Lycodine-Type Alkaloids from *Lycopodium casuarinoides*

by **Sheng Yin**, **Cheng-Qi Fan**, **Xiao-Ning Wang**, and **Jian-Min Yue***

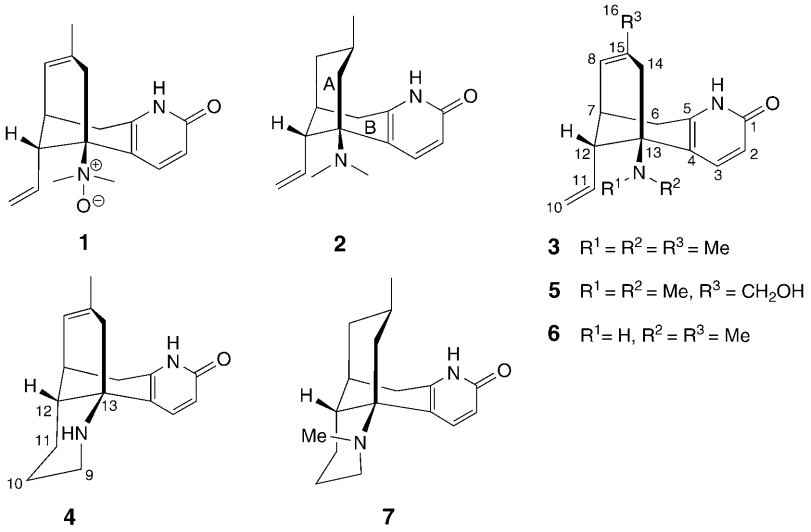
State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai, 201203, P. R. China
(phone: +86-21-50806718; fax: +86-21-50806718; e-mail: jmyue@mail.shcnc.ac.cn)

Two new lycodine-type alkaloids, huperzinine *N*-oxide (**1**) and 8,15-dihydrohuperzinine (**2**) as well as five known compounds, huperzinine (**3**), huperzine B (**4**), huperzine D (**5**), *N*-demethylhuperzinine (**6**), and β-obscurine (**7**), were isolated from the club moss *Lycopodium casuarinoides*. The structures of **1** and **2** were elucidated by spectroscopic methods and chemical transformation. The absolute configuration of **1** was established by chemical correlation with **3**, and that of **2** was determined by its CD spectrum.

Introduction. – *Lycopodium* alkaloids are a group of chemically and pharmacologically interesting secondary metabolites occurring in the family Lycopodiaceae [1], and many of them continue to be the hot points in the aspects of biogenesis [1a] [2] and total synthesis [3]. Since the discovery of potent selective acetylcholinesterase inhibitors huperzines A and B from *Huperzia serrata* [4], a series of research work conducted for their active analogs has led to the identification of over 200 *Lycopodium* alkaloids from *ca.* 54 species of *Lycopodium* (*sensu lato*) [1b]. The present research work on *Lycopodium casuarinoides* Spring, a Chinese folk medicine, allowed the isolation of two new lycodine-type alkaloids, huperzinine *N*-oxide (**1**) and 8,15-dihydrohuperzinine (**2**), along with five known ones, huperzinine (**3**), huperzine B (**4**), huperzine D (**5**), *N*-demethylhuperzinine (**6**), and β-obscurine (**7**). This paper focuses on the isolation and structure elucidation of **1** and **2**.

Results and Discussion. – Huperzinine *N*-oxide (**1**) was obtained as a white powder. The molecular formula $C_{17}H_{22}N_2O_2$ was established by HR-ESI-MS (*m/z* 287.1747, $[M+H]^+$). The spectral data implied that alkaloid **1** was likely a derivative of huperzinine (**3**), a major component also isolated from this plant. Compared with compound **3**, there was one more O-atom in the molecular formula of **1**, which could be attached to the tertiary N-atom. Chemical transformation of huperzinine (**3**) to huperzinine *N*-oxide (**1**) by 3-chloroperbenzoic acid oxidation confirmed the structure of **1**, including its absolute configuration. The full assignment of the ^{1}H- and ^{13}C-NMR data of alkaloid **1** was achieved by comparing them with those of compound **3** and was assisted by using HSQC data (*Table*).

The characteristics of an α-pyridinone (=pyridin-2-(1*H*)-one) were revealed by the spectra of **1** including UV (λ_{max} (MeOH): 306 and 234 nm), IR (1664, 1616, 1556 and 1468 cm^{-1}), and ^{1}H-NMR (2*d*) at δ 9.45, 6.42 J=9.7 Hz)) [4]. An olefinic Me group at δ(H) 1.60 (*s*), 2 Me(N) groups at δ(H)



3.18 (*s*) and 3.28 (*s*), and an *AMX* spin system for the CH_2=CH moiety at δ(H) 5.96 (*ddd*, J = 17.1, 10.2, 10.2 Hz), 5.26 (*d*, J = 10.2) and 5.25 (*d*, J = 17.1), as well as the corresponding carbon signals (*Table*) were also observed. The down-field shifted signals of C(13)[1]) at δ(C) 77.6 and the Me_2N groups at δ(C) 57.1 and 55.3 were diagnostic of the amine oxide moiety.

Also 8,15-dihydrohuperzinine (**2**) is a white powder. HR-EI-MS of **2** exhibited a molecular-ion peak at *m/z* 272.1884, which is two mass units more than that of **3,** and matched the molecular formula $C_{17}H_{24}N_2O$. The UV, IR as well as the ^{1}H-NMR spectra (*Table 1*) of **2** also indicated the presence of an α-pyridinone moiety. The structure of alkaloid **2** was shown to be that of a 8,15-dihydrogenated derivative of **3**. The relative configuration of **2** (*Fig. 1*) was deduced from a ROESY experiment, and the absolute configuration (7*S*,12*R*,13*R*,15*R*)[1]) was assigned by comparing the CD spectrum of **2** (*Fig. 2*) with that of β-obscurine (**7**), whose absolute configuration had been determined before [5].

The ^{1}H-NMR signal of the olefinic H–C(8)[1]) of huperzinine (**3**), δ(H) 5.35 was not observed in the spectrum of **2**, and the signal of Me–C(16) of **2** was shifted upfield to δ(H) 0.85 (*d*, J = 6.0 Hz) as compared to **3** (δ(H) 1.53, (*s*)), suggesting the absence of a C(8)=C(15) bond in **2**. Two additional C-signals at δ(C) 43.9 and 29.0 representing a tertiary and a secondary sp^3 C-atomconfirmed the presence of a CH_2(8)–CH15 moiety of **2**. The ^{1}H-NMR spectrum of **2** showed two aromatic proton signals at δ(H) 7.66 (*d*, J = 9.4 Hz) and 6.41 (*d*, J = 9.4 Hz) assignable to H–C(3) and H–C(2) of the α-pyridinone ring, respectively. The signals for two Me(N) groups overlapped at δ(H) 2.43 (*s*, 6 H), and the signals of a CH_2=CH moiety at δ(H) 5.24 (*d*, J = 17.0 Hz), 5.02 (*d*, J = 10.1 Hz), and 6.00 (*ddd*, J = 17.0, 10.1, 10.1 Hz) were attributed to H_A–C(10), H_B–C(10), and H–C(11), resp.. The signal at δ(H) 2.83 (*dd*, J = 10.1, 3.3Hz) which coupled with H–C(11) was assigned to H–C(12). The correlations H–C(7)/H_α–C(6), H–C(7)/H_{exo}–C(8), and Me(16)/H–C(15) were also observed in the ^{1}H,^{1}H-COSY. The ^{1}H- and ^{13}C-NMR signals (*Table*) of **2** were assigned by a combination of ^{13}C-NMR, ^{1}H,^{1}H-COSY and HSQC data.

---

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

Table. *^{1}H-* and *^{13}C-NMR Data of Compounds* **1**–**3.** δ in ppm, J in Hz. Trivial numbering

	1		**2**		**3**	
	δ(H)[a])	δ(C)[b])	δ(H)[c])	δ(C)[d])	δ(H)[a])	δ(C)[b])
C(1)		165.2		165.6		165.1
H–C(2)	6.42 (*d*, *J* = 9.7)	117.6	6.41 (*d*, *J* = 9.4)	118.6	6.45 (*d*, *J* = 9.5)	117.6
H–C(3)	9.45 (*d*, *J* = 9.7)	143.9	7.66 (*d*, *J* = 9.4)	143.7	7.65 (*d*, *J* = 9.4)	140.1
C(4)		114.3		120.7		118.7
C(5)		143.3		146.6		142.8
H_α–C(6)	3.02 (*dd*, *J* = 17.8, 5.2)	29.0	2.95 (*dd*, *J* = 18.5, 6.9)	31.0	2.98 (*dd*, *J* = 17.5, 5.0)	29.1
H_β–C(6)	2.46 (*d*, *J* = 17.8)		2.26 (*d*, *J* = 18.5)		2.34–2.45 (*m*)	
H–C(7)	2.56 (br. *d*, *J* = 3.9)	39.5	2.02–2.07 (*m*)	39.8	2.38–2.43 (*m*)	38.7
H_{exo}–C(8)	5.38 (br. *d*, *J* = 4.0)	123.4	1.25–1.28 (*m*)	43.9	5.35 (br. *d*, *J* = 5.2)	124.4
H_{endo}–C(8)			1.66–1.70 (*m*)			
H_A–C(10)	5.25 (*d*, *J* = 17.1)	118.7	5.24 (*d*, *J* = 17.0)	116.6	5.21 (*dd*, *J* = 17.1, 1.9)	116.3
H_B–C(10)	5.26 (*d*, *J* = 10.2)		5.02 (*d*, *J* = 10.1)		5.01 (*dd*, *J* = 10.0, 1.9)	
H–C(11)	5.96 (*ddd*, *J* = 17.1, 10.2, 10.2)	138.0	6.00(*ddd*, *J* = 17.0, 10.1, 10.1)	143.1	5.95 (*ddd*, *J* = 17.1, 10.0, 10.0)	142.4
H–C(12)	2.78 (*dd*, *J* = 10.2, 3.9)	46.8	2.83 (*dd*, *J* = 10.1, 3.3)	49.0	2.85 (*dd*, *J* = 10.0, 3.8)	45.8
C(13)		77.6		63.0		59.8
H_{exo}–C(14)	2.84 (br. *s*)	40.3	1.62–1.66 (*m*)	49.0	1.61 (*d*, *J* = 17.2)	44.4
H_{endo}–C(14)	2.84 (br. *s*)		1.31–1.36 (*m*)		2.79 (*d*, *J* = 17.1)	
C(15) or H–C(15)		134.0	1.19–1.24 (*m*)	29.0		134.3
Me–C(16)	1.60 (*s*)	22.8	0.85 (*d*, *J* = 6.0)	22.8	1.53 (*s*)	22.9
Me_AN	3.18 (*s*)	57.1	2.43 (*s*)	40.4	2.41 (*s*)	39.5
Me_BN	3.28 (*s*)	55.3	2.43 (*s*)	40.4	2.41 (*s*)	39.5

[a]) Measured at 400 MHz in $CDCl_3$. [b]) Measured at 100 MHz in $CDCl_3$. [c]) Measured at 400 MHz in CD_3OD. [d]) Measured at 100 MHz in CD_3OD.

Assuming a chair conformation for the six-membered ring A (see *Fig. 1*), the CH_2=CH moiety at C(12) and Me–C(15) are equatorially oriented as shown by the ROESY correlations H–C(12)/H_{exo}–C(8) and H_{exo}–C(14), Me(16)/H_{exo}–C(8) and H_{endo}–C(8). The ROESY interactions H_{endo}–C(8)/H_β–C(6), H–C(7)/H_α–C(6) and H_β–C(6), and MeN/H–C(12) and H–C(11) indicated that H–C(7) and Me_2N–C(13) were equatorially positioned at ring A. The six-membered ring B was also assigned to be in a chair-like conformation. In the CD spectrum of **2** and the related **7** (*Fig. 2*), the *Cotton* effects corresponding to UV absorptions around 221 and 310 nm for both compounds closely matched, indicating that their absolute configurations are the same.

The five known alkaloids, huperzinine (**3**) [6][7], huperzine B (**4**) [4], huperzine D (**5**) [7], *N*-demethylhuperzinine (**6**) [6], and β-obscurine (**7**) [5] were also isolated from this plant and structurally identified by comparison with literature data.

Financial support of the *National Natural Science Foundation* (for *J. M. Yue*, 30025044) and the *Foundation from the Ministry of Science and Technology* (for *J. M. Yue*, 2002CB512807) are gratefully acknowledged. We thank professor *Shi-Man Huang*, Department of Biology, Hainan University, for the collection and identification of the plant material.

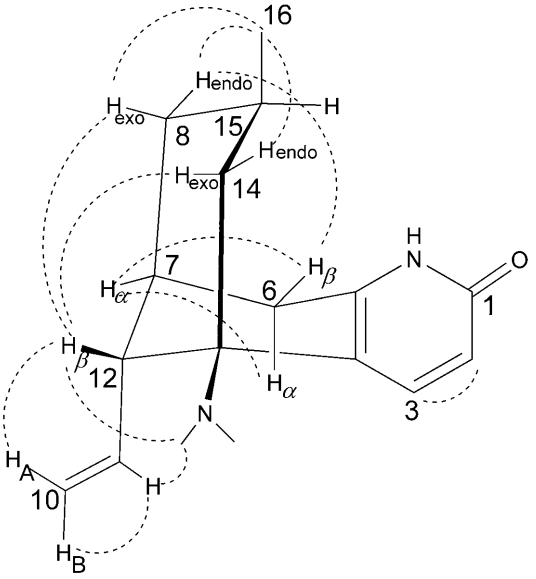


Fig. 1. *Key ROESY correlations observed for* **2**

Experimental Part

General. All solvents used were of anal. grade (*Shanghai Chemical Plants*, Shanghai, P. R. China.). Column chromatography (CC): silica gel (200–300 mesh), silica gel *H60*, C_{18} reversed-phase silica gel (250 mesh, *Merck*), and *MCI* gel (*CHP20P*, 75–150 μm, *Mitsubishi Chemical Industries Ltd.*). TLC: precoated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Plant,* Qingdao, P. R. China). Optical rotation: *Perkin-Elmer 341* polarimeter. UV Spectra: *Hitachi U-2010* λ_{max} (log ε) in nm. CD spectrum: *Jasco J-810* instrument. IR Spectra: *Perkin-Elmer 577* spectrometer; in cm^{-1} NMR Spectra: *Bruker AM-400* spectrometer. EI-MS (70 eV): *Finnigan-MAT 95* mass spectrometer in *m/z* (rel. %).

Plant Material. The club moss *Lycopodium casuarinoides* SPRING. was collected in Hainan province, P. R. China, in July 2003. The plant was authenticated by Prof. *Shi-Man Huang*, Department of Biology, Hainan University. A voucher specimen has been deposited in the Shanghai Institute of Materia Medica (Accession number: LC-2004–1Y).

Extraction and Isolation. The club moss (25 kg) of *L. casuarinoides* was extracted with 95% EtOH at r.t. (3×50 l, each for 5 days). After evaporation, the residue (958 g) was suspended in 4 l acidic H_2O (adjusted with 2N H_2SO_4 to pH *ca.* 1–2), and then immediately partitioned with $CHCl_3$ (3×2 l) to remove the nonalkaloid portion. The acidic soln. was adjusted to pH *ca.* 10 with sat. Na_2CO_3 soln. and extracted with $CHCl_3$ (3×2 l) and then with BuOH (3×1 l). The $CHCl_3$-soluble part (5.2 g) was subject to CC (*RP-18* silica gel, H_2O/MeOH 1:0→0:1): *Fractions 1* and *2*. *Fr. 1* (4.1 g) was kept at r.t. in MeOH to afford crystals of huperzinine (**3**; 3.2 g). The mother liquor was resubjected to CC (silica gel, $CHCl_3$/MeOH 10:1): huperzine B (**4**; 246 mg) and huperzine D (**5**; 320 mg). *Fr. 2* (0.8 g) was purified by CC (amino silica gel, cyclohexane/AcOEt 1:0 → 0:1) 8,15-dihydrohuperzinine (**2**; 8 mg), β-obscurine (**7**; 11 mg), and *N*-demethylhuperzinine (**6**; 511 mg). The BuOH-soluble part (12 g) was separated by CC (*MCI*, H_2O/MeOH 7:3): huperzinine *N*-oxide (**1**; 30 mg).

Huperzinine N-*Oxide* (=*(5*R*,9*R*,11*R*)-5-(Dimethyloxidoamino)-11-ethenyl-5,6,9,10-tetrahydro-7-methyl-5,9- methanocycloocta[*b*]pyridine-2(1H)-one*; **1**). White powder. $[\alpha]_D^{20} = -8.1$ ($c = 0.735$, MeOH). UV (MeOH): 306 (3.84), 234 (4.00). IR (KBr): 3423, 2919, 1664, 1616, 1556, 1468, 1425, 1317, 1101, 941, 833. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS: (pos.) 287.1 (41, $[M+H]^+$), 226.2 (100, $[M-NO]^+$). HR-ESI-MS: 287.1747 ($[M+H]^+$, $C_{17}H_{23}N_2O_2^+$; calc. 287.1760).

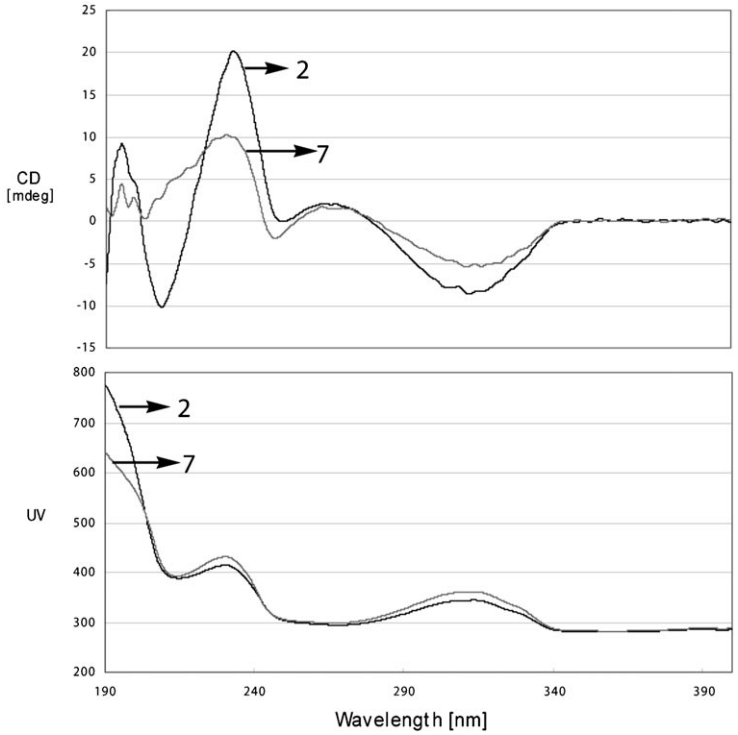


Fig. 2. *CD and UV spectra of* **2** *and* **7**

8,15-Dihydrohuperzinine (=*(5*R*,7*R*,9*S*,11*R*)-5-(Dimethylamino)-11-ethenyl-5,6,7,8,9,10-hexahydro-7-methyl-5,9-methanocycloocta[*b*]pyridine-2(1H)-one*; **2**). White power. $[\alpha]_D^{20} = -68$ (c = 0.11, MeOH). UV (MeOH): 310 (3.82), 221 (4.08). IR (KBr): 3423, 2919, 1668, 1621, 1556, 1460, 1413, 1384, 1126, 1047, 916. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 272 (65, M^+), 257 (100), 229 (36), 215 (97), 205 (37), 189 (28), 163 (32), 146 (15). HR-EI-MS: 272.1884 (M^+, $C_{17}H_{24}N_2O^+$; calc. 272.1889).

Oxidation of Huperzinine (**3**). To a stirred soln. of **3** (10 mg) in $CHCl_3$ (2 ml) at 0°, 3-chloroperbenzoic acid (12 mg) was added. The mixture was stirred at r.t. for 3 h and then evaporated. The resulting yellow solid was then subjected to CC (silica gel, $CHCl_3$/MeOH 10 : 1): **1** (8 mg). ^{1}H-NMR and $[\alpha]_D$: identical to those of natural **1**.

REFERENCES

[1] a) W. A. Ayer, *Nat. Prod. Rep.* **1991**, *8*, 455; b) X. Q. Ma, D. R. Gang, *Nat. Prod. Rep.* **2004**, *21*, 752.

[2] J. Kobayashi, H. Morita, in 'The Alkaloids', Ed. G. A. Cordell, Academic Press, New York, 2005, Vol. 61, p. 292; W. A. Ayer, L. S. Trifonov, in 'The Alkaloids', Ed. G. A. Cordell and A. Brossi, Academic Press, New York, 1994, Vol. 45, p. 233; D. B. Maclean in 'The Alkaloids', Ed. A. Brossi, Academic Press, New York, 1985, Vol. 26, p. 241; T. Hemscheidt, I. D. Spenser, *J. Am. Chem. Soc.* **1993**, *115*, 3020.

[3] C. F. Yen, C. C. Liao, *Angew. Chem., Int. Ed.* **2002**, *41*, 4090; J. Cassayre, F. Gagosz, S. Z. Zard, *Angew. Chem., Int. Ed.* **2002**, *41*, 1783; C. K. Sha, F. K. Lee, C. J. Chang, *J. Am. Chem. Soc.* **1999**, *121*, 9875; J. P. Williams, D. R. St. Laurent, D. Friedrich, E. Pinard, B. A. Roden, L. A. Paquette, *J. Am. Chem. Soc.* **1994**, *116*, 4689; G. C. Hirst, T. O. Johnson, L. E. Overman, *J. Am. Chem. Soc.* **1993**, *115*, 2992.

[4] 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.
[5] W. A. Ayer, J. A. Berezowsky, G. G. Iverach, *Tetrahedron* **1962**, *18*, 567.
[6] Y. C. Shen, C. H. Chen, *J. Nat. Prod*. **1994**, *57*, 824.
[7] J. S. Liu, M. F. Huang, *Phytochemistry* **1994**, *37*, 1759.

Received October 5, 2005