Indole Alkaloids from the Whole Plants of *Ervatamia officinalis*

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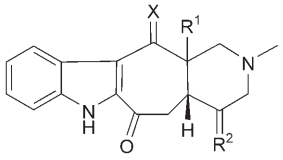
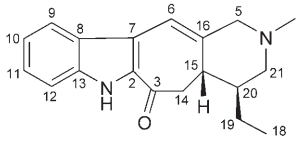
Three new ervatamine-type indole alkaloids, 6-oxo-16,20-episilicine (**1**), 16,20-episilicine (**2**), and 6,16-didehydro-20-episilicine (**6**), along with seven known alkaloids, were isolated from the whole plants of *Ervatamia officinalis*. Their structures were elucidated by spectroscopic methods.

Introduction. – The genus *Ervatamia* (Apocynaceae) comprises *ca.* 120 species distributed mainly in Australia and in the tropical and subtropical areas of Asia [1]. In South China, 15 species and five variants grow, and most of them are used in traditional Chinese medicine [2]. *Ervatamia officinalis*, a shrub growing in the mountain areas of Guangdong, Hainan, and Yunnan provinces, is applied as a folklore herb for the treatment of hypertension, sore throat, and bellyache [2]. Previous chemical studies on this plant had led to the isolation of 16 indole alkaloids, some of which showed antitumor activities [3].

As part of our work on natural products, we carried out a phytochemical re-investigation of *E. officinalis*. Thereby, six ervatamine-type alkaloids were isolated, 6-oxo-16,20-episilicine (**1**), 16,20-episilicine (**2**), 20-episilicine (**3**) [4], methuenine (**4**) [5], 19,20-didehydroervatamine (**5**) [5], 6,16-didehydro-20-episilicine (**6**), and four known vobasine-type alkaloids, tabernaemontanine [6], dregamine [6], vobasine [6], and 16-epiaffinine [7]. We herein report the isolation and structure elucidation of these alkaloids **1**, **2**, and **6**, which are natural products.



	X	R^1	R^2
1	O	α-H	β-Et
2	H_2	α-H	β-Et
3	H_2	β-H	β-Et
4	H_2	β-H	(*E*)-ethylidene
5	H_2	β-MeO_2C	(*E*)-ethylidene



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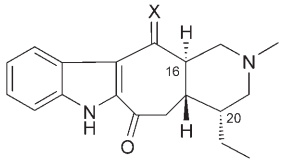

7a X = O
7b X = H_2 (16-episilicine)

Results and Discussion. – 6-Oxo-16,20-episilicine[1]) (**1**), obtained as a yellow, amorphous powder, with $[\alpha]_D^{20} = -46.2$ ($c = 0.13$, $CHCl_3$), has a molecular formula of $C_{19}H_{22}N_2O_2$, as determined by HR-EI-MS (*m/z* 310.1696 (M^+; calc. 310.1681)). Its UV spectrum displayed three typical maxima at λ_{max} (log ε) 220 (4.52), 257 (4.37), and 330 (4.24) nm due to the diacylindole chromophore. The IR spectrum showed an NH absorption at 3304, and strong absorption bands of two conjugated C=O groups at 1664 and 1635 cm^{-1}, respectively. When compared with 6-oxo-16-episilicine (**7a**) [8], the same molecular formula and the similar NMR spectroscopic data (see *Tables 1* and *2* in the *Exper. Part*) suggested that **1** was a stereoisomer of **7a**. The β-configuration of H–C(15) was assigned on the basis of biogenetic considerations [9]. The NMR resonance for H–C(16) at δ(H) 2.92 (*ddd*, $J = 11.1, 11.1, 4.3$ Hz) of **1** is very similar to those of **7a**, indicating that the two alkaloids share the same configuration at C(16). Although the coupling constants of H–C(20) of both alkaloids can not be distinguished due to the complexity of the coupling patterns, the distinctly different H–C(15) signals of **1** at δ(H) 1.77 (*q*, $J = 10.3$ Hz) and of **7a** at δ(H) 2.26 (*td*, $J = 11, 4$ Hz) showed that **1** was the 20-epimer of the latter. Comparison of C(16) at δ(C) 51.8 and C(19) at 18.3 for **7a** with the downfield-shifted C(16) at 57.2 and C(19) at 24.0 for **1**, respectively, supported the absence of a γ-gauche effect between these two groups in the case of **1** (*Fig. 1*). This further confirmed the β-configuration of the 20-Et group. Thus, the structure of alkaloid **1** was undoubtedly identified, and was further confirmed by HMQC and HMBC experiments (*Fig. 2*).

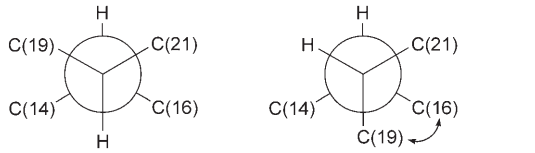

Fig. 1. Newman *projection of* **1** *and* **7a** *viewed along the C(15)–C(20) bond to illustrate potential* γ*-gauche effects*

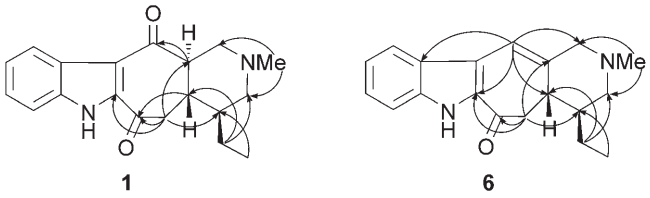

Fig. 2. *Selected HMBC correlations for* **1** *and* **6**

16,20-Episilicine (**2**) showed characteristic UV absorptions at 236 (4.36) and 312 (4.42) nm due to the acylindole chromophore. IR Absorption bands at 3313 (NH) and 1620 cm^{-1} (conjugated C=O) were observed. A molecular formula of $C_{19}H_{24}N_2O$ was derived by HR-EI-MS (*m/z* 296.1895 (M^+; calc. 296.1889)), which was identical with

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

that of 16-episilicine (**7b**) [10]. Analysis of the ^{1}H- and ^{13}C-NMR data (see *Tables 1* and *2*) showed that **2** was closely related to **7b**, which implies that the two compounds were stereoisomers. The ^{1}H- and ^{13}C-NMR data indicated that C(20) was the epimeric center, as judged by the significant chemical-shift changes around C(20). The configuration at C(20) was determined by a NOESY spectrum (*Fig. 3*), in which strong correlations between H–C(15) at δ(H) 1.40 (*m*) and H–C(19) at δ(H) 1.19 (*m*) indicated that H–C(15) and the 20-Et group were on the same side of the molecular plane of **2**. The structure of **2** was, hence, clearly elucidated, and was confirmed by HMQC and HMBC experiments.

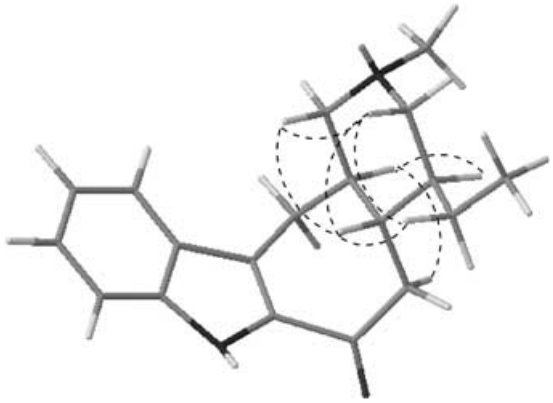

Fig. 3. *Key NOESY correlations for* **2**

6,16-Didehydro-20-episilicine (**6**) had the molecular formula $C_{19}H_{22}N_2O$, as determined by HR-EI-MS (*m*/*z* 294.1720 (M^+; calc. 294.1732)). **2**. Compound **6** exhibited more-complicated UV absorptions than **2**, with λ_{max} (log ε) 217 (4.40), 236 (4.40), 252 (4.45), and 326 (4.06) nm, implying the presence of an extended conjugated system. The IR spectrum showed NH (3288) and conjugated C=O (1633 cm^{-1}) absorption bands. An olefinic signal at δ(H) 6.78 (br. *s*) in the ^{1}H-NMR spectrum (see *Table 1*) of **6** was assigned to H–C(6), and two olefinic signals at δ(C) 116.0 and 142.3 in the ^{13}C-NMR spectrum (see *Table 2*) were attributable to C(6) and C(16), respectively. The aforementioned data indicated that alkaloid **6** was a didehydro derivative of **2**. The location of the C=C bond was confirmed by the HMBC correlations (*Fig. 2*) from H–C(6) at δ(H) 6.78 (br. *s*) to both C(2) at δ(C) 131.8 and C(8) at δ(C) 126.5. The structure of **6** was, therefore, ascertained. HMQC Experiments were also carried out to assign all the H-atoms.

The known alkaloids were identified as 20-episilicine (**3**) [4], methuenine (**4**) [5], 19,20-didehydroervatamine (**5**) [5], tabernaemontanine [6], dregamine [6], vobasine [6], and 16-epiaffinine [7] on the basis of spectroscopic data. 20-Episilicine (**3**), previously reported as a hydrogenation product of methuenine (**4**) [5], was found to be a natural product, and its detailed analytical data are reported in this paper for the first time.

Experimental Part

General. All solvents were of anal. grade (*Shanghai Chemical Reagents Co.*, China). Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Haiyang Chemical Co.*, China); silica gel *H* (60 μm; *Qingdao Haiyang Chemical Co.,* China); *CHP20P* (75–150 μm; *Mitsubishi Chemical Industries, Ltd.*), and C_{18} reverse-phased (*RP-18*) silica gel (150–200 mesh; *Merck*). Thin-layer chromatography (TLC): precoated silica-gel GF_{254} plates (*Qingdao Haiyang Chemical Co.*, China), detection by spraying with *Dragendorff* reagent. Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Hitachi U-2010* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer 577* spectrophotometer, KBr pellets; in cm^{-1}. NMR Spectra: *Varian Mercury-400* instrument; chemical shifts δ in ppm rel. to Me_4Si as internal standard, coupling constants *J* in Hz. EI-MS: *Finnigan MAT-95* mass spectrometer (70 eV); in *m*/*z* (rel. %).

Plant Material. Whole plants of *E. officinalis* were collected in November 2003 in Hainan province, P. R. China. The plant was identified by Prof. *Shi-Man Huang*, Research Center of Biology, Hainan University, China. A voucher specimen (EO-2003-1Y) was deposited at the Shanghai Institute of Materia Medica.

Extraction and Isolation. Powdered *E. officinalis* (8.0 kg) was percolated with 90% EtOH (30 l) at r.t. for 5 d (3×). The extract was evaporated to dryness under reduced pressure, and the residue (400 g) was suspended in H_2O (2 l; pH 1–2, adjusted with 2M H_2SO_4). After removal of the nonalkaloidal products by extraction with Et_2O, the acidic aq. soln. was carefully basified to pH 8–9 with solid Na_2CO_3, and then extracted with $CHCl_3$ (3 × 1 l) to afford the crude alkaloids (24.2 g). They were subjected to CC (SiO_2 (200–300 mesh); petroleum ether/Et_2O/MeOH 50:10:3, 30:10:3, 20:10:3, 10:10:3, and 5:10:3): fractions *Fr. 1–5*. *Fr. 1* (6.01 g) was chromatographed (SiO_2 *H*; $CHCl_3$/MeOH 200:1, 100:1, 50:1, 20:1, 10:1, and 5:1): *Fr. 1.1–1.6*. *Fr. 1.4* was purified further by CC (SiO_2 *H*; petroleum ether/AcOEt/Et_2NH 120:10:3 and 100:10:3), which afforded tabernaemontanine (55 mg). *Fr. 3* (3.10 g) was subjected to CC (SiO_2 *H*; petroleum ether/AcOEt /Et_2NH 100:10:3, 70:10:3, 40:10:3, 20:10:3, and 10:10:3): *Fr. 3.1–3.7*. *Fr. 3.1* was purified by CC (*RP-18*; MeOH/H_2O 8:2) to afford **3** (92 mg). *Fr. 3.3* was re-subjected to extensive CC (1. *RP-18*, MeOH/H_2O 7:3 and 8:2; 2. SiO_2 *H*, $CHCl_3$/MeOH 100:1), and then prep. TLC ($CHCl_3$/MeOH 80:1), to afford **1** (20 mg), **6** (9 mg), dregamine (63 mg), **5** (5 mg), and vobasine (4 mg). *Fr. 4* (4.60 g) was purified by CC (*CHP20P*; MeOH/H_2O 9:1), and then, the major alkaloid fraction was subjected to CC (SiO_2 *H*; petroleum ether/AcOEt/Et_2NH 100:10:3, 70:10:3, 40:10:3, 20:10:3, and 10:10:3): *Fr. 4.1–4.8*. *Fr. 4.2* was re-subjected to CC (*RP-18*; MeOH/H_2O 7:3), and then further purified by prep. TLC (SiO_2; $CHCl_3$/MeOH 50:1 and petroleum ether/AcOEt/Et_2NH 70:10:3) to afford **4** (15 mg) and **2** (12 mg). *Fr. 4.6* was re-subjected to CC (*RP-18*; MeOH/H_2O 6:4 and 7:3), followed by prep. TLC (SiO_2; $CHCl_3$/MeOH 30:1), to afford 16-epiaffinine (6 mg).

6-Oxo-16,20-Episilicine (=*(7a*S,*8*S,*11a*R*)-8-Ethyl-7a,8,9,10,11,11a-hexahydro-10-methylpyrido[3′,4′:4,5]cyclohept[1,2*-b*]indole-6,12(5*H,*7*H*)-dione*; **1**). Yellow, amorphous powder. $[\alpha]_D^{20} = -46.2$ ($c = 0.13$, $CHCl_3$). UV (MeOH): 220 (4.52), 257 (4.37), 330 (4.24). IR (KBr): 3304, 2962, 2875, 2789, 1664, 1635, 1520, 1454, 1429, 1329, 1244, 1146, 1014, 754. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 310 (99, M^+), 293 (100), 282 (23), 265 (20), 239 (25), 210 (20), 180 (22), 144 (26), 124 (40), 115 (21), 84 (97). HR-EI-MS: 310.1696 (M^+, $C_{19}H_{22}N_2O_2^+$; calc. 310.1681).

16,20-Episilicine (=*(7a*S,*8*S,*11a*R*)-8-Ethyl-7,7a,8,9,10,11,11a,12-octahydro-10-methylpyrido[3′,4′:4,5]cyclohept[1,2*-b*]indol-6(5*H*)-one*; **2**). Colorless, amorphous powder. $[\alpha]_D^{20} = -67.1$ ($c = 0.23$, $CHCl_3$). UV (MeOH): 236 (4.36), 312 (4.42). IR (KBr): 3313, 2931, 2875, 2783, 1620, 1576, 1458, 1335, 1254, 743. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 296 (100, M^+), 267 (7), 138 (37), 124 (39). HR-EI-MS: 296.1895 (M^+, $C_{19}H_{24}N_2O^+$; calc. 296.1889).

20-Episilicine (=*(7a*S,*8*S,*11a*S*)-8-Ethyl-7,7a,8,9,10,11,11a,12-octahydro-10-methylpyrido[3′,4′:4,5]cyclohept[1,2*-b*]indol-6(5*H*)-one*; **3**). Colorless, amorphous powder. $[\alpha]_D^{20} = -57.8$ ($c = 0.12$, $CHCl_3$). UV (MeOH): 237 (4.28), 312 (4.45). IR (KBr): 3363, 2935, 2777, 1645, 1539, 1456, 1333, 1240, 748, 546. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 296 (65, M^+), 167 (33), 149 (42), 138 (39), 124 (100).

6,16-Didehydro-20-episilicine (=*(7a*S,*8*S*)-8-Ethyl-7,7a,8,9,10,11-hexahydro-10-methylpyrido[3′,4′:4,5]cyclohept[1,2*-b*]indol-6(5*H*)-one*; **6**). Yellow, amorphous powder. $[\alpha]_D = +76.7$ ($c = 0.21$, $CHCl_3$). UV (MeOH): 217 (4.40), 236 (4.40), 252 (4.45), 326 (4.06). IR (KBr): 3288, 2962, 2933, 1633, 1531, 1460, 1335, 1244, 746. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 294 (100, M^+), 265 (61), 251 (33), 237 (20), 180 (38), 122 (21), 86 (91). HR-EI-MS: 294.1720 (M^+, $C_{19}H_{22}N_2O^+$; calc. 294.1732).

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Table 1. *^{1}H-NMR Data of* **1**–**3** *and* **6**. At 400 MHz in $CDCl_3$; δ in ppm, J in Hz.

Atom	**1**	**2**	**3**	**6**
CH_2(5)	3.63 (*ddd*, J = 11.6, 4.3, 1.9)	3.03 (*ddd*, J = 11.3, 4.3, 1.9)	2.61 (*dd*, J = 11.3, 3.4)	3.71 (br. *d*, J = 14.5)
	1.93 (*dd*, J = 11.6, 11.1)	1.82 (*dd*, J = 11.3, 11.3)	2.01 (*dd*, J = 11.3, 8.0)	2.94–2.98[b])
CH_2(6) or H–C(6)	–	3.26 (*dd*, J = 17.4, 5.2)	3.23 (*dd*, J = 16.5, 4.0)	6.78 (br. *s*)
		2.80 (*dd*, J = 17.4, 8.8)	3.01 (*dd*, J = 16.5, 10.0)	
H–C(9)	8.42 (*d*, J = 8.2)	7.64 (*dd*, J = 8.2, 0.6)	7.69 (*dd*, J = 8.1, 0.7)	7.77 (*d*, J = 8.1)
H–C(10)	7.31 (*ddd*, J = 8.2, 7.0, 1.0)	7.13 (*ddd*, J = 8.2, 6.3, 1.7)	7.14 (*ddd*, J = 8.1, 6.7, 1.2)	7.18 (*ddd*, J = 8.1, 6.2, 1.5)
H–C(11)	7.39–7.43 (*m*)	7.32–7.36 (*m*)[a])	7.32–7.36 (*m*)	7.35–7.39 (*m*)[c])
H–C(12)	7.48 (*d*, J = 8.4)	7.34–7.38 (*m*)[a])	7.36–7.41 (*m*)	7.37–7.41 (*m*)[c])
CH_2(14)	3.15 (*d*, J = 16.6)	3.08 (*dd*, J = 16.8, 1.6)	2.79–2.83 (*m*)	2.80 (*dd*, J = 15.1, 1.8)
	2.78 (*dd*, J = 16.6, 10.1)	2.62 (*dd*, J = 16.8, 9.6)		2.89–2.98[b])
H–C(15)	1.77 (*q*, J = 10.3)	1.37–1.45 (*m*)	1.79–1.86 (*m*)	2.19 (*dd*, J = 11.2, 11.2)
H–C(16)	2.92 (*ddd*, J = 11.1, 11.1 4.3)	2.17–2.26 (*m*)	2.19–2.27 (*m*)	–
Me(18)	0.94 (*t*, J = 7.5)	0.92 (*t*, J = 7.5)	0.89 (*t*, J = 7.5)	0.97 (*t*, J = 7.5)
CH_2(19)	1.69–1.77 (*m*)	1.71 (*dqd*, J = 14.1, 7.5, 2.2)	1.50–1.58 (*m*)	1.69–1.77 (*m*)
	1.17–1.25 (*m*)	1.15–1.23 (*m*)	1.32–1.40 (*m*)	1.21–1.29 (*m*)
H–C(20)	1.50–1.59 (*m*)	1.42–1.50 (*m*)	1.62–1.70 (*m*)	1.58–1.67 (*m*)
CH_2(21)	3.01–3.07 (*m*)	2.99 (*ddd*, J = 11.2, 3.7, 1.9)	2.54 (*dd*, J = 11.7, 5.7)	3.01 (*ddd*, J = 11.5, 4.3, 2.0)
	1.61 (*dd*, J = 11.1, 11.1)	1.60 (*dd*, J = 11.2, 10.7)	2.44 (*dd*, J = 11.7, 3.8)	1.77 (*dd*, J = 11.5, 11.1)
NMe	2.36 (*s*)	2.32 (*s*)	2.29 (*s*)	2.38 (*s*)
NH	10.05 (br. *s*)	9.12 (br. *s*)	9.12 (br. *s*)	9.27 (br. *s*)

[a–c]) Overlapping signals.

Table 2. *^{13}C-NMR Data of* **1**–**3** *and* **6**. At 100 MHz in $CDCl_3$; δ in ppm.

Atom	**1**	**2**	**3**	**6**
C(2)	134.4	132.0	133.2	131.8
C(3)	192.3	193.4	193.9	191.2
C(5)	58.4	63.4	62.0	63.5
C(6)	197.1	29.8	27.0	116.0
C(7)	118.1	122.2	125.9	121.2
C(8)	127.2	127.7	127.4	126.5
C(9)	124.6	120.8	120.9	120.7
C(10)	123.8	120.0	120.1	120.7
C(11)	127.2	126.6	126.3	126.9
C(12)	112.1	111.9	112.1	112.1
C(13)	136.0	136.6	136.4	136.7
C(14)	46.3	47.0	45.7	45.1
C(15)	37.3	40.4	37.4	38.4
C(16)	57.2	1.0	37.6	142.3
C(18)	11.2	11.3	11.3	11.6
C(19)	24.0	24.4	24.4	25.3
C(20)	41.4	42.4	40.7	42.6
C(21)	60.4	60.7	59.0	59.8
NMe	46.2	46.3	46.9	45.9

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