Lycopodium Alkaloids from *Huperzia serrata*

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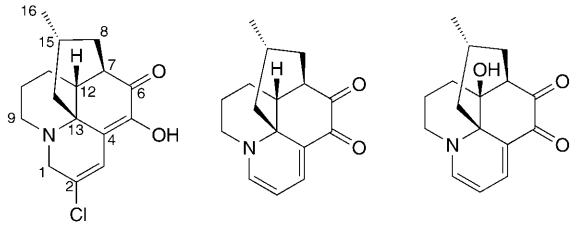
Three new Lycopodium alkaloids, 2-chlorohuperzine E (**1**), huperzines E′ (**2**), and F′ (**3**), along with two known compounds, huperzines E and F, were isolated from *Huperzia serrata* (THUNB.) TREV. Their structures were elucidated by spectroscopic methods.

Introduction. – *Huperzia serrata* (THUNB.) TREV. (Huperziaceae), a moss-like small herb, is a traditional Chinese herbal medicine used to treat contusion, strain, swelling, and schizophrenia [1]. Since the discovery of huperzine A, a potent acetylcholinesterase inhibitor from this plant [2], many searches for analogs have led to the finding of over 200 Lycopodium alkaloids from the plant and its related genera [3]. Within the context of our continuous interest in the title plant [4–10], we examined the petroleum ether/Me_2CO 50:1 (*v*/*v*) soluble fraction of the residue of a large-scale isolation of huperzine A, resulting in the isolation of three new compounds, 2-chlorohuperzine E (**1**), huperzine E′ (**2**), and huperzine F′ (**3**), together with two known alkaloids, huperzines E (**4**) and F (**5**) [4]. This paper focuses on the isolation and structural elucidation of **1**–**3**[1]).



1 **2** **3**

Results and Discussion. – Compound **1** was obtained as yellowish needles. Its IR absorptions at 3426 and 1654 cm^{-1} suggested the presence of an OH and an α,β-unsaturated ketone group, respectively. In the EI-MS, the molecular-ion-peak cluster at *m*/*z*

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

293/295 with a ratio of abundances 3 : 1 revealed the presence of a Cl-atom in the molecule of **1**. A HR-MS measurement on the peak at *m/z* 293.1185 (M^+, $C_{16}H_{20}ClNO_2^+$) indicated the molecular formula $C_{16}H_{20}ClNO_2$. The NMR analyses allowed us to elucidate the structure of **1** as 2-chlorohuperzine E.

The ^{13}C-NMR (DEPT) spectrum of **1** displayed 16 signals: 1 Me, 6 CH_2, and 4 CH groups and 5 quaternary C-atoms. The main difference between the ^{13}C-NMR spectrum of **1** and huperzine E (**4**) was a quaternary C-atom signal at δ 134.1 in the spectrum of **1** instead of the CH(2[1]) signal at δ 128.7 in that of **4**, suggesting **1** to be 2-chlorohuperzine E. This conclusion was in agreement with the ^{1}H-NMR signals of **1** (*Table*) at δ 4.15 (*dd*, *J* = 19.3, 2.6) and 3.09 (*d*, *J* = 19.3) for CH_2(1), because the coupling constants *J*(1α,1β), *J*(1α,2), *J*(1β,2), *J*(1α,3), and *J*(1β,3) of **4** were 19.6, 4.6, 2.3, 0, and 2.3 Hz, respectively. The HMBC and ROESY spectra of **1** (*Fig.*, *a*) supported that **1** had the same relative configuration as **4**.

Table. *^{1}H-NMR Data* ($CDCl_3$) *of* **1**–**3**[1]). δ in ppm, *J* in Hz

	1	**2**	**3**
H–C(1) or CH_2(1)	3.09 (*d*, *J* = 19.3, H_α), 4.15 (*dd*, *J* = 19.3, 2.6, H_β)	6.83 (*d*, *J* = 5.8)	6.81 (*d*, *J* = 5.8)
H–C(2)	–	5.36 (*dd*, *J* = 7.1, 5.9)	5.45 (*dd*, *J* = 7.1, 5.9)
H–C(3)	6.83 (*d*, *J* = 2.5)	7.68 (*d*, *J* = 7.1)	7.64 (*d*, *J* = 7.1)
H–C(7)	2.56–2.59 (*m*)	2.87–2.92 (*m*)	2.82 (*dd*, *J* = 5.3, 2.0)
H_{exo}–C(8)	1.30 (*dd*, *J* = 12.9, 4.6)	1.39 (*td*, *J* = 13.3, 4.8)	1.82 (*m*)[a])
H_{endo}–C(8)	1.79 (*d*, *J* = 13.1)	1.85 (*m*)[a])	1.61–1.64 (*m*)
H_α–C(9)	2.72–2.78 (*m*)	3.83 (*ddd*, *J* = 12.6, 12.5, 5.8)	4.49 (*ddd*, *J* = 12.1, 11.9, 6.0)
H_β–C(9)	2.66 (*d*, *J* = 11.3)	3.33 (*dd*, *J* = 12.6, 6.9)	3.24 (*dd*, *J* = 11.5, 7.3)
H_α–C(10)	1.61–1.66 (*m*)	1.75 (*m*)[a])	1.84 (*m*)[a])
H_β–C(10)	1.61–1.66 (*m*)	1.75 (*m*)[a])	1.74 (*m*)[a])
H_α–C(11)	1.33–1.37 (*m*)	1.77 (*m*)[a])	2.04–2.08 (*m*)
H_β–C(11)	1.47 (*ddd*, *J* = 9.9, 8.3, 3.2)	1.91 (*m*)[a])	1.77 (*m*)[a])
H–C(12)	1.75 (*dd*, *J* = 14.3, 2.2)	2.32–2.37 (*m*)	–
H_{exo}–C(14)	1.05 (*dd*, *J* = 12.3, 12.2)	0.95 (*dd*, *J* = 12.1, 11.8)	1.46 (*dd*, *J* = 11.9, 11.8)
H_{endo}–C(14)	2.07 (*dd*, *J* = 12.4, 4.4)	2.40 (*ddd*, *J* = 11.7, 4.1, 1.5)	2.09 (*dd*, *J* = 12.0, 8.1)
H–C(15)	1.55–1.62(*m*)	1.97–2.03 (*m*)	1.28–1.31 (*m*)
Me(16)	0.92 (*d*, *J* = 6.5)	0.92 (*d*, *J* = 6.3)	0.90 (*d*, *J* = 6.3)
OH–C(5)	6.29 (*s*)	–	–

[a]) Overlapped signals; the δ value is that of the corresponding central position of the HMQC cross-peak.

The HR-EI-MS of **2** gave an empirical molecular formula $C_{16}H_{19}NO_2$ (M^+ at *m/z* 257.1404). The diagnostic EI-MS fragments at *m/z* 214 ($[M-43]^+$), 200 ($[M-57]^+$) suggested **2** to be a lycopodine-type Lycopodium alkaloid [11]. The structure and relative configuration of **2** were elucidated as 5-*O*,1-didehydrohuperzine E on the basis of 1D- and 2D-NMR studies.

The ^{13}C-NMR and DEPT spectra of **2** exhibited 16 C-signals, *i.e.*, 1 Me, 5 CH_2, 3 CH (sp^3), 3 CH (sp^2), 1 C (sp^3), and 3 C (sp^2) signals. Its ^{1}H,^{1}H-COSY, combined with HMQC spectra (*Fig.*, *b*), disclosed two isolated spin systems: $CH_2CH_2CH_2CHCHCH_2CH(Me)CH_2$ and CH=CHCH. The former was consistent with the connectivities from C(9) to C(14[1]) in a lycopodine-type skeleton, and the latter was tentatively attributed to C(1) to C(3). With the structure of **4** as reference, the remaining four quaternary C-atoms

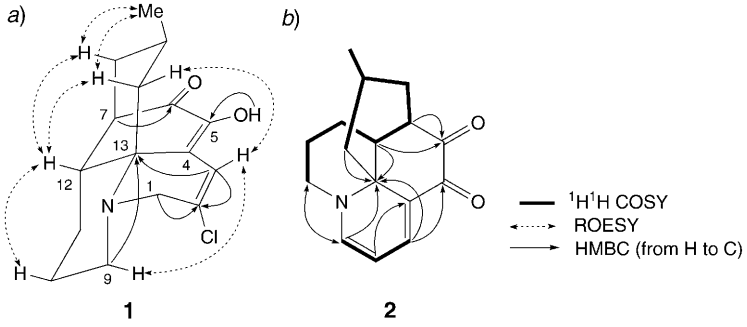


Figure. a) *Key HMBC and ROESY correlations of* **1**, *and* b) *^{1}H,^{1}H COSY and HMBC correlations of* **2**

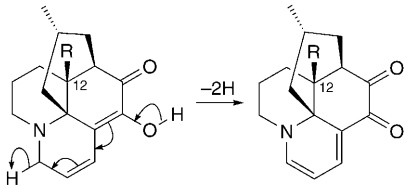
were assigned to C(4) (δ 112.0), C(5) (δ 176.1), C(6) (δ 200.1), and C(13) (δ 61.0). The postulated structure **2** was supported by the HMBC correlations (*Fig.*, *b*). The proposed biogenesis (see below) suggests that **2** has the same relative configuration as **4**.

With the structure of **2** in hand, compound **3** was easily identified as the 12-hydroxylated derivative of **2**. This was consistent with the HR-EI-MS signal at *m*/*z* 273.1356 (M^+, $C_{16}H_{19}NO_3^+$).

Hydroxylation at C(12)[1]) in **2** is expected to increase the chemical shifts of the neighboring C(7), C(11), and C(13) (found: $\Delta\delta = 6.5$, 7.5, and 3.4, resp.) due to deshielding effects, and to decrease the chemical shift of C(8) and C(14) (found: $\Delta\delta = -5.9$ and -7.8, resp.) because of the *γ-gauche* effect, but to have little effect on the remaining C-atoms [12]. These observations, thus, established unambiguously the assignment for structure **3**.

We propose that **2** and **3** are biogenetically formed from huperzine E (**4**) and huperzine F (**5**), respectively (*Scheme*).

Scheme. *Hypothetical Biogenesis of* **2** *and* **3**



4 R = H
5 R = OH

2 R = H
3 R = OH

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Haiyang, Co*., China). M.p.: *Fisher-John*, apparatus; uncorrected. Optical rotation: *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV-240* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Magna-750-FTIR* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AV-500* at instrument 500 (^1H) and 125 MHz (^{13}C); in $CDCl_3$; δ in ppm rel. to $SiMe_4$, *J* in Hz. EI-MS: *MAT-95* mass spectrometer; 70 eV; in *m*/*z* (rel.int. in %).

Plant Material. The fresh whole plant of *H. serrata* (THUNB.) TREV. (Huperziaceae) was collected in Zhejiang Province, P. R. China, September 2004. The plant was identified by Prof. *D.-Y. Zhu*. A voucher sample (No. 04-92) was deposited at the Herbarium in our institute.

Extraction and Isolation. The low polar part (petroleum ether/Me_2CO 50 : 1 (*v*/*v*) soluble; 34 g) of the mother liquor from a large-scale isolation of huperzine A (50 kg of dry whole plant of *H. serrata*) as described in [2], was subjected to CC (SiO_2 (2.0 kg); $CHCl_3$, then gradient $CHCl_3$/MeOH 100 : 1, 50 : 1, 30 : 1, 20 : 1, 10 : 1, 5 : 1): *Fr. 1–7*. *Fr. 1* (with $CHCl_3$; 12 g) was further separated by CC (SiO_2 (1 kg); petroleum ether/Me_2CO 100 : 0 → 1 : 1): *Fr. 1.1–Fr. 1.10*. Repeated CC (SiO_2, petroleum ether/AcOEt 30: 1) of *Fr. 1.2* gave **1** (16 mg). *Fr. 1.4* furnished yellowish needles **4** (310 mg), the remaining mother liquor was repeatedly purified by CC (SiO_2, $CHCl_3$/MeOH 70: 1) to yield **2** (21 mg). Compound **5** (120 mg) was obtained as prisms from *Fr. 1.5*, the remaining mother liquor afforded **3** (7 mg), after repeated CC (SiO_2, $CHCl_3$/MeOH 50 : 1).

2-Chlorohuperzine E ((=*(8a*R,*9*R,*11*R,*12a*R*)-3-Chloro-6,7,8,8a,9,10,11,12-octahydro-14-hydroxy-11-methyl-4*H-*9,1-ethanylylidenebenzo[i]quinolizin-13-one*; **1**): Yellowish needles. M.p. 149–150° (dec.). $[\alpha]_D^{25} = -63.4$ ($c = 0.465$, $CHCl_3$). UV (MeOH): 204 (0.72), 324 (2.53). IR: 3426, 2925, 1654, 1606, 1452, 1384, 1338, 1303, 1184, 1083, 916, 810, 732, 619. ^1H-NMR: *Table*. ^{13}C-NMR (125 MHz, $CDCl_3$)[1]): 196.4 (*s*, C(6)); 142.7 (*s*, C(5)); 134.1 (*s*, C(2)); 121.5 (*s*, C(4)); 118.1 (*d*, C(3)); 56.4 (*s*, C(13)); 55.4 (*t*, C(1)); 50.4 (*t*, C(9)); 48.3 (*d*, C(7)); 46.1 (*d*, C(12)); 41.1 (*t*, C(14)); 37.1 (*t*, C(8)); 26.3 (*t*, C(11)); 25.5 (*d*, C(15)); 25.2 (*t*, C(10)); 21.5 (*q*, C(16)). EI-MS: 295 (6, $M(^{37}Cl)^+$), 293 (18, $M(^{35}Cl)^+$), 252 (4), 250 (12), 238 (33), 237 (21), 236 (100), 174 (16), 222 (5), 208 (9), 201 (7), 173 (3). HR-EI-MS: 293.1185 (M^+, $C_{16}H_{20}ClNO_2^+$; calc. 293.1183).

Huperzine E′ ((=*(8a*R,*9*R,*11*R,*12a*R*)-6,7,8,8a,9,10,11,12-Octahydro-11-methyl-1,9-ethanobenzo[i]quinolizine-13,14-dione*; **2**): Purple amorphous powder. $[\alpha]_D^{23} = -4750$ ($c = 0.004$, MeOH). UV (MeOH): 205 (1.48), 286 (0.78), 547 (2.62). IR: 2925, 2865, 1704, 1598, 1476, 1425, 1303, 1251, 1197, 1074, 896, 673. ^1H-NMR: *Table*. ^{13}C-NMR (125 MHz, $CDCl_3$)[1]): 200.1 (*s*, C(6)); 176.1 (*s*, C(5)); 146.3 (*d*, C(1)); 141.6 (*d*, C(3)); 112.0 (*s*, C(4)); 99.9 (*d*, C(2)); 61.0 (*s*, C(13)); 50.7 (*d*, C(7)); 46.5 (*t*, C(9)); 42.3 (*d*, C(12)); 38.2 (*t*, C(14)); 37.4 (*t*, C(8)); 23.1 (*d*, C(15)); 21.7 (*t*, C(11)); 21.5 (*q*, C(16)); 19.4 (*t*, C(10)). EI-MS: 257 (26, M^+), 229 (8), 214 (3), 200 (55), 186 (20), 173 (29), 172 (100), 158 (21), 133 (19), 132 (12), 117 (8). HR-EI-MS: 257.1404 (M^+, $C_{16}H_{19}NO_2^+$; calc. 257.1416).

Huperzine F′ ((=*(8a*S,*9*R,*11*R,*12a*S*)-6,7,8,8a,9,10,11,12-Octahydro-8a-hydroxy-11-methyl-1,9-ethanobenzo[i]quinolizine-13,14-dione*; **3**): Purple amorphous powder. M.p. 117–119° (dec.). $[\alpha]_D^{23} = -3182$ ($c = 0.0044$, MeOH). UV (MeOH): 204 (1.01), 287 (0.40), 556 (1.23). IR: 3384, 2936, 1702, 1571, 1461, 1421, 1392, 1288, 1211, 1030, 929, 894, 788. ^1H-NMR: *Table*. ^{13}C-NMR (125 MHz, $CDCl_3$)[1]): 199.6 (*s*, C(6)); 175.3 (*s*, C(5)); 147.5 (*d*, C(1)); 141.4 (*d*, C(3)); 112.1 (*s*, C(4)); 101.3 (*d*, C(2)); 72.2 (*s*, C(12)); 63.4 (*s*, C(13)); 58.2 (*d*, C(7)); 47.9 (*t*, C(9)); 31.5 (*t*, C(8)); 30.4 (*t*, C(14)); 29.2 (*t*, C(11)); 22.5 (*d*, C(15)); 21.2 (*q*, C(16)); 17.9 (*t*, C(10)). EI-MS: 273 (45, M^+), 256 (16), 245 (20), 230 (14), 228 (35), 203 (18), 202 (100), 200 (10), 186 (33), 176 (26), 175 (74), 172 (10), 158 (16), 149 (41), 148 (22), 147 (57), 132 (11). HR-EI-MS: 273.1356 (M^+, $C_{16}H_{19}NO_3^+$; calc. 273.1365).

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