Flabelliformides A and B, Two Novel Indole Alkaloids from *Ervatamia flabelliformis*

by **Shuang Liang**, **Hai-Sheng Chen***, **Yun-Heng Shen**, **Li Jin**, and **Wei-Dong Zhang**

College of Pharmacy, Second Military Medical University, Shanghai 200433, P. R. China
(phone: +86-21-25074439; fax: +86-21-25074439; e-mail: haishengc@hotmail.com)

Flabelliformides A (**1**) and B (**2**), containing a novel C_{20} indole core, have been isolated from the stems of *Ervatamia flabelliformis*. The structures of **1** and **2** were elucidated on the basis of spectral analysis and X-ray crystallography.

Introduction. – *Ervatamia flabelliformis* Tsiang (Apocynaceae) [1] is a common plant cultivated in Yunnan and Guanxi provinces in China, whereas its chemical constituents have not been reported yet. As a part of our continuous research on the antiaddictive compounds from the genus *Ervatamia* [2–3], we now report the isolation of the two novel C_{20} indole alkaloids flabelliformides A (**1**) and B (**2**) from the stems of *E. flabelliformis*, and their structures elucidation by spectroscopic methods and X-ray crystallography.

Results and Discussion. – Flabelliformide A (**1**) was obtained as colorless optically active needles (MeOH) which exhibited a positive reaction with the *Dragendoff* reagent. The IR spectrum showed an absorption for an amide group (1653 cm^{-1}). The empirical molecular formula $C_{20}H_{22}N_2O_2$ was established by HR-TOF-MS ($[M+Na]^+$ at m/z 345.1569). The ^{1}H- and ^{13}C-NMR spectra (*Table*) and NOESY data were compatible with the structure of a 1*H*-pyrido[3′,4′:5,6]cycloocta[1,2-*b*]indole derivative for flabelliformide A (**1**). An X-ray crystal-structure analysis confirmed the planar constitution and the relative configuration of **1** (*Fig.*). Although a compound of structure **1** has been obtained in 1975 by *Knox* and *Slobbe* upon treatment of natural tabaernemontanine (**3**) with MeONa [4] (*Scheme*), to the best of our knowledge, flabelliformide A (**1**) is the first indole alkaloid with an 1*H*-pyrido[3′,4′:5,6]cyclooct[1,2-*b*]indole skeleton isolated from a natural source.

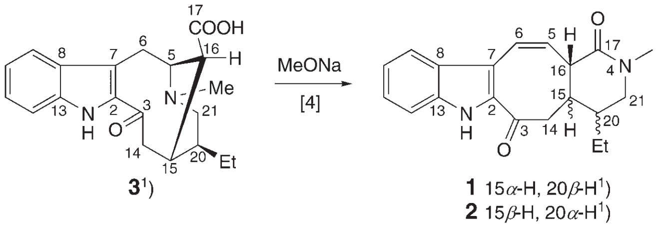
The ^{13}C-NMR spectrum of **1** revealed 20 C-resonances, including those of two Me, three CH_2, and nine CH groups, and of six quaternary C-atoms. Among them, four CH groups (δ(C) 127.0, 121.7, 120.9, and 112.0) and four quaternary C-atoms (δ(C) 136.4, 133.7, 127.2, and 120.0) showed the typical pattern of a 2,3-disubstituted indole alkaloid. In the ^{1}H-NMR spectrum, four aromatic protons at δ(H) 7.71 (*d*, $J=8.0$ Hz, H−C(9)), 7.15 (*t*, $J=8.0$ Hz, H−C(10)), 7.35 (*t*, $J=8.0$ Hz, H−C(11)), and 7.39 (*d*, $J=8.0$ Hz, H−C(12)), together with a signal at δ(H) 9.33 (*s*, H−N(1)), were in good agreement with the assigned indole moiety[1]). The ^{1}H- and ^{13}C-NMR (DEPT) data indicated the presence of a C=O (δ(C) 193.2), an

---

[1]) Trivial atom numbering; for systematic names, see *Exper. Part*.

amide (δ(C) 170.4), an MeN (δ(C) 34.6), and a CH=CH group (δ (C) 132.3 and 124.1). The relative configuration of **1** was obtained through an analysis of coupling constants and the NOESY plot. H–C(16) (δ 3.08), H–C(15) (δ 2.22), and H–C(20) (δ 1.99) were determined to be β-, α-, and β-oriented, respectively, based on the NOE correlation H–C(16)/H–C(20), and the coupling constant ($J = 13$ Hz) between H–C(15) and H–C(16).

Scheme



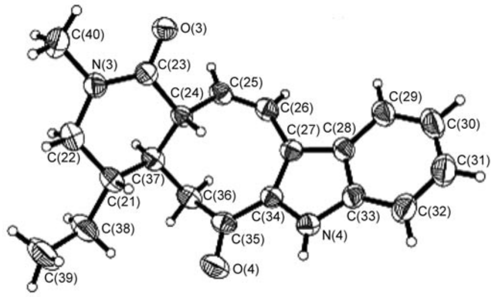


Figure. *X-Ray crystal structure of flabelliformide A* (**1**)[2]). Arbitrary atom numbering.

Flabelliformide B (**2**) was obtained as a white powder, and showed a positive reaction with the *Dragendoff* reagent. The empirical molecular formula $C_{20}H_{22}N_2O_2$ was established by HR-TOF-MS ($[M+H]^+$ at m/z 323.1760). The same planar structure as that of **1** was deduced from the HMQC, HMBC, and ^{1}H,^{1}H-COSY data of **2**. The ^{1}H- and ^{13}C-NMR spectra (*Table*) suggested that **2** may be a diastereoisomer of **1**. Further data confirmed the (15β,20β)-configuration[1]) of flabelliformide B (**2**).

The ^{13}C-NMR and DEPT spectrum of **2** showed 20 C-resonances, including those of two Me, three CH_2, and nine CH groups, and of six quaternary C-atoms. Among them, four CH groups (δ(C) 126.4, 121.0, 120.8, and 112.0) and four quaternary C-atoms (δ(C) 136.4, 132.5, 126.6, and 118.2) pointed to a 2,3-disubstituted indole unit, which was confirmed by proton resonances at δ(H) 7.41 (*d*, $J = 8.0$ Hz, H–C(9)), 7.00 (*t*, $J = 8.0$ Hz, H–C(10)), 7.26 (*t*, $J = 8$, H–C(11)), 7.32 (*d*, $J = 8$, H–C(12)), and 9.92 (*s*, H–N(1))[1]). Also, the ^{1}H- and ^{13}C-NMR (DEPT) data indicated the presence of a C=O (δ(C) 194.8), an amide (δ(C) 171.1), an MeN (δ(C) 34.7), and a CH=CH group (δ(C) 130.8 and 125.9). All the signals in the NMR spectra were very similar to those of **1**, except for the signals of C(14) (δ(C) 41.7 *vs.* 39.7),

---

[2]) Crystal data: $C_{20}H_{22}N_2O_2$, M_r 322.40, monoclinic system, space group $P21$, $a = 8.222(2)$, $b = 8.964(3)$, $c = 23.961(7)$ Å, $V = 1752.7(9)$ Å^3, $Z = 4$, $d = 1.222$mg/m^3. Crystal size $0.15 \times 0.08 \times 0.05$ mm. CCDC-639933 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif.

Table 1. *^{13}C- and ^{1}H-NMR Data* (CDCl$_3$) *of Flabelliformides A* (**1**) *and B* (**2**). δ in ppm, *J* in Hz.

	1^1)		**2**^{1})	
	δ(C)	δ(H)	δ(C)	δ(H)
H−N(1)		9.33 (*s*)		9.92 (*s*)
C(2)	133.7		132.5	
C(3)	193.2		194.8	
H−C(5)	132.3	6.55 (*dd*, *J* = 11.0, 9.0)	130.8	6.08 (*dd*, *J* = 11.0, 9.0)
H−C(6)	124.1	7.27 (*d*, *J* = 11.0)	125.9	7.16 (*dd*, *J* = 11.0, 1.0)
C(7)	120.0		118.2	
C(8)	127.2		126.6	
H−C(9)	121.7	7.71 (*d*, *J* = 8.0)	121.0	7.41 (*d*, *J* = 8.0)
H−C(10)	120.9	7.15 (*t*, *J* = 8.0)	120.8	7.00 (*t*, *J* = 8.0)
H−C(11)	127.0	7.35 (*t*, *J* = 8.0)	126.4	7.26 (*t*, *J* = 8.0)
H−C(12)	112.0	7.39 (*d*, *J* = 8.0)	112.0	7.32 (*d*, *J* = 8.0)
C(13)	136.4		136.4	
CH$_2$(14)	39.7	3.19 (*dd*, *J* = 12.0, 6.0, H$_\alpha$), 2.73 (*dd*, *J* = 12.0, 3.0, H$_\beta$)	41.7	2.87 (*dd*, *J* = 12.0, 10.0, H$_\alpha$), 2.64 (*dd*, *J* = 12.0, 3.0, H$_\beta$)
H−C(15)	49.6	2.22 (*m*)	49.9	2.69 (*m*)
H−C(16)	45.9	3.08 (*dd*, *J* = 13.0, 9.0)	46.0	3.56 (*dd*, *J* = 9.0, 4.5)
C(17)	170.4		171.1	
Me(18)	10.2	0.98 (*t*, *J* = 7.5)	10.4	0.97 (*t*, *J* = 7.5)
CH$_2$(19)	23.9	2.05 (*m*, H$_a$), 1.48 (*m*, H$_b$)	23.1	1.67 (*m*, H$_a$), 1.25 (*m*, H$_b$)
H−C(20)	37.9	1.99 (*m*)	34.7	2.00 (*m*)
CH$_2$(21)	53.3	3.35 (*dd*, *J* = 12.0, 6.0, H$_\alpha$), 3.01 (*dd*, *J* = 12.0, 10.5, H$_\beta$)	54.4	3.42 (*dd*, *J* = 12.5, 5.5, H$_\alpha$), 3.09 (*t*, *J* = 12.0, H$_\beta$)
Me−N(4)	34.6	2.92 (*s*)	34.7	3.01 (*s*)

H−C(15) (δ(H) 2.69 *vs.* 2.22), H−C(16) (δ(H) 3.56 *vs.* 3.08), and C(20) (δ(C) 34.7 *vs.* 37.9). H−C(16) (δ 3.56), H−C(15) (δ 2.69), and H−C(20) (δ 2.00) were determined to be β-, β-, and α-oriented, respectively, based on the NOESY plot. The coupling constant between H−C(15) and H−C(16) was 4.5 Hz, while no NOE correlations H−C(20)/H−C(15) and H−C(16) were observed.

We would like to acknowledge the financial support from the *National Natural Science Foundation of China* (No. 20272081).

Experimental Part

General. Column chromatography (CC): silica gel *H* (10–40 μm) from *Zhifu Huangwu Silica Gel D & R Plant*, Yantai, China; *Sephadex LH-20* and *ODS* from *Pharmacia* and *Merck*, resp. TLC: plates precoated with silica gel *HF$_{254}$* (5–7 μm) from *Zhifu Huangwu Silica Gel D & R Plant*. Optical rotations: *Perkin-Elmer 343* polarimeter; in EtOH. IR Spectra: *Bruker Vector-22* spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker DRX-500* spectrometer; at 500 (^{1}H) and 125 MHz (^{13}C, DEPT); CDCl$_3$ solns. with SiMe$_4$ as internal standard; δ in ppm, *J* in Hz. HR-TOF-MS: ESI mode; *Q-Tof-Micro-Mass* spectrometer; in *m/z* (rel. %).

Plant Material. The plant material was collected in October 2002 in Xishuangbanna, Yunnan province, and identified as *Ervatamia flabelliformis* TSIANG by senior engineer *Wang Hong*, Xishuangbanna Tropical Botanic Garden of the Chinese Academy of Science. A voucher specimen has been deposited in the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai (No. 200210-1).

Extration and Isolation. The air-dried and powdered stems (12 kg) of *E. flabelliformis* were extracted with 95% EtOH under reflux, and the extract was concentrated. The concentrate was dissolved with 2%

HCl soln. and the soln. filtered, and residents (120 g) were given (*Fraction A*). The acidic aq. filtrate was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ layer was dried (Na_2SO_4) and concentrated: 45 g of a crude total alkaloid mixture. *Fr. A* was subjected to CC (silica gel (1 kg), 9 × 100 cm column, petroleum ether/AcOEt 50 : 1, 25 : 1, 10 : 1, 8 : 1, 5 : 1, 3 : 1, and 1 : 1, then $CHCl_3$/MeOH 5 : 1 and 1 : 1): *Fr. 1–24*. *Fr. 6* (5.5 g) was subjected to CC (silica gel (150 g), 6 × 80 cm column, petroleum ether/AcOEt 5 : 1, 3 : 1, and 1 : 1): impure **1** and **2**. The latter were further purified by CC (*Sephadex LH-20* (200 ml), $CHCl_3$/MeOH 1 : 1): **1** (26 mg) and **2** (24 mg).

Flabelliformide A (= rel-*(4*R*,4a*R*,13a*S*)-4-Ethyl-3,4,4a,5,7,13a-hexahydro-2-methyl-1*H*-pyrido[3′,4′:5,6]cyclooct[1,2-*b*]indole-1,6(2*H*)-dione*; **1**): Colorless needles (MeOH). M.p. 264–265°. $[\alpha]_D^{25} = -68.8$ ($c = 0.1$, EtOH). IR (KBr): 3423, 3162, 1653. ^1H- and ^{13}C-NMR: *Table*. EI-MS: 323 (22, $[M+H]^+$), 322 (100, M^+), 294 (20), 265 (14), 251 (25), 183 (14), 180 (22), 154 (16). HR-TOF-MS: 345.1569 ($[M+Na]^+$, $C_{20}H_{22}N_2NaO_2^+$; calc. 345.1579).

Flabelliformide B (= rel-*(4*R*,4a*R*,13a*R*)-4-Ethyl-3,4,4a,5,7,13a-hexahydro-2-methyl-1*H*-pyrido[3′,4′:5,6]cyclooct[1,2-*b*]indole-1,6(2*H*)-dione*; **2**): White powder. M.p. 252–253°. $[\alpha]_D^{25} = -23.3$ ($c = 0.1$, EtOH). IR (KBr): 3440, 2920, 2851, 1731. ^1H- and ^{13}C-NMR: *Table*. EI-MS: 322 (58, M^+), 185 (29), 85 (100), 71 (37), 59 (68), 58 (39), 57 (42), 55 (30). HR-TOF-MS: 323.1760 ($[M+H]^+$, $C_{20}H_{23}N_2O_2^+$; calc. 323.1760).

REFERENCES

[1] P. T. Li, Y. Jiang, 'Flora of China', Science Press, Beijing, 1977, Vol. 16, p. 106.
[2] S. Liang, X. G. Luo, H. S. Chen, X. D. Zhang, *Chin. Chem. Lett.* **2006**, *17*, 662.
[3] S. Liang, H. S. Chen, J. L. Du, *Acad. J. Sec. Mil. Med. Univ.* **2006**, *27*, 892.
[4] J. R. Knox, J. Slobbe, *Aust. J. Chem.* **1975**, *28*, 1843.

Received March 29, 2007