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# Serratezomines D and E, new *Lycopodium* alkaloids from *Lycopodium serratum* var. *serratum*

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ABSTRACT

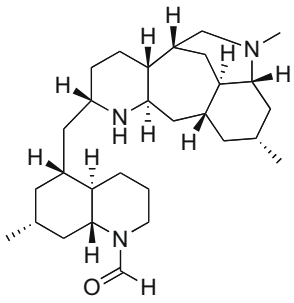
Two new *Lycopodium* alkaloids, serratezomines D (**1**) and E (**2**), were isolated from the club moss *Lycopodium serratum* var. *serratum*. Serratezomine D (**1**) is a new lucidine-type alkaloid, while serratezomine E (**2**) is a new phlegmarane-type alkaloid. The structures and relative stereochemistry of **1** and **2** were elucidated on the basis of spectroscopic data. Serratezomine D (**1**) exhibited an inhibitory activity against acetylcholinesterase.



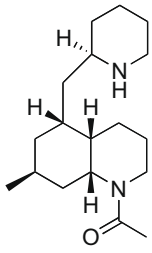
Club moss (Lycopodiaceae) are known to be a rich source of *Lycopodium* alkaloids possessing unique heterocyclic ring systems such as $C_{16}N_1$, $C_{16}N_2$, and $C_{27}N_3$,which have attracted great interest from biogenetic, synthetic, and biological points of view.[1] In our continuing efforts to find new *Lycopodium* alkaloids,[2] we previously isolated a novel seco-serratinine type *Lycopodium* alkaloid, serratezomine A, with two new *Lycopodium* alkaloids, serratezomines B and C, from the club moss *Lycopodium serratum* var. *serratum*.[3] Further investigation of another collection of this plant resulted in the isolation of a new lucidine-type alkaloid, serratezomine D (**1**), and a new phlegmarane-type alkaloid, serratezomine E (**2**). In this article, we describe the isolation and structure elucidation of **1** and **2**.

The club moss *Lycopodium serratum* var. *serratum* (2.6 kg) collected in Nayoro, Hokkaido, were extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. The aqueous phase was adjusted to pH 10 with saturated $Na_2CO_3$ and partitioned with $CHCl_3$. A part of the $CHCl_3$-soluble materials were purified by an amino silica gel column chromatography (*n*-hexane/EtOAc, $CHCl_3$/MeOH, and then MeOH/$NH_3$aq), in which a fraction eluted with $CHCl_3$/MeOH (100:1) was purified by silica gel column chromatographies ($CHCl_3$/MeOH), followed by preparative TLC ($CHCl_3$/MeOH/$H_2O$) to afford serratezomines D (**1**, 0.00003% yield) and E (**2**, 0.000005% yield) together with known *Lycopodium* alkaloids serratezomines A-C,[3] lucidines A and B,[4,5] oxolucidines A and B,[5,6] serratinine,[7] lycovatine A,[8] and lobscurinol.[9]

Serratezomine D (**1**)[10] showed the pseudomolecular ion peak at *m/z* 456 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{29}H_{49}N_3O$, was established by HRESIMS [*m/z* 456.3953, $(M+H)^+$, $\Delta$ −0.1 mmu]. IR absorptions implied the presence of hydroxy and/or amino (3372 $cm^{-1}$) and amide carbonyl (1685 $cm^{-1}$) functionalities. Inspection of the $^1H$ and $^{13}C$ NMR spectra and the HMQC spectrum revealed that **1** consisted of one amide carbonyl, twelve $sp^3$ methines, thirteen $sp^3$ methylenes, and three methyls (Table 1). Among them, four $sp^3$ methines ($\delta_C$ 65.1, 62.5, 49.5, and 47.9), two $sp^3$ methylenes ($\delta_C$ 57.1 and 41.7), and one methyl ($\delta_C$ 43.1) were attributed to those bearing a nitrogen atom.



**1**



**2**

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**Table 1**
$^{1}H$ and $^{13}C$ NMR data of serratezomine D (**1**) in $CDCl_3$[a]

| Position | $\delta_H$ | $\delta_C$ | | Position | $\delta_H$ | $\delta_C$ | |
|---|---|---|---|---|---|---|---|
| 2a | 4.63 (1H, d, 12.6 Hz) | 41.7 | t | 2′a | 2.96 (1H, m) | 57.1 | t |
| 2b | 2.37 (1H, m) | | | 2′b | 2.26 (1H, m) | | |
| 3a | 1.71 (1H, m) | 25.4 | t | 3′ | 2.33 (1H, m) | 43.1 | d |
| 3b | 1.43 (1H, m) | | | 4′a | 2.82 (1H, d, 10.8 Hz) | 28.1 | t |
| 4a | 2.19 (1H, m) | 29.7 | t | 4′b | 1.61 (1H, m) | | |
| 4b | 1.02 (1H, m) | | | 5′ | 1.75 (1H, m) | 25.4 | d |
| 5 | 0.89 (1H, m) | 47.1 | d | 6′ | 2.56 (1H, brs) | 65.1 | d |
| 6 | 2.87 (1H, ddd, 11.1, 11.1, 3.0 Hz) | 62.5 | d | 7′a | 2.06 (1H, m) | 37.6 | t |
| 7a | 2.06 (1H, m) | 37.1 | t | 7′b | 1.15 (1H, m) | | |
| 7b | 1.31 (1H, m) | | | 8′ | 1.54 (1H, m) | 27.8 | d |
| 8 | 1.50 (1H, m) | 31.3 | d | 9′a | 1.93 (1H, br d, 18.6 Hz) | 39.7 | t |
| 9a | 1.78 (1H, m) | 40.8 | t | 9′b | 1.71 (1H, m) | | |
| 9b | 0.65 (1H, ddd, 12.0, 12.0, 12.0 Hz) | | | 10′ | 0.91 (1H, m) | 22.2 | d |
| 10 | 1.10 (1H, m) | 37.6 | d | 11′ | 1.65 (2H, m) | 30.5 | t |
| 11a | 1.97 (1H, m) | 35.5 | t | 12′ | 0.97 (3H, d, 6.6 Hz) | 22.0 | q |
| 11b | 0.84 (1H, m) | | | 13′ | 2.33 (3H, s) | 43.1 | q |
| 12 | 1.01 (1H, d, 6.6 Hz) | 22.6 | q | | | | |
| 13 | 3.07 (1H, br s) | 47.9 | d | | | | |
| 14a | 1.71 (1H, m) | 30.8 | t | | | | |
| 14b | 1.32 (1H, m) | | | | | | |
| 15 | 1.58 (2H, m) | 19.8 | t | | | | |
| 16 | 2.17 (1H, m) | 38.2 | d | | | | |
| 17 | 2.96 (1H, m) | 49.5 | d | | | | |
| 19 | 8.18 (1H, s) | 158.6 | s | | | | |

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

The gross structure of **1** was elucidated from 2D NMR data as shown in Figure 1. Connections of C-2–C-17, C-5 to C-10, C-8 to C-12, C-2′–C-11′, C-8′ to C-12′, C-3′ to C-16, and C-11′ to C-17, which were revealed from analyses of the $^{1}H$–$^{1}H$ COSY, TOCSY, and HMQC-TOCSY spectra of **1**, suggested that **1** possessed a lucidine-type $C_{27}N_3$skeleton. HMBC correlations for a formyl proton ($\delta_H$ 8.18) to C-2 ($\delta_C$ 41.7), H-2 to C-6 ($\delta_C$ 62.5), and H—2 and H—6 to an amide carbonyl carbon ($\delta_C$ 158.6) indicated that C-2 and C-6 were connected through N-1, and a formyl group was attached to N-1. Connections among C-2′, C-6′, and C-13′ via N-1′ were indicated by HMBC cross-peaks of H-13′/C-2′ and H-13′/C-6′. Considering the molecular formula of **1** and $^{1}H$ and $^{13}C$ chemical shifts for two methines at C-13 and C-17, a connection of C-13 and C-17 through N-18 was elucidated. Thus, the gross structure of serratezomine D was assigned as **1**.

The relative stereochemistry of **1** was deduced from NOESY correlations as shown in Figure 2. Inspection of the phase-sensitive NOESY spectrum of **1** revealed that conformations of a piperidine ring (N-1 and C-2–C-6) and a cyclohexane ring (C-5–C-10) of a *trans* decahydroquinoline ring (N-1 and C-2–C-10) were both chair form. The conformations of a piperidine ring (C-13–C-17 and N-18) and a cyclohexane ring (C-5′–C-10′) were assigned as both chair form, while the conformation of a piperidine ring (N-1′ and C-2′–C-6′) of a *trans* decahydroquinoline ring (N-1′ and C-2′–C-10′) was elucidated to be half-chair form. NOESY correlations among H-6, H-8, and H-10 implied that a methyl group at C-8 was in an equatorial position and H-10 was in an axial position. NOESY cross-peaks of H-10/H-13, H-11a/H-17, H-16/H-4′a, H-17/H-9′a, and H-3′/H-4′a implied that H-13 and H-3′ were in equatorial positions and H-16 and H-17 were in axial positions. Axial positions of H-5′, H-6′, H-8′, and H-10′, and an equatorial position of a methyl group at C-8′ with respect to the cyclohexane ring (C-5′–C-10′) were suggested from NOESY correlations between H-4′b and H-6′, H-6′ and H-8′, and H-8′ and H-10′. Relative stereochemistry between unit **a** (N-1, C-2–C-10, C-12, and C-19) and unit **b** (C-13–C-17, N-18, N-1′, and C-2′–C-12′) via C-11 was deduced from NOESY correlations between H-4a and H-11a, H-10 and H-13, and H-11a and H-17 as shown in Figure 2. Thus, the relative stereochemistry of serratezomine D was elucidated to be **1**.

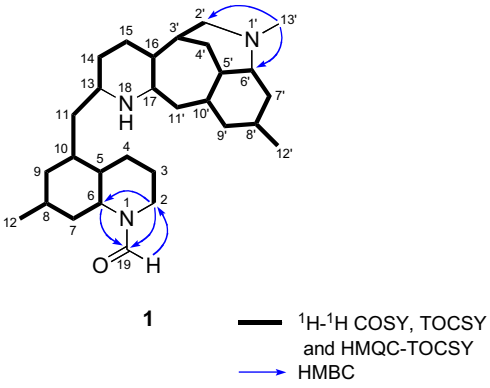


**Figure 1.** Selected 2D NMR correlations for serratezomine D (**1**).

Serratezomine E (**2**)[11] showed the pseudomolecular ion peak at *m/z* 293 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{18}H_{32}N_2O$, was established by HRESIMS [*m/z* 293.2585, $(M+H)^+$, $\Delta$-0.8 mmu]. The IR spectrum suggested the presence of hydroxy

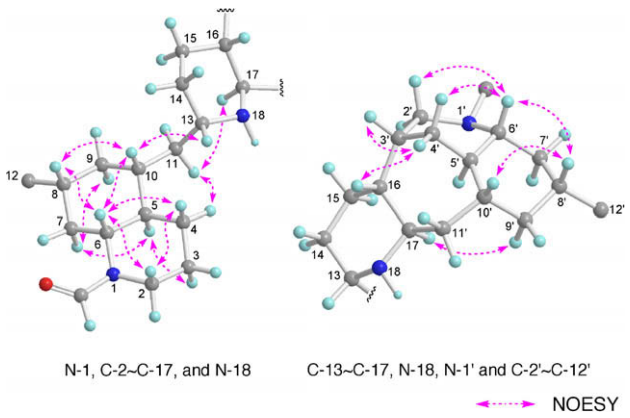


**Figure 2.** Selected NOESY correlations and relative stereochemistry for serratezomine D (**1**). (Hydrogen atoms of methyl groups were omitted).

and/or amino (3385 $cm^{-1}$) and amide carbonyl (1688 $cm^{-1}$) functionalities. Many pairs of signals were observed in the $^{1}H$ and $^{13}C$ NMR spectra of **2** with a ratio of approximately 5:4, suggesting that **2** existed as a mixture of two rotamers due to the rotation of its *N*-acetyl group (Table 2). However, structural elucidation of **2** was carried out using the mixture of the rotation isomers.

Analyses of the $^{1}H$–$^{1}H$ COSY and TOCSY spectra of **2** disclosed connections for C-2–C-17, C-5 to C-10, and C-8 to C-12, indicating that **2** had a phlegmarane-type $C_{16}N_2$ skeleton (Fig. 3). The HMBC correlation for $H_3$-20 to C-19 and NOESY correlations between $H_3$-20 and H-2 (rotamer **a**), and $H_3$-20 and H-6 (rotamer **b**) implied that C-2 and C-6 were connected through N-1 and an acetyl group was attached to N-1. Considering $^{1}H$ and $^{13}C$ chemical shifts for a methine at C-13 and a methylene at C-17 and the molecular formula of **2**, the connection of C-13 and C-17 through N-18 was elucidated. Thus, the gross structure of serratezomine E was assigned as **2**.

The relative stereochemistry of **2** was deduced from correlations observed in the phase-sensitive NOESY spectrum of **2** as depicted in Figure 4. The chair forms of a piperidine ring (N-1 and C-2–C-6) and a cyclohexane ring (C-5–C-10) of a *cis* decahydroquinoline ring (N-1 and C-2–C-10), and an axial position of a methyl group at C-8 were elucidated from NOESY correlations between H-2b and H-7a, H-5 and H-6, H-6 and H-10, and H-6 and $H_3$-12. The conformation of a piperidine ring (C-13–C-17 and N-18) was deduced from NOESY correlations between H-11a and H-17b, and H-15a and H-17b. NOESY cross-peaks of H-4/H-13, H-9a/H-11a, and H-9a/H-17b indicated the relative stereochemistry between a *cis* decahydroquinoline ring (N-1 and C-2–C-10) and a piperidine ring (C-13–C-17 and N-18) through C-11 as shown in Figure 4. Thus, the relative stereochemistry of serratezomine E was elucidated to be **2**.

Serratezomine D (**1**) is a new lucidine-type alkaloid consisting of a *trans* decahydroquinoline ring and a tetracyclic ring system including a *trans* decahydroquinoline ring and a piperidine ring fused to a cycloheptane ring, while serratezomine E (**2**) is a new phlegmarane-type alkaloid having a *cis* decahydroquinoline ring and a piperidine ring. Serratezomine D (**1**) exhibited an inhibitory

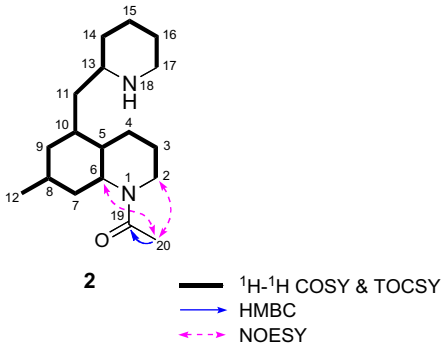


**Figure 3.** Selected 2D NMR correlations for serratezomine E (**2**).

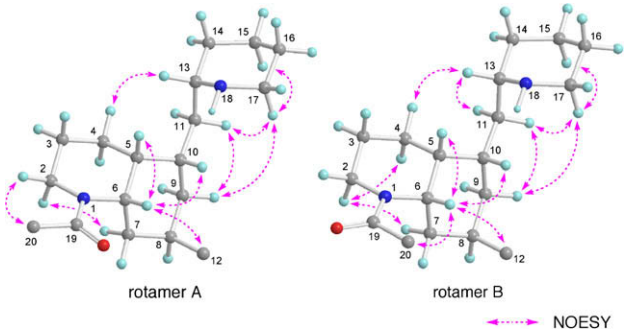


**Figure 4.** Selected NOESY correlations and relative stereochemistry for serratezomine E (**2**). (Hydrogen atoms of methyl groups were omitted).

**Table 2**
$^{1}H$ and $^{13}C$ NMR data of serratezomine E (**2**) in $CDCl_3$[a]

| Rotamer A | | | | Rotamer B | | | |
|---|---|---|---|---|---|---|---|
| Position | $\delta_H$ | $\delta_C$ | | Position | $\delta_H$ | $\delta_C$ | |
| 2a | 3.56 (1H, dd, 13.5, 4.3 Hz) | 41.8 | t | 2a | 4.47 (1H, dd, 12.9, 3.7 Hz) | 36.4 | t |
| 2b | 3.31 (1H, dt, 13.5, 3.0 Hz) | | | 2b | 2.59 (1H, dt, 13.8, 3.0 Hz) | | |
| 3a | 1.75 (1H, m) | 25.9 | t | 3a | 1.75 (1H, m) | 24.9 | t |
| 3b | 1.38 (1H, m) | | | 3b | 1.32 (1H, m) | | |
| 4 | 1.48 (2H, m) | 17.1[b] | t | 4 | 1.47 (2H, m) | 16.9[b] | t |
| 5 | 1.57 (1H, m) | 37.4 | d | 5 | 1.70 (1H, m) | 39.7 | d |
| 6 | 4.85 (1H, dt, 13.8, 4.6 Hz) | 46.0 | d | 6 | 3.90 (1H, dt, 12.6, 4.6 Hz) | 52.0 | d |
| 7a | 1.93 (1H, m) | 27.6 | t | 7a | 2.13 (1H, m) | 29.6 | t |
| 7b | 1.18 (1H, m) | | | 7b | 1.18 (1H, m) | | |
| 8 | 2.13 (1H, m) | 27.2 | d | 8 | 2.13 (1H, m) | 27.2 | d |
| 9a | 1.38 (1H, m) | 33.1 | t | 9a | 1.38 (1H, m) | 32.5 | t |
| 9b | 1.10 (1H, m) | | | 9b | 1.18 (1H, m) | | |
| 10 | 1.94 (1H, m) | 29.6 | d | 10 | 1.94 (1H, m) | 29.6 | d |
| 11a | 1.61 (1H, m) | 37.6 | t | 11a | 1.61 (1H, m) | 37.6 | t |
| 11b | 1.29 (1H, m) | | | 11b | 1.29 (1H, m) | | |
| 12 | 1.09 (3H, d, 7.8 Hz) | 18.8[c] | q | 12 | 1.07 (3H, d, 7.2 Hz) | 18.4[c] | q |
| 13 | 2.76 (1H, m) | 54.0 | d | 13 | 2.76 (1H, m) | 54.0 | d |
| 14a | 1.87 (1H, m) | 23.0 | t | 14a | 1.87 (1H, m) | 23.0 | t |
| 14b | 1.41 (1H, m) | | | 14b | 1.41 (1H, m) | | |
| 15a[d] | 1.94 (1H, m) | 28.4 | t | 15a[d] | 1.83 (1H, m) | 30.2 | t |
| 15b[d] | 1.28 (1H, m) | | | 15b[d] | 1.39 (1H, m) | | |
| 16a | 1.75 (1H, m) | 23.4 | t | 16a | 1.75 (1H, m) | 23.4 | t |
| 16b | 1.67 (1H, m) | | | 16b | 1.67 (1H, m) | | |
| 17a | 3.25 (1H, m) | 45.3 | t | 17a | 3.25 (1H, m) | 45.3 | t |
| 17b | 2.59 (1H, m) | | | 17b | 2.59 (1H, m) | | |
| 19 | | 169.0[e] | s | 18 | | 168.8[e] | s |
| 20 | 2.04 (3H, s) | 22.1[f] | q | 20 | 2.06 (3H, s) | 21.3[f] | q |

[a] $^{1}H$ and $^{13}C$ NMR spectra were recorded at 600 MHz and 150 MHz, respectively.
[b–f] Assignments are interchangeable.

activity against acetylcholinesterase ($IC_{50}$ 0.6 mM),[12–14] while lucidines A and B,[4,5] and oxolucidines A and B[5,6] showed less or no activity.

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10. *Serratezomine D* (**1**): colorless amorphous solid; $[\alpha]_D^{23}$ + 5.4 (*c* 0.37, $CHCl_3$); IR (film) $\nu_{max}$ 3372, 2927, 1685, 1647, 1457, 1200, and 1129 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; ESIMS *m/z* 456 $(M+H)^+$; HRESIMS *m/z* 456.3953 (M+H; calcd for $C_{29}H_{50}N_3O$, 456.3954).
11. *Serratezomine E* (**2**): colorless amorphous solid; $[\alpha]_D^{22}$ + 67.2 (*c* 0.06, $CHCl_3$); IR (film) $\nu_{max}$ 3385, 2925, 1688, 1623, 1436, 1200, and 1129 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data see Table 2; ESIMS *m/z* 293 $(M+H)^+$; HRESIMS *m/z* 293.2585 (M+H; calcd for $C_{18}H_{33}N_2O$, 293.2593).
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