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LYCOPLADINE E, A NEW C₁₆N₁-TYPE ALKALOID FROM *LYCOPODIUM COMPLANATUM*

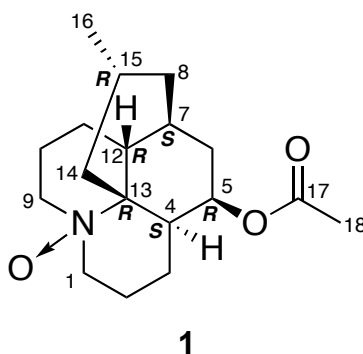
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Abstract - A new C₁₆N₁-type alkaloid, lycopladine E (**1**), has been isolated from the club moss *Lycopodium complanatum*, and the structure and absolute stereochemistry were elucidated on the basis of spectroscopic data and chemical correlation.

INTRODUCTION

Club moss (Lycopodiaceae) are known to be a rich source of *Lycopodium* alkaloids possessing unique heterocyclic ring systems such as C₁₆N₁, C₁₆N₂, and C₂₇N₃, 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 C₁₆N₁-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**.



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 Na₂CO₃, were partitioned with CHCl₃. CHCl₃-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, then CHCl₃/MeOH), in which a fraction eluted with hexane/EtOAc (10:1) was purified by LH-20 (MeOH) and silica gel (CHCl₃/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**, [α]_D²³ -28.8 (*c* 0.1, MeOH)} was revealed to have the molecular formula, C₁₈H₃₀N₁O₃, by HRESIMS data [*m/z* 308.2204, (M+H)⁺, Δ -2.1 mmu]. IR absorptions implied the presence of a

Table 1. ¹H and ¹³C NMR Data of Lycopladine E (**1**) in CD₃OD.

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

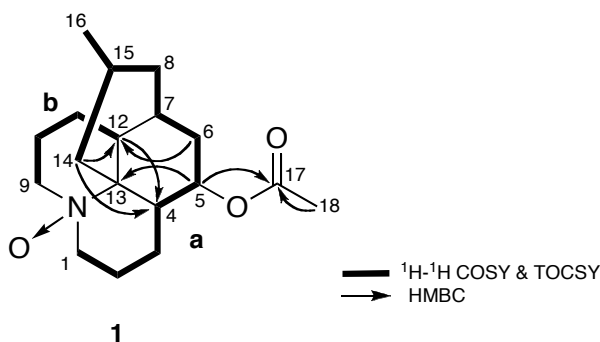


Figure 1. Selected 2D NMR correlations for lycopladine E (**1**).

carbonyl group (1730 cm^{-1}). The ^1H 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 ($\delta_{\text{C}} 75.0$) and two methylenes ($\delta_{\text{C}} 64.6$, and 60.6) were ascribed to those bearing a nitrogen atom.

The ^1H - ^1H 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 ($\delta_{\text{H}} 5.22$) to C-13 ($\delta_{\text{C}} 75.0$), H-6b ($\delta_{\text{H}} 1.49$) to C-12 ($\delta_{\text{C}} 39.2$), H-12 ($\delta_{\text{H}} 2.23$) to C-4 ($\delta_{\text{C}} 36.7$), and H_2 -14 ($\delta_{\text{H}} 2.15$) to C-4 ($\delta_{\text{C}} 36.7$) and C-12 ($\delta_{\text{C}} 39.2$) suggested connectivities among C-4, C-12, and C-14 via C-13. HMBC cross-peaks of an oxymethine proton H-5 ($\delta_{\text{H}} 5.22$) and a singlet methyl proton H_3 -18 ($\delta_{\text{H}} 2.04$) to an ester carbonyl carbon C-17 ($\delta_{\text{C}} 170.6$) revealed that an acetoxy group was attached to C-5. ^1H 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 lycopladiene 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 C-9 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 lycopladiene E (**1**) was elucidated by chemical correlation as follows. Lycopodine (**2**)³ was converted into dihydrolycopodine (**3**)^{8,9} by the reported procedure⁹, which was

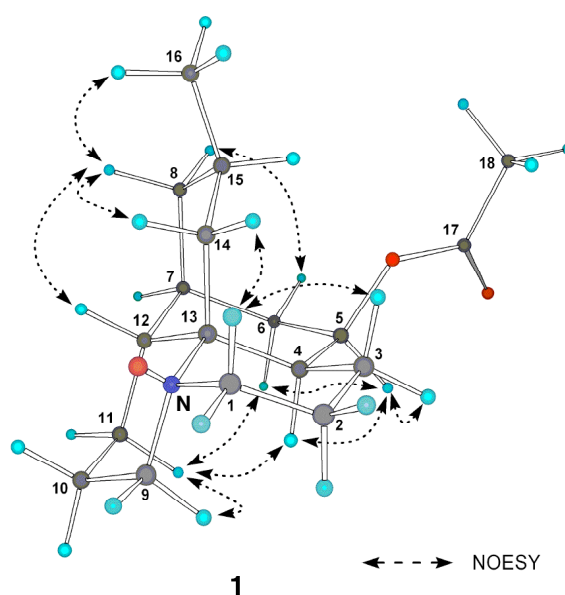
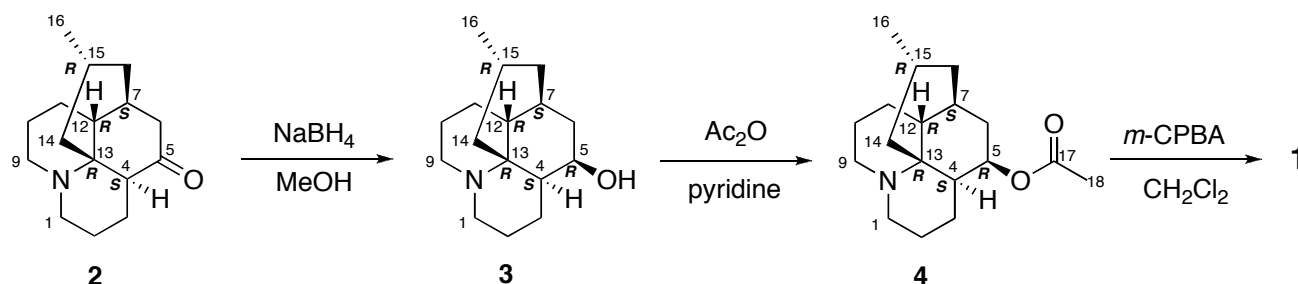


Figure 2. Selected NOESY correlations and relative stereochemistry for lycopladiene E (**1**).



Scheme 1. Chemical conversion of lycopodine (**2**) to lycoplamine 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 $\{[\alpha]_D^{23} -29.4 (c\ 0.5, \text{MeOH})\}$ were coincident with those of natural lycoplamine E (**1**). Thus, the absolute configurations at six chiral centers in lycoplamine E (**1**) were established to be *4S*, *5R*, *7S*, *12R*, *13R*, and *15R*.

Lycoplamine E (**1**) is a new C₁₆N-type alkaloid having a lycopodane-skeleton with N-oxide and acetoxo group at C-5. Effects of lycoplamine 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 CD₃OD were used as internal references for ¹H and ¹³C 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 $\rightarrow$ 1:1 and then CHCl_3 /MeOH, 1:0 $\rightarrow$ 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 $\rightarrow$ 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 ($\text{M}+\text{H}$)⁺; HRESIMS m/z 308.2204 ($\text{M}+\text{H}$; calcd for $\text{C}_{18}\text{H}_{30}\text{N}_1\text{O}_3$, 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 $\rightarrow$ 1:1) to afford lycopladine E (**1**, 3.16 mg, 0.010 mmol).

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