Serratezomines A−C, New Alkaloids from *Lycopodium serratum* var. *serratum*

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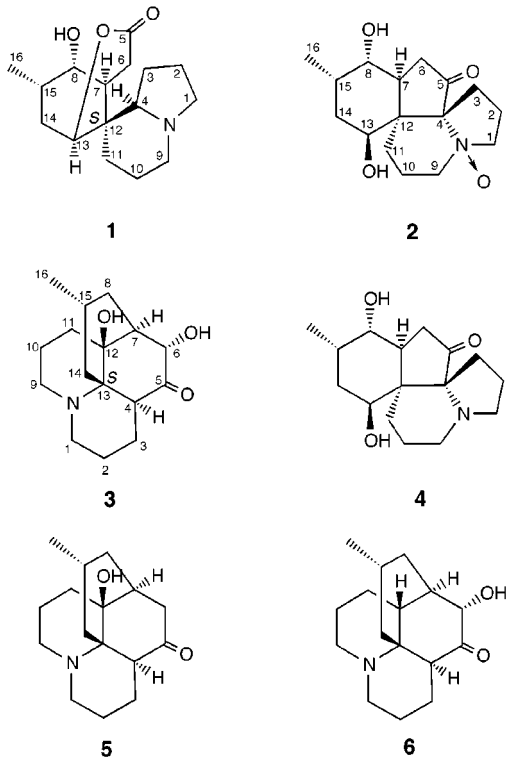
Received April 28, 2000

Lycopodium alkaloids[1] such as serratinine (**4**) and lycodoline (**5**), possessing a common formula of $C_{16}N$, and lucidines A and B, with that of $C_{30}N_3$, are a class of natural products with unique ring systems, which have attracted great interest from biogenetic,[1,2] synthetic,[1,3] and biological[4] points of view. These unique skeletons have prompted us extensive phytochemical work. In our search for biogenetic intermediates of *Lycopodium* alkaloids, three new alkaloids, serratezomines A (**1**), B (**2**), and C (**3**), with a *seco*-serratinine-type, a serratinine-type, and a lycodoline-type skeletons, respectively, were isolated from the club moss *Lycopodium serratum* var. *serratum*. In this paper, we describe the isolation and structure elucidation of **1**−**3**.

The club moss *L. serratum* var. *serratum*[5] collected in Sapporo 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 a C_{18} column (CH_3CN/0.1% CF_3CO_2H, 1:4 → 4:1), in which a fraction eluted with CH_3CN/0.1% CF_3CO_2H (1:4) was purified by an amino silica gel column ($CHCl_3$/MeOH, 1:0 → 1:1) followed by C_{18} HPLC (CH_3CN/0.1% CF_3CO_2H, 13:87) to afford serratezomines A (**1**, 0.0002% yield), B (**2**, 0.002%), and C (**3**: 0.0002%) as colorless solids together with known related alkaloids, serratinine (**4**, 0.02%),[6] lycodoline (**5**, 0.004%),[7] and L20 (**6**, 0.004%).[8]



The molecular formula, $C_{16}H_{25}NO_3$, of serratezomine A (**1**) was established by HRFABMS [*m*/*z* 280.1926, $(M + H)^+$, Δ +1.3 mmu]. IR absorptions implied the presence of hydroxyl (3400 cm^{-1}) and ester carbonyl (1730 cm^{-1}) functionalities. ^1H and ^{13}C NMR data (Table 1) disclosed the existence of one ester carbonyl, one sp^3 quaternary carbon, five sp^3 methines, eight sp^3 methylenes, and one secondary methyl. Among them, two methines (δ_C 76.24; δ_H 3.77 and δ_C 83.57; δ_H 4.32) were ascribed to those bearing an oxygen, while one methine (δ_C 66.80; δ_H 3.81) and two methylenes (δ_C 56.04; δ_H 3.35 and 3.54 and δ_C 48.76; δ_H 2.98 and 3.26) were ascribed to those attached to a nitrogen. Since one out of five unsaturations were accounted for, **1** was inferred to possess 4 rings. 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 (Figure 1).

Connectivities of C-1−C-4 and C-9−C-11 were revealed by the $^1H-^1H$ COSY and HOHAHA spectra. Both C-4 and C-11 were connected to C-12 from HMBC correla-

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(2) (a) Hemscheidt, T.; Spenser, I. D. *J. Am. Chem. Soc.* **1996**, *118*, 1799−1800. (b) Hemscheidt, T.; Spenser, I. D. *J. Am. Chem. Soc.* **1993**, *115*, 3020−3021.

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(4) The club moss *L. serratum* has been used as Chinese traditional medicine, in which the constituent alkaloids, hupderzines A and B, show pronounced anticholinesterase activity and are under clinical investigation in China for the treatment of myasthenia gravis and senile memory loss now recognized as symptoms of Alzheimer's dementia (AD): Liu, J. S.; Zhu, Y. L. Yu, C. M.; Zhou, Y. Z.; Han, Y. Y.; Wu, F. W.; Qi, B. F. *Can J. Chem.* **1986**, *64*, 837−839.

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10.1021/jo000661e CCC: $19.00
Published on Web 09/15/2000

Table 1. ^{1}H and ^{13}C NMR Data of Serratezomine A (1) in CD_3OD at 300 K

	δ_H	δ_C	HMBC (^{1}H)
1a	3.35 (1 H, m)	56.04	
1b	3.54 (1 H, ddd, 9.2, 9.2, 9.2)		
2	2.20 (2 H, m)	19.70	1, 3a, 3b
3a	2.25 (1 H, m)	22.05	4
3b	2.15 (1 H, m)		
4	3.81 (1 H, dd, 6.0, 10.9)	66.80	1a, 9b, 11
5		173.15	6a, 6b, 7, 13
6a	2.46 (1 H, d, 20.0)	34.32	7
6b	3.14 (1 H, dd, 8.0, 20.0)		
7	2.61 (1 H, m)	37.15	6a, 6b, 8, 11a, 13
8	3.77 (1 H, t, 3.4)	76.24	6a, 6b, 7, 16
9a	2.98 (1 H, dt, 3.5, 13.0)	48.76	1b, 11
9b	3.26 (1 H, m)		
10a	1.84 (1 H, m)	20.63	
10b	2.03 (1H, m)		
11a	1.40 (1 H, dt, 3.3, 13.6)	23.63	3a, 9b, 10b
11b	2.83 (1 H, brd, 13.6)		
12		37.35	4, 8, 6b, 11a, 13
13	4.32 (1 H, brd, 2.7)	83.57	7
14	1.83 (2 H, m)	32.28	7, 8, 16
15	1.78 (1 H, m)	27.05	13, 14, 16
16	1.01 (3 H, d, 6.4)	17.28	

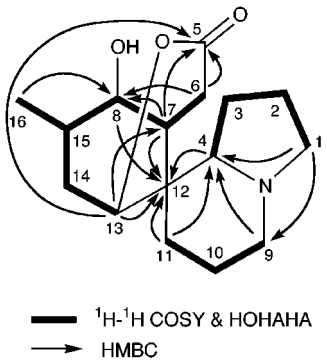

Figure 1. Selected 2D NMR correlations for serratezomine A (**1**).

tions of H-4 and H_a-11 to C-12, and H_2-11 to C-4. HMBC cross-peaks of H_b-1 to C-9, H_a-1 to C-4, and H_b-9 to C-4 indicated connections among C-1 (δ_c 56.04), C-4 (δ_c 66.80), and C-9 (δ_c 48.76) through N-1, constructing an indolizidine ring system.

Connectivities of C-6−C-8, C-13−C-16, and C-8 to C-15 were revealed by the ^{1}H−^{1}H COSY and HOHAHA spectra. The hydroxy group at C-8 (δ_c 76.24) was inferred by HMBC correlations of H_2-6, H-7, and H_3-16 to C-8. The connection between C-7 and C-13 through C-12 to form a cyclohexane ring was deduced from HMBC correlations of H-7 and H-13 to C-12, and H-13 to C-7. On the other hand, the C-6 methylene (δ_c 34.32; δ_H 2.46 and 3.14) was attached to a carbonyl group at C-5 (δ_c 173.15), which was connected to C-13 (δ_c 83.57) through an ester linkage to form a δ-lactone ring, since HMBC correlations of H_2-6, H-7, and H-13 to C-5 were observed. These results indicated the presence of a 2-oxabicyclo[3.3.1]-nonan-3-one ring system[9] connected to the indolizine ring through the spiro carbon at C-12. Thus, the gross structure of serratezomine A was assigned as **1**.

(9) Inspection of molecular models, which were obtained by MMFF calculations using MacroModel version 6.0 developed by C. Still, Columbia University: Halgren, T. *J. Am. Chem. Soc.* **1990**, *112*, 4710−4723, clearly demonstrated that the dihedral angles between H-7 and H_a-6 was approximately 90°, resulting in one of the coupling constant being nearly zero.

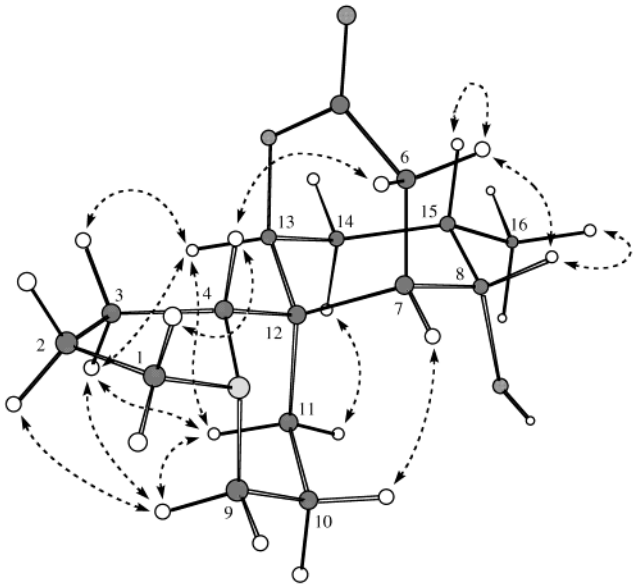

Figure 2. Selected NOESY correlations (dotted arrows) and relative configurations for serratezomine A (**1**).

The relative stereostructure of **1** as shown in computer-generated 3D drawing (Figure 2) was deduced from cross-peaks observed in the phase sensitive NOESY spectrum. NOESY correlations of H_3-16/H-8, H_a-6/H-8, and H_a-6/H-15 indicated that both methyl group at C-15 and the hydroxyl group at C-8 were α-oriented. The proton coupling constants ($J_{7,8}$ = 3.4 Hz and $J_{8,15}$ = 3.4 Hz) in the cyclohexane ring and a W-type long-range coupling between H-7 and H-13, which were both equatorial, supported the proposed relative stereochemistry and a chair form of the cyclohexane ring in the 2-oxabicyclo-[3.3.1]nonan-3-one ring (Figure 2). The *cis*-fused ring junction[10] in the indolizidine ring and a chair form of the piperidine ring were deduced from NOESY correlations of H_a-11/H_a-3, H_a-9/H_a-3, and H_a-9/H_a-2. The NOEs of H-4/H_b-6, H_b-3/H-13, H-7/H_b-10, and H_a-14/H_b-11 argued well the stereochemistry of the spiro carbon at C-12.

Furthermore, the relative configuration at C-12 was estimated by the floating chirality method[11] as follows. Floating chirality method allows the distance constraints to guide the molecule into configurations consistent with its NOE data.[12] For molecules possessing complex ring systems, high-temperature dynamics alone may fail to invert certain chiral centers with sufficient frequency. This chiral inversion is caused by the application of NOE constraints. Inversion of chirality about C-12 could be detected by monitoring the distances of H_b-10−H-7 and H-4−H_b-6. A significant change in these interproton distances would indicate that an inversion about this

(10) The lowest energy conformation of serratezomine A (**1**) obtained by molecular modeling[9] indicated that the indolizidine moiety with cis ring junction was 1.81 kcal/mol lower in energy than that with trans ring junction, although an indolizidine alkaloid, slaframine, has been reported to have trans ring juncture: Pearson, W. H.; Bergmeier, S. C.; Williams, J. P. *J. Org. Chem.* **1992**, *57*, 3997−3987.

(11) Falk, M.; Spierenburg, P. F.; Walter, J. A. *J. Comput. Chem.* **1996**, *17*, 409−417.

(12) Interatomic distances were classified into five ranges, ≤ 2.5, ≤3.0, ≤3.5, ≤ 4.0, and ≤5.0 Å, corresponding to the integrated volumes of the 32 NOESY cross-peaks. Interproton distances were calculated from the integrated volumes of the corresponding cross-peaks using the distance between the two H-6 protons as a reference (1.8 Å). Because of the lack of stereospecific assignments in some methylene protons, the upper distances of these methylene and methyl protons were further relaxed by means of the pseudoatoms corrections.

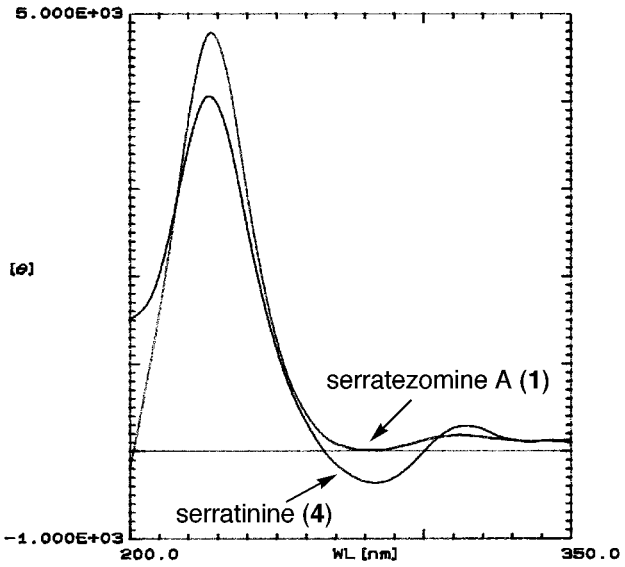

Figure 3. CD spectra of serratezomine A (**1**) and serratinine (**4**).

chiral center has occurred. When started from *S** configuration at C-12 of **1**, no inversion of the chiral center at C-12 was observed at any time during the course of the simulation, whereas simulation starting from *R** isomer at C-12 led to conversion into *S** configuration.[13] These results provided additional proof corroborating well the relative stereochemistry and the stable conformer of **1** in CD_3OD. The CD spectra (Figure 3) of **1** showed a similar CD curve ($[\theta]_{230}$ +4800, and $[\theta]_{312}$ +200) to that of serratinine (**4:** $[\theta]_{230}$ +4050, $[\theta]_{303}$ -400, and $[\theta]_{313}$ +350), whose absolute stereostructure has been established by X-ray analysis.[14] Therefore, the absolute configurations of **1** were assigned as 4*R*,7*S*,8*S*,12*S*,13*S*, 15*S*.[15]

HRFABMS data [*m*/*z* 296.1863, $(M + H)^+$, Δ +0.1 mmu] of serratezomine B (**2**) indicated the molecular formula, $C_{16}H_{25}NO_4$, which was larger than that of **4** by one oxygen unit. IR absorptions implied the presence of hydroxy (3420 cm^{-1}) and ketone (1750 cm^{-1}) functionalities. ^1H and ^{13}C NMR (Table 2) spectra of **2** revealed signals due to 3 quaternary carbons ($sp^2 \times 1$ and $sp^3 \times 2$), 4 methines (sp^3), 8 methylenes, and 1 methyl, implying that the structure of **2** was similar to that of serratinine (**4**).[6] Detailed analyses of 2D NMR (^1H-^1H COSY, HOHAHA, HMQC, and HMBC) spectra of **2** and comparison of the ^{13}C chemical shifts of C-1, C-4, and C-9 (δ 68.56, 87.44, and 63.20, respectively) in **2** with

Table 2. ^1H and ^{13}C NMR Data of Serratezomine B (2) in CD_3OD at 300 K

	δ_H	δ_C	HMBC (^1H)
1a	3.90 (1 H, brt, 9.7)	68.56	
1b	4.04 (1 H, ddd, 10.8, 10.8, 10.8)		
2a	2.12 (1 H, m)	19.02	1b, 3a, 3b
2b	2.38 (1 H, m)		
3a	2.27 (1 H, ddd, 5.0, 11.5, 15.0)	23.71	1a
3b	2.67 (1 H, ddd, 4.6, 11.5, 15.0)		
4		87.44	1a, 9, 10b
5		210.98	6, 3a
6	2.42 (2 H, d, 10.9)	41.42	7
7	3.23 (1 H, brt, 10.9)	41.33	6, 13, 14a
8	3.79 (1 H, brs)	71.68	6a, 6b, 7, 16
9	3.56 (2 H, m)	63.20	1b, 10b
10a	1.42 (1 H, m)	28.26	9, 11b
10b	2.57 (1 H, dt, 14.0, 3.3)		
11a	1.82 (1 H, dd, 1.5, 12.7)	16.98	9, 10a, 10b
11b	2.46 (1 H, m)		
12		45.66	3a, 6, 7, 8, 11a, 14a
13	3.59 (1 H, brs)	74.40	7, 14a
14a	1.39 (1 H, m)	32.60	8, 16
14b	1.84 (1 H, dd, 1.4, 12.9)		
15	2.23 (1 H, m)	24.78	7, 13, 14b, 16
16	1.00 (3 H, d, 7.0)	17.90	8

Table 3. ^1H and ^{13}C NMR Data of Serratezomine C (3) in CD_3OD at 300 K

	δ_H	δ_C	HMBC (^1H)
1a	2.48 (1 H, brd, 11.8)	47.30	3b
1b	3.25 (1 H, m)		
2a	2.01 (1 H, m)	18.59	3a, 3b
2b	1.42 (1 H, m)		
3a	1.63 (1 H, m)	22.33	
3b	1.99 (1 H, m)		
4	3.48 (1 H, brd, 11.9)	41.23	3, 6
5		213.00	6
6	3.85 (1 H, d, 1.5)	79.10	7, 8
7	1.97 (1 H, m)	49.42	8
8a	1.32 (1 H, m)	34.26	6, 14a, 16
8b	2.00 (1 H, m)		
9a	3.39 (1 H, m)	47.89	11b
9b	2.61 (1 H, brd, 11.7)		
10a	1.61 (1 H, m)	21.36	
10b	2.22 (1 H, m)		
11a	2.79 (1 H, dt, 3.3, 14.5)	33.05	
11b	1.48 (1 H, brd, 14.5)		
12		70.40	6, 7, 10b, 14a
13		64.68	1
14a	2.25 (1 H, dd, 4.9, 13.0)	35.66	16
14b	1.58 (1 H, t, 13.0)		
15	1.27 (1 H, m)	26.24	7, 8b, 14a, 14b,
16	0.87 (3 H, d, 6.1)	23.11	8b, 14b

those (δ 53.23, 79.35, and 51.32, respectively) of serratinine (**4**) indicated the presence of an *N*-oxide functionality for **2**. Oxidation of serratinine (**4**) with *m*-chloroperbenzoic acid (*m*-CPBA) afforded the *N*-oxide derivative, whose spectral data and the $[\alpha]_D$ value were identical with those of natural serratezomine B (**2**). Thus, serratezomine B (**2**) was concluded to be the *N*-oxide of serratinine (**4**).

HRESIMS data [*m*/*z* 280.1914, $(M + H)^+$, Δ +0.1 mmu] of serratezomine C (**3**) revealed the same molecular formula, $C_{16}H_{25}NO_3$, as that of **1**. The ^1H and ^{13}C NMR (Table 3) spectra of **3** gave signals including three quaternary carbons ($sp^2 \times 1$ and $sp^3 \times 2$), four methines (sp^3), eight methylenes, and one methyl, suggesting that **3** had a backbone skeleton similar to that of lycodoline (**5**). In the ^{13}C NMR spectrum of **3**, signals due to the oxygen bearing carbons at δ_C 79.10 (d) and 70.40 (s) and a ketone carbonyl carbon at δ_C 213.00 appeared. The structure of **3** was elucidated by 2D NMR ($^1H-^1H$ COSY,

(13) This chiral inversion is caused by the application of NOE constraints. When relatively large force constants for the NOE derived distance constraints are used, these constraints become energetically much more important than the torsion and angle energies which are responsible for maintaining the chirality. Once the chirality is inverted simultaneously at both centers, all angles, torsions, and distance constraints are satisfied. This floating chirality technique is much more convenient and goes one step further to increase the level of confidence in the assignment of relative configuration.

(14) The absolute stereostructure of serratinine (**4**) has been established on the basis of the Bijvoet method using X-ray crystal structure of 13-acetyl-8-*p*-bromobenzoyl derivative of **4**: Nishio, K.; Fujiwara, T.; Tomita, K.; Ishii, H.; Inubushi, Y.; Harayama, T. *Tetrahedron Lett.* **1969**, 861−864. In this study, by using direct methods in X-ray analysis, the stereostructure of **4** was confirmed to be the same as that previously reported (see the Supporting Information).

(15) Determination of the absolute configuration at C-8 of **1** by using the modified Mosher's method failed (all of the $\Delta\delta_S - \Delta\delta_R$ values were negative). Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

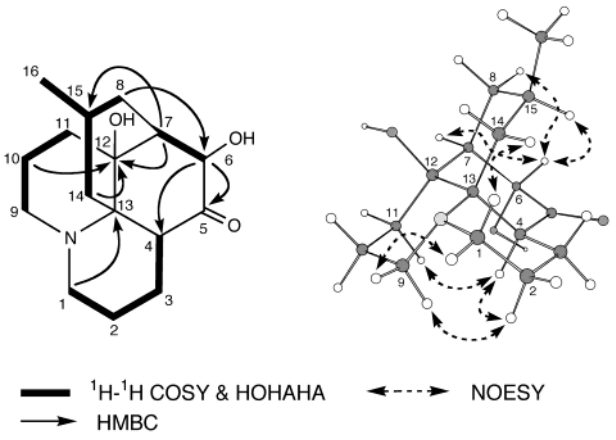

Figure 4. Selected 2D NMR correlations and relative configurations for serratezomine C (**3**).

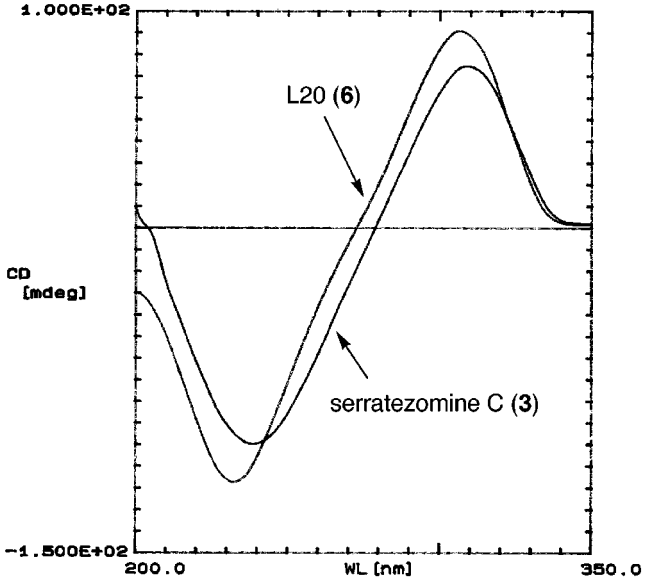

Figure 5. CD spectra of serratezomine C (**3**) and L20 (**6**).

HOHAHA, HMQC, and HMBC) data (Figure 4). The ^{1}H−^{1}H COSY and HOHAHA spectra revealed connectivities of C-1 to C-4, C-6 to C-7, C-9 to C-11, and C-8 to C-15, C-14, and C-16. These four partial units were connected on the basis of HMBC correlations of H-7 to C-12 and C-15, H-10 and H-14 to C-12, and H-1 to C-13. The presence of an α-hydroxy ketone at C-5 and C-6 like that of L20 (**6**) was revealed by HMBC correlations of H-8 to C-6, H-6 to C-5 and C-4. Thus serratezomine C was elucidated to be 6-hydroxy form of lycodoline (**5**).

The relative stereochemistry of **3** was deduced from NOESY correlations (Figure 4). The CD spectra of **3** in MeOH (Figure 5) showed similar CD curves [λ_{max} 240 (θ −100) and 309 (+75) nm] to those [λ_{max} 233 (θ -120) and 307 (+90) nm] of L20 (**6**), whose stereochemistry was confirmed by X-ray analysis.[16] Thus, the absolute stereostructure of serratezomine C was assigned as **3**.

Serratezomine A (**1**) is a novel structural type alkaloid consisting of a 2-oxabicyclo[3.3.1]nonan-3-one and an indolizidine ring connected through a spiro carbon, while serratezomine B (**2**) is the first example of the *N*-oxide of serratinine-type alkaloid. A plausible biogenetic path for serratezomine A (**1**) is proposed as shown in Scheme 1. It is known that lycodoline-type skeleton such as serratezomine C (**3**) and lycodoline (**5**) is biosynthesized from L-lysine via pelletierine, of which the acetate-derived C3 fragments are introduced via acetonedicarboxylic acid.[17] Furthermore, a biogenesis of lycodoline (**5**) to serratinine (**4**) has been proposed by Inubushi et al. (Scheme 1).[18] Serratezomine A (**1**) might be derived from serratinine (**4**) through its N-oxidation to generate serratezomine B (**2**), formation of a hemiacetal linkage between the hydroxyl at C-13 and the ketone at C-5, and subsequent cleavage of the C-4 − C-5 bond. Serratezomines A (**1**) and B (**2**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50}, 9.7 and 7.2 μg/mL, respectively) and human epidermoid carcinoma KB cells (IC_{50}, >10 and 5.1 μg/mL, respectively) in vitro, while **3** was not cytotoxic (>10 μg/mL).

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD on a 600 MHz and a 500 MHz spectrometers (Bruker AMX600 and ARX500) at 300 K equipped with an ×32 computer and an Eurotherm temperature control unit. 1D NMR spectra were measured at 300 K, which were multiplied by a Gaussian filter and zero filled to 32 K data points before Fourier transformation. 2D NMR spectra were measured at 300 K. NOESY and HOHAHA spectra in the phase sensitive mode were recorded using the TPPI method. HOHAHA spectra were recorded by spin-lock field preceded and followed by 2.5 ms trim pulses. NOESY spectra were measured with mixing times of 400, 600, and 800 ms. Since NOESY spectra gave no indications of spin diffusion at 600 ms, NOE intensities at this mixing time were used in the calculations. Typically 256 FID's of 2 K data points, and 32 scans each were employed. Chemical shifts were presented using residual CD_3OD (δ_H 3.31 and δ_C 49.50) as internal standards. Standard pulse sequences were employed for 2D NMR experiments. HMBC spectra were recorded using a 50 ms delay time for long-range C−H coupling with *Z*-axis PFG. FABMS was measured by using glycerol as matrix.

Material. The club moss *L. serratum* var. *serratum* was collected in Sapporo in 1999. The botanical identification was made by Mr. N. Yoshida, Graduate School of Pharmaceutical Sciences, Hokkaido University. A voucher specimen has been deposited in the herbarium of Hokkaido University.

Isolation. The club moss *L. serratum* var. *serratum* (1 kg) was crushed and extracted with MeOH (5 L × 3). The MeOH extract (135 g) 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 (4 g). A portion (2 g) of the fraction was subjected to C_{18} column chromatography (CH_3CN/0.1% CF_3CO_2H, 1:4 → 4:1), in which a fraction eluted with CH_3CN/0.1% CF_3CO_2H (1:4) was purified by an amino silica gel column ($CHCl_3$/MeOH, 1:0 → 1:1) followed by C_{18} HPLC (13% CH_3CN/0.1% CF_3CO_2H) to afford serratezomines A (**1**, 0.0002% yield), B (**2**, 0.002%), and C (**3**, 0.0002%) as colorless solid together with serratinine (**4**, 0.02%), lycodoline (**5**, 0.004%), and L20 (**6**, 0.004%).

Serratezomine A (1): colorless solid; $[\alpha]_D$ +13° (*c* 0.5, MeOH); IR (neat) ν_{max} 3400, 2940, 2870, 1730, 1430, 1190, and 1130 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); FABMS *m/z* 280 (M + H)$^+$; HRFABMS *m/z* 280.1926 (M + H; calcd for $C_{16}H_{26}NO_3$, 280.1913).

(16) Absolute stereostructure of L20 (**6**) has been established on the basis of chemical correlation with lycopodine (See ref 8). In this study, by X-ray analysis, the stereostructure of **6** was directly confirmed to be the same as that previously reported (see the Supporting Information).

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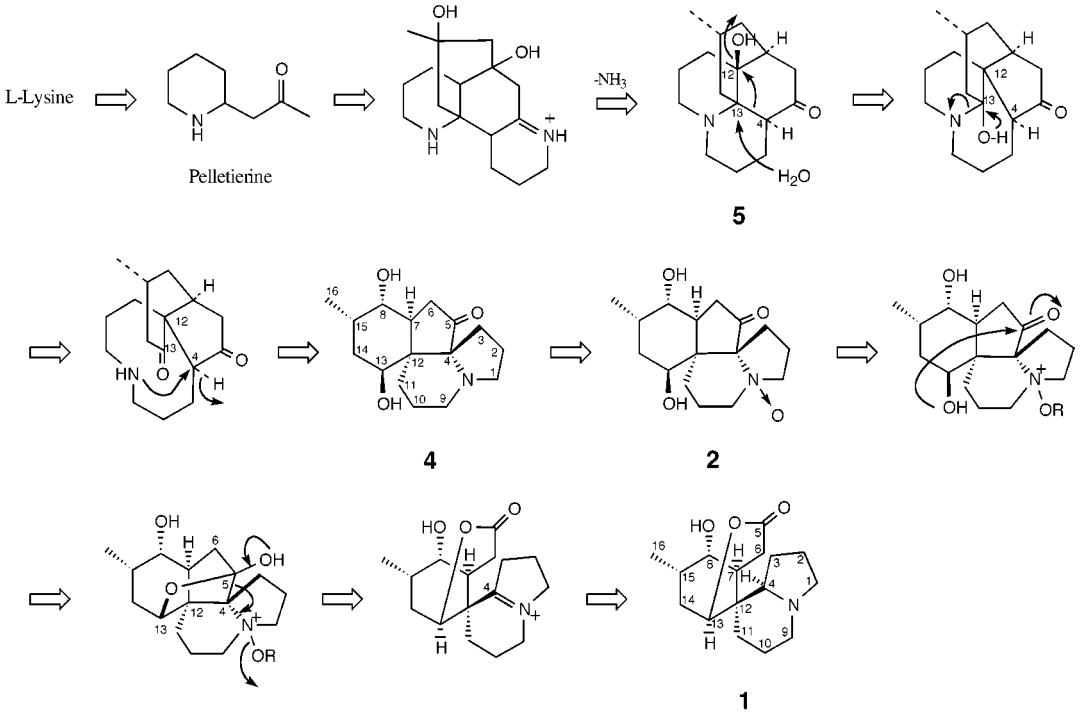
Scheme 1



Serratezomine B (2): colorless solid; $[\alpha]_D$ +7° (*c* 1.3, MeOH); IR (neat) ν_{max} 3420, 2930, 1750, 1680, and 1200 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); FABMS *m*/*z* 296 $(M+H)^+$; HRFABMS *m*/*z* 296.1863 (M + H; calcd for $C_{16}H_{26}NO_4$, 296.1862).

Serratezomine C (3): colorless solid; $[\alpha]_D$ −8° (*c* 0.3, MeOH); IR (neat) ν_{max} 3400, 2930, 1710, 1570, and 1460 cm^{-1}; ^1H and ^{13}C NMR data (Table 3); ESIMS *m*/*z* 280 $(M + H)^+$; HRESIMS *m*/*z* 280.1914 (M + H; calcd for $C_{16}H_{26}NO_3$, 280.1913).

Oxidation of Serratinine (4). *m*-Chloroperbenzoic acid (4 mg) was added to a stirred solution of serratinine (**4**, 5.0 mg) in CH_2Cl_2 (0.2 mL) at room temperature. The mixture was stirred at room temperature for 1 day, washed with 20% Na_2SO_3 (2 mL) and then H_2O (4 mL), and concentrated to give a pale yellow oil (5.5 mg). The oil was subjected to amino silica gel column chromatography ($CHCl_3$/MeOH, 15:1) to give the *N*-oxide derivative (2.1 mg), whose spectral data and $[\alpha]_D$ value were identical with those of serratezomine B (**2**).

Computational Methods. Molecular modeling was carried out using Macromodel ver. 6.0 and SYBYL ver. 6.5 programs for **1**. Each conformer was finally minimized by molecular mechanics calculation of the MMFF force field.[9] Floating chirality method by NOE restrained molecular dynamics simulations was performed employing a time step of 1.0 fs for the integration of Newton's equation of motion for a duration of 100 ps each. The atomic velocities were applied following a Boltzmann distribution about the center of mass at a temperature of 2000 K. Structures were sampled every 100 fs and analyzed, leading to an ensemble of 1000 structures per trajectory. The NOE derived range constraints were applied as a biharmonic constraining function $[k(\theta - \theta_0)$ added to the force field $(k = 200)]$. An initial structure satisfying the experimental restraints was embedded by distance geometry calculations, followed by minimization with TRIPOS force field.[19] An epimer with *R* configuration at C-12 was built up with molecular modeling from that with *S* configuration, followed by minimization.

Acknowledgment. We thank Dr. M. Shiro, Rigaku Corp., for useful advice in X-ray analysis. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

Supporting Information Available: 1D and 2D NMR spectra for compounds **1**−**3**. X-ray crystallographic data for compounds **4** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO000661E

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