Lycopodium Alkaloids from *Lycopodium obscurum* L.

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Two new *Lycopodium* alkaloids, named obscurumines F and G (**1** and **2**, resp.), together with eleven known alkaloids, were isolated from the club moss *Lycopodium obscurum* L. Their structures were elucidated on the basis of spectroscopic analyses.

Introduction. – Lycopodiaceae are known as a rich source of *Lycopodium* alkaloids with unique heterocyclic ring systems of $C_{11}N$, $C_{16}N$, $C_{16}N_2$, and $C_{27}N_3$ type and diverse biological activities such as acetylcholinesterase inhibitory activity [1]. The fascinating properties of this genus are still attracting great interest from chemists and biologists in search for novel bioactive compounds. *Lycopodium obscurum* is a traditional Chinese medicine which has been used for many years for the treatment of contusion, dysmenorrhea, quadriplegia, and arthritic pain [2]. Several new alkaloids with lycopodine- and fawcettimine-related structures have been isolated recently from this plant [3–5]. In the course of our efforts to discover further *Lycopodium* alkaloids in this plant, we have purified and identified 13 alkaloids, **1**–**13**, with lycopodine-, lycodine-, fawcettimine-related skeletons (*Fig. 1*). Among all isolated alkaloids, two new alkaloids, named obscurumines F and G (**1** and **2**, resp.) were identified by 1D- and 2D-NMR techniques, and HR-EI-MS analysis. Herein, we report the isolation and structure elucidation of these new compounds.

Result and Discussion. – Compound **1** was obtained as white powder, its molecular formula was determined as $C_{18}H_{25}NO_3$ by HR-EI-MS (*m*/*z* 303.1843; calc. 303.1834). The NMR spectra of **1** showed the signals of an AcO group at δ(C) 169.9 and 20.8, and δ(H) 1.96 (*s*, 3 H). Further, the ^{13}C-NMR spectrum of **1** (*Table 1*) exhibited 16 signals including those of three quaternary C-atoms (one ketone, one olefinic, and one sp^3 quaternary C-atom), of five CH (one olefinic C-atom) and seven CH_2 groups, and of one Me group. In the EI-MS spectrum, the abundance of the fragment-ion peak at *m*/*z* 233 ($C_{14}H_{19}NO_2^+$, $[M-70]^+$) is higher than that of the fragment-ion peak at *m*/*z* 232 ($C_{14}H_{18}NO_2^+$, $[M-71]^+$). These data suggested the structure of a lycopodine-type alkaloid with a C(8)=O group and devoid of the usual bridgehead H-atom at C(12) [6]. Comparison of its ^{13}C-NMR data with those of acrifoline [7], disclosed that they had

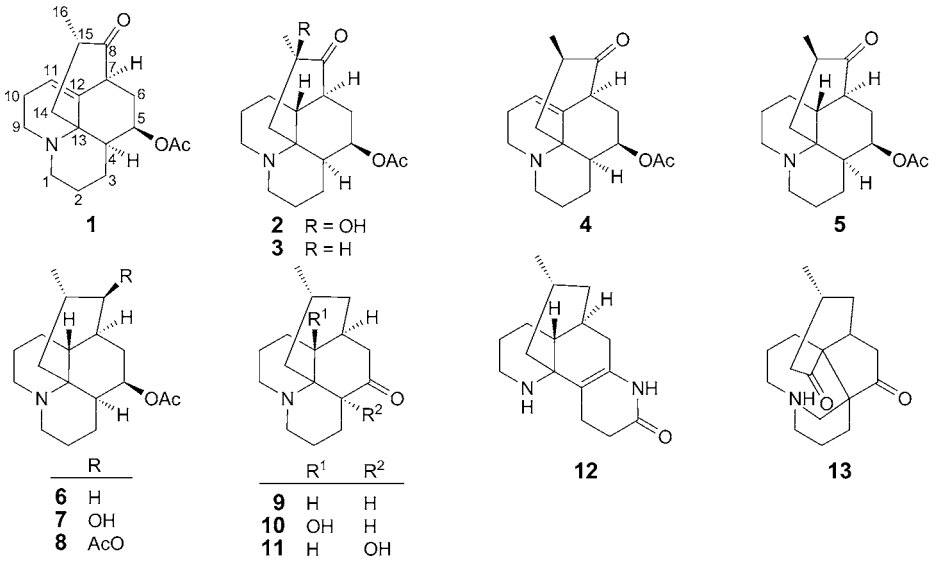

Fig. 1. *Structures of compounds* **1**–**13**

almost the same chemical shifts except for downfield shifts for C(5) and C(16) ($\Delta\delta$(C) = +2.4 and +7.2, resp.), and that **1** had an additional AcO group. Therefore, the constitution of **1** is the same as for acetylacrifoline [8]. There are two types of lycopodine alkaloids based on the configuration at C(15). Most of the lycopodine alkaloids have an α-Me group corresponding to a (15*S*)-configuration, as the lycoposerramines F–O. The ^{13}C-NMR signal of Me(16) appears at *ca.* 23 ppm [9]. So far, four alkaloids, acrifoline, acetylacrifoline, annofoline, and acetylannofoline, with a β-Me group corresponding to a (15*R*)-configuration have been isolated from Lycopodiaceae plants [10]. In comparison with those of (15*S*)-configurated lycopodine alkaloids, the upfield shifts is induced for Me(16) ($\Delta\delta$(C) $\approx$ –7.0) in (15*R*)-configurated lycopodine alkaloids [7], indicating that **1** should be the 15-*epi* form of acetylacrifoline. The relative configuration was further confirmed by an analysis of the ROESY spectrum (*Fig. 2*). From the above data, the structure of **1** was elucidated as depicted in *Fig. 1*, and it was given the trivial name obscurumine F.

Compound **2** was obtained as white powder. Its molecular formula was deduced as $C_{18}H_{27}NO_4$ from HR-EI-MS (*m*/*z* 321.1939; calc. 321.1940). By the ^{1}H- and ^{13}C-NMR data of **2**, one AcO group was identified and confirmed by the observation of

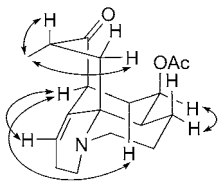

Fig. 2. *ROESY* (H ↔ H) *Correlations of* **1**

Table 1. *NMR Data of Compounds* **1** *and* **2**. In $CDCl_3$; δ in ppm, J in Hz.

Position	Obscurumine F (**1**)		Obscurumine G (**2**)	
	δ(H)	δ(C)	δ(H)	δ(C)
1	2.54–2.64 (*m*), 2.70–2.82 (*m*)	48.5	2.52–2.56 (*m*), 3.12 (*td*, *J* = 14.7, 3.0)	47.5
2	1.44–1.52 (*m*), 1.72–1.78 (*m*)	23.1	1.26 (br. *d*, *J* = 13.5), 1.88–1.92 (*m*)	18.7
3	1.58–1.64 (*m*)	25.7	1.36–1.40 (*m*), 1.50–1.60 (*m*)	22.8
4	1.98–2.02 (*m*)	46.9	2.34–2.42 (*m*)	31.0
5	4.91 (br. *d*, *J* = 2.4)	72.1	4.91(br. *d*, *J* = 3.1)	73.1
6	1.86–1.92 (*m*), 2.26–2.34 (*m*)	37.3	1.90–2.00 (*m*)	30.2
7	2.96–3.04 (*m*)	50.7	2.38–2.44 (*m*)	44.9
8		217.0		211.1
9	2.60–2.72 (*m*)	45.0	2.46–2.54 (*m*), 3.04–3.14 (*m*)	46.3
10	2.04–2.06 (*m*), 2.40–2.42 (*m*)	26.0	1.64–1.80 (*m*)	25.4
11	5.60 (*dd*, *J* = 5.4, 2.4)	119.8	1.36–1.44 (*m*), 1.62–1.68 (*m*)	23.2
12		138.5	3.04–3.10 (*m*)	35.5
13		56.4		54.2
14	1.42–1.48 (*m*), 2.50–2.62 (*m*)	29.1	2.64 (*d*, *J* = 15.3), 2.02 (*d*, *J* = 15.3)	44.5
15	2.64–2.70 (*m*)	42.1		73.3
16	1.20 (*d*, *J* = 7.2)	22.8	1.41 (*s*)	26.0
AcO	1.96 (*s*)	20.8, 169.9	1.94 (*s*)	21.4, 169.9

a H-atom signal at δ(H) 1.94 (*s*, 3 H), and two C-atom signals at δ(C) 21.4 and 169.9. Further, the ^{13}C-NMR and DEPT data of **2** exhibited 16 additional C-atom signals, including those of a ketone C=O group, two sp^3 quaternary C-atoms, four CH and eight CH_2 groups, and one Me group. On the basis of the NMR data discussed above, compound **2** can be considered as a lycopodine alkaloid. By comparison of the NMR data with those of obscurumine B [3], it was found that the major differences were the absence of the signals at δ(H) 1.28 (*d*, *J* = 7.7, Me) and at δ(C) 40.2 (C(15)) and the appearance of the signal δ(H) 1.41 (s, 3 H) and that of one oxygenated quaternary C-atom at δ(C) 73.3. Furthermore, the ^{13}C-NMR signals of C(14) (δ(C) 44.5), C(15) (δ(C) 73.3), and C(16) (δ(C) 26.0) in **2** were shifted downfield compared with those of C(14) (δ(C) 38.1), C(15) (δ(C) 40.2), and C(16) (δ(C) 22.5) of obscurumine B, suggesting that **2** should be 15-hydroxyobscurumine B, which was confirmed by the HMBC correlations of Me(16) with C(15) (δ(C) 73.3) and C(8) (δ(C) 211.1). The relative configuration of **2** was deduced from the ROESY spectrum (*Fig. 3*). In the ROESY spectrum of **2**, correlations from H–C(5) to H_α–C(4) and H_α–C(3), and from Me (16) to H–C(12) and H_α–C(14) were observed, indicating that both AcO–C(5) and

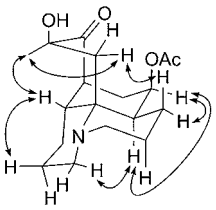

Fig. 3. *ROESY* (H ↔ H) *Correlations of* **2**

Table 2. *^{13}C-NMR Data of Compounds* **4**–**8**. In $CDCl_3$.

Position	**4**	**5**	**6**	**7**	**8**
1	48.8 (*t*)	47.7 (*t*)	46.9 (*t*)	47.2 (*t*)	47.3 (*t*)
2	24.0 (*t*)	18.4 (*t*)	20.0 (*t*)	19.6 (*t*)	19.8 (*t*)
3	23.0 (*t*)	22.3 (*t*)	22.7 (*t*)	22.4 (*t*)	22.7 (*t*)
4	46.3 (*d*)	32.0 (*d*)	31.1 (*d*)	30.8 (*d*)	30.7 (*d*)
5	72.2 (*d*)	72.1 (*d*)	69.8 (*d*)	69.4 (*d*)	69.7 (*d*)
6	36.0 (*t*)	29.4 (*t*)	30.9 (*t*)	24.1 (*t*)	24.6 (*t*)
7	50.5 (*d*)	45.9 (*d*)	34.8 (*d*)	41.2 (*d*)	37.3 (*d*)
8	217.0 (*s*)	215.5 (*s*)	41.4 (*t*)	78.3 (*d*)	80.5 (*d*)
9	45.2 (*t*)	46.5 (*t*)	46.8 (*t*)	46.5 (*t*)	46.6 (*t*)
10	26.0 (*t*)	24.2 (*t*)	25.7 (*t*)	25.8 (*t*)	26.0 (*t*)
11	119.0 (*d*)	22.2 (*t*)	24.2 (*t*)	24.1 (*t*)	23.9 (*t*)
12	138.0 (*s*)	37.9 (*d*)	44.8 (*d*)	43.2 (*d*)	42.8 (*d*)
13	58.0 (*s*)	58.0 (*s*)	55.7 (*s*)	54.8 (*s*)	54.1 (*s*)
14	30.5 (*t*)	36.3 (*t*)	42.0 (*t*)	41.0 (*t*)	41.2 (*t*)
15	43.2 (*d*)	40.0 (*d*)	23.4 (*d*)	31.5 (*d*)	28.7 (*d*)
16	14.3 (*q*)	14.4 (*q*)	24.1(*q*)	20.7 (*q*)	20.4 (*q*)
5-AcO	21.2 (*q*), 170.0 (*s*)	21.2 (*q*), 169.8 (*s*)	21.5 (*q*), 170.5 (*s*)	21.3 (*q*), 170.4 (*s*)	21.0 (*q*), 170.7 (*s*)
8-AcO					21.3 (*q*), 170.2 (*s*)

OH at (15) are β-oriented. Therefore, the structure of **2** was elucidated as depicted in *Fig. 1*, named obscurumine G.

The eleven known alkaloids were identified as obscurumine B (**3**) [3], acetylacrifoline (**4**) [8], acetylannofoline (**5**) [8], acetyldihydrolycopodine (**6**) [11], fawcettiine (**7**) [11], acetylfawcettine (**8**) [11], lycopodine (**9**) [12], lycodoline (**10**) [12], flabelliformine (**11**) [12], des-*N*-methyl-α-obscurine (**12**) [13], and lycoflexine (**13**) [14][15] by comparison of the spectroscopic data with those reported in the literature. However, as ^{13}C-NMR data of **4**–**8** were not available in the literature, they are included in *Table 2*. The yet undetermined assignment of all C-atoms was accomplished by 2D-NMR techniques. These alkaloids were also evaluated for their *in vitro* cytotoxicities against KB cell lines using MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay. All of them showed an IC_{50} values higher than 50 μM and were, therefore, considered inactive against KB cell lines.

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Experimental Part

General. TLC: Precoated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Co., Ltd.*, P. R. China). Column Chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, P. R. China) and C_{18} reversed-phase (RP) silica gel (*YMC Co., Ltd.*, Japan). HPLC: *Ultimate 3000* HPLC system (*Dionex Co. California*, USA); *Ultimate 3000* pump; *Ultimate 3000* variable wavelength; column *Waters 5C_{18}-MS-II* (10 × 250 mm). Optical rotations: *P-1020* digital polarimeter in MeOH (*JASCO*; Tokyo, Japan) ^{1}H-, ^{13}C-, and 2D-NMR spectra: *Bruker-AM-400* instrument; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS and HR-EI-MS: *Finnigan-MAT-95* mass spectrometer (70 eV); in *m*/*z* (rel. %).

Plant Material. The club moss *Lycopodium obscurum* L. was collected in Jianshi County, Hubei Province, P. R. China, and identified by Prof. *Ding-Rong Wan*, College of Pharmacy, South Central University for Nationalities. The voucher specimen was deposited with the herbarium of College of Pharmacy, South Central University for Nationalities, P. R. China.

Extraction and Isolation. The air-dried whole plants of *L. obscurum* L. (12.5 kg) were extracted with MeOH three times. The combined MeOH extract (1,650 g) was dissolved in 3% aq. tartaric acid soln., and then partitioned with AcOEt. The aq. layer was adjusted to pH 10 with sat. aq. Na_2CO_3 soln., and extracted with AcOEt. The AcOEt extract was concentrated *in vacuo* to give a crude alkaloid residue (36 g), which was subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0, 99:1, 95:5, 9:1, 8:2, 1:1) to give seven fractions, *Fr. 1 – Fr. 7*. Compound **9** (46 mg) was crystallized from *Fr. 1*. *Fr. 2* (1.77 g) was purified by CC (SiO_2; cyclohexane/$NH_2CH_2CH_2NH_2$ 200:1, 400:3) and was further purified by prep. HPLC (MeOH/H_2O 6:4): **1** (4 mg) at t_R 16.89 min and **4** (5 mg) at t_R 18.78 min. *Fr. 3* (0.72 g) was submitted to CC (SiO_2; petroleum ether (PE) $NH_2CH_2CH_2NH_2$ 100:1 → 100:10) to give compound **3** (23 mg). *Fr. 4* (0.77 g) was purified by CC (*ODS*; H_2O/MeOH 7:3 → 0:1) to give two major fraction, *Fr. 4-A* and *4-B*. *Fr. 4-A* was further purified by prep. HPLC (MeOH/H_2O 6:4) to give **5** (29 mg) at t_R 9.54 min, and *Fr. 4-B* was further purified by prep. HPLC (MeOH/H_2O 1:1) to give **13** (8 mg) at t_R 29.6 min. *Fr. 5* (2.5 g) was subjected to repeated CC (SiO_2; cyclohexane/$NH_2CH_2CH_2NH_2$ 200:1 → 200:20) and to prep. HPLC to give compound **8** (50 mg), **12** (35 mg). *Fr. 6* (3.4 g) was purified by repeated CC (SiO_2; cyclohexane/$NH_2CH_2CH_2NH_2$ 100:1 → 100:5) and prep. HPLC to give compound **2** (12 mg), **6** (32 mg), **10** (8 mg), and **11** (35 mg). *Fr. 7* (3.5 g) was submitted to repeated CC (SiO_2; cyclohexane/$NH_2CH_2CH_2NH_2$ 200:1 → 200:10) to give compound **7** (110 mg).

Obscurumine F (=*(5β,15*S*)-15-Methyl-8-oxolycopod-11-en-5-yl Acetate*; **1**). White powder. $[\alpha]_D^{20} = -209^\circ$ ($c = 0.20$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS (70 eV): 303 (6), 275 (61), 233 (73), 231 (9), 173 (100). HR-EI-MS: 303.1843 ($C_{18}H_{25}NO_3^+$; calc. 303.1834).

Obscurumine G (=*(5β,15*R*)-15-Hydroxy-15-methyl-8-oxolycopodan-5-yl Acetate*; **2**). White powder. $[\alpha]_D^{20} = -83.8^\circ$ ($c = 0.60$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS (70 eV): 321 (9), 304 (5), 278 (26), 234 (100), 174 (89). HR-EI-MS: 321.1939 ($C_{18}H_{27}NO_4^+$; calc. 321.1940).

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