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LYCOPLADINE E, A NEW $C_{16}N_1$-TYPE ALKALOID FROM *LYCOPODIUM COMPLANATUM*

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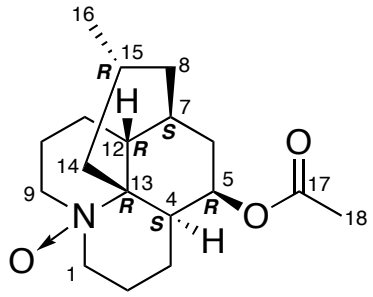
Abstract - A new $C_{16}N_1$-type alkaloid, lycopladine E (**1**), has been isolated from the club moss *Lycopodium complanatum*, and the structure and absolute stereochemistry were elucidated on the basis of spectroscopic data and chemical correlation.

INTRODUCTION

Club moss (Lycopodiaceae) are known to be a rich source of *Lycopodium* alkaloids possessing unique heterocyclic ring systems such as $C_{16}N_1$, $C_{16}N_2$, and $C_{27}N_3$, which have attracted great interest from biogenetic, synthetic, and biological points of view.[1] In our continuing efforts to find new *Lycopodium* alkaloids,[2] a new $C_{16}N_1$-type alkaloid, lycopladine E (**1**), was isolated from the club moss *Lycopodium complanatum*. In this paper, we describe the isolation and structure elucidation of **1**.



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This paper is dedicated to Professor Dr. Ekkehard Winterfeldt on the occasion of his 75th birthday.

RESULTS AND DISCUSSION

The club moss *L. complanatum* collected in Nayoro, Hokkaido, were extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 10 with saturated Na_2CO_3, were partitioned with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, then $CHCl_3$/MeOH), in which a fraction eluted with hexane/EtOAc (10:1) was purified by LH-20 (MeOH) and silica gel ($CHCl_3$/MeOH) columns to afford lycopladine E (**1**, 0.000008% yield), lycopodine (**2**),[3] lycodine,[4] lycopladines A~D,[5] complanadines A, C, and D,[6] and lyconadines A and B.[5b, 7]

Lycopladine E {**1**, $[\alpha]_D^{23}$ -28.8 (*c* 0.1, MeOH)} was revealed to have the molecular formula, $C_{18}H_{30}N_1O_3$, by HRESIMS data [*m*/*z* 308.2204, $(M+H)^+$, Δ -2.1 mmu]. IR absorptions implied the presence of a

Table 1. ^1H and ^{13}C NMR Data of Lycopladine E (**1**) in CD_3OD.

Position	δ_H	δ_C		Position	δ_H	δ_C	
1a	3.64 (1H, m)	64.6	t	9a	4.27 (1H, dt, 12.8, 3.2)	60.6	t
1b	2.99 (1H, dd, 13.2, 4.8)			9b	2.85 (1H, brd, 12.7)		
2a	2.00 (1H, m)	22.9	t	10a	2.37 (1H, m)	20.9	t
2b	1.83 (1H, m)			10b	1.25 (1H, m)		
3a	1.83 (1H, m)	21.9	t	11a	1.83 (1H, m)	23.1	t
3b	1.45 (1H, m)			11b	1.38 (1H, brd, 12.0)		
4	2.92 (1H, m)	36.7	d	12	2.23 (1H, m)	39.2	d
5	5.22 (1H, t, 6.8)	70.4	d	13		75.0	s
6a	2.25 (1H, m)	30.9	t	14	2.15 (2H, m)	33.5	t
6b	1.49 (1H, d, 16.4)			15	2.80 (1H, m)	24.9	d
7	1.88 (1H, m)	36.7	d	16	0.99 (3H, d, 6.3)	24.4	q
8a	1.68 (1H, m)	41.9	t	17		170.6	s
8b	1.25 (1H, m)			18	2.04 (3H, s)	21.1	q

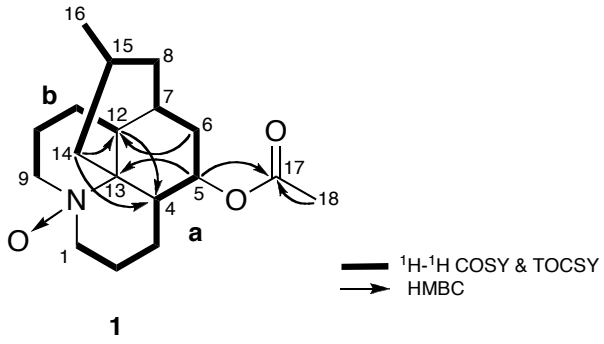

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Figure 1. Selected 2D NMR correlations for lycopladine E (**1**).

carbonyl group (1730 cm^{-1}). The ^{1}H and ^{13}C NMR (Table 1) spectra of **1** showed signals due to one sp^2 quaternary carbon, one sp^3 quaternary carbon, five sp^3 methines, nine sp^3 methylenes, and two methyls. Among them, one methine (δ_C 75.0) and two methylenes (δ_C 64.6, and 60.6) were ascribed to those bearing a nitrogen atom.

The ^{1}H-^{1}H COSY and TOCSY spectra revealed two structural units **a** (C-1 to C-8, C-14 to C-16, and C-8 to C-15) and **b** (C-9 to C-12). HMBC correlations of H-5 (δ_H 5.22) to C-13 (δ_C 75.0), H-6b (δ_H 1.49) to C-12 (δ_C 39.2), H-12 (δ_H 2.23) to C-4 (δ_C 36.7), and H_2-14 (δ_H 2.15) to C-4 (δ_C 36.7) and C-12 (δ_C 39.2) suggested connectivities among C-4, C-12, and C-14 via C-13. HMBC cross-peaks of an oxymethine proton H-5 (δ_H 5.22) and a singlet methyl proton H_3-18 (δ_H 2.04) to an ester carbonyl carbon C-17 (δ_C 170.6) revealed that an acetoxy group was attached to C-5. ^{1}H and ^{13}C NMR data suggested connections of C-1, C-9, and C-13 via the remaining oxygenated nitrogen atom. Thus, the gross structure of lycopladine E was elucidated to be **1**.

The relative stereochemistry of **1** was deduced from cross-peaks observed in the NOESY spectrum as shown in computer-generated 3D drawing (Figure 2). These NOESY correlations indicated the relative stereochemistry of C-5 and C-15 as well as chair conformations of the two six-membered rings in the trans quinolizidine ring (N-1, C-1 to C-4, and C9 to C-13) and two cyclohexane rings in bicyclo[3.3.1]nonane moiety (C-4 to C-8 and C-12 to 15).

The absolute configuration of lycopladine E (**1**) was elucidated by chemical correlation as follows. Lycopodine (**2**)[3] was converted into dihydrolycopodine (**3**)[8,9] by the reported procedure[9], which was

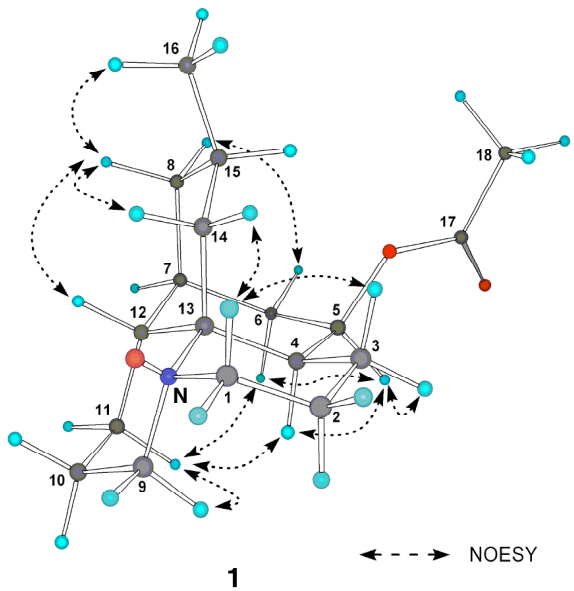
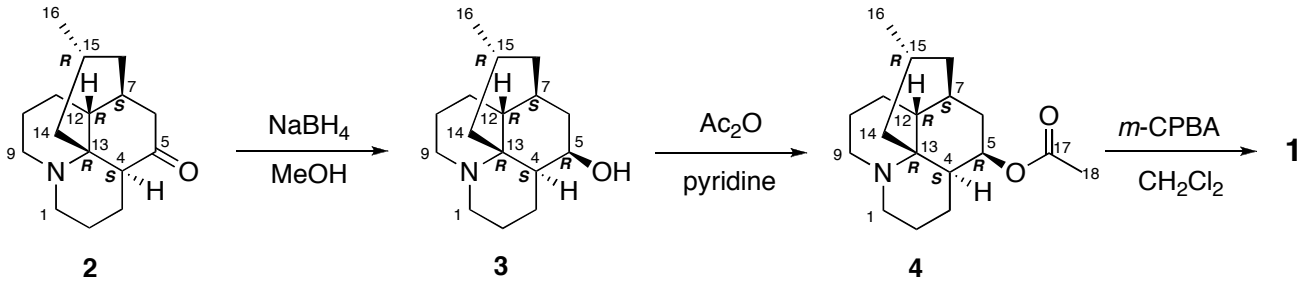


Figure 2. Selected NOESY correlations and relative stereochemistry for lycopladine E (**1**).



Scheme 1. Chemical conversion of lycopodine (**2**) to lycopladine E (**1**).

treated with pyridine and acetic anhydride to afford 5-*O*-acetyldihydrolycopodine (**4**)[10]. Compound **4** was oxidized by *m*-CPBA to give compound **1** as a single product (Scheme 1), whose spectral data and $[\alpha]_D$ value {$[\alpha]_D^{23}$ -29.4 (*c* 0.5, MeOH)} were coincident with those of natural lycopladine E (**1**). Thus, the absolute configurations at six chiral centers in lycopladine E (**1**) were established to be *4S, 5R, 7S, 12R, 13R,* and *15R*.

Lycopladine E (**1**) is a new $C_{16}N$-type alkaloid having a lycopodane-skeleton with N-oxide and acetoxy group at C-5. Effects of lycopladine E (**1**) on neurotrophic factor biosynthesis in 1321N1 human astrocytoma cells were examined by a semiquantitative RT-PCR method[11,12] to find that the mRNA expressions for NGF were enhanced by **1**.

EXPERIMENTAL

General Experimental Procedures

Optical rotation was recorded on a JASCO P-1030 polarimeter. IR spectrum was recorded on a JASCO FT/IR-230 spectrometer. NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells (Shigemi Co., Ltd). The 3.31 and 49.0 ppm resonances of residual CD_3OD were used as internal references for ^1H and ^{13}C NMR spectra, respectively. Positive-mode ESIMS was obtained on a JEOL JMS 700-TZ spectrometer using a sample dissolved in MeOH.

Plant Material

The club moss *Lycopodium complanatum* was collected at Nayoro in Hokkaido in 2001. The botanical identification was made by Mr. N. Yoshida, Health Sciences University of Hokkaido. A voucher specimen has been deposited in the herbarium of Hokkaido University.

Extraction and Isolation

The club moss *Lycopodium complanatum* (3 kg) was crushed and extracted with MeOH. The MeOH extract was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was

treated with saturated Na_2CO_3 (aq) to pH 10 and extracted with $CHCl_3$ to give a crude alkaloidal fraction. A part of the alkaloidal fraction was purified by an amino silica gel column (hexane/EtOAc, 50:1→1:1 and then $CHCl_3$/MeOH, 1:0→0:1), in which a fraction eluted with hexane/EtOAc (10:1) was purified by LH-20 ($CHCl_3$/MeOH, 1:1) and silica gel columns ($CHCl_3$/MeOH, 1:0→1:1) to afford lycopladine E (**1**, 0.2 mg, 0.000008% yield), lycopodine (**2**),[3] lycodine,[4] lycopladines A~D,[5] complanadines A, C, and D,[6] and lyconadines A and B[5b, 7]

Lycopladine E (1): A colorless amorphous solid; $[\alpha]_D^{23}$ -28.8 (*c* 0.1, MeOH); IR (KBr) ν_{max} 1730 and 1230 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 308 $(M+H)^+$; HRESIMS *m*/*z* 308.2204 (M+H; calcd for $C_{18}H_{30}N_1O_3$, 308.2225).

Lycopladine E (1) derived from lycopodine (2): Lycopodine (**2**, 19.0 mg, 0.077 mmol) was converted to dihydrolycopodine (**3**, 11.0 mg) by the reported procedure, part of which (8.7 mg) was treated with pyridine (1.0 mL) and acetic anhydride (1.0 mL) at 90 °C for 5 h. The reaction mixture was evaporated in vacuo to give 5-*O*-acetyldihydrolycopodine (**4**, 14.2 mg), part of which (12.8 mg) was treated with CH_2Cl_2 (1.0 mL) and *m*-CPBA (14.2 mg, 0.082 mmol) at 4 °C for 1 h. The reaction mixture was partitioned between $CHCl_3$ and saturated Na_2CO_3 (aq), and the $CHCl_3$ layer was evaporated in vacuo. The residue was purified by a silica gel column ($CHCl_3$/MeOH, 1:0→1:1) to afford lycopladine E (**1**, 3.16 mg, 0.010 mmol).

ACKNOWLEDGEMENTS

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