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Cephalezomines A–F, Potent Cytotoxic Alkaloids from *Cephalotaxus harringtonia* var. *nana*

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Abstract—Six new alkaloids, cephalezomines A–F (**1**–**6**), have been isolated together with known related alkaloids **7**–**16** from the leaves of *Cephalotaxus harringtonia* var. *nana* and the structures were elucidated by spectroscopic data and chemical methods. Cephalezomines A–F (**1**–**6**) showed potent cytotoxicity against tumor cells.

Cephalotaxus alkaloids such as cephalotaxine (**9**) and harringtonine (**13**) are a family of cytotoxic alkaloids from higher plants of the genus *Cephalotaxus*, some of which showed potent antileukemic activity by intraperitoneal injection to mice.[1] Recently, clinical studies of **13** in China have shown that it has a certain effect on various types of acute leukemia by intravenous administration.[2] Furthermore, patients who had become resistant to treatment with other chemotherapeutic drugs have been reported to respond to treatment with the cephalotaxine esters.[3] In our search for structurally unique and bioactive alkaloids from northern plants in Hokkaido, we have isolated six new cytotoxic alkaloids, cephalezomines A–F (**1**–**6**), from the leaves of *Cephalotaxus harringtonia* var. *nana*. In this paper we describe the isolation and structure elucidation of **1**–**6**, and the cytotoxicity of **1**–**6** and known related alkaloids **7**–**16** (Scheme 1).

The leaves of *C. harringtonia* var. *nana* collected in Sapporo were extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials were adjusted at pH 9 with sat. Na_2CO_3 and partitioned with $CHCl_3$. $CHCl_3$-soluble materials were subjected to a C_{18} column (MeOH/0.03 M $(NH_4)_2CO_3$, 3:7→1:0) followed by C_{18} HPLC (20–50% CH_3CN/0.03 M $(NH_4)_2CO_3$) and/or a silica gel column ($CHCl_3$/MeOH, 10:1) to afford cephalezomines A (**1**, 0.0001%), B (**2**, 0.00005%), C (**3**, 0.002%), D (**4**, 0.0003%), E (**5**, 0.0001%), and F (**6**, 0.0002%) as colorless solid together with known related alkaloids, drupacine (**7**),[4] demethylcephalotaxinone (**8**),[5] cephalotaxine (**9**),[6] 11-hydroxycephalotaxine (**10**),[4] epischellhammericine B (**11**),[7] 3-epischellhammericine (**12**),[8] harringtonine (**13**),[9] homodeoxyharringtonine (**14**),[10] deoxyharringtonine (**15**),[9] and isoharringtonine (**16**).[9]

Cephalezomine A (**1**) showed the pseudomolecular ion at *m/z* 562 $(M+H)^+$ in the FABMS, and the molecular formula, $C_{29}H_{39}NO_{10}$, was established by HRFABMS (*m/z* 562.2652, $(M+H)^+$, Δ −0.1 mmu). IR absorptions implied the presence of hydroxyl (3500 cm^{-1}) and ester carbonyl (1740 cm^{-1}) groups. Analysis of ^1H and ^{13}C NMR data (Tables 1 and 2) and the HMQC spectrum provided evidence that **1** possessed 29 carbon signals including 10 quaternary carbons (sp^2×6 and sp^3×4), 5 methines (sp^2×2 and sp^3×3), 10 methylenes, and 4 methyls. Among them, three sp^3 quaternary carbons (δ_c 108.04, 74.30, and 70.74), two methine (δ_c 78.16 and 74.77), and one methylene (δ_c 101.02) were ascribed to those bearing an oxygen atom, and the carbons at δ_c 173.91 and 170.87 were assigned to ester carbonyls. The ^1H–^1H COSY spectrum revealed the connectivities of C-3 to C-4, C-6 to C-8, C-10 to C-11, and C-1″ to C-3″. In the HMBC spectrum, long-range ^1H–^{13}C correlations (Fig. 1) indicated that **1** possessed a drupacine-type framework. HMBC cross-peaks of H-3′ to C-1′ and C-1″, H_3–OMe (5′) to C-4′, and H-5″ and H-6″ to C-3″ revealed the presence of the methyl ester of 2-hydroxy-isohexyanoylbutanedioic acid. The connectivity between C-3 and C-1′ was supported by HMBC correlations of H-3 and H-3′ to C-1′. Thus, the structure of cephalezomine A was assigned as **1**.

The relative stereochemistry of **1** was deduced from NOESY data as shown in Fig. 2. The hexacyclic core in **1** was elucidated to have the same relative stereochemistry as that of **7**.[4]

The CD spectrum ($[\theta]_{215}$=+2700, $[\theta]_{255}$=−3000, and $[\theta]_{290}$=+1600) of **1** showed Cotton effects similar to those ($[\theta]_{220}$=+1300, $[\theta]_{250}$=−6500, and $[\theta]_{290}$=+3000)

Keywords: alkaloids; cytotoxicity; *Cephalotaxus*; NMR.

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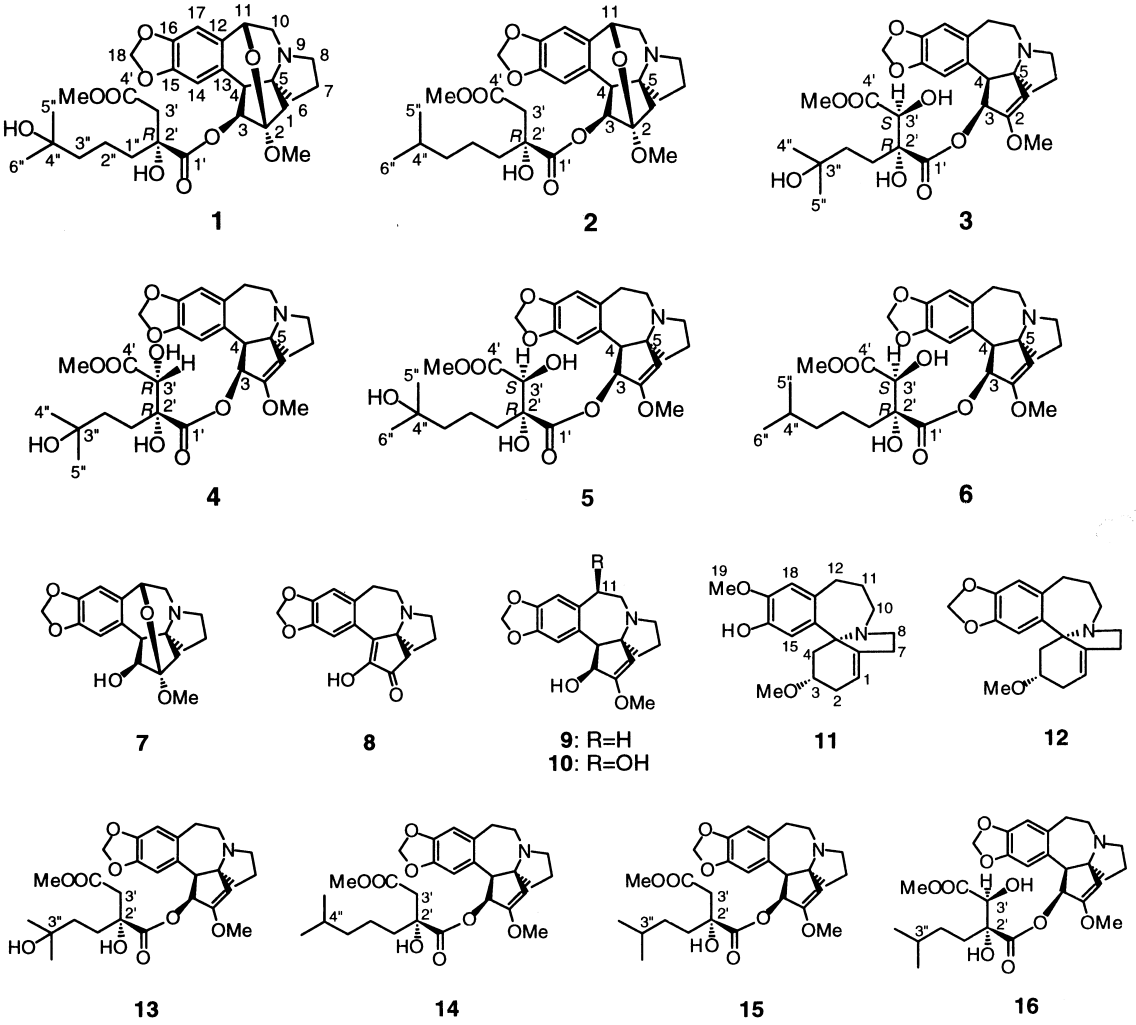

Scheme 1.

of drupacine (**7**). Furthermore, the CD spectrum for the molybdate complex of the dicarboxy acid moiety (C-1′–C-4′ and C-1″–C-6″), which was derived from the acid hydrolysates of **1**,[11] showed a negative Cotton effect at 270 nm. Therefore the absolute stereochemistry of **1** was elucidated to be 2*R*, 3*S*, 4*S*, 5*R*, 11*R* and 2′*R*.

The molecular formula of cephalezomine B (**2**) was determined to be $C_{29}H_{39}NO_9$ by HRFABMS (*m/z* 546.2715, $(M+H)^+$, Δ +1.1 mmu), which was smaller than that of **1** by one oxygen atom. The IR spectrum implied the presence of hydroxyl (3450 cm^{-1}) and ester (1740 cm^{-1}) functionalities. ^1H and ^{13}C NMR data (Tables 1 and 2) suggested that **2** had the same drupacine-type framework as **1**. The ^1H signals due to the two methyl groups (δ 0.82 and 0.83) of **2**, which resonated at higher field than the two methyl groups (δ 1.17 and 1.18) of **1**, were assigned as the terminal carbons (C-5″ and C-6″) of the side chain. Interpreting the ^1H–^1H COSY and HMBC spectra revealed a drupacine-type framework with the methyl ester of 2-hydroxy-isohexyl-butanedioic acid at C-3. Thus the structure of cephalezomine B was elucidated to be **2**. The relative stereostructure was elucidated by NOESY correlations which were almost the same as those observed for **1** (Fig. 2). The CD spectrum of **2** showed Cotton effects ($[\theta]_{215}$=+1200, $[\theta]_{250}$=−2700, and $[\theta]_{290}$=+870) similar to those of drupacine (**7**), and the CD spectrum for the molybdate complex of the dicarboxy acid part (C-1′–C-4′ and C-1″–C-6″) of **2** obtained by the same procedure as described for **1** exhibited a negative Cotton effect at 270 nm.[11] Hence the absolute stereochemistry of **2** was elucidated to be 2*R*, 3*S*, 4*S*, 5*R*, 11*R* and 2′*R*.

Cephalezomines C (**3**, $[\alpha]_D^{24}$=−122° (*c* 2.3, MeOH)) and D (**4**, $[\alpha]_D^{24}$=−97° (*c* 13.7, MeOH)) showed the same pseudomolecular ion at *m/z* 548 $(M+H)^+$, and each molecular formula, $C_{28}H_{37}NO_{10}$, was established by HRFABMS (**3**, *m/z* 548.2478, $(M+H)^+$, Δ −1.8 mmu; **4**, *m/z* 548.2495, $(M+H)^+$, Δ −0.1 mmu). IR absorptions of **3** and **4** were attributed to hydroxyl (3500 cm^{-1}) and ester carbonyl (1740 cm^{-1}) groups, respectively. The FABMS spectra of **3** and **4** showed a common fragment ion peak at *m/z* 298, characteristic for cephalotaxine-type skeleton with a side chain at C-3.[12] ^1H and ^{13}C NMR data (Tables 1 and 2) of **3** and **4** corresponded well to those of 3′S-hydroxyharringtonine.[13] The different CD spectra of **3** and **4** (**3**: $[\theta]_{220}$=−17500, $[\theta]_{247}$=+3600, and $[\theta]_{288}$=−2700; **4**: $[\theta]_{225}$=+19300 and $[\theta]_{288}$=−7000) suggested that the

Table 1. ^{1}H NMR data [δ_H (J, Hz)] of cepharezomines A–F (**1**–**6**) in $CDCl_3$ at 300 K (600 MHz)

Position	1	2	3	4	5	6
1a	1.55 d (14.0)	1.53 d (14.0)	5.10 s	5.09 s	5.08 s	5.07 s
1b	2.67 d (14.0)	2.66 d (14.0)				
3	5.23 d (9.5)	5.16 d (9.4)	6.00 d (9.7)	6.04 d (9.8)	5.99 d (9.8)	5.99 d (9.8)
4	3.56 d (9.5)	3.57 d (9.4)	3.82 d (9.7)	3.79 d (9.8)	3.80 d (9.8)	3.80 d (9.8)
6a	2.19 m	2.19 m	1.93 br m	1.94 br m	1.90 br m	1.90 br m
6b	2.04 m	2.03 m	2.06 br m	2.06 br m	2.04 m	2.03 m
7	1.77 m	1.76 m	1.78 br m	1.79 br m	1.77 br m	1.76 br m
8a	3.05 m	3.05 m	2.62 br m	2.66 br m	2.61 br m	2.60 br m
8b	2.41 dd (8.6, 17.5)	2.40 dd (8.6, 17.5)	3.14 br m	3.14 br m	3.09 m	3.10 m
10a	3.11 dd (4.9, 13.1)	3.10 dd (4.9, 12.9)	2.62 br m	2.66 br m	2.61 br m	2.62 br m
10b	2.97 d (13.1)	2.97 d (12.9)	2.98 br m	2.98 br m	2.96 m	2.97 m
11	4.86 d (4.9)	4.85 d (4.9)	2.44 br m	2.43 dd (6.6, 14.2)	2.42 dd (6.9, 14.2)	2.42 dd (6.7, 14.3)
			3.14 br m	3.14 br m	3.13 m	3.14 br m
14	6.45 s	6.44 s	6.61 s	6.55 s	6.61 s	6.61 s
17	6.78 s	6.64 s	6.64 s	6.65 s	6.65 s	6.65 s
18	5.87 s	5.78 s	5.85 s	5.85 s	5.84 s	5.84 s
	5.91 s	5.91 s	5.86 s	5.81 s	5.86 s	5.87 s
2-OMe	3.41 s	3.39 s	3.71 s	3.70 s	3.68 s	3.67 s
3′	1.96 d (16.5)	1.97 d (16.5)	3.48 br s	3.39 s	3.41 br s	3.40 br s
	2.29 d (16.5)	2.30 d (16.5)				
4′-OMe	3.67 s	3.66 s	3.76 s	3.61 s	3.74 s	3.74 s
1″	1.45 m	1.40 m	1.19 m	1.28 m	1.51 m	1.47 m
			1.55 dt (5.1, 13.0)	1.48 m	1.57 m	1.52 m
2″	1.45 m	1.31 m	1.67 dt (5.1, 13.0)	1.62 m	1.18 m	1.34 m
	1.15 m	1.08 m	1.77 m	1.99 m	1.41 m	1.10 m
3″	1.37 m	1.07 m			1.41 m	1.10 m
4″		1.47 m	1.14 s	1.16 s		1.45 m
5″	1.18 s	0.83 d (6.5)	1.17 s	1.17 s	1.18 s	0.84 d (6.5)
6″	1.17 s	0.82 d (6.5)			1.19 s	0.85 d (6.5)

absolute configurations at C-2′ and/or C-3′ were different from each other. To determine the absolute configuration at C-3′, **3** and **4** were converted into their (*S*)- and (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) esters. The values of $\Delta\delta$ [δ(*S*-MTPA ester)−δ(*R*-MTPA ester)] obtained from the ^{1}H NMR spectra of the MTPA esters suggested that the absolute configurations at C-3′ of **3** and **4** were *S* and *R*, respectively. Furthermore, the CD spectrum for the molybdate complex of each dicarboxy acid moiety (C-1′–C-4′ and C-1″–C-5″) derived from the acid hydrolysates of **3** and **4** showed a negative Cotton effect at 270 nm, indicating the absolute configurations at C-2′ of **3** and **4** were both *R*. On the other hand, that of **3** showed a positive Cotton effect at 215 nm, while that of **4** exhibited a negative one, supporting the different configurations at C-3′ of **3** and **4**.[14] Therefore the absolute stereochemistry of **3** was elucidated to be 3*S*, 4*S*, 5*R*, 2′*R*, and 3′*S*, and that of **4** to be 3*S*, 4*S*, 5*R*, 2′*R*, and 3′*R*.

Table 2. ^{13}C NMR data (δ_C) of cephlezomines A–F (**1**–**6**) in $CDCl_3$ at 300 K (125 MHz)

Position	1	2	3	4	5	6
1	35.87	36.03	101.01	100.88	100.94	100.55
2	108.04	107.63	156.51	157.49	157.18	157.52
3	74.77	74.83	75.50	74.70	74.65	74.75
4	57.07	57.08	55.86	55.84	55.98	55.88
5	65.75	65.79	70.53	70.81	70.64	70.75
6	43.33	43.34	43.18	43.27	43.44	43.34
7	22.33	22.33	20.25	20.30	20.35	20.32
8	53.99	54.02	53.84	53.87	53.94	53.88
10	56.78	56.91	48.52	48.48	48.60	48.54
11	78.16	78.18	31.12	31.26	31.40	31.78
12	131.54	131.69	132.88	133.33	132.97	133.14
13	130.54	130.44	127.83	128.22	128.35	128.08
14	111.05	111.10	112.94	112.75	112.86	112.82
15	146.90	146.88	148.98	146.77	146.99	146.98
16	145.95	145.96	146.23	145.75	146.13	146.13
17	107.63	107.61	109.84	109.97	109.82	109.85
18	101.02	101.00	101.01	100.88	100.94	100.93
2-OMe	52.10	51.66	57.41	57.33	57.48	57.27
1′	173.91	174.12	172.69	172.89	172.73	172.84
2′	74.30	74.52	79.64	79.17	79.53	79.63
3′	42.36	42.37	74.75	75.05	75.58	75.48
4′	170.87	170.94	171.30	171.60	171.32	171.43
4′-OMe	52.10	51.79	52.51	52.39	52.46	52.42
1″	39.41	39.06	37.22	36.65	34.62	34.44
2″	17.96	20.72	28.68	29.80	18.16	20.79
3″	43.46	38.92	70.15	70.35	43.95	39.08
4″	70.74	27.96	29.71	29.10	70.87	27.79
5″	29.24	22.53	28.48	29.15	29.35	22.39
6″	29.16	22.61			28.94	22.68

Cephalezomine E (**5**, $[\alpha]_D^{24}=-131°$ (*c* 2.9, MeOH)) showed

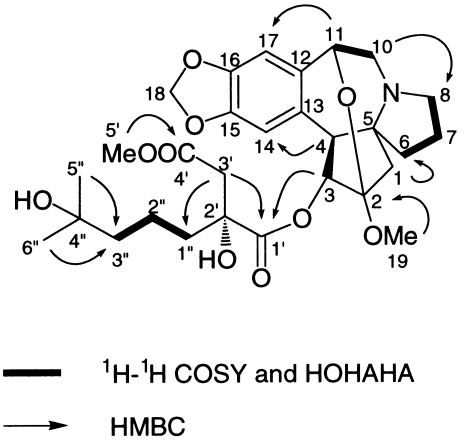

Figure 1. Selected 2D NMR correlations of cephalezomine A (**1**).

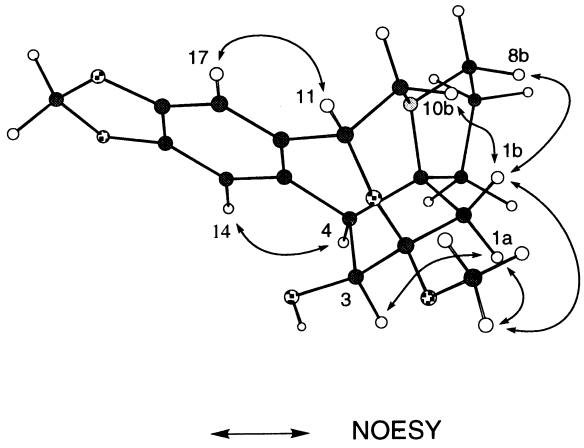

Figure 2. Relative stereochemistry of hexacyclic core in cephalezomine A (**1**).

the pseudomolecular ion at *m/z* 562 $(M+H)^+$ and the molecular formula, $C_{29}H_{39}NO_{10}$, was established by HRFABMS (*m/z* 562.2629, $(M+H)^+$, Δ −2.4 mmu), which was larger than those of **3** and **4** by a CH_2 unit. The molecular formula, $C_{29}H_{39}NO_9$, of cephalezomine F (**6**, $[\alpha]_D^{24}=-61°$ (*c* 0.2, MeOH)) was established by HRFABMS (*m/z* 546.2704 $(M+H)^+$, Δ 0.0 mmu)], which was smaller than that of **5** by one oxygen atom. In the FABMS spectra, **5** and **6** gave the fragment ion at *m/z* 298 characteristic of cephalotaxine-type skeleton with a side chain at C-3.[12] Detailed analyses of the ^1H–^1H COSY, HOHAHA, HMQC, and HMBC spectra of **5** and **6** led to assignments of cephalotaxine-type backbone like **3** and **4**, and of the side chains at C-3 corresponding to those of **1** and **2**, respectively. The CD spectra (**5**: $[\theta]_{215}=-26500$, $[\theta]_{250}=+2700$, and $[\theta]_{290}=-2900$; **6**: ($[\theta]_{215}=-17900$, $[\theta]_{245}=+2000$, and $[\theta]_{290}=-1700$) of **5** and **6** were similar to those of **3**. To determine the absolute configuration at C-3′, **5** and **6** were converted into their (*S*)- and (*R*)-MTPA esters. The values of $\Delta\delta$ obtained from the ^1H NMR spectra suggested that the absolute configurations at C-3′ of **5** and **6** were both *S*. Furthermore, the molybdate complex of the dicarboxy acid moiety (C-1′–C-4′ and C-1″–C-6″) derived from the acid hydrolysates of **5** and **6** showed a negative Cotton effect at 270 nm, indicating that the absolute configurations at C-2′ were both *R*. Thus, the absolute configurations of **5** and **6** were elucidated to be 3*S*, 4*S*, 5*R*, 2′*R*, and 3′*S*.

Table 3. Cytotoxicity of cephalezomines A–F (**1**–**6**) and known related alkaloids **7**–**16** against murine lymphoma L1210 and human epidermoid carcinoma KB cells

Compounds	IC_{50} (μg/ml)	
	L1210	KB
1	0.067	0.020
2	0.030	0.024
3	0.88	0.078
4	7.6	0.40
5	0.68	0.18
6	0.10	0.084
7	0.84	0.99
8	3.8	0.87
9	3.0	0.90
10	2.4	0.75
11	3.7	1.3
12	0.84	0.61
13	2.0	0.74
14	0.014	0.010
15	0.0082	0.0079
16	0.14	0.22

Cephalezomines A–F (**1**–**6**) are new *Cephalotaxus* alkaloids having drupacine- or cephalotaxine-type skeletons with different side chains at C-3, among which cephalezomine D (**4**) is the first alkaloid possessing 2′*R* and 3′*R*-configurations. The cytotoxicity of cephalezomines A–F (**1**–**6**) and known related alkaloids **7**–**16** against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells is shown in Table 3. These compounds belong to five groups, drupacine-type ones with a side chain (**1** and **2**) or without it (**7**), cephalotaxine-type ones with a side chain (**3**, **4**, **5**, **6**, **13**, **14**, **15**, and **16**) or without it (**8**, **9**, and **10**), and homoerythrina-type ones (**11** and **12**). In comparison of cytotoxicities among these compounds, drupacine- and cephalotaxine-type ones with a side chain (**1**–**6** and **13**–**16**) showed potent cytotoxicity, whereas those without a side chain (**7**–**12**) exhibited relatively weak cytotoxicity. It is noted that most of the alkaloids showed more potent cytotoxicity against KB cells rather than L1210 cells.

Experimental

General methods

^1H and 2D NMR spectra were recorded in $CDCl_3$ on a 600 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 125 MHz spectrometer. Chemical shifts were reported using residual $CDCl_3$ (δ_H 7.26 and δ_C 77.03) as internal standards. Standard pulse sequences were employed for the 2D NMR experiments. HMBC spectra were recorded using a 50 ms delay time for long-range C–H coupling with Z-axis PFG. NOESY spectra were measured with a mixing time of 800 ms. FABMS spectra were measured by using glycerol matrix.

Material

The leaves of *Cephalotaxus harringtonia* var. *nana* were collected in Sapporo (Hokkaido, Japan) in 1998. The botanical identification was made by Mr N. Yoshida, Faculty of Pharmaceutical Sciences, Hokkaido University. A voucher specimen has been deposited in the herbarium of Hokkaido University.

Extraction and isolation

The leaves of *C. harringtonia* var. *nana* (2.6 kg) were crashed and extracted with MeOH (10 L) three times to give a MeOH extract (546 g), which was treated with 3% tartaric acid to adjust pH 2 and then partitioned with EtOAc. The aqueous layer was treated with sat. aq. Na_2CO_3 to adjust pH 9 and extracted with $CHCl_3$ to give a crude alkaloidal fraction (5.7 g), which was subjected to C_{18} column chromatography (MeOH/0.03 M $(NH_4)_2CO_3$, 3:7→1:0) to yield four fractions **a**–**d**. The fraction **a** was purified by silica gel column ($CHCl_3$/MeOH, 10:1) followed by C_{18} HPLC (20% CH_3CN/0.03 M $(NH_4)_2CO_3$) to afford

drupacine (**7**, 0.01%), demethylcephalotaxine (**8**, 0.00003%), and cephalotaxine (**9**, 0.0001%). The fraction **b** was subjected to C_{18} HPLC (30% CH_3CN/0.03 M $(NH_4)_2CO_3$) and/or silica gel column ($CHCl_3$/MeOH/EtOAc, 9:1:2) to give cephalezomines A (**1**, 0.0001%), C (**3**, 0.0003%), D (**4**, 0.002%), and E (**5**, 0.0001%), epischellhammericine B (**11**, 0.0002%), and harringtonine (**13**, 0.0004%). The fraction **c** was subjected to C_{18} HPLC (50% CH_3CN/0.03 M $(NH_4)_2CO_3$) to afford cephalezomine F (**6**, 0.0002%) and isoharringntonine (**16**, 0.0002%). The fraction **d** was purified by silica gel column followed by C_{18} HPLC (50% CH_3CN/0.03 M $(NH_4)_2CO_3$) and/or silica gel column ($CHCl_3$/MeOH/EtOAc, 16:1:5) to afford cephalezomines B (**2**, 0.00005%) and F (**6**, 0.0002%), 3-epischellhammericine (**12**, 0.001%), homodeoxyharringtonine (**14**, 0.0005%), and deoxyharringtonine (**15**, 0.0003%).

Cephalezomine A (1). Colorless solid; $[\alpha]_D=-42°$ (*c* 0.9, MeOH); ^1H and ^{13}C NMR (Tables 1 and 2); FABMS *m/z* 562 $(M+H)^+$; HRFABMS *m/z* 562.2652 (M+H; calcd for $C_{29}H_{40}NO_{10}$, 562.2653); IR (neat) ν_{max} 3500, 2930, 1740, 1490, 1230, and 1040 cm^{-1}; UV (MeOH) λ_{max} 289 nm (ϵ 1900); CD (MeOH) $[\theta]_{215}=+2700$, $[\theta]_{225}=-6000$, $[\theta]_{255}=-3000$, and $[\theta]_{290}=+1600$.

Cephalezomine B (2). Colorless solid; $[\alpha]_D=-125°$ (*c* 4.9, MeOH); ^1H and ^{13}C NMR data (Tables 1 and 2); FABMS *m/z* 546 $(M+H)^+$; HRFABMS *m/z* 546.2715 (M+H; calcd for $C_{29}H_{40}NO_9$, 546.2704); IR (neat) ν_{max} 3450, 2920, 1740, and 1060 cm^{-1}; UV (MeOH) λ_{max} 291 nm (ϵ 1400); CD (MeOH) $[\theta]_{215}=+1200$, $[\theta]_{250}=-2700$, and $[\theta]_{290}=+870$.

Cephalezomine C (3). Colorless solid; $[\alpha]_D=-122°$ (*c* 2.3, MeOH); ^1H and ^{13}C NMR data (Tables 1 and 2); FABMS *m/z* 548 $(M+H)^+$; HRFABMS *m/z* 548.2478 (M+H; calcd for $C_{28}H_{38}NO_{10}$, 548.2496); IR (neat) ν_{max} 3500, 2960, 1740, 1490, and 1220 cm^{-1}; UV (MeOH) λ_{max} 291 nm (ϵ 2100); CD (MeOH) $[\theta]_{220}=-17500$, $[\theta]_{247}=+3600$, and $[\theta]_{288}=-2700$.

Cephalezomine D (4). Colorless solid; $[\alpha]_D=-97°$ (*c* 13.7 MeOH); ^1H and ^{13}C NMR data (Tables 1 and 2); FABMS *m/z* 548 $(M+H)^+$; HRFABMS *m/z* 548.2495 (M+H; calcd for $C_{28}H_{38}NO_{10}$, 548.2496); IR (neat) ν_{max} 3500, 2960, 1740, 1490, and 1220 cm^{-1}; UV (MeOH) λ_{max} 291 nm (ϵ 2100); CD (MeOH) $[\theta]_{225}=+19300$ and $[\theta]_{288}=-7000$.

Cephalezomine E (5). Colorless solid; $[\alpha]_D=-131°$ (*c* 2.9, MeOH); ^1H and ^{13}C NMR data (Tables 1 and 2); FABMS *m/z* 562 $(M+H)^+$; HRFABMS *m/z* 562.2629 (M+H; calcd for $C_{29}H_{40}NO_{10}$, 562.2653); IR (neat) ν_{max} 3500, 2960, 1740, 1490, and 1220 cm^{-1}; UV (MeOH) λ_{max} 291 nm (ϵ 2100); CD (MeOH) $[\theta]_{215}=-26500$, $[\theta]_{250}=+2700$, and $[\theta]_{290}=-2900$.

Cephalezomine F (6). Colorless solid; $[\alpha]_D=-61°$ (*c* 0.2, MeOH); ^1H and ^{13}C NMR data (Tables 1 and 2); FABMS *m/z* 546 $(M+H)^+$; HRFABMS *m/z* 546.2704 (M+H; calcd for $C_{29}H_{40}NO_9$, 546.2704); IR (neat) ν_{max} 3410, 2940, 1740, 1650, 1220, and 1030 cm^{-1}; UV (MeOH) λ_{max} 291 nm (ϵ 1800); CD (MeOH) $[\theta]_{215}=-17900$, $[\theta]_{245}=+2000$, and $[\theta]_{290}=-1700$.

(*R*)- and (*S*)-MTPA esters of cephalezomines C–F (3–6)

To a solution of **3** (0.5 mg) in CH_2Cl_2 (50 μl) was added (−)- or (+)-MTPACl (1.1 μl), triethylamine (1.3 μl) and *N*,*N*-dimethylamino pyridine (0.2 mg). The mixture was allowed to stand at room temperature for 2 h. *N*,*N*-Dimethylamino-1,3-propandiamine (1.0 μl) was added, and after evaporation of solvent, the residue was applied to a silica gel column ($CHCl_3$–MeOH, 19:1) to give the (*S*)-MTPA ester of **3** (0.6 mg, 87 %). The other (*S*)- and (*R*)-MTPA esters were prepared according to the same procedure as described above.

(*S*)-MTPA ester of 3. FABMS *m/z* 764 $(M+H)^+$; HRFABMS *m/z* 764.2905 (M+H; calcd for $C_{38}H_{45}NO_{12}F_3$, 764.2894); ^1H NMR ($CDCl_3$) δ 5.06 (s, H-1), 5.69 (d, 9.8, H-3), 3.66 (d, 9.8, H-4), 1.89 and 2.02 (m, H-6), 1.75 (m, H-7), 2.55 and 3.08 (m, H-8), 2.58 and 3.13 (m, H-10), 2.41 and 2.92 (m, H-11), 6.35 (s, H-14), 6.69 (s, H-17), 5.93 (s, H-18), 3.75 (s, 2-OMe), 3.99 (s, H-3′), 3.58 (s, 4′-OMe), 1.21 (m, H-1″), 1.55 (m, H-2″), 1.14 (s, H-4″), 1.14 (s, H-5″).

(*R*)-MTPA ester of 3. FABMS *m/z* 764 $(M+H)^+$; HRFABMS *m/z* 764.2857 (M+H; calcd for $C_{38}H_{45}NO_{12}F_3$, 764.2894); ^1H NMR ($CDCl_3$) δ 5.08 (s, H-1), 5.85 (d, 9.8, H-3), 3.64 (d, 9.8, H-4), 1.89 and 2.00 (m, H-6), 1.74 (m, H-7), 2.55 and 3.07 (m, H-8), 2.58 and 3.14 (m, H-10), 2.43 and 2.91 (m, H-11), 6.15 (s, H-14), 6.69 (s, H-17), 5.92 (s, H-18), 3.70 (s, 2-OMe), 4.08 (s, H-3′), 3.48 (s, 4′-OMe), 1.25 (m, H-1″), 1.64 (m, H-2″), 1.15 (s, H-4″), 1.15 (s, H-5″).

(*S*)-MTPA ester of 4. FABMS *m/z* 764 $(M+H)^+$; HRFABMS *m/z* 764.2806 (M+H; calcd for $C_{38}H_{45}NO_{12}F_3$, 764.2894); ^1H NMR ($CDCl_3$) δ 5.11 (s, H-1), 6.07 (d, 9.8, H-3), 3.80 (d, 9.8, H-4), 1.91 and 2.05 (m, H-6), 1.78 (m, H-7), 2.60 and 3.09 (m, H-8), 2.61 and 3.13 (m, H-10), 2.60 and 2.94 (m, H-11), 6.56 (s, H-14), 6.71 (s, H-17), 5.87 (s, H-18), 3.69 (s, 2-OMe), 4.77 (s, H-3′), 3.51 (s, 4′-OMe), 1.30 (m, H-1″), 1.52 (m, H-2″), 1.13 (s, H-4″), 1.13 (s, H-5″).

(*R*)-MTPA ester of 4. FABMS *m/z* 764 $(M+H)^+$; HRFABMS *m/z* 764.2899 (M+H; calcd for $C_{38}H_{45}NO_{12}F_3$, 764.2894); ^1H NMR ($CDCl_3$) δ 5.10 (s, H-1), 6.03 (d, 9.8, H-3), 3.80 (d, 9.8, H-4), 1.91 and 2.06 (m, H-6), 1.64 (m, H-7), 2.59 and 3.07 (m, H-8), 2.62 and 3.11 (m, H-10), 2.46 and 2.95 (m, H-11), 6.56 (s, H-14), 6.70 (s, H-17), 5.93 (s, H-18), 3.67 (s, 2-OMe), 4.87 (s, H-3′), 3.60 (s, 4′-OMe), 1.15 (m, H-1″), 1.41 (m, H-2″), 0.98 (s, H-4″), 0.98 (s, H-5″).

(*S*)-MTPA ester of 5. FABMS *m/z* 778 $(M+H)^+$; HRFABMS *m/z* 778.2999 (M+H; calcd for $C_{39}H_{47}NO_{12}F_3$, 778.3051); ^1H NMR ($CDCl_3$) δ 5.05 (s, H-1), 5.67 (d, 9.7, H-3), 3.65 (d, 9.7, H-4), 1.89 and 2.02 (m, H-6), 1.75 (m, H-7), 2.56 and 3.07 (m, H-8), 2.60 and 3.09 (m, H-10), 2.42 and 2.94 (m, H-11), 6.35 (s, H-14), 6.70 (s, H-17), 5.93 (s, H-18), 3.64 (s, 2-OMe), 3.91 (s, H-3′), 3.73 (s, 4′-OMe), 1.17 (s, H-5″), 1.17 (s, H-6″).

(*R*)-MTPA ester of 5. FABMS *m/z* 778 $(M+H)^+$;

HRFABMS *m/z* 778.3073 (M+H; calcd for $C_{39}H_{47}NO_{12}F_3$, 778.3051); ^{1}H NMR ($CDCl_3$) δ 5.07 (s, H-1), 5.81 (d, 9.8, H-3), 3.64 (d, 9.8, H-4), 1.89 and 2.02 (m, H-6), 1.74 (m, H-7), 2.58 and 3.08 (m, H-8), 2.60 and 3.12 (m, H-10), 2.43 and 2.94 (m, H-11), 6.20 (s, H-14), 6.69 (s, H-17), 5.92 (s, H-18), 3.69 (s, 2-OMe), 4.06 (s, H-3′), 3.65 (s, 4′-OMe), 1.17 (s, H-5″), 1.18 (s, H-6″).

(*S*)-MTPA ester of 6. FABMS *m/z* 762 $(M+H)^+$; HRFABMS *m/z* 762.3094 (M+H; calcd for $C_{39}H_{47}NO_{11}F_3$, 764.3102); ^{1}H NMR ($CDCl_3$) δ 5.04 (s, H-1), 5.66 (d, 9.7, H-3), 3.64 (d, 9.7, H-4), 1.89 and 2.03 (m, H-6), 1.75 (m, H-7), 2.58 and 3.09 (m, H-8), 2.59 and 3.09 (m, H-10), 2.59 and 2.95 (m, H-11), 6.35 (s, H-14), 6.69 (s, H-17), 5.92 (s, H-18), 3.63 (s, 2-OMe), 3.91 (s, H-3′), 3.73 (s, 4′-OMe), 1.37 (m, H-4″), 0.84 (d, 6.0, H-5″), 0.85 (d, 6.0, H-6″).

(*R*)-MTPA ester of 6. FABMS *m/z* 762 $(M+H)^+$; HRFABMS *m/z* 762.3148 (M+H; calcd for $C_{39}H_{47}NO_{11}F_3$, 764.3102), ^{1}H NMR ($CDCl_3$) δ 5.06 (s, H-1), 5.81 (d, 9.7, H-3), 3.63 (d, 9.7, H-4), 1.89 and 2.02 (m, H-6), 1.75 (m, H-7), 2.57 and 3.08 (m, H-8), 2.59 and 3.10 (m, H-10), 2.43 and 2.90 (m, H-11), 6.18 (s, H-14), 6.69 (s, H-17), 5.92 (s, H-18), 3.64 (s, 2-OMe), 4.04 (s, H-3′), 3.69 (s, 4′-OMe), 1.48 (m, H-4″), 0.85 (d, 6.0, H-5″), 0.85 (d, 6.0, H-6″).

Molybdate complexes of hydrolysates of cephalezomines A–F (1–6)

Each of compounds **1**–**6** (1 mg) was hydrolyzed with 3N HCl (1 ml) under reflux for 4 days. After cooling, 3 M NH_4OH was added and the alkaline phase was extracted with $CHCl_3$. Excess NH_4OH was neutralized and the solvent was evaporated under reduced pressure. The residue was used directly in the preparation of solution for CD measurement, which contained 3 mM each hydrolysates of **1**–**6** and 2.7 mM Na molybdate. HCl and NaOH solution were added until pH 2.9–3.1 was reached. Measurements of CD spectra were carried out in a 1 mm cell 5 days after the solution had been prepared.

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