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Senepodines B–E, new $C_{22}N_2$ alkaloids from *Lycopodium chinense*

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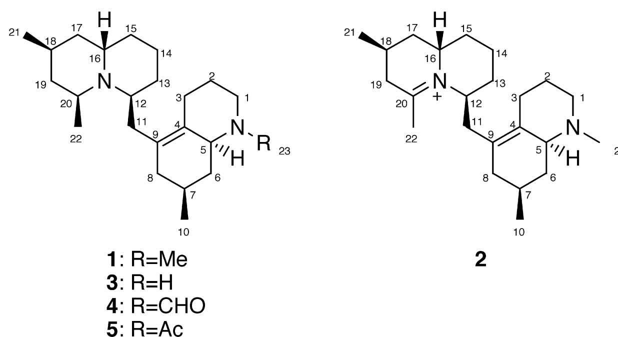
Abstract—Four new $C_{22}N_2$ *Lycopodium* alkaloids, senepodines B–E (**2**–**5**), consisting of an octahydroquinoline ring and a quinolizidine ring have been isolated together with senepodine A (**1**) from the club moss *Lycopodium chinense*. The relative and absolute stereochemistry of **2**-**5** were determined by combination of NOESY correlations and chemical transformation, while the absolute configuration of **1** was assigned by exciton chirality method. © 2003 Elsevier Science Ltd. All rights reserved.

A number of *Lycopodium* alkaloids with fascinating heterocyclic frameworks of $C_{16}N$, $C_{16}N_2$, and $C_{27}N_3$ types have been isolated from club moss of the genus *Lycopodium*.[1] These unique skeletons have attracted great interest not only from biogenetic[1,2] and biological[3] points of view but also as challenging targets for total synthesis.[4] Recently, we have isolated serratezomine A[5] with a seco-serratinine-type skeleton from *Lycopodium serratum* var. *serratum*, complanadine A[6] with a lycodine-dimeric skeleton, lyconadin A[7] from *L. complanatum*, and lyconesidine A[8] and senepodine A[9] from *Lycopodium chinense*. Biomimetic transformation from serratinine into serratezomine A through a modified Polonovski reaction has also been reported.[10] Our interest has been focused on isolation of structurally interesting alkaloids and biosynthetic intermediates to clarify the biogenetic pathway. Further investigation on extracts of *L. chinense* (Lycopodiaceae) resulted in the isolation of four new $C_{22}N_2$ alkaloids, senepodines B–E (**2**–**5**), as well as a known related alkaloid, senepodine A (**1**).[9] This paper describes the isolation and structure elucidation of **2**–**5** and the absolute configuration of **1**.

*Isolation of senepodines B (**2**)–E (**5**).* The club moss of *L. chinense* was extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 10 with sat. Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-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 eluted with hexane/EtOAc (3:2) was purified by a silica gel column ($CHCl_3$/MeOH→$CHCl_3$/MeOH/TFA) and then C_{18} HPLC to afford senepodines B (**2**, 0.0004%), C (**3**, 0.003%), D (**4**, 0.1%), and E (**5**, 0.01%) together with senepodine A (**1**, 0.003%) and known $C_{16}N$ type alkaloids, lyconesidines A[8] (0.002%), B[8] (0.005%), and C[8] (0.003%), lycodoline[11] (0.001%), and lucidine B[12] (0.001%).



*Absolute stereochemistry of senepodine A (**1**).* Although the gross structure and the relative stereochemistry of senepodine A (**1**) have been reported previously,[10] the absolute stereochemistry remains to be defined. The absolute stereochemistry of **1** was elucidated by applying exciton chirality method[13] after oxidation of allylic position at C-3 or C-8 with selenium dioxide, followed by introduction of *p*-bromobenzoyl group into the hydroxyl group at C-3 or C-8 as follows. Treatment of **1** with selenium dioxide in acetic anhydride and H_2O at 75°C for 5 days gave two oxidized products (**1a**, 11% yield; **1b**, 8%) (Scheme 1). The oxidative position and its relative stereochemistry of the hydroxy group of **1a** and **1b** were elucidated by NOESY correlations (Fig. 1) to be 8α and 3α, respectively. Inspection of molecular models giving the same results was obtained by Monte Carlo simulation[14] using MMFF force field.[15] Treatment of **1a** with *p*-bromobenzoyl chloride

Keywords: *Lycopodium chinense*; alkaloids; exciton chirality method.
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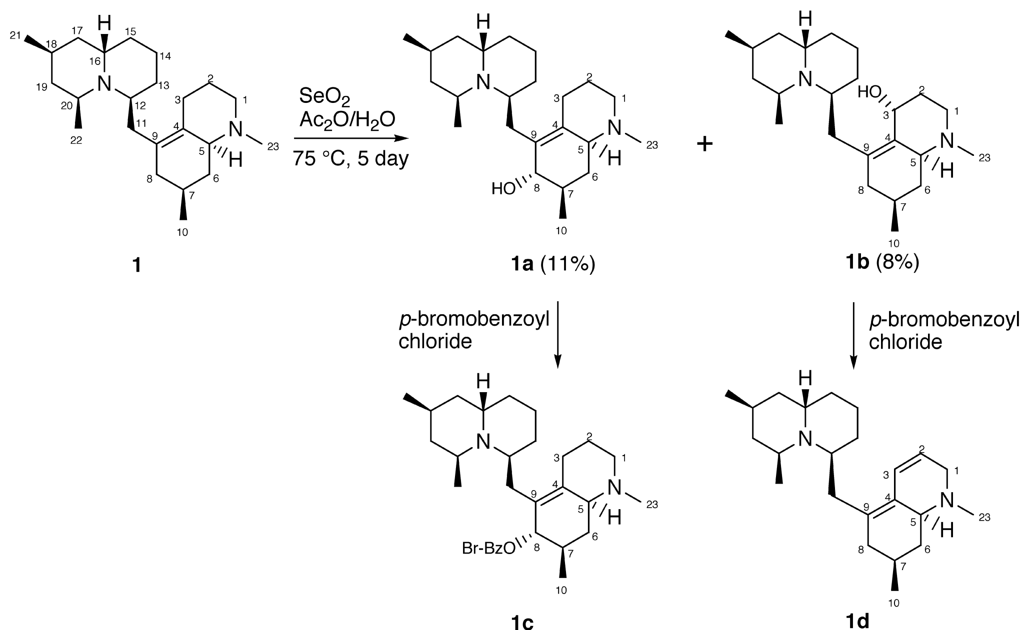


Scheme 1.

afforded the corresponding *p*-bromobenzoyl ester (**1c**), whereas in the case of **1b** with the axially oriented hydroxyl at C-3, dehydration proceeded by the same treatment to give the diene (**1d**). Sign of the first Cotton effect [λ_{max} 244 nm ($\Delta\varepsilon$ -9.2)] for **1c** was negative (Fig. 2), indicating that the chirality between the *p*-bromobenzoyl group at C-8 and the olefin at C-4-C-9 was as shown in Figure 2 (left-handed screw). Thus, the absolute configuration at C-8 of the oxidative compound (**1a**) was assigned as *S*, indicating that the absolute structure of senepodine A (**1**) was as shown in Scheme 1.

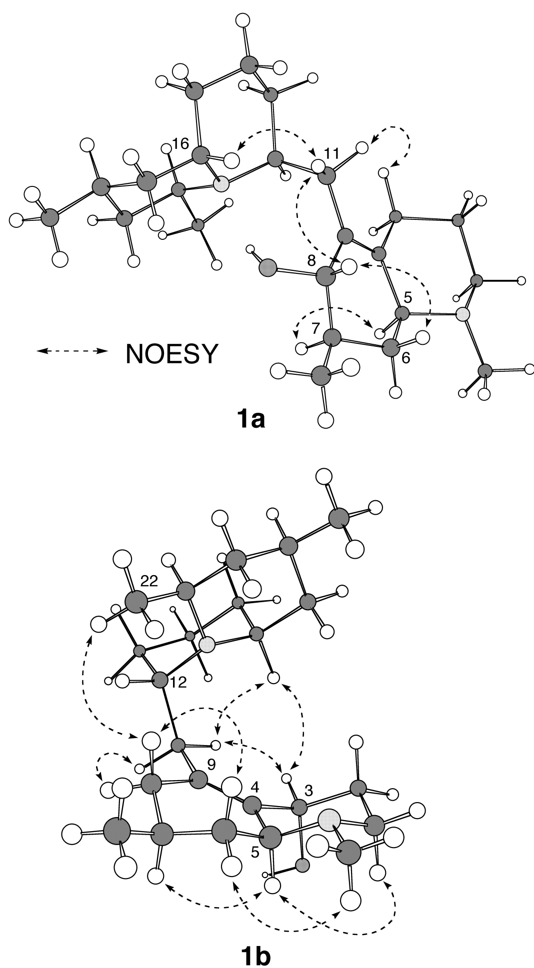


Figure 1. Selected NOESY correlations for 8-hydroxysenepodine A (**1a**) and 3-hydroxysenepodine A (**1b**).

*Structures of senepodines B–E (**2**–**5**).* Senepodine B (**2**) was shown to have the molecular formula of $C_{23}H_{39}N_2$ by HRFABMS [*m/z* 343.3133, $(M)^+$, Δ +2.0 mmu]. The IR spectrum was indicative of the presence of imine ($1680\ cm^{-1}$) functionality. ^1H and ^{13}C NMR data including DEPT experiments (Tables 1 and 2) disclosed three sp^2

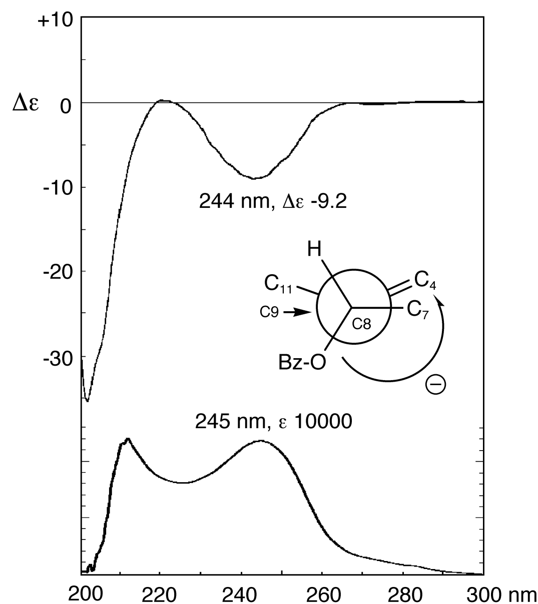


Figure 2. CD and UV spectra of *p*-bromobenzoate (**1c**) of 8-hydroxysenepodine A (**1a**) and rotation model for the C8–C9 bond.

quaternary carbons, five sp^3 methines, eleven sp^3 methylenes, and four methyl groups, and the chemical shift [δ_C 189.17 (s)] of C-20 remarkably shifted to lower field as compared with that [δ_C 50.14 (d)] of **1**. The presence of an iminium carbon (C-20) was elucidated by HMBC correlations for H_3-22 to C-20. In addition, the ^{1}H signals at 12-, 16-, 19-, and 22-positions and the ^{13}C signals at 12-, 16-, and 22-positions around the imine functionality were observed at lower field due to deshielding effects (Tables 1 and 2). 2D NMR data of **2** including the ^{1}H–^{1}H COSY, HOHAHA, HMQC, and HMBC spectra, and FABMS/MS fragmentation (Fig. 3) corroborated well with those of the imine (C-20 and N) form of **1**. Treatment of **2** with $NaBH_4$ afforded senepodine A (**1**) by stereoselective attack of a hydride outside the cage form of **1**.[10]

HRFABMS data [*m/z* 331.3108, $(M+H)^+$, Δ −0.5 mmu] of senepodine C (**3**) established the molecular formula to be $C_{22}H_{38}N_2$, which was smaller than that of senepodine A (**1**) by a CH_2 unit. ^{1}H and ^{13}C NMR data (Tables 1 and 2, respectively) of **3** were analogous to those of **1** in the quinolizidine part, although a methyl signal (δ_H 2.30) at C-23 lacking for **3** was observed for **1**. The gross structure of **3** was elucidated by 2D NMR (^{1}H–^{1}H COSY, HOHAHA, HMQC, and HMBC) data. Treatment of **3** with CH_3I gave senepodine A (**1**). Thus, the structure of senepodine C was assigned as **3**.

Table 1. ^{1}H NMR data [δ_H (J, Hz)] of senepodines A (**1**), B (**2**), and C (**3**) in CD_3OD at 300 K

	1	2	3
1a	2.31 (1H, m)	3.19 (1H, m)	3.09 (1H, ddd, 12.9, 12.8, 3.0)
1b	2.92 (1H, brd, 11.8)	3.53 (1H, m)	3.38 (1H, brd, 12.8)
2a	1.54 (1H, m)	1.74 (1H, m)	1.66 (1H, m)
2b	1.73 (1H, m)	2.02 (1H, m)	2.00 (1H, m)
3a	1.75 (1H, m)	2.02 (1H, m)	2.93 (1H, brd, 14.5)
3b	2.86 (1H, brd, 13.6)	2.98 (1H, m)	2.05 (1H, m)
4			
5	2.63 (1H, dd, 9.2, 8.0)	3.82 (1H, m)	3.80 (1H, dd, 9.2, 8.0)
6a	1.04 (1H, ddd, 12.5, 12.5, 9.2)	1.35 (1H, m)	1.28 (1H, ddd, 12.3, 12.3, 9.2)
6b	2.09 (1H, m)	2.34 (1H, m)	2.11 (1H, m)
7	1.58 (1H, brd, 14.0)	1.69 (1H, m)	1.75 (1H, brd, 13.8)
8a	1.79 (1H, m)	1.96 (1H, m)	1.92 (1H, m)
8b	1.92 (1H, brd, 16.1)	2.09 (1H, brd, 15.6)	2.02 (1H, brd, 16.8)
9			
10	0.99 (3H, d, 6.6)	1.07 (3H, d, 6.4)	1.03 (3H, d, 6.4)
11a	2.13 (1H, brd, 12.5)	2.43 (1H, brd, 11.5)	2.37 (1H, brd, 12.2)
11b	3.03 (1H, dd, 12.5, 12.2)	3.11 (1H, dd, 11.5, 11.1)	3.17 (1H, dd, 12.5, 12.2)
12	3.48 (1H, m)	4.70 (1H, m)	3.79 (1H, m)
13a	1.34 (1H, brd, 12.6)	1.87 (1H, m)	1.55 (1H, brd, 10.6)
13b	1.70 (1H, m)	1.87 (1H, m)	1.84 (1H, m)
14a	1.68 (1H, m)	1.72 (1H, m)	1.70 (1H, m)
14b	1.77 (1H, m)	1.87 (1H, m)	1.83 (1H, m)
15a	1.28 (1H, brd, 15.5)	1.88 (1H, m)	1.60 (1H, brd, 16.1)
15b	2.04 (1H, ddd, 15.5, 13.1, 4.1)	1.88 (1H, m)	2.17 (1H, m)
16	3.46 (1H, m)	4.28 (1H, m)	3.85 (1H, brd, 13.3)
17a	1.47 (1H, ddd, 13.1, 12.8, 4.9)	1.73 (1H, m)	1.60 (1H, m)
17b	1.59 (1H, brd, 12.8)	1.87 (1H, m)	1.70 (1H, m)
18	1.82 (1H, m)	1.96 (1H, m)	1.98 (1H, m)
19a	1.13 (1H, ddd, 12.5, 12.5, 12.5)	2.54 (1H, m)	1.38 (1H, m)
19b	1.72 (1H, m)	3.03 (1H, m)	1.93 (1H, m)
20	3.44 (1H, m)		3.89 (1H, m)
21	0.90 (3H, d, 6.5)	1.04 (3H, d, 6.3)	0.94 (3H, d, 6.3)
22	1.18 (3H, d, 6.1)	2.49 (3H, s)	1.38 (3H, d, 6.3)
23	2.30 (3H, s)	2.89 (3H, s)	

Table 2. ^{13}C NMR data (δ_C) of senepodines A (**1**), B (**2**), and C (**3**) in CD_3OD at 300 K

	1	2	3
1	58.22	57.00	45.36
2	26.53	24.48	24.48
3	29.17	27.19	27.01
4	131.74	128.62	128.82
5	66.02	66.59	57.61
6	38.95	35.52	37.01
7	29.02	28.93	28.49
8	40.45	39.17	39.67
9	130.91	135.24	133.66
10	22.44	21.66	21.58
11	35.37	35.13	33.21
12	53.88	62.07	55.89
13	19.24	28.58	19.51
14	20.07	19.66	18.41
15	24.54	34.06	24.23
16	52.93	58.45	55.33
17	39.88	35.13	38.13
18	26.10	21.20	25.03
19	44.11	43.80	42.00
20	50.14	189.17	53.32
21	22.44	20.18	21.61
22	20.28	24.54	18.02
23	43.29	41.39	

HRFABMS data [*m/z* 359.3067, $(M+H)^+$, Δ +0.5 mmu] of senepodine D (**4**) established the molecular formula, $C_{23}H_{38}N_2O$. The IR spectrum was indicative of the presence of amide carbonyl (1667 cm^{-1}). ^{1}H and ^{13}C NMR data of **4** were analogous to those of senepodine A (**1**), although ^{1}H NMR signals were broad. The presence of an amide carbonyl carbon [C-23, δ_C 164.21 (s)] was elucidated by HMBC correlations for H-23 (δ_H 8.07) to C-1 and C-5, and H-1 and H-5 to C-23 through a nitrogen atom. Treatment of **4** with $LiAlH_4$ afforded senepodine A (**1**), while hydrolysis of **4** with 1N HCl at 100°C for 6 h gave senepodine C (**3**) (Scheme 2). Thus, senepodine D (**4**) was concluded to be the *N*-formyl form at C-23 of senepodine A (**1**).

HRFABMS data [*m/z* 373.3223, $(M+H)^+$, Δ +0.4 mmu] of senepodine E (**5**) established the molecular formula, $C_{24}H_{40}N_2O$. The IR and NMR spectra were indicative of the presence of an *N*-acetyl group (1640 cm^{-1}; δ_H 2.09; δ_C 171.88). Acetylation of senepodine C (**3**) afforded senepodine E (**5**) (Scheme 2). Thus, senepodine E (**5**) was concluded to be the *N*-acetyl form at C-23 of senepodine A (**1**).

Plausible biogenesis of senepodines B–E (2–5). Senepodines B–E (**2–5**) are $C_{22}N_2$ *Lycopodium* alkaloids, consisting of an octahydroquinoline and a quinolizidine ring. A plausible biogenetic pathway for senepodines B (**2**)–E (**5**) as well as senepodine A[10] (**1**) is proposed as shown in Scheme 3. Biogenetically, the decahydroquinoline and quinolizidine units in **2–5** may be derived from an intermediate A with loss of a carbon (path **a**), which have been proposed previously,[10] although an alternative path **b**,

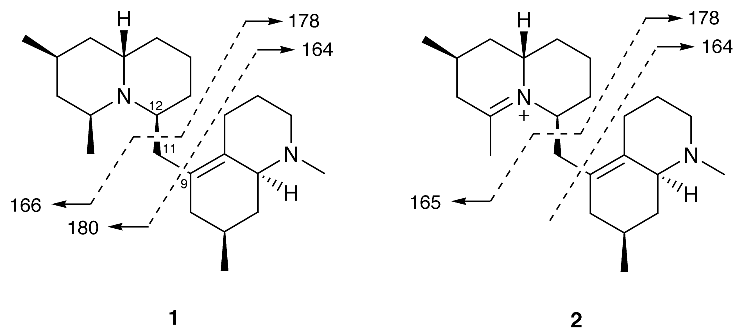
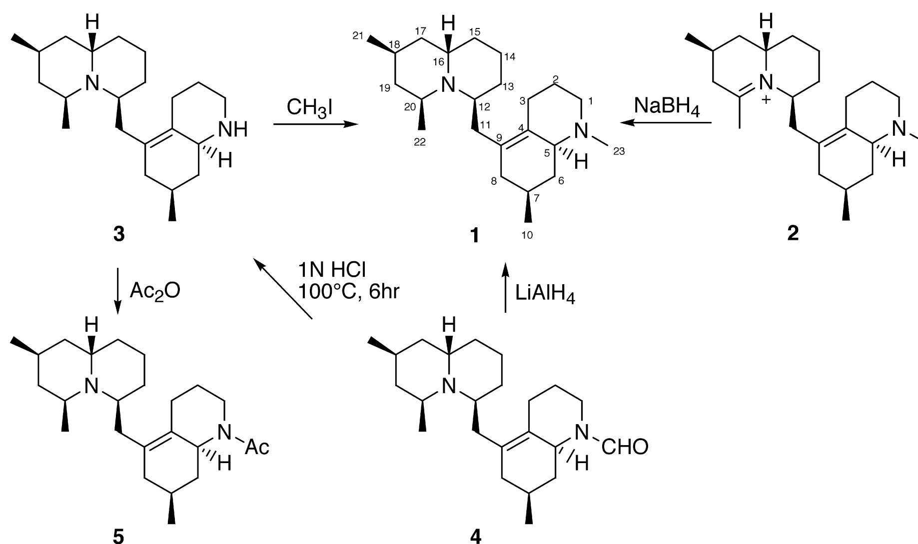
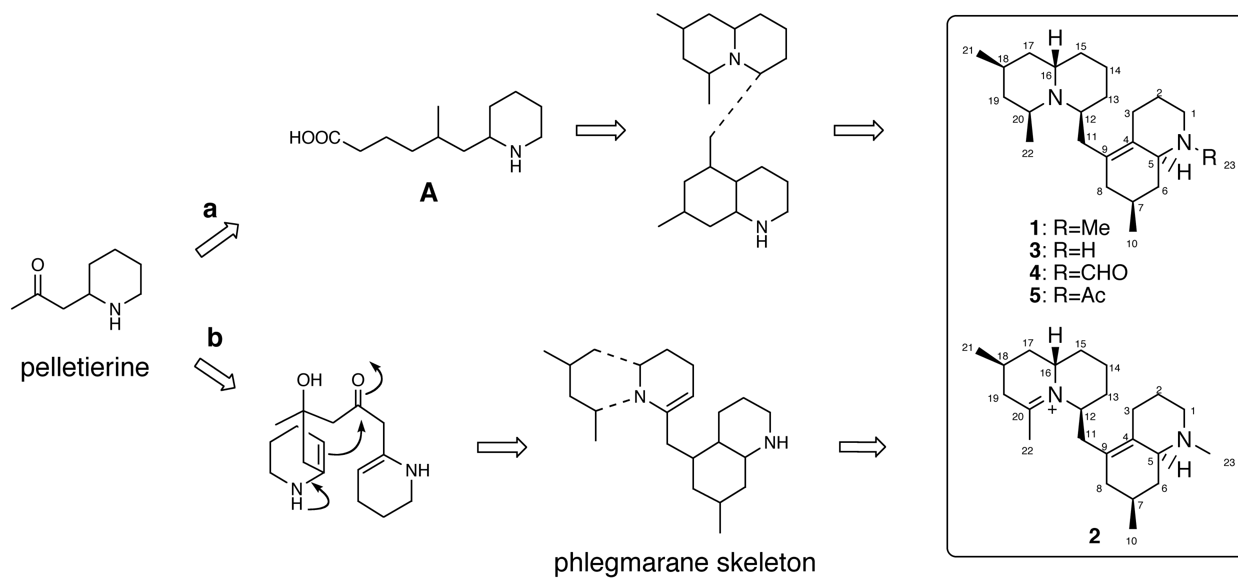


Figure 3. Fragmentation patterns observed in positive ion FABMS/MS spectra of senepodines A (**1**) (precursor ion, *m*/*z* 344) and B (**2**) (*m*/*z* 343).



Scheme 2.



Scheme 3.

which form the C-20–N and C-17–C-16 connectivities of senepodines B–E with a C6 unit, is also possible.

*Bioactivity of senepodines A–E (**1–5**).* Senepodines A–E (**1–5**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50} 0.1, 0.1, 1.2, 0.8, and 0.6 μg/ml, respectively) in vitro.

1. Experimental

1.1. General methods

^{1}H and 2D NMR spectra were recorded on a 600 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. Each NMR sample of senepodines A–E (**1–5**) 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 an internal standard. Standard pulse sequences were employed for the 2D NMR experiments. ^{1}H–^{1}H 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 1 K 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 1 K 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.

1.2. Material

The club moss *L. chinense* was collected at Kiyosato in Hokkaido in 2001. The botanical identification was made by Mr N. Yoshida, Health Sciences University of Hokkaido. A voucher specimen has been deposited in the herbarium of Hokkaido University.

1.3. Extraction and isolation

The club moss (2 kg) of *L. chinense* was extracted with MeOH (10 l×3). The MeOH extract (146 g) was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, after being adjusted at pH 10 with sat. Na_2CO_3, were partitioned with $CHCl_3$. $CHCl_3$-soluble materials (2.4 g) were subjected to an amino silica gel column (hexane/EtOAc, 1:0→0:1, and then $CHCl_3$/MeOH, 1:0→0:1). The fraction eluted with hexane/EtOAc (3:2) was separated by a silica gel column ($CHCl_3$/MeOH/EtOAc, 10:1:0.5→MeOH/TFA, 10:1) and, then C_{18} HPLC (Mightysil RP-18 GP 250-20, 5 μm, Kanto Chemical Co., 10×250 mm; eluent, 14–35% CH_3CN/0.1–0.2% TFA; flow rate, 2 ml/min; UV detection at 205 nm) to afford senepodine A (**1**, 0.003%), B (**2**, 0.0004%), C (**3**, 0.003%), D (**4**, 0.1%), and E (**5**, 0.01%), together with lyconesidines A (0.002%), B (0.005%), C (0.003%), lycodoline (0.001%), and lucidine B (0.001%).

1.3.1. Senepodine B (2). Colorless solid; $[\alpha]_D^{27}=-38°$ (*c* 0.2, MeOH) (with TFA); IR (neat) ν_{max} 3450, 3295, 2927, 1680, 1195, and 1128 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); FABMS *m/z* 343 $(M)^+$; HRFABMS *m/z* 343.3133 (M^+; calcd for $C_{23}H_{39}N_2$, 343.3113).

1.3.2. Senepodine C (3). Colorless solid; $[\alpha]_D^{25}=-15°$ (*c* 1.0, MeOH); IR (neat) ν_{max} 3260, 2920, 1700, 1450, and 1115 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); FABMS *m/z* 331 $(M+H)^+$; HRFABMS *m/z* 331.3108 (M+H; calcd for $C_{22}H_{39}N_2$, 331.3113).

1.3.3. Senepodine D (4). Colorless solid; $[\alpha]_D^{23}=-33°$ (*c* 0.5, MeOH); IR (neat) ν_{max} 2920, 1667, 1450, and 1370 cm^{-1}; ^{1}H NMR (CD_3OD) δ: 0.96 (3H, d, *J*=6.3 Hz), 1.02 (3H, d, *J*=6.1 Hz), 1.20 (1H, m), 1.36 (1H, m), 1.39 (3H, d, *J*=6.2 Hz), 2.70 (1H, dd, *J*=13.0, 8.4 Hz), 3.51 (1H, dd, *J*=12.6, 8.4 Hz), 3.90 (3H, m), 4.62 (1H, m), 8.07 (1H, s); ^{13}C NMR (CD_3OD) δ: 18.90, 19.81, 21.97, 22.20, 23.50, 25.47, 26.51, 28.95, 33.96, 35.39, 38.84, 39.91, 40.85, 42.83, 51.99, 54.32, 55.36, 56.23, 132.06, 133.15, 164.21. FABMS *m/z* 359 $(M+H)^+$; HRFABMS *m/z* 359.3067 (M+H; calcd for $C_{23}H_{39}N_2O$, 359.3062).

1.3.4. Senepodine E (5). Colorless solid; $[\alpha]_D^{22}=-106°$ (*c* 1.0, MeOH); IR (neat) ν_{max} 2920, 1640, 1430, and 1370 cm^{-1}; ^{1}H NMR (CD_3OD) δ: 0.87 (3H, d, *J*=6.3 Hz), 0.98 (3H, d, *J*=3.5 Hz), 1.07 (3H, d, *J*=6.0 Hz), 1.34 (2H, m), 1.52 (1H, brd, *J*=12.8 Hz), 2.09 (3H, s), 2.71 (1H, dd, *J*=13.4, 9.0 Hz), 2.89 (1H, dd, *J*=12.0, 12.0 Hz), 2.99 (1H, m), 3.18 (1H, m), 4.70 (1H, m); ^{13}C NMR (CD_3OD) δ: 19.59, 21.02, 21.56, 22.41, 22.71, 23.64, 24.60, 25.79, 26.59, 29.01, 35.37, 35.60, 40.30, 40.79, 41.54, 45.26, 51.92, 53.15, 58.04, 130.65, 133.10, 171.88. FABMS *m/z* 373 $(M+H)^+$; HRFABMS *m/z* 373.3223 (M+H; calcd for $C_{24}H_{41}N_2O$, 373.3219).

1.3.5. Oxidation of Senepodine A (1). To a solution of **1** (15.9 mg) in water (1.0 ml) at 75°C was added selenium oxide (1.0 g) and acetic anhydride (75 μl). The mixture was allowed to stand at 75°C for 5 days. The residue was dissolved in Na_2CO_3 aq. and extracted with $CHCl_3$. After evaporation of solvent, the residue was applied to C_{18} HPLC (Phenomenex LUNA C18(2), 5 μm, Shimadzu, 10×250 mm; eluent, 15% CH_3CN/0.1% TFA; flow rate, 2 ml/min; UV detection at 205 nm) to give compound **1a** (1.8 mg) and 1b (1.3 mg).

*Compound **1a**.* $[\alpha]_D^{27}=-20°$ (*c* 0.3, MeOH) (with no TFA), ^{1}H NMR (CD_3OD with TFA) δ 0.95 (3H, brd, *J*=3.1 Hz, H-21), 1.08 (3H, brd, *J*=3.1 Hz, H-10), 1.25 (1H, m, H-19a), 1.30 (1H, m, H-2a), 1.35 (3H, brd, *J*=5.8 Hz, H-22), 1.64 (1H, m, H-15a), 1.67 (1H, m, H-17a), 1.71 (2H, m, H-13a, 14a), 1.72 (1H, m, H-2b), 1.73 (1H, m, H-6a), 1.76 (1H, m, H-7), 1.78 (1H, m, H-17b), 1.95 (3H, m, H-13b, 14b, 19b), 1.98 (1H, m, H-18), 2.06 (1H, m, H-6b), 2.12 (1H, m, H-3a), 2.15 (1H, m, H-15b), 2.66 (1H, dd, *J*=14.3, 8.0 Hz, H-11a), 2.80 (1H, brd, *J*=14.1 Hz, H-3b), 2.93 (3H, s, H-23), 3.04 (1H, brd, *J*=13.0 Hz, H-11b), 3.20 (1H, brt, *J*=11.5 Hz, H-1a), 3.55 (1H, brd, *J*=9.9 Hz, H-1b),

3.75 (1H, m, H-5), 3.86 (1H, m, H-20), 3.89 (1H, m, H-8), 3.90 (1H, m, H-12), 3.95 (1H, brd, J=11.8 Hz, H-16).

*Compound **1b***. $[\alpha]_D^{26}=+0.4°$ (c 0.4, MeOH) (with TFA), ^{1}H NMR (CD_3OD with TFA) δ 0.95 (3H, d, J=6.2 Hz, H-21), 1.06 (3H, d, J=6.4 Hz, H-10), 1.32 (1H, m, H-6a), 1.35 (1H, m, H-19a), 1.39 (3H, d, J=6.1 Hz, H-22), 1.51 (1H, brd, J=14.6 Hz, H-13a), 1.63 (1H, m, H-15a), 1.65 (1H, m, H-17a), 1.72 (1H, m, H-7), 1.77 (3H, m, H-14, 17b), 1.85 (1H, m, H-13b), 1.94 (1H, m, H-19b), 1.97 (1H, m, H-2a), 1.98 (1H, m, H-18), 1.99 (1H, m, H-8a), 2.04 (1H, m, H-8b), 2.07 (1H, m, H-2b), 2.17 (1H, m, H-15b), 2.35 (1H, m, H-6b), 2.52 (1H, brd, J=12.6 Hz, H-11a), 2.91 (3H, s, H-23), 3.17 (1H, t, J=12.2 Hz, H-11b), 3.37 (1H, m, H-1a), 3.55 (1H, t, J=12.0 Hz, H-1b), 3.88 (1H, m, H-16), 3.89 (1H, m, H-12), 3.92 (1H, m, H-20), 4.17 (1H, m, H-5), 4.90 (1H, m, H-3).

1.3.6. *p*-Bromobenzoate of 8α-hydroxysenepodine A (1a). To a solution of **1a** (0.45 mg) in CH_2Cl_2 (200 μl) was added *p*-bromobenzoyl chloride (5.0 mg), *N*,*N*-dimethylamino pyridine (0.1 mg), and Et_3N (3 μl). The mixture was allowed to stand at 20°C for 12 h. The residue was dissolved in $CHCl_3$ and washed with Na_2CO_3 aq. After evaporation of solvent, the residue was applied to LH-20 column ($CHCl_3$/MeOH 1:1) to give a compound **1c** (0.1 mg). When **1b** was treated according to the same procedure as described above, the dehydration derivative **1d** (0.2 mg) was produced.

*Compound **1c***. FABMS m/z 543 $(M+H)^+$; HRFABMS m/z 543.2579 (M+H; calcd for $C_{30}H_{44}N_2O_2Br$, 543.2587). UV (MeOH) λ_{max} 245 (ε 10000), 212 (ε 10000): CD (MeOH) $\Delta\varepsilon_{244}$ −9.2 and $\Delta\varepsilon_{203}$ −35.5; ^{1}H NMR (CD_3OD) δ 0.92 (3H, d, J=6.6 Hz), 1.14 (3H, brd, J=5.1 Hz), 1.29 (3H, brs), 2.40 (3H, s), 2.66 (1H, dd, J=10.3, 6.4 Hz), 2.71 (1H, brt, J=12.5 Hz), 2.91 (1H, brd, J=14.6 Hz), 2.97 (1H, brd, J=11.7 Hz), 5.55 (1H, brs), 7.70 (2H, d, J=8.6 Hz), 8.04 (2H, d, J=8.6 Hz).

*Compound **1d***. ^{1}H NMR (CD_3OD) δ 0.93 (3H, d, J=6.4 Hz), 1.05 (3H, d, J=7.0 Hz), 1.18 (3H, brs), 2.14 (1H, brd, J=19.0 Hz), 2.42 (3H, s), 2.65 (2H, m), 2.80 (1H, t, J=12.4 Hz), 2.91 (1H, dd, J=11.4, 5.9 Hz), 5.54 (1H, brs), 5.91 (1H, brs).

1.3.7. Reduction of senepodine B (2). To a solution of **2** (0.1 mg) in MeOH (100 μl) was added sodium borohydride (0.2 mg). The mixture was allowed to stand for 5 min. The residue was dissolved in $CHCl_3$ and washed with Na_2CO_3 aq. After evaporation of solvent, the residue was applied to LH-20 column ($CHCl_3$/MeOH 1:1) to give a compound (0.1 mg), which was identical with senepodine A (**1**).

1.3.8. Methylation of senepodine C (3) with MeI. A solution of **3** (0.1 mg), MeI (0.1 ml), and K_2CO_3 (0.1 mg) in acetone (0.1 ml) was heated under reflux for 6 h, and then concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ and washed with sat. NaCl aq., and then dried over anhydrous Na_2SO_4. Removal of the solvent afforded the compound (0.1 mg), which was identical with senepodine A (**1**).

1.3.9. Reduction of senepodine D (4). To a solution of **4** (0.5 mg) in THF (125 μl) was added lithium aluminum hydride (3 mg). The mixture was allowed to stand at 70°C for 4 h. After addition of EtOAc, H_3PO_4 buffer, and Na_2CO_3 aq. the mixture was extracted with $CHCl_3$. After evaporation of solvent, the residue was applied to C_{18} HPLC (Mightysil C-18, 5 μm, Kanto Chemical Co., 4.6×250 mm; eluent, 25% CH_3CN/0.1% TFA; flow rate, 1 ml/min; UV detection at 205 nm) to give a compound (0.3 mg), which was identical with senepodine A (**1**).

1.3.10. Hydrolysis of senepodine D (4). Compound **4** (5.0 mg) in 1N HCl (100 μl) was allowed to stand at 100°C for 6 h. After evaporation of solvent, the residue was applied to C_{18} (Phenomenex LUNA C18(2), 5 μm, Shimadzu, 10×250 mm; eluent, 25% CH_3CN/0.1% TFA; flow rate, 2 ml/min; UV detection at 210 nm) to give a compound (4.0 mg), which was identical with senepodine C (**3**).

1.3.11. Acetylation of senepodine C (3). To a solution of **3** (4.0 mg) in pyridine (1 ml) was added acetic anhydride (0.25 ml). The mixture was allowed to stand for 12 h. After addition of H_2O, the mixture was extracted with $CHCl_3$. After evaporation of solvent, the residue was applied to C_{18} HPLC (Mightysil C-18, 5 μm, Kanto Chemical Co., 4.6×250 mm; eluent, 35% CH_3CN/0.1% TFA; flow rate, 1 ml/min; UV detection at 205 nm) to give a compound (4.0 mg), which was identical with senepodine E (**5**).

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