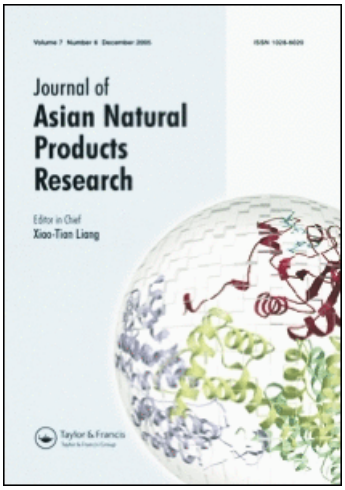

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TWO NEW METHYL CHANOFRUTICOSINATES FROM *KOPSIA FLAVIDA* BLUME

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Two new indole alkaloids with the methyl chanofruticosinate skeletal system viz., methyl 3-oxo-12-methoxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate (**1**) and methyl 3-oxo-11,12-methylenedioxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate (**2**), together with four known compounds, methyl 12-methoxy-N^1-decarbomethoxychanofruticosinate, methyl 12-methoxychanofruticosinate, methyl 11,12-dimethoxychanofruticosinate and methyl 11,12-methylenedioxy-N^1-decarbomethoxychanofruticosinate, were isolated in continuing studies on the leaves of *Kopsia flavida* Blume. The structures of the new indole alkaloids were assigned by NMR spectral data using various 2D-techniques.

Keywords: *Kopsia flavida* Blume; Apocynaceae; Indole alkaloids; Methyl chanofruticosinate skeletal system

INTRODUCTION

The genus *Kopsia* (family: Apocynaceae), widely distributed throughout tropical Asia, is known to be a source of novel indole alkaloids with intriguing structures and useful biological activities [1–8]. There are about 18 *Kopsia* species in Malaysia including four species in Sarawak and north Borneo [9–11]. In Peninsular Malaysia, *Kopsia flavida* Blume is widespread in the lowland forests but it has been grown as a garden tree particularly for its attractive white flowers [12]. In Malaysia, the roots of several *Kopsia* species are known to be used for poulticing ulcerated noses in tertiary syphilis [13].

Previously, we have reported the isolation of a series of new aspidofractinine-type alkaloids with the methyl chanofruticosinate skeletal system from the leaves of *Kopsia flavida* Blume [14]. Alkaloids with this skeletal system have been reported before this in *K. officinalis* and *K. arborea* but their occurrence in the genus is not common [15,16]. In continuation of our phytochemical investigation on this plant, we further describe

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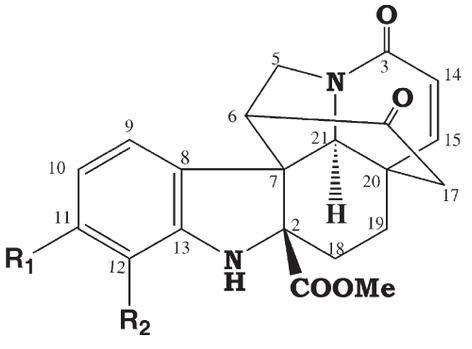
the isolation and structural elucidation of two new indole alkaloids with the methyl chanofruticosinate skeletal system, viz., methyl 3-oxo-12-methoxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate (**1**) and methyl 3-oxo-11,12-methylenedioxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate (**2**). In addition to the new indole alkaloids, we also isolated four known compounds, methyl 12-methoxy-N^1-decarbomethoxychanofruticosinate, methyl 12-methoxychanofruticosinate, methyl 11,12-dimethoxychanofruticosinate and methyl 11,12-methylenedioxy-N^1-decarbomethoxychanofruticosinate, whose structures have been described previously [14].

RESULTS AND DISCUSSION

The alkaloidal extract of the leaves of *K. flavida* was obtained in the usual manner as described in the Experimental section to afford two new compounds. The structures of the compounds were elucidated by a combination of FAB mass spectrometry, ^{1}H and ^{13}C NMR spectra in combination with 2D NMR techniques (COSY-45, HMQC and HMBC). The UV spectra of the compounds showed absorption maxima typical of a dihydroindole chromophore while the mass spectra exhibited fragmentation patterns of indole alkaloids with a methyl chanofruticosinate skeletal system, with a typical base peak corresponding to $[M-CO_2Me]^+$ fragment.



	R_1	R_2
1	H	OMe
2	-O-CH_2-O	

The FABMS of compounds **1** and **2** showed molecular ions at *m/z* 395 $[M + H]^+$ and 409 $[M + H]^+$, corresponding to the formulae $C_{22}H_{23}N_2O_5$ and $C_{22}H_{21}N_2O_6$, respectively. Based on the ^{1}H and ^{13}C NMR spectra (Tables I and II, respectively), compounds **1** and **2** were very similar to each other except for the presence of a methoxy moiety in compound **1** and a methylenedioxy group in compound **2**. The connectivities between protons and carbons in the HMBC and HMQC spectra indicated that the proton singlets at δ 3.87 in compound **1** were due to the methoxyl group attached to the 12 position in the benzene ring using the following data. In the aromatic region the two doublets at δ 6.92 ($J = 7.6$ Hz) and δ 6.77 ($J = 8.2$ Hz) were assigned to H-9 and H-11 protons, respectively, and the doublet of doublets at δ 6.81 ($J = 7.6$, 8.2 Hz) was due to H-10. All the characteristic aromatic proton signals were unambiguously assigned to the protons at C-9, C-11 and C-10, respectively, from the HMQC spectrum. These spectroscopic properties were similar to those of the known compound methyl 12-methoxychanofruticosinate [14]. The presence of the methylenedioxy group in compound **2** at C-11 and C-12 was confirmed by the observation of two pairs of AB doublets at δ 5.98 and 5.92 (OCH_2O, $J = 0.9$, 0.9 Hz), by the connectivity of these protons to the carbon resonance at δ 101.4 and by the coupling between

TABLE I ^{1}H-NMR (600 MHz, $CDCl_3$) spectral data for compounds **1** and **2**

	*Compound **1***		*Compound **2***	
Proton	*δ (ppm)*	*J (Hz)*	*δ (ppm)*	*J (Hz)*
H-5 α	3.91 (d)	12.8	3.88 (d)	12.8
H-5 β	4.42 (dd)	12.8, 4.9	4.37 (dd)	12.8, 4.9
H-6	3.49 (d)	4.6	3.43 (d)	4.9
H-9	6.92 (d)	7.6	6.78 (d)	8.1
H-10	6.81 (dd)	7.6, 8.2	6.35 (d)	8.1
H-11	6.77 (d)	8.2	–	–
H-14	5.98 (d)	9.7	5.98 (d)	9.7
H-15	6.66 (d)	9.7	6.66 (d)	9.7
H-17 α	2.32 (d)	18.4	2.31 (d)	18.3
H-17 β	2.48 (d)	18.4	2.47 (d)	18.3
H-18 α	1.82–1.89 (m)	–	1.82–1.92 (m)	–
H-18 β	2.02–2.09 (m)	–	1.99–2.03 (m)	–
H-19 α	1.75–1.77 (m)	–	1.75–1.77 (m)	–
H-19 β	2.09–2.17 (m)	–	2.05–2.11 (m)	–
H-21	3.59 (s)	–	3.56 (s)	–
N-*H*	4.70 (s)	–	4.50 (s)	–
$Ar_{12}O$*Me*	3.87 (s)	–	–	–
COO*Me*	3.65 (s)	–	3.70 (s)	–
O*C*H_2O	–	–	5.98 (d)	0.9
			5.92 (d)	0.9

Assignments were by COSY, HMQC and HMBC 2D-NMR experiments.

two aromatic protons centered at δ 6.78 (H-9, $J = 8.1$ Hz) and δ 6.35 (H-10, $J = 8.1$ Hz). The characteristic proton signals observed as a doublet at δ 5.98 (1H, d, $J = 9.7$ Hz) and δ 6.66 (1H, d, $J = 9.7$ Hz) in the HMQC spectra for both compounds were unambiguously assigned to be the proton at C-14 and C-15, respectively. The proton at δ 5.98 showed J^3

TABLE II ^{13}C-NMR (100 MHz, $CDCl_3$) spectral data for compounds **1** and **2**

Carbon	***1***	***2***
2	73.9	74.2
3	167.8	167.8
5	51.5	51.6
6	53.4	53.4
7	56.6	55.9
8	131.0	126.4
9	115.7	116.4
10	121.0	100.7
11	110.8	148.9
12	146.0	132.4
13	137.6	130.7
14	126.0	126.0
15	151.0	151.0
16	205.2	205.0
17	47.1	47.0
18	27.0	26.9
19	28.4	28.3
20	38.4	38.6
21	68.2	68.1
$Ar_{12}O$*Me*	55.5	–
*C*OOMe	174.4	174.1
COO*Me*	52.7	52.8
O*C*H_2O	–	101.4

correlations with carbon atoms at δ 38.4 (C-20) while the proton at δ 6.66 showed J^3 correlations with carbon atoms at δ 167.8 (C-3), δ 28.4 (C-19) and δ 68.2 (C-21). The chemical shift value observed at δ 167.8 was assigned as a carbonyl group attached to C-3. All these signals suggested that **1** and **2** have the derivatives of methyl chanofruticosinate skeletal system containing one carbonyl group attached at C-3 and one unsaturated bond attached at C-14 and C-15. Therefore, the structure of compounds **1** and **2** were confirmed as methyl 3-oxo-12-methoxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate and methyl 3-oxo-11,12-methylenedioxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures

UV spectra were recorded in MeOH using a Hitachi U 3400 while optical rotations were determined in MeOH at 24°C, using a JASCO DIP-140. The ^{1}H and ^{13}C-NMR spectral data and also ^{1}H–^{1}H COSY, HMQC and HMBC experiments were measured with a JEOL JNM A-500 spectrometer at 500 and 125.65 MHz, respectively, using $CDCl_3$ as solvent and TMS as internal standard. The low resolution FAB-MS were obtained on a JEOL JMS-AM20 spectrometer, using a direct probe insert at 70 eV while HRFAB-MS were recorded using a JEOL JMS-HX110. Adsorption flash chromatography and column chromatography were performed with Si gel 60 (Merck, 230–400 mesh) while preparative-TLC were carried out on Si gel 60 GF_{254} (Merck 7730, 0.5 mm thick). The TLC analysis was performed on Merck precoated Kieselgel 60 F_{254} aluminium sheets. The alkaloids were visualized under UV and by spraying with Dragendorff reagent.

Plant Material

The leaves of *K. flavida* were collected from the Forest Research Institute of Malaysia (FRIM), Malaysia, and a voucher specimen has been deposited at the Herbarium of FRIM, Malaysia.

Extraction and Isolation

The dry powdered sample of the leaves (624.5 g) was extracted with MeOH (2 l) for 3 days at room temperature (30°C) and concentrated under reduced pressure to yield 48.5 g of the crude extract which was then triturated in 5% aqueous H_2SO_4 (150 ml) followed by basification with 10% Na_2CO_3 and then extraction into $CHCl_3$. The organic layer was washed with distilled water, dried (Na_2SO_4) and concentrated to yield 2.9 g of crude alkaloids. The alkaloid mixture (2.5 g) was purified by flash column chromatography (silica gel, Merck 230–400 mesh) using *n*-hexane–EtOAc (1:1; 200 ml), $CHCl_3$ (100%; 150 ml), EtOAc (100%, 150 ml) and then MeOH (100%, 100 ml) as eluents. Several fractions (50 ml each) were collected, analyzed by TLC and grouped accordingly. The *n*-hexane–EtOAc (1:1) eluates were subjected to repeated column chromatography followed by SiO_2 medium-pressure liquid chromatography (acetone–$CHCl_3$; 1:25) to afford methyl 12-methoxy-N^1-decarbomethoxychanofruticosinate (1.5 mg). Further purification of the same fraction using ODS reverse phase column chromatography (H_2O–MeOH; 1:1) gave methyl 11,12-methylenedioxy-N^1-decarbomethoxychanofruticosinate (4.5 mg). Methyl 12-methoxychanofruticosinate (54.8 mg) and methyl 11,12-dimethoxychanofruticosinate

(2.5 mg), were obtained from the EtOAc fractions (100%) after further purification by preparative TLC (SiO_2, *n*-hexane–EtOAc; 1:1). While the $CHCl_3$ (100%) eluents were subjected to extensive column chromatography and preparative TLC (SiO_2, $CHCl_3$–EtOAc; 10:1) to afford two other new indole alkaloids with the methyl chanofruticosinate skeletal system viz., methyl 3-oxo-12-methoxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate (**1**) (10.4 mg) and methyl 3-oxo-11,12-methylenedioxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate (**2**) (26.6 mg).

Methyl 3-oxo-12-methoxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate (**1**): yellowish amorphous powder; UV (MeOH), λ_{max} nm (log ϵ): 210 (4.33), 293 (3.30); FABMS (70 eV), *m/z* (rel. int.): 395 ($C_{22}H_{23}N_2O_5$, 5), 394 (28), 335 (100), 212 (15); HR-FABMS, $[M + H]^+$, found: 395.1609, calcd. for $C_{22}H_{23}N_2O_5$: 395.1607; CD (0.27 mM, MeOH, 24°C), λ nm ($\Delta\epsilon$): 330 (0), 297 (+2.3), 283 (0), 269 (−2.8), 249 (0), 230 (+8.3), 217 (0), 211 (−6.7); ^{1}H-NMR and ^{13}C-NMR: Tables I and II.

Methyl 3-oxo-11,12-methylenedioxy-N^1-decarbomethoxy-14,15-didehydrochanofruticosinate (**2**): orange amorphous powder; UV (MeOH), λ_{max} nm (log ϵ): 218 (4.37), 246 (3.65); FABMS (70 eV), *m/z*(rel. int.): 409 ($C_{22}H_{21}N_2O_6$, 100), 349 (95), 307 (15), 289 (12), 154 (75), 136 (49); HR-FABMS, $[M + H]^+$, found: 409.1380, calcd. for $C_{22}H_{21}N_2O_6$: 409.1400; CD (0.27 mM, MeOH, 24°C), λ nm ($\Delta\epsilon$): 318 (0), 295 (+1.6), 277 (+0.5), 249 (+8.7), 226 (0), 211 (−9.7); ^{1}H-NMR and ^{13}C-NMR: Tables I and II.

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