Three New Indole Alkaloids from the Leaves of *Kopsia officinalis*

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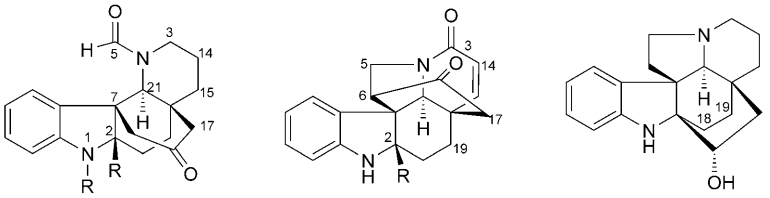
Three new indole alkaloids, 11,12-de(methylenedioxy)danuphylline (**1**), methyl (2β,11β,12β,19α)-6,7-didehydro-8,21-dioxo-11,21-cycloaspidospermidine-2-carboxylate (**2**), and (2β,5β)-aspidofractinin-16-ol (**3**) were isolated from *Kopsia officinalis*, together with 16 known compounds. Their structures were determined by spectroscopic methods. The isolated known compound (−)-12-methoxykopsinaline displayed antimanic effects in Drosophila, with an IC_{50} value of 12.5 μg/ml.

Introduction. – Plants belonging to the genus *Kopsia*, which has its stronghold in Southeast Asia, produce a number of biologically active alkaloids [1][2]. Cultivated *K. officinalis*, *e.g.*, is used in China for the treatment of rheumatoid arthritis, dropsy, and tonsillitis. In Malaysia, the roots of several *Kopsia* species are used for the treatment of ulcerated noses in tertiary syphilis [3]. In our research, leaf extracts have shown antimanic effects in *Drosophila*; at the same time, (−)-12-methoxykopsinaline was identified as the bioactive component, with an IC_{50} value of 12.5 μg/ml.

In this paper, we report the isolation and structure elucidation of the three new alkaloids **1**–**3**, together with 16 known compounds: (−)-12-methoxykopsinaline [4], kopsamine [5][6], kopsiflorine [5][6], kopsilongine [5][6], kopsinine *N*(4)-oxide [6], (+)-(19*R*)-19-hydroxyeburnamine [6], rhazinicine [6], (−)-(19*R*)-19-hydroxyisoeburnamine [6], 11-methoxykopsilongine [4], kopsinine [4], (+)-eburnamonine [7], (+)-isoeburnamine [7], larutenine [7], (−)-kopsininic acid [8], methyl 11,12-(methylenedioxy)chanofruticosinate [9], and methyl chanofruticosinate [9].



1 R = MeOOC **2** **3**

Arbitrary atom numbering[1])

[1]) For systematic names, see the *Exper. Part.*

Table 1. *^{13}C-NMR Data for Compounds* **1**–**3**. At 125 MHz in $CDCl_3$; δ in ppm. Arbitrary atom numbering.

Position	**1**	**2**	**3**	Position	**1**	**2**	**3**
2	78.9	73.4	72.4	14	19.3	126.0	15.4
3	34.9	167.8	47.6	15	29.8	150.9	28.9
5	165.6	51.6	50.6	16	206.6	205.0	68.9
6	39.4	53.3	39.6	17	46.0	47.0	33.1
7	52.8	55.9	56.3	18	23.4	26.8	33.4
8	129.1	130.1	135.1	19	39.1	28.3	33.8
9	123.8	123.3	124.5	20	34.4	38.4	31.9
10	123.2	120.3	122.2	21	62.1	68.0	67.0
11	129.8	129.1	127.9	CO_2Me	170.4	174.3	–
12	115.7	111.2	111.7		54.3	52.6	–
13	142.9	148.5	148.0	NCO_2Me	155.1	–	–

Results and Discussion. – 1. *Structure Elucidation*. Compound **1** had the molecular formula $C_{23}H_{26}N_2O_6$, as deduced by HR-ESI-MS (*m*/*z* 449.1686 ($[M+Na]^+$; calc. 449.1688), indicating twelve degrees of unsaturation. The UV spectrum of **1** was characteristic of an indole derivative, with absorption maxima at 243, 279, and 286 nm. The IR spectrum showed bands due to two COO functions at 1739 and 1669 cm^{-1}, respectively.

The ^{13}C-NMR spectrum of **1** (*Table 1*) showed a total of 23 signals, in agreement with the molecular formula. The ^{1}H-NMR spectrum (*Table 2*) showed signals typical of an indole moiety (δ(H) 6.90 (*dd*, *J* = 7.8, 1.0, H–C(9)); 7.08 (*dt*, *J* = 7.8, 1.0, H–C(10)); 7.34 (*dt*, *J* = 7.8, 1.0, H–C(11)); 7.78 (*dd*, *J* = 7.8, 1.0 Hz, H–C(12)); and δ(C) 129.1 (C(8)), 123.8 (C(9)), 123.2 (C(10)), 129.8 (C(11)), 115.7 (C(12)), 141.4 (C(13))). The ^{13}C-NMR data further indicated a CO_2Me substituent at N(1) (δ(C) 155.1), a keto C=O group (206.6), an ester function (170.4), and an aldehyde moiety (δ(C) 165.6; δ(H) 6.37). ^{1}H,^{1}H-COSY and HMBC Experiments showed the presence of the fragments $NCH_2CH_2CH_2$, CH_2CH_2, and $CH_2C(=O)CH_2$. These data implied that **1** possessed a danuphyllin-type skeleton [10]. The ^{1}H- and ^{13}C-NMR spectra of **1** were identical to those of danuphyllin proper, except for the absence of the methylenedioxy signals.

From the above data, in combination with HMBC experiments (*Fig. 1*), the structure of compound **1** was elucidated as 11,12-de(methylenedioxy)danuphyllin[1]).

Compound **2** had the molecular formula $C_{21}H_{20}N_2O_4$, on the basis of HR-ESI-MS data (*m*/*z* 387.1325 ($[M+Na]^+$, calc.387.1320)). The UV spectrum showed maxima at 192, 241, and 289 nm, and the IR spectrum indicated two COO functions (1731 and 1659 cm^{-1}).

The ^{13}C-NMR (DEPT) spectrum of **2** (*Table 1*) showed 21 carbon signals: one Me, four CH_2, and eight CH groups, and eight quaternary C-atoms. The signal at δ(C) 167.8 was assigned to C(3), and the olefinic resonances were due to a double bond between C(14) and C(15). The downfield ^{1}H-NMR shift and the vicinal coupling constant (9.5 Hz) of the olefinic H-atoms suggested that the C=C bond was part of a six-membered lactam ring. This conclusion was supported by the absence of peaks normally associated with the aminomethylene H-atoms at C(3), the presence of a lactam resonance at δ(C) 167.8, as well as a marked downfield shift for H–C(15) at δ(H) 6.65, characteristic of a β-H-atom of an α,β-unsaturated carbonyl moiety.

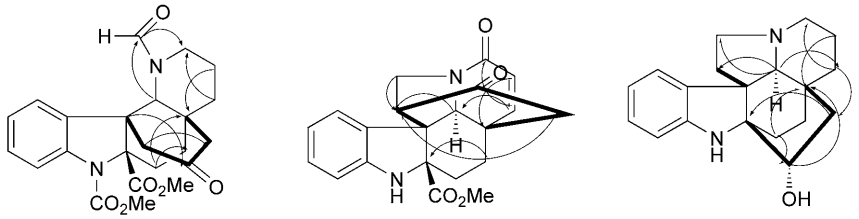


Fig. 1. *Key HMBC correlations for compounds* **1**–**3**

Table 2. *^{1}H-NMR Data for Compounds* **1**–**3**. At 500 MHz in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering.

Position	**1**	**2**	**3**
3	2.50–2.53 (*m*)	–	3.26–3.68 (*m*)
	4.54 (*dd*, *J* = 14.0, 9,6)		3.38–3.42 (*m*)
5	6.37 (*s*)	3.92 (*d*, *J* = 12.5)	3.48–3.52 (*m*)
		4.43 (*dd*, *J* = 12.5, 5.0)	3.31–3.34 (*m*)
6	2.40 (*d*, *J* = 17.1)	3.49 (*s*)	2.12–2.16 (*m*)
	2.86 (*d*, *J* = 17.1)		1.90–1.95 (*m*)
9	6.90 (*dd*, *J* = 7.8, 1.0)	7.28 (*dd*, *J* = 7.7, 1.0)	8.23 (*dd*, *J* = 7.9, 1.0)
10	7.08 (*dt*, *J* = 7.8, 1.0)	6.83 (*dt*, *J* = 7.7, 1.0)	6.93 (*dt*, *J* = 7.9, 1.0)
11	7.34 (*dt*, *J* = 7.8, 1.0)	7.17 (*dt*, *J* = 7.7, 1.0)	7.05 (*dt*, *J* = 7.9, 1.0)
12	7.78 (*dd*, *J* = 7.8, 1.0)	6.83 (*dd*, *J* = 7.7, 1.0)	6.70 (*dd*, *J* = 7.9, 1.0)
14	1.64–1.66 (*m*)	5.98 (*d*, *J* = 9.5)	1.85–1.91 (*m*)
	1.72–1.75 (*m*)		
15	1.25 (*dt*, *J* = 13.6, 9.0)	6.65 (*d*, *J* = 9.5)	1.15–1.18 (*m*)
	1.60–1.63 (*m*)		
16	–	–	4.24–4.28 (*m*)
17	2.49 (*d*, *J* = 20)	2.48 (*d*, *J* = 15.0)	1.62–1.65 (*m*)
	2.74 (*d*, *J* = 20)	2.32 (*d*, *J* = 15.0)	1.34–1.37 (*m*)
18	3.49 (*dt*, *J* = 16.7, 3.5)	1.98–2.00 (*m*)	3.88–3.92 (*m*)
	3.27 (*dd*, *J* = 16.5, 12.8)	2.05–2.07 (*m*)	1.89–1.92 (*m*)
19	1.60–1.64 (*m*)	1.78–1.82 (*m*)	1.58–1.61 (*m*)
			1.28–1.32 (*m*)
21	3.34 (*s*)	3.58 (*s*)	3.78 (*s*)
CO_2Me	3.59 (*s*)	3.65 (*s*)	–
NCO_2Me	3.90 (*s*)	–	–

The above data indicated that **2** was a methyl chanofruticosinate derivative [11]. Its structure was finally elucidated as methyl (2β,11β,12β,19α)-6,7-didehydro-8,21-dioxo-11,21-cycloaspidospermidine-2-carboxylate, as confirmed through HMBC experiments (*Fig. 1*).

Compound **3** had the molecular formula $C_{19}H_{24}N_2O$, as determined by HR-ESI-MS (*m*/*z* 297.1961 ($[M+1]^+$, calc. 297.1966)).

The ^{13}C-NMR (DEPT) data of **3** (*Table 1*) displayed signals for eight CH_2, six CH, and five quaternary C-atoms. A ^{1}H,^{1}H-COSY experiment revealed the presence of CH_2CH_2, CH_2CH, and $CH_2CH_2CH_2N$ fragments, in agreement with the HMBC spectrum (*Fig. 1*). The above data suggested

an aspidofractinine-type skeleton [12]. The NMR data of **3** were very similar to those of aspidofractinine, except for the notable absence of one CH_2 resonance, which was replaced by an oxygenated CH resonance at δ(H) 68.9. This assignment was established by HMBC correlations (*Fig. 1*) of H–C(16) at δ(H) 4.25 to C(17), C(18), and C(20) at δ(C) 33.1, 33.4, and 31.9, resp.

The relative configuration of **3** was determined by a 2D-ROESY NMR experiment (*Fig. 2*). ROESY Cross-peaks were observed between H–C(16) and H_β–C(18), and between H_α–C(18) and H–C(21). On the basis of the above discussion, compound **3** was, thus, identified as (2β,5β)-aspidofractinin-16-ol.

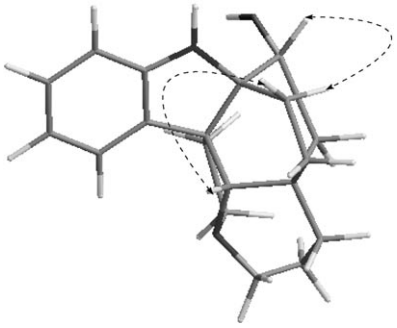


Fig. 2. *Model of compound* **3**, *and key ROESY correlations* (arrows)

2. *Biological Studies.* All compounds were subjected to a bioassay described by *Zhong* and co-workers [13][14] to determine their antimanic activities in *Drosophila*. Our studies confirmed that the known compound (–)-12-methoxykopsinaline was the main bioactive constituent, with an IC_{50} value[2]) of 12.5 μg/ml.

Experimental Part

General. Solvents were distilled before use. Thin-layer (TLC) and column chromatography (CC): silica gel *F254* and *H*, resp. (*Qingdao Haiyang Chemical Co.*). Optical rotations: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *577* spectrometer; in *m*/*z*. 1D- and 2D-NMR Spectra: *Bruker AM 500* spectrometer; δ in ppm, *J* in Hz. EI- and HR-ESI-MS Experiments: *VG Auto Spec 3000*; in *m*/*z*.

Plant Material. The leaves of *Kopsia officinalis* were collected in the Xishuangbanna district, Yunnan province, P. R. China, in February 2005. The plant was identified by Prof. *De Ding Tao*, Kunming Institute of Botany. A specimen was deposited at the Kunming Institute of Botany, Kunming, P. R. China.

Extraction and Isolation. The dried leaves (8 kg) of *K. officinalis* were ground, and then extracted with 8 l each of refluxing 95% EtOH for 4, 3, 2 and 1 h, resp. After evaporation of the solvent, the dry residue was extracted with 1% aq. HCl. The acid-soluble fraction (300 g) was washed with $CHCl_3$, and the acidic soln. was basified to pH 10 with 25% aq. NH_3, and then re-extracted with $CHCl_3$ to give a crude alkaloid fraction (30 g). The latter was purified by CC (SiO_2; increasing proportions of MeOH in $CHCl_3$): four fractions (*Fr. 1–4*). *Fr. 1* contained (–)-12-methoxykopsinaline (30 mg), rhazinicine (10 mg), larutenine (3 mg), and compounds **1** (5 mg) and **2** (6 mg). *Fr. 2* was subjected to repeated CC (SiO_2; $CHCl_3$/MeOH 10:0.5) to afford 11-methoxykopsilongine (8 mg), methyl 11,12-(methylenedioxy)chanofruticosinate (14 mg), methyl chanofruticosinate (10 mg), and compound **3** (3 mg). *Fr. 3* was purified further by CC (SiO_2; AcOEt/MeOH 10:1.5) to afford kopsamine (15 mg), kopsiflorine

[2]) The concentration required to put 50% of the *Drosophila* flies at sleep.

(30 mg), kopsilongine (8 mg), (+)-eburnamonine (9 mg), (+)-isoeburnamine (11 mg), (−)-(19*R*)-19-hydroxyisoeburnamine (8 mg), (+)-(19*R*)-19-hydroxyeburnamine (10 mg), and kopsinine (30 mg). Purification of *Fr. 4* by CC (SiO_2; $CHCl_3/CH_3OH$ 10:1.5) furnished kopsinine *N*(4)-oxide (5 mg) and (−)-kopsininic acid (200 mg)

11,12-De(methylenedioxy)danuphylline (= *Dimethyl (1*R,*9*S,*12*R,*17*S*)-16-Formyl-19-oxo-8,16-diazapentacyclo[10.5.3.0^{1,9}.0^{2,7}.0^{12,17}]icosa-2,4,6-triene-8,9-dicarboxylate*; **1**). Colorless, amorphous powder. UV ($CHCl_3$): 243, 279, 286. $[\alpha]_D^{26} = +28.9$ ($c = 0.36$, $CHCl_3$). IR (KBr): 3443, 2956, 2931, 1739, 1712, 1669, 1463, 1340, 1263, 1153, 790, 757. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. ESI-MS: 427 (100, $[M+1]^+$). HR-ESI-MS: 449.1686 ($[M+Na]^+$, $C_{23}H_{26}N_2NaO_6^+$; calc. 449.1688).

Methyl (2β,11β,12β,19α)-6,7-Didehydro-8,21-dioxo-11,21-cycloaspidospermidine-2-carboxylate (**2**). Colorless powder. $[\alpha]_D^{26} = +167.5$ ($c = 0.24$, $CHCl_3$). UV ($CHCl_3$): 289, 241, 192. IR (KBr): 3433, 2954, 2924, 2853, 1731, 1657, 1462, 1242, 785, 745. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. ESI-MS: 365 (100, $[M+1]^+$). HR-ESI-MS: 387.1325 ($[M+Na]^+$, $C_{21}H_{20}N_2NaO_4^+$; calc. 387.1320).

(2β,5β)-Aspidofractinin-16-ol (**3**). Colorless, amorphous powder. UV ($CHCl_3$): 391, 290, 242. $[\alpha]_D^{26} = -15.7$ ($c = 0.33$, $CHCl_3$). IR (KBr): 3439, 2955, 2918, 2849, 1463, 1019, 729. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. ESI-MS: 297 (100, $[M+1]^+$). HR-ESI-MS: 297.1961 ($[M+1]^+$, $C_{19}H_{25}N_2O^+$; calc. 297.1966).

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