Two New Lycopodine Alkaloids from *Huperzia serrata*

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Two new lycopodine alkaloids, (12β)-12-hydroxyhuperzine G (**1**) and (5β,6β,15α)-15-methyllycopodane-5,6-diol (**2**), were isolated from the whole plants of *Huperzia serrata*, together with six known compounds, huperzines A, B, and G, phlegmariurine B, (8β)-8-hydroxyphlegmariurine B, and lycoposerramine D. Their structures were elucidated on the basis of spectroscopic analysis, including HR-ESI-MS, ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY data.

Introduction. – *Huperzia serrata* (THUNB. EX MURRAY) TREV. (Huperziaceae) is one of the most commonly used traditional Chinese herbal medicines for the treatment of contusion, strain, swelling, and schizophrenia [1]. Previous investigations have shown that it contains many alkaloids with novel and diverse structures including huperzine A, which was reported to increase efficiency for learning and memory in animals and show promise in the treatment of *Alzheimer*'s disease and myasthenia gravis [2–5]. Further, numerous efforts on the isolation of new potent alkaloids from *H. serrata* and other related plants have been carried out by many research groups [6–8]. Up to date, most of the isolated alkaloids are liposoluble. As a part of our research of structurally unique and biologically active compounds from medicinal plants of Yunnan, China, we isolated and identified two new alkaloids, (12β)-12-hydroxyhuperzine G (**1**) which is hydrosoluble and (5β,6β,15α)-15-methyllycopodane-5,6-diol (**2**), as well as six known compounds, *i.e.*, huperzines A, B, and G (**3**–**5**) [2][9][10], phlegmariurine B (**6**) [11], (8β)-8-hydroxyphlegmariurine B (**7**) [12], and lycoposerramine D (**8**) [13], from *H. serrata*. This article focuses on the isolation and structural elucidation of **1** and **2**.

Results and Discussion. – The alkaloid fraction was obtained by extraction of the whole plant of *H. serrata* with aqueous tartaric acid, basification to pH 10 with saturated sodium carbonate solution, and partition with $CHCl_3$ and BuOH. The BuOH layer was separated and purified by repeated chromatography to afford the two new alkaloids (12β)-12-hydroxyhuperzine G[1]) and (5β,6β,15α)-15-methyllycopodane-5,6-diol[1]) (**1** and **2**, resp.; *Fig. 1*), along with huperzine G (**5**) and lycoposerramine D (**8**). The $CHCl_3$ fraction yielded the known huperzines A and B (**3** and **4**, resp.),

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

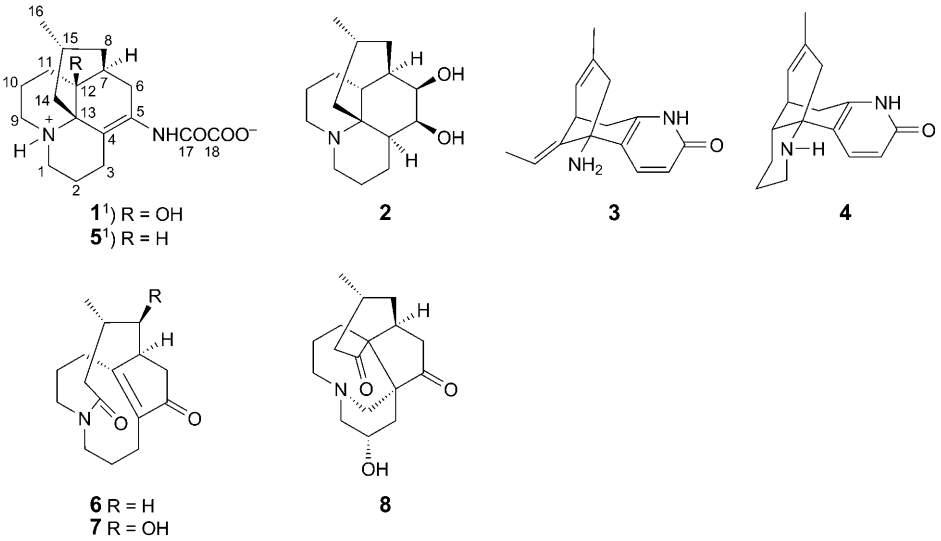

Fig. 1. *Alkaloids isolated from* Huperzia serrata

phlegmariurine (**6**), and (8β)-8-hydroxyphlegmariurine B (**7**) after repeated chromatography.

(12β)-12-Hydroxyhuperzine G (**1**) was obtained as a colorless amorphous powder, which could easily be dissolved in H_2O. Its molecular formula was established as $C_{18}H_{26}N_2O_4$ by HR-ESI-MS analysis. The IR absorptions at 3387, 3361, 1698, 1626, and 788 cm^{-1} suggested the presence of OH, carboxy, and amide groups. The ^{13}C-NMR and DEPT spectra of **1** displayed 18 signals, including one Me group, nine sp^3 CH_2 groups, two sp^3 CH groups, four sp^2 quaternary C-atoms including two CO groups, and two sp^3 quaternary C-atoms. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** (*Table*) with those of the known huperzine G (**5**), which was also obtained in this study as a hydrosoluble component, showed that the two compounds were similar. From further data, including a HMBC spectrum (*Fig. 2*), the structure of compound **1** was identified as a novel alkaloid, which was given the name (12β)-12-hydroxyhuperzine G. The only difference between the NMR data of **1** and **5** is the replacement of the signal of H–C(12) of **5** by

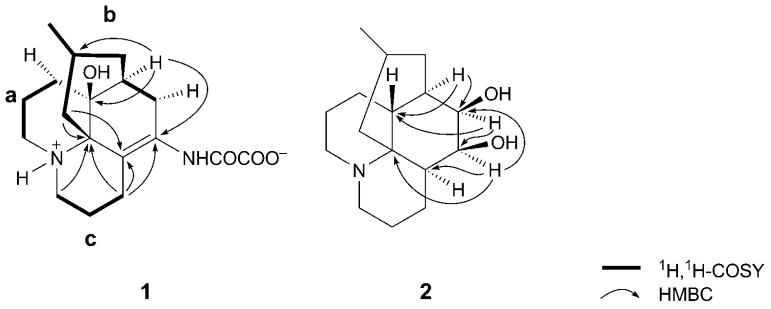

Fig. 2. *^{1}H,^{1}H-COSYs and key HMBCs* (H → C) *for* **1** *and* **2**

an oxygenated C-signal of **1**. ^{1}H,^{1}H-COSY and HSQC analyses indicated three fragments, **a** – **c** (*Fig. 2*). HMBCs from H–C(7) at δ(H) 1.89 – 1.92 (*m*) to C(5) at δ(C) 134.0, C(12) at δ(C) 69.5, and C(15) at δ(C) 27.3, and from CH_2(3) at δ(H) 2.33 – 2.36 and 1.69 – 1.74 to C(13) at δ(C) 70.2 indicated that the O-bearing quaternary C-atom was C(12). Thus, the structure of **1** was determined as shown in the formula.

Table. *^{1}H- and ^{13}C-NMR Data* (CD_3OD; 500 and 125 MHz, resp.) *of Compounds* **1** *and* **2**[a]). δ in ppm, *J* in Hz.

	1[1])		**2**[1])	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	3.68 – 3.72 (*m*), 2.99 (*d*, *J* = 12.1)	48.0	3.69 – 3.73 (*m*), 2.93 – 2.96 (*m*)	49.1[a])
CH_2(2)	2.15 – 2.17 (*m*), 1.77 – 1.81 (*m*)	17.4	2.09 – 2.12 (*m*), 1.86 – 1.88 (*m*)	20.5
CH_2(3)	2.33 – 2.36 (*m*), 1.69 – 1.74 (*m*)	20.2	1.99 – 2.01 (*m*), 1.61 (*d*, *J* = 13.5)	22.6
C(4) or H–C(4)		121.7	2.73 – 2.76 (*m*)	32.0
C(5) or H–C(5)		134.0	3.89 (*d*, *J* = 6.1)	74.1
CH_2(6) or H–C(6)	1.53 (*d*, *J* = 13.3), 2.11 – 2.17 (*m*)	32.0	3.73 (*s*)	78.7
H–C(7)	1.89 – 1.92 (*m*)	41.2	2.09 – 2.12 (*m*)	44.4
CH_2(8)	2.15 – 2.17 (*m*), 1.89 – 1.92 (*m*)	35.3	1.73 – 1.76 (*m*), 1.27 – 1.29 (*m*)	40.6
CH_2(9)	3.67 – 3.70 (*m*), 3.06 (*d*, *J* = 11.3)	51.9	3.82 – 3.85 (*m*), 3.03 – 3.05 (*m*)	49.0[a])
CH_2(10)	2.60 (*d*, *J* = 13.3), 2.12 – 2.15 (*m*)	21.8	2.31 – 2.33 (*m*), 1.85 – 1.89 (*m*)	25.6[b])
CH_2(11)	2.68 – 2.72 (*m*), 2.26 – 2.30 (*m*)	35.6	3.03 – 3.05 (*m*), 1.94 – 1.97 (*m*)	25.5[b])
C(12) or H–C(12)		69.5	1.73 – 1.76 (*m*)	46.0
C(13)		70.2		66.1
CH_2(14)	1.47 (*d*, *J* = 7.9), 1.85 – 1.87 (*m*)	37.8	1.20 (*dd*, *J* = 12.3, 12.3), 2.68 (*dd*, *J* = 12.3, 5.9)	41.2
H–C(15)	1.86 – 1.91 (*m*)	27.3	1.54 – 1.56 (*m*)	25.8
Me(16)	1.00 (*d*, *J* = 6.4)	22.3	0.94 (*d*, *J* = 6.4)	24.2
C(17)		164.9		
C(18)		166.8		

[a]) Assignment may be reversed. [b]) Assignment may be reversed.

(5β,6β,15α)-15-Methyllycopodane-5,6-diol (**2**) was obtained as a colorless amorphous solid, and the molecular formula $C_{16}H_{27}NO_2$ was established by HR-ESI-MS. The IR absorption bands at 3385 cm^{-1} indicated the presence of an OH group. ^{13}C-NMR and DEPT data implied the presence of 16 C-atoms, including one Me group, eight sp^3 CH_2 groups, six sp^3 CH groups (including two O-bearing CH groups at δ(C) 78.7 and 74.1), and a sp^3 quaternary C-atom. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** (*Table*) with those of the known deacetyllycoclavine established that they possessed the same planar structure, which was confirmed by the HMBC spectrum (*Fig. 2*) [14]. Correlations between H–C(4) at δ(H) 2.73 – 2.76 and H–C(5) at δ(H) 3.89, H–C(5) and H–C(6) at δ(H) 3.73, and H–C(6) and H–C(7) at δ(H) 2.09 – 2.12 in the ROESY plot (*Fig. 3*) suggested that the orientation of the OH groups at both C(5) and C(6) were β. Thus, the structure of **2** was elucidated as the 6β-epimer of deacetyllycoclavine and named (5β,6β,15α)-15-methyllycopodane-5,6-diol.

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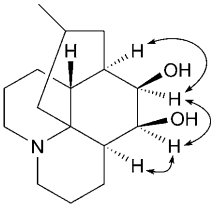

2 Fig. 3. *Major ROESY correlations for* **2**

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Experimental Part

General. Solvents were of industrial purity and distilled prior to use. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Haiyang Chemical Factory*, Qingdao, P. R. China), silica gel *H* (10–40 μm; *Qingdao Haiyang Chemical Factory*), *MCI* gel *CHP20P* (75–150 μm; *Mitsubishi Chemical Corporation*, Tokyo, Japan), *RP-18* (40–75 μm; *Fuji Chemical Industrial Co., Ltd.*, Tochigi, Japan), and *Sephadex LH-20* (25–100 μm; *Pharmacia Fine Chemical Co., Ltd.*). TLC: silica gel *GF254* (*Yantai Jiangyou Silica Gel Co. Ltd.*, Yantai, P. R. China). Optical rotations: *Horiba-SEAP-300* spectropolarimeter. IR: *Perkin-Elmer-241* polarimeter; $\tilde{\nu}$ in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR Spectra: *Bruker-DRX-AV-500* spectrometer; δ in ppm, J in Hz. MS: *Finnigan-MAT-95* instrument and *VG-Auto-Spec-3000* spectrometer; in m/z (rel. int.).

Plant Material. The whole plants of *H. serrata* were collected from Pingbian County of Yunnan Province, P. R. China, in February 2007, and identified by Prof. *Shugang Lu*, School of Life Science, Yunnan University, P. R. China, where a voucher specimen (No. 02152007) is deposited.

Extraction and Isolation. Air-dried powdered whole plants (5 kg) were extracted 4 × with 2% aq. tartaric acid soln. at r.t., and the concentrated acidic extract was basified to pH 10 with sat. Na_2CO_3 soln., and then partitioned with $CHCl_3$ and BuOH, resp. The BuOH fraction (30 g) was subject to CC (*MCI* gel, gradient H_2O/MeOH): *Fractions 1–4*. *Fr. 2* (5 g) was further subjected to CC (*RP-18*, H_2O/MeOH 8:2): **1** (50 mg) and **5** (45 mg). *Fr. 3* (3 g) was separated by CC (SiO_2, $CHCl_3$/MeOH 10:1 → 1:1; then *Sephadex LH-20*, MeOH/H_2O 8:2): **2** (25 mg) and **8** (14 mg). The $CHCl_3$ fraction (15 g) was separated by CC (SiO_2, gradient $CHCl_3$/MeOH): *Fractions 1–4*. *Fr. 2* (0.5 g) was purified by CC (SiO_2, $CHCl_3$/MeOH 30:1 → 1:1) and then TLC ($CHCl_3$/MeOH 20:1): **3** (15 mg) and **4** (20 mg). *Fr. 3* (3 g) was separated by CC (SiO_2, $CHCl_3$/MeOH 20:1 → 1:1) and then TLC ($CHCl_3$/MeOH 5:1): **6** (15 mg) and **7** (23 mg).

(*12β*)*-12-Hydroxyhuperzine G* (=*2-{[(8a*S*,9*S*,11*R*,12a*S*)-3,4,6,7,8,8a,9,10,11,12-Decahydro-8a-hydroxy-11-methyl-2*H*-9,1-ethanylylidenebenzo[*i*]quinolizin-14-yl]amino}-2-oxoacetic Acid*; **1**): Colorless amorphous powder. $[\alpha]_D^{25} = -74.00$ ($c = 0.0025$, H_2O). IR (KBr): 3387, 3361, 3284, 2933, 1698, 1627, 1458, 1364, 788. ^1H- and ^{13}C-NMR: *Table.* FAB-MS: 335 ($[M+H]^+$). HR-ESI-MS: 335.1969 ($[M+H]^+$, $C_{18}H_{27}N_2O_4^+$; calc. 335.1970).

(5β,6β,15α)-15-Methyllycopodane-5,6-diol (=*(1*S*,8a*R*,9*R*,11*R*,12a*R*,13*R*,14*S*)-Dodecahydro-11-methyl-1,9-ethanobenzo[i]quinolizine-13,14-diol*; **2**): Colorless amorphous powder. $[\alpha]_D^{25} = -85.00$ ($c =$ 0.001, MeOH). IR (KBr): 3376, 3324, 2934, 2657, 2588, 1650, 1453, 1378, 740. ^1H- and ^{13}C-NMR: *Table.* EI-MS: 265 (14, M^+), 208 (100), 209 (16), 205 (19), 162 (25), 148 (14), 137 (9). HR-ESI-MS: 266.2131 ($[M+H]^+$, $C_{16}H_{28}NO_2^+$; calc. 266.2120).

REFERENCES

[1] Jiangsu New Medical College, 'The Dictionary of Traditional Chinese Medicine', Shanghai Sci & Tech Press, Shanghai, 1985, p. 1138.
[2] J.-S. Liu, Y.-L. Zhu, C.-M. Yu, Y.-Z. Zhou, Y.-Y. Han, F.-W. Wu, B.-F. Qi, *Can. J. Chem.* **1986**, *64*, 837.
[3] X.-C. Tang, Y.-F. Han, X.-P. Chen, X.-D. Zhu, *Acta Pharmacol. Sin.* **1986**, *7*, 507.
[4] K. Katakawa, N. Kogure, M. Kitajima, H. Takayama, *Helv. Chim. Acta* **2009**, *92*, 445.
[5] K. Katakawa, A. Nozoe, N. Kogure, M. Kitajima, M. Hosokawa, H. Takayama, *J. Nat. Prod.* **2007**, *70*, 1024.
[6] T. Kubota, H. Yahata, S. Yamamoto, S. Hayashi, T. Shibata, J. Kobayashi, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3577.
[7] Y. Hirasawa, T. Tanaka, K. Koyama, H. Morita, *Tetrahedron Lett.* **2009**, *50*, 4816.
[8] J. He, X.-Q. Chen, M.-M. Li, Y. Zhao, G. Xu, X. Cheng, L.-Y. Peng, M.-J. Xie, Y.-T. Zheng, Y.-P. Wang, Q.-S. Zhao, *Org. Lett.* **2009**, *11*, 1397.
[9] B.-N. Zhou, D.-Y. Zhu, M.-F. Huang, L.-J. Lin, L.-Z. Lin, X.-Y. Han, G. A. Cordell, *Phytochemistry* **1993**, *34*, 1425.
[10] B. D. Wang, S. H. Jiang, W. Y. Gao, D. Y. Zhu, X. M. Kong, Y. Q. Yang, *Acta Bot. Sin.* **1998**, *40*, 842.
[11] C. H. Tan, X. Q. Ma, H. Zhou, S. H. Jiang, D. Y. Zhu, *Acta Bot. Sin.* **2003**, *45*, 118.
[12] S. Q. Yuan, Y. M. Zhao, *Acta Pharmacol. Sin.* **2003**, *38*, 596.
[13] H. Takayama, K. Katakawa, M. Kitajima, K. Yamaguchi, N. Aimi, *Tetrahedron Lett.* **2002**, *43*, 8307.
[14] O. M. Muñoz, M. Castillo, A. S. Feliciano, *J. Nat. Prod.* **1990**, *53*, 200.

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