Two Novel *Lycopodium* Alkaloids from *Huperzia serrata*

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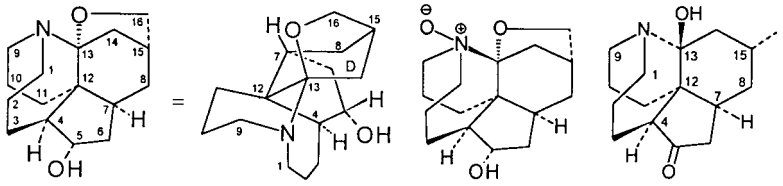
Huperzine Q (**1**) and *N*-oxyhuperzine Q (**2**), two novel irregular fawcettimine-type *Lycopodium* alkaloids were isolated from the $CHCl_3$ fraction of the basic material of the whole plant of the Chinese medicinal herb *Huperzia serrata*. Their structures were determined as 13-epi-13β,16-epoxydihydrofawcettimine (**1**) and *N*-oxy-13-epi-13β,16-epoxydihydrofawcettimine (**2**) by means of spectroscopic studies and X-ray crystallographic analysis.

Introduction. – *Huperzia serrata* (Thunb.) Trev. (Huperziaceae) is one of the most commonly used traditional Chinese herbal medicines for the treatment of contusion, strain, swelling, and schirophrema [1]. The discovery that huperzine A, a *Lycopodium* alkaloid isolated from this plant, was a potent acetylcholinesterase inhibitor [2] has prompted us reinvestigate the chemical constituents of this plant. As a continuation of our work [3], we re-examined the $CHCl_3$ extract of the basic materials of dry whole plants (10 kg), and obtained huperzine Q (**1**) and *N*-oxyhuperzine Q (**2**), two novel compounds that represent a unique structural type among the *Lycopodium* alkaloids [4]. In the present paper, we report the isolation and structural elucidation of the above compounds.



1 **2** **3**

Results and Discussion. – Huperzine Q (**1**), showed a positive response with *Dragendorff*'s reagent and was attributed the molecular formula $C_{16}H_{25}NO_2$ based on the HR-EI-MS spectrum. The fragmentation pattern of **1** in the EI-MS is quite different from that of previously reported *Lycopodium* alkaloids [5]. The ^{13}C-NMR spectrum displayed 16 sp^3-C signals, which were resolved into ten methylene, four methine, and two quaternary C-atoms through DEPT experiments. As signals representing double bonds from the ^{13}C-NMR spectrum are lacking, the molecule of **1** must be pentacyclic, one cycle more than common fawcettimine-type *Lycopodium* alkaloids, such as fawcettimine (**3**) [6]. The ^{1}H-^{1}H-COSY and HMQC spectra indicated

the presence of two isolated spin systems (*Fig. 1*). The HMBC spectrum (*Fig. 1*) exhibited the long-range correlations between C(12) and H–C(4), H–C(7), and H–C(11), and between C(13) and H_{exo}–C(16), H_{exo}–C(14), H_{α}–C(9), and H_{α}–C(11). Thus, the planar structure of **1**, as shown in *Fig. 1*, was established. The relative configuration of **1** was defined by a NOESY experiment (*Fig. 2*). Significant NOEs were observed between H_{endo}–C(14) and H_{β}–C(5), H_{β}–C(1), and H_{β}–C(3), and H–C(4) and H_{α}–C(2) and H_{α}–C(10). Hence, the relative stereochemistry of **1** as shown in *Fig. 2* was proved, and was confirmed by crystal-structure analyses[1]). *Fig. 3* shows the ORTEP view of **1**. Huperzine Q, a pentacyclic alkaloid that has reversed D ring and an ether linkage between C(13) and C(16), represents a new structural type among the *Lycopodium* alkaloids.

Compound **2** was isolated as white amorphous powder; the molecular formula was established as $C_{16}H_{25}NO_3$ by HR-EI-MS. In the EI-MS, the fragment peak at *m*/*z* 263 was due to the loss of one O-atom and suggested the presence of an *N*-oxide function; other signals were similar to those of compound **1**. Important differences between the

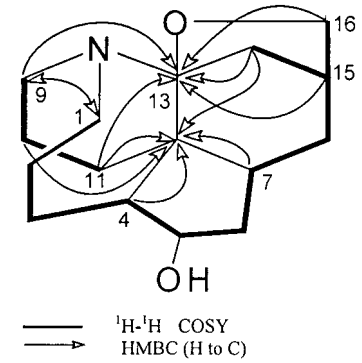


Fig. 1. *^{1}H-^{1}H-COSY and important HMBC correlations of* **1**

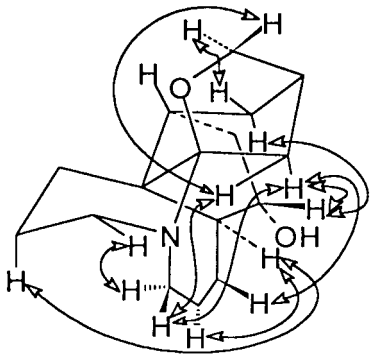


Fig. 2. *Significant NOESY correlations of* **1**

[1]) The crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-155176. Copies of data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (1223) 336033; e-mail: deposit@ccdc.ac.uk).

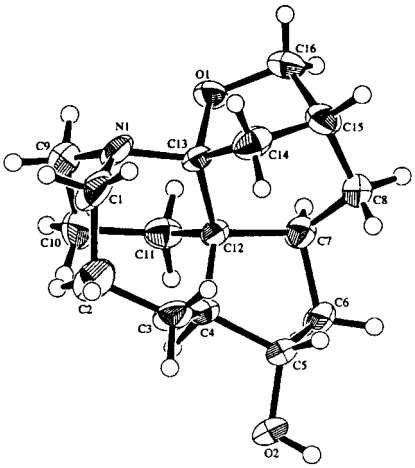


Fig. 3. *ORTEP View of* **1**

Table. *^{13}C-* (100 MHz) *and ^{1}H-* (400 MHz) *NMR Data of* **1** *and* **2** (in $CDCl_3$, δ in ppm, J in Hz)

Position	^{1}H-NMR		^{13}C-NMR	
	1	**2**	**1**	**2**
1α	54.06 (*t*)	72.20 (*t*)	3.02 (*dt*, J = 15.2, 1.9)	3.62 (br. *d*, J = 14.4)
1β	–	–	3.12 (*ddd*, J = 15.2, 12.8, 2.8)	3.33 (br. *t*, J = 14.4)
2α	29.71 (*t*)	26.11 (*t*)	1.84[a])	2.08[a])
2β	–	–	1.63[a])	1.87[a])
3α	31.47 (*t*)	28.26 (*t*)	2.19[a])	2.29[a])
3β	–	–	1.69[a])	1.58[a])
4	54.16 (*d*)	53.64 (*d*)	1.64[a])	1.56[a])
5	80.01 (*d*)	78.44 (*d*)	3.95 (*ddd*, J = 10.0, 6.7, 5.5)	4.00 (*ddd*, J = 9.8, 6.6, 5.6)
6α	38.27 (*t*)	37.71 (*t*)	1.81[a])	1.77 (*dd*, J = 11.1, 6.6)
6β	–	–	1.69[a])	1.67[a])
7	40.41 (*d*)	43.36 (*d*)	1.82[a])	2.02[a])
8*endo*	37.58 (*t*)	37.18 (*t*)	1.81[a])	1.88[a])
8*exo*	–	–	1.24 (*m*)	1.31 (br. *t*, J = 13.6)
9α	47.86 (*t*)	66.10 (*t*)	2.73 (*dd*, J = 14.0, 5.4)	3.40 (*dd*, J = 13.6, 4.0)
9β	–	–	3.45 (*td*, J = 14.0, 4.2)	3.76 (*td*, J = 13.6, 3.8)
10α	22.68 (*t*)21.07 (*t*)	1.83[a])	2.08[a])	
10β	–	–	1.44 (br. *d*, J = 13.5)	1.65[a])
11α	37.82 (*t*)	35.28 (*t*)	1.48 (br. *d*, J = 12.8)	1.43 (br. *d*, J = 12.8)
11β	–	–	2.19 (*td*, J = 12.8, 4.2)	2.19 (*td*, J = 12.8, 3.1)
12	52.29 (*s*)	53.16 (*s*)	–	–
13	96.53 (*s*)	106.74 (*s*)	–	–
14*endo*	40.90 (*d*)	33.39 (*t*)	1.87 (*dd*, J = 11.2, 5.0)	2.87 (*ddd*, J = 9.8, 3.2, 1.3)
14*exo*	–	–	2.06 (*d*, J = 11.2)	1.68 (*d*, J = 9.8)
15	36.58 (*d*)	36.66 (*d*)	2.49 (*q*, J = 4.1)	2.54 (br. *s*)
16*endo*	70.70 (*t*)	73.29 (*t*)	4.00 (*ddd*, J = 7.5, 4.9, 1.5)	4.14 (*ddd*, J = 7.2, 3.6, 2.4)
16*exo*	–	–	3.74 (*d*, J = 7.5)	3.85 (*d*, J = 7.2)

[a]) Overlapping signals.

^{13}C-NMR spectra (*Table*) of **1** and **2** were three C(N) signals (δ C(9) = 47.86, δ C(1) = 54.06, and δ C(13) = 96.53) of **1** shifted upfield to 66.10, 72.20, and 106.74; respectively, in **2**, and the δ value for C(14) shifted from 40.90 in **1** to 33.39 in **2**. These shifts are consistent with the assignment of **2** as *N*-oxyhuperzine Q: C(9), C(1), and C(13) are deshielded by conduction from *N*-oxide group, and the difference in the results from a C(14) signal results from γ-gauche effect from *N*-oxide group. These results prove that **2** possesses the same configuration as **1**, also consistent with the formulation of **2** as *N*-oxyhuperzine Q.

Experimental Part

General. Column chromatography (CC): silica gel (200–300, 400 mesh' *Qingdao Haiyang Chemical Group Co*., China). M.p.: *Fisher-John*, uncorrected. $[\alpha]_D$: *P.E. 241* MC polarimeter. IR Spectra: *Nicolet Magna-750* FT-IR spectrophotometer, KBr pellets; ν in cm^{-1}. EI-MS: *MAT-95* spectrometer; 70 eV; *m*/*z* (rel. int. in %). NMR Spectra: *Bruker AM-400* instrument, $CDCl_3$ as solvent with residual $CHCl_3$ peak (δ (H): 7.26; δ (C): 77.10) as reference; chemical shifts δ in ppm, *J* in Hz.

Plant Material. Fresh whole plants of *Huperzia serrata* (Thunb) Trev. were collected in Zhejiang Province, China and identified by Dr. *Xiao-Qiang Ma*. Voucher sample (No. 97-63) was deposited in the Herbarium of this institute.

Extraction and Isolation. The air-dried whole plants (10 kg) were powdered and extracted with 1% aq. tartaric acid at r.t. and the pH of the concentrated acidic extract adjusted to 9 with conc. NH_3 soln., then extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated to give the $CHCl_3$ fraction of the total alkaloid extract, which was subjected to silica-gel CC (eluted with solvents of increasing polarity ($CHCl_3$, $CHCl_3$/MeOH (50:1, 20:1, 10:1, and 5:1)), and MeOH). Compound **1** (48 mg) was obtained from the $CHCl_3$/CH_3OH 10:1 fraction, and **2** (13 mg) from $CHCl_3$/MeOH 5:1, after being purified by repeated silica-gel CC.

Data of **1**: Colorless prisms from Me_2CO. M.p. 192–194°. $[\alpha]_D^{25}$: −0.275 (*c* 0.44, $CHCl_3$). IR: 3415 (OH), 3205, 2938, 1460, 1354, 1336, 1205, 1082, 928, 827. ^{13}C- and ^{1}H-NMR: see *Table.* EI-MS: 263 (100, M^+), 246 (57), 232 (17), 222 (18), 204 (7), 192 (9), 180 (12), 162 (14), 152 (26), 123 (8). HR-EI-MS: 263.1894 ($C_{16}H_{25}NO_2$; calc.: 263.1886).

X-Ray Crystal-Structure Analysis of **1**. $C_{16}H_{25}NO_2$ (MW: 263.38), Monoclinic $P2_1$ (#4): a = 7.322 (2) Å, b = 12.040 (2) Å, c = 16.067 (3) Å, β = 91.01 (2)°, V = 1416.1 (4) Å^3, Z = 4; D_{calc} = 1.235 g·cm^{-3}, R = 0.054, R_W = 0.059. From a crystal of size 0.20 × 0.20 × 0.30 mm, 2090 reflexions were measured on a *Rigaku AFC7R* diffractometer with Mo-Kα radiation (graphite monochromator λ = 0.71069 Å) at 273 ± 1 K. The structure was solved by direct methods and expanded by means of *Fourier* techniques. The non-H-atoms were refined anisotropically. H-Atoms were included but not refined. Drawings of the molecule were made with ORTEP.

Data of **2**: Amorphous powder. $[\alpha]_D^{25}$ + 0.06 (*c* 0.12, $CHCl_3$). IR: 3415 (OH), 3215 (intermolecular H-bond, N–OH), 2937, 1637 (N=O), 1456, 1348, 1335, 1204, 1094, 1028. ^{13}C- and ^{1}H-NMR: see Table. EI-MS: 279 (20, M^+), 263 (48), 246 (21), 232 (10), 222 (11), 205 (8), 191 (10), 167 (26), 150 (36), 149 (100), 123 (22). HR-EI-MS: 279.1850 ($C_{16}H_{25}NO_3$; calc.: 279.1834).

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