
4a	118.4 (+)
5a	117.3 (+)
5	121.0 (-)
6	109.2 (-)
7	159.8 (+)
8	96.1 (-)
8a	143.0 (+)
3-CHO	196.2 (-)
7-OCH ₃	55.5 (-)

Mass spectrum (EIMS) (figure 25 p. 177)

m/z (% relative intensity)

241 (M⁺, 100), 226 (54.6), 210 (24.2), 149 (93.1)

Therefore it is concluded that MS-3 is 2-hydroxy-3-formyl-7-methoxycarbazole(18), the structure of which is shown below.



2-Hydroxy-3-formyl-7-methoxycarbazole
(MS-3)

4.4 Characterization of MS-4

MS-4 was obtained as a pale yellow solid.

hRf value (figure 8-12 p. 160-164)

17 on TLC solvent system a
35 on TLC solvent system b
22 on TLC solvent system c
20 on TLC solvent system d
42 on TLC solvent system e

Melting point

219-220°C

Molecular weight

255 (Mass spectrometry)

UV spectrum (figure 26 p. 178)

λ_{\max} = 298 nm (log ϵ = 4.69)
344 nm (log ϵ = 4.14)

IR spectrum (in CCl_4) (figure 27 p. 179)

$\bar{\nu}_{\max}$ (cm^{-1})

3340, 2964, 1687, 1662, 1632, 1148, 1063

^1H NMR spectrum (figure 28 p. 180)

Proton	Chemical shift (ppm) (δ)		
	Acetone- d_6	CDCl_3	Benzene- d_6
1	7.11 (s)	6.85	6.13
4	8.37 (s)	8.45	8.82
5	7.97 (d, 8.3)	7.87	7.56
6	6.83 (dd, 8.3, 2.2)	6.88	6.74
8	7.02 (d, 2.2)	6.90	6.62
CHO	10.43 (s)	10.50	10.93
NH	10.64 (br. s)	8.11	6.58
7-OCH ₃	3.85 (s)	3.90	3.46
2-OCH ₃	3.99 (s)	3.99	3.32

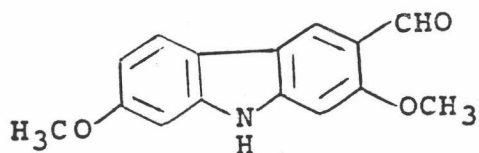
 ^{13}C NMR spectrum (in acetone- d_6) (figure 29 p. 181)

<u>Carbon</u>	<u>Chemical shift (ppm) (δ)</u>
1a	146.6 (+)
1	93.9 (-)
2	161.7 (+)
3	116.1 (+)
4	121.5 (-)
4a	119.4 (+)
5a	118.2 (+)
5	120.2 (-)
6	109.5 (-)
7	159.7 (+)
8	96.2 (-)
8a	143.0 (+)
2-OCH ₃	56.3 (-)
3-CHO	188.6 (-)
7-OCH ₃	55.8 (-)

Mass spectrum (EIMS) (figure 30 p. 182)

m/z (% relative intensity)
 255 (M^+ , 80.7), 240 (33.2), 212 (9.6), 169 (15.8),
 159 (26.7)

Therefore it is concluded that MS-4 is 3-formyl-2,7-dimethoxycarbazole, the structure of which is shown below.



3-Formyl-2,7-dimethoxycarbazole (MS-4)

4.5 Characterization of MS-5

MS-5 was obtained as white crystals.

hRf value (figure 8-12 p. 160-164)

59 on TLC solvent system a
77 on TLC solvent system b
62 on TLC solvent system c
54 on TLC solvent system d
81 on TLC solvent system e

Melting point

127-128°C

Molecular weight

258 (Mass spectrometry)

IR spectrum (KBr disc) (figure 31 p. 183)

$\bar{\nu}$ max (cm⁻¹)

3090, 2980, 2872, 1725, 1638, 1610

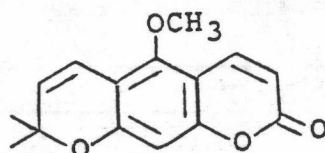
^1H NMR spectrum (in acetone- d_6) (figure 32 p. 184)

<u>Proton</u>	<u>Chemical shift (ppm) (δ)</u>
3	6.20 (d, 9.6)
4	7.84 (d, 9.6)
8	6.55 (s)
3'	5.70 (d, 10.2)
4'	6.56 (d, 10.8)
OCH ₃	3.86 (s)
CH ₃	1.46 (s)
CH ₃	1.46 (s)

Mass spectrum (EIMS) (figure 33 p. 185)

258 (M^+ , 9.30), 243 (over), 228 (over), 172 (1.49),
144 (1.49), 129 (1.38), 115 (1.85), 107 (2.02)

Therefore it is concluded that MS-5 is xanthoxyletin(134), the structure of which is shown below.



Xanthoxyletin (MS-5)

4.6 Characterization of MS-6

MS-6 was obtained as light yellow crystals.

hRf value (figure 8-12 p. 160-164)

55 on TLC solvent system a
56 on TLC solvent system b
49 on TLC solvent system c
43 on TLC solvent system d
74 on TLC solvent system e

Melting point

235.5-236.0°C

Molecular weight

211 (Mass spectrometry)

IR spectrum (in CHCl₃) (figure 34 p. 186) $\bar{\nu}$ max (cm⁻¹)

3680, 3454, 1637, 1611, 1158

¹H NMR spectrum (figure 35 p. 187)

Proton	Chemical shift (ppm) (δ)		
	Acetone-d ₆	CDCl ₃	Benzene-d ₆
1	6.89 (s)	6.88	6.59
4	8.43 (s)	8.18	7.42
5	8.07 (d, 7.6)	7.99	7.75
6	7.22 (t, 7.6)	7.28	7.18
7	7.38 (t, 7.6)	7.40	7.26
8	7.48 (d, 7.6)	7.40	6.88
CHO	9.98 (s)	9.95	9.45
NH	10.72 (br.s)	8.22	6.31
OH	11.46 (s)	11.46	12.16

¹³C NMR spectrum (in acetone-d₆) (figure 36 p.188)

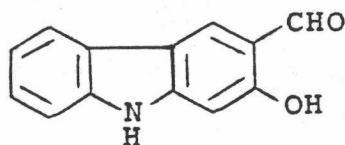
<u>Carbon</u>	<u>Chemical shift (ppm) (δ)</u>
1a	146.8 (+)
1	97.1 (-)
2	161.7 (+)
3	116.1 (+)
4	128.3 (-)
4a	118.6 (+)
5a	124.0 (+)
5	121.0 (-)
6	120.4 (-)
7	126.5 (-)
8	111.8 (-)
8a	141.7 (+)
3-CHO	196.1 (-)

Mass spectrum (EIMS) (figure 37 p. 189)

m/z (% relative intensity)

211 (M^+ , 100), 195 (8.5), 154 (11.0), 127 (5.5)

Therefore it is concluded that MS-6 is mukonal (12),
the structure of which is shown below.



Mukonal (MS-6)

4.7 Characterization of MS-7

MS-7 was obtained as a yellow crystalline solid.

hRf value (figure 8-12 p. 160-164)

45 on TLC solvent system a
51 on TLC solvent system b
46 on TLC solvent system c
36 on TLC solvent system d
70 on TLC solvent system e

Melting point

211-213°C

Molecular weight

307 (Mass spectrometry)

UV spectrum (figure 38 p. 190)

$\lambda_{\max} = 306 \text{ nm}$ ($\log \epsilon = 4.58$)

354 nm ($\log \epsilon = 4.09$)

IR spectrum (in CHCl_3) (figure 39 p. 191)

$\bar{\nu}_{\max}$ (cm^{-1})

3461, 1666, 1628, 1603, 1590, 1156

^1H NMR spectrum (figure 40 p. 192)

Proton	Chemical shift (ppm)(δ)		
	Acetone- d_6	CDCl_3	Benzene- d_6
4	8.23 (s)	8.30	8.69
5	7.97 (d, 8.4)	7.84	7.53
6	6.83 (dd, 8.4, 2.2)	6.86	6.73
8	6.97 (d, 2.2)	6.90	6.55
9	6.91 (d, 9.8)	6.61	6.03
10	5.90 (d, 9.8)	5.80	5.32
12	1.54 (s)	1.55	1.26
13	1.54 (s)	1.55	1.26
CHO	10.45 (s)	10.49	10.92
NH	10.68 (br.s)	8.14	6.55
7-OCH ₃	3.85 (s)	3.89	3.46

^{13}C NMR spectrum (in acetone- d_6) (figure 41 p. 193)

<u>Carbon</u>	<u>Chemical shift (ppm) (δ)</u>
1a	141.6 (+)
1	105.2 (+)
2	154.4 (+)
3	117.2 (+)
4	121.5 (-)
4a	119.1 (+)
5a	118.0 (+)
5	118.4 (-)
6	109.3 (-)
7	160.1 (+)
8	96.2 (-)
8a	143.2 (+)
3-CHO	188.3 (-)
7-OCH ₃	55.7 (-)

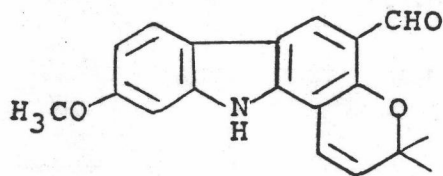
<u>Carbon</u>	<u>Chemical shift (ppm) (δ)</u>
9	130.8 (-)
10	117.4 (-)
11	77.7 (+)
12	27.6 (-)
13	27.6 (-)

Mass spectrum (EIMS) (figure 42 p. 194)

m/z (% relative intensity)

307 (M^+ , 16.5), 292 (29.9), 277 (20.1), 255
(30.8)

Therefore it is concluded that MS-7 is 7-methoxymurrayacine, the structure of which is shown below.



7-Methoxymurrayacine (MS-7)

4.8 Characterization of MS-8

MS-8 was obtained as a cream-colored solid.

hRf value (figure 8-12 p. 160-164)

40 on TLC solvent system a
45 on TLC solvent system b
40 on TLC solvent system c
31 on TLC solvent system d
60 on TLC solvent system e

Melting point

189-189.5°C

Molecular weight

225 (Mass spectrometry)

UV spectrum (figure 43 p. 195) $\lambda_{\max} = 296 \text{ nm}$ ($\log \epsilon = 4.64$)350 nm ($\log \epsilon = 4.19$)IR spectrum (in CHCl_3) (figure 44 p. 196) $\bar{\nu}_{\max}$ (cm^{-1})

3461, 1668, 1628, 1606, 1580, 1460, 1324, 1165, 1153

 ^1H NMR spectrum (figure 45 p. 197)

Proton	Chemical shift (ppm) (δ)		
	Acetone- d_6	CDCl_3	Benzene- d_6
1	7.12 (s)	6.88	6.10
4	8.50 (s)	8.56	8.88
5	8.10 (d, 8.0)	8.00	7.70
6	7.15 (t, 7.1)	7.25	7.29
7	7.35 (t, 7.1)	7.38	7.25
8	7.47 (d, 8.0)	7.40	6.96
CHO	10.45 (s)	10.49	10.89
NH	10.61 (s)	8.88	6.65
OCH_3	4.00 (s)	3.99	3.30

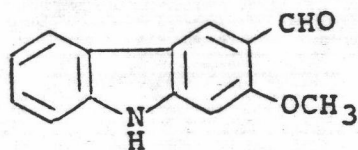
^{13}C NMR spectrum (in acetone- d_6) (figure 46 p. 198)

<u>Carbon</u>	<u>Chemical shift (ppm) (δ)</u>
1a	146.2 (+)
1	53.5 (-)
2	162.2 (+)
3	117.7 (+)
4	121.2 (-)
4a	119.3 (+)
5a	124.2 (+)
5	120.7 (-)
6	120.5 (-)
7	126.2 (-)
8	111.7 (-)
8a	141.4 (+)
2-OCH ₃	56.1 (-)
3-CHO	188.4 (-)

Mass spectrum (EIMS) (figure 47 p. 199)

m/z (% relative intensity)
 225 (M^+ , 100), 208 (11.6), 179 (18.3), 168 (8.0),
 154 (13.4)

Therefore it is concluded that MS-8 is O-methylmukonal, the structure of which is shown below.



O-Methylmukonal (MS-8)