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LYCOVATINE A, a $C_{16}N$-TYPE QUATERNARY ALKALOID FROM *LYCOPODIUM CLAVATUM* VAR. *ROBUSTUM*

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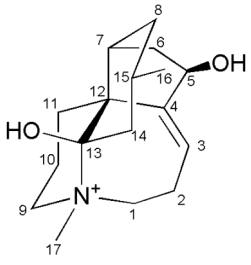
Abstract - A new $C_{16}N$-type quaternary alkaloid, lycovatine A (**1**), has been isolated from the club moss *Lycopodium clavatum* var. *robustum*, and the structure including the relative stereochemistry was elucidated on the basis of spectroscopic data.

INTRODUCTION

Club moss (Lycopodiaceae) are known to be a rich source of *Lycopodium* alkaloids possessing unique heterocyclic ring systems such as $C_{16}N$, $C_{16}N_2$, 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 *N*-methylated $C_{16}N$-type quaternary alkaloid, lycovatine A (**1**), was isolated from the club moss *Lycopodium clavatum* var. *robustum*. In this paper, we describe the isolation and structure elucidation of **1**.



lycovatine A (**1**)

RESULTS AND DISCUSSION

The club moss *L. clavatum* var. *robustum* collected at Nayoro in 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 purified by an amino silica gel column (hexane/EtOAc and then $CHCl_3$/MeOH) followed by C_{18} HPLC (CH_3CN/0.1% CF_3CO_2H, 15:85) to afford lycovatine A (**1**, 0.0002% yield) together with a known alkaloid, lycodoline [3].

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

Position	δ_H		δ_C	
1a	3.79	(1H, m)	59.5	t
1b	3.47	(1H, m)		
2a	3.12	(1H, m)	28.2	t
2b	2.63	(1H, m)		
3	6.07	(1H, d, 6.7)	125.9	d
4			149.1	s
5	4.65	(1H, d, 7.4)	75.6	d
6a	2.28	(1H, m)	38.3	t
6b	1.45	(1H, d, 14.7)		
7	2.20	(1H, m)	48.2	d
8a	1.65	(1H, m)	39.9	t
8b	1.53	(1H, m)		
9a	3.69	(1H, m)	59.4	t
9b	3.47	(1H, m)		
10a	2.17	(1H, m)	21.2	t
10b	1.75	(1H, m)		
11a	2.18	(1H, m)	28.7	t
11b	1.53	(1H, m)		
12			57.3	s
13			100.7	s
14a	2.06	(1H, brd)	36.3	t
14b	1.98	(1H, t, 11.4)		
15	1.98	(1H, m)	30.4	d
16	1.02	(3H, d, 5.6)	32.3	q
17	3.00	(3H, s)	51.3	q

Lycovatine A (**1**) was revealed to have the molecular formula, $C_{17}H_{28}N_1O_2$, by HRESIMS data [*m/z* 278.2120, $(M)^+$, ±0.0 mmu]. IR absorptions implied the presence of hydroxy group (3400 cm^{-1}). The ^1H and ^{13}C NMR (Table 1) spectra of **1** showed signals due to one sp^2 quaternary carbon, two sp^3 quaternary carbons, one sp^2 methine, three sp^3 methines, eight sp^3 methylenes, and two methyls. Among them, one methyl (δ_C 51.3) and two methylenes (δ_C 59.4, and 59.5) were ascribed to those bearing a nitrogen atom, while one sp^3 quaternary carbon (δ_C 100.7) was ascribed to that bearing an oxygen and a nitrogen atoms.

The ^1H-^1H COSY and HOHAHA spectra revealed connectivities of C-1 to C-3, C-5 to C-6, C-7 to C-8, C-14 to C-16, C-8 to C-15, and C-9 to C-11. These partial units were connected to each other on the basis of HMBC correlations of H-3 to C-5 and C-12, H-5 to C-7, H-6 to C-4 and C-12, and H-1, H-9, H-11, and H-14 to C-13. The presence of an *N*-methyl group (C-17) was revealed by HMBC correlations of H-17 to C-1, C-9 and C-13. Thus, the gross structure of lycovatine A was elucidated to be **1** (Figure 1).

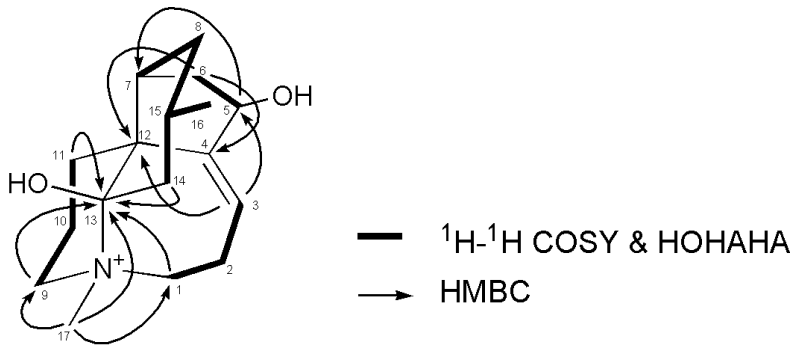


Figure 1. Selected 2D NMR correlations for lycovatine A (**1**)

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 forms of a cyclohexane (C-7 to C-8 and C-12 to C-15) and a piperidine (N-1 and C-9 to C-13) rings in the trans fused decahydroquinoline moiety.

The absolute configuration of lycovatine A (**1**) was elucidated by applying the modified Mosher's method.[4] To elucidate the absolute configuration at C-5, **1** was converted into its (*S*)- and (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) ester of the hydroxy group at C-5. The Δδ [δ(*S*-MTPA ester)-δ(*R*-MTPA ester)] values obtained from the ^1H NMR spectra of the MTPA esters suggested that the absolute configuration at C-5 was *R*.

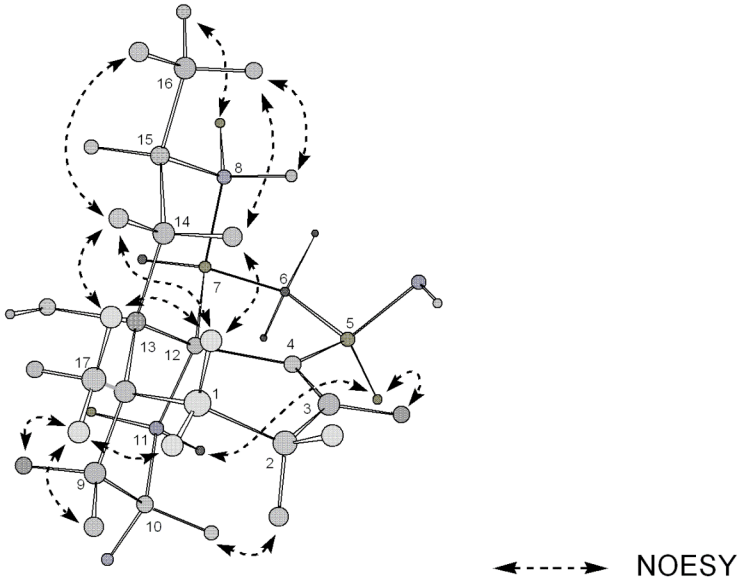
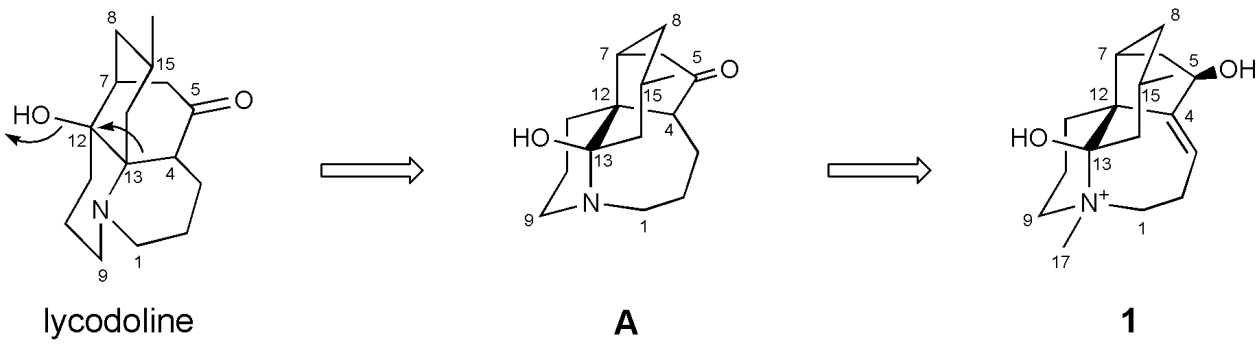


Figure 2. Selected NOESY correlations and relative stereochemistry for lycovatine A (**1**)

Lycovatine A (**1**) is a rare *N*-methyl fawcettidane-type[5] quaternary alkaloid from *Lycopodium clavatum* var. *robustum*, although some quaternary alkaloids such as senepodines B, D, and H[6, 7] from club moss (Lycopodiaceae) have been reported. A plausible biogenetic path of lycovatine A (**1**) was proposed as shown in Scheme 1. Biogenetically, lycovatine A (**1**) might be derived from lycodoline[3] through a fawcettidane-type intermediate **A**. Effects of lycovatine A (**1**) on neurotrophic factor biosynthesis in 1321N1 human astrocytoma cells were examined by a semiquantitative RT-PCR method[8, 9] to find that the mRNA expressions for NGF were enhanced by **1**. Lycovatine A (**1**) did not show cytotoxicity against murine leukemia L1210 cells and human epidermoid carcinoma KB cells (IC_{50} > 10 μg/mL). Lycovatine A (**1**) exhibited antimicrobial activity against *Cryptococcus neoformans* (MIC, 0.52 μg/ml) and *Aspergillus niger* (MIC, 2.05 μg/ml).



Scheme 1. Plausible biogenetic path of lycovatine A (**1**)

EXPERIMENTAL

General Experimental Procedures.

IR spectra were recorded on a JASCO FT/IR-230 and a Shimadzu UV-1600PC spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells (Shigemi Co., Ltd). The 3.31 and 49.5 ppm resonances of residual CD_3OD were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ spectrometer.

Plant Material.

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

Extraction and Isolation.

The club moss *Lycopodium clavatum* var. *robustum* (0.9 kg) was crushed and extracted with MeOH. A portion of 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 $CHCl_3$. The $CHCl_3$ soluble materials were subjexted to an amino silica gel column (hexane/EtOAc to $CHCl_3$/MeOH) followed by C18 HPLC (15% CH_3CN/0.1% CF_3CO_2H) to afford lycovatine A (**1**, 0.0002% yield) and lycodoline[3] (0.0006% yield).

Lycovatine A (1): colorless amorphous solid; $[\alpha]_D^{23}$ -8° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3420 and 2920 cm-1; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 278 $(M)^+$; HRESIMS *m*/*z* 278.212 (M; calcd for $C_{17}H_{28}N_1O_2$, 278.212).

***(R)*- and *(S)*-MTPA Esters of Lycovatine A (1).** To a pyridine solution (50 μL) of **1** (0.1 mg) was added 4-dimethylaminopyridine (50 μg), triethylamine (2 μL), and (*S*)-(+)-MTPACl (1 μL), and stirring was continued at 40 °C for 23 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (1 μL) and H_2O (100 μL), the mixture was extracted with EtOAc (100 μL x 3), and the extract was concentrated in *vacuo* to afford the (*R*)-MTPA ester of **1** (0.1 mg). The *(S)*-MTPA ester of **1** was prepared according to the same procedure as described above.

***(S)*-MTPA ester of 1:** ^{1}H NMR ($CDCl_3$) δ5.95 (1H, H-3), 5.72 (1H, H-5), 3.55 (3H, H_3-17), 3.17 (1H, H-9a), 3.07 (1H, H-9b), 2.91 (2H, H_2-1), 2.64 (1H, H-2a), 2.38 (1H, H-7), 2.34 (1H, H-2b), 2.33 (1H, H-11a), 2.29 (1H, H-10a), 2.29 (1H, H-14a), 2.22 (1H, H-14b), 2.15 (1H, H-6a), 1.93 (1H, H-15), 1.92 (1H, H-10b), 1.72 (1H, H-6b), 1.47 (1H, H-11b), 1.47 (1H, H-8a), 1.27 (1H, H-8b), 0.86 (3H, H_3-16); ESIMS *m*/*z* 494 $(M+H)^+$.

***(R)*-MTPA ester of 1:** ^{1}H NMR ($CDCl_3$) δ6.08 (1H, H-3), 5.79 (1H, H-5), 3.54 (3H, H_3-17), 3.18 (1H, H-9a), 3.17 (1H, H-1a), 3.04 (1H, H-1b), 2.99 (1H, H-9b), 2.74 (1H, H-2a), 2.40 (1H, H-2b), 2.23 (1H, H-7), 2.44 (1H, H-14a), 2.40 (1H, H-11a), 2.25 (1H, H-6a), 2.24 (1H, H-14b), 2.07 (1H, H-10a), 1.88 (1H, H-15), 1.80 (1H, H-10b), 1.57 (1H, H-6b), 1.56 (1H, H-11a), 1.47 (1H, H-8a), 1.01 (1H, H-8a), 0.79 (3H, H_3-16); ESIMS *m*/*z* 494 $(M+H)^+$.

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