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Lycojapodine A, a Novel Alkaloid from *Lycopodium japonicum*

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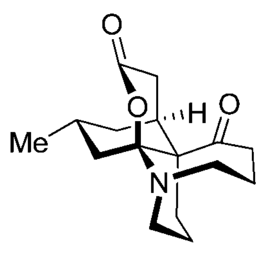
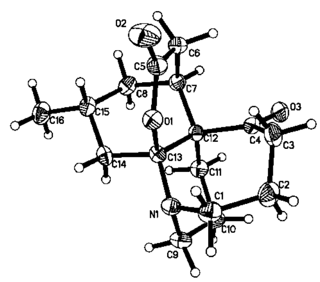
ABSTRACT



Lycojapodine A (**1**)



Lycojapodine A, a novel $C_{16}N$-type *Lycopodium* alkaloid with an unprecedented 6/6/6/7 tetracyclic ring system, was isolated from the club moss *Lycopodium japonicum*. The structure and relative stereochemistry were elucidated on the basis of spectroscopic data and were further confirmed by X-ray analysis. A possible biosynthetic pathway for 1 was proposed. Its inhibitory activity on acetylcholinestrease and anti-HIV-1 activity were also evaluated.

Lycopodium alkaloids, elaborated by plants of the genus *Lycopodium* (Lycopodiaceae), are a group of structurally diverse alkaloids[1] that often possess unusual skeletons and exhibit potent acetylcholinesterase inhibitory activity.[2] Many of them, such as fawcettimine,[3] cernuine,[4] nakaurines A,[5] and lyconadin A,[6] continue to be challenging targets for total synthesis. *Lycopodium japonium* THUNB. ex Murray, abundant in Guangdong, Guangxi, Yunnan, and Guizhou province, People's Republic of China, was historically used as a traditional folk medicine for the treatment of contusion, strains, and myasthenia.[7] Its chemical constituents have been widely investigated, and a large number of compounds such as diterpenoids, triterpenoids, flavones, and anthraquinones have been isolated and reported.[8] However, only a few *Lycopodium* alkaloids were reported from this plant up to now.[9] As a part of our serach for biologically active *Lycopodium* alkaloids, lycojapodine A, a novel alkaloid with

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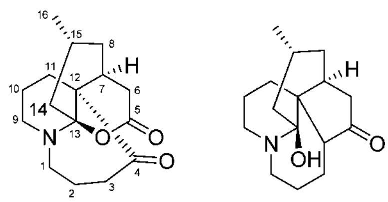
an unprecedented 6/6/6/7 tetracyclic ring system, was isolated from this plant, together with the known compounds fawcettimine (**2**),[10] lycoflexine,[11] lycopodine, and huperine E.[12] Its interesting to note that the structure of **1** was remarkable for its skeleton, since it never existed in *lycopodium* alkaloids, which was probably derived from fawcettimine (**2**) by C−C bond cleavage. Reported herein are the isolation, structure elucidation, and bioactivities of **1**.



1 **2**

The whole plant of *L. japonicum* was collected in Simao of Yunnan province and identified by Prof. Xiao Cheng at Kunming Institute of Botany, Chinese Academy of Sciences (voucher no. 2006-8-17). The air-dried and powdered sample (50 kg) was extracted with 95% EtOH (24 h × 3), the extract was partitioned between EtOAc and 0.5% HCl/H_2O. Water-soluble materials, which were adjusted to pH 10 with 17% ammonia solution, were extracted with $CHCl_3$ to give an alkaloidal extract (67 g). The latter was subjected to a silica gel column (petrolecum ether/actone, 1:0 to 0:1) to afford fractions I−VI. Fraction I (7 g) was chromatographed over repeated silica gel columns (petrolecum ether/EtOAc) and finally purified by HPLC, with 85% MeOH in H_2O as the mobile phase, to yield compound **1** (4 mg) and huperine E (7 mg). Fraction II (14 g) was purified by sequential silica gel columns (petroleum ether/actone, 9:1) and Sephadex LH-20 and recrystallization to afford lycopodine (11 mg). Fraction V (11 g) was subjected to repeated silica gel columns eluted with $CHCl_3$/MeOH (9.5/0/5) and then further purified by RP-18 column (MeOH/H_2O, 4/6) to give compound **2** (26 mg) and lycoflexine (13 mg).

Lycojapodine A (**1**) was isolated as colorless crystals (MeOH).[13] Its molecular formula, $C_{16}H_{23}NO_3$, was established on the basis of HRESIMS for the $[M + H]^+$ ion at *m*/*z* 278.1755 (calcd 278.1756), indicating six degrees of unsatuation. The IR absorptions at 1739 and 1685 cm^{-1} implied the presence of the carbonyls of ketone and lactone groups. Analysis of the ^1H and ^{13}C NMR spectra of **1** (Table 1) revealed 16 carbon signals due to four quaternary carbons, two tertiary carbons, nine methylenes, and one methyl group. Among them, one sp^3 quaternary carbon (δ_C 93.3) was ascribed to the carbon (C-13) bearing both an oxygen atom and a nitrogen atom, and two sp^2 quaternary carbons were

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1** in $CDCl_3$ (δ in ppm, *J* in Hz)

	δ_H	δ_C
1a	3.79 (1H, m)	50.4 (t)
1b	2.91 (1H, dt, 15.2, 1.6)	
2a	1.97 (1H, m)	26.6 (t)
2b	1.62 (1H, m)	
3a	2.68 (1H, m)	46.6 (t)
3b	2.62 (1H, m)	
4		217.4 (s)
5		170.7 (s)
6a	2.43 (1H, m)	35.9 (t)
6b	2.40 (1H, m)	
7	2.26 (1H, m)	36.4 (d)
8a	1.48 (1H, m)	34.8 (t)
8b	1.46 (1H, m)	
9a	3.35 (1H, m)	49.1 (t)
9b	3.03 (1H, dd, 15.2, 5.3)	
10a	1.44 (1H, m)	24.0 (t)
10b	1.41 (1H, m)	
11a	2.05 (1H, m)	31.4 (t)
11b	2.00 (1H, m)	
12		54.9 (s)
13		93.3 (s)
14a	2.18 (1H, dd, 19.1, 12.2)	41.3 (t)
14b	1.69 (1H, m)	
15	1.77 (1H, m)	24.3 (s)
16	0.95 (3H, d, 6.3)	21.2 (q)

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(13) Lycojapodine A (**1**): colorless crystals (MeOH); mp 167−168 °C; $[\alpha]^{24.7}_D$ −140.98 (*c* 0.2, $CHCl_3$). UV ($CHCl_3$) λ_{max} (log ε): 219 (2.87), 226 (2.84), 240 (3.28) nm. IR (KBr) ν_{max}: 2957, 2870, 1739, 1685, 1180, 1127 cm^{-1}. ^1H and ^{13}C NMR data: see Table 1. ESMS *m*/*z* 277; HRESIMS *m*/*z* 278.1755 $[M + H]^+$ ($C_{16}H_{23}NO_3$ calcd 278.1756).

attributable to the ketone group (δ_C 217.4) and the lactone group (δ_C 170.7). Furthermore, its $^1H-^1H$ COSY spectrum revealed the presence of three fragments: **a** (C-1/C-2/C-3), **b** (C-9/C-10/C-11), and **c** (C-6/C-7/C-8/C-15/C-14 and C-15/C-16) as shown in Figure 1. In the HMBC spectrum (Figure 1),

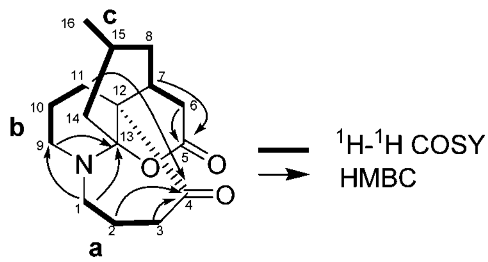

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Figure 1. Selected 2D NMR correlations of **1**.

cross-peaks of H-1/C-9, H-1/C-13, and H-9/C-13 established the connections of C-1, C-9, and C-13 through a nitrogen atom.

In the HMBC, the connections from H-2 and H-3 to the ketone group (δ_C 217.4) indicated that the ketone group was stationed at C-4; meanwhile, the correlations between H-11 and C-4 displayed the linkage of C-4 and C-12. Furthermore, the HMBC connections of H-6 and H-7 with the lactone group were also observed, which suggested that the carbonyl of lactone group was assigned to C-5. Then, to fulfill the unsaturation degrees and MS analysis, a six-membered lactone ring formed from C-5 to C-13. Thereby, compound **1** likely possessed a fancy skeleton that never existed in any other fawcettine-type alkaloids, and its planar structure is shown in Figure 1 as a novel 6/6/6/7 tetracyclic ring system.

In the ROESY spectrum of **1**, the NOE correlations of H-14a with H-9a and Me-16 were observed. However, because of the overlap of H-11a/11b with H-2a, H-14a with H-2a, and H-8a/8b with H-10b, the ROESY spectra could not provide sufficient information to elucidate the stereochemistry of **1**.

Because of the abnormal change of the C−C bond and the limited ROESY information, a single X-ray diffraction study was made to validate the planar structure and the confirmed configuration of **1**. The X-ray structure of **1** not only revealed the unique 6/6/6/7-tetracyclic ring system as deduced above but also established the relative configuration of C-7, C-12, C-13, and Me-16 (Figure 2).[14]

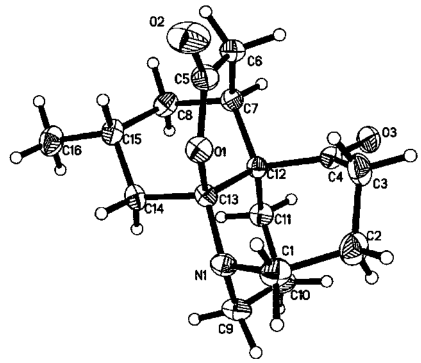
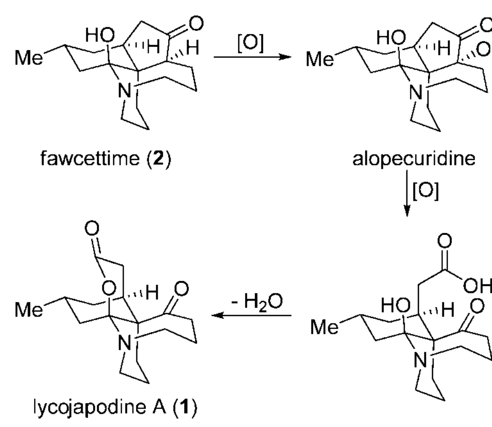


Figure 2. X-ray structure of lycojapodine A (**1**).

Structural comparison of compound **1** and fawcettimine (**2**) revealed that the two compounds had a similar carbinolamine moiety. The C-4 of **1** is a ketone group instead of a tertiary carbon in **2**, while the C-5 of **1** is a locatone ketone instead of an isolated ketone in **2**. These relationships between **1** and **2** implied that compound **1** could be derived from fawcettimine (**2**) via the C−C bond cleavage.

A plausible biogenetic pathway for **1** is proposed as shown in Scheme 1. As shown, compound **2** underwent an oxidation

Scheme 1. Plausible Biogenetic Pathway of Lycojapodine A (**1**)



to produce alopecuridine, which further underwent a retrograde aldol reaction and subsequent esterification reaction to produce **1** under the catalyzed base.[15,16]

Lycojapodine A (**1**) inhibited acetycholinesterase with an IC_{50} value of 90.3 μM, which was comparable to that of (−)-huperzine A,[17] and its anti-HIV-1 activity was also tested

(14) Crystal data for lycojapodinaeA (**1**): $C_{16}H_{23}NO_3$, MW = 277.35; monoclinic, space group $P2_1$; a = 7.1643(8) Å, b = 10.6250(12) Å, c = 9.4329(11) Å, α = 90.00, β = 97.1230(10), γ = 90.00, V = 712.50(14) Å^3, Z = 2, d = 1.293 g/cm^3, crystal dimensions 0.32 × 0.28 × 0.25 nm was used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 6136, of which 5485, were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0430, wR_2 = 0.0925. The crystal structure of **1** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 661199). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax: (+44) 1223-336-033; or desposit@ccdc.cam.ac.uk).

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using the MTT method as previously repored,[18] showing an EC_{50} value of 85 μg/mL.

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Supporting Information Available: 1D and 2D NMR spectra, MS spectra, and X-ray crystallographic data in CIF format of lycojapodine A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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