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Lycospidine A, a New Type of *Lycopodium* Alkaloid from *Lycopodium complanatum*

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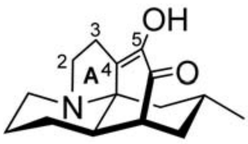
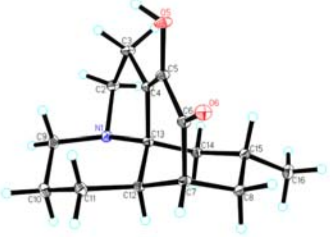
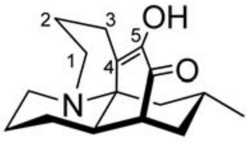
ABSTRACT



Lycospidine A (**1**)



1



12-deoxyhuperzine O (**2**)

Lycospidine A (1), the first example of a *Lycopodium* alkaloid which contains an unprecedented five-membered A ring, was isolated from *Lycopodium complanatum*. The unique five-membered A ring in 1 indicates that carbons 2–5 in 1 are presumably derived from proline instead of the lysine biosynthetically, which suggests that 1 represent a new class of *Lycopodium* alkaloid. In addition, the unique structural feature and biosynthetic origin of 1 shed new insight into the structural diversity of *Lycopodium* alkaloid analogue libraries potentially accessible by engineered biosynthesis.

Club moss (Lycopodiaceae) is known to be a rich source of *Lycopodium* alkaloids possessing complex heterocyclic ring systems.[1] Among *Lycopodium* alkaloids, huperzine A is a promising agent to treat Alzheimer's disease with highly specific and potent inhibitory activity against acetylcholinesterase (AChE).[2] As a consequence, these structurally complex *Lycopodium* alkaloids have attracted great interest from chemists as challenging targets for total synthesis[3] as well as for biosynthetic studies.[4]

Lycopodium complanatum (L.) Holub, abundant in the temperate and subtropical regions of the world,[5] was used as a traditional Chinese herbal medicine for the treatment of arthritic pain, quadriplegia, and contusion.[6] Its chemical constituents have been widely investigated, and a large number of *Lycopodium* alkaloids with fascinating structures, such as lyconadin A,[7] complanadine A,[8] lycopladine A,[9] and lycopladine H,[10] were isolated. In our continuing

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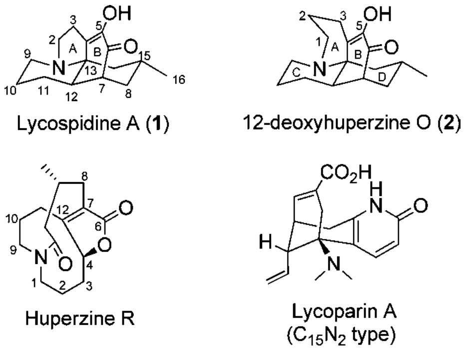
efforts for structurally novel and bioactive *Lycopodium* alkaloids from the genus *Lycopodium* to expand the structure diversity of this fascinating family of natural products and to exploit their utility for drug discovery,[11] a novel $C_{15}N$ alkaloid with an unprecedented 5/6/6/6 tetracyclic ring system was isolated from *L. complanatum*, together with the known 12-deoxyhuperzine O (**2**).[12] *Lycopodium* alkaloids with a C_{15} skeleton are rare. To the best of our knowledge, only 14 natural C_{15} *Lycopodium* alkaloids have been reported so far, of which 13 belong to the $C_{15}N_2$-type (Figure 1).[2,13] Huperzine R was the only *Lycopodium* alkaloid possessing a $C_{15}N$ skeleton due to the carbon-5 loss (Figure 1).[13d] Reported herein are the isolation, structure elucidation, and proposed biosynthetic pathway of the new type of *Lycopodium* alkaloid lycospidine A (**1**).



Lycospidine A (**1**)

12-deoxyhuperzine O (**2**)

Huperzine R

Lycoparin A ($C_{15}N_2$ type)

Figure 1. Chemical structures of **1**, **2**, and other two C_{15} *Lycopodium* alkaloids.

The air-dried and powdered whole plant of *L. complanatum* was extracted with MeOH three times. The extract was partitioned between EtOAc and 1% HCl/H_2O. The pH of the water-soluble portion was adjusted to pH 9 with saturated Na_2CO_3 aq. Then, it was extracted with $CHCl_3$ to afford an alkaloidal extract. Further column chromatography on MCI gel, silica gel, and semipreparative HPLC led to the isolation of compounds **1** (8 mg) and **2** (13 mg).

Table 1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1** in CD_3OD (δ in ppm, J in Hz)

no.	δ_H	δ_C
2a	3.84 (1H, ddd, 12.0, 9.7, 9.7)	52.4 (t)
2b	3.34 (1H, overlapped)	
3a	3.03 (1H, ddd, 19.0, 9.3, 9.3)	23.6 (t)
3b	2.85 (1H, m)	
4		124.5 (s)
5		147.6 (s)
6		195.9 (s)
7	2.57 (1H, dt, 3.9, 1.4)	50.6 (d)
8a	1.70 (1H, overlapped)	37.7 (t)
8b	1.34 (1H, m)	
9a	3.34 (overlapped)	52.0 (t)
9b	2.84 (overlapped)	
10a	1.80 (1H, td, 14.6, 3.7)	23.5 (t)
10b	1.63 (1H, br.d 14.6)	
11a	1.57 (1H, td, 13.1, 3.7)	24.7 (t)
11b	1.31 (1H, m)	
12	2.18 (1H, ddd, 11.9, 2.9, 2.9)	46.7 (d)
13		72.6 (s)
14a	2.07 (1H, m)	39.9 (t)
14b	1.36 (1H, m)	
15	1.70 (1H, overlapped)	28.4 (d)
16	0.92 (3H, d, 6.3)	21.5 (q)

Lycospidine A (**1**)[14] was obtained as optically active colorless needle crystals {$[\alpha]_D^{17.7}$ −8.8 (c = 0.3, MeOH)}. It showed the pseudomolecular ion peak at m/z 248 $[M + H]^+$ in the ESI mass spectrum, and the molecular formula, $C_{15}H_{21}NO_2$, was established by HR-EI-MS [m/z 247.1574 (calcd 247.1572)]. The IR spectrum showed absorptions for a hydroxy and a carbonyl group at 3435 and 1686 cm^{-1}, respectively. Analysis of the ^1H and ^{13}C NMR spectra of **1** (Table 1) revealed 15 carbon signals due to one sp^3 and three sp^2 quaternary carbons, three methines, seven methylenes, and a methyl group. Among them, one sp^3 quaternary carbon (δ_C 72.6 ppm) and two sp^3 methylene carbons (δ_C 52.4 and 52.0 ppm) were attributed to those attached to a nitrogen atom. And three sp^2 quaternary carbons (δ_C 124.5, 147.6, and 195.9 ppm) were attributable to an enolic ketone moiety similar to that of **2**.[10]

Furthermore, its HSQC and $^1H-^1H$ COSY spectra revealed the presence of two spin systems **a** (C-2/C-3) and **b** (C-9–C-12/C-7/C-8/C-15 and C-14/C-15/C-16) (Figure 2). HMBC cross-peaks of H-2/C-9, H-2/C-13, and H-9/C-13 (Figure 2) established the connections of C-2, C-9, and C-13 through a nitrogen atom (Figure 2). The HMBC cross-peaks of H-3/C-4 and H-3/C-5 indicated that the enolic part of the enolic ketone moiety was connected to fragment **a** (Figure 2). The connectivity of the ketone part of the enolic ketone moiety with C-7 of fragment **b** can be deduced from the HMBC cross-peaks of H-7/C-6, H-7/C-5, and H-8/C-6. The above evidence, along with the ^1H and ^{13}C NMR data comparison between **1** and **2**, led to the conclusion that the structures of both

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(14) Lycospidine A (**1**): colorless needle crystals (EtOH); mp 179−181 °C; $[\alpha]^{17.7}{}_D$ −8.8°(c 0.3, MeOH); UV (MeOH) λ_{max} (log ε): 331 (2.58), 278 (2.75), 205 (2.85) nm; IR (KBr) ν_{max}: 3435, 2927,1686, 1450, 1348, 1208, 1141 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data (CD_3OD), see Table 1; HR-EI-MS m/z 247.1754 $[M]^+$ (calcd for $C_{15}H_{21}NO_2$, 247.1752).

compounds were quite similar and the five-membered A ring is evident for **1** instead of the six-membered A ring in **2**.

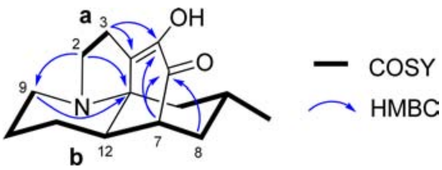

Figure 2. Selected COSY and HMBC correlations of **1**.

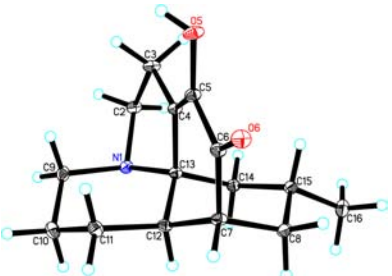

Figure 3. X-ray crystal structure of lycospidine A (**1**).

In the ROESY spectrum of **1**, there were no solid correlations which can be used to establish the relative configurations of C-7, C-12, C-13, and C-15 except for the ROESY correlations of H-12/H-8a/H-14a that indicated the same orientations of H-12, H-8a, and H-14a. Thus, a single X-ray diffraction was necessary to assign the stereochemistry of chiral centers in **1** and to validate the planar structure. Fortuitously, a single crystal of **1** was obtained from ethanol and the X-ray diffraction not only revealed the unique five-membered A ring in **1** as deduced above but also established the relative configurations of C-7, C-12, C-13, and C-15 (Figure 3).[15]

Lycopodium alkaloids biosynthesis, particularly the lycopodine-type alkaloids,[16] have been investigated in *L. tristachyum* featuring the putative lysine decarboxylase and aminotransferase catalyzed biosynthesis of Δ^1-piperideine from amino acid L-lysine and the subsequent two rounds of condensations: (i) Δ^1-piperideine with acetonedicarboxylic acid or its CoA esters to form the 4-(2-piperidyl) acetoacetate (4PAA) or 4-(2-piperidyl) acetoacetyl-CoA (4PAACoA) and (ii) 4PAA or 4PAACoA with the 4PAA decarboxylated intermediate pelletierine to generate the phlegmarine which is a general intermediate for all *Lycopodium* alkaloids (Scheme 1a). The remaining steps involved the ring closure of phlegmarine to yield the lycodane skeleton and the A ring opening and recyclization with the nitrogen atom in the C ring to form lycopodine. Modeled on the partially established paradigm for *Lycopodium* alkaloid biosynthesis, compound **2** could be simply viewed as a C-6 oxidation product of lycopodine in *L. complanatum*. Based on the proposed pathway in Scheme 1a, the five carbon atoms from C-1 to C-5 in **2** are all derived from L-lysine. There is a carbon loss of the A ring in **1** compared with that of **2**. We therefore rationally propose that the four carbon atoms from C-2 to C-5 in **1** are presumably derived from proline instead of lysine. Thus, as depicted in Scheme 1b, the precursor proline underwent the decarboxylation to generate the five-membered 1-pyrroline instead of the six-membered Δ^1-piperideine and proceeded in the coupling reaction with acetonedicarboxylic acid or its CoA esters to afford the 4-(2-pyrrolidyl) acetoacetate (4PYAA) or 4PYAACoA, subsequently following pathways similar to the biosynthesis pathways for **2** to finally generate **1** (Scheme 1).

Except for the four major classes (phlegmarine,[17] lycodine,[18] lycopodine,[19] and fawcettimine[20]),[21] there were plenty of structurally diverse and complex *Lycopodium* alkaloids that have been isolated from the genus of *Lycopodium*.[22] However, all the reported alkaloids are derived from the four major classes through carbon skeleton rearrangements, chemical bond cleavage, and further cyclization.[23] Lycospidine A (**1**) is independent from all of the classes and subgroups biosynthetically, therefore representing a new class of *Lycopodium* alkaloid.

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(15) Crystal data for lycospidine A (**1**): $C_{17}H_{26}F_3NO_6$ ($C_{15}H_{21}NO_2\cdot CF_3COOH\cdot 2H_2O$), M_W = 397.39; monoclinic, space group $P2_12_12_1$; a = 9.2410 (17) Å, b = 7.3317 (13) Å, c = 14.239 (3) Å, α = γ = 90.00, β = 106.149 (3), V = 1123.4 (3) Å^3, Z = 2, d = 1.42.4 g/cm^3, crystal dimensions 0.15 × 0.24 × 0.70 mm^3 were used for measurement on a Bruker APEX DUO with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 9702, of which 4420 were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0370, wR_2 = 0.0970. The crystal structure of **1** was solved by direct method SHLXS-97 (Sheldrick, 1990). Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 931623). 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, UK.; fax: (+44) 1223-336-033; or desposit@ccdc.cam.ac.uk).

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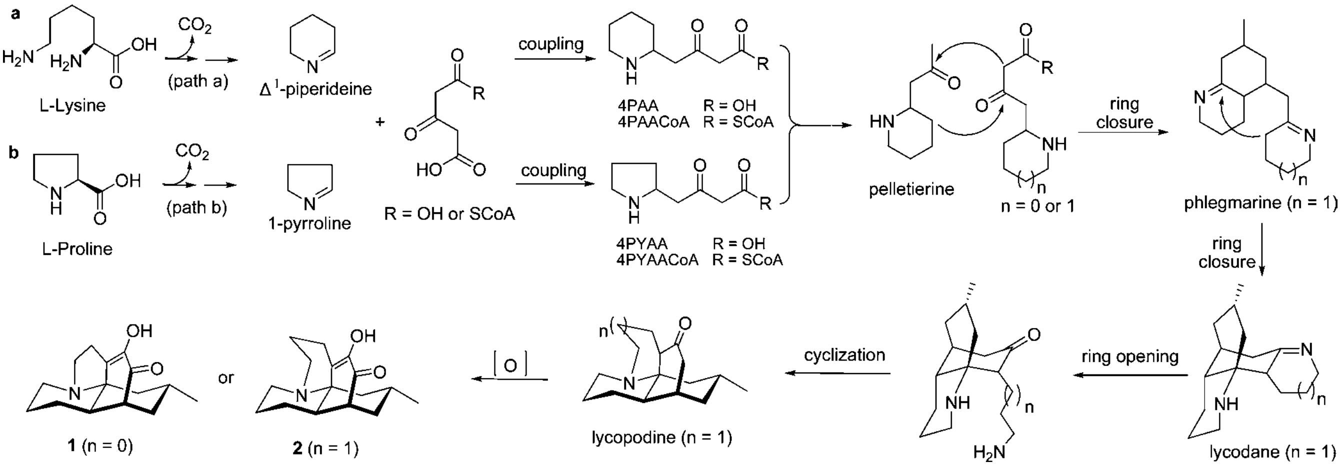
Scheme 1. Hypothetical Biosynthetic Pathways of **1** and **2**^{a}



^{a}(a) Biosynthesis of 4PAA or 4PAACoA from L-lysine; (b) biosynthesis of 4PYAA or 4PYAACoA from L-proline.

The discovery of **1** and **2** in the same organism provides new insight into *Lycopodium* alkaloid biosynthesis. The first condensation enzyme in Scheme 1 is generally considered to act preferentially on Δ^1-piperideine originated from L-lysine based on the structures of all the reported *Lycopodium* alkaloids. The isolation of **1** not only suggests that this enzyme can act equally well on the five-membered 1-pyrroline originated from proline but also indicates all the downstream enzymes in the lycopodine biosynthetic machinery possess significant substrate promiscuities which could be readily exploited for natural product structural diversity via engineered biosynthesis of *Lycopodium* alkaloids.

Both **1** and **2** were inactive (IC$_{50}$ >200 μM) against AChE using the improved Ellman method.^{24} Compounds **1** and **2** were also evaluated for cytotoxicity against five human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480),^{25} but showed no activity with IC$_{50}$ values of >40 μM.

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

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The authors declare no competing financial interest.