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Coumarins from the heartwoods of *Mansonia gagei* Drumm.

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Abstract

Three coumarins and three known mansonones were isolated from the heartwood of *Mansonia gagei* Drumm. The structures of the three coumarins were elucidated as 3,8-dimethyl-5-isopropyl-6-methoxycoumarin (mansonrin A) 3,8-dimethyl-5-isopropyl-6-hydroxycoumarin (mansorin B) and 2,3-dihydro-3,6,9-trimethyl naphtho[1,8-bc]pyran-7-oxa-8-one (mansorin C) by analyses of physical properties and spectroscopic data. The cytotoxicity of the isolated compounds against brine shrimp *Artemia salina* Linn. was also evaluated. © 2002 Published by Elsevier Science Ltd.

Keywords: *Mansonia gagei*; Sterculiaceae; Coumarin; Mansonone

1. Introduction

Mansonia gagei Drumm., a traditional medicinal plant in the Sterculiaceae family found in Thailand, has been used as cardiac stimulant, onilivertigo, antiemitic, antidepressant and refreshment agent (Pongboonrod, 1976). Literature surveys on the chemical constituents of the *Mansonia* genus revealed that the heartwood of *Mansonia altissima* have been examined. Constituents that have been previously isolated include primarily 1,2-naphthoquinones of the mansonone type, mansonones A–H (Marini Bettolo et al., 1965; Tanaka et al., 1966), I (Shimada et al., 1967) and L (Galeffi et al., 1969). Two additional cardiac glycosides (stophanthidin-2,3-di-*O*-methyl-6-deoxy-β-D-glucopyranoside and stophanthidin-3-*O*-methyl-6-deoxy-β-D-gluco-pyranoside) were reported in 1967 (Allgeier et al., 1967). However, to the best of our knowledge, there have been no reports concerning the chemical constituents of *Mansonia gagei* Drumm. We report in this study the isolation and structural elucidation of constituents of the heartwood of *Mansonia gagei* and evaluation of their toxicity to brine shrimp.

2. Results and discussion

The hexane and dichloromethane crude extracts of the heartwood of *Mansonia gagei* were screened for cytotoxicity against brine shrimp *Artemia salina* Linn. The fact that the LC_{50} was 23.69 and 22.83 μg/ml, respectively, prompted further investigation of both extracts for active principles.

Extensive chromatography of the hexane and dichloromethane crude extracts on silica gel column yielded three new coumarins (**1**–**3**) along with three known mansonones (**4**–**6**). The known mansonones were mansonones C (**4**) (Marini Bettolo et al., 1965), G (**5**) (Tanaka et al., 1966; Letcher and Shirley, 1992) and H (**6**) (Tanaka et al., 1966). The structures of these compounds were elucidated on the basis of spectroscopic analysis.

Compound **1** was isolated as a pale yellow crystal and having a molecular formula of $C_{15}H_{18}O_3$ as established by EIMS and elemental analysis. The molecular ion was observed at m/z 246 $[M]^+$. The IR absorption of **1** showed the presence of an α, β-unsaturated lactone at 1711 cm^{-1} and an aromatic moiety at 1600 cm^{-1} (Pretsch et al., 1989b). The ^{13}C NMR and DEPT spectra indicated that **1** possessed a coumarin skeleton based on a total of 15 carbons, comprising a carbonyl lactone carbon at δ_C 169.1, eight olefinic and aromatic carbons between δ_C 116.7 and 153.7, four methyl carbons at δ_C 15.7, 17.6 (2C) and 21.4, a methoxy carbon at δ_C 56.2, and a methine carbon at δ_C 26.6. In the ^1H NMR spectrum of **1** (Table 1), an isopropyl (δ_H 1.38, 6H, *d*, $J=7.3$ Hz; 3.56, 1H, *m*), two methyl (δ_H 2.23, 3H, *s*; 2.42, 3H, *s*) and one methoxy (δ_H 3.83, 3H, *s*) groups were observed. The complete assignments of these protons were established from analysis of the HMBC and ^1H–^1H NOESY NMR spectral data. In the HMBC spectrum, the carbonyl lactone carbon at δ_C

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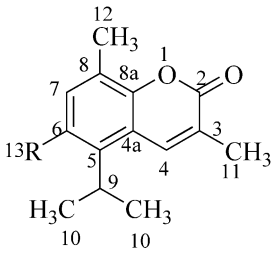

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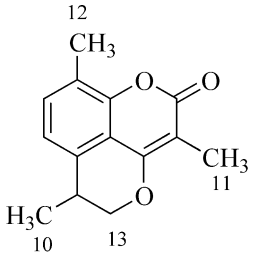

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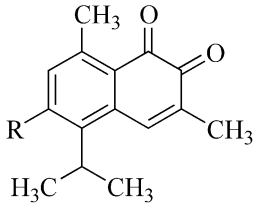
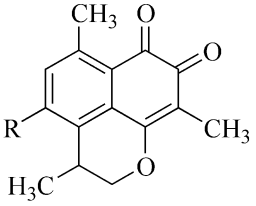


4 R = H

5 R = OH



6 R = H

7 R = OH

162.1 (C-2) showed correlations with H-4, whereas the quaternary carbon at δ_C 146.6 (C-8a) showed correlations with H-4, H-7 and Me-8. The quaternary carbon at δ_C 129.6 (C-5) revealed $^3J_{C\text{-}H}$ interactions with $(Me)_2$-9 and H-7. Moreover, the ^1H–^1H NOESY spectrum supplied important data to prove the actual structure of this compound. Significant correlations between H-7, Me-3, $(Me)_2$-9 and H-9, as well as between H-7, Me-8 and OMe-6, were observed. Therefore, compound **1**, named mansorin A, was determined as 3,8-dimethyl-5-isopropyl-6-methoxycoumarin (Fig. 1).

Table 1
^1H NMR and ^{13}C NMR spectral data of compounds **1** and **2** in $CDCl_3$[a]

	1		**2**	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
2	–	162.1	–	162.6
3	–	124.6	–	124.3
4	7.90, *s*, 1H	136.7	7.90, *s*, 1H	137.1
4a	–	117.8	–	117.9
5	–	129.6	–	126.7
6	–	153.7	–	150.0
7	6.90, *s*, 1H	116.7	6.79, *s*, 1H	120.9
8	–	123.8	–	124.1
8a	–	146.6	–	147.6
9	3.56, *m*, 1H	26.6	3.51, *m*, 1H	26.6
10	1.38, *d*, 6H (7.3)	17.6 (2C)	1.40, *d*, 6H (8.0)	21.9 (2C)
11	2.23, *s*, 3H	21.4	2.22, *d*, 3H (1.2)	17.6
12	2.42, *s*, 3H	15.7	2.31, *s*, 3H	15.3
13	3.83, *s*, 3H	56.2	5.67, *s*, 1H	–

[a] Assignments are based on DEPT, HMBC, HMQC and ^1H–^1H NOESY experiments, and chemical shift values.

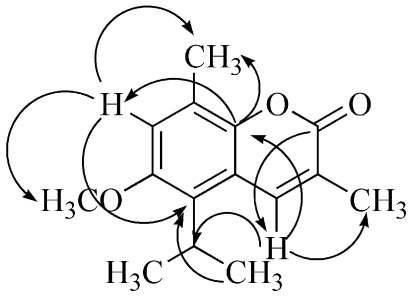


Fig. 1. HMBC and ^1H–^1H NOESY correlations of **1**.

The ^1H and ^{13}C NMR spectra of compound **2**, $C_{14}H_{16}O_3$, were similar to those obtained for compound **1**, except for the absence of the methoxyl group signal and the presence of an additional signal at δ_H 5.67 (1H, *s*), assignable to a hydroxyl group at position 6. In the ^1H–^1H NOESY spectrum, H-7 (δ_H 6.79) showed correlations with Me-8 (δ_H 2.31) and OH-6 (δ_H 5.67). Thus, the structure of **2**, another natural occuring compound named mansorin B, could be deduced to be 3,8-dimethyl-5-isopropyl-6-hydroxycoumarin.

Compound **3** (white crystals with the molecular formula of $C_{14}H_{14}O_3$) also possessed a coumarin skeleton based on its ^{13}C NMR (Table 2) and IR spectra. The latter revealed an α,β-unsaturated lactone absorption at 1705 cm^{-1}. The ^1H and ^{13}C NMR spectral data of **3** was similar to that of mansonone E **(7)** (Table 2) except for **3** having one carbon less than that reported for mansonone E (Chen et al., 1990). In addition, the structure of **3** was confirmed by the *para*-substituted aromatic pattern signals

Table 2
^1H NMR and ^{13}C NMR spectral data of compound **3** and mansonone E **(7)** in $CDCl_3$[a]

	3		Mansonone E	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	–	–		182.3
2	–	164.2	–	180.3
3	–	102.5	–	116.3
4	–	159.4	–	162.4
4a	–	120.0	–	127.4
5	–	110.8	–	126.9
6	7.28, *d*, 1H (7.6)	124.0	7.35, *d*, 1H (7.9)	132.6
7	6.98, *d*, 1H (7.6)	132.4	7.26, *d*, 1H (7.9)	134.8
8	–	134.4	–	142.9
8a	–	150.1	–	136.9
9	3.15, *m*, 1H	31.0	3.09, *m*, 1H	31.2
10	1.32, *d*, 3H (7.4)	15.4	1.37, *d*, 3H (7.0)	17.6
11	2.04, *s*, 3H	8.9	1.95, *s*, 3H	7.8
12	2.39, *s*, 3H	17.0	2.65, *s*, 3H	22.5
13	4.10, *dd*, 1H (6.7, 11.0) 4.39, *dd*, 1H (4.0, 10.7)	72.5	4.41, *dd*, 1H (4.0, 10.7) 4.23, *dd*, 1H (5.2, 10.7)	71.4

[a] Assignments are based on DEPT, HMBC, HMQC and ^1H–^1H NOESY experiments, and chemical shift values.

(Pretsch et al., 1989a): two doublet signals, at δ_H 6.98 (H-7) and 7.28 (H-6) with $J = 7.6$ Hz. In the ^1H NMR spectrum (Table 2), two doublet of doublet signals centered at δ_H 4.10 (1H, $J = 6.7$ and 11.0 Hz) and 4.39 (1H, $J = 4.0$ and 10.7 Hz) were assigned to a –CH–C**H$_2$**–O– group. From the ^1H–^1H NOESY spectrum, H-7 (δ_H 6.98) was coupled with H-6 (δ_H 7.28) and Me-10 (δ_H 1.32). The latter protons also showed a correlation between the methine proton at δ_H 3.15 (H-9) and the methylene protons at δ_H 4.10 and 4.39 (CH_2-13). These data established **3** to be 2,3-dihydro-3,6,9-trimethylnaphtho [1,8-bc]pyran-7-oxa-8-one which was named mansorin C.

Three additional compounds were identified as mansonones C (**4**), G (**5**) and H (**6**). The identification of these known compounds was carried out by comparing their physical and spectral data with previously reported values (Marini Bettolo et al., 1965; Tanaka et al., 1966; Letcher and Shirley, 1992).

The results of brine shrimp *Artemia salina* Linn. cytotoxicity tests of isolated compounds are shown in Table 3. Compounds **2** and **4** displayed very high cytotoxicity against brine shrimp *Artemia salina* Linn which may be an indication of antitumor activity (Meyer et al., 1982).

3. Experimental

3.1. General

Mps: uncorr. NMR: Bruker model ACF 200 spectrometer and a Joel model JNM-A500. MS: Fission Instrument model Trio 2000. CC: silica gel 60 (Merck).

3.2. Plant material

The dried heartwood of *Mansonia gagei* Drumm. was collected from Saraburi province, Thailand in 1997. The identity of this plant was compared with a voucher specimen No. 43281 at the herbarium of the Royal Foresty Department of Thailand.

Table 3
Brine shrimp cytotoxicity of compounds **1–6**

Compound	LC_{50} at 24 h	Activity
1	24.18	Medium
2	0.61	High
3	187.85	Low
4	2.08	High
5	64.36	Medium
6	50.70	Medium

High activity, $LC_{50} < 10$ μg/ml. Medium activity, $LC_{50} < 100$ μg/ml. Low activity, $LC_{50} < 1000$ μg/ml.

3.3. Extraction and isolation

Dried heartwood of *Mansonia gagei* Drumm. (8 kg) was milled and extracted with hexane at room temperature for 4 days. The hexane extract was filtered and concentrated under reduced pressure to obtain a dark-brown oil (15.5 g). The residue remaining after hexane extraction was triturated with dichloromethane for 4 days and concentrated to obtain a brown oil (219 g). Methanol was used for further extraction and a dark-black residue (470 g) was obtained after evaporation.

The hexane extract (15.5 g) was subjected to silica gel column chromatography using a gradient eluent hexane-dichloromethane system. The fraction which was eluted with hexane-dichloromethane 9:1–8:2 gave 3,8-dimethyl-5-isopropyl-6-methoxycoumarin **(1)** (90 mg) and 2,3-dihydro-3,6,9-trimethyl naphtho[1,8-bc]pyran-7-oxa-8-one **(3)** (40 mg). The separation of the dichloromethane crude extract (60 g) was performed in the same fashion using hexane-ethyl acetate as a solvent system yielding **1** (0.3 g), 3,5-dimethyl-5-isopropyl-6-hydroxycoumarin **(2)** (40 mg), mansonone C (80 mg), mansonone G (2 g) and mansonone H (80 mg), respectively.

3.4. 3,8-Dimethyl-5-isopropyl-6-methoxycoumarin (*1*)

Pale yellow crystals, mp 135–137 °C. UV λ_{max} nm (log ε): 232 (4.15), 292 (4.13), 344 (3.43) nm; IR ν_{max} cm^{-1}: 1711, 1600, 1450, 1300, 1200, 1035. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) spectral data: Table 1. EIMS 70 eV *m*/*z* (rel.int.%): 246 [M^+] (62), 231 (100), 203 (5). (Found: C, 72.98; H, 7.59. $C_{15}H_{18}O_3$ requires: C, 73.17; H, 7.31.)

3.5. 3,8-Dimethyl-5-isopropyl-6-hydroxycoumarin (*2*)

Pale yellow crystals, mp 202–204 °C. UV λ_{max} nm (log ε): 232 (4.06), 290 (4.12), 342 (3.49). IR ν_{max} cm^{-1}: 3300–3400, 1695, 1600, 1300, 1025. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Table 1. EIMS 70 eV *m*/*z* (rel.int.%): 232 [M^+] (81), 217 (100), 189 (13). (Found: C, 72.66; H, 7.10. $C_{14}H_{16}O_3$ requires: C, 73.41; H, 6.89.)

3.6. 2,3-Dihydro-3,6,9-trimethyl naphtho[1,8-bc]pyran-7-oxa-8-one (*3*)

White crystals, mp 150–151 °C. UV λ_{max} nm (log ε): 230 (3.95), 284(4.01), 294 (4.04), 312 (3.91). IR ν_{max} cm^{-1}: 1705, 1605, 1608, 1320, 1200. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): Table 2. EIMS 70 eV *m*/*z* (rel.int.%): 230 [M^+] (100), 215 (25), 20 (20).

3.7. Bioassays

Compounds **1–6**, isolated from *M. gagei*, were tested for their cytotoxic activity against *Artemia salina* Linn (Meyer et al., 1982; Solis et al., 1992). Brine shrimp eggs were hatched in artificial seawater prepared from dissolved 0.017 M NaCl. A plastic divider with several 2 mm holes was clamped in the dish to make two unequal compartments. The eggs were sprinkled into the darkened larger compartment and the smaller compartment was illuminated with the 20-watt lamp. Following incubation at 22–29 °C for 24 h, the phototropic nauplii were deshelled and were collected with a micropipette from the illuminated side.

Serial dilution of samples (10, 100 and 1000 μg/ml) were made in the wells of a 24 well microplate with 100 μl artificial seawater. Control wells containing DMSO were included in each experiment. A suspension of nauplii containing 5 organisms per 100 μl was added to each well with the whole then covered and the plate was incubated with the 20 W lamp at 22–29 °C under illumination for 24 h. Plates were examined under binocular microscope to count the number of dead (non-motile) nauplii in each well at 24 h after initiation of the test. LC_{50} values were calculated by Probit analysis.

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