Two New Nitrone Alkaloids from *Huperzia serrata*

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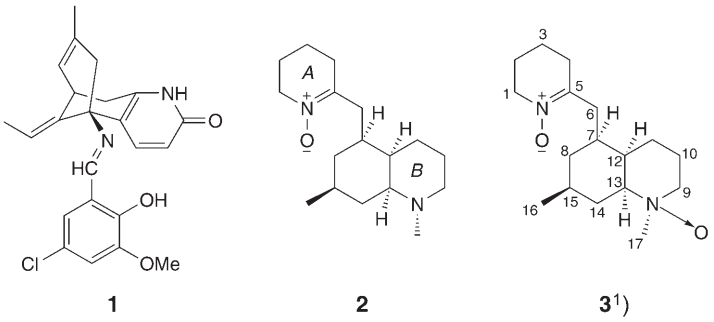
Two new nitrone alkaloids were isolated from the whole plant of *Huperzia serrata* (THUNB.) TREV. They are both phlegmarine-type lycopodium alkaloids with a nitrone moiety. Their structures were elucidated on the basis of spectral evidences, and their configurations were established on the basis of optical rotation, CD, and NOESY-NMR data.

Introduction. – The family of nitrone compounds is a kind of spin-trapping composition, which reacts with the free radical to form a compound called a spin adduct. Once the adduct is formed, it is relatively stable, and the radical thus becomes inactivated and unable to damage cellular tissues or biochemical processes [1][2]. Studies have reported that the nitrone spin trap α-phenyl *tert*-butyl nitrone can significantly ameliorate neuronal cell loss and neurologic deficits induced by stroke in a gerbil model of global ischemia [3]. *Huperzia serrata* (THUNB.) TREV. is one of the most commonly used traditional Chinese herbal medicines for the treatment of contusions, strains, and swellings [4]. Up to now, some tens of lycopodium alkaloids with different structural variations have been isolated from this plant including huperzine A [5–7], a compound with anti-acetylcholinesterase (AChE) activity and memory-enhancing effect [8]. Schiprine (**1**; *Fig. 1*) is one of the derivatives of huperzine A that has recently



1 **2** **3**[1])

Fig. 1. *Chemical structures of* **1**, **2**, *and* **3**

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

been prepared by this research group. It showed stronger anti-AChE activity and lower toxicity than the mother compound [9] and is now in the clinical trial in Europe. In our further investigations of the plant, seeking for a better AChE inhibitor, two new nitrone alkaloids of the phlegmarine type were isolated, and, in this work, the structures of them, namely huperzines M and N (**2** and **3**, resp.), will be disclosed.

Results and Discussion. – The crude alkaloid mixture was obtained in accordance with the standard method from the whole plant of *H. serrata* [10]. SiO_2 Chromatography of the crude alkaloids and elution with $CHCl_3$/acetone followed by MeOH afforded two alkaloid-rich fractions. Repeated chromatography of the MeOH fraction over neutral Al_2O_3 and SiO_2 furnished two novel lycopodium alkaloids with a nitrone moiety (**2** and **3**) which were assigned the trivial names huperzines M and N, respectively.

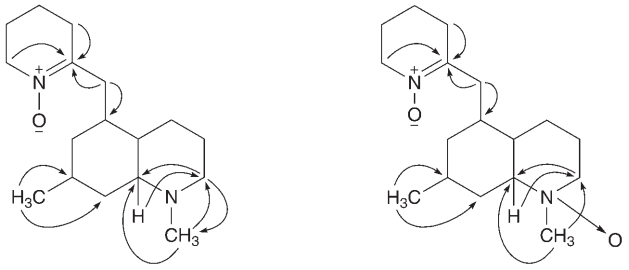
Huperzine M (**2**) was obtained as a colorless oil, with $[\alpha]_D^{20} = -13.4$ ($c = 0.04$, $CHCl_3$). It was assigned the molecular formula $C_{17}H_{30}N_2O$ as established from its HR-EI-MS (*m/z* 278.2347; calc. 278.2349), and NMR data. The CD spectrum of **2** showed a positive *Cotton* effect ($\Delta\varepsilon = -0.65$, 221 nm; $\Delta\varepsilon = 1.1$, 252 nm). The IR spectrum showed the presence of a C=N group (1637 cm^{-1}). The EI-MS displayed a molecular ion at *m/z* 278 and a base peak at *m/z* 166. The ^{1}H-NMR spectrum of **2** (*Table*)

Table. *NMR Data of* **2** *and* **3**. δ in ppm, J in Hz.

Position[1])	**2**		**3**	
	δ(H)[a])	δ(C)[b])	δ(H)[a])	δ(C)[b])
1	3.78 (*t*, *J* = 6)	58.1 (*t*)	3.75 (*t*, *J* = 6)	58.2 (*t*)
2	1.86–1.94 (*m*)	23.1 (*t*)	1.84–1.92 (*m*)	23.1 (*t*)
3	1.70–1.74 (*m*)	18.8 (*t*)	1.64–1.69 (*m*)	18.8 (*t*)
4	2.37 (*t*, *J* = 6)	29.9 (*t*)	2.34 (*t*, *J* = 6)	30.0 (*t*)
5		148.6 (*s*)		148.0 (*s*)
6	2.78 (*dd*, *J* = 13, 4, H_a), 2.26 (*d*, *J* = 13, H_b)	35.6 (*t*)	2.96 (*dd*, *J* = 12, 3, H_a), 1.91 (*d*, *J* = 12, H_b)	36.4 (*t*)
7	1.93–1.96 (*m*)	32.8 (*d*)	2.10–2.17 (*m*)	32.3 (*d*)
8	1.83–1.87 (*m*, H_a), 1.36–1.41 (*m*, H_b)	35.4 (*t*)	1.34 (*ddd*, *J*= 12, 8, 4, H_a), 1.29 (br. *d*, 12, H_b)	36.6 (*t*)
9	2.86 (br. *d*, *J* = 12, H_a), 2.08–2.13 (*m*, H_b)	57.5 (*t*)	3.35 (br. *d*, *J* = 12, H_a), 3.14 (*ddd*, *J* = 12, 11, 3, H_b)	69.0 (*t*)
10	1.63–1.73 (*m*)	25.1 (*t*)	2.34–2.43 (*m*, H_a), 1.57 (br. *d*, *J* = 14, H_b)	20.1 (*t*)
11	2.02 (br. *d*, *J* = 14, H_a), 1.00–1.05 (*m*, H_b)	28.3 (*t*)	2.01–2.06 (*m*, H_a), 1.08–1.13 (*m*, H_b)	27.0 (*t*)
12	1.04–1.12 (*m*)	46.7 (*d*)	1.78–1.83 (*m*)	40.8 (*d*)
13	1.82 (br. *d*, *J* = 13)	63.3 (*d*)	2.89 (*ddd*, *J* = 11, 10, 3)	73.4 (*d*)
14	1.28–1.35 (*m*)	37.5 (*t*)	2.06–2.17 (*m*, H_a), 1.67–1.72 (*m*, H_b)	30.0 (*t*)
15	2.03–2.11 (*m*)	27.1 (*d*)	2.16–2.25 (*m*)	26.8 (*d*)
16	0.98 (*d*, *J* = 8)	19.3 (*q*)	0.93 (*d*, *J* = 7)	19.0 (*q*)
17	2.24 (*s*)	42.5 (*q*)	3.04 (*s*)	57.6 (*q*)

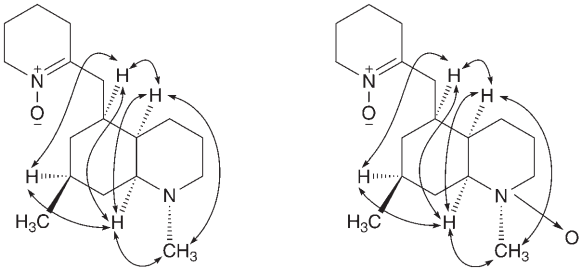
[a]) At 400 MHz, referenced to residual solvent $CHCl_3$ (δ(H) 7.26). [b]) At 100 MHz, referenced to $CDCl_3$ (δ(C) 77.0), with multiplicities determined by DEPT and HMQC.

exhibited a MeN *singlet* at δ(H) 2.24, a Me *doublet* at δ(H) 0.98, and two CH_2 *triplet*s at δ(H) 3.78 and 2.37. The ^{13}C-NMR data of this compound (*Table*) showed 17 C-atom signals, and one of them appeared at δ(C) 148.6 (*s*) which, together with its IR, indicated the presence of a nitrone moiety in ring *A*. The other 16 signals were all at $\delta(C) < 70$ ppm (4 CH, 10 CH_2, and 2 Me groups). This evidence suggested that **2** possessed the skeletal structure of a phlegmarine-type lycopodium alkaloid [11]. Further analysis of the ^{1}H-NMR data confirmed the existence of the nitrone moiety because $CH_2(1)^1$) was shifted downfield (δ(H) 3.78) compared to $CH_2(9)$, H–C(13) and Me(17), which were shifted upfield. The ^{1}H,^{1}H-COSY, HMQC, and HMBC (*Fig. 2*) data of huperzine M confirmed the structure of **2**. In addition, the NOESY spectrum of **2** (*Fig. 3*) showed correlations between H–C(7) and H–C(12), H–C(13) and H–C(15), between H–C(12) and H–C(13) and Me(17), and between H–C(13) and H–C(15). Based on the NOESY results and compared with the published compounds [12], the configuration of huperzine M could be elucidated as shown in *Fig. 1* for **2**.



2 **3**

Fig. 2. *HMBC Correlations of* **2** *and* **3**



2 **3**

Fig. 3. *NOESY Correlations of* **2** *and* **3**

Huperzine N (**3**) was obtained as a yellowish oil, with $[\alpha]_D^{20} = -25.6$ ($c = 0.17$, $CHCl_3$). Its molecular formula, $C_{17}H_{30}N_2O_2$, was deduced from the HR-EI-MS (*m*/*z* 294.2311; calc. 294.2298) and NMR data. The CD spectrum of **3** showed the same

positive *Cotton* effect ($\Delta\varepsilon = -1.4$, 222 nm; $\Delta\varepsilon = 0.62$, 254 nm) as that of **2** and its IR spectrum was also similar to **2**. Except for the molecular ion at *m/z* 294, the other EI-MS peaks of **3** were identical to those of **2**. The ^{1}H-NMR spectrum (*Table*) exhibited a quaternary MeN *singlet* at δ(H) 3.04, a Me *doublet* at δ(H) 0.93, and two CH_2 *triplet*s at δ(H) 3.75 and 2.34. In the ^{13}C-NMR spectrum (*Table*), 17 C-atom signals were found, and one of them appeared at δ(C) 148.0 (*s*), which indicated the presence of a nitrone moiety. The other 16 signals were all at $\delta(C) < 75$ (4 CH, 10 CH_2, and 2 Me groups). All these evidences suggested that **3** was an analogue of **2**, and the ^{1}H,^{1}H-COSY, HMQC, and HMBC (*Fig. 2*) data confirmed that huperzine N had the structure of **3**. Comparison of the NMR data of positions 1–6[1]) of **2** and **3** (*Table*) confirmed the existence of the nitrone moiety in **3**. Further comparison of the NMR data at positions 9, 13, and 17 of **3** and **2** (*Table*) showed that the signals of these positions exhibited obvious downfield shifts in the spectrum of **3** (^{1}H-NMR: $\Delta(C(9a)) = \delta(\mathbf{3}) - \delta(\mathbf{2}) = 0.49$, $\Delta(C(9b)) = 1.03$, $\Delta(C(13)) = 1.07$ and $\Delta(C(17)) = 0.80$; ^{13}C-NMR: $\Delta(9) = \delta(\mathbf{3}) - \delta(\mathbf{2}) = 11.5$, $\Delta(C(13)) = 10.1$ and $\Delta(C(17)) = 15.1$), indicating that the N-atom in the *B* ring was oxidized [13]. Based on the NOESY (*Fig. 3*) results and compared with the published compounds [12], the configuration of huperzine N could be deduced as shown in *Fig. 1* for **3**.

Experimental Part

General. Precoated plates of SiO_2 (*HSGF$_{254}$*) and neutral Al_2O_3 were used for detection. Column chromatography (CC) was carried out on SiO_2 (200–300 mesh) and neutral Al_2O_3 (200–300 mesh). Optical rotations: *Jasco DIP-181* polarimeter. CD Spectra: *Jasco 500A* polarimeter. IR Spectra: *Perkin-Elmer 599B* spectrophotometer; in cm^{-1}. 1D- and 2D-NMR: *Bruker AM-400* NMR spectrometer; in $CDCl_3$; δ in ppm, *J* in Hz. MS: *MAT-711* and *MAT-95* mass spectrometers; in *m/z*.

Plant Material. The whole plants of *H. serrata* were collected at Xianju, Zhejiang province in August, 2005 and were identified by Dr. *Xiaoqiang Ma* of the Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences. A voucher specimen has been deposited at the herbarium of the institute (No. 97–36).

Extraction and Isolation. About 50 kg of dry plants were extracted 5 times with 1% HCl. The combined HCl extracts were concentrated under vaccum to *ca.* 2 l and alkalized with concentrated ammonia water to pH 9–10. The basic soln. was then extracted repeatedly with $CHCl_3$ (5 × 1 l) until no alkaloids detectable in the H_2O layer. After removal of $CHCl_3$ under reduced pressure, the procedure described above was repeated once more and *ca.* 5 kg of crude alkaloids were obtained, which were divided into ten portions, and each portion was submitted to CC (2 kg of SiO_2; 20 × 100 cm column) and eluted with *ca.* 100 l of $CHCl_3$/acetone (from 10:1 to 1:1), then with *ca.* 5 l of MeOH. Concentration of the MeOH fraction under reduced pressure gave a mixture of *ca.* 50 g, which was separated repeatedly by CC on 1 kg of neutral Al_2O_3 ($CHCl_3$/acetone, 20:1 to 10:1) to give two parts: part *A* ($CHCl_3$/acetone, 20:1, 80 ml) and part *B* ($CHCl_3$/acetone, 15:1 to 10:1, 120 ml). Part *B* was purified on 160 g SiO_2 to give **2** (55 mg, $CHCl_3$/MeOH/NH_4OH, 6:1:0.1, 140 ml) and **3** (48 mg, $CHCl_3$/MeOH/NH_4OH, 4:1:0.1, 130 ml).

Huperzine M (=*(4a*R,*5*S,*7*S,*8a*S*)-Decahydro-1,7-dimethyl-5-[(3,4,5,6-tetrahydro-1-oxidopyridin-2-yl)methyl]quinoline*; **2**). Colorless oil. $[\alpha]_D^{20} = -13.4$ ($c = 0.04$, $CHCl_3$). CD ($c = 4.5 \times 10^{-3}$, MeOH): $\Delta\varepsilon^{18} = -0.65$ (221 nm), $\Delta\varepsilon^{18} = 1.1$ (252 nm). IR: 2947, 2775, 1637, 1440, 1243, 1168. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 278 (18, M^+), 262 (3, $[M-16]^+$), 261 (68, $[M-17]^+$), 166 (100), 164 (77), 152 (40), 123 (24), 97 (28). HR-EI-MS: 278.2347 ($C_{17}H_{30}N_2O^+$; calc. 278.2349), 261.2330 ($C_{17}H_{29}N_2^+$; calc. 261.2322), 166.1599 ($C_{11}H_{20}N^+$; calc. 166.1590), 164.1440 ($C_{11}H_{18}N^+$; calc. 164.1432).

Huperzine N (=*(1*R,*4a*R,*5*S,*7*S,*8a*S*)-Decahydro-1,7-dimethyl-5-[(3,4,5,6-tetrahydro-1-oxidopyridin-2-yl)methyl]quinoline 1-Oxide*; **3**). Yellowish oil. $[\alpha]_D^{20} = -25.6$ ($c = 0.17$, $CHCl_3$). CD ($c = 1.7 \times 10^{-2}$,

MeOH): $\Delta\varepsilon^{18} = -1.4$ (222 nm), $\Delta\varepsilon^{18} = 0.62$ (254 nm). IR: 2967, 2775, 1645, 1446, 1244, 1133. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 294 (3, M^+), 278 (9, $[M-16]^+$), 277 (2, $[M-17]^+$), 264 (18), 261 (20, $[M-16-17]^+$), 247 (9), 205 (22), 166 (51), 164 (36), 152 (100), 150 (50), 149(47), 57 (83). HR-EI-MS: 294.2311 ($C_{17}H_{30}N_2O_2^+$; calc. 294.2298), 278.2340 ($C_{17}H_{30}N_2O^+$; calc. 278.2349), 264.2226 ($C_{16}H_{28}N_2O^+$; calc. 264.2193), 261.2334 ($C_{17}H_{29}N_2^+$; calc. 261.2322), 167.1670 ($C_{11}H_{21}N^+$; calc. 167.1668), 166.1598 ($C_{11}H_{20}N^+$; calc. 166.1590), 164.1435 ($C_{11}H_{18}N^+$; calc. 164.1432), 152.1444 ($C_{10}H_{18}N^+$; calc. 152.1434).

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