Lyconadins D and E, and complanadine E, new *Lycopodium* alkaloids from *Lycopodium complanatum*

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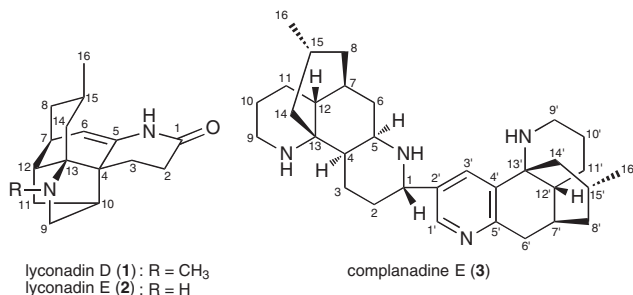
ABSTRACT

Three new *Lycopodium* alkaloids, lyconadins D (1) and E (2), and complanadine E (3), were isolated from the club moss *Lycopodium complanatum*. Lyconadin D (1) was the first example of fastigiatine-type alkaloid isolated from *Lycopodium complanatum*. The structures and relative stereochemistry of 1–3 were elucidated on the basis of spectroscopic data. Complanadine E (3) enhanced mRNA expression for NGF.

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1. Introduction

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 fastigiatine[6]-type alkaloids, lyconadins D (1) and E (2), and a new dimeric alkaloid, complanadine E (3), were isolated from the club moss *Lycopodium complanatum*. In this paper, we describe the isolation and structure elucidation of 1–3.

lyconadin D (1) : R = CH₃
lyconadin E (2) : R = H

complanadine E (3)

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2. Results and discussion

The club moss *L. complanatum* (2.33 kg) collected at Nayoro in Hokkaido was extracted with MeOH aq, and the extracts were partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, adjusted at pH 9 with satd Na_2CO_3 aq, were partitioned with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (*n*-hexane/EtOAc, and then $CHCl_3$/MeOH/H₂O). A fraction eluted with *n*-hexane/EtOAc and 100% $CHCl_3$ was purified by silica gel columns to afford lyconadin D (1, 0.00012%). A fraction eluted from an amino silica gel column with 100% $CHCl_3$ was purified by silica gel columns to afford lyconadin E (2, 0.00001%). A part of $CHCl_3$-soluble materials was purified by a Sephadex LH-20 column ($CHCl_3$/MeOH), a silica gel column, and a C_{18} HPLC to give complanadine E (3, 0.00025%).

Lyconadin D (1) showed the pseudomolecular ion peak at *m/z* 273 (M+H)⁺ in the FABMS, and the molecular formula, $C_{17}H_{24}N_2O$, was established by HRFABMS [*m/z* 273.1949, (M+H)⁺, Δ −1.8 mmu]. IR absorptions implied the presence of amide carbonyl (1670 cm⁻¹) functionality. ¹H and ¹³C NMR data (Table 1) and the HMQC spectrum revealed the existence of 17 carbons due to one carbonyl carbon, one sp² quaternary carbon, one sp² methine, two sp³ quaternary carbons, four sp³ methines, six sp³ methylenes, and two methyl groups. Among them, one sp³ quaternary carbon (δ_C 65.6), one sp³ methylene carbon (δ_C 59.5), and one methyl carbon (δ_C 35.0) were attributed to those attached to a nitrogen atom.

Table 1
^{1}H and ^{13}C NMR data of lyconadins D (**1**) and E (**2**) in CDCl$_3$ at 300 K[a]

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1		170.4		169.7
2a	2.52 (1H, ddd 17.4, 4.2, 1.8 Hz)	29.7	2.57 (1H, br d 18.0 Hz)	29.7
2b	2.27 (1H, dd 14.4, 5.4 Hz)		2.32 (1H, ddd 18.0, 18.0, 5.4 Hz)	
3a	2.03 (1H, ddd 13.8, 4.8, 2.4 Hz)	19.6	1.79 (1H, m)	19.5
3b	1.67 (1H, td 14.4, 4.8 Hz)		1.67 (1H, m)	
4		49.7		48.4
5		136.8		136.7
6	4.70 (1H, d 5.4 Hz)	103.8	4.63 (1H, d 5.4 Hz)	103.4
7	2.32 (1H, m)	39.2	2.29 (1H, m)	39.4
8a	1.49 (1H, br d)	37.8	1.54 (1H, m)	38.0
8b	0.92 (1H, dt 12.6, 3.0 Hz)		1.03 (1H, ddd 13.2, 13.2, 3.0 Hz)	
9a	3.29 (1H, dt 9.0, 3.0 Hz)	59.5	3.06 (1H, ddd 9.6, 3.0, 3.0 Hz)	49.7
9b	2.22 (1H, d 9.0 Hz)		2.74 (1H, d 9.6 Hz)	
10	2.15 (1H, m)	42.0	2.31 (1H, m)	43.6
11a	1.56 (1H, m)	33.8	1.60 (1H, m)	33.3
11b	1.36 (1H, dd 12.6, 7.8 Hz)		1.55 (1H, m)	
12	2.13 (1H, m)	38.6	1.80 (1H, m)	46.8
13		65.6		63.0
14a	1.58 (1H, dd 14.4, 4.8 Hz)	34.5	1.71 (1H, m)	36.9
14b	1.15 (1H, br t 13.2 Hz)		1.11 (1H, dd 12.6, 12.6 Hz)	
15	1.95 (1H, m)	24.7	1.90 (1H, m)	24.9
16	0.86 (3H, d 6.6 Hz)	22.3	0.88 (3H, d 6.6 Hz)	22.2
17	2.29 (3H, s)	35.0		
NH	8.89 (1H, s)		7.71 (1H, s)	

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

The gross structure of **1** was elucidated by analysis of 2D NMR data including the ^{1}H–^{1}H COSY, TOCSY, HMQC, and HMBC spectra (Fig. 1). The ^{1}H–^{1}H COSY and TOCSY spectra of **1** disclosed two structural units **a** (C-2–C-3) and **b** (C-6–C-8, C-9–C-12, and C-14–C-16). Connectivities of C-9, C-13, and C-17 through N-9 were revealed by HMBC correlations for H$_3$-17 to C-9 (δ_C 59.5) and C-13 (δ_C 65.6).

HMBC cross-peaks of H$_2$-2 (δ_H 2.52, 2.27) and H-3a (δ_H 2.03) to C-1 (δ_C 170.4) and N–H (δ_H 8.89) to C-2 (δ_C 29.7) indicated the connectivity of C-1 and C-2. HMBC correlations for H-2a to C-4 (δ_C 49.7) and H-3a to C-4, C-5 (δ_C 136.8), and C-10 (δ_C 42.0) suggested that C-3 (δ_C 19.6), C-5, and C-10 were connected via C-4. Connectivities of C-4, C-12 (δ_C 38.6), and C-14 (δ_C 34.5) through C-13 and connectivity of C-5 and N-1 were elucidated by HMBC correlations for H-14a (δ_H 1.58) to C-12, H-14b (δ_H 1.15) to C-4 and C-13, and N–H to C-4. An HMBC correlation for H-6 (δ_H 4.70) to C-5 revealed the connectivity of C-5 and C-6 (δ_C 103.8). Thus, the gross structure of lyconadin D was elucidated to be **1** (Fig. 1).

The NOESY spectrum of **1** showed cross-peaks as shown in Figure 2. The chair form of the cyclohexane ring (C-7–C-8 and C-12–C-15) was revealed from NOESY correlations for H-8b/H-12 and H-12/H-14b. The methyl group at C-15 was assigned as

equatorial by ^3J value (13.2 Hz) between H-14b and H-15. NOESY cross-peaks of H$_2$-2/H-3a, H-3a/H-9a, H-7/H-12, H-9b/H-11b, and H-14b/H$_3$-17 indicated that the relative stereochemistry of **1** was the same as that of fastigiatine.[6]

Thus, the relative stereochemistry of lyconadin D was assigned as **1** (Fig. 2).

Lyconadin E (**2**) showed the pseudomolecular ion peak at m/z 259 (M+H)$^+$ in the ESIMS, and the molecular formula, C$_{16}$H$_{22}$N$_2$O, was established by HRFABMS [m/z 259.1807, (M+H)$^+$, Δ +0.2 mmu]. IR absorptions implied the presence of amide carbonyl (1670 cm^{-1}) functionality. ^{1}H and ^{13}C NMR data of **2** was closed to those of **1** except for lack of signals for an N-methyl group (Table 1). Analyzes of 2D NMR data including the ^{1}H–^{1}H COSY, TOCSY, HMQC, and HMBC spectra revealed that **2** was des-N-methyl form of **1**. Inspection of NOESY spectrum of **2** suggested that the relative stereochemistry of **2** was the same as that of **1**. Thus, the structure of lyconadin E, including relative stereochemistry, was assigned as **2**.

Complanadine E (**3**) showed the pseudomolecular ion peak at m/z 489 (M+H)$^+$ in the ESIMS, and the molecular formula, C$_{32}$H$_{48}$N$_4$, was established by HRESIMS [m/z 489.3945, (M+H)$^+$,
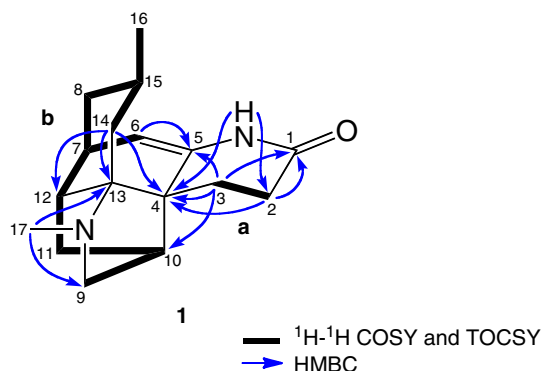
Figure 1. Selected 2D NMR correlations for lyconadin D (**1**).
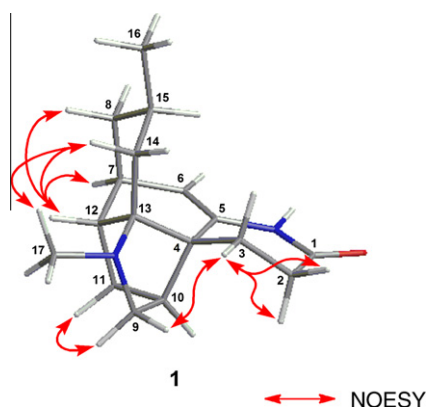
Figure 2. Selected NOESY correlations and relative stereochemistry for lyconadin D (**1**).

Δ −1.2 mmu]. IR absorptions implied the presence of amino or hydroxy (3285 cm^{-1}) functionality. ^{1}H and ^{13}C NMR data (Table 2) and the HMQC spectrum revealed the existence of 32 carbons due to three sp^2 quaternary carbons, two sp^2 methines, two sp^3 quaternary carbons, nine sp^3 methines, fourteen sp^3 methylenes, and two methyl groups. Among them, two sp^3 quaternary carbons (δ_C 60.6, 62.7), two sp^3 methine carbons (δ_C 50.3, 53.1), and two sp^3 methylene carbons (δ_C 41.4, 41.9) were attributed to those attached to nitrogen atoms.

The gross structure of **3** was elucidated by analysis of 2D NMR data including the ^{1}H–^{1}H COSY, TOCSY, HMQC, HMQC-TOCSY, and HMBC spectra (Fig. 3). The ^{1}H–^{1}H COSY, TOCSY, and HMQC-TOCSY spectra of **3** disclosed four structural units **a** (C-1–C-7), **b** (C-9–C-12), **c** (C-8, C-14–C-16), and **d** (C-6′–C-12′, C-14′–C-16′). HMBC correlations for H-5 (δ_H 4.59) to C-1 (δ_C 53.1) and H-9 (δ_H 3.28) to C-13 (δ_C 60.6) indicated the connectivity of C-1 and C-5 through N-1, and the connectivities of C-9 and C-13 through N-9. Connectivities of C-7 (δ_C 33.2) and C-8 (δ_C 43.2) were revealed by HMBC cross-peaks of H-8b (δ_H 1.30) to C-6 (δ_C 24.8). HMBC correlations for H-14a (δ_H 2.14) to C-12 (δ_C 45.7) and C-13, H-14b (δ_H 1.20) to C-4 (δ_C 34.3), and H-7 (δ_H 2.33) to C-13 (δ_C 60.6) suggested that C-7 connected with C-12, and C-4, C-12, and C-14 (δ_C 45.1) was connected via C-13. HMBC cross-peaks of H-9′a (δ_H 3.29) to C-13′ (δ_C 62.7) indicated the connectivity of C-9′ and C-13′ through N-9′. Connectivities of C-4′ (δ_C 131.3), C-12′ (δ_C 42.4) and C-14′ (δ_C 48.1) via C-13′ were revealed by HMBC correlations for H-7′ (δ_H 2.37) and H-14′b (δ_H 1.64) to C-13′, and H-14′b to C-4′. HMBC cross-peaks of H$_2$-6′ (δ_H 3.30, 2.84) to C-4′ and C-5′ (δ_C 160.5) indicated the connectivity of C-5′ and C-6′ (δ_C 34.8). HMBC correlations for H-1′ (δ_H 8.68) to C-2′ (δ_C 133.2) and C-5′ (δ_C 160.5), and H-3′ (δ_H 8.45) to C-1′ (δ_C 149.1), C-5′, and C-13′ suggested the presence of a tri-substituted pyridine. The HMBC cross-peak of H-3′ to C-1 (δ_C 53.1) revealed the connection between C-1 and C-2′. Thus, the gross structure of complanadine E was elucidated to be **3** (Fig. 3).

The NOESY spectrum of **3** showed cross-peaks as shown in Figure 4. The chair form of the cyclohexane ring (C-7–C-8 and C-12–C-15) was revealed from a NOESY cross-peak of H-8b/H-12. The methyl group at C-15 was assigned as equatorial by a NOESY
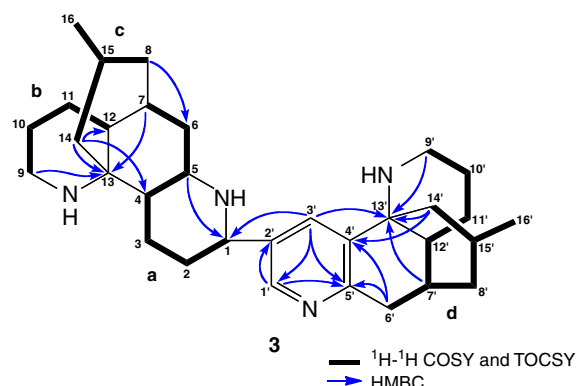

Figure 3. Selected 2D NMR correlations for complanadine E (**3**).

correlation for H-8b/H$_3$-16. NOESY cross-peaks of H-4/H-9 and H-5/H-11b suggested that a decahydroquinoline ring (C-7–C-15, N-9) was trans-fused, and the piperidine ring (C-9–C-13, N-9) and the cyclohexane ring (C-4–C-7, C-12–C-13) were chair form and boat form, respectively. A NOESY correlation for H-1/H-6b and ^3J values (10.2 and 3.6 Hz) between H-1 and H$_2$-2 revealed that C-2′ was assigned as equatorial. Thus, the relative stereochemistry of C-1–C-16 part was assigned as Figure 4. Inspection of the NOESY spectrum revealed that the relative stereochemistry of the C-1′–C-16′ part was the same as that of lycodine.[7]

Lyconadins D (**1**) and E (**2**) were the first new fastigiatine[6]-type alkaloids from *L. complanatum*. Complanadine E (**3**) was a new unsymmetrical dimeric alkaloid, which was the first example forming a piperidine ring at C-1–C-5 and N-1 of lycodine-type alkaloids.

Effects of lyconadins D (**1**) and E (**2**), and complanadines A,[8a] B,[8b] D,[8c] and E (**3**) on neurotrophic factor biosynthesis in 1321N1 human astrocytoma cells were examined by a quantitative RT-real time PCR method.[9] Among complanadines, complanadine A showed the strongest NGF expression (3.9-fold at 100 μM) and the effects were concentration-dependent. Complanadines B, D,

Table 2
^{1}H and ^{13}C NMR data of complanadine E (**3**) in CD$_3$OD at 300 K[a]

Position	δ_H	δ_C	Position	δ_H	δ_C
1	4.56 (1H, dd 10.2, 3.6 Hz)	53.1	1′	8.68 (1H, s)	149.1
2a	2.21 (1H, m)	30.1	2′		133.2
2b	2.13 (1H, m)				
3a	2.19 (1H, m)	21.7	3′	8.45 (1H, s)	133.8
3b	2.13 (1H, m)				
4	2.77 (1H, m)	34.3	4′		131.3
5	4.59 (1H, m)	50.3	5′		160.5
6a	2.23 (1H, m)	24.8	6′a	3.30 (1H, m)	34.8
6b	1.89 (1H, m)		6′b	2.84 (1H, d 19.2 Hz)	
7	2.33 (1H, m)	33.2	7′	2.37 (1H, m)	33.8
8a	1.82 (1H, m)	43.2	8′a	1.88 (1H, m)	43.3
8b	1.30 (1H, m)		8′b	1.47 (1H, ddd 12.6, 12.6, 3.6 Hz)	
9	3.28 (2H)	41.4	9′a	3.29 (1H, m)	41.9
			9′b	2.92 (1H, ddd 12.0, 12.0, 4.2 Hz)	
10a	1.98 (1H, m)	23.3	10′	1.89 (2H)	23.8
10b	1.74 (1H, m)				
11a	1.83 (1H, m)	28.4	11′a	1.74 (1H, m)	25.0
11b	1.74 (1H, m)		11′b	1.33 (1H, m)	
12	1.63 (1H, m)	45.7	12′	2.10 (1H, m)	42.4
13		60.6	13′		62.7
14a	2.14 (1H, m)	45.1	14′a	1.90 (1H, m)	48.1
14b	1.20 (1H, m)		14′b	1.64 (1H, m)	
15	2.34 (1H, m)	26.9[b]	15′	1.20 (1H, m)	27.0[b]
16	1.06 (3H, d 6.0 Hz)	22.2	16′	0.86 (3H, d 6.0 Hz)	21.6

[a] ^{1}H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively.
[b] Exchangeable.
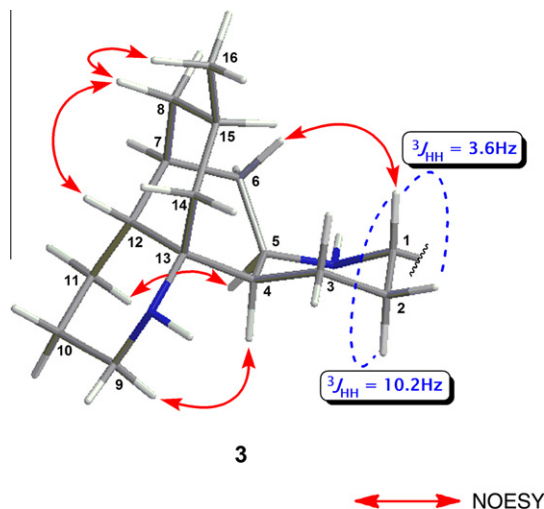
Figure 4. Selected NOESY correlations and relative stereochemistry for C-1–C-16 part of complanadine E (**3**).

and E (**3**) induced NGF expression level by 2.3-, 2.4-, and 2.8-fold, respectively (Fig. 5). Lyconadins D (**1**) and E (**2**) did not show such significant activity.

3. Experimental

3.1. General experimental procedures

Optical rotation was recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on a JASCO FT/IR-230 and a Shimadzu UV-1600PC spectrophotometer, respectively. ^{1}H-, ^{13}C-, and 2D-NMR spectra were recorded on a Bruker AMX-600 and a JEOL ECA-500 spectrometers. Each NMR sample of lyconadins D (**1**) and E (**2**), and complanadine E (**3**) was prepared by dissolving in 50 μl of CDCl$_3$ or CD$_3$OD in 2.5 mm micro cells (Shigemi Co. Ltd) and chemical shifts were reported using residual CDCl$_3$ (δ_H 7.26 and δ_C 77.0) and CD$_3$OD (δ_H 3.31 and δ_C 49.0) as internal standard. Standard pulse sequences were employed for the 2D NMR experiments (Bruker AMX-600). ^{1}H–^{1}H COSY, TOCSY, 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 TOCSY 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 in the phase sensitive mode and HMBC spectra, a total of 256 increments of 1 K data points were collected. For HMBC spectra with Z-axis PFG, 20, 50, and 80 ms delay time were 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. For HMQC spectra in the phase sensitive mode (JEOL ECA-500), a total of 128 increments of 1K data points were collected. FAB mass spectra were obtained on a JEOL JMS-HX110 spectrometer (glycerol and 3-nitrobenzyl alcohol as matrix). ESI mass spectra were obtained on a JEOL JMS-700TZ spectrometer and a Shimadzu ESIMS LCMS-QP-8000 spectrometer.

3.2. Plant material

The club moss *L. complanatum* was collected in Nayoro, Hokkaido in Japan, in 2007. The botanical identification was made by Dr. T. Shibata, Hokkaido Division, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation. A voucher specimen has been deposited in the herbarium of Hokkaido University.

3.3. Extraction and isolation

The club moss *L. complanatum* (2.33 kg) collected at Nayoro in Hokkaido was extracted with MeOH aq, and the extracts were partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, adjusted at pH 9 with sat. Na$_2$CO$_3$ aq, were partitioned with CHCl$_3$. CHCl$_3$-soluble materials were subjected to an amino silica gel column (*n*-hexane/EtOAc, 1:0 → 1:1, CHCl$_3$/MeOH, 1:0 → 1:1, and then CHCl$_3$/MeOH/H$_2$O, 6:4:1). The HMQC spectrum (CDCl$_3$) of the fraction eluted with *n*-hexane/EtOAc (1:1) and CHCl$_3$ showed cross-peaks at δ_H 3.29/δ_C 59.4 and δ_H 4.70/δ_C 103.0,[10] which did not ascribe to any known alkaloids from *L. complanatum*, indicating the existence of a new alkaloid. Therefore, the fraction was further purified by a silica gel column (CHCl$_3$/MeOH, 1:0 → 4:1). The fraction eluted with CHCl$_3$/MeOH (14:1) was separated by a silica gel column (CHCl$_3$/acetone/MeOH, 1:0:0 → 0:1:0 → 0:0:1) to afford lyconadin D (**1**, 0.00012%). The fraction eluted from an amino silica gel column with CHCl$_3$ was purified with a silica gel column (CHCl$_3$/MeOH, 1:0 → 1:1).

The fraction eluted with CHCl$_3$/MeOH (1:1) was further purified by a silica gel column (acetone/MeOH, 1:0 → 1:4) to afford lyconadin E (**2**, 0.00001%). A part of CHCl$_3$-soluble materials was subjected to a Sephadex LH-20 column (CHCl$_3$/MeOH, 1:1) to obtain seven fractions. The 3rd–5th fractions were separated with a silica gel FL100D (Fuji Silysia) column (CHCl$_3$/MeOH, 1:0 → 1:1, and then CHCl$_3$/MeOH/H$_2$O/TFA, 6:4:1:0 → 6:4:1:0.01). The fraction eluted with CHCl$_3$/MeOH/H$_2$O/TFA (6:4:1:0.01) was further purified by a C$_{18}$ HPLC (MeCN/H$_2$O/TFA, 14:86:0.1) to give complanadine E (**3**, 0.00025%).

3.4. Lyconadin D (1)

Colorless amorphous solid; $[\alpha]_D^{22}$ +76 (*c* 0.52, MeOH); IR (film) ν_{max} 2920, 1670, and 1460 cm^{-1}; UV (MeOH) λ_{max} 240 (ε 6800) nm; FABMS *m/z* 273 (M+H)$^+$; HRFABMS *m/z* 273.1949 [calcd for C$_{17}$H$_{25}$N$_2$O (M+H)$^+$, 273.1967]; ^{1}H and ^{13}C NMR data (Table 1).

3.5. Lyconadin E (2)

Colorless amorphous solid; $[\alpha]_D^{23}$ +26 (*c* 0.11, MeOH); IR (film) ν_{max} 2920, 1670, and 1460 cm^{-1}; UV (MeOH) λ_{max} 240 (ε 5200) nm; ESIMS *m/z* 259 (M+H)$^+$; HRESIMS *m/z* 259.1807 [calcd for C$_{16}$H$_{23}$N$_2$O (M+H)$^+$, 259.1805]; ^{1}H and ^{13}C NMR data (Table 1).
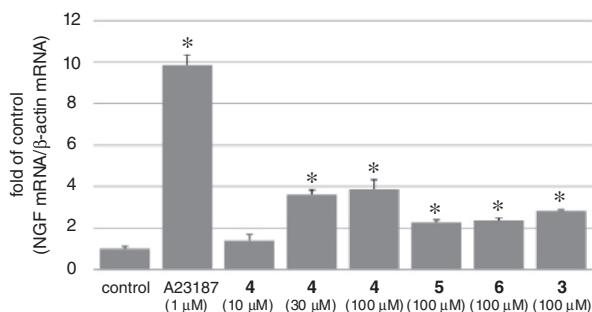
Figure 5. Effects of complanadines A (**4**), B (**5**), D (**6**), and E (**3**) on the NGF gene expression in 1321N1 cells. Values are means ± standard error of mean of three independent experiments. Calcium ionophore A23187 was used as a positive control. *P <0.05 versus control (without drug).

3.6. Complanadine E (3)

Colorless amorphous solid; $[\alpha]_D^{23}$ +3 (*c* 0.79, MeOH); IR (film) ν_{max} 3285, 2920, 1682, and 1455 cm^{-1}; UV (MeOH) λ_{max} 274 (ε 5000) nm; ESIMS *m/z* 489 (M+H)$^+$; HRESIMS *m/z* 489.3945 [calcd for $C_{32}H_{49}N_4$ (M+H)$^+$, 489.3957]; ^{1}H and ^{13}C NMR data (Table 2).

3.7. Quantitative RT-real time PCR method[9]

Total RNA from 1321N1 cells was extracted by using a total RNA extraction kit, and quantitative RT-real time PCR was carried out by using a real time PCR kit. NGF mRNA expression was examined as described previously.[9]

Acknowledgements

References and notes

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