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Sieboldine A, a Novel Tetracyclic Alkaloid from *Lycopodium sieboldii*, Inhibiting Acetylcholinesterase

Yusuke Hirasawa,[†] Hiroshi Morita,[†] Motoo Shiro,[‡] and Jun'ichi Kobayashi*,[†]

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, and X-ray Research Laboratory, Rigaku Corporation, Akishima, Tokyo 196-8666, Japan

jkobay@pharm.hokudai.ac.jp

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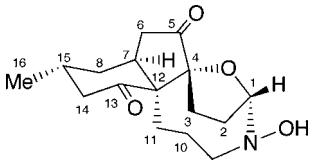
ABSTRACT



sieboldine A (**1**)

A novel *Lycopodium* alkaloid with an unprecedented fused-tetracyclic ring system consisting of an aza-cyclononane ring having a *N*-hydroxy group, a cyclohexanone, a cyclopentanone, and a tetrahydrofuran ring, sieboldine A (1), was isolated from the club moss *Lycopodium sieboldii*. The structure and relative stereochemistry were elucidated on the basis of 2D NMR data and X-ray analysis. Sieboldine A (1) exhibited a potent inhibitory activity against acetylcholinesterase and modest cytotoxicity.

Plants of *Lycopodium* species (Lycopodiaceae) produce a number of structurally diverse alkaloids[1] that often possess unusual skeletons, and many of them continue to be of interest from biogenetic[2] and biological[1a,1b] points of view, as well as challenging targets for total synthesis.[3] Recently we have isolated serratezomines A, B, and C[4] with seco-serratinine-type, serratinine-type, and lycodoline-type skeletons, respectively, from the club moss *L. serratum* var. *serratum*, complanadine A[5] with a lycodine-dimeric skeleton and lyconadin A[6] consisting of a fused pentacyclic ring system from *L. complanatum*, and senepodines A−E,[7] lyconesidines A−C,[8] and himeradine A[9] from *L. chinense*. In our search for biogenetically interesting intermediates of

[†] Hokkaido University.
[‡] Rigaku Corporation.

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Lycopodium alkaloids, sieboldine A (**1**), a novel alkaloid with an unprecedented skeleton consisting of an aza-cyclononane ring having an *N*-hydroxy group, a cyclohexanone, a cyclopentanone, and a tetrahydrofuran ring, was isolated from the club moss *L. sieboldii*. In this paper we describe the isolation and structure elucidation of **1**.

The club moss *L. sieboldii* collected in Kagoshima 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 saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an LH-20 column ($CHCl_3$/MeOH, 1:1), followed by a silica gel column ($CHCl_3$/MeOH, 80:1), to afford sieboldine A[10] (**1**, 10 mg, 0.003% yield), together with a known, related alkaloid, alopecuridine[11] (**2**, 0.03%).

Sieboldine A (**1**) showed a pseudomolecular ion peak at *m/z* 294 $(M + H)^+$ in the FABMS, and the molecular formula $C_{16}H_{23}NO_4$ was established by HRFABMS [*m/z* 294.1709, $(M + H)^+$, Δ + 0.4 mmu]. IR absorptions implied the presence of hydroxyl (3400 cm^{-1}) and cyclopentanone and cyclohexanone (1750 and 1695 cm^{-1}, respectively) functionalities. ^{13}C NMR data (Table 1) revealed 16 carbon

Table 1. ^1H and ^{13}C NMR Data of Sieboldine A (**1**) in CD_3OD

	δ_H	δ_C
1	4.89 (1H, m)	98.5
2a	1.98 (1H, m)	31.4
2b	2.12 (1H, m)	
3a	2.08 (1H, m)	26.1
3b	2.40 (1H, m)	
4		92.8
5		212.6
6a	1.93 (1H, dd, 19.6, 10.9)	37.2
6b	2.45 (1H, dd, 19.6, 9.2)	
7	3.25 (1H, m)	38.7
8a	1.76 (1H, m)	31.8
8b	1.77 (1H, m)	
9a	2.91 (1H, ddd, 14.8, 8.0, 3.7)	54.5
9b	3.19 (1H, m)	
10a	1.63 (1H, m)	19.4
10b	2.57 (1H, m)	
11a	1.77 (1H, m)	28.3
11b	2.46 (1H, m)	
12		62.3
13		216.5
14a	2.03 (1H, m)	47.7
14b	2.54 (1H, dd, 12.7, 12.7)	
15	2.06 (1H, m)	32.5
16	1.06 (3H, d, 6.2)	22.5

signals due to two carbonyls, two sp^3 quaternary carbons, three sp^3 methines, eight sp^3 methylenes, and one methyl. Among them, one quaternary carbon (δ_C 92.8) was ascribed to that bearing an oxygen atom, and one methine (δ_C 98.5; δ_H 4.89) was ascribed to that bearing both an oxygen and a nitrogen atom.

The $^1H-^1H$ COSY and HOHAHA spectra revealed connectivities of three partial structures **a** (C-6 to C-8, C-8 to C-15, and C-14 to C-16), **b** (C-9 to C-11), and **c** (C-1 to C-3) as shown in Figure 1. HMBC correlations were

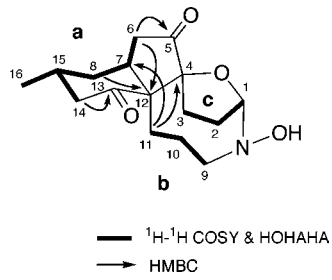

Figure 1. Selected 2D NMR correlations for sieboldine A (**1**).

observed for H-6b and H-8a to C-12 (δ_C 62.3) and H-11b to C-7 (δ_C 38.7), suggesting that C-7 and C-11 were connected to each other through C-12. The connectivity of C-4 to C-12 was implied by an HMBC correlation for H-11b to C-4 (δ_C 92.8). HMBC cross-peaks for H_2-6 to C-5 (δ_C 212.6) and H_2-14 to C-13 (δ_C 216.5) indicated that two carbonyls were attached to C-6 and C-14, respectively. Since ^1H and ^{13}C NMR signals at C-2, -3, -10, and -11 were broadened, further connections could not be clarified by the NMR data.

The X-ray crystal structure[12] (Figure 2) of sieboldine A (**1**) revealed a unique, fused-tetracyclic ring system consisting

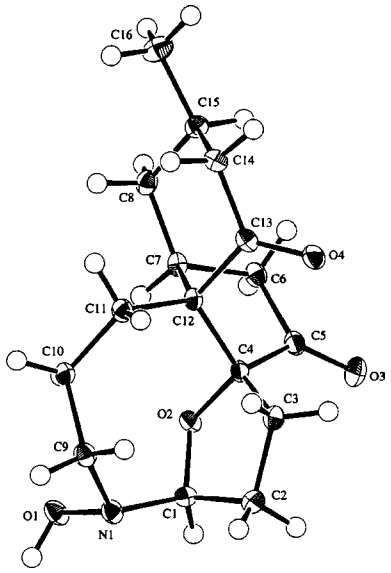

Figure 2. X-ray structure of sieboldine A (**1**).

of an aza-cyclononane ring (C-1−C-4, C-9−C-12, and N-1) having an *N*-hydroxy group, a tetrahydrofuran ring (C-1−

(10) Sieboldine A (**1**): colorless needles; dec 160 °C (from CH_3OH), $[\alpha]_D$ +139° (*c* 0.3, CH_3OH); IR (neat) ν_{max} 3400, 2940, 1750, 1695, 1450, and 1060 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); FABMS *m/z* 294 $(M + H)^+$; HRFABMS *m/z* 294.1709 (M + H; calcd for $C_{16}H_{24}NO_4$, 294.1705).

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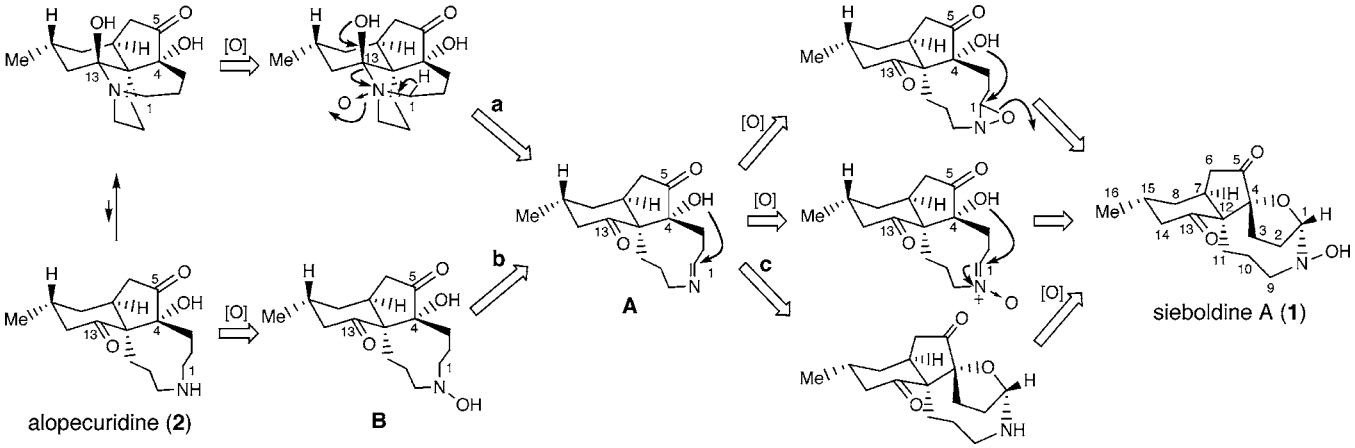
Scheme 1. Plausible Biogenetic Path for Sieboldine A (**1**)



C-4 and O-2) connected to a *cis*-5,13-dioxo-octahydroindene ring (C-4−C-8 and C-12−C-15) with a methyl (C-16) group at C-15 through the spiro carbon at C-4.

A plausible biogenetic pathway for sieboldine A (**1**) is proposed as shown in Scheme 1. Alopecuridine (**2**),[11] which might be derived from lycopodane skeleton,[1] as well as fawcettimine,[13] may exist in either an aminoacetal form or an amino ketone form. The aminoacetal form of **2** was confirmed directly by X-ray analysis (Figure 3).[14] Sieboldine A (**1**) might be generated from a fawcettimine-type alkaloid such as alopecuridine[11] (**2**) as follows. Cleavage of the C-13−N-1 bond of an N-oxidative product of **2** followed by Polonovski-type reaction[15] (path **a**) might result in an iminium intermediate **A** with a nine-membered ring system, although an alternative path through a hydroxylamine derivative **B** is also possible (path **b**). Oxidation of the imine **A** to produce an oxaziridine ring or a nitrone followed by attack of the hydroxy group at C-4 to C-1 will give sieboldine A (**1**) with a hydroxylamine and a tetrahydrofuran ring, although an alternative path (path **c**) is also possible.

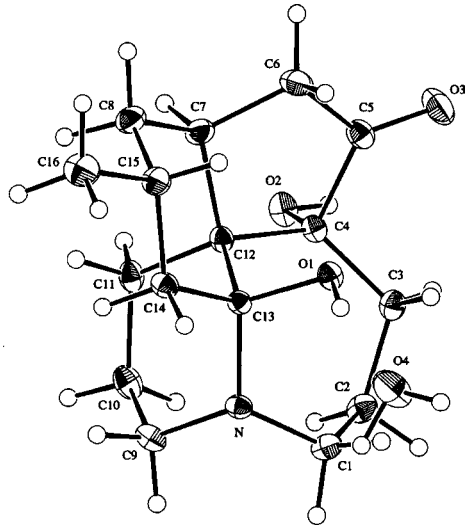


Figure 3. X-ray structure of alopecuridine (**2**). One H_2O molecule is contained in the crystal.

Sieboldine A (**1**) inhibited acetylcholinesterase (from electric eel) with an IC_{50} value of 2.0 μM, which was comparable to that (IC_{50} 1.6 μM) of (±)-huperzine A,[16,17] and exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50} 5.1 μg/mL) in vitro,[18] whereas **2** did not show such activity (IC_{50} >10 μg/mL).

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

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(12) Crystal data for sieboldine A (**1**): dec 160 °C (from CH_3OH), $C_{16}H_{23}NO_4$, M_r = 293.36, monoclinic, space group $P2_12_12_1$ (No. 19), a = 7.8245 (7), b = 10.4867(9), c = 17.601(1) Å, V = 1444.2(2) Å^3, Z = 4, D_{calc} = 1.349 g/cm^3. The final R value was 0.053 (R_W = 0.078) for 2398 reflections [$I > -3.00\sigma(I)$].

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(14) Crystal data for alopecuridine (**2**): mp 170−171 °C (from CH_3OH), $C_{17}H_{21}NO_4$, M_r = 303.36, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 13.329 (1), b = 15.113(1), c = 7.459(2) Å, V = 1502.5(4) Å^3, Z = 4, D_{calc} = 1.341 g/cm^3. The final R value was 0.047 (R_W = 0.090) for 1983 reflections [$I > -3.00\sigma(I)$].

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