Two New *Lycopodium* Alkaloids from *Lycopodium obscurum*

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Two new *Lycopodium* alkaloids, (+)-cermizine D *N*-oxide (**1**) and (8β)-8-(acetyloxy)obscurumine A (**2**), along with five known compounds, were isolated from the crude alkaloid portion of *Lycopodium obscurum*. Their structures were elucidated on the basis of spectroscopic data and chemical correlation. All of these alkaloids were tested in an assay for acetylcholine esterase (AChE) inhibitory activity.

Introduction. – The genus *Lycopodium*, which produces a class of structurally complex alkaloids, has been extensively studied for many years. To date, more than 200 *Lycopodium* alkaloids have been identified, and some of them exhibit remarkable biological activities [1][2]. *L. obscurum* is a widely used folk medicine for the treatment of contusion, strain, and swelling in China. Its alkaloids have been the subject of research since the 1940s. From *L. obscurum* collected in Canada, *Ayer* and *Kasitu* isolated α-obscurine and β-obscurine, the first α-pyridone-containing *Lycopodium* alkaloids [3]. They also reported the structure of hydroxypropyllycodine, the first C_{19} *Lycopodium* alkaloid [3]. A more recent study on alkaloids of this plant was performed by *Kobayashi* and co-workers in 2005, and two novel lycopodine-type alkaloids, obscurumines A and B, were described [4]. As a part of our investigation on alkaloids from the genus *Lycopodium*, we examined the crude base fraction of *L. obscurum* collected in Northeast China. As a result, two new alkaloids, (+)-cermizine D *N*-oxide (**1**) and 8β-(acetyloxy)obscurumine A (**2**), along with five known compounds, (+)-cermizine D (**3**) [5], clavolonine (**4**) [6], isofawcettiine (**5**) [7], deacetyllycofawcine (**6**) [8], and lycodine (**7**) [9] were isolated. We now report the isolation and structure elucidation of **1** and **2**.

Results and Discussion. – The crude alkaloid fraction obtained from the whole plant of *L. obscurum* was subjected to repeated column chromatography on silica gel to afford the two new *Lycopodium* alkaloids **1** and **2**, together with the five known compounds **3**–**7** (*Fig. 1*).

Compound **1** was isolated as a pale yellow oil. Its molecular formula, $C_{16}H_{30}N_2O$, was established by positive-ion-mode HR-ESI-MS (*m*/*z* 267.2431 ($[M + H]^+$)). The ^{1}H- and ^{13}C-NMR data (*Table*) showed signals due to four sp^3 CH, eleven sp^3 CH_2, and one Me group. Detailed analyses of ^{1}H,^{1}H-COSY, TOCSY, HSQC, and HMBC data (*Fig. 2*) revealed that the structure of **1** was very similar to that of cermizine D (=(2*S*,4*R*,9a*S*)-octahydro-2-methyl-4-[(2*S*)-piperidin-2-ylmethyl]-2*H*-quinolizine; **3**)

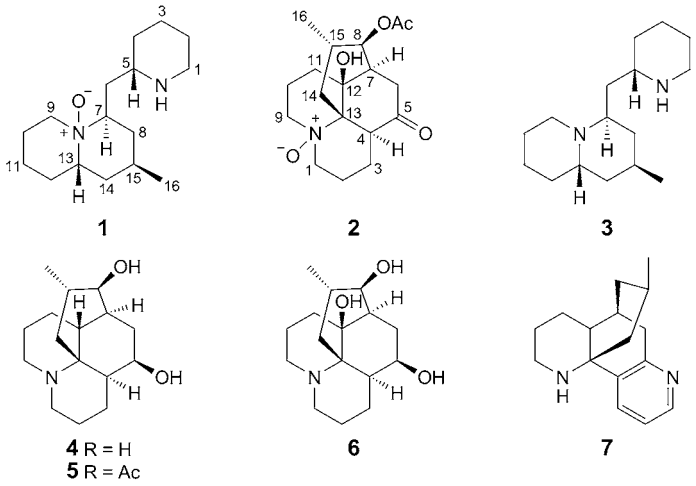

Fig. 1. *Compounds* **1**–**7**, *isolated from* Lycopodium obscurum

[5][10], which was also isolated in the present study. The molecular structure of **1** contains one more O-atom than that of **3**. Additionally, the ^{13}C-NMR signals of C(7) (δ(C) 59.8), C(9) (65.8), and C(13) (75.0) of **1** were shifted downfield compared to those of **3** (C(7) (δ(C) 51.5), C(9) (50.0), and C(13) (62.4)) [10], implying that **1** was the *N*-oxide derivative of **3**. Natural cermizine D, originally isolated by *Kobayashi* and co-workers from *L. cernuum*, is a cernuane type *Lycopodium* alkaloid exhibiting cytotoxicity against murine lymphoma L1210 cells ($IC_{50} = 7.5$ μg/ml) [5]. To clarify the absolute configuration of **3**, several groups accomplished its asymmetric total synthesis [10–12]. Although ^{1}H- and ^{13}C-NMR data of synthetic **3** were in good agreement with those reported for the natural product, its optical rotation showed an opposite sign (synthetic **3** · CF_3COOH salt, $[\alpha]_D^{20} = +24.2$ ($c = 0.50$, MeOH); natural **3**, $[\alpha]_D^{25} = -33$ ($c = 0.60$, MeOH)) [10]. Consequently, the absolute configuration of natural **3** still remained a question. In the present study, **3** was isolated from *L. obscurum*, and its spectroscopic properties, particularly the optical rotation (**3** · CF_3COOH salt, $[\alpha]_D^{25} = +22.9$ ($c = 0.30$, MeOH)), were in agreement with those of synthetic dextrorotatory **3** with established absolute configuration. Oxidation of **3** with *m*-chloroperbenzoic acid (*m*-CPBA) afforded an *N*-oxide derivative (*Fig. 2*), whose spectroscopic data and $[\alpha]_D$ value were identical with those of natural **1**. Therefore, **1** was elucidated to be (+)-cermizine D *N*-oxide.

Compound **2** was obtained as a colorless solid. Its molecular formula was established as $C_{18}H_{27}NO_5$ by positive-ion-mode HR-ESI-MS (*m*/*z* 338.1968 ($[M + H]^+$)). The ^{1}H- and ^{13}C-NMR data (*Table*) revealed the presence of 18 C-atoms, including four sp^3 CH, eight sp^3 CH_2, four quaternary C-atoms, and two Me groups, indicating that **2** has a similar structure to that of obscurumine A (=(1*S*,8a*S*,9*S*,11-*R*,12a*S*)-dodecahydro-11-methyl-1,9-ethanobenzo[*i*]quinolizin-14-one). The major difference consisted in **2** having one more AcO group than obscurumine A [4]. The constitutional formula of **2** was elucidated by extensive analyses of ^{1}H,^{1}H-COSY,

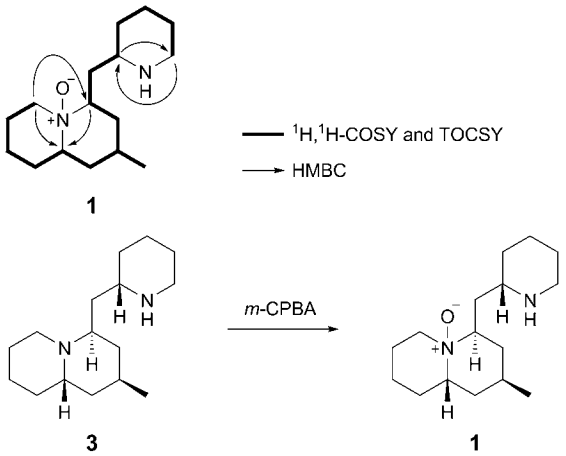

Fig. 2. *Key 2D-NMR correlations of* **1***, and chemical correlation of* **1** *and* **3**

HSQC, and HMBC data. The ^{1}H,^{1}H-COSY plot revealed the presence of three fragments (*Fig. 3*). The linkages of these partial structures were elucidated by the HMBCs CH_2(1)/C(9) and C(13), H–C(4)/C(5), CH_2(6)/C(4), C(5), C(7), and C(12), H–C(7)/C(11) and C(12), and CH_2(14)/C(12) and C(13) (*Fig. 3*). The HMBC plot also exhibited a cross-peak H–C(8)/Me*C*OO, indicating that the AcO group was positioned at C(8). The relative configuration of **2** was deduced by a ROESY experiment (*Fig. 3*). Thus, compound **2** was determined to be (8*β*)-8-(acetyloxy)obscurumine A.

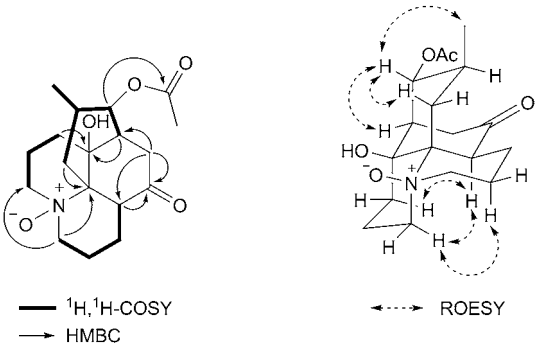

Fig. 3. *Key 2D-NMR correlations of* **2**

The AChE inhibitory activities of compounds **1**–**7** were evaluated by means of a modification of *Ellman*'s method [13]. However, all of them were found to be inactive ($IC_{50} > 200$ μM).

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

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	3.06[a]), 2.58 (*td*, J = 12.5, 2.5)	46.8 (*t*)	3.59 (*td*, J = 13.9, 4.7), 2.91 (*dd*, J = 12.1, 3.3)	63.4 (*t*)
CH_2(2)	1.55 – 1.60), 1.35 – 1.40 (2*m*)	26.9 (*t*)	1.90 – 1.96, 1.84 – 1.88 (2*m*)	21.2 (*t*)
CH_2(3)	1.76 – 1.81, 1.35 – 1.43 (2*m*)	24.8 (*t*)	2.20 – 2.25, 1.65 – 1.75 (2*m*)	17.5 (*t*)
CH_2(4) or H–C(4)	1.61 – 1.66, 1.15 – 1.20 (2*m*)	34.3 (*t*)	2.98 (*d*, J = 10.0)	49.7 (*d*)
H–C(5) or C(5)	2.54 (*td*, J = 15.0, 5.0)	53.8 (*d*)		205.9 (*s*)
CH_2(6)	1.77 – 1.83, 1.67 – 1.72 (2*m*)	35.5 (*t*)	2.70 (*d*, J = 16.0), 2.38 (*dd*, J = 10.0, 3.0)	37.8 (*t*)
H–C(7)	3.68 – 3.73 (*m*)	59.8 (*d*)	2.39 – 2.44 (*m*)	43.9 (*d*)
CH_2(8) or H–C(8)	1.76 – 1.82, 1.61 – 1.67 (2*m*)	34.5 (*t*)	5.39 (*dd*, J = 11.0, 4.3)	75.3 (*t*)
CH_2(9)	3.84 (br. *d*, J = 13.5), 3.06[a])	65.8 (*t*)	4.06 (*td*, J = 12.8, 3.5), 3.10 (*dd*, J = 12.3, 4.6)	59.7 (*t*)
CH_2(10)	1.70 – 1.76, 1.62 – 1.68 (2*m*)	23.1 (*t*)	2.90 – 3.00, 1.78 – 1.86 (2*m*)	16.0 (*t*)
CH_2(11)	1.77 – 1.85, 1.45 – 1.50 (*m*)	23.4 (*t*)	2.24 (*td*, J = 13.5, 4.4), 1.70 – 1.80 (*m*)	29.6 (*t*)
CH_2(12) or C(12)	2.02 – 2.10, 1.60 – 1.65 (2*m*)	28.0 (*t*)		73.1 (*s*)
H–C(13) or C(13)	3.34 (br. *d*, J = 13.0)	75.0 (*d*)		72.7 (*s*)
CH_2(14)	2.41 – 2.48 (*m*), 1.29 (*d*, J = 14.5)	33.8 (*t*)	3.00 (*t*, J = 10.0), 2.00 (*dd*, J = 13.6, 5.4)	28.6 (*t*)
H–C(15)	1.78 – 1.84 (*m*)	24.6 (*d*)	1.47 – 1.56 (*m*)	29.7 (*d*)
Me(16)	0.94 (*d*, J = 6.0)	21.8 (*q*)	0.98 (*d*, J = 6.1)	18.8 (*q*)
AcO			2.04 (*s*, 3 H)	21.1 (*q*), 170.1 (*s*)

[a]) Overlapped signals.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 300–400 mesh; *Qingdao Marine Chemical Group Co.*, Qingdao, P. R. China); *RP-18* (150–200 μm; *Merck*). Optical rotation: *Jasco-P-1020* polarimeter. IR Spectra: *Bruker-Tensor-27* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker-ACF-500* instrument; at 500 (^1H) and 125 MHz (^{13}C); in $CDCl_3$ or CD_3OD; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Agilent-Micro-Q-TOF* mass spectrometer; in *m*/*z*.

Plant Material. The whole plants of *L. obscurum* were purchased in Heilongjiang Province, China, in January 2011. The botanical identification was made by one of the authors, *J.-G. L.* A voucher specimen was deposited with the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The air-dried whole plants of *L. obscurum* (4 kg) were extracted with MeOH three times at r.t. The residue fo the MeOH extract was dissolved in 3% tartaric acid and the soln. filtered and then extracted with AcOEt. The aq. layer was adjusted to pH 10 with Na_2CO_3, and extracted by $CHCl_3$. The $CHCl_3$ extract was concentrated to give a crude alkaloid-containing residue (3 g), which was subjected to CC (*RP-18*, MeOH/H_2O 0:1, 3:7, 1:1, 1:0): *Fractions A–D. Fr. D* was further subjected to CC (SiO_2, $CHCl_3$ sat. with $NH_3 \cdot H_2O$/MeOH 1:0 → 0:1): **1** (5 mg) and **3** (15 mg). *Frs. A* and *B* were combined (TLC analysis) and subjected to repeated CC (SiO_2, $CHCl_3$/MeOH 1:0 → 1:1): **2** (5 mg), **4** (3 mg), **5** (5 mg), **6** (3 mg), and **7** (3 mg).

(+)-Cermizine D N-*Oxide* (= *(2S,4R,9aS)-Octahydro-2-methyl-4-[(2*S*)-piperidin-2-ylmethyl]-2*H-*quinolizine 5-Oxide*; **1**): Pale yellow oil. $[\alpha]_D^{25} = +8.6$ ($c = 0.30$, $CHCl_3$). IR (KBr): 3445, 2926, 2853, 1645, 1456, 695. ^1H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 267 ($[M + H]^+$). HR-ESI-MS: 267.2431 ($[M + H]^+$, $C_{16}H_{31}N_2O^+$; calc. 267.2431).

(8β)-8-(Acetyloxy)obscurumine A (= (rel-*(1*R*,8a*R*,9*S*,10*S*,11*R*,12a*R*)-10-(Acetyloxy)-dodecahydro-8a-hydroxy-11-methyl-1,9-ethanobenzo[i]quinolizin-14-one 5-Oxide*; **2**): Colorless solid. $[\alpha]_D^{25} = +13$ ($c = 0.20$, $CHCl_3$). IR (KBr): 3444, 2924, 1729, 1245. ^1H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 338 ($[M + H]^+$). HR-ESI-MS: 338.1968 ($[M + H]^+$, $C_{18}H_{28}NO_5$; calc. 338.1961).

Chemical Correlation of **3** *with* **1**. To a stirred soln. of **3** (3.2 mg, 0.0128 mmol) in dry CH_2Cl_2 (0.6 ml) was added *m*-CPBA (85%, 3.0 mg). After 2 h at 0°, the mixture was directly subjected to CC (Al_2O_3, $CHCl_3$/MeOH 1:0 → 0:1): semi-synthetic **1** (1.2 mg). Spectroscopic data and $[\alpha]_D$ value: in agreement with those of natural **1**.

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