Two New Lycodine Alkaloids from *Lycopodiastrum casuarinoides*

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Two new lycodine alkaloids, 11β-methoxyhuperzine B (**1**) and 16-oxohuperzinine (**2**), together with seven known ones, huperzinine *N*-oxide, huperzine D, casuarinine A, huperzine B, casuarinine B, huperzinine, and *N*-methyllycodine, were isolated from whole plants of *Lycopodiastrum casuarinoides*. Their structures were elucidated by spectroscopic methods, including NMR and MS experiments.

Introduction. – *Lycopodium* alkaloids are compounds, isolated from plants of Lycopodiaceae and Huperziaceae, with diverse structures including many unusual skeletons of interest from biogenetic and biological points of view, and challenging targets for total synthesis [1–4]. *Lycopodiastrum casuarinoides* (SPRING) HOLUB (Lycopodiaceae) is a climbing plant distributed mainly in southern China [5]. Previous research indicated that this plant is a rich source of lycodine alkaloids possessing inhibitory activity against acetylcholinesterase [6–9]. As a part of our search for structurally unique and biologically active *Lycopodium* alkaloids from medicinal plants of Yunnan, China [9][10], we have isolated and identified two new lycodine-type alkaloids, 11β-methoxyhuperzine B (**1**) and 16-oxohuperzinine (**2**), from whole plants of *L. casuarinoides*, as well as seven known ones, *i.e.*, huperzinine *N*-oxide (**3**) [11], huperzine D (**4**) [6], casuarinine A (**5**) [6], huperzine B (**6**) [12], casuarinine B (**7**) [6], huperzinine (**8**) [8], and *N*-methyllycodine (**9**) [13] (*Fig. 1*). Herein, we report the isolation and structure elucidation of these compounds.

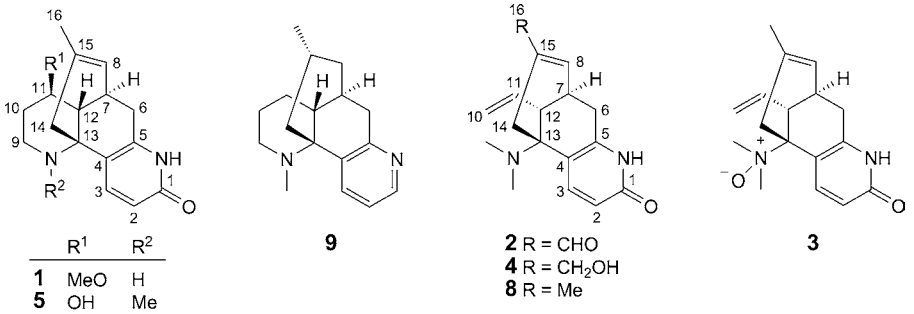

Fig. 1. *Alkaloids isolated from* L. casuarinoides

Results and Discussion. – The alkaloid fraction was obtained by extraction of whole plants of *L. casuarinoides* with MeOH, acidification with aqueous H_2SO_4, partition with AcOEt to remove neutral components, basification to pH 9 with saturated Na_2CO_3, and extraction with $CHCl_3$. The $CHCl_3$ layer was separated and purified by repeated chromatography to afford two new alkaloids, 11β-methoxyhuperzine B and 16-oxohuperzinine (**1** and **2**, resp.; see *Fig. 1*), along with the known compounds **3**–**9**.

11β-Methoxyhuperzine B (**1**) was obtained as white powder, and the molecular formula $C_{17}H_{22}N_2O_2$ was established by HR-EI-MS. The IR absorption band at 1668 cm^{-1} indicated the presence of an α,β-unsaturated δ-lactam group. The ^{1}H-NMR spectrum (*Table*) exhibited signals of an olefinic Me group (δ(H) 1.46 (*s*)), a MeO group at 3.26 (*s*), an olefinic H-atom (5.42 (*d*, $J = 5.0$)), and two aromatic *ortho*-H-atoms (δ(H) 6.38 and 7.67 (*d*, $J = 9.0$, each 1 H)). The ^{13}C-NMR (*Table*) and DEPT spectra exhibited 17 C-atom signals, including those of a Me and a MeO group, four sp^3 CH_2, three sp^2, and three sp^3 CH groups, and four sp^2 (including one NHC=O), and one sp^3 C_q-atom. Comparison of the NMR data of **1** with those of the known huperzine B (**6**) [12], which was also isolated in this study, revealed that these two compounds were similar. The only difference between the NMR data of **1** and **6** was the replacement of CH_2(11) signal of **6** by an O-bearing CH signal in **1**, which was supported by ^{1}H,^{1}H-COSY analyses (*Fig. 2*). The HMBCs (*Fig. 2*) of MeO (δ(C) 56.0) with H–C(11) indicated that the MeO group is located at C(11). Thus, the structure of **1** was determined as the 11-MeO derivative of **6**. The large coupling constant ($J = 10.5$) between H–C(11) (δ(H) 2.96) and H–C(12) (δ(H) 1.53) suggested that both H-atoms assumed axial orientations. In the ROESY plot (*Fig. 2*), correlations between H–C(11)

Table. *^{1}H- and ^{13}C-NMR Data* (at 500 and 125 MHz, resp., in $CDCl_3$) *of* **1** *and* **2**. δ in ppm, *J* in Hz. Atom numbering as indicated in *Fig. 1*.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1		165.4		165.1
2	6.38 (*d*, $J = 9.0$)	117.6	6.46 (*d*, $J = 9.0$)	118.4
3	7.67 (*d*, $J = 9.0$)	140.3	7.70 (*d*, $J = 9.0$)	142.5
4		118.5		118.1
5		143.8		141.4
6	2.83 (overlapped), 2.45 (*d*, $J = 17.5$)	29.2	3.17 (*dt*, $J = 18.0, 5.5$), 2.57 (*d*, $J = 18.0$)	27.8
7	2.87–2.89 (*m*)	29.3	2.78–2.80 (*m*)	39.5
8	5.42 (*d*, $J = 5.0$)	125.7	6.71 (*d*, $J = 5.5$)	151.0
9	2.83 (overlapped), 2.24 (*t*, $J = 12.5$)	40.0		
10	2.02 (overlapped), 1.14–1.17 (*m*)	32.2	5.27 (*d*, $J = 17.0$), 5.14 (*d*, $J = 10.0$)	117.6
11	2.96 (*dt*, $J = 10.5, 4.0$)	76.2	5.95–6.03 (*m*)	138.3
12	1.53 (*dd*, $J = 10.5, 2.5$)	46.5	2.94 (*dd*, $J = 10.0, 3.0$)	45.6
13		54.3		59.4
14	2.02 (overlapped), 1.83 (*d*, $J = 17.0$)	47.8	2.85 (*d*, $J = 18.0$), 2.16 (*d*, $J = 18.0$)	36.0
15		131.8		141.2
16	1.46 (*s*)	22.8	9.38 (*s*)	193.4
MeO	3.26 (*s*)	56.0		
MeN			2.43 (*s*)	39.5

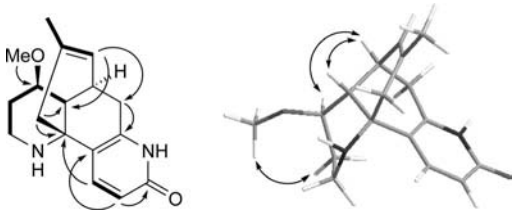

Fig. 2. *Selected $^1H,^1H$-COSY* (—), *ROESY* (H ↔ H) *correlations and HMBCs* (H → C).

and H–C(12), and H–C(11) and H–C(7) at (δ(H) 2.87 – 2.89) suggested that both the MeO group at C(11) and H–C(12) are β-oriented. Thus, the structure of **1** was elucidated as 11β-methoxyhuperzine B.

16-Oxohuperzinine (**2**) was obtained as white powder and had the molecular formula $C_{17}H_{20}N_2O_2$ as deduced by HR-EI-MS. The IR spectra exhibited strong absorptions at 3441 and 1670 cm^{-1}, suggesting the presence of NH_2 and C=O groups. The ^{1}H-NMR spectra (*Table*) showed signals at δ(H) 5.14 (*d*, *J* = 10.0, 1 H), 5.27 (*d*, *J* = 17.0, 1 H), and 5.95 – 6.03 (*m*, 1 H), which were attributed to a monosubstituted vinyl group (CH_2=CH–R). Signals of an olefinic H-atom at δ(H) 6.71 (*d*, *J* = 5.5), and two aromatic *ortho* H-atoms at 6.46 and 7.70 (*d*, *J* = 9.0, each 1 H) were also observed. The signal at δ(H) 9.38 (*s*, 1 H) suggested the presence of a CHO group. The ^{13}C-NMR (*Table*) and DEPT data displayed 17 C-atom signals, *i.e.*, those of two MeN groups, one sp^2 and two sp^3 CH_2 groups, two sp^3 and five sp^2 CH groups (including a CHO group (δ(C) 193.4)), and one sp^3 and four sp^2 C_q-atoms. These NMR data showed general features similar to those of huperzinine (**8**) [8], which was also obtained in this study. Differing from **8**, **2** had a CHO group instead of the Me group, which was confirmed by observed HMBCs (*Fig. 3*) of H–C(16) (δ(H) 9.38) with C(8) (δ(C) 151.0), C(14) (36.0), and C(15) (141.2). The structure of **2**, therefore, was elucidated as 16-oxohuperzinine. The complete assignment of all H- and C-atoms was established by extensive use and interpretation of ^{1}H,^{1}H-COSY, HMQC, HMBC, and ROESY spectra (*Fig. 3*).

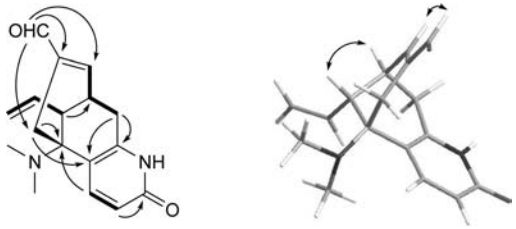

Fig. 3. *Selected $^1H,^1H$-COSY* (—), *ROESY* (H ↔ H) *correlations and HMBCs* (H → C)

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Experimental Part

General. TLC: Silica gel GF_{254} (SiO_2; *Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, P. R. China). Column chromatography (CC): SiO_2 (200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*) and *RP-18* (40–75 μm; *Fuji Chemical Industrial Co., Ltd.*, Tochigi, Japan). Optical rotations: *Horiba-SEAP-300* spectropolarimeter. IR Spectra: *PerkinElmer-241* polarimeter; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-Avance-500* NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. MS: *VG-Auto-Spec-3000* spectrometer; in m/z.

Plant Material. Whole plants of *L. casuarinoides* were collected in Pingbian, Yunnan Province, P. R. China, in August 2011. A voucher specimen (No. 1108019) of this herb was identified by Prof. *Shu-Gang Lu* at School of Life Science, Yunnan University.

Extraction and Isolation. The air-dried and powdered plant (7 kg) was extracted four times with MeOH at r.t., and the extract was partitioned between AcOEt (2 l) and 1% aq. H_2SO_4 (2 l). The H_2O-soluble portion, adjusted to pH 9 with sat. Na_2CO_3, was extracted with $CHCl_3$ (2 l). The crude alkaloids (10 g) were chromatographed over *RP-18* (MeOH/H_2O 1:1 → 10:0): *Frs. 1–4*. *Fr. 1* (2.7 g) was subjected to CC (*RP-18*; MeOH/H_2O 1:1; then SiO_2; $CHCl_3$/MeOH 5:1) to furnish **1** (25 mg), **2** (20 mg), **3** (28 mg), **4** (50 mg), **5** (16 mg), and **6** (39 mg). Compounds **7** (150 mg), **8** (2.1 g), and **9** (60 mg) were crystallized from *Fr. 2* (1.0 g), *Fr. 3* (3.5 g), and *Fr. 4* (0.5 g), resp.

11β-Methoxyhuperzine B (*=(4*R*,4a*R*,5*R*,10b*R*)-2,3,4,4a,5,6-Hexahydro-4-methoxy-12-methyl-1*H*-5,10b-prop[1]eno-1,7-phenanthrolin-8(7*H*)-one*; **1**). Colorless amorphous powder. $[\alpha]_D^{22} = -77.3$ ($c =$ 0.0048, MeOH). IR (KBr): 3441, 2915, 2892, 1668, 1603, 1458, 1429, 1104. ^{1}H- and ^{13}C-NMR: *Table.* HR-EI-MS: 286.1679 (M^+, $C_{17}H_{22}N_2O_2^+$; calc. 286.1681).

16-Oxohuperzinine (*=(5*R*,9*R*,11*R*)-5-(Dimethylamino)-11-ethenyl-1,2,5,6,9,10-hexahydro-2-oxo-5,9-methanocycloocta[*b*]pyridine-7-carbaldehyde*; **2**). Colorless amorphous powder. $[\alpha]_D^{26} = -96.5$ ($c =$ 0.0016, MeOH). IR (KBr): 3441, 2935, 2907, 2800, 1670, 1610, 924, 837. ^{1}H- and ^{13}C-NMR: *Table.* HR-EI-MS: 284.1520 (M^+, $C_{17}H_{20}N_2O_2^+$; calc. 284.1525).

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