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A New Type of Lycopodium Alkaloid, Lycoposerramine-A, from *Lycopodium serratum* Thunb

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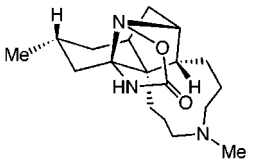
ABSTRACT



Lycoposerramine-A (**1**)

A novel alkaloid, lycoposerramine-A (1), which has a 1,2,4-oxadiazolidin-5-one residue in the molecule, was isolated from the club moss *Lycopodium serratum* Thunb. The structure was determined by spectroscopic and X-ray analyses.

The genus *Lycopodium* (Lycopodiaceae), which produced a potential therapeutic agent, huperzine A, for treatment of Alzheimer's disease has been extensively studied in recent years,[1] resulting in the isolation of several new alkaloids having biological activities.[2] Further, numerous efforts on the medicinal chemistry and pharmacological investigations using *Lycopodium* alkaloids in order to develop clinically useful drugs for patients suffering from various memory disorders have been carried out by many research groups.[3] In the course of our chemical and pharmacological studies on biologically active alkaloids in medicinal plants,[4] we embarked on an investigation of constituents in the lycopodiaceous plants. In this communication, we report the isolation and structure elucidation of a new class of alkaloid, lycoposerramine-A (**1**), isolated from *Lycopodium serratum* Thunb = *Huperzia serrata* (Thunb.) Trev.

The crude basic fraction obtained by the usual procedure from the MeOH extract of the club moss *L. serratum* collected in Boso Peninsula in Japan was purified by a combination of normal- and reverse-phase SiO_2 column chromatography to afford new alkaloid **1** (0.031% based on the crude base) together with known *Lycopodium* alkaloids such as huperzine A and B, lycodine, and serratidine.[5] Compound **1**, named lycoposerramine-A, was obtained as colorless needles (mp 169−171 °C).[6] High-resolution FABMS

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analysis gave *m*/*z* 320.2345 $(M + H)^+$ and established the molecular formula as $C_{18}H_{29}N_3O_2$, which indicated **1** to have the unsaturated number of six and to be the first case of a three-nitrogen-containing C_{16}-type of *Lycopodium* alkaloid.[5] The ^1H and ^{13}C NMR data (Table 1) disclosed the presence

Table 1. ^1H and ^{13}C Data of Lycoposerramine-A (**1**) in $CDCl_3$

	δ_H	δ_C	HMBC
1a	1.99 (1H, dd, 3.5, 13.9)	47.9	C-3,N-*C*H$_3$
1b	2.66 (1H, ddd, 4.1, 13.0)		C-2,9,N-*C*H$_3$
2a	1.12 (1H, *brt*, 13.3, 13.3)	27.6	
2b	1.66 (1H, m)		C-1
3a	1.84 (1H, m)	19.5	C-2,4,5
3b	2.40 (1H, m)		C-2,4,12
4	1.61 (1H, *brs*)	52.1	C-2,3,5,7,12,13
5	3.66 (1H, d, 4.3)	69.4	C-3,4,6,7,12
6a	1.84 (1H, m)	30.4	C-7,8
6b	1.28 (1H, dd, 4.6, 13.1)		C-3,4,5,7,8
7	2.06 (1H, m)	35.1	C-6,8,12,13,15
8a	1.02 (1H, ddd, 3.4, 13.1, 13.1)	31.8	C-6,7,15,16
8b	1.42 (1H, m)		C-7,15
9a	2.28 (1H, m)	56.0	C-10,11,N-*C*H$_3$
9b	2.40 (1H, m)		C-1,11
10a	1.42 (1H, m)	21.2	
10b	1.56 (1H, m)		C-9,12
11a	1.56 (1H, m)	18.6	C-4,10,12
11b	1.84 (1H, m)		C-9,10,12
12		53.1	
13		88.6	
14a	1.20 (1H, dd, 12.7, 12.7)	40.5	C-8,13,15,16
14b	1.84 (1H, m)		C-8,12,13,15
15	1.93 (1H, m)	22.6	C-8,14,16
16	0.91 (3H, d, 6.4)	21.4	C-8,13,14,15
18		157.0	
N-*C*H$_3$	2.25 (3H, s)	44.2	C-1,9
N*H*	5.08 (1H, *brs*)		

of one secondary methyl, nine sp^3 methylenes, four sp^3 methines, two quaternary carbons, and one carbonyl carbon. Among them, the chemical shift of the carbonyl carbon (δ_C 157.0) indicated the existence of a novel urethane, oxime, or a guanidine function in the molecule. Further, a characteristic signal at δ_C 88.6 implied the presence of an sp^3 carbon that had two geminal nitrogen atoms or an aminoacetal function. The gross structure of **1** was elucidated by analyses of $^1H-^1H$ COSY, FG-HMQC, and FG-HMBC spectra, as follows. The presence of two fragments of the three-carbon methylene chain (C1−C3 and C9−C11) was revealed by $^1H-^1H$ COSY spectrum (Figure 1). The HMBC spectrum (Table 1) indicated that one of the terminal carbons (C1 and C9) of each unit was attached to a nitrogen atom of an *N*-Me function. The $^1H-^1H$ COSY and HMQC spectra disclosed the presence of a long connectivity, -$CH_2CH(CH_3)CH_2$-$CHCH_2CH$-. The terminal carbon (C5) of this fragment bore a heteroatom because of the chemical shift of H-5 (δ 3.66).

(6) Colorless needles, mp 169−171 °C (from AcOEt); UV (EtOH) end absorption; FABMS *m*/*z* 320 $(M + H)^+$; HRFABMS *m*/*z* 320.2345 (M + H, calcd for $C_{18}H_{30}N_3O_2$, 320.2338); ORD (*c* 0.00058 g/mL, $CHCl_3$) $[\Phi]_{589}$ −16°, $[\Phi]_{339}$ −154°, $[\Phi]_{256}$ −632°, $[\Phi]_{245}$ 0°, $[\Phi]_{230}$ +342°, $[\Phi]_{213}$ +89°.

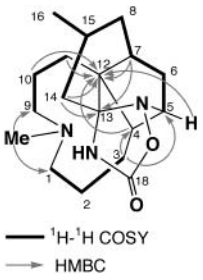

Figure 1. Selected 2D NMR correlations for lycoposerramine-A (**1**).

HMBC cross-peaks of H5 to C12, H4 to C12, and H7 to C12 indicated connection between C4 and C12, constructing a cyclopentane ring consisted of C4−C7 and C12. Further, a cyclohexane ring with C12−C15 and C8−C7 could be constructed on the basis of the HMBC cross-peaks of H7 to C13, H14 to C13, H14 to C12. HMBC cross-peaks of H3 to C4, C5, and C12 as well as those of H10 to C12 and H11 to C12 revealed the respective connection between C3 and C4 and between C11 and C12. These data enabled us to construct the basic skeleton of **1** possessing a fused tricyclic ring system with five- and six-membered cycloalkanes and a 1-azacyclononane, which retains the fundamental backbone of the known alkaloid, fawcettimine (**2**).[7] For constructing the final structure of **1** by incorporating the remaining elements, i.e., one carbonyl, two nitrogens, and one oxygen atoms, several candidates consisting of spectroscopic data above could be nominated, i.e., 1,2,4-oxadiazolidine-5-one, 1,2,4-oxadiazolidine-3-one, 1,3,4-oxadiazolidine-2-one, etc.

At this stage, X-ray crystallographic analysis of **1** was carried out.[8] As shown in Figure 2, it became clear that **1**

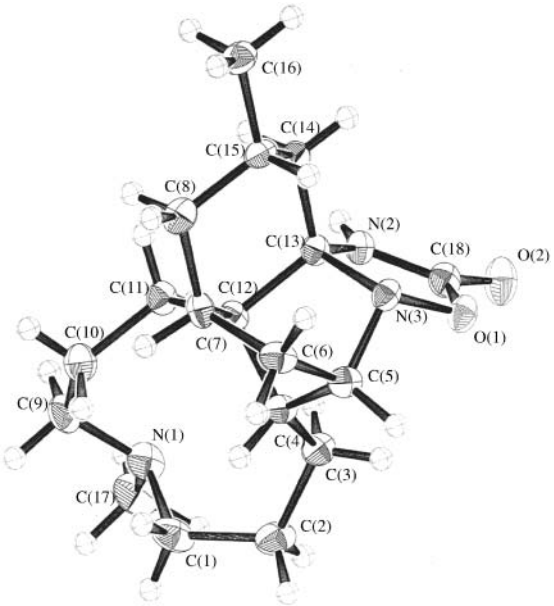

Figure 2. X-ray structure of lycoposerramine-A (**1**).

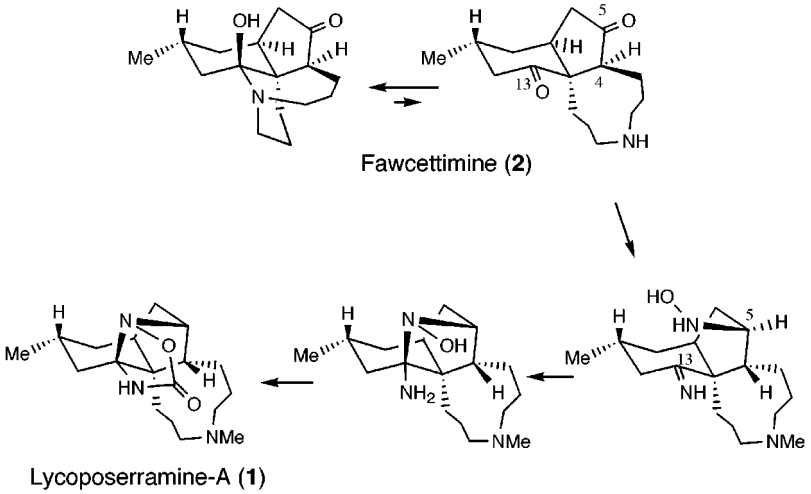

Figure 3. Hypothetical biogenetic route from fawcettimine to lycoposerramine-A.

had a novel 1,2,4-oxadiazolidine-5-one residue and one of their nitrogen atoms connected to the C5 position. Although the absolute configuration of **1** was not determined by the present study, it would be easily supposed to be the same as that of other *Lycopodium* alkaloids from a biogenetic viewpoint. Thus, lycoposerramine-A would be biogenetically derived from fawcettimine (**2**)[7] via the following hypothetical route. (Figure 3) The C5 and C13 carbonyl functions in **2** would be converted to hydroxylamine and imine, respectively, accompanied by *N*-methylation and C4 epimerization. Then, a diaminoacetal would form on C13 by transannulation from the resulting nitrogen atom attached on C5, followed by the formation of cyclic carbamate through the incorporaton of an external carbonyl unit.

To the best of our knowledge, lycoposerramine-A is the first example of a natural product that contains a 1,2,4-oxadiazolidin-5-one residue in the molecule. Our preliminary biological tests by the Ellman method[9] showed that compound **1** exhibited no effect on the inhibition of acetylcholinesterase (from bovine erythrocytes) at the concentration of 200 μmol/L, while the IC_{50} of huperzine A was 1 μmol/L under the experimental conditions. Further investigation of the minor constituents of this plant and biological evaluation of the alkaloids using different assay systems are in progress in our laboratory.

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(8) **Crystal Data for 1.** Data were acquired with a Bruker SMART1000 CCD diffractometer Mo Kα radiation (λ = 0.71069 Å), graphite monochromated, orthorhombic, $C_{18}H_{29}N_3O_2$ (MW 319.45), space group $P2_12_12_1$ with a = 8.721(1) Å, b = 9.711(2) Å, c = 19.469(3) Å, V = 1660.3(4) Å^3, Z = 4, and D_{calc}=1.278 g/cm^3 The final R value was 0.039 (Rw = 0.045) for 1237 reflections ($I > 3\sigma(I)$).

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