Two New Aspidosperma Indole Alkaloids from Yunnan *Kopsia arborea*

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Received January 9, 2010; accepted April 14, 2010; published online April 15, 2010

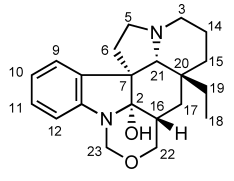
Two new indole alkaloids, kopsiyunnanines G (1) and H (2), possessing the Aspidosperma skeleton were isolated from the aerial part of Yunnan *Kopsia arborea* Blume (Apocynaceae). Their structures and stereochemistry were elucidated by means of MS and 2D NMR analyses.

Key words Apocynaceae; *Kopsia*; indole alkaloid; structure elucidation; NMR

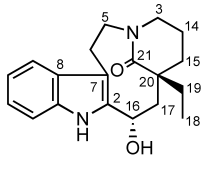
Kopsia plants are widely distributed from South China and Burma to northern Australia and Vanuatu[1)] and are noted for producing a great variety of monoterpenoid indole alkaloids. Phytochemical work on various *Kopsia* plants, particularly those indigenous to Malaysia, has led to the isolation of more than 180 alkaloids.[2)] The alkaloids often possess unusual skeletons and useful bioactivities,[3—8)] and many of them are intriguing targets of total synthesis.[9—11)] As part of our ongoing search for bioactive novel indole alkaloids from various botanical resources,[12—18)] we have reported the isolation of a series of novel alkaloids kopsiyunnanines A—F from Yunnan *Kopsia arborea* Blume (Apocynaceae).[19—21)] We describe herein the structure elucidation of two new minor compounds having an Aspidosperma skeleton (Fig. 1).

Results and Discussion

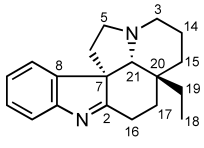
The MeOH extract of the aerial part of Yunnan *K. arborea* was subjected to a conventional procedure[19—21)] to give the crude base, which was roughly separated by SiO_2 column chromatography into 9 fractions using $CHCl_3$–MeOH gradient as eluent. The 10—40% MeOH/$CHCl_3$ fractions from the first column chromatography were subjected to repeated column chromatography to give two new indole alkaloids, kopsiyunnanine G (**1**) and kopsiyunnanine H (**2**), together with 9 known compounds: eburenine (**3**),[22)] (−)-eburnamine,[23)] (−)-methyleburnamine,[24)] (−)-ethyleburnamine,[25)] (+)-isoeburnamine,[23)] (+)-methylisoeburnamine,[24)] (+)-ethylisoeburnamine,[26)] (+)-eburnamenine,[27)] and (+)-eburnamonine.[28)] The identification of known compounds was done by analyzing spectroscopic data and comparing their physicochemical data with those published in the literature.



Kopsiyunnanine G (**1**)



Kopsiyunnanine H (**2**)



Eburenine (**3**)

Fig. 1. Structures of Compounds **1**—**3**

Compound **1**, named kopsiyunnanine G, was obtained as a colorless amorphous solid. The UV spectrum exhibited absorption maxima at λ_{max} 291.5, 246.0, and 208.0 nm, suggesting a typical indoline chromophore. It displayed a molecular ion peak at *m/z* 340.2157 [M^+] (Calcd 340.2151) in the HR-electron ionization-mass spectra (EI-MS) spectrum, which corresponded to the molecular formula $C_{21}H_{28}N_2O_2$ requiring 9 degrees of unsaturation. The ^{1}H-NMR spectrum of **1** (Table 1) showed signals assignable to four aromatic protons (δ_H 7.09, dd, H-9; δ_H 6.79, ddd, H-10; δ_H 7.12, ddd, H-11; δ_H 6.71, br d, H-12) in the benzene ring and an ethyl side chain (judging from the triplet signal for the methyl function δ_H 0.71 with J=7.5 Hz). ^{13}C-NMR (Table 1) and distortionless enhancement by polarization transfer (DEPT) spectra demonstrated a total of 21 carbons, including 2 sp^2 quaternary carbons, 4 sp^2 methine groups, 3 sp^3 quaternary carbons, 2 sp^3 methines, 9 sp^3 methylene groups, and 1 methyl group, in agreement with the molecular formula.

To establish the connections among the above-described structural units, heteronuclear multiple bond connectivity (HMBC) and ^{1}H–^{1}H correlation spectroscopy (COSY) analyses were carried out. ^{1}H–^{1}H COSY data enabled the determination of several sets of connections as indicated by bold bonds in Fig. 2. Furthermore, the fragment C(21)–N(4)–C(3)–C(14)–C(15) was defined through a ^3J long-range HMBC correlation from H-21 to C-3 and C-15. Another fragment C(21)–N(4)–C(5)–C(6)–C(7) was suggested by the correlations of H-21/C-5 and H-5/C-7. The chemical shifts of the two proton signals resonating unusually downfield (δ_H 5.08, 4.87 with geminal coupling constant of J=11.0 Hz) were assigned to one sp^3 methylene group (δ_C 72.6) that was adjacent to both nitrogen and oxygen atoms. This was confirmed by the HMBC correlations from H-23 to C-2 and C-13. The chemical shifts of two other protons resonating downfield at δ_H 3.91 and δ_H 3.48 with a geminal coupling constant of J=11.0 Hz indicated the existence of an oxygenated sp^3 methylene group (δ_C 67.8). This group associated with the same oxygen atom as the C-23 methylene

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Table 1. ^{1}H-NMR (J in Hz) and ^{13}C-NMR Data for **1** and **2** in $CDCl_3$

Position	**1**		**2**	
	δ_H (mult, Hz)[a]	δ_C[b]	δ_H (mult, Hz)[c]	δ_C[b]
NH			8.38 (br s)	
2		93.0		138.0
3	3.06 (m)	53.4	2.78 (m)	52.0
	1.97 (ddd, 13.5, 11.0, 3.0)		1.78 (overlapped)	
5	3.04 (ddd, 13.5, 9.5, 3.5)	52.0	4.20 (dd, 13.0, 7.0)	47.2
	2.23 (m)		2.61 (ddd, 13.0, 12.0, 6.4)	
6	2.72 (ddd, 13.5, 9.5, 7.0)	30.3	3.48 (ddd, 13.8, 12.0, 7.0)	19.7
	1.29 (ddd, 13.5, 11.0, 3.5)		2.88 (dd, 13.8, 6.4)	
7		55.5		108.0
8		135.1		128.4
9	7.09 (dd, 7.5, 1.0)	123.3	7.56 (d, 7.6)	118.0
10	6.79 (ddd, 7.5, 7.5, 1.0)	120.0	7.12 (ddd, 7.6, 7.6, 1.2)	119.6
11	7.12 (ddd, 7.5, 7.5, 1.0)	127.7	7.19 (ddd, 7.6, 7.6, 1.2)	122.2
12	6.71 (br d, 7.5)	109.4	7.36 (d, 7.6)	111.2
13		147.3		135.4
14	1.72 (m)	21.5	1.28 (m)	19.6
	1.50 (m)		1.05 (m)	
15	1.64 (overlapped)	34.2	1.64 (m)	31.3
	1.06 (overlapped)		0.95 (ddd, 14.4, 12.0, 3.0)	
16	2.18 (m)	35.1	5.22 (dd, 10.8, 6.0)	64.5
17	1.63 (overlapped)	23.3	2.44 (dd, 13.2, 6.0)	49.5
	0.80 (dd, 13.0, 2.0)		1.88 (dd, 13.2, 10.8)	
18	0.71 (3H, t, 7.5)	7.0	0.89 (3H, t, 7.6)	8.7
19	1.66 (overlapped)	30.8	1.81 (overlapped)	32.0
	1.11 (m)		1.74 (overlapped)	
20		36.9		44.1
21	2.41 (s)	70.4		175.8
22	3.91 (dd, 11.0, 11.0)	67.8		
	3.48 (dd, 11.0, 3.5)			
23	5.08 (d, 11.0)	72.6		
	4.87 (d, 11.0)			
OH			2.21 (br s)	

a) Measured at 500 MHz. *b*) Measured at 125 MHz. *c*) Measured at 600 MHz.

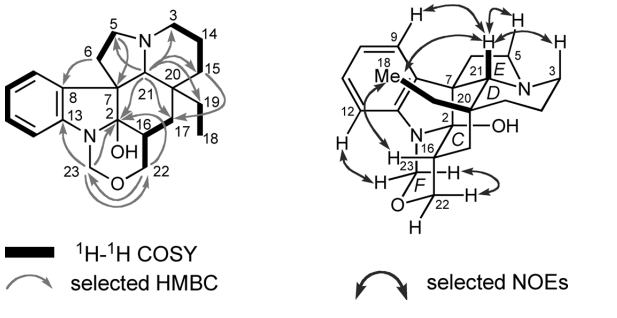

Fig. 2. HMBC, ^{1}H–^{1}H COSY, and NOE Correlations of **1**

group and extended its connection to C-17 and C-2, as suggested by the HMBC correlations from H-22 to C-23 and C-2 as well as the ^{1}H–^{1}H COSY correlation of H_2-22/H-16/H_2-17, respectively. In addition, the C-20 quaternary carbon with an ethyl side chain was linked to C-21, C-17, and C-15, as shown by HMBC correlations of H-15/C-17, H-21/C-17, and H-21/C-19. Other correlations in the HMBC spectrum were in complete accord with the proposed structure.

The relative configurations of compound **1** at the stereogenic centers were determined by analyzing ^{1}H–^{1}H coupling constants and nuclear Overhauser effect (NOE) correlations, as discussed below. As a result of the rigid character of the Aspidosperma skeleton, the relative configurations at C-7, C-20, and C-21 were restricted, *i.e.*, 7*S**, 20*R**, and 21*R**,

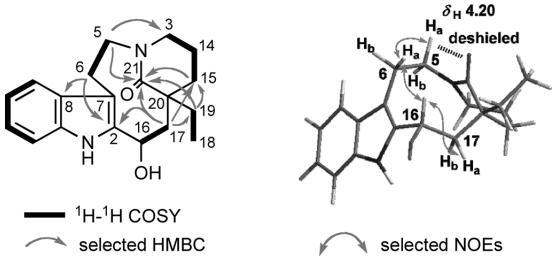

Fig. 3. HMBC, ^{1}H–^{1}H COSY, and NOE Correlations of **2**

which were supported by the NOE correlations between H-21 and H-9 and between H-21 and H_3-18 (Fig. 2). Furthermore, NOE observed between H_3-18 and the angular methine proton on C-16 and a large coupling constant (J=11.0 Hz) between this proton (H-16) and Hα-22 indicated the *trans* fusion of the *C*/*F* ring. Therefore, alkaloid **1** possesses a novel hexacyclic skeleton incorporating a 1,3-oxazinane ring with relative configurations of 2*S**, 7*S**, 16*R**, 20*R**, and 21*R**.

Compound **2**, named kopsiyunnanine H, was obtained as a light yellow amorphous solid. Its molecular formula was established as $C_{19}H_{24}N_2O_2$ from HR-EI-MS (*m*/*z* 312.1838 [M^+]). The UV and ^{1}H-NMR spectra showed a typical indole chromophore.[19] The ^{1}H- and ^{13}C-NMR data (Table 1) revealed the presence of an α,β-disubstituted indole moiety, an amide function (δ_C 175.8), an oxymethine (δ_H 5.22, δ_C 64.5), and an ethyl side chain [δ_H 0.89 (t, J=7.6 Hz), δ_C 8.7].

Further 2D NMR experiments were carried out to connect the above partial fragments. As the HMBC correlations of H-19/C-15, H-19/C-21, and H-17/C-19 suggested, the ethyl side chain was attached to C-15, C-17, and C-21 *via* C-20 (Fig. 3). Fragments C(5)–C(6) and C(3)–C(14)–C(15) revealed by ^{1}H–^{1}H COSY correlations were both linked to C-21 of the amide group. HMBC correlations from H-5 to C-3 and C-21, and from H-15 to C-21 supported the above linkages. On the other hand, fragment C(5)–C(6) was attached to the indole moiety, as indicated by the HMBC correlations from H-6 to C-8 and C-2. Fragment C(16)–C(17) was also attached to the indole moiety, based on the HMBC correlation observed from H-17 to C-2. C-16 of the sp^3 methine group gave a downfield chemical shift of δ_C 64.5, suggesting one hydroxyl group substitution. Thus, the structure of new compound **2** was elucidated as shown.

The relative configuration of the hydroxyl group on C-16 was established by analyzing the coupling constants and the NOE correlations (Fig. 3), as follows. One of the protons on C-5 showed a characteristic downfield chemical shift (δ_H 4.20), strongly deshielded by the anisotropy effect of the amide carbonyl group. Observation of the NOE correlation between that particular proton (H-5a) and one of the protons on C-6 (H-6a: δ_H 3.48) indicated that they were on the same side of the ring. This was supported by the large coupling constant (J=12.0 Hz) between H-5b and H-6a, which was characteristic of an *anti* relationship (Fig. 4). On the other hand, the NOE correlation between H-6a and H-16, an oxymethine proton, was clearly observed, the latter of which possessed coupling constants J=10.8 Hz and 6.0 Hz that were attributed to vicinal coupling with the two methylene protons on C-17. The *anti* relationship of H-16 and H-17b (J=10.8 Hz) was proved by the NOE correlation between H-16 and H-17a (J=6.0 Hz). Although compound **2** had a con-

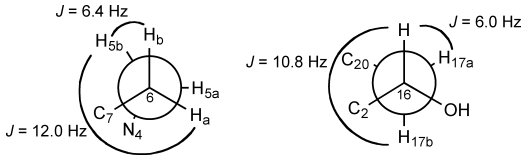

Fig. 4. Analysis of Coupling Constant of **2**

formationally flexible nine-membered ring, taking together all the data mentioned above, the hydroxyl group on C-16 might have an alpha orientation. The positive Cotton effect at approximately λ_{max} 230 nm in the circular dichroism (CD) spectrum could be used to deduce the *R* absolute configuration at C-20 in compound **2**,[20] leading to the *S* configuration at C-16.

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO P-1020 polarimeter. CD was recorded on a JASCO J-720WI spectrometer. UV spectra were measured with a JASCO V-560 spectrophotometer. NMR spectroscopic data were recorded on a JNM A-500 or a JNM ECP-600 spectrometer, where *J* values are given in Hz. EI-MS and HR-EI-MS were recorded on a JEOL JMS GC-mate spectrometer with direct probe insertion at 70 eV. TLC was done on precoated silica gel 60 F254 plates (Merck, 0.25 mm thick) or precoated amino-silica gel plates (Fuji Silysia Chemical Ltd.). Column chromatography was carried out over silica gel 60 (Merck, 70—230 mesh) or amino-silica gel (Fuji Silysia Chemical Ltd., Chromatorex NH 100—200 mesh).

Plant Material *Kopsia arborea* Blume (*Kopsia officinalis* Tsiang *et* P. T. Li) was collected from Xishuangbanna, Yunnan Province, China and identified by one of the authors, Professor Dr. Rongping Zhang. A voucher specimen (no. 20060401) was deposited at the Faculty of Pharmaceutical Sciences, Kunming Medical University.

Extraction and Isolation Extraction of the aerial part of *K. arborea* Blume (*K. officinalis* Tsiang *et* P. T. Li) (9.0 kg, dry weight) was carried out in the usual manner as has been described in detail before.[20] The alkaloids were partitioned primarily by silica open column chromatography using gradient MeOH/$CHCl_3$ solvent system. Eburenine (**3**, 7.1 mg) was obtained from the fraction eluted with 10—20% MeOH/$CHCl_3$. The fraction eluted with 30—40% MeOH/$CHCl_3$ was subjected to rechromatography on silica gel and the fraction eluted with 1—2.5% MeOH/$CHCl_3$ gave (+)-eburnamonine (5.6 mg). Successive separation of the fraction eluted with 3% MeOH/$CHCl_3$ on a silica gel open column (80% *n*-hexane/EtOAc) gave kopsiyunnanine G (**1**, 1.2 mg), (−)-eburnamine (15.0 mg), (−)-methyleburnamine (1.2 mg), (−)-ethyleburnamine (1.9 mg), (+)-isoeburnamine (4.3 mg), (+)-methylisoeburnamine (2.3 mg), (+)-ethylisoeburnamine (1.8 mg), and (+)-eburnamenine (3.5 mg). On the other hand, separation of the fraction eluted with 4% MeOH/$CHCl_3$ on an amino-silica gel open column (*n*-hexane/EtOAc in gradient) gave kopsiyunnanine H (**2**, 2.1 mg).

Kopsiyunnanine G (**1**): Colorless amorphous solid, $[\alpha]_D^{25}$ −72.9 (*c*=0.07, $CHCl_3$). EI-MS *m/z* (%): 340 (M^+), 80 (100). HR-EI-MS *m/z*: 340.2157 $[M]^+$ (Calcd for $C_{21}H_{28}N_2O_2$: 340.2151). UV λ_{max} (MeOH) nm (log ε): 291.5 (3.59), 246.0 (3.96), 208.0 (4.40). CD (*c*=0.36 mM, MeOH, 25 °C) $\Delta\varepsilon$ (λ nm): −11.7 (211), −1.8 (233), 0 (250), +0.5 (262), 0 (275), −2.8 (297), 0 (320). ^{1}H- and ^{13}C-NMR data are shown in Table 1.

Kopsiyunnanine H (**2**): Light yellow amorphous solid, $[\alpha]_D^{18}$ −98.6 (*c*=0.09, $CHCl_3$). EI-MS *m/z*: 312 (M^+). HR-EI-MS *m/z*: 312.1838 $[M]^+$ (Calcd for $C_{19}H_{24}N_2O_2$: 312.1828). UV λ_{max} (MeOH) nm (log ε): 292.5 (3.73), 283.5 (3.80), 224.0 (4.44), 204.0 (4.33). CD (*c*=0.3 mM, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm): −17.9 (211), 0 (222), +21.4 (229), 0 (243), −3.5 (270), 0 (309). ^{1}H- and ^{13}C-NMR data are shown in Table 1.

Eburenine (**3**): The detailed spectroscopic data of **3** have not been published so far and thus, we present them here. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.51 (1H, d, *J*=7.5 Hz, H-12), 7.33 (1H, d, *J*=7.5 Hz, H-9), 7.29 (1H, ddd, *J*=7.5, 7.5, 1.0 Hz, H-11), 7.16 (1H, ddd, *J*=7.5, 7.5, 1.0 Hz, H-10), 3.19 (1H, overlapped, H-5a), 3.18 (1H, overlapped, H-3a), 3.11 (1H, ddd, *J*=14.0, 14.0, 5.0 Hz, H-16a), 2.76 (1H, ddd, *J*=14.0, 10.0, 3.5 Hz, H-16b), 2.60 (1H, m, H-5b), 2.47 (1H, ddd, *J*=13.5, 13.5, 3.0 Hz, H-17a), 2.41 (1H, s, H-21), 2.18 (1H, overlapped, H-3b), 2.17 (1H, overlapped, H-6a), 1.86 (1H, m, H-14a), 1.65 (1H, dd, *J*=12.5, 5.0 Hz, H-6b), 1.61 (1H, m, H-17b), 1.54 (1H, m, H-14b), 1.48 (1H, br d, *J*=14.0 Hz, H-15a), 1.01 (1H, ddd, *J*=13.5, 13.5, 5.0 Hz, H-15b), 0.64 (2H, m, H_2-19), 0.50 (3H, t, *J*=7.5 Hz, H_3-18). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 192.4 (C-2), 154.5 (C-13), 147.1 (C-8), 127.4 (C-11), 125.1 (C-10), 121.0 (C-9), 120.1 (C-12), 78.9 (C-21), 61.2 (C-7), 54.5 (C-5), 52.0 (C-3), 36.5 (C-20), 35.1 (C-6), 33.2 (C-15), 29.7 (C-19), 27.2 (C-17), 23.7 (C-16), 22.0 (C-14), 7.2 (C-18). CD (*c*=0.37 mM, MeOH, 25 °C) $\Delta\varepsilon$ (λ nm): +10.8 (203), 0 (220), −3.2 (226), 0 (230), +1.0 (237), 0 (241), −0.3 (244), 0 (246), +10.3 (270), 0 (382).

Acknowledgements This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and The Uehara Memorial Foundation.

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