8-Methoxycapnolactone and stigmasterol From *Micromelum minutum*

8-Metoksikapnolakton dan stigmasterol dari *Micromelum minutum*

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Abstrak

Suatu kumarin, 8-metoksikapnolakton dan stigmasterol diisolasi dari daun *Micromelum minutum* (Rutaceae) yang dikoleksi dari Sepilok, Sabah, Malaysia dan strukturnya ditentukan dengan menggunakan metode spektroskopi.
Kata kunci: *Micromelum minutum*; Kumarin; 8-Metoksikapnolakton, Stigmasterol

Abstract

The coumarin, 8-methoxycapnolactone and stigmasterol were isolated from the leaves of *Micromelum minutum* (Rutaceae) which collected from Sepilok, Sabah, Malaysia and their structures were characterized by spectroscopic methods.
Key words: *Micromelum minutum*; Coumarin; 8-Methoxycapnolactone, Stigmasterol

Introduction

Micromelum minutum (G. Forst.) Wight & Arn (Rutaceae) is a tall shrub or small to medium-sized tree commonly found in the forests and limestone areas in Malaysia. It is a species with four varieties, the most widespread of which, var. *minutum*, occurs in Sabah and Sarawak (Jones, 1995). The leaves were traditionally used in the treatment of fever and giddiness and a poultice of the boiled roots for ague (Burkill, 1935). The leaves and stems of *Micromelum* species are known to contain numerous coumarins (Cassady *et al.*, 1979; Das *et al.*, 1984; Tantishaiyakul *et al.*, 1986; Kong *et al.*, 1988; Nakamura *et al.*, 1998; Rahmani *et al.*, 1993, 1994). Previous study on this plant collected in Sepilok, Sabah, Malaysia afforded five new coumarins, 3″,4″-dihydrocapnolactone, 2',3'-epoxyisocapnolactone, 8-hydroxyisocapnolactone-2′,3′-diol, 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol and 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol and two known triterpenes, 5(6)-gluten-3-one and 5(6)-gluten-3α-ol which have not been previously reported to occur in the genus *Micromelum* (Rahmani *et al.*, 2003; Susidarti *et al.*, 2006). A reinvestigation of the leaves of this plant resulted in the isolation of 8-methoxycoumarin (Figure 1) and stigmasterol (Figure 2). This coumarin was new for the genus *Micromelum*.

Methodology

General

Melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were recorded using KBr discs on Perkin Elmer FTIR spectrophotometer model 1275X. The ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker DRX-500 spectrometer in $CDCl_3$ operating at 500 MHz and 125 MHz, respectively. Chemical shifts are shown in δ values (ppm) with tetramethylsilane as an internal standard. Mass

spectra were performed with Direct Induction Probe (DIP) using a Shimadzu GCMS-QP5050 spectrometer with ionisation induced by electron impact at 70 eV. High Performance Liquid Chromatography (HPLC) was performed on a Waters Assoc. (Milford, MA, USA) liquid chromatograph model 510 equipped with two solvent delivery systems, a model Rheodyne injector, a model 486 absorbance detector which were linked by Waters System Interface Module to Maxima 820 Chromatography Workstation. The semipreparative Alltech Econosil C18 column (25 cm x 0.7 cm, 10 μm) was used and eluting with degassed and filtered HPLC grade methanol (Fisher) at flow rate of 1 mL/minute. Detection was carried out at 254 nm, with a detector sensitivity of 1.0 a.u.f.s. Samples were injected through a microliter 1710 syringe (Hamilton, Reno, NV, USA).

Plant material

The leaves of *Micromelum minutum* were collected from Sepilok, Sabah, Malaysia and a voucher specimen was deposited at Forest Research Centre, Sepilok, Sabah.

Extraction and isolation

The dried ground leaves (200,0 g) were extracted successively with petroleum ether, $CHCl_3$ and MeOH gave 6,7; 2.0 and 2,5 g of dark gummy semisolid extracts, respectively. The pet. ether extract (6.5 g) was fractionated by vacuum chromatography over silica gel eluted with different mixtures of pet. ether-chloroform and chloroform-methanol gave 27 fractions. Fractions 23 and 24 were combined and further fractionated by silica gel column gave 31 fractions. Fractions of intermediate polarity (10-25) were combined and then fractionated by preparative TLC plates triply eluted with chloroform. The greenish-blue band was scraped and washed with chloroform. Evaporation of the solvent yielded a yellow semisolid, which was shown by TLC analysis to contain a mixture of two compounds. The material was further separated by PLC doubly eluted with chloroform-ethyl acetate (8:2). The greenish fluorescent more polar bands afforded colorless semisolid (6.4 mg) of 8-methoxycapnolactone (Figure 1).

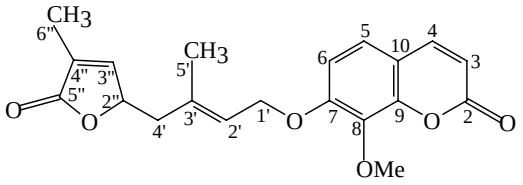

Figure 1. 8-Methoxycapnolactone

IR (KBr disc) cm^{-1}: 2926, 1816, 1793, 1758, 1728, 1607, 1460, 1382, 1291, 1096. EIMS m/z (% intensity): 356 (0.58), 192 (100.00), 177 (7.28), 164 (17.36), 163 (10.85), 147 136 (9.06), 121 (6.87), 99 (16.42), 97 (71.04).

^{1}H-NMR (500 MHz, Pyridin-d_5): δ 1.77 (*s*, H-5′), 1.84 (*s*, H-6″), 2.41 (*m*, H-4′), 3.99 (*s*, 8-OMe), 4.77 (*dd*, *J* = 5.9; 11Hz, H-1′), 4.93 (H-2″), 5.73 (*m*, H-2′), 6.36 (*d*, *J* = 9.5 Hz, H-3), 7.06 (*d*, *J* = 8.6 Hz, H-6), 7.06 (H-3″), 7.26 (*d*, *J* = 8.6 Hz, H-5), 7.70 (*d*, *J* = 9.5 Hz, H-4).

^{13}C-NMR (125 MHz, $CDCl_3$): δ 10.8 (C-6″), 17.6 (C-5′), 43.3 (C-4′), 66.0 (C-1′), 79.7 (C-2″), 110.3 (C-6), 113.7 (C-3), 114.0 (C-10), 122.8 (C-5), 123.3 (C-2′), 134.2 (C-4″), 136.3 (C-3′), 136.8 (C-8), 143.7 (C-4), 148.3 (C-9, C-3″), 154.7 (C-7), 160.2 (C-2), 174.0 (C-5″).

The chloroform extract (2.0 g) was subjected to column chromatography using chloroform as solvent gave 27 fractions. The combined fraction 8-10 was further submitted to high performance liquid chromatography on a semipreparative Alltech Econosil C18 (25cm x 0.7cm, 10μm) HPLC column. The samples (50 μL) was injected into the column and eluted with methanol at 2 mL/min. The fraction with retention time 15.00 minute was collected to afford stigmasterol (Figure 2) as white solid (5.2 mg), m.p. 158°-160°C.

IR (KBr disc) cm^{-1}: 3351 (br), 2934, 1656, 1459, 1379, 1093, 1049, 970, 841, 747. EIMS m/z (% intensity): 412 ([M^+], 59.72), 394 (5.48), 379 (8.70), 351 (17.77), 300 (26.94), 271 (40.90), 255 (52.94), 213 (36.51), 159 (55.91), 133 (57.83), 119 (45.21), 105 (65.01), 95 (63.98), 81 (96.32).

^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.14 (H-22), 5.02 (H-23), 5.36 (H-6), 3.52 (H-3), 2.40 - 0.67 (other protons).

Result And Discussion

Coumarin 8-methoxycapnolactone (Figure 1) was isolated as colourless semisolid. The EI mass spectrum showed a molecular ion peak at m/z 356 consistent with the molecular formula $C_{20}H_{20}O_6$. The IR spectrum was informative with the presence of γ and δ lactones carbonyl functionalities at 1758 and 1728 cm^{-1}, respectively. A pair of doublets, each with coupling constant 9.5 Hz at δ 6.36 and 7.70 in the ^{1}H-NMR spectrum was typical of H-3 and H-4 of the coumarin nucleus, respectively. Another pair of doublets each with coupling constant 8.6 Hz at δ 7.26 and 7.06 was

assigned to H-5 and H-6, respectively. A strong singlet signal at δ 3.99 was typical of a methoxy group. These findings suggested the existence of 7-oxygenated-8-methoxy coumarin nucleus.

The ^{1}H-NMR, ^{13}C-NMR and mass spectral data (Table) of 8-methoxycapnolactone were in a close agreement with those reported for clauslactone-K previously isolated from the twigs and leaves of *Clausena excavata* from Bengkulu Province, Indonesia (Nakamura *et al*., 1998). The presence of partial structure –CH_2-$C(CH_3)$=CH-CH_2-O- between the coumarin nucleus and γ-lactone ring was represented by the proton signals at δ 4.77 (*dd*, *J* = 5.9; 11 Hz, H_2-1′), 5.73 (*m*, H-2′), 2.41 (*m*, H-4′) and 1.77 (*s*, H_3-5′) and carbon signals at δ 66.0 (C-1′), 123.3 (C-2′), 136.3 (C-3′), 43.3 (C-4′) and 17.6 (C-5′). The signals due to H-2″, H-3″, H-6″ of the 3″,4″-unsaturated γ-lactone ring appeared at δ 4.93, 7.06 (overlapped with H-3 signal) and 1.84, respectively. The signals due to C-2″, C-3″, C-4″, C-5″ and C-6″ was observed to occur at δ 79.7, 148.3, 134.2, 174.0 and 10.8, respectively.

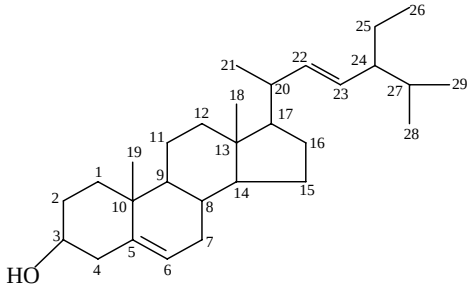

Figure 2. Stigmasterol

In the mass spectrum of 8-methoxy-capnolactone, the cleavage of the bond between oxygen atom at C-7 and the side chain (involving transfer of a hydrogen atom from C-1′ to oxygen atom) resulted in the formation

Table NMR spectral data of 8-methoxycapnolactone

C/H	δ_C	δ_H	δ_C*	δ_H*
2	160.7		160.5	
3	113.7	6.36 (*d*, *J* = 9.5 Hz)	113.7	6.26
4	143.7	7.70 (*d*, *J* = 9.5 Hz)	143.6	7.61
5	122.8	7.26 (*d*, *J* = 8.6 Hz)	122.7	7.15
6	110.3	7.06 (*d*, *J* = 8.6 Hz)	110.2	6.85
7	154.7		154.7	
8	136.8		136.8	
9	148.3		148.2	
10	114.0		113.9	
1′	66.0	4.77 (*dd*, *J* = 5.9; 11Hz)	65.9	4.71
2′	123.3	5.73 (*m*)	123.4	5.63
3′	136.3		136.0	
4′	43.3	2.41 (*m*)	43.2	2.41
5′	17.6	1.77 (*s*)	17.5	1.84
2″	79.7	4.93	79.5	5.00
3″	148.3	7.06 (*m*)	148.1	7.00
4″	134.2		134.0	
5″	174.0		173.9	
6″	10.8	1.84 (*s*)	10.6	1.91
8-OMe	61.6	3.99 (*s*)	61.5	3.99

* Nakamura *et al*., 1998

of stable 7-hydroxy-8-methoxycoumarin ion at m/z 192 (base peak). The loss of a methyl group of this ion gave a fragment at m/z 177, while the successive loss of CO afforded fragments at m/z 164 and 136. The loss of a methyl group from the later gave a fragment at m/z 121. In addition, the γ-lactone ring ion gave a peak at m/z 97.

Stigmasterol (Figure 2) was isolated from the chloroform extract of the leaves of *M. minutum* by HPLC. This compound was obtained as white powder with m.p. 158-160 °C from fraction with retention time 15.00 minutes. The EI mass spectrum showed the molecular ion peak at m/z 412 consistent with the molecular formula $C_{29}H_{48}O$. The IR spectrum showed a broad strong band at 3351 cm^{-1} was due to the presence of a hydroxyl group. The absorption band at 1656 cm^{-1} was typical of C-C double bond.

Since the molecular formula indicated six units of saturation, this compound was concluded to be stigmasterol. This was further supported by the presence of a pair of doublets at δ 5.14 and 5.02 in the ^{1}H-NMR spectrum, which is the characteristic of the sp^2 methine protons at C-22 and C-23 of the side chain, respectively. Another sp^2 methine proton at C-6 was represented by a triplet signal at δ 5.36. The multiplet signal at δ 3.52 was assigned to the resonance of methine proton at C-3.

In the mass spectrum of stigmasterol the molecular ion of this compound was observed at m/z 412. The loss of water from the molecular ion was indicated by the presence of a fragment ion at m/z 394. This implied the existence of a hydroxyl group at C-3. The subsequent fragment at m/z 379 was due to the loss of a methyl group. The characteristic feature of this fragmentation is the presence of a fragment ion peak at m/z 271 due to the loss of the side chain of stigmasterol followed by the loss of two hydrogen atoms. Based of the above spectral data, this compound was identified as stigmasterol.

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