# Three New Monoterpenoid Indole Alkaloids from *Ervatamia flabelliformis*

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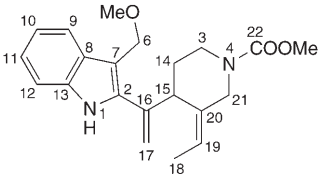
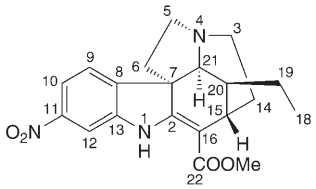
The three new monoterpenoid indole alkaloids **1**–**3** were isolated from the stems of *Ervatamia flabelliformis*. The structures were elucidated on the basis of spectral analysis and chemical derivatization.

**Introduction.** – In our previous paper [1], we have reported the isolation of two novel indole alkaloids, flabelliformides A and B, from the stems of *Ervatamia flabelliformis* TSIANG (Apocynaceae), a common plant cultivated in Yunnan and Guangxi provinces in China [2][3]. In the present work, we isolated three new monoterpenoid indole alkaloids, flabelliformine[1]) (**1**), 11-nitrotubotaiwine[1]) (**2**), and sodium dregaminate[1]) (**3**). Herein we report the isolation and structural elucidation of the three new compounds.




**1**[1])

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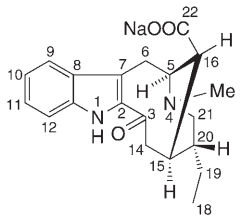



**3**[1])

**Results and Discussion.** – Flabelliformine (**1**) was obtained as a yellow powder which exhibited a positive reaction with the *Dragendoff* reagent. The empirical molecular formula $C_{21}H_{26}N_2O_3$ was established by HR-TOF-MS ($[M + Na]^+$ at $m/z$ 377.1844). The $^1$H- and $^{13}$C-NMR (*Table*), HMBC, $^1$H,$^1$H-COSY, and NOE data

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

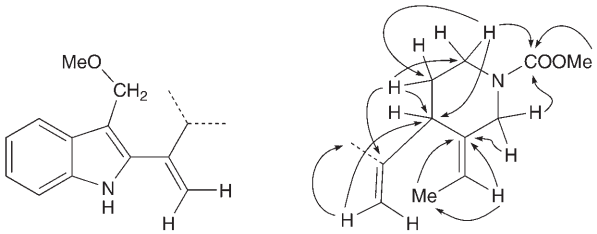
Table. *$^{13}C$- and $^{1}H$-NMR Data* ($(D_6)$DMSO) *of Compounds* **1**–**3**[1]). $\delta$ in ppm, $J$ in Hz.

| | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) |
| H–N(1) | – | 8.36 (*s*) | – | 10.20 (*s*) | – | 11.49 (*s*) |
| C(2) | 137.4 | – | 165.8 | – | 135.3 | – |
| $CH_2$(3) or C(3) | 40.8 | 3.07–3.12, 3.52–3.56 (2*m*) | 44.7 | 2.30–2.33, 2.81–2.85 (2*m*) | 193.0 | – |
| $CH_2$(5) or H–C(5) | – | – | 53.5 | 2.69–2.72, 3.02–3.06 (2*m*) | 55.3 | 3.71 (*t*, *J* = 9.0) |
| $CH_2$(6) | 65.4 | 4.66, 4.62 (2*d*, *J* = 12.0) | 44.5 | 1.64–1.67, 2.72–2.77 (2*m*) | 19.0 | 3.27 (*d*, *J* = 9.0) |
| C(7) | 110.7 | – | 54.9 | – | 120.8 | – |
| C(8) | 128.6 | – | 137.3 | – | 127.7 | – |
| H–C(9) | 119.1 | 7.70 (*d*, *J* = 8.0) | 109.5 | 7.18 (*d*, *J* = 9.0) | 120.3 | 7.73 (*d*, *J* = 8.0) |
| H–C(10) | 120.2 | 7.14 (*t*, *J* = 8.0) | 125.0 | 8.08 (*dd*, *J* = 9.0, 2.4) | 119.1 | 7.04 (*t*, *J* = 8.0) |
| H–C(11) or C(11) | 122.5 | 7.19 (*t*, *J* = 8.0) | 140.6 | – | 125.1 | 7.24 (*t*, *J* = 8.0) |
| H–C(12) | 110.7 | 7.32 (*d*, *J* = 8.0) | 115.6 | 8.17 (*d*, *J* = 2.4) | 112.3 | 7.38 (*d*, *J* = 8.0) |
| C(13) | 135.2 | – | 150.3 | – | 136.4 | – |
| $CH_2$(14) | 28.4 | 1.72–1.76 (*m*) | 27.8 | 1.64–1.67 (*m*) | 39.0 | 2.31 (*dd*, *J* = 12.0, 7.0), 3.17 (*t*, *J* = 12.0) |
| H–C(15) | 39.5 | 3.85–3.88 (*m*) | 31.0 | 3.01–3.03 (*m*) | 30.1 | 2.57–2.61 (*m*) |
| C(16) or H–C(16) | 139.2 | – | 98.3 | – | 44.7 | 2.01 (*s*) |
| $CH_2$(17) | 118.9 | 5.48, 5.34 (2 *s*) | – | – | – | – |
| Me(18) | 13.0 | 1.67 (*d*, *J* = 7.0) | 11.3 | 0.74 (*t*) | 11.1 | 0.87 (*t*, *J* = 7.0) |
| H–C(19) or $CH_2$(19) | 124.5 | 5.72–5.75 (*m*) | 23.5 | 0.76–0.79 (*m*) | 23.0 | 1.14–1.19 (*m*) |
| C(20) or H–C(20) | 133.2 | – | 40.2 | 1.84–1.88 (*m*) | 36.6 | 2.20–2.25 (*m*) |
| $CH_2$(21) or H–C(21) | 48.5 | 3.81 (*d*, *J* = 14.0) | 64.8 | 3.91 (*s*) | 48.2 | 2.23–2.27, 2.57–2.61 (2*m*) |
| C(22)=O | 156.0 | – | 165.6 | – | 176.4 | – |
| *Me*O–C(6) | 57.6 | 3.40 (*s*) | – | – | – | – |
| COO*Me* | 52.5 | 3.68 (*s*) | 51.1 | 3.72 (*s*) | – | – |
| MeN | – | – | – | – | 42.1 | 2.36 (*s*) |

established the structure of **1** which was named flabelliformine. To the best of our knowledge, **1** is the first indole alkaloid with a 2-[1-(piperidin-4-yl)ethenyl]-3-(methoxymethyl)-1*H*-indole skeleton isolated from a natural source.

The $^{13}$C-NMR and DEPT spectrum of **1** revealed 21 C-resonances, including those of three Me, three $CH_2$, and nine CH groups, and of six quaternary C-atoms. Among them, four CH groups ($\delta$(C) 122.5, 120.2, 119.1, and 110.7) and four quaternary C-atoms ($\delta$(C) 137.4, 135.2, 128.6, and 110.7) showed the typical pattern of a 2,3-disubstituted indole alkaloid, which was confirmed by the resonances at $\delta$(H) 7.70 (*d*, *J* = 8.0 Hz, H–C(9)), 7.14 (*t*, *J* = 8.0 Hz, H–C(10)), 7.19 (*t*, *J* = 8.0 Hz, H–C(11)), 7.32 (*d*, *J* = 8.0 Hz, H–C(12)), and 8.36 (*s*, H–N(1)). The $^{1}$H- and $^{13}$C-NMR (DEPT) data indicated the presence of an amide ($\delta$(C) 156.0), two MeO groups ($\delta$(C) 52.5 and 57.6; $\delta$(H) 3.68 and 3.40), a CH=C group ($\delta$(C) 124.5 and 133.2), and a CH=$CH_2$ group ($\delta$(C) 139.2 and 118.9). In the HMBC plot, the protons at $\delta$(H)

4.66 and 4.62 showed correlations with the signals at $\delta$(C) 57.6 (MeO), 137.4 (C(2)), 110.7 (C(7)), and 128.6 (C(8)), and the protons at $\delta$(H) 5.48 and 5.34 showed correlations with the signals at $\delta$(C) 137.4 (C(2)) and 39.5 (C(15)), revealing the presence of structural unit **A** (*Fig.*). The interpretation of the $^1$H- and $^{13}$C-NMR data of **1**, together with the analysis of the $^1$H,$^1$H-COSY and HMQC data, allowed the assignment of a $-CH_2-CH_2-CH-C=CH_2$ fragment due to the signals at $\delta$(H) 3.07–3.12 and 3.52–3.56 (2 *m*, each 1 H, $CH_2$(3)), 1.72–1.76 (*m*, $CH_2$(14)), 3.85–3.88 (*m*, H–C(15)), and 5.48 and 5.34 (2*s*, each 1 H, $CH_2$(17)). Furthermore, the protons at $\delta$(H) 3.07–3.12 (1 H of $CH_2$(3)) and 3.81 (*d*, $J$ = 14.0 Hz, $CH_2$(21)) showed correlation with the signal at $\delta$(C) 156.0 (C(22)=O), and the protons at $\delta$(H) 1.67 (Me(18)) showed correlations with the signals at $\delta$(C) 133.2 (C(20)) in the HMBC plot, which indicated the structure of a piperidine ring with a 1-carboxylate and a 3-ethylidene group (see **B**, in *Fig.*). The configuration of the C(19)=C(20) bond was assigned to be (*E*) through the observation of an NOE correlation between Me(18) ($\delta$(H) 1.67) and one proton of $CH_2$(17) ($\delta$(H) 5.34).




**A** **B**

Figure. *Structures of units* **A** *and* **B** (key HMBC correlations) *of* **1**

Compound **2** was obtained as a yellow powder which exhibited a positive reaction with the *Dragendoff* reagent. The empirical molecular formula $C_{20}H_{23}N_3O_4$ was established by HR-TOF-MS ($[M+H]^+$ at $m/z$ 370.1763). Comparison of the NMR spectra of **2** (*Table*) with those of tubotaiwine [3] suggested that compound **2** was 11-substituted tubotaiwine. The molecular mass of **2** was 45 units higher than that of tubotaiwine, indicating that compound **2** had an additional $NO_2$ group. All $^1$H,$^1$H-COSY, HMQC, HMBC, and NOESY data were consistent with the proposed structure of **2** which was named 11-nitrotubotaiwine. This is the first time that the isolation of a nitro-substituted indole alkaloid from *Ervatamia* genus is reported.

The $^1$H-NMR spectrum of **2** displayed a NH *s* at 10.06 (H–N(1)) and an aromatic *ABX* coupling system at $\delta$(H) 8.17 (*d*, $J$ = 2.4 Hz, H–C(12)), 8.08 (*dd*, $J$ = 2.4, 9.0 Hz, H–C(10)), and 7.18 (*d*, $J$ = 9.0 Hz, H–C(9)), which showed the typical pattern of a 10- or 11-substituted indole alkaloid. The NOESY correlation $\delta$ (H) 8.17 (H–C(12))/$\delta$(H) 10.06 (NH) indicated that the substituting group was linked to C(11).

Compound **3**, a white powder, exhibited a positive reaction with the *Dragendoff* reagent. The empirical molecular formula $C_{20}H_{24}N_2NaO_3$ was established by HR-TOF-MS ($[M+H]^+$ at $m/z$ 363.1686). The NMR spectra of **3** (*Table*) were very similar to those of dregamine [4], except that the MeO signal of dregamine was absent in **3**. Considering the molecular mass of **3**, it was deduced that **3** was sodium dregaminate. To determine its structure furthermore, compound **3** was treated with $MeOH/H_2SO_4$. The NMR data of the obtained ester were in good agreement with those of dregamine.

Consequently, the structure of **3** was established and the compound was named sodium dregaminate.

The $^{13}$C-NMR and DEPT spectrum of **3** revealed 20 C-resonances, including those of two Me, four $CH_2$, and eight CH groups, and of six quaternary C-atoms. Among them, four CH groups ($\delta$(C) 125.1, 120.3, 119.1, and 112.3) and four quaternary C-atoms ($\delta$(C) 136.4, 135.3, 127.7, and 120.8) showed the typical pattern of a 2,3-disubstituted indole alkaloid, which was confirmed by the resonances at $\delta$(H) 7.73 (*d*, *J* = 8.0 Hz, H–C(9)), 7.04 (*t*, *J* = 8.0 Hz, H–C(10)), 7.24 (*t*, *J* = 8 Hz, H–C(11)), 7.38 (*d*, *J* = 8 Hz, H–C(12)), and 11.49 (*s*, H–N(1)).

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* (Yantai, China). NMR Spectra: *Bruker DRX-500* spectrometer; at 500 ($^1$H) and 125 MHz ($^{13}$C and DEPT); ($D_6$)DMSO solns. with $SiMe_4$ as internal standard; $\delta$ in ppm, *J* in Hz. HR-TOF-MS: ESI mode; *Micromass Q-Tof* 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 was deposited in the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai (No. 200210-1).

*Extraction 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 from solid material (120 g). The aq. acidic 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 alkaloid mixture. The $CHCl_3$ extract 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. 13* (5.5 g) was subjected to CC (silica gel (150 g), 6 × 80 cm, $CHCl_3$/MeOH 20:1 and 15:1): impure **1**. The latter was further purified by CC (*Sephadex LH-20* (200 ml), $CHCl_3$/MeOH 1:1): **1** (30 mg). *Fr. 17* (2.5 g) was subjected to CC (silica gel (75 g), 6 × 80 cm, $CHCl_3$/MeOH 10:1 and 8:1): impure **2**. The latter was further purified by CC (*Sephadex LH-20* (200 ml), $CHCl_3$/MeOH 1:1): **2** (15 mg). *Fr. 19* (700 mg) was subjected to CC (*ODS* (100 g), MeOH/$H_2O$ 45:55): **3** (35 mg).

*Flabelliformine* (= *Methyl (3*E*,4*S*)-3-Ethylidene-4-{1-[3-(methoxymethyl)-1*H*-indol-2-yl]ethenyl}piperidine-1-carboxylate*; **1**): White powder (MeOH). $[\alpha]_D^{25} = -29.0$ (*c* = 0.20, EtOH). $^1$H- and $^{13}$C-NMR (($D_6$)DMSO): *Table*. EI-MS: 354 (10, $M^+$), 322 (51), 307 (98), 279 (23), 220 (57), 221 (52), 208 (40), 59 (100). HR-TOF-MS: 377.1844 ($[M + Na]^+$, $C_{21}H_{26}N_2NaO_3^+$; calc. 377.1841).

*11-Nitrotubotaiwine* (= *Methyl (3*S*,3a*R*,4*S*,5*S*,11b*S*)-4-Ethyl-1,2,3a,4,5,7-hexahydro-9-nitro-3,5-ethano-3*H*-pyrrolo[2,3-*d*]carbazole-6-carboxylate*; **2**): Yellow powder (MeOH). $[\alpha]_D^{25} = -13.9$ (*c* = 0.20, EtOH). $^1$H- and $^{13}$C-NMR (($D_6$)DMSO): *Table*. HR-TOF-MS: 370.1763 ($[M + H]^+$, $C_{20}H_{25}N_3O_4^+$; calc. 370.1767).

*Sodium Dregaminate* (= *(2*S*,5*R*,6*S*,14*S*)-5-Ethyl-2,3,4,5,6,7,8,9-octahydro-3-methyl-8-oxo-2,6-methano-1*H*-azecino[5,4-*b*]indole-14-carboxylic Acid Sodium Salt* = *(20α)-19,20-Dihydro-3-exovobasan-17-oic Acid Sodium Salt*; **3**): White powder (MeOH). $[\alpha]_D^{25} = +24.1$ (*c* = 0.20, EtOH). $^1$H- and $^{13}$C-NMR (($D_6$)DMSO): *Table*. HR-TOF-MS: 363.1686 ($[M + H]^+$, $C_{20}H_{25}N_3NaO_4^+$; calc. 363.1685).

*Esterification of* **3**. To a suspension of **3** (10 mg) in MeOH (10 ml), a drop of conc. $H_2SO_4$ was added and the mixture was heated under reflux for 4 h. Then the mixture was basified with 5% $K_2CO_3$ soln. to pH 9–10. After evaporation of the MeOH, the residue was extracted with $CHCl_3$ (20 ml × 3) and the

extract concentrated to afford a white power (6.2 mg). NMR: in good agreement with those of dregamine.

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*Received September 15, 2007*