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Lycopladines B–D and lyconadin B, new alkaloids from *Lycopodium complanatum*

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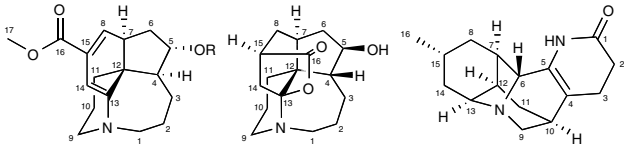
Abstract—Four new alkaloids, lycopladines B–D (**1**–**3**) and lyconadin B (**4**), have been isolated from the club moss *Lycopodium complanatum* and the structures including the stereochemistry were elucidated on the basis of spectral data and modified Mosher's method. Lyconadin B (**4**) elevated NGF mRNA expression in 1321N1 human astrocytoma cells.

1. 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] lycopladine A[3] (**5**) and lyconadin A[4] (**6**) were previously isolated from the club moss *Lycopodium complanatum*. Further investigation allowed us to isolate four new *Lycopodium* alkaloids, lycopladines B–D (**1**–**3**) and lyconadin B (**4**) from the club moss *L. complanatum*. In this paper, we describe the isolation and structure elucidation of **1**–**4**.



2. 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, in which a fraction eluted with hexane/EtOAc (10:1) was purified by LH-20 and silica gel columns to afford lycopladine C (**2**, 0.00003% yield). The fraction eluted with hexane/EtOAc (1:1) from an amino silica gel column was purified by a silica gel column to yield lycopladine B (**1**, 0.0001% yield), while two fractions eluted with $CHCl_3$/MeOH (1:0 and 50:1) and $CHCl_3$/MeOH (10:1) from an amino silica gel column were purified by silica gel columns to give lycopladines A[3] (**5**, 0.0001% yield) and D (**3**, 0.0001% yield), and lyconadins A[4] (**6**, 0.0009% yield) and B (**4**, 0.00007% yield), respectively.

Lycopladine B (**1**) showed the pseudomolecular ion peak at *m*/*z* 290 $(M+H)^+$ in the ESI-MS, and the molecular formula, $C_{17}H_{23}NO_3$, was established by HRESIMS [*m*/*z* 290.1759, $(M+H)^+$, Δ +0.3 mmu]. IR absorptions implied the presence of hydroxy (3380 cm^{-1}) and ester carbonyl (1710 cm^{-1}) functionalities. ^{13}C NMR data of **1** (Table 1) revealed 17 carbon signals due to one carbonyl carbon, two sp^2 quaternary carbons, two sp^2 methines, one sp^3 quaternary carbon, three sp^3 methines, seven sp^3 methylenes, and one *O*-methyl group.

The gross structure of **1** was elucidated by analyses of 2D NMR data including ^1H–^1H COSY, HOHAHA, HMQC, and HMBC spectra in CD_3OD (Fig. 1).

Keywords: *Lycopodium* alkaloids; Lycopladines B–D; Lyconadin B; NGF expression; Astrocytoma cells.
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Table 1. ^{1}H and ^{13}C NMR data of lycopladine B (**1**) in CD_3OD

Position	δ_H		δ_C	
1a	3.26	(1H, br d, 13.6)	62.8	t
1b	3.09	(1H, m)		
2a	1.84	(1H, m)	30.2	t
2b	1.76	(1H, m)		
3a	2.10	(1H, m)	32.1	t
3b	1.21	(1H, q, 12.3)		
4	1.73	(1H, m)	59.9	d
5	3.59	(1H, m)	80.8	d
6a	2.08	(1H, m)	40.3	t
6b	1.96	(1H, dd, 10.3, 5.9)		
7	2.76	(1H, br s)	47.4	d
8	6.51	(1H, d, 2.0)	139.7	d
9	3.11	(2H, m)	53.5	t
10a	2.04	(1H, m)	23.0	t
10b	1.61	(1H, m)		
11a	2.02	(1H, m)	42.1	t
11b	1.82	(1H, m)		
12			48.2	s
13			147.9	s
14	6.22	(1H, s)	114.9	d
15			127.6	s
16			167.1	s
17	3.74	(3H, s)	52.3	q

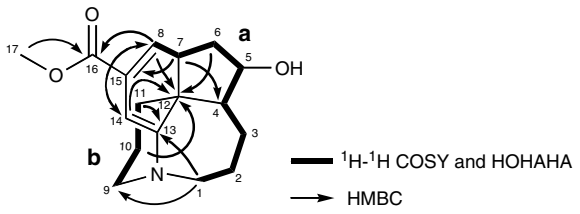


Figure 1. Selected 2D NMR correlations for lycopladine B (**1**).

^{1}H–^{1}H COSY and HOHAHA spectra of **1** revealed two structural units **a** (C-1 to C-8) and **b** (C-9 to C-11). In unit **a**, C-5 was deduced to be connected to a hydroxy group from the ^{1}H and ^{13}C NMR chemical shifts (δ_H 3.59 and δ_C 80.8). HMBC correlations of H_2-6 (δ_H 2.08 and 1.96) and H-8 (δ_H 6.51) to C-12 (δ_C 48.2), and H-7 (δ_H 2.76) to C-4 (δ_C 59.9) suggested a connection of C-7 to C-4 through C-12, indicating the presence of a cyclopentane ring (C-4 to C-7 and C-12). HMBC cross-peaks of H-1b (δ_H 3.09) to C-9 (δ_C 53.5) and H-1a to C-13 revealed connections of C-1 to C-9, C-1 to C-13, and C-9 to C-13 through a nitrogen atom. While HMBC cross-peaks of H-10b (δ_H 1.61) and H-14 (δ_H 6.22) to C-12 (δ_C 48.2) and H-11a (δ_H 2.02) to C-13 (δ_C 147.9) indicated a connection of C-11 to C-14 through C-12 and C-13. HMBC cross-peaks of H-8 (δ_H 6.51) to C-14 and C-16 (δ_C 114.9 and 167.1, respectively) and H-7 (δ_H 2.76) to C-15 (δ_C 127.6) suggested that C-8 was connected to C-14 and C-16 through C-15. The presence of a methyl ester group was elucidated by the HMBC correlation of H-17 (δ_H 3.74) to C-16 (δ_C 167.1). Thus, the gross structure of lycopladine B was elucidated to be **1**.

The NOESY spectrum of **1** showed cross-peaks as shown in computer-generated 3D drawing (Fig. 2).

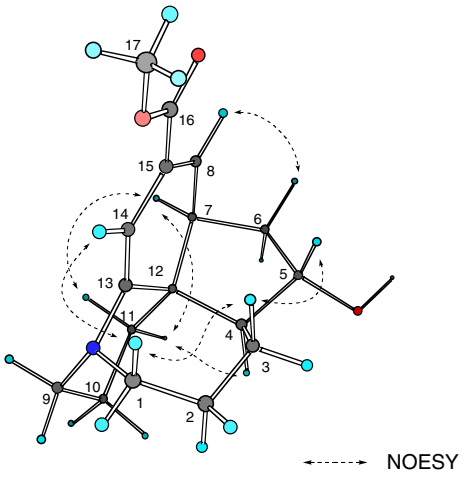


Figure 2. Selected NOESY correlations and relative stereochemistry for lycopladine B (**1**).

The relative stereochemistry of H-5 was deduced to be a β-configuration from NOESY correlations of H-1b/H-3b, H-1b/H-14, and H-3b/H-5. While the relative stereochemistry of H-4 was deduced to be an α-configuration from the NOESY correlation of H-4/H-11a. A chair-like conformation of the piperidine ring (N-1 and C-9 to C-13) and an α-configuration of H-7 were suggested by NOESY correlations of H-7/H_2-11.

The absolute configuration of C-5 in lycopladine B (**1**) was elucidated by the modified Mosher's method for the MTPA esters of **1**.[5] The values of $\Delta\delta$[δ(*S*-MTPA ester)-δ(*R*-MTPA ester)] for H_2-6, H-7, and H-8 were negative, while the values of $\Delta\delta$ for H_2-1, H_2-2, H_2-3, H-4, H_2-9, H_2-10, and H_2-11 were positive. These data suggested that the absolute configuration at C-5 was *S*. Thus, the absolute stereochemistry of **1** was assigned as shown.

Lycopladine C (**2**) showed the pseudomolecular ion peak at *m*/*z* 332 $(M+H)^+$ in the ESI-MS, and the molecular formula, $C_{19}H_{25}NO_4$, was established by HRESIMS [*m*/*z* 332.1858, $(M+H)^+$, Δ −0.4 mmu]. IR absorptions implied the presence of ester carbonyl (1735 cm^{-1}) functionality. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** (Table 1) suggested that they were almost identical with each other except for the presence of signals for an acetate group [δ_H 2.04 (s, 3H) and δ_C 170.8 and 21.2] and low field shift of signal of H-5 (δ_H 4.67) in **2**. The spectral data of the product which was obtained by acetylation of **1** were identical with those of **2**. Thus, lycopladine C (**2**) was assigned as 5-*O*-acetyl form of lycopladine B (**1**).

Lycopladine D (**3**) showed the pseudomolecular ion peak at *m*/*z* 278 $(M+H)^+$ in the ESI-MS, and the molecular formula, $C_{16}H_{23}NO_3$, was established by HRESIMS [*m*/*z* 278.1773, $(M+H)^+$, Δ +1.7 mmu]. IR absorptions implied the presence of hydroxy (3380 cm^{-1}) and lactone carbonyl (1780 cm^{-1}) functionalities. ^{13}C NMR data of **3** (Table 2) revealed 16 carbon signals due to one ester carbonyl carbon, two sp^3 quater-

Table 2. ^{1}H and ^{13}C NMR data of lycopladine D (**3**) in CD_3OD

Position	δ_H		δ_C	
1a	3.46	(1H, dt, 15.4, 4.2)	48.8	t
1b	2.62	(1H, ddd, 15.4, 5.2, 1.6)		
2a	2.02	(1H, m)	26.8	t
2b	1.73	(1H, m)		
3a	2.04	(1H, m)	21.0	t
3b	1.52	(1H, m)		
4	2.19	(1H, m)	48.5	d
5	3.43	(1H, m)	74.0	d
6a	1.96	(1H, m)	43.9	t
6b	1.22	(1H, m)		
7	1.70	(1H, m)	41.2	d
8a	2.09	(1H, m)	27.8	t
8b	1.67	(1H, m)		
9a	3.20	(1H, m)	46.6	t
9b	3.05	(1H, dd, 14.2, 6.1)		
10a	1.88	(1H, m)	21.2	t
10b	1.37	(1H, m)		
11a	1.91	(1H, m)	34.1	t
11b	1.86	(1H, m)		
12			46.1	s
13			101.3	s
14a	2.62	(1H, d)	37.7	t
14b	2.00	(1H, m)		
15	2.73	(1H, br s)	41.4	d
16			179.0	s

nary carbons, four sp^3 methines, and nine sp^3 methylenes.

The gross structure of **3** was elucidated by analyses of 2D NMR data including the ^{1}H–^{1}H COSY, HOHAHA, HMQC, and HMBC spectra in CD_3OD (Fig. 3). The ^{1}H–^{1}H COSY and HOHAHA spectra of **3** revealed two structural units **a** (C-1 to C-8, C-14 to C-15, and C-8 to C-15) and **b** (C-9 to C-10). In unit **a**, C-5 was deduced to be connected to a hydroxy group from the ^{1}H and ^{13}C NMR data (δ_H 3.43, δ_C 74.0). HMBC cross-peaks of H-1a (δ_H 3.46) to C-9 (δ_C 46.6), and H-1b (δ_H 2.62) and H-9b (δ_H 3.05) to C-13 (δ_C 101.3) suggested connections of C-1 to C-9, C-1 to C-13, and C-9 to C-13 through a nitrogen atom. The δ_C value (101.3 ppm) suggested that C-13 was ascribed to aminoacetal carbon. HMBC correlations of H-8a (δ_H 2.09) and H-14a (δ_H 2.62) to C-16 (δ_C 179.0) revealed that the ester carbonyl group was connected to C-15. The connection of C-10 to C-11 was suggested by the HMBC cross-peak of H-11a (δ_H 1.91) to C-10 (δ_C 21.2). HMBC correlations of H-7 (δ_H 1.70) to C-11 (δ_C 34.1) and C-12 (δ_C 46.1) indicated that C-7 was connected to C-11 through C-12. HMBC cross-peaks of H-4 (δ_H 2.19) to C-12 (δ_C 46.1) and C-13 (δ_C 101.3), and H-14b (δ_H 2.00) to C-13 (δ_C 101.3) revealed that C-4 was connected to C-14 through C-12 and C-13. Thus, the gross structure of lycopladine D was elucidated to be **3**.

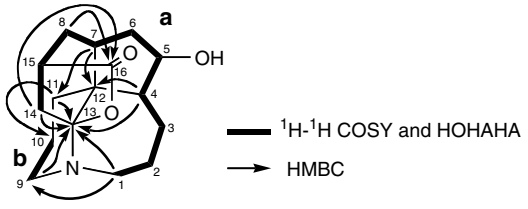


Figure 3. Selected 2D NMR correlations for lycopladine D (**3**).

The NOESY spectrum of **3** showed cross-peaks as shown in computer-generated 3D drawing (Fig. 4). The relative stereochemistry of H-4 was deduced to be a β-configuration from NOESY correlations of H-1b/H-4, H-4/H-6b, and H-6b/H-8b, while the relative stereochemistry of H-5 and H-7 was deduced to be both α-configurations from NOESY correlations of H-3b/H-5, H-5/H-6a, H-5/H-7, and H-5/H-11a. A chair-like conformation of the piperidine ring (N-1 and C-9 to C-13) and the cyclohexane ring (C-7 to C-8 and C-12 to C-15) and relative stereochemistry of H-15 were suggested by NOESY correlations of H-8b/H-6b H-8a/H-14a, H-9a/H-11a, and H-9a/H_2-14. Thus, the relative stereochemistry of lycopladine D (**3**) was elucidated as shown in Figure 4.

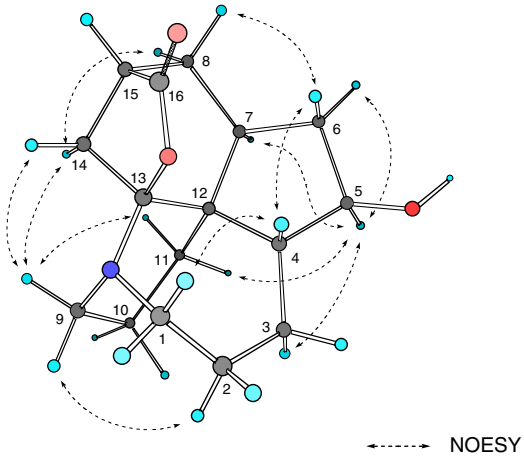


Figure 4. Selected NOESY correlations and relative stereochemistry for lycopladine D (**3**).

Table 3. ^{1}H and ^{13}C NMR data of lyconadin B (**4**) in CD_3OD

Position	δ_H		δ_C	
1			173.3	s
2	2.47	(2H, m)	31.5	t
3	2.37	(2H, m)	24.0	t
4			120.7	s
5			135.8	s
6	3.45	(1H, S)	63.6	d
7	2.25	(1H, d, 4.8)	49.9	d
8a	1.84	(1H, m)	40.9	t
8b	0.94	(1H, t, 13.2)		
9a	3.25	(1H, m)	61.9	t
9b	2.83	(1H, d, 12.0)		
10	2.10	(1H, m)	34.6	d
11a	1.95	(1H, m)	33.4	t
11b	1.68	(1H, d, 13.5)		
12	1.89	(1H, s)	48.2	d
13	3.26	(1H, d, 3.1)	72.5	d
14a	1.95	(1H, m)	41.2	t
14b	1.04	(1H, ddd, 12.6, 12.6, 2.4)		
15	1.74	(1H, m)	26.2	d
16	0.89	(3H, d, 6.5)	22.1	q

The absolute configuration of lycopladine D (**3**) was elucidated by the modified Mosher's method for the MTPA ester of **3**. The values of $\Delta\delta[\delta(S\text{-MTPA ester})\text{-}\delta(R\text{-MTPA ester})]$ for H_2-6, H_2-8, and H-15 were positive, while the values of $\Delta\delta$ for H_2-1, H_2-2, H_2-3, H-4, H_2-9, H_2-10, and H-14a were negative, suggesting that the absolute configuration at C-5 was *R*. Thus, the absolute configuration of **3** was assigned as shown.

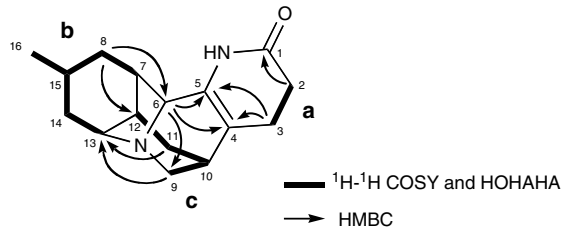


Figure 5. Selected 2D NMR correlations for lyconadin B (**4**).

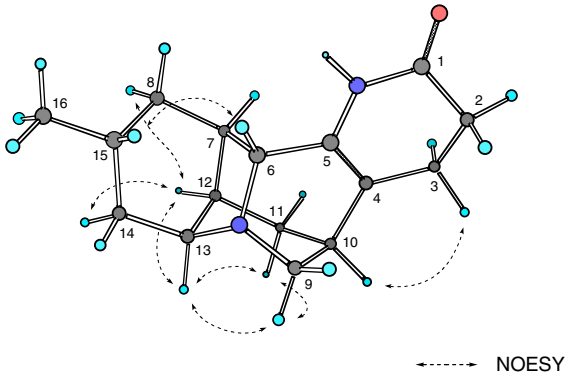


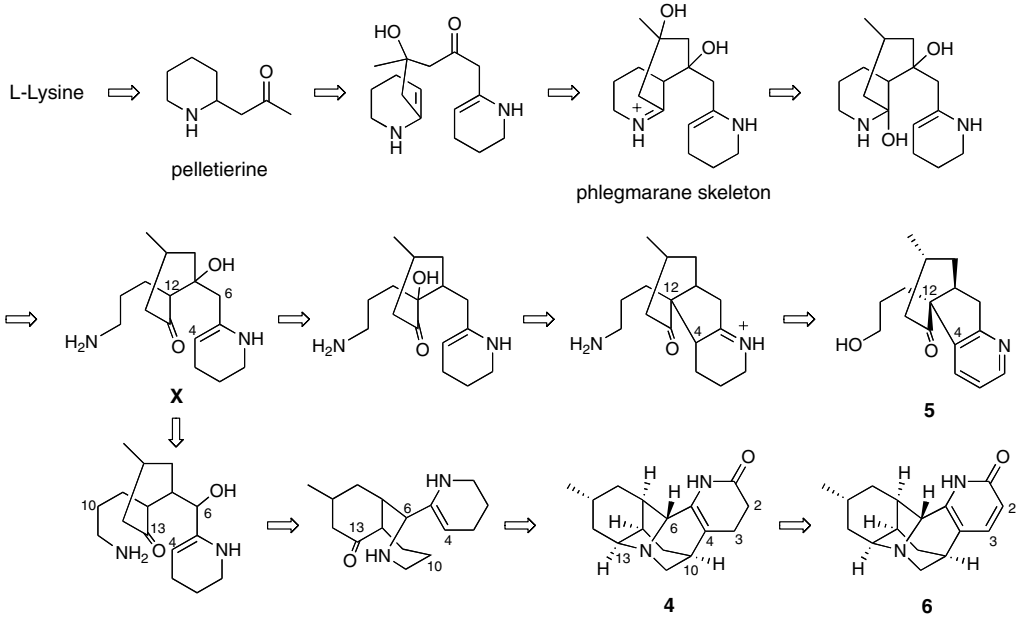
Figure 6. Selected NOESY correlations and relative stereochemistry for lyconadin B (**4**).

Lyconadin B (**4**) showed the pseudomolecular ion peak at *m*/*z* 259 $(M+H)^+$ in the ESI-MS, and the molecular formula, $C_{16}H_{22}N_2O$, was established by HRESIMS [*m*/*z* 259.1805, $(M+H)^+$ Δ −0.5 mmu]. IR absorptions implied the presence of amide carbonyl (1675 cm^{-1}) functionality. The ^{13}C NMR (Table 3) spectra of **4** gave signals due to one amide carbonyl, one tetrasubstituted olefin, six sp^3 methylenes, six sp^3 methines, and one methyl group, implying that the structure of **4** was similar to that of lyconadin A (**6**).[4] The ^{1}H–^{1}H COSY and HOHAHA spectra of **4** revealed connectivities of three partial structures **a** (C-2 to C-3), **b** (C-8 to C-7 and C-15, and C-13 to C-16), and **c** (C-9 to C-12), which were connected to each other on the basis of HMBC correlations as shown in Figure 5.

The relative stereochemistry of **4** was deduced from NOESY correlations as shown in computer-generated 3D drawing (Fig. 6). These NOESY correlations indicated the relative stereochemistry of C-6, C-7, C-10, C-12, C-13, C-15, and a chair conformation of the cyclohexane (C-7 to C-8 and C-12 to C-15) moiety. Thus, lyconadin B (**4**) was assigned as 2,3-dihydro form of lyconadin A (**6**).

An alternative plausible biogenetic path of lyconadin A (**6**)[4] is proposed by isolation of lycopladine A (**5**) as shown in Scheme 1. Biogenetically, lyconadins A (**6**) and B (**4**) might be derived from L-lysine via pelletierine, plegmarane skeleton,[6,7] and an intermediate X from which lycopladine A (**5**) may be also provided.

Effects of lycopladines A (**5**) and B–D (**1**–**3**), and lyconadins A (**6**) and B (**4**) on neurotrophic factor biosynthesis in 1321N1 human astrocytoma cells were examined by determining NGF mRNA expression. The mRNA expressions of NGF in 1321N1 cells were examined by



Scheme 1. Plausible biogenetic path of lycopladine A (**5**) and lyconadins A (**6**) and B (**4**).

a semiquantitative RT-PCR method.[8,9] The mRNA expressions for NGF were enhanced by lyconadins A (**6**) and B (**4**). Lycopladines B–D (**1**–**3**) and lyconadin B (**4**) did not show cytotoxicity against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells in vitro ($IC_{50} > 10$ μg/mL).

3. Experimental

3.1. General

IR spectra were recorded on a JASCO FT/IR-230 and a Shimadzu UV-1600PC spectropolarimeter. ^1H 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 ^1H and ^{13}C NMR spectra, respectively. ESI-mass spectra were obtained on a JEOL JMS-700TZ spectrometer.

3.2. Plant material

The club moss *Lycopodium complanatum* were collected in Nayoro, Hokkaido, in 2004. The botanical identification was made by Mr. N. Yoshida, Health Sciences University of Hokkaido.

3.3. Extraction and isolation

The club moss *Lycopodium complanatum* were 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 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). The fraction eluted with $CHCl_3$ was further purified by a silica gel column (hexane/EtOAc, 10:1 → 1:1) to afford lycopladine C (**2**, 0.00003% yield). The fraction eluted with hexane/EtOAc (1:1) from an amino silica gel column was purified by a silica gel column ($CHCl_3$/MeOH, 1:0 → 1:1) to afford lycopladine B (**1**, 0.0001% yield). The fraction eluted with $CHCl_3$/MeOH (1:0 and 50:1) from an amino silica gel column was purified by a silica gel column ($CHCl_3$/MeOH, 1:0 → 4:1) to yield lycopladines A[3] (**5**, 0.0001% yield) and D (**3**, 0.0001% yield). The fraction eluted with $CHCl_3$/MeOH (10:1) from an amino silica gel column was purified by a silica gel column ($CHCl_3$/MeOH, 1:0 → 1:4) to give lyconadins A[4] (**6**, 0.0009% yield) and B (**4**, 0.00007% yield).

3.4. Lycopladine B (1)

Colorless amorphous solid; $[\alpha]_D^{23}$ +202° (*c* 0.2, MeOH); IR (neat) ν_{max} 3380, 1710, and 1600 cm^{-1}; UV (MeOH) λ_{max} 315 nm (ε 1200); ^1H and ^{13}C NMR data (Table 1); ESI-MS *m/z* 290 $(M+H)^+$; HRESIMS *m/z* 290.1759 (M+H; calcd for $C_{17}H_{24}NO_3$, 290.1756).

To a solution of **1** (0.1 mg) in pyridine (50 μL) was added aceticacidanhydride (5 μL). The mixture was allowed to stand at room temperature for 12 h. After evaporation of the solvent, the residue was applied to a silica gel column to give lycopladine C (**3**).

3.5. Lycopladine C (2)

Colorless amorphous solid; $[\alpha]_D^{23}$ +121° (*c* 0.3, MeOH); IR (neat) ν_{max} 2925, 2850, and 1735 cm^{-1}; UV (MeOH) λ_{max} 316 nm (ε 760); ^1H NMR ($CDCl_3$) δ: 6.43 (1H, H-8), 6.19 (1H, H-14), 4.67 (1H, H-5), 3.74 (3H, H-17), 3.15 (2H, H-9), 3.10 (1H, H-1a), 3.01 (1H, H-1b), 2.76 (1H, H-7), 2.13 (1H), 2.04 (3H), 2.03 (1H), 2.00–1.80 (5H), 1.72 (1H), 1.64 (1H), 1.55 (1H, H-10b), 1.30 (1H, H-3b); ^{13}C NMR ($CDCl_3$) δ: 170.8 (C-18), 166.0 (C-16), 145.2 (C-13), 135.8 (C-8), 127.3 (C-15), 113.2 (C-14), 81.1 (C-5), 61.4 (C-1), 55.7 (C-4), 51.7 (C-17), 51.7 (C-9), 47.2 (C-12), 46.3 (C-7), 41.0, 36.9 (C-3), 36.9, 31.2 (C-2), 22.6 (C-10), 21.2 (C-19); ESI-MS *m/z* 332 $(M+H)^+$; HRESIMS *m/z* 332.1858 (M+H; calcd for $C_{19}H_{26}NO_4$, 332.1862).

3.6. Lycopladine D (3)

Colorless amorphous solid; $[\alpha]_D^{23}$ −17° (*c* 1.0, MeOH); IR (neat) ν_{max} 3380 and 1780 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); ESI-MS *m/z* 278 $(M+H)^+$; HRESIMS *m/z* 278.1773 (M+H; calcd for $C_{16}H_{24}NO_3$, 278.1756).

3.7. Lyconadin B (4)

Colorless amorphous solid; $[\alpha]_D^{23}$ −66° (*c* 0.5, MeOH); IR (neat) ν_{max} 3220, 2920, and 1670 cm^{-1}; ^1H and ^{13}C NMR data (Table 3); ESI-MS *m/z* 259 $(M+H)^+$; HRESIMS *m/z* 259.1805 (M+H; calcd for $C_{16}H_{23}N_2O$, 259.1810).

3.8. (*R*)- and (*S*)-MTPA esters of lycopladine B (1)

To a solution of **1** (0.1 mg) in CH_2Cl_2 (50 μL) were added (*R*)-MTPACl (0.8 μL), triethylamine (1.0 μL), and *N*,*N*-demethyl-aminopyridine (20 μg). The mixture was allowed to stand at room temperature for 31 h. After evaporation of the solvent, the residue was applied to a silica gel column to give the (*S*)-MTPA ester of **1**. The (*R*)-MTPA ester of **1** was prepared according to the same procedure as described above.

(*S*)-MTPA ester of **1**: ^1H NMR (CD_3OD) δ6.52 (1H, H-8), 6.18 (1H, H-14), 4.85 (1H, H-5), 3.76 (1H, H-17), 3.18 (1H, H-1a), 3.06 (1H, H-1b), 3.03 (2H, H-9a), 2.75 (1H, H-7), 2.17 (1H, H-6a), 2.11 (1H, H-6b), 2.09 (1H, H-4), 1.93 (1H, H-11a), 1.91 (1H, H-10a), 1.90 (1H, H-3a), 1.86 (1H, H-2a), 1.73 (1H, H-11b), 1.69 (1H, H-2b), 1.53 (1H, H-10b), 1.34 (1H, H-3b); ESI-MS *m/z* 506 $(M+H)^+$; HRESIMS *m/z* 506.2159. (M+H; calcd for $C_{27}H_{31}NO_5F_3$, 506.2154).

(*R*)-MTPA ester of **1**: ^{1}H NMR (CD_3OD) δ6.53 (1H, H-8), 6.17 (1H, H-14), 4.85 (1H, H-5), 3.76 (1H, H-17), 3.15 (1H, H-1a), 3.03 (1H, H-1b), 3.01 (2H, H-9a), 2.81 (1H, H-7), 2.23 (1H, H-6a), 2.20 (1H, H-6b), 1.98 (1H, H-4), 1.84 (1H, H-11a), 1.81 (1H, H-10a), 1.77 (1H, H-2a), 1.75 (1H, H-3a), 1.72 (1H, H-11b), 1.62 (1H, H-2b), 1.50 (1H, H-10b), 1.27 (1H, H-3b); ESI-MS *m*/*z* 506 $(M+H)^+$; HRESIMS *m*/*z* 506.2160. (M+H; calcd for $C_{27}H_{31}NO_5F_3$, 506.2154).

3.9. (*R*)- and (*S*)-MTPA esters of lycopladine D (3)

The (*S*)- and (*R*)-MTPA esters of **3** were prepared according to the same procedure as described above.

(*S*)-MTPA ester of **3**: ^{1}H NMR (CD_3OD) δ4.78 (1H, H-5), 3.35 (1H, H-1a), 3.19 (1H, H-9a), 3.04 (1H, H-9b), 2.88 (1H, H-1b), 2.76 (1H, H-15), 2.63 (1H, H-14a), 2.46 (1H, H-4), 2.27 (1H, H-6a), 2.13 (1H, H-8a), 2.02 (1H, H-14b), 1.95 (1H, H-2a), 1.92 (1H, H-3a), 1.87 (1H, H-7), 1.82 (1H, H-10a), 1.69 (1H, H-8b), 1.54 (1H, H-2b), 1.52 (1H, H-3b), 1.38 (1H, H-10b), 1.31 (1H, H-6b); ESI-MS *m*/*z* 494 $(M+H)^+$; HRESIMS *m*/*z* 494.2160. (M+H; calcd for $C_{26}H_{31}NO_5F_3$, 494.2154).

(*R*)-MTPA ester of **3**: ^{1}H NMR (CD_3OD) δ4.78 (1H, H-5), 3.41 (1H, H-1a), 3.21 (1H, H-9a), 3.06 (1H, H-9b), 2.92 (1H, H-1b), 2.74 (1H, H-15), 2.64 (1H, H-14a), 2.51 (1H, H-4), 2.24 (1H, H-6a), 2.12 (1H, H-8a), 2.02 (1H, H-14b), 2.01 (1H, H-2a), 2.01 (1H, H-3a), 1.87 (1H, H-7), 1.87 (1H, H-10a), 1.71 (1H, H-2b), 1.66 (1H, H-8b), 1.63 (1H, H-3b), 1.40 (1H, H-10b), 1.16 (1H, H-6b); ESI-MS *m*/*z* 494 $(M+H)^+$; HRESIMS *m*/*z* 494.2170. (M+H; calcd for $C_{26}H_{31}NO_5F_3$, 494.2154).

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