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Lycopladines F and G, new $C_{16}N_2$-type alkaloids with an additional C_4N unit from *Lycopodium complanatum*

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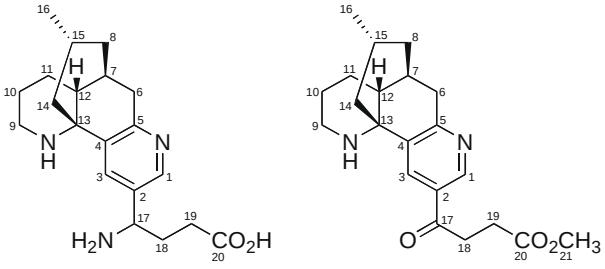
ABSTRACT

Two new *Lycopodium* alkaloids, lycopladines F (**1**) and G (**2**), have been isolated from the club moss *Lycopodium complanatum*, and the structures and relative stereochemistries of **1** and **2** were elucidated on the basis of spectroscopic data. Lycopladine F (**1**) is a rare $C_{16}N_2$-type *Lycopodium* alkaloid possessing an amino acid residue (C_4N).

Club moss (Lycopodiaceae) is known to be a rich source of *Lycopodium* alkaloids[1] 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[2], synthetic[3], and biological[4] points of view. In our continuing efforts to find new *Lycopodium* alkaloids[5], two new $C_{16}N_2$-type alkaloids, lycopladines F (**1**) and G (**2**), were isolated from the club moss *Lycopodium complanatum*. In this Letter, we describe the isolation and structure elucidation of **1** and **2**.



1 (17*S* / 17*R*, 3.5 : 1)

2

The club moss *L. complanatum* collected at Nayoro in Hokkaido was extracted with MeOH, and the MeOH extracts were partitioned between EtOAc and 3% aqueous tartaric acid. Water-soluble materials, adjusted at pH 9 with satd Na_2CO_3, were partitioned with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an LH-20 column ($CHCl_3$/MeOH, 1:1), followed by a SiO_2 column ($CHCl_3$/MeOH, 1:0→1:1 and then $CHCl_3$/MeOH/H_2O/TFA, 6:4:1:0→6:4:1:0.01). The fraction eluted with $CHCl_3$/MeOH/H_2O/TFA (6:4:1:0.01) was purified by a C_{18} HPLC (MeCN/H_2O/TFA, 14:86:0.01) to yield lycopladine F (**1**, 0.00016%), while a fraction eluted with $CHCl_3$/MeOH (100:1 and 50:1) was purified by a C_{18} HPLC (MeCN/ H_2O/TFA, 19:81:0.01) to give lycopladine G (**2**, 0.00010%).

Lycopladine F (**1**)[6] {$[\alpha]_D^{21}$ +8 (*c* 0.5, MeOH)} showed the pseudomolecular ion peak at *m/z* 344 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{20}H_{29}N_3O_2$, was established by HRESIMS [*m/z* 344.2331, $(M+H)^+$, Δ −0.7 mmu]. IR absorptions implied the presence of amino and/or hydroxy (3400 cm^{-1}) and carbonyl (1683 cm^{-1}) functionalities. ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum of **1** revealed 20 carbon signals due to one carbonyl carbon, three sp^2 quaternary carbons, two sp^2 methines, one sp^3 quaternary carbon, four sp^3 methines, eight sp^3 methylenes, and one methyl group. Several pairs of signals were observed in ^1H NMR spectrum of **1** with a ratio of 3.5:1 (Table 1), indicating that **1** was a mixture of epimeric or isomeric isomers.

The gross structure of **1** was elucidated by analyses of 2D NMR data including ^1H–^1H COSY, TOCSY, HMQC, and HMBC spectra in CD_3OD (Fig. 1). ^1H–^1H COSY and TOCSY spectra of **1** revealed two structural units **a** (C-6–C-8, C-9–C-12, C-14–C-16) and **b** (C-17–C-19). An HMBC correlation for H-9a (δ_H 3.28) to C-13 (δ_C 62.7) suggested the connectivity from C-9 (δ_C 41.9) to C-13 through a nitrogen atom. The connectivities of C-4 (δ_C 131.0), C-12 (δ_C 42.4), and C-14 (δ_C 48.2) via C-13 were elucidated by HMBC correlations for H-12 to C-13, and H-14b to C-4 and C-13. HMBC

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Table 1
^{1}H and ^{13}C NMR Data of lycopladines F (**1**) and G (**2**) in CD_3OD[a]

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1	8.59 (0.78H, s), 8.61 (0.22H, s)	149.4 d	9.07 (1H, s)	149.1 d
2	—	132.3 s	—	128.5 s
3	8.24 (0.78H, s), 8.16 (0.22H, s)	133.8 d	8.50 (1H, s)	133.6 d
4	—	131.0 s	—	132.4 s
5	—	160.8 s	—	164.3 s
6a	3.28 (1H, m)	35.2 t	3.28 (1H, m)	35.8 t
6b	2.82 (0.78H, 19.2 Hz), 2.83 (0.22H, d, 19.2 Hz)		2.86 (1H, d, 19.8 Hz)	
7	2.35 (1H, m)	33.9 d	2.34 (1H, m)	34.0 d
8a	1.87 (1H, m)	43.4 t	1.88 (1H, m)	43.5 t
8b	1.47 (1H, ddd, 13.2, 12.6, 3.6 Hz)		1.47 (1H, ddd, 12.6, 12.6, 3.6 Hz)	
9a	3.28 (1H, m)	41.9 t	3.21 (1H, br d, 13.2 Hz)	41.9 t
9b	2.94 (1H, ddd, 13.2, 12.6, 3.6 Hz)		2.83 (1H, ddd, 13.2, 13.2, 4.2 Hz)	
10	1.88 (2H, m)	23.8 t	1.84 (2H, m)	24.5 t
11a	1.73 (1H, br d, 13.2 Hz)	25.0 t	1.71 (1H, br d, 13.8 Hz)	25.4 t
11b	1.34 (1H, m)		1.29 (1H, m)	
12	2.09 (1H, br d, 12.6 Hz)	42.4 d	2.05 (1H, br d, 12.6 Hz)	42.8 d
13	—	62.7 s	—	61.7 s
14a	1.89 (1H, m)	48.2 t	1.83 (1H, m)	48.8 t
14b	1.63 (1H, dd, 12.0, 12.0 Hz)		1.60 (1H, dd, 12.0, 12.0 Hz)	
15	1.23 (1H, m)	27.0 d	1.23 (1H, m)	26.9 d
16	0.87 (2.34H, d, 6.6 Hz), 0.88 (0.66H, d, 6.6 Hz)	21.7 t	0.88 (3H, d, 6.6 Hz)	21.8 t
17	4.50 (0.78H, m), 4.51 (0.22H, m)	53.8 d	—	198.3 s
18	2.38 (2H, m)	30.4 t	3.39 (2H, m)	34.6 t
19a	2.42 (1H, m)	29.8 t	2.78 (2H, t, 6.0 Hz)	28.6 t
19b	2.36 (1H, m)			
20	—	175.8 s	—	175.0 s
21	—	—	3.68 (3H, s)	52.3 t

[a] ^{1}H and ^{13}C NMR spectra were recorded at 600 MHz and 150 MHz, respectively.

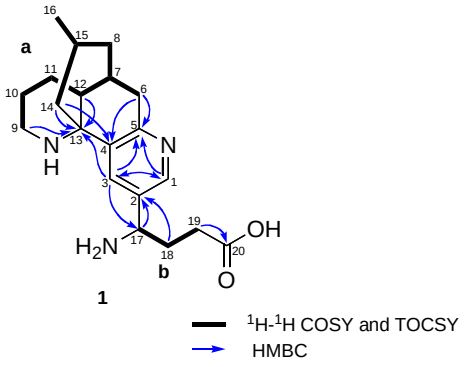

Figure 1. Selected 2D NMR correlations for lycopladine F (**1**).

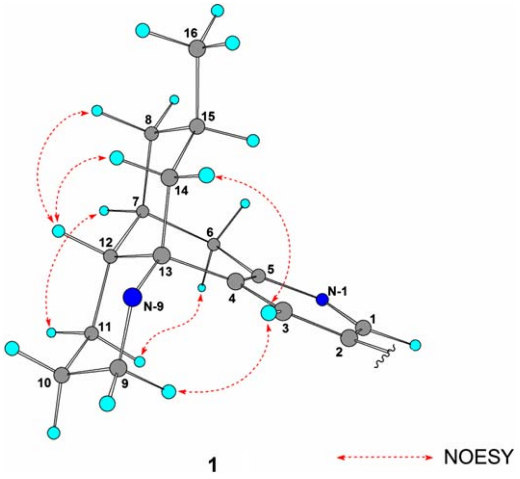

Figure 2. Selected NOESY correlations and relative stereochemistry for C-1–C-16 moiety of lycopladine F (**1**).

cross-peaks of H_2-6 to C-4 (δ_C 131.0) and C-5 (δ_C 160.8) indicated the connectivity from C-6 (δ_C 35.2) to C-4. HMBC correlations observed for H-1 and H-3 to C-5, and H-3 to C-13 suggested the presence of a tri-substituted pyridine ring, which constituted a 2-substituted lycodine[7] with unit **a**. HMBC correlations for H-3 to C-17 (δ_C 53.8), and H-17 and H-18 to C-2 (δ_C 132.3) revealed the connectivity from C-17 to C-2. An HMBC correlation for H-19b to C-20 (δ_C 175.8) indicated the connectivity of a carboxyl group to C-19 (δ_C 29.8). Finally, the molecular formula of **1** and chemical shifts of C-17 (δ_H 4.50, δ_C 53.8) suggested that the primary amino group was attached to C-17. Thus, the gross structure of lycopladine F was elucidated to be **1**.

The phase-sensitive NOESY spectrum showed cross-peaks as shown in 3D drawing of **1**, obtained from the molecular mechanics calculation using the MM2 force field on Chem3D Ultra (ver. 7.0.0) (Fig. 2). NOESY correlations for H-12/H-8b and H-12/H-14b revealed that a cyclohexane ring (C-7–C-8, C-12–C-15) was chair form. The methyl group at C-15 was assigned as equatorial by ^{3}J value (12.0 Hz) between H-14b and H-15. NOESY cross-peaks of H-3/H-9b and H-6b/H-11b suggested that a decahydro quinoline ring (C-7–C-15, N-9) was trans-fused, and the piperidine ring (C-9–C-13, N-9) and the cyclohexene ring (C-4–C-7, C-12–C-13) were chair form and half-chair form, respectively. Thus, the relative stereochemistry for C-1–C-16 moiety of lycopladine F (**1**) was assigned as shown in Figure 2. Since the relative stereochemistry of C-1–C-16 moiety was single, **1** was deduced to be a mixture of diastereomers at C-17.

The absolute configuration at C-17 of lycopladine F (**1**) was inspected by the modified Mosher's method[8] for the MTPA amides of methylester derivative of **1**.[9] The values of $\Delta\delta$[δ(*S*-MTPA amide) – δ(*R*-MTPA amide)] of major isomer of **1** are shown in Figure 3. The $\Delta\delta$ values for H-17, H_2-18, H_2-19, and CO_2Me of major isomer were negative, while the $\Delta\delta$ values for H-1 and H-3 were po-

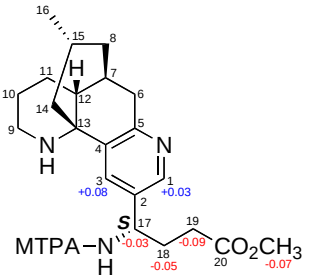

Figure 3. $\Delta\delta$ values [$\Delta\delta$(in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA amides of methyl ester derivative of the major isomer of lycopladine F (**1**).

sitive. These data suggested that the absolute configuration at C-17 of major isomer of **1** was *S*. The $\Delta\delta$ values for H-1, H-3, H-17, H_2-18, H_2-19, and CO_2Me of minor isomer were opposite in sign to those of major isomer, suggesting that the absolute configuration at C-17 of minor isomer of **1** was *R*.[10]

Lycopladine G (**2**)[11] {$[\alpha]_D^{23}$ +4 (*c* 0.3, MeOH)} showed the pseudomolecular ion peak at *m/z* 357 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{21}H_{28}N_2O_3$, was established by HRESIMS [*m/z* 357.2174, $(M+H)^+$, Δ −0.4 mmu]. IR absorptions implied the presence of amino (3428 cm^{-1}), ester carbonyl (1731 cm^{-1}), and conjugated keto carbonyl (1684 cm^{-1}) functionalities. ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum of **2** revealed 21 carbon signals due to two carbonyl carbons, three sp^2 quaternary carbons, two sp^2 methines, one sp^3 quaternary carbon, three sp^3 methines, eight sp^3 methylenes, and two methyl groups.

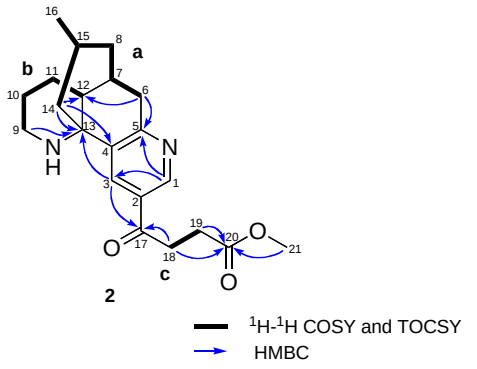

Figure 4. Selected 2D NMR correlations for lycopladine G (**2**).

Analyses of 2D NMR data including the ^1H–^1H COSY, TOCSY, HMQC, and HMBC spectra in CD_3OD (Fig. 4) revealed that **2** possessed a 2-substituted lycodine[7] moiety. HMBC correlations for H-3 and H-18 to C-17 (δ_C 198.3) suggested that C-18 (δ_C 34.6) was connected to C-2 (δ_C 128.5) through C-17, while HMBC correlations for H-19 and H-21 to C-20 (δ_C 175.0) indicated that a methoxy carbonyl group was attached to C-19. Inspection of phase-sensitive NOESY spectrum of **2** revealed that the relative stereochemistry of C-1–C-16 moiety of **2** was same as that of **1**. Thus, the structure of lycopladine G (**2**), including relative stereochemistry, was assigned as **2**.

Lycopladine F (**1**) is a rare $C_{16}N_2$-type *Lycopodium* alkaloid possessing an amino acid residue (C_4N). Plausible biogenetic path of **1** and **2** was proposed as shown in Scheme 1. Though the origin of γ-aminobutyric acid moiety (C_4N) attached to C-2 of **1** was unknown, it was known that the origin of pyrrolidine ring of nicotine was L-ornithine and nicotine was metabolized to γ-(3-pyridyl)-γ-methylaminobutyric acid.[12] Lycopladine F (**1**) could be derived from lycodine[7] and L-ornithine via hypothetical intermediate X, while lycopladine G (**2**) might be derived from **1** by oxidation. Biological activity of **1** and **2** is currently investigated.

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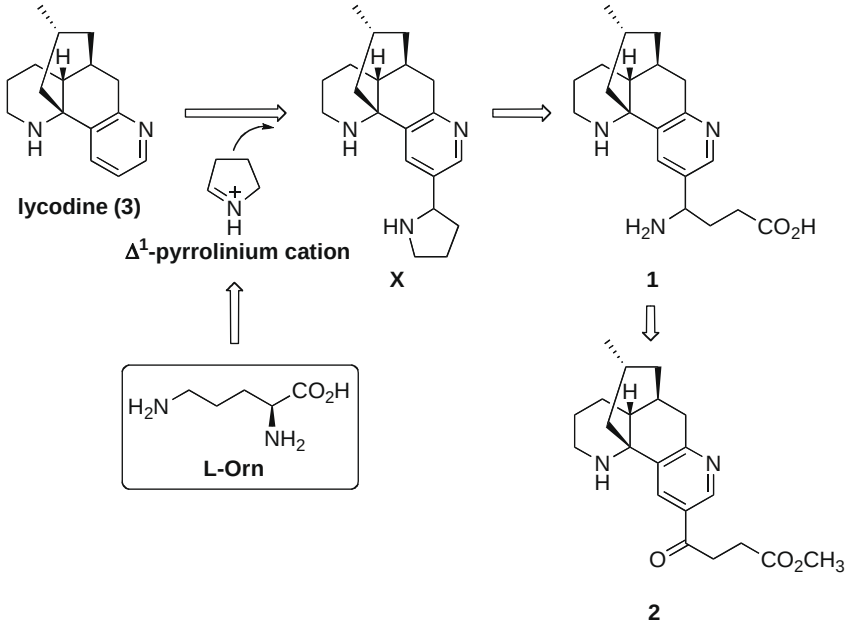

Scheme 1. Plausible biogenetic path of lycopladines F (**1**) and G (**2**).

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6. *Lycopladine F* (**1**): colorless amorphous solid; $[\alpha]_D^{21}$ +8 (*c* 0.5, MeOH); IR (film) ν_{max} 3400, 1683, and 1574 cm^{-1}; UV (MeOH) λ_{max} 272 nm (ε 1600); ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 344 $(M+H)^+$; HRESIMS *m/z* 344.2331 (M+H; calcd for $C_{20}H_{30}N_3O_2$, 344.2338).
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9. The methyl ester derivative of **1** was obtained by treatment of **1** with trimethyl silyl diazomethane.
10. The relative stereochemistry between C-1–C-16 moiety and C-17 of **1** was not elucidated.
11. *Lycopladine G* (**2**): colorless amorphous solid; $[\alpha]_D^{23}$ +4 (*c* 0.3, MeOH); IR (film) ν_{max} 3428, 1731, and 1684 cm^{-1}; UV (MeOH) λ_{max} 280 nm (ε 3300); ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 357 $(M+H)^+$; HRESIMS *m/z* 357.2174 (M+H; calcd for $C_{21}H_{29}N_2O_3$, 357.2178).
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