A New Alkaloid from *Lycopodium japonicum* THUNB.

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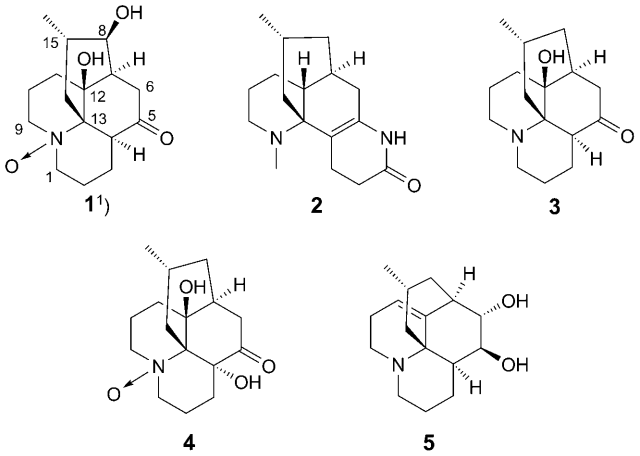
A new alkaloid, miyoshianine C (**1**), was isolated from the whole plants of *Lycopodium japonicum* THUNB., together with the known four compounds α-obscurine (**2**), lycodoline (**3**), miyoshianine A (**4**), and lucidioline (**5**). Their structures were elucidated on the basis of in-depth spectroscopic analysis.

Introduction. – The plants of Lycopodiaceae are very important medicinal plants for the treatment of rheumatism, anesthesia, and trauma. Previous chemical studies established the occurrence of alkaloids, terpenes, anthraquinones, and organic acids in this plant family [1–10]. *Lycopodium japonicum* THUNB., a traditional folk medicine, is widely distributed in China. Our studies of *L. japonicum* led to the isolation of a new alkaloid, named miyoshianine C (**1**) and carrying an *N*-oxide functionality, besides four known alkaloids, *i.e.* α-obscurine (**2**) [11], lycodoline (**3**) [11], miyoshianine A (**4**) [1], and lucidioline (**5**) [2].



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

4 **5**

Results and Discussion. – Miyoshianine C (**1**) was isolated from *L. japonicum* as a white crystalline solid with MeOH. The molecular formula $C_{16}H_{25}NO_4$ was established

[1]) Trivial atom numbering; for the systematic name, see *Exper. Part.*

by FAB-MS, combined with HR-ESI-MS (*m/z* 296.1863 ($[M+H]^+$, $C_{16}H_{26}NO_4^+$). The IR spectrum of **1** showed absorption bands characteristic of OH (3385 cm^{-1}) and C=O groups (1701 cm^{-1}). Comparison of the ^{1}H- and ^{13}C-NMR data of **1** (*Table*) with the known compound miyoshianine A (**4**), which was also isolated in this study, showed that the two compounds are similar. From further data, including a HMBC spectrum (*Fig.*), the structure of compound **1** was identified as a novel alkaloid, which was given the name miyoshianine C.

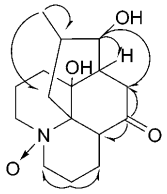


Figure. *Key HMBC* (C → H) *for* **1**

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; C_5D_5N) *of* **1**[1]). δ in ppm, J in Hz.

	δ(C)	δ(H)		δ(C)	δ(H)
CH_2(1)	60.29 (*t*)	2.65 (*dd*, J = 5.3, 12.3, H_α), 3.40 (*dt*, J = 3.8, 12.8, H_β)	CH_2(9)	56.99 (*t*)	2.89 (*dd*, J = 2,7 13.1, H_α), 3.72 (*td*, J = 3.0, 11.9, H_β)
CH_2(2)	18.90 (*t*)	2.13–2.38 (*m*, H_α), 1.62 (*d*, J = 14.2, H_β)	CH_2(10)	16.34 (*t*)	1.43–1.46 (*m*, H_α), 2.09 (*d*, J = 14.2, H_β)
CH_2(3)	26.51 (*t*)	1.50–1.53 (*m*, H_α), 3.07 (*t*, J = 3.3, H_β)	CH_2(11)	32.55 (*t*)	2.34–2.38 (*m*, H_α), 1.87 (*dd*, J = 4.1, 12.3, H_β)
H–C(4)	46.17 (*d*)	3.87 (*t*, J = 3.3)	C(12)	72.09 (*s*)	–
C(5)	211.81 (*s*)	–	C(13)	73.32 (*s*)	–
CH_2(6)	45.03 (*t*)	3.12 (*dd*, J = 5.4, 16.0, H_α), 2.00 (*d*, J = 16.0, H_β)	CH_2(14)	36.19 (*t*)	1.35 (*dd*, J = 4.8, 13.3, H_α), 2.23–2.33 (*m*, H_β)
H–C(7)	41.68 (*d*)	2.25–2.31 (*m*)	H–C(15)	25.28 (*d*)	1.39–1.46 (*m*)
H–C(8)	72.04 (*d*)	4.01 (*dd*, J = 2.8, 11.9)	Me(16)	21.56 (*q*)	0.79 (*d*, J = 6.1)

The ^{1}H-NMR spectrum of **1** showed signals for a Me group at a tertiary C-atom, and the ^{13}C-NMR spectrum exhibited signals for 16 C-atoms, *i.e.* three quaternary C-atoms (including a carbonyl group and an oxygenated one) and four CH (one oxygenated), eight CH_2, and a Me group. The only difference between the NMR data of **1** and **4** is the replacement of the CH_2(8) and the C(4) signal of **4** by a CH (oxygenated) and a CH signal for **1**. In the HMBC spectrum of **1**, the signals at δ(H) 2.25–2.31 (H–C(7)), 3.12 (H–C(6)), and 1.35 (H_α–C(14)) were correlated with the signal at δ(C) 72.04 (C(8)), which supported the presence of an OH–C(8) in **1**.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical*, *Inc.*, China), *Lichroprep RP-18* (40–63 μm; *Merck*), or *Sephadex LH-20* (*Pharmacia*). M.p.: *X-4* apparatus; uncorrected. Optical rotations: *Horiba SEAP-300* spectropolarimeter. NMR Spectra: *Bruker AV-400* or

DRX-500 instruments; chemical shifts δ in ppm rel. to Me_4Si, coupling constants J in Hz. FAB-MS: *VG-Autospec-3000* spectrometer; in m/z (rel. %).

Plant Material. The whole plants of *Lycopodium japonicum* were purchased from the Kunming medicine market, Yunnan province, P. R. China, in August 2003. The plant was identified by Prof. *Wu Sugong*, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen was deposited with the Laboratory of Phytochemistry, Kunming Institute of Botany.

Extraction and Isolation. The chipped whole plants of *L. japonicum* (20 kg) were extracted with EtOH. The extract was filtered, concentrated *in vacuo* to a suitable volume, suspended in H_2O, and then extracted with AcOEt. The aq. phase was subjected to macroporous resin CC with MeOH. Then the concentrated MeOH eluate was suspended in H_2O, and NH_4OH was added to adjust the solvent pH to 8–9. The mixture was extracted with $CHCl_3$, the extract concentrated, and the residue subjected to CC (silica gel). Further purification by CC (*RP-18* gel and *Sephadex LH-20*) yielded **1**, **2**, **3**, **4**, and **5**.

Miyoshianine C (=*(8a*S*,9*R*,11*S*,12a*S*)-Dodecahydro-8a,10-dihydroxy-11-methyl-1,9-ethanobenzo[*i*]*quinolizin-14-one 5-Oxide*; **1**): White crystalline solid. M.p. 264°. $[\alpha]_D^{25} = -25.00$ (c = 1, MeOH). IR (KBr): 3400, 3385, 2966, 2826, 1701, 1456, 1378, 1077, 988. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 296.1863 ($[M+H]^+$, $C_{16}H_{26}NO_4^+$; calc. 296.1861).

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