Lycopodatines A−C, $C_{16}N$ Alkaloids from *Lycopodium inundatum*

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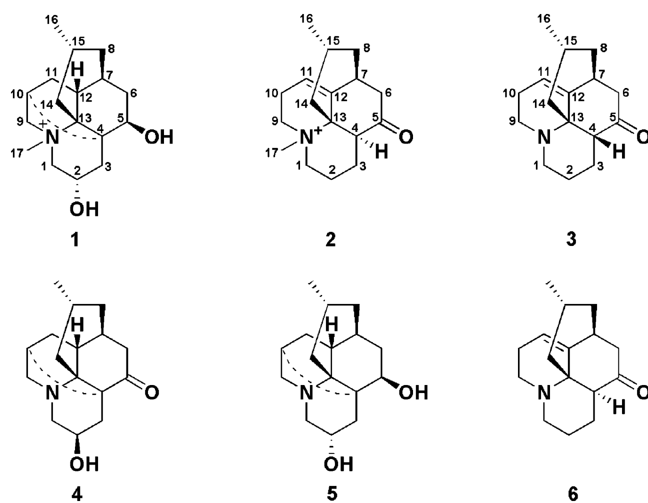
Three new alkaloids, lycopodatines A (**1**), B (**2**), and C (**3**), have been isolated from the club moss *Lycopodium inundatum*, and the structures and absolute configuration were elucidated on the basis of 2D NMR data and chemical transformation.

Lycopodium alkaloids[1] with unique heterocyclic frameworks of $C_{11}N$, $C_{16}N$, $C_{16}N_2$, and $C_{27}N_3$ types have attracted great interest from biogenetic[1,2] and biological[3] points of view. A common feature in all *Lycopodium* alkaloids is a polycyclic carbon skeleton with varying levels of oxidation. These unique skeletons have also been challenging targets for total synthesis.[4] Huperzine A,[3] a representative *Lycopodium* alkaloid, is a highly specific and potent inhibitor of acetylcholinesterase (AChE), and the inherent inhibition of AChE has promoted the pursuit of the total synthesis and SAR studies of this alkaloid.[5,6] Recently we have isolated new types of alkaloids such as sieboldine A,[7] serratezomine A,[8] complanadine A,[9] lyconadin A,[10] senepodine A,[11] lyconesidine A,[12] himeradine A,[13] cermizine A,[14] and nankakurine A[15] from various *Lycopodium* species. Our interest has been focused on the isolation of structurally interesting alkaloids and biosynthetic intermediates to clarify the biogenetic pathway. Further investigation on extracts of *L. inundatum* (Lycopodiaceae) resulted in the isolation of new $C_{16}N$ type alkaloids, lycopodatines A−C (**1**−**3**), as well as known related alkaloids, inundatine (**4**),[16] debenzoylalopecurine (**5**),[17] and anhydrolycodoline (**6**).[16] This paper describes the isolation and structure elucidation of **1**−**3**.



The club moss *L. inundatum* was extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$ followed by *n*-BuOH. The water layer was subjected to SPE chromatography using a Diaion HP-20 column ($MeOH/H_2O$, 0:1 → 1:0), in which a fraction eluting with 100% MeOH was purified by C_{18} HPLC to afford lycopodatines A (**1**, 0.00006%) and B (**2**, 0.00006%). The $CHCl_3$ extract was subjected to an amino silica gel column (hexane/EtOAc, 1:0 → 0:1, and then $CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with hexane/EtOAc (3:2) was purified by a silica gel column ($CHCl_3$/MeOH → MeOH) to afford lycopodatine C (**3**, 0.0006%) and known $C_{16}N$ type alkaloids, inundatine (**4**, 0.001%),[16] debenzoylalopecurine (**5**, 0.0004%),[17] and anhydrolycodoline (**6**, 0.0006%).[16]

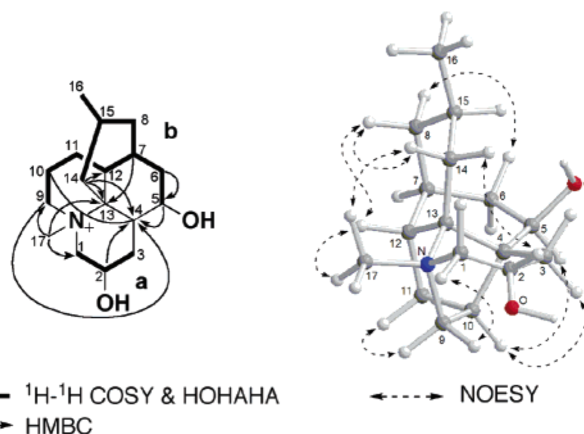


Figure 1. Selected two-dimensional NMR correlations and relative configuration for lycopodatine A (**1**).

Lycopodatine A (**1**) had a molecular formula of $C_{17}H_{28}NO_2$ by HRESIMS [*m/z* 278.2117, $(M)^+$, Δ −0.3 mmu]. The IR spectrum was indicative of the presence of a hydroxy group (3440 cm^{-1}). ^1H and ^{13}C NMR data of **1** were analogous to those of debenzoylalopecurine (**5**), although the three carbons C-1 (δ_C 67.7; δ_H 3.25, 3.82), C-9 (δ_C 72.1; δ_H 3.04, 4.66), and C-13 (δ_C 80.7) were remarkably shifted to lower field as compared to those of **5**. Furthermore, a methyl signal (δ_C 3.00; δ_H 45.4) was observed in the ^1H and ^{13}C NMR spectra of **1**. The molecular structure of **1** was deduced from extensive analyses of the two-dimensional NMR data, including the ^1H−^1H COSY, HOHAHA, HMQC, and HMBC spectra in CD_3OD (Figure 1). The ^1H−^1H COSY and HOHAHA spectra revealed connectivities of two partial structures **a** (C-1−C3) and **b** (C-5−C-8, C-9−C-12, and C-14−C-16), as shown in Figure 1. Connectivities of C-17 to C-1, C-9, and C-13 through a nitrogen atom were implied by HMBC correlations for H_3-17 to C-1, C-9, and C-13. HMBC correlations were observed for H-2, H-5, and H-9a to C-4 (δ_C 54.6), suggesting that C-3, C-5, and C-10

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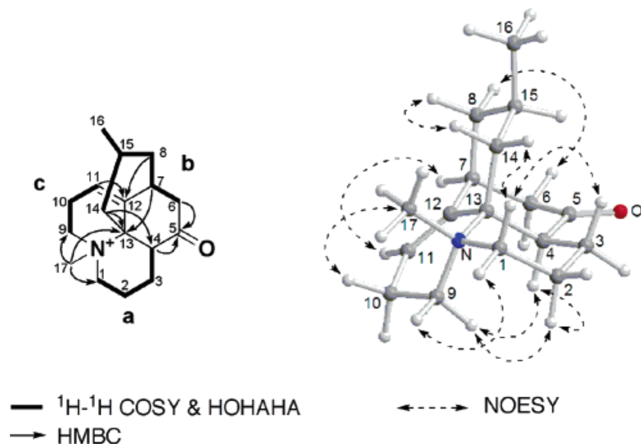


Figure 2. Selected two-dimensional NMR correlations and relative configuration for lycopodatine B (**2**).

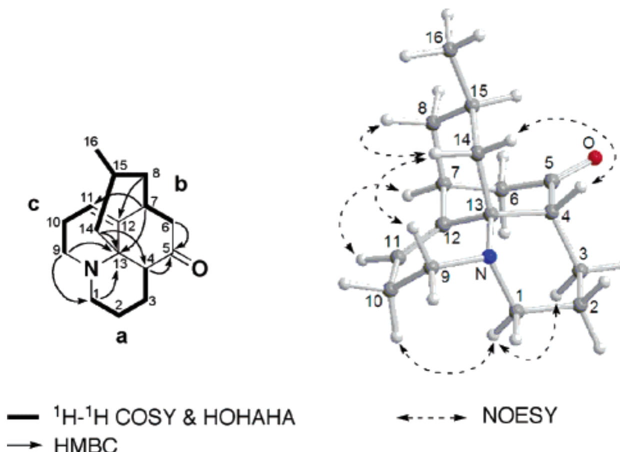


Figure 3. Selected two-dimensional NMR correlations and relative configuration for lycopodatine C (**3**).

were connected through C-4. HMBC cross-peaks for H-14 to C-4, C-12, and C-13 indicated that C-4, C-12, and C-14 were connected through C-13. Thus, the molecular structure of lycopodatine A was elucidated to be **1**, possessing an alopecurane skeleton with two hydroxy groups at C-2 and C-5 and an *N*-methyl group.

The relative configuration of **1** was elucidated by NOESY correlations and $^{3}J_{H-H}$ couplings as depicted in the computer-generated three-dimensional drawing (Figure 1). The chair conformation of the cyclohexane ring (C7–C8, C-12–C-15) was deduced from NOESY correlations, as shown in Figure 1. The methyl group at C-15 was assigned to be equatorial from the large ^{3}J coupling (12.4 Hz) between H-14a and H-15. The NOESY correlation of H-2/H-14b and H-5/H-10 indicated that the hydroxy groups at C-2 and C-5 were α- and β-orientated, respectively.

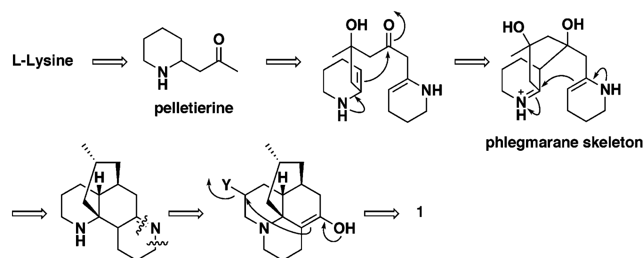
Treatment of debenzoylalopecurine (**5**)[17] with methyl iodide afforded an *N*-methyl derivative, whose spectroscopic data and specific rotation were identical with lycopodatine A (**1**). Thus, the absolute configuration of lycopodatine A was assigned as **1**.

Lycopodatine B (**2**) had a molecular formula of $C_{17}H_{26}NO$ by HRESIMS [*m/z* 260.2037, $(M)^+$, Δ +2.3 mmu]. The IR spectrum indicated the presence of a carbonyl group (1770 cm^{-1}). ^1H and ^{13}C NMR data of **2** were similar to those of anhydrolycodoline (**6**),[16] although the three carbons C-1 (δ_C 61.8; δ_H 3.40, 3.79), C-9 (δ_C 54.9; δ_H 3.23, 4.29), and C-13 (δ_C 72.5) were remarkably shifted to lower field. Furthermore, a methyl signal (δ_H 3.16; δ_C 48.9) was observed in the ^1H and ^{13}C NMR of **2**. Two-dimensional NMR data, including the $^1H-^1H$ COSY, HOHAHA, HMQC, HMBC, and NOESY spectra in CD_3OD (Figure 2), supported the structure of **2**. Thus, the structure of lycopodatine B was assigned as the *N*-methylammonium form of anhydrolycodoline (**6**).

Lycopodatine C (**3**) had a molecular formula of $C_{16}H_{23}NO$ by HRESIMS [*m/z* 246.1855 $(M + H)^+$, Δ −0.3 mmu]. IR absorptions implied the presence of a carbonyl (1700 cm^{-1}) group. $^1H-^1H$ COSY, HOHAHA, HMQC, and HMBC spectra suggested that **3** had the same tetracyclic backbone framework as that of anhydrolycodoline (**6**),[16] although the ^1H and ^{13}C NMR chemical shifts of lycopodatine C (**3**) were not identical to **6**, indicating that it was a diastereomer of anhydrolycodoline (**6**).

The relative configuration of **3** was elucidated by NOESY correlations and $^{3}J_{H-H}$ couplings (Figure 3). A chair conformation of the cyclohexane ring (C-7, C-8, and C-12–C-15) was suggested by NOESY correlations of H-14b to H-8a and H-9b and the large ^{3}J coupling (12.4 Hz) between H-8a and H-15. The methyl group at C-16 was assigned to be equatorial. A NOESY cross-peak for H-4 to H-14a was observed in the case of **3**, but not for **6**. Furthermore, a *W*-type long-range coupling was observed between H-4 and H-6a. This suggested that lycopodatine C (**3**) was the 4-*epi* form of anhydrolycodoline (**6**). Thus, the relative configuration of lycopodatine C (**3**) was assigned as shown in Figure 3.

Scheme 1. Plausible Biogenetic Formation of Lycopodatine A (**1**)



A plausible biogenetic path for lycopodatine A is proposed in Scheme 1. It may be derived from L-lysine via pelletierine and then a phlegmarane intermediate.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-4 polarimeter. ^1H and 2D NMR spectra were recorded on a 600 MHz spectrometer (Bruker) at 300 K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. Each NMR sample of lycopodatines was prepared by dissolving 1.0 mg in 30 μL of CD_3OD in 2.5 mm micro cells (Shigemi Co. Ltd.), and chemical shifts were reported using residual CD_3OD (δ_H 3.31 and δ_C 49.0) as internal standard. Standard pulse sequences were employed for the 2D NMR experiments. COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. NOESY and HOHAHA spectra in the phase-sensitive mode were measured with a mixing time of 800 and 30 ms, respectively. For HMQC spectra in the phase-sensitive mode and HMBC spectra, a total of 256 increments of 1K data points were collected. For HMBC spectra with *Z*-axis PFG, a 50 ms delay time was used for long-range C–H coupling. Zero-filling to 1K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation. FABMS was measured by using glycerol as a matrix.

Plant Material. The club moss *L. inundatum* was collected at Nayoro in Hokkaido in 2002. The botanical identification

Table 1. ^{1}H and ^{13}C NMR Data of Lycopodatine A (**1**) in CD_3OD at 300 K

	δ_H	δ_C	HMBC (^{1}H)
1a	3.25 (1H, dd, 14.2, 1.8)	67.7	17
1b	3.82 (1H, dd, 14.2, 6.9)		
2	4.33 (1H, m)	61.5	1a, 3a,
3a	1.58 (1H, m)	31.7	
3b	3.08 (1H, dd, 15.9, 8.9)		
4		54.6	2, 5, 9a, 14a
5	3.95 (1H, d, 8.6)	68.4	6a
6a	1.42 (1H, d, 16.2)	35.2	5, 8a
6b	2.57 (1H, ddd, 16.2, 8.1, 8.1)		
7	2.20 (1H, m)	38.2	5, 6
8a	1.14 (1H, ddd, 13.0, 13.0, 3.0)	40.9	6, 16
8b	1.65 (1H, m)		
9a	3.04 (1H, d, 11.4)	72.1	1b, 17
9b	4.66 (1H, ddd, 11.4, 3.8, 3.8)		
10	2.30 (1H, m)	45.6	3a, 5, 9a
11a	1.62 (1H, m)	33.1	9
11b	1.89 (1H, brd, 13.9)		
12	2.37 (1H, m)	40.4	14b
13		80.7	1a, 3a, 5, 7, 14,
14a	1.37 (1H, dd, 12.6, 12.4)	31.2	16
14b	1.93 (1H, dd, 12.6, 5.3)		
15	2.70 (1H, m)	25.8	14, 16
16	1.01 (3H, d, 6.4)	22.9	14a
17	3.00 (3H, s)	45.4	9a

Table 2. ^{1}H and ^{13}C NMR Data of Lycopodatine B (**2**) in CD_3OD at 300 K

	δ_H	δ_C	HMBC (^{1}H)
1a	3.40 (1H, brd, 13.6)	61.8	17
1b	3.79 (1H, ddd, 13.6, 13.6, 3.7)		
2a	1.94 (1H, m)	20.7	
2b	2.00 (1H, m)		
3a	1.70 (1H, m)	18.8	1a
3b	2.18 (1H, dd, 14.9, 2.0)		
4	3.37 (1H, m)	56.8	6a, 14a
5		207.6	4, 6, 7
6a	2.51 (1H, d, 16.5)	50.0	8a
6b	2.72 (1H, dd, 16.5, 6.6)		
7	3.04 (1H, m)	40.5	6, 11
8a	1.37 (1H, ddd, 12.9, 12.8, 3.2)	41.0	16
8b	1.81 (1H, brd, 12.9)		
9a	3.23 (1H, dd, 12.6, 6.1)	54.9	1b, 10b, 11, 17
9b	4.29 (1H, ddd, 12.6, 12.3, 4.7)		
10a	2.52 (1H, m)	22.2	9b, 11
10b	2.78 (1H, m)		
11	5.90 (1H, d, 5.5)	117.2	9a, 10b
12		138.8	8b, 10a
13		72.5	3b, 4, 7, 9a, 11, 14, 17
14a	1.66 (1H, dd, 11.8, 11.8)	35.1	4, 8b, 16
14b	2.50 (1H, m)		
15	1.73 (1H, m)	26.6	14a, 16
16	0.99 (3H, d, 5.8)	22.1	14a
17	3.16 (3H, s)	48.9	9b

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 *L. inundatum* was extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$ followed by *n*-BuOH. The water layer was subjected to a Diaion HP-20 column (MeOH/H_2O, 0:1 and 1:0), in which the fraction eluted with 100% MeOH was purified by C_{18} HPLC to afford lycopodatines A (**1**, 0.00006%) and B (**2**, 0.00006%). $CHCl_3$-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, 1:0 → 0:1, and then $CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluting with hexane/EtOAc (3:2) was purified by a silica gel column ($CHCl_3$/MeOH → MeOH) to afford lycopodatine C (**3**, 0.0006%), inundatine (**4**, 0.001%), debenzoylalopecurine (**5**, 0.0004%), and anhydrolycodoline (**6**, 0.0006%).

Table 3. ^{1}H and ^{13}C NMR Data of Lycopodatine C (**3**) in CD_3OD at 300 K

	δ_H	δ_C	HMBC (^{1}H)
1a	2.79 (1H, m)	49.8	9a
1b	3.01 (1H, ddd, 12.0, 12.0, 2.9)		
2a	1.62 (1H, m)	25.2	1b
2b	1.80 (1H, m)		
3a	1.42 (1H, dddd, 13.2, 13.2, 13.2, 3.5)	29.8	1b, 4
3b	1.65 (1H, m)		
4	2.42 (1H, dd, 13.6, 3.6)	60.2	3a, 6a
5		215.9	4, 6a, 7
6a	2.28 (1H, d, 18.3)	44.1	
6b	2.78 (1H, m)		
	2.79 (1H, m)	42.0	6a
8a	1.28 (1H, ddd, 12.4, 12.4, 4.2)	45.9	6a, 16
8b	1.82 (1H, m)		
9a	2.76 (1H, m)	46.2	
9b	3.43 (1H, ddd, 13.6, 11.7, 4.9)		
10a	1.90 (1H, m)	20.3	11
10b	2.61 (1H, m)		
	5.81 (1H, d, 5.7)	119.4	7, 9a, 10b
12		139.8	6a, 14
13		60.2	1a, 3a, 9a
14a	1.72 (1H, m)	52.3	4, 16
14b	1.83 (1H, m)		
	1.83 (1H, m)	27.2	16
16	0.89 (3H, d, 5.7)	22.4	

Lycopodatine A (1): colorless solid, $[\alpha]^{33}_{D}$ −16 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3440 (OH) cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESITOFMS *m/z* 278 $(M)^+$; HRESITOFMS *m/z* 278.2117 $(M)^+$, calcd for $C_{17}H_{28}NO_2$ 278.2120.

Lycopodatine B (2): colorless solid; $[\alpha]^{33}_{D}$ −36 (*c* 0.3, MeOH); IR (KBr) ν_{max} 1700 (C=O) cm^{-1}; ^{1}H and ^{13}C NMR (Table 2); ESITOFMS *m/z* 260 $(M)^+$; HRESITOFMS *m/z* 260.2037 $(M)^+$, calcd for $C_{17}H_{26}NO$ 260.2014.

Lycopodatine C (3): colorless solid; $[\alpha]^{33}_{D}$ −118 (*c* 1.0, MeOH); IR (KBr) ν_{max} 1700 (C=O) cm^{-1}; ^{1}H and ^{13}C NMR (Table 3); ESITOFMS *m/z* 246 $(M + H)^+$; HRESITOFMS *m/z* 246.1855 $(M + H)^+$, calcd for $C_{16}H_{24}NO$ 246.1858.

Chemical Conversion of Debenzoylalopecurine (5) into Lycopodatine A (1). To a solution of debenzoylalopecurine (**5**) (1.0 mg) in acetone (0.2 mL) was added methyl iodide (20 μL), and the mixture was kept at 50 °C for 1 h. After evaporation, the residue was applied to a silica gel column ($CHCl_3$/MeOH/TFA, 4:1:0.5) to give a compound (0.7 mg) whose spectroscopic data and $[\alpha]_D$ value were identical with those of natural lycopodatine A (**1**).

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