

Lycoperine A, A Novel C₂₇N₃-Type Pentacyclic Alkaloid from *Lycopodium hamiltonii*, Inhibiting Acetylcholinesterase

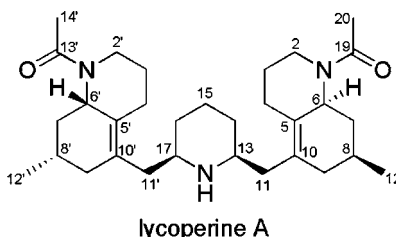
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ABSTRACT



lycoperine A

A novel C₂₇N₃-type pentacyclic *Lycopodium* alkaloid, lycoperine A (1) consisting of two octahydroquinoline rings and a piperidine ring, was isolated from the club moss *Lycopodium hamiltonii*. The structure and relative stereochemistry were elucidated on the basis of 2D NMR data and chemical transformation. Lycoperine A (1) exhibited an inhibitory activity against acetylcholinesterase.

Plants of the *Lycopodium* species (Lycopodiaceae) produce a number of structurally diverse alkaloids,¹ which often possess unusual skeletons, and many of them continue to be of interest from biogenetic² and biological¹ points of view, as well as challenging targets for total synthesis.³ Recently, we isolated new types of alkaloids, such as sieboldine A,⁴

serratezomine A,⁵ complanadine A,⁶ lyconadin A,⁷ senepodine A,⁸ lyconesidine A,⁹ himeradine A,¹⁰ cermizine A,¹¹ and nankakurine A,¹² from various *Lycopodium* spp. In our search for biogenetically interesting *Lycopodium* alkaloids, lyco-

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perine A (**1**), a novel C₂₇N₃-type alkaloid consisting of two octahydroquinoline rings and a piperidine ring, was isolated from the club moss *Lycopodium hamiltonii*. In this paper, we describe the isolation and structure elucidation of **1**.

The club moss *L. hamiltonii* 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 aqueous Na₂CO₃, were extracted with CHCl₃. CHCl₃-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, 1:0 → 0:1, and then CHCl₃/MeOH, 1:0 → 0:1), in which a fraction eluted with hexane/EtOAc (3:2) was purified by a silica gel column (CHCl₃/MeOH, 10:1) to afford lycoperine A (**1**, 0.009%)¹³ and a known C₁₆N₂-type alkaloid, nankakurine A (0.003%).¹²

Lycoperine A (**1**) was shown to have the molecular formula C₃₁H₄₉N₃O₂ by HRFABMS [*m/z* 496.3907, (M + H)⁺, Δ +0.4 mmu]. The IR spectrum was indicative of amide carbonyl (1629 cm⁻¹) and amine (3440 cm⁻¹) functionalities. ¹H and ¹³C NMR spectra showed broad signals because of rotation of its *N*-acetyl moiety. Treatment of **1** with LiAlH₄ afforded tetrahydrodeoxylycoperine A (**2**), which provided sharp signals on the ¹H and ¹³C NMR spectra.

Tetrahydrodeoxylycoperine A (**2**) showed the pseudo-molecular ion peak at *m/z* 468 (M + H)⁺. Analysis of the ¹H and ¹³C NMR data (Table 1) and the HMQC spectrum of **2** revealed the presence of six sp³ methines, seventeen sp³ methylenes, four sp² quaternary carbons, and four methyl groups. Among them, four sp³ methylenes (δ_C 52.5, δ_H 2.17 and 2.91; δ_C 52.4, δ_H 2.13 and 2.85; δ_C 47.3, δ_H 2.42 and 2.83; δ_C 47.2, δ_H 2.43 and 2.78) and four sp³ methines (δ_C 61.7, δ_H 2.84; δ_C 61.6, δ_H 2.75; δ_C 56.6, δ_H 2.70; δ_C 55.6, δ_H 2.69) were ascribed to those bearing a nitrogen atom.

The gross structure of **2** was deduced from extensive analyses of the 2D NMR data, including the ¹H–¹H COSY, HOHAHA, HMQC, and HMBC spectra in C₆D₆ (Figure 1).

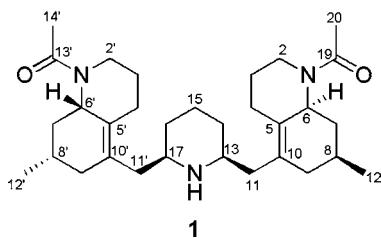


Figure 1. Structure for lycoperine A (**1**).

The ¹H–¹H COSY and HOHAHA spectra in C₆D₆ revealed connectivities of seven partial structures, **a** (C-2 ~ C-4), **b** (C-6 ~ C-9, C-12), **c** (C-11, C-13 ~ C-17, C-11'), **d** (C-2' ~ C-4'), **e** (C-6' ~ C-9', C-12'), **f** (C-19 ~ C-20), and **g** (C-13' ~ C-14'), as shown in Figure 2.

(13) Lycoperine A (**1**): colorless solid; [α]_D²⁵ –238 (c 0.1, MeOH); IR (neat) ν_{max} 3440, 2930, 1630, 1430 cm⁻¹; FABMS *m/z* 496 (M + Na)⁺; HRMS–FAB *m/z* 496.3907 (M + H) calcd for C₃₁H₅₀N₃O₂, 496.3903.

Table 1. ¹H and ¹³C NMR Data for Tetrahydrodeoxylycoperine A (**2**) in C₆D₆ (Bruker AMX600)

	δ _H	δ _C
2a	2.17 (1H, ddd, 11.6, 11.3, 2.6)	52.5
2b	2.91 (1H, brd, 11.3)	
3a	1.68 (1H, m)	26.3
3b	1.68 (1H, m)	
4a	1.76 (1H, m)	28.8
4b	2.99 (1H, brd, 13.2)	
5		128.3
6	2.84 (1H, m)	61.7
7a	1.27 (1H, m)	38.4
7b	2.01 (1H, m)	
8	1.62 (1H, m)	28.5
9a	1.78 (1H, m)	39.9
9b	1.98 (1H, m)	
10		132.0
11a	2.26 (1H, dd, 13.1, 6.5)	41.1
11b	2.37 (1H, m)	
12	0.98 (3H, m)	22.5
13	2.70 (1H, m)	56.6
14a	1.26 (1H, m)	32.5
14b	1.71 (1H, m)	
15a	1.35 (1H, m)	25.6
15b	1.83 (1H, m)	
16a	1.32 (1H, m)	33.5
16b	1.58 (1H, m)	
17	2.69 (1H, m)	55.6
19a	2.42 (1H, m)	47.3
19b	2.83 (1H, m)	
20	1.00 (3H, m)	10.1
2a'	2.13 (1H, ddd, 11.2, 11.2, 1.6)	52.4
2b'	2.85 (1H, m)	
3a'	1.62 (1H, m)	26.5
3b'	1.71 (1H, m)	
4a'	1.58 (1H, m)	28.6
4b'	3.04 (1H, brd, 13.9)	
5'		128.3
6'	2.75 (1H, m)	61.6
7a'	1.22 (1H, m)	38.2
7b'	1.98 (1H, m)	
8'	1.55 (1H, m)	28.3
9a'	1.85 (1H, m)	39.7
9b'	1.98 (1H, m)	
10'		132.3
11a'	1.92 (1H, brd, 12.8)	41.7
11b'	2.56 (1H, dd, 12.8, 10.0)	
12'	0.97 (3H, m)	22.4
13a'	2.43 (1H, m)	47.2
13b'	2.78 (1H, m)	
14'	1.00 (3H, m)	9.7

In the octahydroquinoline moiety, the connectivity of partial structures **a** and **b** revealed by the ¹H–¹H COSY and HOHAHA spectra was analyzed by the HMBC spectrum. HMBC correlations from H₂–19 to C-2 (δ_C 52.5) and C-6 (δ_C 61.7) established the connection among C-2, C-6, and C-19 through a nitrogen atom. HMBC cross-peaks of H-4, H-7, and H-11 to C-5, of H-4 and H-11 to C-10, and of H-11 to C-9 indicated the connection among partial structures **a**, **b**, **c**, and four-substituted olefinic carbons assigned to C-5 and C-10, constructing the octahydroquinoline ring (C-2 ~

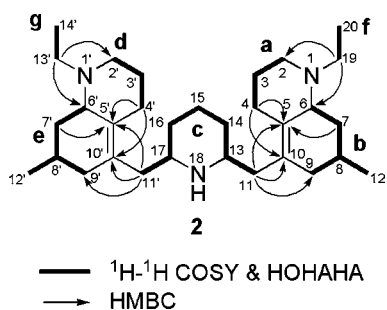


Figure 2. Selected 2D NMR correlations for tetrahydrodeoxylycoperine A (**2**).

C-10 and N-1) with a methyl group (C-12) at C-8. Another octahydroquinoline moiety (C-2' ~ C-10' and N-1') was analyzed in the same way as that mentioned above. Thus, the gross structure of tetrahydrodeoxylycoperine A was assigned as **2**.

The relative stereochemistry of **2** was elucidated by NOESY correlations and by comparison of chemical shifts with known piperidine derivatives. The NOESY correlation of H-6 to H-8 suggested that H-6 and H-8 were oriented to the same side (Figure 3). A similar incident was observed

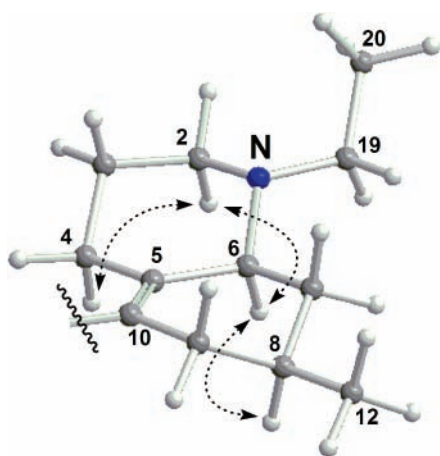


Figure 3. Selected NOESY correlations (dotted arrow) and relative configurations for an octahydroquinoline ring of tetrahydrodeoxylycoperine A (**2**).

for another octahydroquinoline moiety (C-2' ~ C-10' and N-1'). The relative stereochemistry of the piperidine moiety (C-13 ~ C-17 and N-18) was deduced from comparison of chemical shifts with andrachamine (**3**) and its analogue (**4**).¹⁴

The ¹H signals assigned to the piperidine ring of **2** were observed at δ 2.70 (H-13) and δ 2.69 (H-17), and the ¹³C signals were observed at δ 56.6 (C-13), δ 25.6 (C-15), and δ 55.6 (C-17) (see Table 1). The ¹³C signals of the trans-

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substituted piperidine analogue (**4**) of andrachamine were observed at higher fields than those of **2** and **3** (see Figure 4). On the other hand, andrachamine (**3**) with a cis-substituted

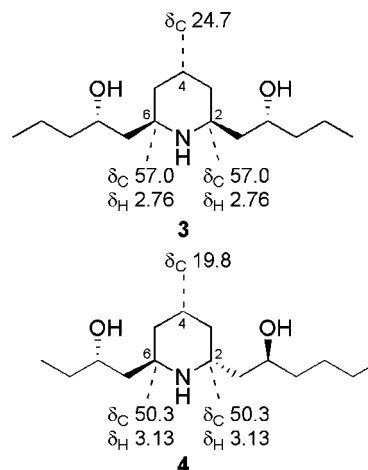


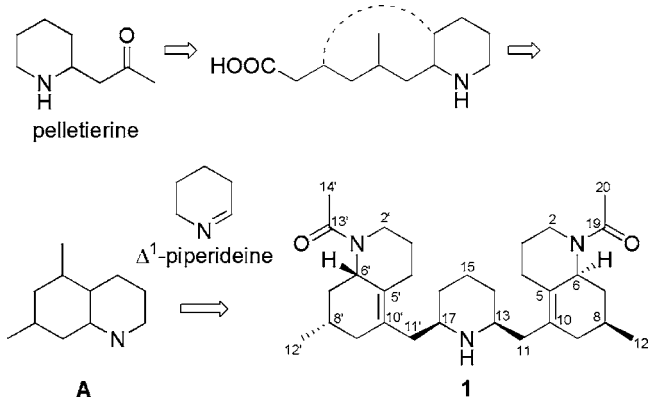
Figure 4. Partial NMR chemical shifts of andrachamine (**3**) and its analogue (**4**).

piperidine ring showed ¹H and ¹³C chemical shifts similar to those of **2**. Thus, the piperidine ring (C-13 ~ C-17 and N-18) of tetrahydrodeoxylycoperine A (**2**) was assigned as cis configuration. The gross relative stereochemistry of **2** was deduced from the ¹³C NMR spectrum. Because **2** is not a symmetrical structure (26 signals were observed in the ¹³C NMR spectrum of **2**), the absolute configurations of the two octahydroquinoline moieties were elucidated to be the same.

Consequently, the relative stereochemistry of lycoperine A consisting of the two octahydroquinoline rings with the same absolute stereochemistry and a piperidine ring was assigned as **1**.

A plausible biogenetic pathway for lycoperine A (**1**) is proposed as shown in Scheme 1. Lycoperine A (**1**) might

Scheme 1. Plausible Biogenetic Pathway for Lycoperine A (**1**)



be generated from two octahydroquinoline units (**A**) and a Δ^1 -piperideine unit.

Lycoperine A (**1**) inhibited acetylcholinesterase (from bovine erythrocyte) with IC_{50} , $60.9 \mu M$.¹⁵

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

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