CHAPTER 3
RESULTS AND DISCUSSION

The Results of Extraction

The heartwoods of *Mansonia gagei* Drumm. were extracted according to the procedure described in Chapter 2. The results of extraction can be summarized as shown in Scheme 3.1.

The Results of Biological Activity Screening Tests

Brine Shrimp Cytotoxicity Test

Each crude extract of *M. gagei* was preliminarily screened for cytotoxicity against brine shrimp (*Artemia salina* Linnaeus) according to the procedure described in Chapter 2. The results are reported in Table 3.1.

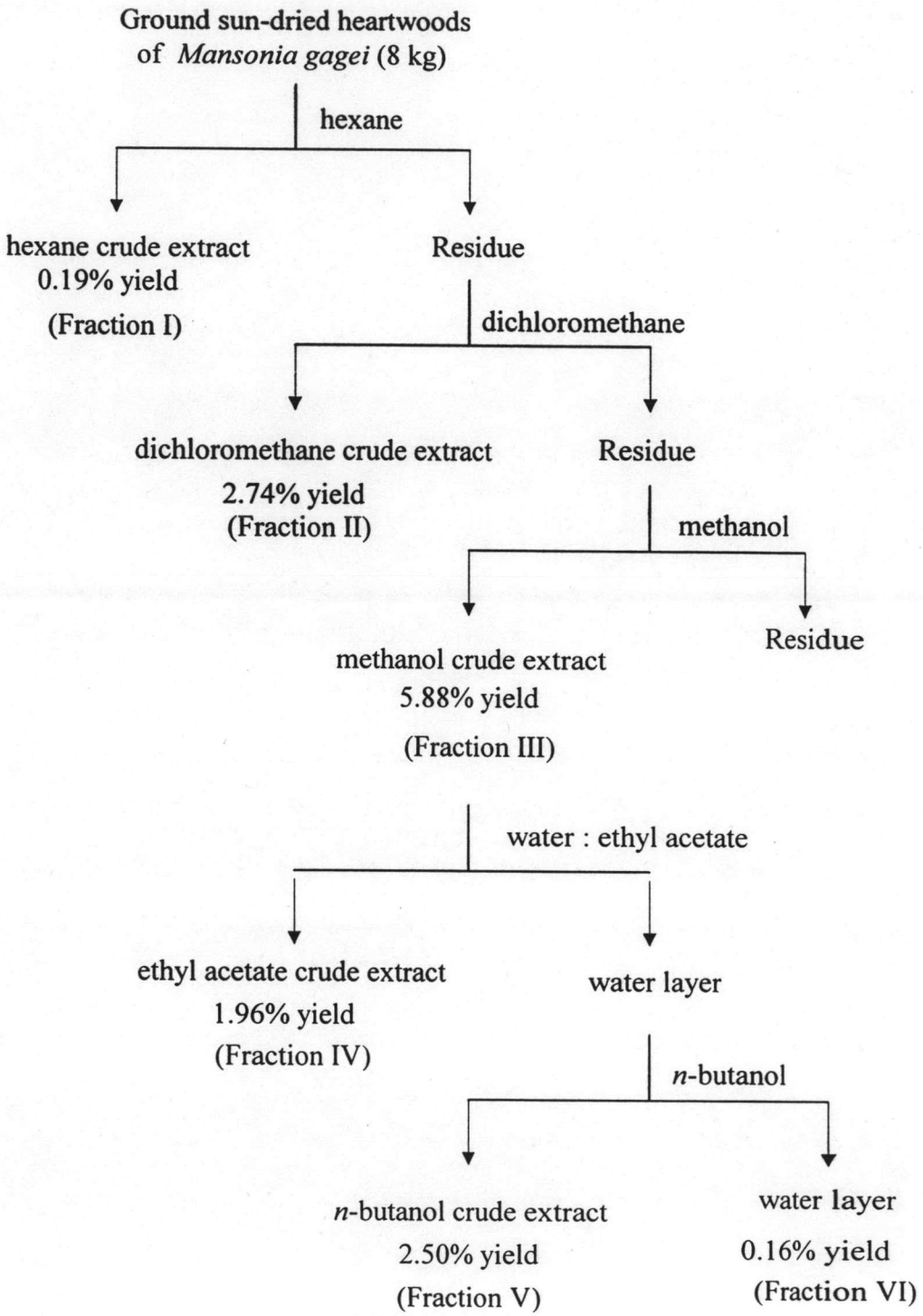
Table 3.1 The results of brine shrimp cytotoxicity test

Fraction / Solvent extract	LC_{50}	Activity
I (hexane)	23.69	medium activity
II (dichloromethane)	22.83	medium activity
III (methanol)	279.13	low activity
IV (ethyl acetate)	26.52	medium activity
V (*n*-butanol)	115.10	low activity
VI (water)	518.81	low activity

Note : High Activity (LC_{50} < 10 μg/mL)

: Medium Activity (LC_{50} < 100 μg/mL)

: Low Activity (LC_{50} < 1000 μg/mL)



Scheme 3.1 The extraction procedure

Anticell Line Cytotoxicity Test[16]

The crude extracts, Fraction I, II and III, of *Mansonia gagei* were tested for cytotoxicity against various cell lines, *i.e.*, *Nasopharyngeal carcinoma* (KB), *Bladder carcinoma* (BIU), *Erythroleukemia carcinoma* (K-562), *Gastric carcinoma* (BGC-823), *Leukemia carcinoma* (HL-60), *Hepatocellular carcinoma* (Bel-7402), *Colon carcinoma* (HCT-8). The results are reported in Table 3.2-3.8.

Table 3.2 The results of cytotoxicity test against *Nasopharyngeal carcinoma* (KB) cell line

Sample	Concentration (μg/mL)	Inhibition (%)	Estimation
Fraction I	1	2.35	
	10	2.73	+
	100	99.20	
Fraction II	1	48.52	
	10	57.70	++
	100	89.67	
Fraction III	1	18.29	
	10	-6.09	-
	100	6.68	

Table 3.3 The results of cytotoxicity test against *Bladder carcinoma* (BIU) cell line

Sample	Concentration (μg/mL)	Inhibition (%)	Estimation
Fraction I	1	0.35	
	10	14.49	+
	100	74.94	
Fraction II	1	15.49	
	10	48.12	+
	100	91.13	
Fraction III	1	14.14	
	10	44.83	+
	100	61.26	

Table 3.4 The results of cytotoxicity test against *Erythroleukemia carcinoma* (K-562) cell line

Sample	Concentration (μg/mL)	Inhibition (%)	Estimation
Fraction I	1	-39.49	
	10	-1.84	+
	100	83.93	
Fraction II	1	-51.27	
	10	48.96	+
	100	80.83	
Fraction III	1	-26.78	
	10	-18.70	+
	100	90.76	

Table 3.5 The results of cytotoxicity test against *Gastric carcinoma* (BGC-823) cell line

Sample	Concentration (μg/mL)	Inhibition (%)	Estimation
Fraction I	1	-27.53	
	10	-7.85	+
	100	90.16	
Fraction II	1	48.81	
	10	97.62	++
	100	97.74	
Fraction III	1	11.40	
	10	95.84	++
	100	98.69	

Table 3.6 The results of cytotoxicity test against *Leukemia carcinoma* (HL-60) cell line

Sample	Concentration (μg/mL)	Inhibition (%)	Estimation
Fraction I	1	-25.46	
	10	41.20	+
	100	93.11	
Fraction II	1	-33.21	
	10	30.38	+
	100	91.88	
Fraction III	1	25.42	
	10	74.43	++
	100	88.02	

Table 3.7 The results of cytotoxicity test against *Hepatocellular carcinoma* (Bel-7402) cell line

Sample	Concentration (μg/mL)	Inhibition (%)	Estimation
Fraction I	1	-5.99	
	10	8.81	+
	100	94.73	
Fraction II	1	-7.57	
	10	0.97	-
	100	1.71	
Fraction III	1	67.48	
	10	93.64	+++
	100	95.11	

Table 3.8 The results of cytotoxicity test against *Colon carcinoma* (HCT-8) cell line

Sample	Concentration (μg/mL)	Inhibition (%)	Estimation
Fraction I	1	12.22	
	10	18.96	+
	100	78.56	
Fraction II	1	6.17	
	10	6.23	+
	100	77.76	
Fraction III	1	51.54	
	10	84.68	+++
	100	96.68	

The results of biological activity screening tests revealed that both hexane and dichloromethane crude extracts showed medium cytotoxic activity against brine shrimp. The same trend for anticell line cytotoxicity of dichloromethane crude extract was observed. The hexane crude extract also displayed cytotoxicity against certain cell lines, while the methanol crude extract showed promising results against *Hepatocellular carcinoma* (Bel-7402) and *Colon carcinoma* (HCT-8).

Fractionation of Dichloromethane Crude Extract by Quick Column Chromatography for Activity Test

Based upon these results, the dichloromethane crude extract, which was the most promising, 100 g was further separated into small fractions by quick column chromatography using silica gel 60G Art. 7731 as an adsorbent. The column was initially eluted with hexane and gradually changed to a mixture of dichloromethane and hexane, dichloromethane and a mixture of dichloromethane and methanol. Approximately 3 L of solvent was collected for each fraction and then concentrated to about 30 mL. The results of the separation of dichloromethane crude extract are shown in Table 3.9.

Table 3.9 The results of the separation of dichloromethane crude extract by quick column chromatography

Eluent (% volume by volume)	Fraction No.	Remarks	Weight (g)
hexane	1	yellow oil	1.72
10% CH_2Cl_2 in hexane	2	yellow oil	2.48
20% CH_2Cl_2 in hexane	3	red oil	2.15
40% CH_2Cl_2 in hexane	4	brown oil	2.66
60% CH_2Cl_2 in hexane	5	brown oil	3.78
80% CH_2Cl_2 in hexane	6	solid in brown oil	5.41
100% CH_2Cl_2	7	solid in brown oil	7.11
2% MeOH in CH_2Cl_2	8	solid in brown oil	20.87
10% MeOH in CH_2Cl_2	9	solid in brown oil	50.44

Brine Shrimp Cytotoxicity Test for Dichloromethane Crude Extract

Each small fraction of dichloromethane crude extract derived from the separation by quick column chromatography was further subjected to brine shrimp cytotoxicity test. The results of brine shrimp cytotoxicity test are shown in Table 3.10.

Table 3.10 The results of brine shrimp cytotoxicity test of various fractions derived from the separation of dichloromethane crude extract

Fraction	LC_{50}
hexane	*
10% CH_2Cl_2 in hexane	*
20% CH_2Cl_2 in hexane	*
40% CH_2Cl_2 in hexane	13.88
60% CH_2Cl_2 in hexane	7.58
80% CH_2Cl_2 in hexane	21.82
100% CH_2Cl_2	71.26
2% MeOH in CH_2Cl_2	13.23
10% MeOH in CH_2Cl_2	15.23

* not test because the crude extract did not dissolve in ethanol

It could be clearly seen from the brine shrimp cytotoxicity test that the most active fraction of the dichloromethane crude extract was the 60% CH_2Cl_2 in hexane fraction. Other fractions displayed moderate cytotoxic activity. These preliminary biological activity tests assured that at least the active principles should exist in the dichloromethane fraction. This fraction was therefore needed for further investigation.

Separation

Separation of Hexane Crude Extract (Fraction I)

The hexane crude extract was obtained as dark-brown oil, 15.53 g (see Scheme 3.1). The technique used for separating 8.0 g of the hexane crude extract into various fractions was column chromatography using silica gel as an adsorbent. About 500 mL of solvent was collected for each fraction. The solution in each fraction was evaporated to about 10 mL and checked by TLC. The combined fractions of the separation of crude hexane are shown in Table 3.11.

Table 3.11 The results of the separation of hexane crude extract

Eluent (% volume by volume)	Fraction No.	Remarks	Weight (g)
hexane	1-3	yellow oil + white amorphous (Mixture **1**)	2.22
2% CH_2Cl_2 in hexane	4-18	yellow oil	0.95
5% CH_2Cl_2 in hexane	19-32	yellow oil	1.25
7% CH_2Cl_2 in hexane	33-54	yellow oil	1.52
10% CH_2Cl_2 in hexane	55-65	yellow solid in yellow oil (Compound **2**)	1.65
15% CH_2Cl_2 in hexane	66-73	yellow solid in yellow oil (Compound **3**)	1.47
30% CH_2Cl_2 in hexane	74-75	solid in brown oil	0.35
50% CH_2Cl_2 in hexane	76-85	solid in brown oil	0.59
70% CH_2Cl_2 in hexane	86-92	brown oil	0.47
100% CH_2Cl_2	93-100	brown oil	0.45

Separation of Dichloromethane Crude Extract (Fraction II)

The dichloromethane crude extract (60 g) was chromatographed on silica gel using column chromatography. Hexane, a mixture of hexane and ethyl acetate, ethyl acetate and a mixture of ethyl acetate and methanol were used as eluents. About 800 mL of solution was collected for each fraction and then concentrated to about 50 mL. They were subsequently transfered to small flasks and evaporated on a water bath to about 10 mL. Each fraction was monitored by TLC plate. The combined fractions from the separation of dichloromethane crude extract are shown in Table 3.12.

Table 3.12 The results of the separation of dichloromethane crude extract

Eluent (% volume by volume)	Fraction No.	Remarks	weight (g)
Hexane	1-7	yellow oil	2.82
5% EtOAc in hexane	8-22	yellow oil	0.74
	23-28	yellow solid in red oil (Fraction II A)	0.82
	29-37	red oil + red solid (Compound **4**)	0.62
	38-49	yellow oil (Fraction II B)	0.75
	50-112	red oil	7.82
10% EtOAc in hexane	113-123	red solid (Fraction II C)	0.03
	124-133	red oil (Fraction II D)	1.56
	134-135	white solid in red oil (Mixture **6**)	0.25
	136-140	orange crystal in red oil (Compound **8**)	2.65
15% EtOAc in haxane	141-200	brown solid	11.20
20% EtOAc in hexane	201-325	brown solid in brown oil	6.38
40% EtOAc in hexane	326-358	brown solid in brown oil	5.13
	359-388	brown solid (Compound **9**)	1.35
	389-398	pale brown solid (Compound **10**)	0.09
60% EtOAc in hexane	399-409	brown solid	1.11
80% EtOAc in hexane	410-418	brown solid	1.22
100% EtOAc	419-441	brown solid	1.44
3% MeOH in EtOAc	442-459	black solid	1.77
5% MeOH in EtOAc	460-466	black solid	1.75

Table 3.12 (Continued)

Eluent (% volume by volume)	Fraction No.	Remarks	weight (g)
10% MeOH in EtOAc	467-475	black solid	0.97
20% MeOH in EtOAc	476-486	black solid	0.99
40% MeOH in EtOAc	487-495	balck solid	0.18

Separation of Fraction II A

The yellow crystals in red oil derived from fraction 23-28 (0.82 g) (see also Table 3.12) was flash chromatographed on silica gel. Hexane and a mixture of hexane and ethyl acetate were used as eluents. About 10 mL of solution was collected for each fraction and checked by TLC. The results of the separation of Fraction II A are presented in Table 3.13.

Table 3.13 Separation of Fraction II A

Eluent (% volume by volume)	Fraction no.	Remarks	Weight (g)
hexane	1-15	yellow oil	0.02
5% EtOAc in hexane	16-20	yellow oil + yellow solid (Compound **2**)	0.08
10% EtOAc in hexane	21-69	red oil	0.35
20% EtOAc in hexane	70-115	brown solid	0.27

The Separation of Fraction II B

Fraction II B (0.75 g) was further purified by flash column chromatography on silica gel (25 g). Hexane and a mixture of hexane and dichloromethane were used as eluents. About 10 mL of solution was collected for each fraction and checked the similarity by TLC. The results of the separation of Fraction II B are tabulated in Table 3.14.

Table 3.14 Separation of Fraction II B

Eluent (% volume by volume)	Fraction no.	Remarks	Weight (g)
hexane	1-36	yellow oil	0.01
10% CH_2Cl_2 in hexane	37-43	yellow oil	0.04
30% CH_2Cl_2 in hexane	44-72	yellow oil	0.07
50% CH_2Cl_2 in hexane	73-114	yellow oil	0.10
	115-131	yellow oil	0.07
	132-150	yellow oil + white solid (Compound **3**)	0.03
	151-200	yellow oil	0.11
	201-234	red oil	0.14
100% CH_2Cl_2	235-276	brown solid	0.17

Separation of Fraction II C

The red solid obtained from Fraction II C (0.03 g) was chromatographed on silica gel PF_{254} Art.7749.1000 (45 g) using a chromatotron. Hexane, a mixture of hexane and ethyl acetate were used as eluents. Fractions of about 50 mL of solution were collected and checked for similarity by TLC. The results of the separation of Fraction II C are shown in Table 3.15.

Table 3.15 Separation of Fraction II C

Eluent (% volume by volume)	Fraction no.	Remarks	Weight (g)
hexane	1-4	yellow oil	0.08
5% EtOAc in hexane	5-6	yellow solid (Compound **5**)	0.02
10% EtOAc in hexane	7-10	red oil	0.20

Separation of Fraction II D

The red oil of Fraction II D (1.56 g) was chromatographed C-Gel C560 (0.015-0.035 mm) using medium pressure chromatography. Hexane and a mixture of hexane and ethyl acetate were used as eluents. Fractions of about 50 mL each were collected and checked by TLC. The results of the separation of Fraction II D are shown in Table 3.16.

Table 3.16 Separation of Fraction II D

Eluent (% volume by volume)	Fraction no.	Remarks	Weight (g)
hexane	1-3	yellow oil	0.75
5% EtOAc in hexane	4-5	yellow oil + yellow solid (Compound **5**)	0.20
15% EtOAc in hexane	6-9	red oil + white solid (Mixture **7**)	0.61

Purification, Properties and Structural Elucidation of Mixture 1

The combined fractions no.1-3 of the separation of Fraction I (see also Table 3.11) contained a white amorphous solid and a pale yellow oil. The oil was removed by washing with methanol. The remaining white solid was recrystallized from methanol yielding Mixture **1**, 5 mg (6.25×10^{-5}% wt. by wt. of the dried wood), m.p. 65-67 °C.

The IR spectrum (Fig 3) of Mixture **1** showed the absorption band of C=O stretching vibration of an ester at 1736 cm^{-1} (s) and C-O stretching vibration at 1183 cm^{-1} (m). The C-H stretching vibration and C-H bending vibration of $-CH_2-$, $-CH_3$ were observed at 2847 and 1463 cm^{-1}, respectively. The additional absorption peak at 722 cm^{-1} was due to $-CH_2-$ (for chain, 4 carbons).[17-18] From this spectroscopic data, the mixture was identified as a saturated long chain aliphatic esters.

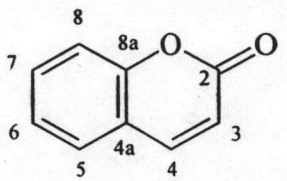
Purification, Properties and Structural Elucidation of Compound 2

Compound **2** as pale yellow crystals, m.p. 135-137 °C, 0.39 g (4.87×10^{-3}% wt. by wt. of the dried wood) was collected from the separation of hexane soluble and dichloromethane soluble crude extracts. This compound showed a single spot on TLC with an R_f value of 0.40 (silica gel/hexane:ethyl acetate (8:2)).

The molecular formula of this compound was proposed to be $C_{15}H_{18}O_3$ according to the elemental analysis results (Found %C 72.98 and % H 7.59 ; Calcd. for $C_{15}H_{18}O_3$ MW. 246.30; % C 73.17 and % H 7.31). This proposed formula implied a high degree of unsaturation ,*i.e.*, DBE = 7.

The FT-IR spectrum (Fig 4) revealed strong absorption band at 1711 cm^{-1} which corresponds to the C=O stretching vibration of, possibly, an α,β-unsaturated ester (lactone). The characteristic absorption peak due to an aromatic moiety was observed at 1600 cm^{-1}.[18] The UV-Vis spectrum (Fig 5) exhibited λ_{max} (CH_2Cl_2) at 232, 292 and 344 nm (log ε = 4.15, 4.13 and 3.43). This data clearly showed the presence of a conjugated system, such as α,β-unsaturated carbonyl moiety, in this molecule.[19]

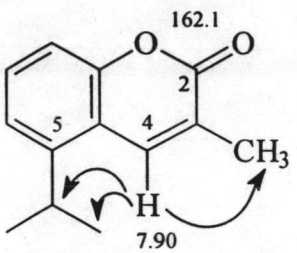
These results suggested that Compound 2 may be classified as a coumarin.



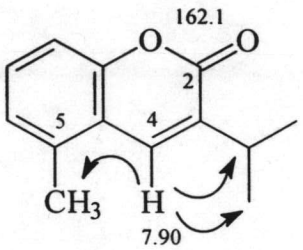
The crucial information for the structural elucidation of this compound was obtained from the ^{1}H and ^{13}C-NMR spectra. From the ^{1}H-NMR spectrum (Fig 6), the obvious pattern of an isopropyl group was observed at 1.38 ppm (d, J = 7.3 Hz, 6H) assigned for two methyl groups. The methine proton of this moiety was detected as a multiplet (1H) at 3.56 ppm. Other two singlet signals at 2.23 and 2.42 ppm with 3H integration each were, no doubt, methyl groups. The singlet signal at 3.83 ppm (3H) was attributed to methoxy protons. In addition, two singlet signals with intensity corresponding to 1H each, were observed at 6.91 and 7.90 ppm and could be tentatively assigned to an aromatic proton and an olefinic proton, respectively.[20] The ^{1}H-NMR spectrum therefore clearly implied that there were four substituents in the coumarin structure of Compound **2** : two methyl, one methoxy and one isopropyl groups.

The ^{13}C-NMR spectrum (Fig 7) exhibited a total of fourteen signals. The DEPT-90 spectrum (Fig 8), showed signals for three methine carbons at 26.6, 116.7 and 136.7 ppm. The first signal was assigned to the methine carbon of the isopropyl group. The remaining two signals were ascribed to an olefinic carbon and an aromatic carbon, respectively. The DEPT-135 spectrum (Fig 8) displayed seven signals of >CH- , $-CH_3$ which were assigned to four methyl carbons at 15.7, 17.6 (2 carbons) and 21.4 ppm and one methoxy carbon at 56.2 ppm. There was no signal corresponding to $-CH_2-$ observed in this spectrum. The remaining seven signals in the ^{13}C-NMR spectrum were compatible with quarternary carbons. A signal at 162.1 ppm was likely to be a carbonyl carbon of an ester or lactone. Others were observed at 153.7, 146.6, 129.6, 124.6, 123.8 and 117.8 ppm corresponding to either olefinic or aromatic carbons.

The useful information to be ascertained for the appropriate positions of substituents was derived from the HMBC spectrum (Fig 9A). According to the HMBC spectrum, the C-4 position of the coumarin should be unsubstituted. This is because the signal of C-2 (at 162.1 ppm) which was assigned to a carbonyl lactone carbon was clearly coupled with an olefinic proton at 7.90 ppm. Moreover, the ^{1}H-^{1}H NOESY spectrum (Fig 10) clearly showed that the previously mentioned proton had a coupling interaction with the methyl proton signal at 2.23 ppm and also with the two signals belonging to an isopropyl group : the methyl and methine protons at 1.38 ppm and 3.56 ppm, respectively. Consequently, the substituent located at C-3 may be either methyl (Structure I) or isopropyl group (Structure II). Possible sub-structure of this compound are shown below.

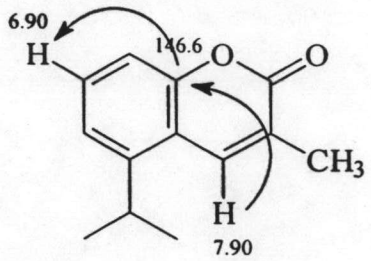


(I)

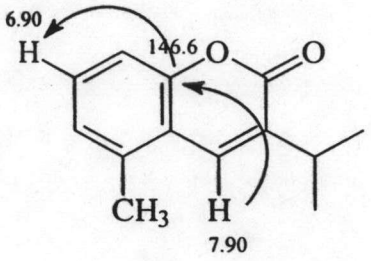


(II)

Further information was obtained from the HMBC spectrum (Fig 9A). Besides the coupling between the proton signal at 7.90 ppm and a lactone carbon signal at 162.1 ppm, this proton signal was additionally coupled with another carbon signal (at 146.6 ppm) which was assigned as C-8a of the coumarin moiety. This carbon signal, in turn, was also long-range coupled with the proton signal at 6.90 ppm which was an aromatic proton. All the above data clearly implied that the C-7 position was not substituted (Structures III or IV).

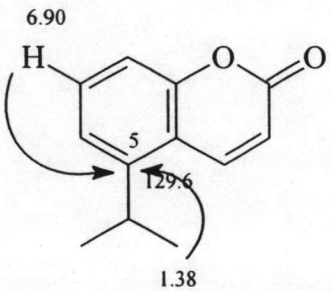


(III)

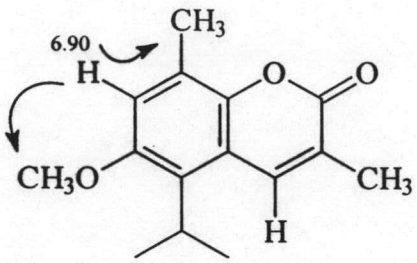


(IV)

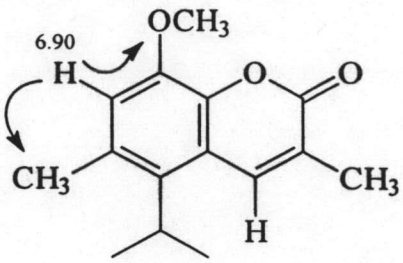
In addition, the HMBC spectrum (Fig 9B) provided further informative spectroscopic data. The proton signal at 1.38 ppm which was assigned to the two methyl groups of the isopropyl moiety, was coupled with the carbon signal at 129.6 ppm. The proton signal at 6.90 ppm was also found to couple with this carbon signal. This observation strongly indicated that this quarternary carbon (from DEPT-90 and DEPT-135 spectra) should be located at C-5. Therefore, a possible sub-structure of Compound **2** must be structure I.



Two missing substituents : one methyl and one methoxy group, could be placed at C-6 and C-8 or *vice versa*. This was also supported by the ^{1}H-^{1}H NOESY spectrum (Fig 10). To illustrate this, the proton signal at 6.90 ppm was coupled with both signals observed at 2.42 ppm (methyl protons) and at 3.83 ppm (methoxy protons). Thus, a plausible structure for Compound **2** could be deduced as shown below.

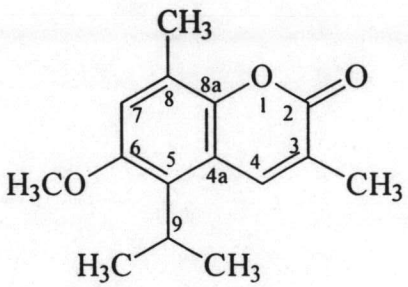


(V)



(VI)

The HMBC spectrum (Fig 9C) also supplied important data to distinguish between structures V and VI. The C-8a signal which was detected at 147.0 ppm, coupled with the proton signals of the methyl group at 2.42 ppm. Therefore, the C-8 should be substituted by a methyl group.



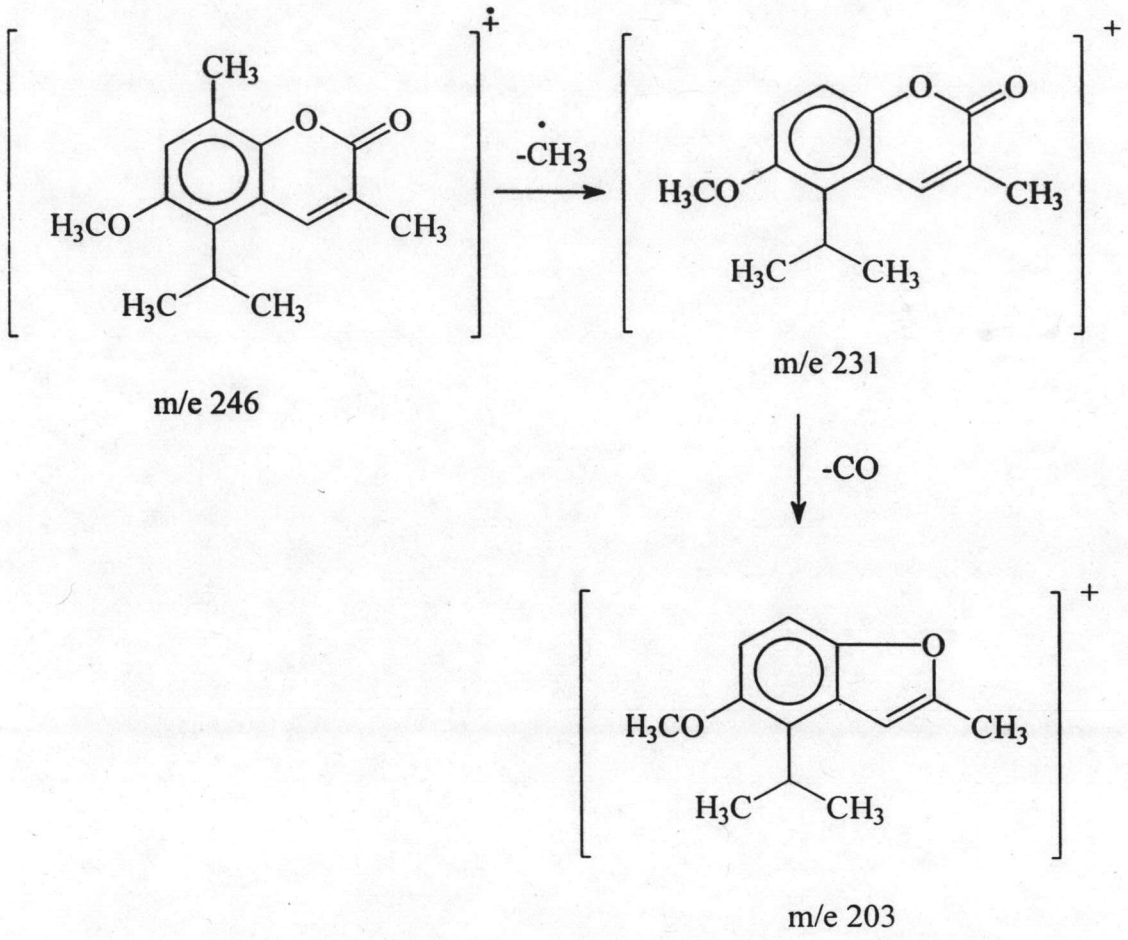
Compound **2**

According to all spectroscopic data, the tentative ^{1}H and ^{13}C-NMR assignment can be tabulated in Table 3.17.

Table 3.17 Tentative ^{1}H and ^{13}C-NMR assignment of Compound **2**

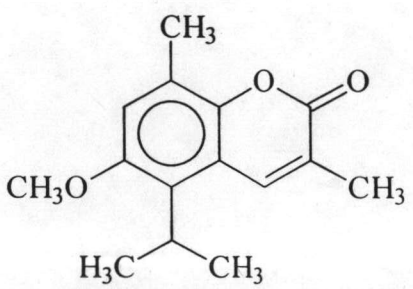
Position	Chemical shift (ppm)	
	^{1}H-NMR	^{13}C-NMR
2	-	162.1
3	-	124.6
4	7.90 (s)	136.7
4a	-	117.8
5	-	129.6
6	-	153.7
7	6.90 (s)	116.7
8	-	123.8
8a	-	146.6
9	3.56 (m)	26.6
3-CH_3	2.23 (s)	21.4
8-CH_3	2.42 (s)	15.7
6-OCH_3	3.83 (s)	56.2
$(CH_3)_2$-CH-	1.38 (d)	17.6 (2C)

The mass spectrum (Fig 11) displayed the molecular ion peak at m/e (% rel. int.) 246 (62) (Calcd. for $C_{15}H_{18}O_3$: MW. 246.31). Other fragmentations were detected at m/e 231 (100) (M^+-CH_3) and 203 (5) (M^+-C_2H_3O). The proposed fragmentation pattern of Compound **2** is shown in Scheme 3.2.



Scheme 3.2 The proposed fragmentation pattern of Compound **2**

Supported by spectroscopic data, it was obvious to conclude that Compound **2** was 3,8-dimethyl-5-isopropyl-6-methoxy coumarin. The structure is shown below.



Compound **2** : 3,8-dimethyl-5-isopropyl-6-methoxy coumarin

This proposed structure for Compound **2** was finally confirmed its structure by X-ray crystallography.[21] The ORTEP derived from X-ray crystallography is presented in Fig 12.

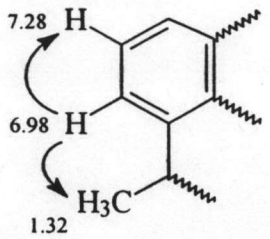
A literature search on the coumarins having a molecular formula $C_{15}H_{18}O_3$ was carried out and it was found that there was no previous report of Compound **2** as either a natural or a synthetic coumarin. Therefore, this compound is a new naturally occuring coumarin.

Purification, Properties and Structural Elucidation of Compound 3

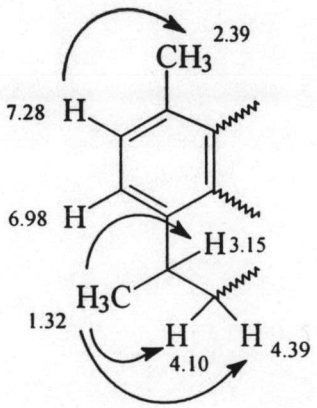
Compound **3** was obtained from the separation of the hexane crude extract (Fraction I) (see Table 3.11) and dichloromethane crude extract (Fraction II) (see Table 3.12). After multiple recrystallization from a mixture of hexane and ethyl acetate, Compound **3** was obtained as white crystal, m.p. 150-151 °C, 0.04 g (5×10^{-4} % wt. by wt. of the dried wood). This compound showed a single spot on TLC with R_f value 0.25 (silica gel/hexane:dichloromethane (3:7)).

The ^{1}H-NMR spectrum of this compound (Fig 13) was found to be very close to that of Compound **2**. The doublet signal appeared at 1.32 ppm (3H) with J=7.4 Hz was attributed to the methyl protons. Other two singlet signals at 2.04 and 2.39 ppm with 3H integration each were also compatible with methyl protons. The methine proton was detected as a multiplet signal (1H) at 3.15 ppm. Two doublet of doublet signals centered at 4.10 ppm (J=6.7 and 11.0 Hz) and 4.39 ppm (J= 4.0 and 10.7 Hz) were assigned to a -CH-C<u>H</u>$_2$-O- group. Two doublet signals (1H each) centered at 6.98 and 7.28 ppm with J=7.6 Hz were suggestive of *p*-substituted aromatic moiety being present in the molecule.[20]

The certain assignment of substituents in this compound could be performed based upon the spectral data derived from the ^{1}H-^{1}H NOESY spectrum (Fig 14). To illustrate this, the signal of the aromatic protons at 6.98 ppm coupled with two signals; one was observed at 7.28 ppm(aromatic proton) and the other at 1.32 ppm (methyl protons. Therefore, a possible sub-structure of this compound could be drawn as shown below.

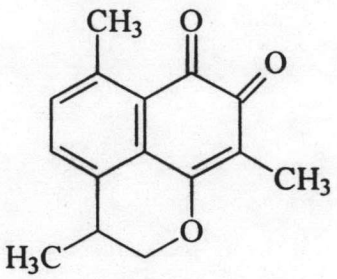


In addition, the data obtained from the ^{1}H-^{1}H NOESY spectrum revealed that the aromatic proton signal at 7.28 ppm was coupled with the signal at 2.39 ppm which was assigned to methyl protons. Moreover, the methyl proton signals at 1.32 ppm was coupled with both the methine proton signal at 3.15 and methylene protons at 4.10 and 4.39 ppm.



The ^{13}C-NMR spectrum (Fig 15) and DEPT 90, DEPT 135 (Fig 16) showed 14 signals at δ (ppm) as follows : methyl carbons at 8.9, 15.4 and 17.0, methylene carbon at 72.5, and methine carbons at 31.0, 119.9 and 132.4. The signal at 31.0 ppm could be assigned for a methine carbon of CH_3-C**H**-CH_2-. The remaining two signals were ascribed to aromatic carbons. Quarternary carbons observed at 164.2, 159.4, 150.1, 134.3, 124.3, 110.8 and 102.5 ppm corresponded to either olefinic or aromatic carbons.

Compound **3** was first thought to be mansonone E or 2,3-dihydro-3,6,9-trimethyl-naphtho[1,8-bc]pyran-7,8-dione[22] due to its widely distribution in *Mansonia* genus as well as some well-matched spectroscopic data.

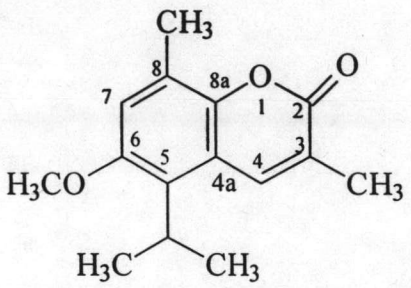


mansonone E

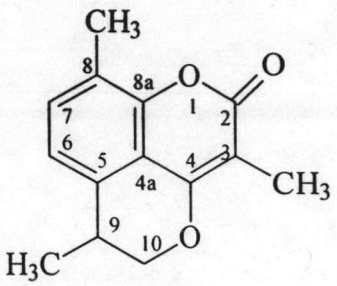
However, there was solid evidence to confirm that this compound should be a coumarin rather than a 1,2-naphthoquinones (such as mansonone E). The IR spectrum of Compound **3** (Fig 17) showed a strong absorption band corresponding to the C=O stretching vibration of an α,β-unsaturated ester (lactone) at 1705 cm^{-1}, whereas the absorption band for a conjugated carbonyl group of 1,2-naphthoquinone is generally observed around 1650 cm^{-1} and for an aromatic moiety is observed around 1608 cm^{-1}.[18] In addition, the ^{13}C-NMR spectrum revealed the signal at 164.2 ppm. which was likely to be a carbonyl carbon of an ester (lactone) much more than being a carbonyl of quinone generally detected around 178-182 ppm.[22] The UV spectrum (Fig 18) exhibited λ_{max} (CH_2Cl_2) at 230, 284, 294 and 312 nm (log ε = 3.95, 4.01, 4.04 and 3.91). The comparison with the maximum absorption of mansonone E[22], Compound **2** and Compound **3** is shown in Table 3.18.

Table 3.18 The comparison of the maximum absorption of mansonone E, Compound **2** and Compound **3**

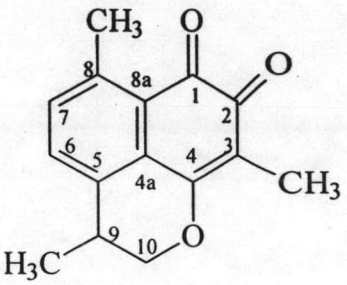
Compound **2**		Compound **3**		mansonone E	
λ_{max}	log ε	λ_{max}	log ε	λ_{max}	log ε
232	4.15	230	3.95	219	4.25
292	4.13	284	4.01	264	4.31
344	3.43	294	4.04	370	3.20
		312	3.91	445	3.38



Compound **2**



Compound **3**



mansonone E

The ^{1}H- and ^{13}C-NMR data of mansonone E[22], Compound **2** and Compound **3** are presented in Table 3.19.

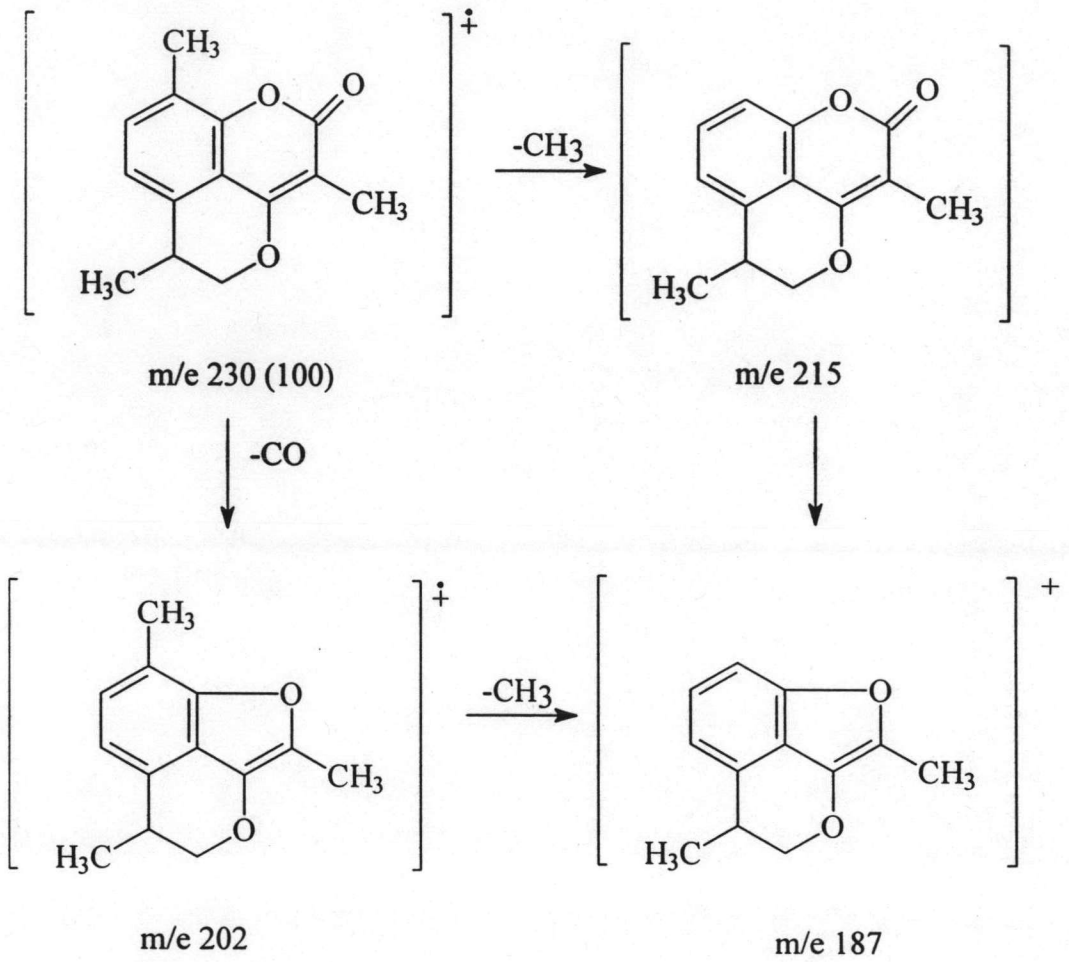
Table 3.19 The comparison of the ^{1}H and ^{13}C-NMR data of mansonone E, Compound **2** and Compound **3**

position	Chemical shift (ppm)					
	Compound **2**		Compound **3**		mansonone E	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	-	-	-	-	-	182.3
2	-	162.1	-	164.2	-	180.3
3	-	124.6	-	102.5	-	116.3
4	7.90 (s)	136.7	-	159.4	-	162.4
4a	-	117.8	-	120.0	-	127.4
5	-	129.6	-	110.8	-	126.9
6	-	153.7	7.28 (d)	124.0	7.32 (d)	132.6
7	6.90 (s)	116.7	6.98 (d)	132.4	7.23 (d)	134.9
8	-	123.8	-	134.3	-	142.9
8a	-	146.6	-	150.1	-	136.9
9	3.56 (m)	26.6	3.15 (m)	31.0	3.67 (m)	31.2
10	*	*	4.39 (dd), 4.10 (dd)	72.5	4.38 (dd), 4.20 (dd)	71.4
3-CH_3	2.23 (s)	21.4	2.04 (s)	8.9	1.92 (s)	7.8
8-CH_3	2.42 (s)	15.7	2.39 (s)	17.0	2.62 (s)	22.5
9-CH_3			1.32 (d)	15.4	1.34 (d)	17.6
6-OCH_3	3.83 (s)	56.2	*	*	*	*
$(CH_3)_2$-CH-	1.38 (d)	17.6 (2C)	*	*	*	*

* no position in the corresponding structure

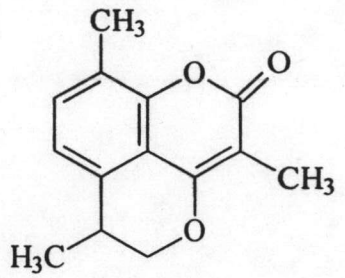
Moreover, the mass spectrum of mansonone E ($C_{15}H_{14}O_3$) must reveal a molecular ion peak at 242. The mass spectrum of Compound **3** (Fig 19) exhibited the molecular ion peak at 230 (100) corresponding to the molecular formular $C_{14}H_{14}O_3$. Other important ions were detected at m/e (% rel int.) 215 (25) (M^{+}-CH_3), 202 (20)

(M^+-CO), 187 (20) (M^+-C_2H_3O). The proposed fragmentation pattern of Compound **3** is shown in Scheme 3.3.



Scheme 3.3 The proposed fragmentation pattern of Compound **3**

According to various spectroscopic data, Compound **3** has obviously structure of 2,3-dihydro-3,6,9-trimethyl naphtho[1,8-bc]pyran-7-oxa-8-one. The structure is shown below.



Compound **3** : 2,3-dihydro-3,6,9-trimethyl naphtho[1,8-bc]pyran-7-oxa-8-one

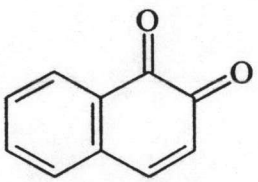
A literature search based upon the compound having the formula $C_{14}H_{14}O_3$ and containing a coumarin nucleus was carried out. It was found that there was no previous report of this compound in chemical literature as either natural or synthetic coumarin. Compound **3** is thus another new naturally occuring coumarin derived from *Mansonia gagei*.

Purification, Properties and Structural Elucidation of Compound 4

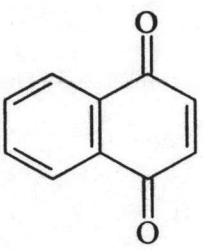
Compound **4** was obtained from the separation of the dichloromethane crude extract (Fraction II) (see also Table 3.12). After multiple recrystallization from a mixture of hexane and ethyl acetate for several times, Compound **4,** as red needles, was obtained 0.08 g (1×10^{-3}% wt. by wt. of the dried wood), m.p.135-136 °C. This compound showed a single spot on TLC with R_f value 0.5 (silica gel ; hexane : ethyl acetate (7:3)).

The molecular formula of this compound was proposed to be $C_{15}H_{16}O_2$ according to the elemental analysis result : Found %C 79.10 and % H 6.80 ; calcd. for $C_{15}H_{16}O_2$ MW. 228.29 : % C 78.94 and % H 7.01).

The FT-IR spectrum (Fig 20) revealed a strong absorption band at 1664 cm^{-1} which corresponded to the C=O stretching vibration of α,β-unsaturated ketone (quinone).[23] The characteristic absorption peak due to an aromatic moiety was observed at 1550 cm^{-1}. In addition, the ^{13}C-NMR spectrum (Fig 21) showed two carbonyl carbons at 181.9 and 182.3 ppm. This abovementioned result implied that Compound **4** might be a 1,2-naphthoquinone (I) or a 1,4-naphthoquinone (II).



I



II

One of the main features to distinguish between 1,2- and 1,4-naphthoquinones is the UV-visible absorption data. In general, in the UV-visible spectrum of a 1,2-naphthoquinone (mansonone C)[4] absorbances are exhibited at λ_{max} (EtOH) 206, 258 and 432 nm. (log ε = 4.14, 4.24 and 3.39) whereas that of a 1,4-naphthoquinone reveals λ_{max} (EtOH) at 250, 268 and 360 nm (log ε = 4.18, 3.92 and 3.53).[24] The UV-Vis spectrum of this compound (Fig 22) showed λ_{max} at 260 and 430 (log ε = 3.95 and 3.25) which were corresponded to a 1,2-naphthoquinones rather than the other. Besides the information obtained from the UV-Vis spectrum that could be used to differentiate the 1,2- and 1,4-naphthoquinone structures, the mass spectrum pattern was also found to be another informative tool in this aspect. Generally, in the mass spectra of 1,2-naphthoquinones, the molecular ion peaks (M^+) are weak or sometimes missing, the signals corresponding to $(M+2)^+$ are normally more intense.[25] In contrast, in those of 1,4-naphthoquinone the molecular ion peaks are always observed.[25] The mass spectrum of this compound did not show the molecular ion peak at m/e 228, but clearly displayed the $(M+2)^+$ peak at m/e 230 instead. Therefore, Compound **4** should be classified as a 1,2-naphthoquinone group.

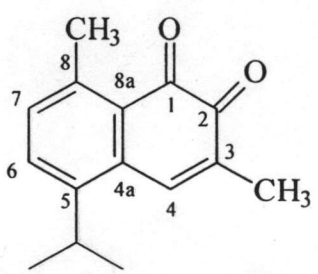
The ^{1}H-NMR spectrum (Fig 23) also displayed the typical characteristic pattern of an isopropyl group, *i.e.*, two methyl groups were observed at 1.26 ppm. (d, J=7.0 Hz, 6H) and a multiplet signal at 3.36 ppm (1H) assigned for the methine proton of this moiety. Another two signals with 3H integration each detected at 2.05 ppm (d, J=2.0 Hz, 3H) and 2.60 ppm (s) could be ascribed for methyl protons. Two doublet signals (1H each) centered at 7.16 and 7.29 ppm with J=8.2 Hz was suggestive of *p*-substituted aromatic moiety present in the molecule.[4] The other signal detected at 7.65 ppm (d, J=1.5 Hz, 1H) could be an olefinic proton.

From the above spectroscopic data, it was found that there were three substituents in this proposed 1,2-naphthoquinone structure ; *i.e.*, one isopropyl group and two methyl groups.

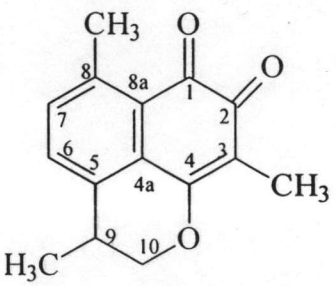
The ^{13}C-NMR spectrum (Fig 21) showed a total of fourteen carbon signals comprising four methyl carbons and a methine carbon in the range of 15.9 to 28.3 ppm. The aromatic and olefinic carbon signals (8 carbons) were in the range of 131.9 - 143.0 ppm. The two carbon signals detected at 181.9 and 182.3 ppm. could be assigned to carbonyl carbons of an *o*-quinone compound.[22] The assigned carbon signals based on the ^{13}C-NMR spectrum were confirmed by the information obtained from the DEPT-90 (Fig 24) and DEPT-135 spectra (Fig 25).

These two spectra clearly exhibited the presence of four methyl carbons at 15.9, 22.8 (two signals) and 23.7 ppm. There was no signal corresponding to a methylene carbon observed in these spectra. Four methine carbons were detected at 28.3, 131.9, 134.1 and 138.0 ppm. The remaining seven carbon signals were observed at 129.3, 132.4, 135.0, 143.0, 145.3, 181.9 and 182.3 ppm.

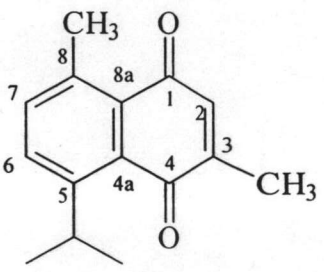
According to a literature search, naphthoquinones, particularly 1,2-naphthoquinones, are often found occurred as constituents of Mansonia plants (see also Table 1.1). The reported "mansonone C or 3,5-dimethyl-5-isopropyl-1,2-naphthoquinone" was found to possess both physical properties and proton signals in the ^{1}H-NMR spectrum very close to those of Compound **4**. The comparison of proton signals of mansonone C[4], Compound **4** and cadalenequinone I[24] is illustrated in Table 3.20.



Mansonone C



Mansonone E



Cadalenequinone I

Table 3.20 The comparison of proton signals of mansonone C[4], Compound **4** and Cadalenequinone I[24]

position	Chemical shift (ppm)		
	mansonone C	Compound **4**	Cadalenequinone I
4	7.67 (m,J=1 Hz, 1H)	7.63 (d, J=1.5 Hz, 1H)	6.68 (q, J=1.5 Hz, 1H)
6, 7	7.31 (J= 8.0 Hz, 2H)	7.16 (d, J=8.2 Hz, 1H)	7.34, 7.44 (AB, J= 9.0 Hz, 2H)
		7.40 (d, J=8.2 Hz, 1H)	
C**H**$_3$	2.08 (J=1.0 Hz, 3H)	2.05 (d, J=2.0 Hz, 3H)	2.13 (d, J=1.5 Hz, 3H)
	2.62 (3H)	2.60 (s, 3H)	2.68 (s, 3H)
$(CH_3)_2$-C**H**-	3.40 (m,1H)	3.36 (m,1H)	4.30 (m, 1H)
$(CH_3)_2$-CH-	1.30 (d, J=7.0 Hz, 6 H)	1.27 (d, J= 7.0 Hz, 6H)	1.27 (d, J=6.0, 6H)

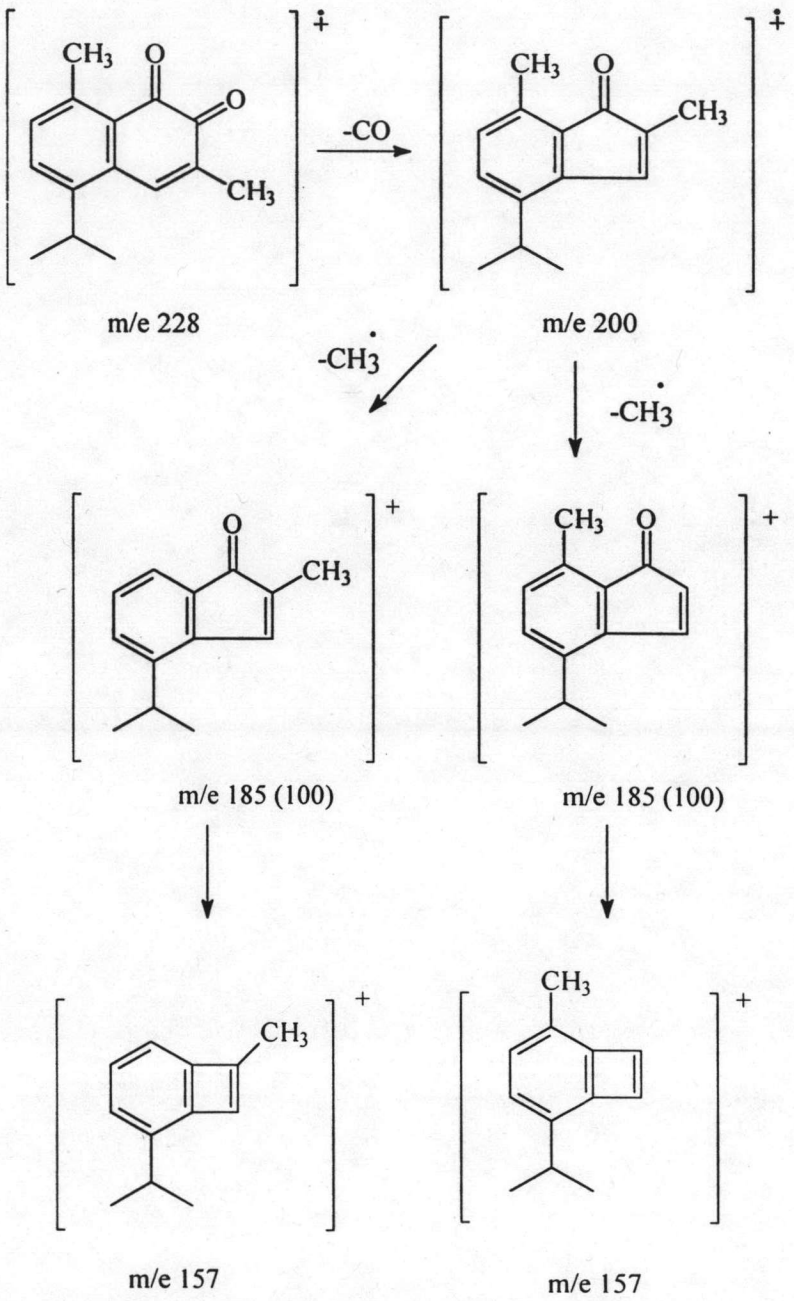
However, there was no report of the carbon signal assignment of mansonone C in previous literatures. The tentative assignment for carbon signals was consequently performed as presented in Table 3.21 by comparing with another known compound, mansonone E.[22]

Table 3.21 The comparison of carbon signals of mansonone E[22] and Compound **4**

position	Chemical shift (ppm)	
	mansonone E	Compound **4**
1	182.3	182.3
2	180.3	181.9
3	116.3	129.3
4	162.4	138.0
4a	127.4	135.0
5	126.9	132.4
6	132.6	131.9
7	134.9	134.1
8	142.9	145.3
8a	136.9	143.0
9	31.2	28.3
10	71.4	*
3-CH_3	7.8	16.0
8-CH_3	22.5	22.9
9-CH_3	17.6	*
$(CH_3)_2$-CH-	*	23.7 (2C)

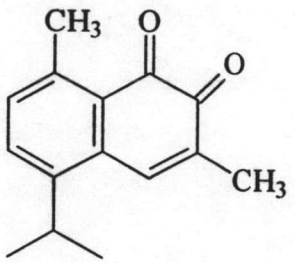
* no position in the corresponding to structure

The mass spectrum (Fig 26) displayed the molecular ion peaks at m/e (% rel. int.) 228 (2) (Calcd. for $C_{15}H_{16}O_2$: MW. 228.29). Other significant fragmentation peaks were detected at m/e 200 (61) (M^+-CO), 185 (100) (M^+-C_2H_3O) and 157 (10) (M^+-$C_3H_3O_2$). The possible fragmentation pattern of Compound **4** is proposed as shown in Scheme 3.4.



Scheme 3.4 The proposed fragmentation pattern of Compound **4**

According to all spectral evidence, it was clear that Compound **4** was 3,8-dimethyl-5-isopropyl-1,2-naphthoquinone or mansonone C. The structure is shown below.



Compound **4** : 3,8-dimethyl-5-isopropyl-1,2-naphthoquinone (mansonone C)

Purification, Properties and Structural Elucidation of Compound 5

Compound **5** was obtained from the separation of dichloromethane crude extract (Fraction II) (see also Table 3.12). After multiple recrystallization from hexane and ethyl acetate, Compound **5** was obtained as pale yellow crystals, m.p. 202-204 °C, 0.04 g (5×10^{-4} % wt. by wt. of the dried wood). This compound showed a single spot on TLC with R_f value 0.40 (silica gel ; hexane : ethyl acetate (7:3)).

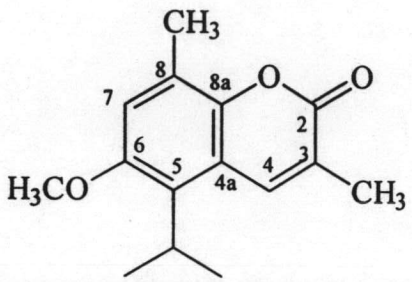
The molecular formula of this compound was proposed to be $C_{14}H_{16}O_3$ according to the elemental analysis result : Found %C 72.66 and % H 7.10 ; Calcd. for $C_{14}H_{16}O_3$ MW 232.28 : % C 72.41 and % H 6.89.

The UV-Vis spectrum (Fig 27) displayed characteristic absorption peaks of an α,β-unsaturated carbonyl of ester[19] at λ_{max} (CH_2Cl_2) 232, 290 and 342 nm (log ε = 4.06, 4.12 and 3.49). The FT-IR spectrum of this compound (Fig 28) was found to be similar to that of Compound **2**, *i.e.*, the strong absorption peak due to the >C=O stretching vibration of lactone at 1695 cm^{-1}. However, the presence of a broad absorption band at 3300-3400 cm^{-1} of O-H stretching vibration[26] in the IR spectrum of this compound was not found in that of Compound **2**. This implied that there should also be an O-H group as a substituent in Compound **5**.

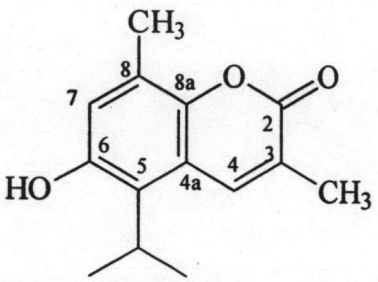
The ^{1}H-NMR spectrum of Compound **5** (Fig 29) exhibited a singlet signal at 2.31 ppm (3H) which coincided with methyl protons. Another signal with 3H integration detected at 2.22 ppm (d, J=1.2 Hz) could be assigned to methyl protons. The doublet

signal at 1.40 ppm (6H, J=8.0 Hz) revealed two methyl protons of an isopropyl group and the multiplet signal at 3.51 ppm (1H) was consistent with a methine proton of an isopropyl group. An additional singlet signal appeared at 5.67 ppm (1H) was attributed to a hydroxy proton. Moreover, two singlet signals at 6.79 and 7.90 ppm (1H each) were consistent with an aromatic proton and an olefinic proton, respectively.[20]

The comparison of ^{1}H-NMR signals of Compound **2** and Compound **5** is presented in Table 3.22.



Compound **2**



Compound **5**

Table 3.22 The comparison of the ^{1}H-NMR signals of Compound **2** and Compound **5**

position	Chemical shift (ppm)	
	Compound **2**	Compound **5**
4	7.90 (s)	7.90 (s)
7	6.90(s)	6.79 (s)
3-CH_3	2.23 (s)	2.22 (s)
8-CH_3	2.42 (s)	2.31 (s)
6-OCH_3	3.83 (s)	*
6-OH	*	5.67 (s)
$(CH_3)_2$-CH-	3.56 (m)	3.51 (m)
$(CH_3)_2$-CH-	1.38 (d)	1.40 (d)

* no position in the corresponding structure

The ^{13}C-NMR spectrum (Fig 30) exhibited the carbonyl of ester at 162.6 ppm. Six quarternary carbon signals were detected at 150.0, 147.6, 126.7, 124.3, 124.1 and 117.9 ppm were assigned to eiher olefinic or aromatic carbons. The signals at 26.6, 120.9 and 137.1 ppm which were observed from in DEPT-90 spectrum (Fig 31) showed signals for three methine carbons. The first signal could be assigned to a methine carbon of an isopropyl group. The remaining two signals were assigned to an olefinic carbon and an aromatic carbon, respectively. Four methyl carbons at 15.3, 17.6 (2 carbons) and 21.9 ppm were observed in the DEPT-135 spectrum (Fig 32) which displayed a total of seven signals of >CH- and -CH_3. There was no signal corresponding to CH_2 observed in this spectrum. In addition, the ^{13}C-NMR spectrum of this compound (Fig 30) was found to be similar to that of Compound **2**. The comparison of the ^{13}C-NMR signals of Compounds **2** and **5** is presented in Table 3.23.

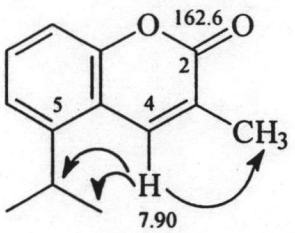
Table 3.23 The comparison of the ^{13}C-NMR signals of Compound **2** and Compound **5**

position	Chemical shift (ppm)	
	Compound **2**	Compouns **5**
2	162.1	162.6
3	124.6	124.3
4	136.7	137.1
4a	117.8	117.9
5	129.6	126.7
6	153.7	150.0
7	116.7	120.9
8	123.8	124.1
8a	146.6	147.6
3-CH_3	21.4	17.6
8-CH_3	15.7	15.3
6-OCH_3	56.2	*
$(CH_3)_2$-**C**H-	26.6	26.6
(C$H_3)_2$-CH-	17.6 (2C)	21.9 (2C)

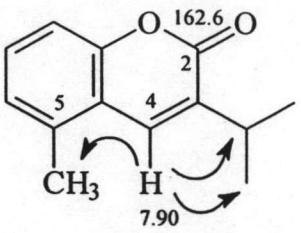
* no position in the corresponding structure

Additional important information for the structural elucidation of Compound **5** was obtained from the HMBC spectrum (Fig 33A). The signal of C-2 (at 162.6 ppm) which was detected and assigned to a carbonyl lactone carbon was clearly coupled with an olefinic proton at 7.90 ppm. This indicated that the C-4 position of this compound should be unsubstituted. Moreover, the ^{1}H-^{1}H NOESY spectrum (Fig 34) clearly showed that the former proton had a coupling interaction with the methyl proton signal at 2.22 ppm and also with other two signals of methyl protons (1.40 ppm) and methine proton

(3.51 ppm) belonging to an isopropyl group. Therefore, the substituent located at C-3 or C-5 may either be methyl (Structure I) or isopropyl group (Structure II). A possible sub-structure of this compound can be proposed as shown below.

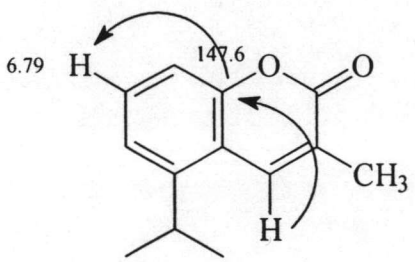


(I)

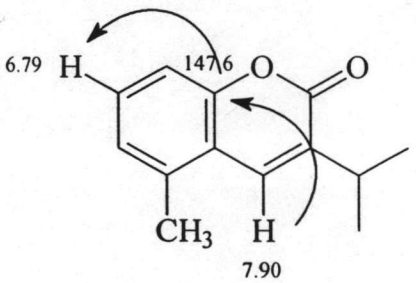


(II)

Further information was derived from the HMBC spectrum (Fig 33A). The carbon signal at 147.6 ppm was assigned for C-8a of the coumarin. This was because the proton signal at 7.90 ppm coupled with the carbon signal at 147.6 ppm. This carbon signal was also long-ranged coupled with the proton signal at 6.79 ppm which was an aromatic proton. All the above data may lead to the possible structures as shown below.

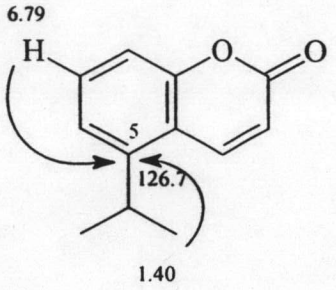


(III)



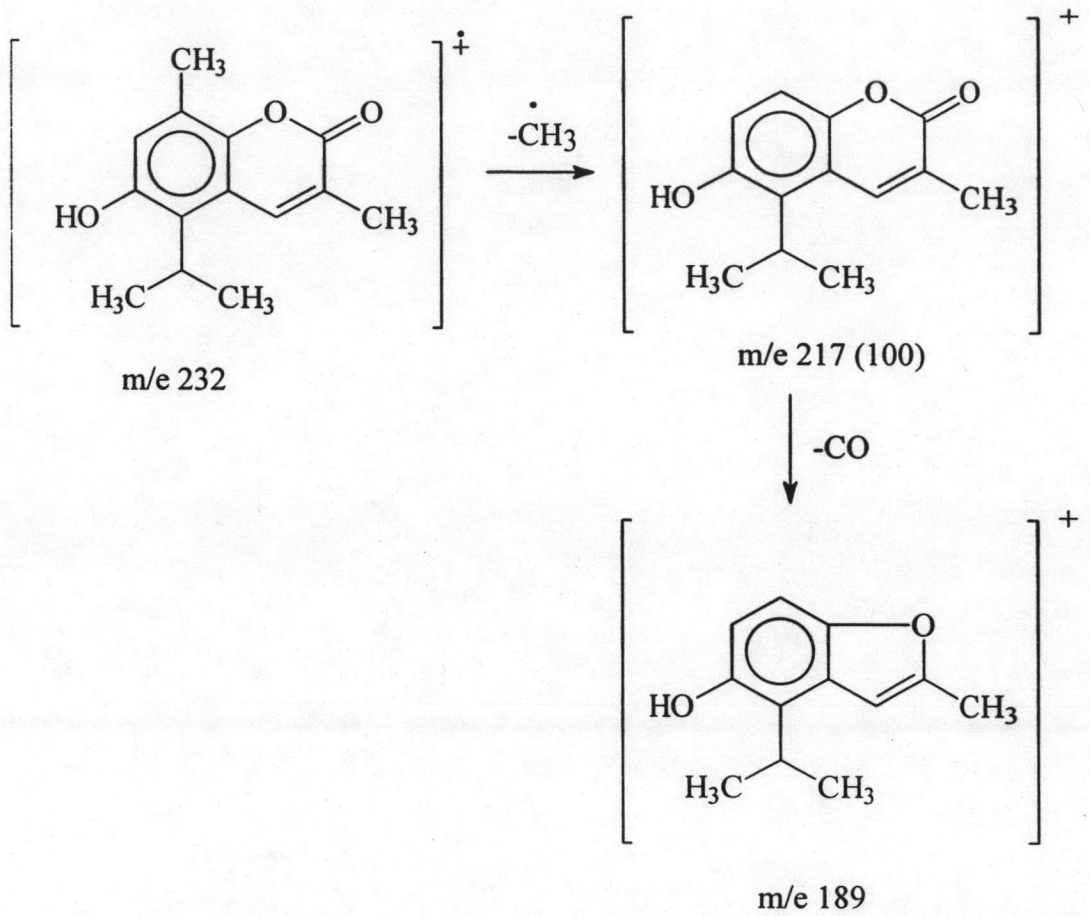
(IV)

Further informative spectroscopic evidence from the HMBC spectrum (Fig 33A) was the coupling interaction of two methyl protons of isopropyl group and the carbon signal at 126.7 ppm. The proton signal at 6.79 ppm was also found to couple with this carbon signal. All the above data strongly indicated that the C-5 position carried an isopropyl group. Therefore, a part of possible structure of Compound **5** must be I.



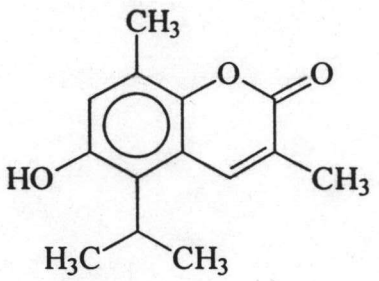
The C-6 and C-8 positions could be substituted by either methyl or hydroxy groups. This was clarified by the ^1H-^1H NOESY spectrum. The proton signal at 6.79 ppm coupled with both signals observed at 2.31 ppm (methyl protons) and at 5.67 ppm (hydroxy proton). However, the HMBC spectrum (Fig 33B) provided useful evidence to indicate that the C-9 signal which was detected at 147.6 ppm coupled with the proton signals of methyl group at 2.31 ppm. Therefore, the C-8 should be substituted by a methyl group.

The mass spectrum (Fig 35) displayed the molecular ion peak at m/e (% rel int.) 232 (81). Other important fragmentation peaks were observed at m/e 217 (100) (M^+-CH_3) and 189 (13) (M^+-C_2H_3O). The proposed fragmentation pattern of Compound **5** is shown in Scheme 3.5.



Scheme 3.5 The proposed fragmentation pattern of Compound **5**

Supported by spectroscopic data, it was obvious to conclude that Compound **5** was 3,8-dimethyl-5-isopropyl-6-hydroxy coumarin. The structure is shown below.



Compound **5** : 3,8-dimethyl-5-isopropyl-6-hydroxy coumarin

A literature search for the structures of coumarin compounds with a molecular formula $C_{14}H_{16}O_3$ was conducted. However, no reported compounds could match all of the obtained spectroscopic data. Therefore, this compound is another new naturally occuring coumarin derived from this plant.

Purification, Properties and Structural Elucidation of Component 6

The solid in red oil was obtained from the combination of fractions no.134-140, from the separation of dichloromethane fraction (Fraction II). The red oil was removed with methanol. The remained solid was recrystallized from methanol several times to yield a white amorphous solid designated as Component **6**, 0.005 g (6.25×10^{-5}% wt. by wt. of the dried wood), m.p. 65-67 °C.

The IR spectrum (Fig 36) of Component **6** clearly revealed the absorption band of a C=O stretching vibration of a ketone at 1705 cm^{-1} (s). The C-H stretching vibration and C-H bending vibration of $-CH_2-$, $-CH_3$ were present at 2852 and 1465 cm^{-1}, respectively. The additional absorption peak at 722 cm^{-1} (w) due to $-CH_2-$ (for chain > 4 carbons)[17-18] was observed. Therefore, this component should be a saturated long chain aliphatic ketones. Due to a limit amount of this substance obtained, no further structural elucidation was made.

Purification, Properties and Structural Elucidation of Mixture 7

Mixture **7** was a white solid which was separated from Fraction IID by medium pressure liquid chromatography. It was recrystallized from a mixture of dichloromethane and methanol for several times to give white needles, m.p. 160-162 °C, 0.001 g (1.25×10^{-5}% wt. by wt. of the dried wood). This mixture showed a single spot of R_f value 0.46 (silica gel/dichloromethane).

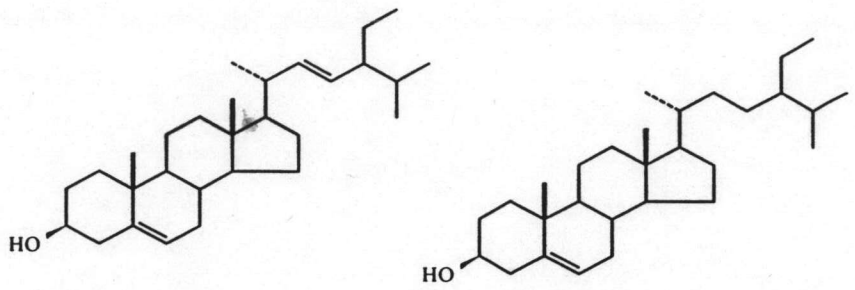
After monitoring by colour tests, this substance gave a deep green colour with Liebermann-Burchard's reagent which suggested the presence of a steroidal ring system.

Mixture **7** was then analyzed by GLC and the chromatogram of the mixture was compared with that of three standard steroids : campesterol, stigmasterol and β-sitosterol (Fig 37). The results of GLC analysis indicated that Mixture **7** was in fact a mixture of stigmasterol and β-sitosterol. The composition of Mixture **7** is presented in Table 3.24.

Table 3.24 The composition of steroids in Mixture 7

Steroid	Retention time of standard steroid (min)	Retention time of Mixture 7 (min)	Composition (%)
campesterol	16.99	-	-
stigmasterol	17.81	17.81	21.83
β-sitosterol	20.57	20.19	78.17

From this data, it could be concluded that Mixture **7** was a mixture of stigmasterol and β-sitosterol. The major component in this mixture was β-sitosterol.



stigmasterol β-sitosterol

Purification, Properties and Structural Elucidation of Compound 8

Compound **8** was obtained from the separation of the dichloromethane crude extract (Fraction II) (see also Table 3.12). After recrystallization from a mixture of hexane and ethyl acetate several times, Compound **8** as orange needle 2.0 g (0.025% wt. by wt. of the dried wood), melting point at 208-210 °C (dec) was obtained. This compound showed a single spot on TLC with R_f value 0.15 (silica gel ; hexane : ethyl acetate (7:3)).

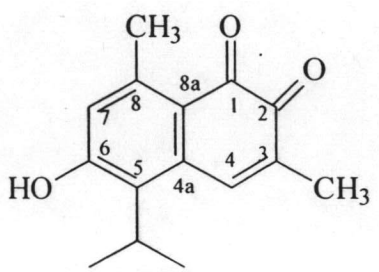
The molecular formula of this compound was proposed to be $C_{15}H_{16}O_3$ according to the elemental analysis result : Found % C 73.45 and % H 6.74 ; calcd. for $C_{15}H_{16}O_3$ MW. 244.29 : % C 73.77 and % H 6.55.

According to literature search studies, the chemicals belonging to the mansonone group which possessed the main common feature as 1,2-naphthoquinone were always found in *Mansonia* genus. That is also true for *Mansonia gagei* Drumm. since mansonone C (Compound **4**) was isolated from this particular species. Compound **8** was more polar than mansonone C. There was spectroscopic evidence showing some common characteristics of 1,2-naphthoquinones, *i.e.*, the FT-IR spectrum (Fig 38) revealed a strong absorption band at 1644 cm^{-1} which corresponded to the C=O stretching vibration of an α,β-unsaturated ketone (quinone). The characteristic absorption peaks due to an aromatic moiety were observed at 1585 and 1551 cm^{-1}. Another crucial piece of

information derived from the FT-IR spectrum was a strong absorption band at 3100-3300 cm^{-1} which revealed an O-H stretching vibration of a hydroxy functional group. The UV-vis absorption spectrum of this compound (Fig 39) displayed λ_{max} (CH_2Cl_2) at 240, 264 and 398 nm (log ε = 5.68, 5.72 and 5.90) which was well-matched to the characteristic bands of an α,β-unsaturated ketone.[27] Additionally, the mass spectrum (Fig 40) exhibited a less intense molecular ion peak at m/e 244. The latter was the characteristic of 1,2-naphthoquinone, but not of 1,4-naphthoquinone.[25]

The ^{1}H-NMR spectrum (Fig 41) showed the following characteristic signals : the signals attributed to the isopropyl group, *i.e.*, two methyl groups at 1.42 ppm (d, J=7.0 Hz, 6H) and the multiplet signal at 3.58 ppm (1H) could be assigned to the methine proton of this moiety. Two signals with 3H integration each were detected at 2.06 ppm (d, J=1.2 Hz, 3H) and 2.58 ppm (d, J=0.6 Hz, 3H) and could be assigned to methyl protons. The singlet signal appeared at 5.76 ppm (1H) was fitted to a phenolic hydroxy proton. In addition, two singlet signals with intensity corresponding to 1H each observed at 6.50 and 7.70 ppm could be tentatively assigned as an aromatic proton and an olefinic proton, respectively.

The literature search revealed that mansonone G which was reported as a chemical constituent of *Mansonia altissima*[27], was found to have both physical properties and various spectroscopic data such as ^{1}H and ^{13}C-NMR spectra, very close to those of Compound **8**. The comparison of the ^{1}H-NMR signals of mansonone G and those of Compound **8** is tabulated in Table 3.25.



mansonone G

Table 3.25 The comparison of the ^{1}H-NMR signals of Compound **8** and mansonone G[27]

Position	Chemical shift (ppm)	
	mansonone G	Compound **8**
4	7.69 (s, 1H)	7.70 (s, 1H)
7	6.49 (s, 1H)	6.50 (s, 1H)
3-CH_3	1.98 (s, 3H)	2.06 (s, 3H)
8-CH_3	2.47 (s,3H)	2.58 (s, 3H)
$(CH_3)_2$-**CH**-	3.48 (m,1H)	3.58 (m,1H)
$(CH_3)_2$-CH-	1.38 (d, J=7.0 Hz, 6H)	1.42 (d, J=7.0 Hz, 6H)

The ^{13}C-NMR spectrum (Fig 42) exhibited a total of fourteen carbon signals. In the range of 10-30 ppm, there were four methyl carbons and a methine carbon at 15.7, 21.4 (2 carbons), 23.6 and 28.1 ppm, respectively. The carbonyl carbons of an *o*-quinone[22] were observed at 181.2 and 183.8 ppm. The assigned carbon signals based on the ^{13}C-NMR spectrum were confirmed by the information obtained from the DEPT-90 and DEPT-135 spectra (Fig 43). There was no signal corresponding to a methylene carbon observed in the above spectra. Three methine carbons were detected at 28.1, 120.6 and 140.4 ppm, respectively. The remaining eight carbon signals could be observed at 122.9, 134.7, 135.7, 136.6, 147.8, 164.4, 181.2 and 183.8 ppm.

Since the ^{13}C-NMR signal assignment of mansonone G was lacking in the chemical literature, the tentative ^{13}C-NMR signal assignment was performed by comparison with that of the reported mansonone E.[22] The ^{13}C-NMR signals of both mansonone E and Compound **8** were compared as shown in Table 3.26.

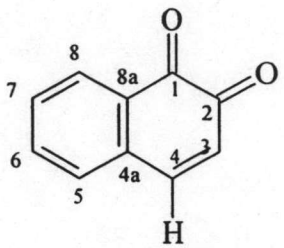
Table 3.26 The comparison of the ^{13}C-NMR signals of mansonone E and Compound **8**

Position	Chemical shift (ppm)	
	mansonone E	Compound **8**
1	182.3	181.2
2	180.3	183.8
3	116.3	135.7
4	162.4	140.4
4a	127.4	122.9
5	126.9	134.7
6	132.6	164.4
7	134.9	120.6
8	142.9	136.6
8a	136.9	147.8
9	31.2	28.1
10	71.4	*
3-CH_3	7.8	15.7
8-CH_3	22.5	23.6
9-CH_3	17.6	*
$(CH_3)_2$-CH-	*	21.4 (2C)

* no position in the corresponding structure

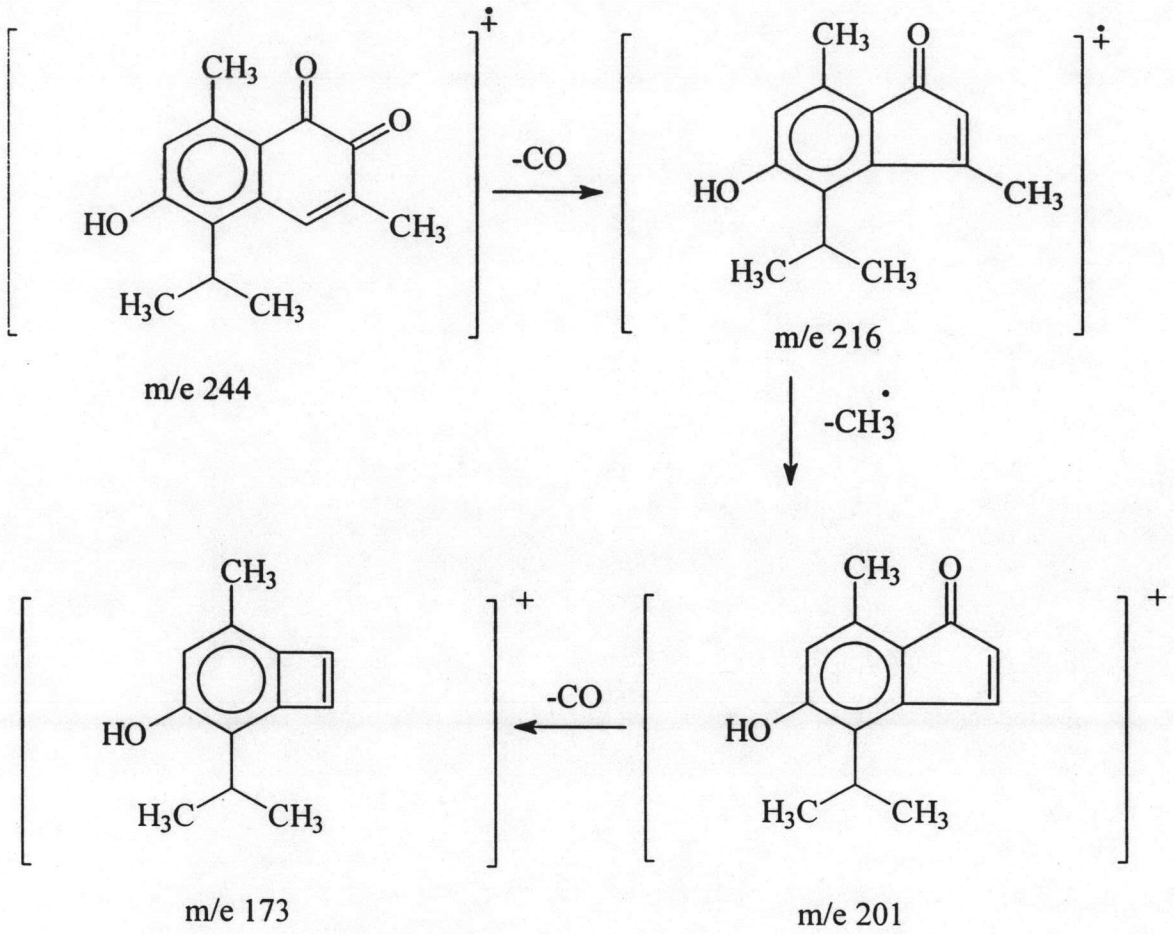
In addition to the spectroscopic data obtained from 1D-NMR, the 2D-NMR of Compound **8** was also conducted to provide complementary information for structural elucidation of this compound. The appropiate positions of the substituents of Compound **8** were confirmed by the HMBC spectrum (Fig 44A). To illustrate this, from the HMBC spectrum, the carbonyl carbon signal at 183.8 ppm coupled with the proton signal at 7.70 ppm while the carbon signal at 181.2 ppm was not coupled with any signal. This

information clearly revealed that the assignment of C-1 and C-2 of Compound 8 should be at 183.8 and 181.2 ppm, respectively.



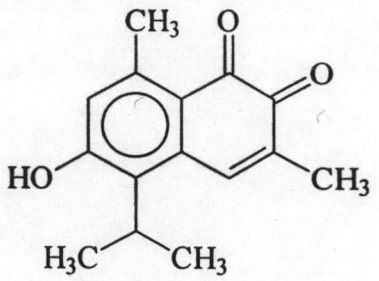
The information obtained from the NOESY spectrum (Fig 45) also confirmed the structure of Compound **8**. The positions of two methyl, an isopropyl and a hydroxy groups were assigned by comparison with the NOESY spectrum of Compound **2** (Fig **10**). The proton signal at 7.70 ppm coupled with methyl proton signal at 2.06 ppm, the methine proton of isopropyl group signal at 3.58 ppm and the methyl protons of isopropyl group signal at 1.42 ppm. This data implied that the substituents located at C-3 or C-5 may be either methyl or isopropyl group. The proton signal at 7.70 ppm coupled with the carbon signal at 147.8 ppm and the carbon signal at 147.8 ppm coupled with the proton signal of methyl group at 2.58 ppm. The proton signal at 6.50 ppm coupled with the proton signal of methyl group at 2.58 ppm. This information clearly indicated that the hydroxy substituent might be at C-6 position. The signal of the proton of hydroxy group at 5.76 ppm coupled with the signal of methine of isopropyl group at 3.58 ppm and the signal of methyl of isopropyl group at 1.42 ppm. According to all evidence obtained from tne NOESY spectrum, the substituents at C-5 should therefore be an isopropyl group and that at C-3 ought to be a methyl group.

The mass spectrum (Fig 40) displayed the molecular ion peak at m/e (% rel int.) 244 (Calcd. for $C_{15}H_{16}O_3$: MW. 244.29). Other important fragmentation pattern of the molecule was detected at m/e 216 (M^+-CO), 201 (M^+-C_2H_3O) and 173 (M^+-$C_3H_3O_2$). The possible fragmentation pattern of Compound **8** is shown in Scheme 3.6.



Scheme 3.6 The possible fragmentation pattern of Compound **8**

Supported by spectroscopic data and comparison with reported mansonone G, it was concluded that Compound **8** was 3,8-dimethyl-5-isopropyl-6-hydroxy-1,2-naphthalenedione. The structure is shown below.



Compound **8** : 3,8-dimethyl-5-isopropyl-6-hydroxy-1,2-naphthalenedione coumarin

Moreover, the structure of Compound **8** was confirmed by chemical means. The methylation of Compound **8** was carried out by usual methylation procedure[13] to obtain the methylated product was designated as Compound **8A** (20% yield), m.p. 155-157 °C.

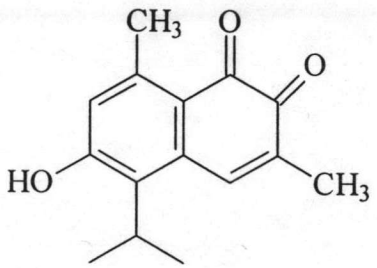
The FT-IR spectrum of Compound **8A** (Fig 46) revealed the characteristic absorption peaks very similar to those of Compound **8**, except for the disappearance of the OH absorption peak in the former. The mass spectrum of this methylated product (Fig 47) did not display the molecular ion peak. Other important fragmentation pattern of the molecule was observed at m/e (% rel. int) 230 (22) (M^+-CO) and 215 (73) (M^+-C_2H_3O).

The ^{1}H-NMR spectrum of Compound **8A** (Fig 48) clearly showed the methoxy protons signal at 3.88 ppm instead of the hydroxy proton at 5.76 ppm. The remaining signals were almost the same as those in Compound **8**. The comparison of ^{1}H-NMR signals of Compound **8** and Compound **8A** are recorded in Table 3.27.

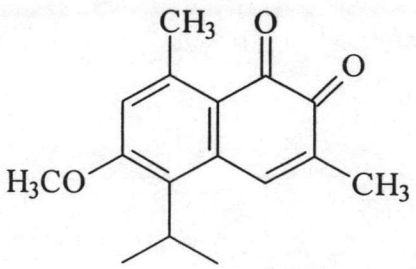
Table 3.27 The comparison of the ^{1}H-NMR signals of Compound **8** and Compound **8A**

Position	Chemical shift (ppm)	
	Compound **8**	Compound **8A**
4	7.70 (s, 1H)	7.69 (s, 1H)
7	6.50 (s, 1H)	6.59 (s, 1H)
3-CH_3	2.06 (s, 3H)	2.04 (s, 3H)
8-CH_3	2.58 (s, 3H)	2.62 (s, 3H)
6-OCH_3	*	3.88 (s, 3H)
$(CH_3)_2$-CH-	3.58 (m,1H)	3.58 (m, 1H)
$(CH_3)_2$-CH-	1.42 (d, J=7.0 Hz, 6H)	1.35 (d, J=7.1 Hz, 6H)

* no position in the corresponding structure



Compound **8**



Compound **8A**

Purification, Properties and Structural Elucidation of Compound 9

Compound **9** was obtained from the separation of the dichloromethane crude extract (Fraction II) (see also Table 3.12) as brown crystals, melting point at 272-275 °C (dec). After recrystallization from methanol several times 0.08 g (1×10^{-3}% wt. by wt. of the dried wood) was obtained. This compound showed a single spot on TLC with R_f value 0.33 (silica gel; hexane : ethyl acetate (3:7)).

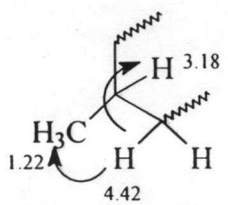
The molecular formula of this compound was proposed to be $C_{15}H_{14}O_4$ according to the elemental analysis result : Found %C 69.41 and % H 5.67 ; calcd. for $C_{15}H_{14}O_4$ MW. 258.27 : % C 69.76 and % H 5.42.

The FT-IR spectrum of Compound **9** (Fig 49) gave the following major absorption peaks to those of Compound **8** (Fig 38) which indicated that Compound **9** should be a 1,2-naphthoquinone compound (ν_{max} 1670 cm^{-1} (C=O), 3100-3300 cm^{-1} (O-H) and 1564 cm^{-1} (C=C aromatic)). The UV-Vis spectrum of this compound (Fig 50) showed λ_{max} (CH_2Cl_2) at 230, 272, 300 and 374 nm (log ε = 4.68, 4.80, 4.49 and 4.24) indicating an α,β-unsaturated ketone and aromatic moiety.[22] In addition, the mass spectrum (Fig 51) gave the weak molecular ion peak at m/e (% rel int.) 258 (23).

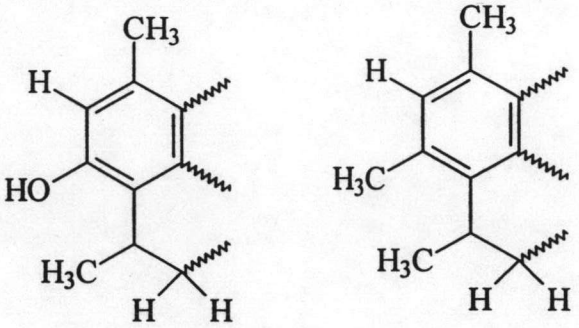
The significant information for the structural elucidation of this compound was obtained from the ^{1}H-NMR spectrum (Fig 52). The doublet signal appeared at 1.22 ppm (3H) with J=7.0 Hz was attributed to the methyl protons. Two singlet signals at 1.81 and 2.48 ppm with 3H integration each were, no doubt, methyl protons. The methine proton was detected as a multiplet signal (1H) at 3.18 ppm. Two double doublet signals of -CH-CH_2-O- group were centered at 4.29 ppm (J=10.7 and 3.3 Hz) and 4.42 ppm (J=10.7 and 0.9 Hz). A singlet signal (1H) at 6.75 ppm was indicative of an aromatic proton present in the molecule.

From the above spectroscopic data, it was found that there were three substituents in this structure : three methyl groups and a hydroxy group.

From the NOESY spectrum (Fig 53), it was observed that the methylene proton signal at 4.42 ppm coupled with the methyl proton signal at 1.22 ppm and the methine proton at 3.18 ppm. A part of possible structure can therefore be drawn as follows:



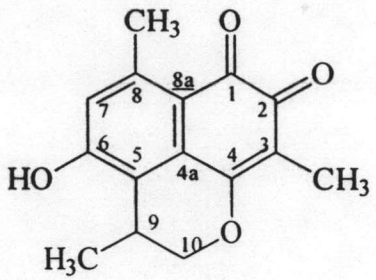
In addition, the aromatic proton signal at 6.75 ppm coupled with the methyl proton signal at 2.48 ppm. However, the latter signal was found not to have any long range interaction with other proton signals in the spectrum. The suitable substituent at C-6 may be either a hydroxy group (I) or another methyl group (II). Therefore, the possible structures of Compound **9** could be limited as shown below :



I II

The ^{13}C-NMR spectrum (Fig 54) exhibited a total of fourteen signals. The DEPT-90 spectrum (Fig 55) showed signals for two methine carbons at 25.5 and 119.0 ppm. The first signal was assigned to a methine carbon of CH_3-C**H**-CH_2-. The other signal was assigned to an aromatic carbon. The DEPT-135 spectrum (Fig 56) displayed six signals of >CH-, -CH_2- and -CH_3. Three methyl carbons were detected at 7.8, 16.9 and 22.6 ppm. There was also a signal corresponding to a methylene carbon observed in this spectrum at 71.6 ppm. The remaining nine signals in the ^{13}C-NMR spectrum were compatable with quarternary carbons which were observed at 114.5 (2C), 125.2, 127.9, 144.5, 159.4, 161.5, 179.5 and 180.0 ppm, corresponding to either olefinic or aromatic carbons.

According to the above spectroscopic data together with the information obtained from the literature search on chemical constituents of plants in *Mansonia* genus, it was seen that Compound **9** resembled mansonone H.[22] The comparison of the ^{1}H and ^{13}C-NMR signals of both mansonone H and Compound **9** was thereby performed as shown in Tables 3.28 and 3.29, respectively.



mansonone H

Table 3.28 The comparison of the ^{1}H-NMR signals of mansonone H[22] and Compound **9**

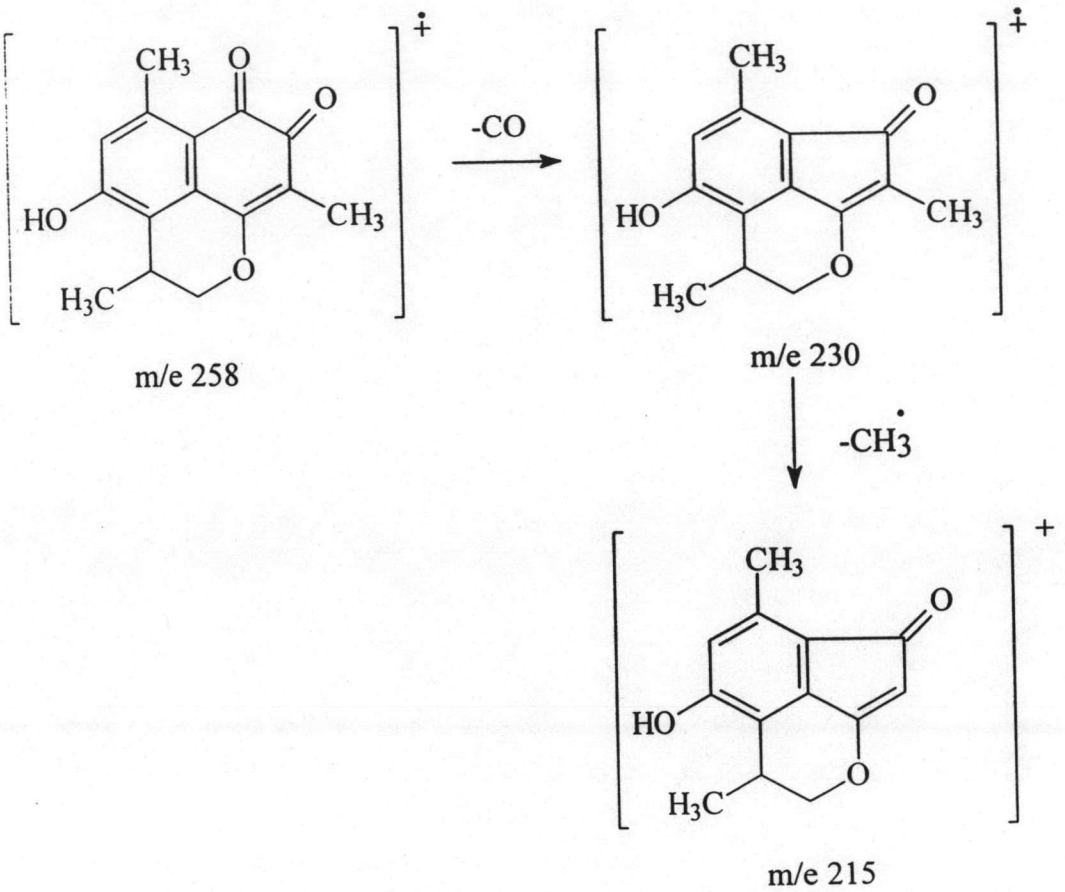
position	Chemical shift (ppm)	
	mansonone H[19]	Compound **9**
7	6.75 (s, 1H)	6.75 (s, 1H)
9	3.16 (m, 1H))	3.18 (m, 1 H)
10a	4.40 (br d, J=10.8 Hz, 1H)	4.42 (dd, J=10.7, 0.9 Hz, 1H)
10b	4.26 (dd, J=10.8, 3.0 Hz, 1H))	4.29 (dd, J=10.7, 3.3 Hz, 1H)
3-CH_3	1.80 (s, 3H)	1.95 (s, 3H)
8-CH_3	2.48 (s, 3H)	2.64 (s, 3H)
9-CH_3	1.20 (d, J=7.0 Hz)	1.22 (d, J= 7.0 Hz, 3H)
6-OH	11.17 (br s)	*

* no position in the corresponding structure

Table 3.29 The comparison of the ^{13}C-NMR signals of mansonone H[22] and Compound **9**

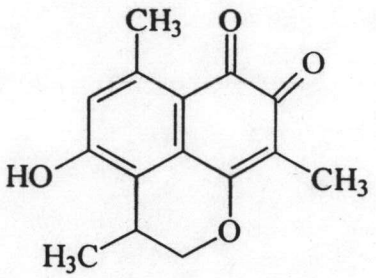
position	Chemical shift (ppm)	
	mansonone H	Compound **9**
1	180.2	180.0
2	179.7	179.5
3	114.7	114.5
4	161.7	161.5
4a	125.5	125.2
5	119.1	119.0
6	159.6	159.3
7	119.1	119.0
8	144.7	144.5
8a	128.1	127.9
9	25.6	25.5
10	71.7	71.6
3-CH_3	7.9	7.8
8-CH_3	22.7	22.6
9-CH_3	17.0	16.9

The mass spectrum of Compound **9** exhibited a molecular ion peak at m/e (% rel int.) 258 (23). Other significant fragmentation peaks were detected at m/e 230 (100) (M^+-CO) and 215 (22) (M^+-C_2H_3O). The possible fragmentation pattern of Compound **9** is shown in Scheme 3.7.



Scheme 3.7 The proposed fragmentation pattern of Compound **9**

Supported by various spectroscopic data and comparison with reported mansonone H, Compound **9** was assigned the structure of 2,3-dihydro-4-hydroxy-3,6,9-trimethyl naphtho[1,8-bc]pyran-7,8-dione. The structure is shown below.



Compound **9** : 2,3-dihydro-4-hydroxy-3,6,9-trimethyl naphtho[1,8-bc]pyran-7,8-dione

Purification, Properties and Structural Elucidation of Compound 10

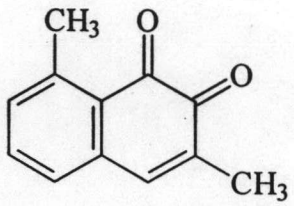
Compound **10** was isolated from the separation of the dichloromethane crude extract (Fraction II) (see also Table 3.12). After recrystallization from methanol several times, Compound **10,** as pale brown solid, was obtained 0.004 g (5×10^{-5}% wt. by wt. of the dried wood), melting point at 283-284 °C (dec). This substance presented a single spot on TLC with R_f value 0.33 (silica gel; hexane : ethyl acetate (3:7)).

The TLC experiment displayed that this compound was more polar than Compound **9** (mansonone H). Nevertheless, the mass spectrum (Fig 56) revealed its molecular ion peak (M^+) at m/e 258 which was the same as that of mansonone H. In addition, the FT-IR spectrum (Fig 57) gave the absorption peaks close to those of Compound **9** (ν_{max} 3100-3450 cm^{-1} (O-H), 1685 cm^{-1} (C=O) and 1588 cm^{-1} (C=C aromatic). All observations implied that this compound should be classified as a 1,2-naphthoquinone.

The ^{1}H-NMR spectrum of this compound (Fig 58) exhibited three methyl groups at 1.69, 1.77 and 2.52 ppm, respectively. A singlet signal at 6.72 ppm (1H) could be assigned for an aromatic proton. The ^{13}C-NMR spectrum (Fig 59) revealed three methyl carbon signals at 7.9, 20.0 and 25.5 ppm, respectively which corresponded to the spectroscopic data obtained from the ^{1}H-NMR. The DEPT 90 and 135 spectra (Fig 60) exhibited methine carbon signals at 25.5 and 120.0 ppm. There was no signal

corresponding to a methylene carbon in this spectrum. Ten quarternary carbons were detected at 95.8, 107.2, 116.1, 130.3, 135.7, 145.9, 157.0, 137.3, 176.9 and 179.9 ppm. In addition, the ^{13}C-NMR spectrum exhibited in total fourteen carbon signals. However, the number of carbons in this molecule should be fifteen carbons because the signal at 25.5 ppm was a methine carbon signal possibly overlapped with a methyl signal.

All the above spectral data provided a partial structure of Compound **10** as shown below :



Unfortunately, the amount of this compound that was obtained was insufficient for full structural elucidation. Therefore, the further characterization of the structure of this compound was not made.

Cardiac Glycoside Tests for Methanol Crude Extract (Fraction III), Ethyl Acetate Crude Extract (Fraction IV) and Butanol Crude Extract (Fraction V)

Samples of crude extracts (5 mL) were submitted for cardiac glycoside tests according to the procedure described in Chapter 2. The results are shown in Table 3.30

Table 3.30 The results of cardiac glycoside test for Fractions III, IV and V

Crude Extract	Liebermann-Burchard reaction	Kedde's reaction	Keller-Kiliani reaction
methanol (Fraction III)	+	+	+
ethyl acetate (Fraction IV)	+	+	+
butanol (Fraction V)	+	+	+

(+) : positive result

From these preliminary screening results for cardiac glycosides, it was found that both ethyl acetate and butanol fractions clearly exhibited the possibility of the presence of cardiac glycosides. An attempt to isolate the cardiac glycoside was therefore performed as described below.

Separation of Butanol Crude Extract (Fraction V)

The butanol crude extract (10 g) was chromatographed on silica gel 60G Art. 7734 (150 g.) using column chromatography. The lower phase of a mixture of dichloromethane : methanol : water (65:35:10) was used as the eluent. The results of separation are presented in Table 3.31.

Table 3.31 The results of the separation of butanol crude extract (Fraction V)

Eluent (% volume by volume)	Fraction no.	Remarks	Weight (g)
CH_2Cl_2 : CH_3OH : H_2O (65:30:10)	1-10	black solid (Fraction V A)	2.45
	11-20	black solid	3.17
	21-30	black solid	3.49

Reseparation of Fraction VA

After combination of fractions 1-10 (Fraction VA), a black solid was obtained. A red-brown solution was gained by dissolving the black solid in ethyl acetate. This solution showed 1 spot and 1 band on TLC (silica gel, solvent system; ethyl acetate : methanol : water (81:11:8) using Kedde's reagent as a detecting agent). Therefore an attempt was made to reseparately of the mixture by column chromatography using sephadex LH_{20} as an adsorbent. A mixture of ethyl acetate : methanol : water (81:11:8) was used as an eluent. The results of the separation are shown in Table 3.32.

Table 3.32 The results of the separation of fraction 1-10 (Fraction VA)

Fraction no.	Remarks	Weight (g)
1-7	yellow solid	0.27
8-17	yellow oil	0.34
18-23	brown oil	0.39

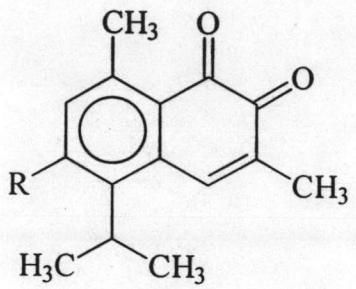
After combination of fraction 1-7, a yellow solid were obtained. However, there were 4 spots showed on TLC (silica gel, solvent system; ethyl acetate : methanol : water (81:11:8), using Kedde's reagent as a detecting agent). From this result, it could be seen that there were at least 4 cardiac glycosides present in the butanol fraction. The aid of modern techniques of separation such as MPLC or HPLC may need to be applied for the better resolution of this separation.

Study on Biological Activity of Isolated Compounds

Literature survey of the biological activity of 1,2-naphthoquinones revealed that this group of compounds exhibited a wide spectrum of biological activity. For instance, in 1970 Overeen and coworkers reported the fungicide activity against *Ceratocystis ulmi* of mansonone E and F.[28] In 1983, Dumas and his colleagues isolated six mansonones, namely mansonones A, C, D, E, F and G, from *Ulmus amercicana* and found that these compounds also possessed antifungal activity.[29] In addition, Chen *et al* investigated the root barks of *Helicteres angustifolia* in 1990 and reported the isolation of mansonones E, F, H and M. These compounds were claimed to be antitumor agents according to the folkoric medicinal uses.[30] In the same year, Villamil and his colleagues studied effects of mansonones C, E and F derived from *Mansonia altissima* Chev. on lipid peroxidation, P450 monooxygenase activity and superoxide anion generation by rat liver microsomes.[31] They found that mansonone C had a greater effect than mansonones E and F on NADPH-dependent lipid peroxidation, O^{-}_{2} production and ascorbate oxidation, whereas mansonone E was more effective than mansonones C and F on aniline 4-hydroxylase activity. Mansonones E and F did not inhibit hydroxyperoxide-dependent lipid peroxidation, cytochrome P450 destruction, or microsomal aniline 4-hydroxylase activity. Mansonone C inhibited to a limited degree *tert*-butyl hydroxyperoxide-dependent lipid peroxidation, this inhibition being increased by NADPH. Mansonone A was in all respects relatively less effective than mansonones C, E and F. It is postulated that mansonones C, E and F inhibited microsomal lipid peroxidation, and cytochrome P450-catalyzed reactions by diverting reducing equivalent from NADPH to dioxygen, but mansonone C (including its reduced form) may also exert direct antioxidant activity.

The Results of Brine Shrimp (*Artemia salina* Linnaeus) Cytotoxicity Test of Isolated Compounds

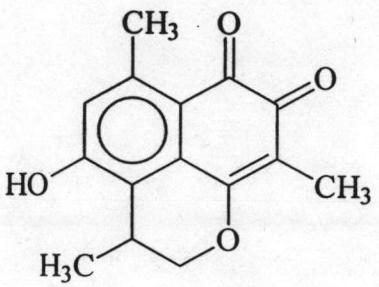
According to the preliminary cytotoxicity screening test (see also Table 3.1), the dichloromethane crude extract was selected for further study and searching for bioactive compounds that were toxic to brine shrimp. The isolated compounds were therefore submitted to the brine shrimp test in order to confirm that biological activity. The results are shown in Table 3.33.



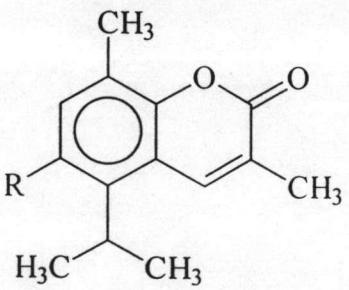
Compound **4** R=H

Compound **8** R= OH

Compound **8A** R=OCH_3

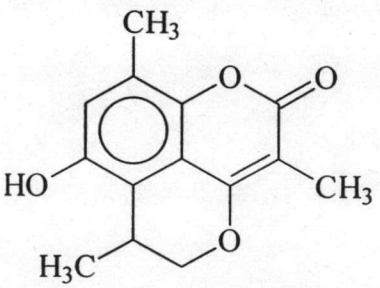


Compound **9**



Compound **5** R= OH

Compound **2** R= OCH_3



Compound **3**

Table 3.33 The results of brine shrimp cytotoxicity test of isolated compounds

Sample	LC_{50}			
	6 hr.	Activity	12 hr.	Activity
Compound **2**	-	no activity	3166.93	no activity
Compound **3**	1419.40	no activity	187.85	low activity
Compound **4**	92.27	medium activity	8.20	high activity
Compound **5**	-	no activity	19.6	medium activity
Compound **8**	511.31	low activity	8.29	high activity
Compound **8A**	-	no activity	24.12	medium activity
Compound **9**	-	no activity	50.70	medium activity

The compounds obtained from *M. gagei*, can be categorized into two main groups, *i.e.*, 1,2-naphthoquinones and coumarin-based compounds. From the results of brine shrimp cytotoxicity tests, it was found that 1,2-naphthoquinone-type compounds revealed much higher toxicity than those of the others.

The substituents on the parent compounds were found to have great influence on the activity. To illustrate this, a series of 1,2-naphthoquinone compounds, Compounds **4**, **8** and **8A** can be examined. The activity results clearly showed that when the substituents were H or OH they would provide much more cytotoxic activity against brine shrimp than that having OCH_3. This same trend could be observed in coumarin-type structures, *i.e.*, Compounds **2** and **5**. Whereas the former bearing a OCH_3 did not reveal any cytotoxic activity, the latter with an -OH substituent, showed much better activity. Therefore, the structure-activity relationship (SAR) study is definitely called for further investigation.

The Results of Antitumor against *Hepatocellular carcinoma* (Bel-7402) of Isolated Compounds

The isolated compounds, Compounds **2**, **8** and **9** were also tested for antitumor activity against *Hepatocellular carcinoma* and the results are shown in Table 3.34.

Table 3.34 The results of antitumor activity against *Hepatocellular carcinoma* (Bel 7402)

Sample	Concentration (μmol/l)	Inhibition (%)	Estimation
Compound **2**	0.1	21.30	
	1.0	56.80	++
	10.0	60.35	
Compound **8**	0.1	50.29	
	1.0	49.70	+
	10.0	74.55	
Compound **9**	0.1	17.75	
	1.0	-	-
	10.0	5.32	

For this specific antitumor test against *Hepatocellular carcinoma*, it could be observed that coumarin-type compound, Compound **2,** showed quite promising results. Among two 1,2-naphthoquinones tested, a simple 1,2-naphthoquinone, Compound **8,** gave a better result than 2,3-dihyro-4-hydroxy-3,6,9-trimethyl-naphtho[1,8-bc]pyran-7,8-dione (Compound **9**).