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# Lycoperine A, A Novel $C_{27}N_3$-Type Pentacyclic Alkaloid from *Lycopodium hamiltonii*, Inhibiting Acetylcholinesterase

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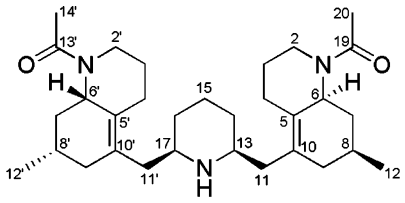
## ABSTRACT




lycoperine A

**A novel $C_{27}N_3$-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 biogenetic[2] and biological[1] 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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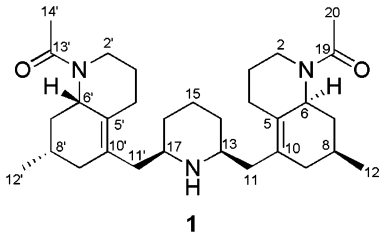
perine A (**1**), a novel $C_{27}N_3$-type alkaloid consisting of two octahydroquinoline rings and a piperidine ring, was isolated from the club moss *Lycopodium hamiltonii*. In this paper, we describe the isolation and structure elucidation of **1**.

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 $Na_2CO_3$, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, 1:0 → 0:1, and then $CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with hexane/EtOAc (3:2) was purified by a silica gel column ($CHCl_3$/MeOH, 10:1) to afford lycoperine A (**1**, 0.009%)[13] and a known $C_{16}N_2$-type alkaloid, nankakurine A (0.003%).[12]

Lycoperine A (**1**) was shown to have the molecular formula $C_{31}H_{49}N_3O_2$ by HRFABMS [*m*/*z* 496.3907, $(M + H)^+$, $\Delta$ +0.4 mmu]. The IR spectrum was indicative of amide carbonyl (1629 $cm^{-1}$) and amine (3440 $cm^{-1}$) functionalities. $^1H$ and $^{13}C$ NMR spectra showed broad signals because of rotation of its *N*-acetyl moiety. Treatment of **1** with $LiAlH_4$ afforded tetrahydrodeoxylycoperine A (**2**), which provided sharp signals on the $^1H$ and $^{13}C$ NMR spectra.

Tetrahydrodeoxylycoperine A (**2**) showed the pseudomolecular ion peak at *m*/*z* 468 $(M + H)^+$. Analysis of the $^1H$ and $^{13}C$ NMR data (Table 1) and the HMQC spectrum of **2** revealed the presence of six $sp^3$ methines, seventeen $sp^3$ methylenes, four $sp^2$ quaternary carbons, and four methyl groups. Among them, four $sp^3$ methylenes ($\delta_C$ 52.5, $\delta_H$ 2.17 and 2.91; $\delta_C$ 52.4, $\delta_H$ 2.13 and 2.85; $\delta_C$ 47.3, $\delta_H$ 2.42 and 2.83; $\delta_C$ 47.2, $\delta_H$ 2.43 and 2.78) and four $sp^3$ methines ($\delta_C$ 61.7, $\delta_H$ 2.84; $\delta_C$ 61.6, $\delta_H$ 2.75; $\delta_C$ 56.6, $\delta_H$ 2.70; $\delta_C$ 55.6, $\delta_H$ 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 $C_6D_6$ (Figure 1).




**1**

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

The $^1H-^1H$ COSY and HOHAHA spectra in $C_6D_6$ 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; $[\alpha]^{24}_D$ −238 (*c* 0.1, MeOH); IR (neat) $\nu_{max}$ 3440, 2930, 1630, 1430 $cm^{-1}$; FABMS *m*/*z* 496 $(M + Na)^+$; HRMS−FAB *m*/*z* 496.3907 (M + H) calcd for $C_{31}H_{50}N_3O_2$, 496.3903.

**Table 1.** $^1H$ and $^{13}C$ NMR Data for Tetrahydrodeoxylycoperine A (**2**) in $C_6D_6$ (Bruker AMX600)

| | $\delta_H$ | $\delta_C$ |
|---|---|---|
| 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 $H_2$-19 to C-2 ($\delta_C$ 52.5) and C-6 ($\delta_C$ 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 ~

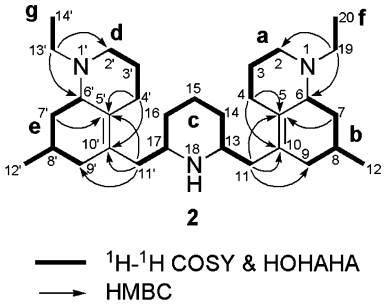



**Figure 2.** Selected 2D NMR correlations for tetrahydrodeoxylycoperine A (**2**).

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 metioned 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

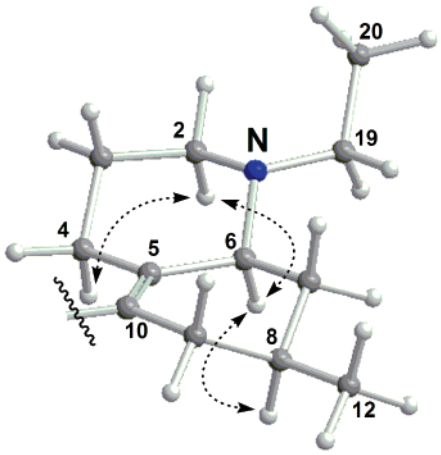



**Figure 3.** Selected NOESY correlations (dotted arrow) and relative configurations for an octahydroquinoline ring of tetrahydrodeoxylycoperine A (**2**).

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

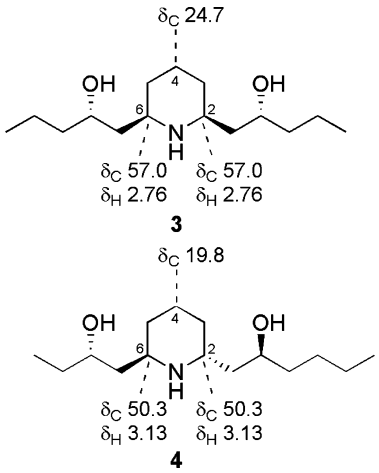


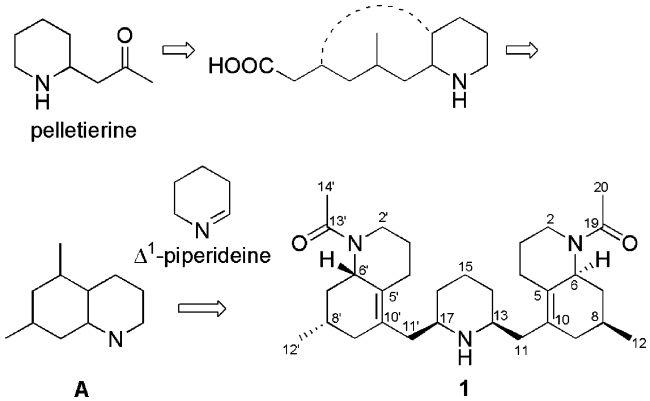

**Figure 4.** Partial NMR chemical shifts of andrachamine (**3**) and its analogue (**4**).

piperidine ring showed $^1$H and $^{13}$C 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 $^{13}$C NMR spectrum. Because **2** is not a symmetrical structure (26 signals were observed in the $^{13}$C 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 piperdine 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 $\Delta^1$-piperideine unit.

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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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