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Lycojaponicumins D and E: Two New Alkaloids from *Lycopodium japonicum*

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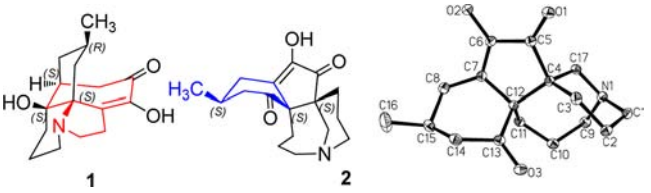
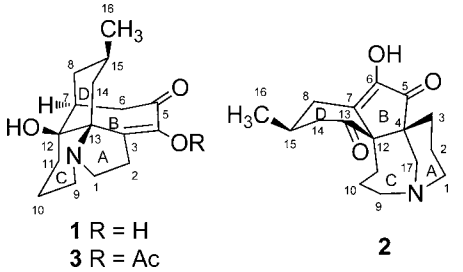
ABSTRACT



Two new alkaloids, lycojaponicumins D (1) and E (2), were isolated from the club moss *Lycopodium japonicum*. Their structures were elucidated by spectroscopic methods, calculated ECD, CD experiments and X-ray diffraction analysis. Lycojaponicumin D (1) possesses an unprecedented 5/7/6/6 tetracyclic skeleton formed by an unusual C3−C13 linkage, which is first reported in *Lycopodium* alkaloids. The plausible biogenetic pathway of 1 is proposed.

Plants of the *Lycopodium* species (Lycopodiaceae) are known to be a rich source of *Lycopodium* alkaloids possessing unique heterocyclic frameworks, such as huperzine A, fawcettimine, and serratinine, which have attracted great interest from biogenetic, synthetic, and biological perspectives.[1] As a representative plant of the Lycopodiaceae family, *Lycopodium japonicum* Thunb. ex Murray has historically been used as a traditional Chinese medicine for the treatment of contusions, strains, and myasthenia.[2] Previously, we reported three trace *Lycopodium* alkaloids, lycojaponicumins A and B with a 5/5/5/5/6 pentacyclic ring system and lycojaponicumin C with a 6/5/5/6 tetracyclic ring system, isolated from this plant.[3] In our continued research on the discovery of structurally unique alkaloids, lycojaponicumin D (**1**), with an unprecedented 5/7/6/6 tetracyclic skeleton formed by an unusual C3−C13 linkage, and lycojaponicumin E (**2**), the second example of fawcettimine-type alkaloids possessing a boat conformation for ring D with a β-oriented methyl group substituted at C-15, were obtained. Their structures were determined by extensive spectroscopic methods, CD experiments, ECD calculations, and X-ray crystallography. In this paper, we describe the structure elucidation of **1** and **2**, the plausible biogenetic pathway, and the biological activity of **1**.



Lycojaponicumin D (**1**) was obtained as a white amorphous powder, and its molecular formula was established to be $C_{16}H_{23}NO_3$ by HRESIMS at m/z 278.1747 $[M + H]^+$ (calcd 278.1751, $C_{16}H_{24}NO_3$), accounting for 6 degrees of unsaturation. The IR spectrum of **1** showed the absorption of hydroxyl groups at 3391 cm^{-1} and that of α,β-unsaturated

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keto group at 1677 cm^{-1}. ^1H and ^{13}C NMR spectra [Figures S1 and S2, Supporting Information (SI)] revealed the presence of one α,β-unsaturated keto group (δ_C 195.8), one tetrasubstituted olefinic functional group (δ_C 143.1, 133.0), two sp^3 quaternary carbons (δ_C 73.7, 70.7), two sp^3 methines (δ_C 41.4, 25.1), eight methylenes including two aminomethylenes (δ_C 51.1, 49.3, 42.6, 37.7, 36.0, 32.0, 29.1, 20.4), and one methyl (δ_C 21.5) in the structure. All of these functional groups accounted for 2 degrees of unsaturation, indicating a tetracyclic ring system in the structure.

$^1H-^1H$ COSY and HSQC analysis (Figures S3 and S4, SI) revealed the presence of three isolated spin systems: (a) $C(1)H_2-C(2)H_2$, (b) $C(6)H_2-C(7)H-C(8)H_2-C(15)H-[C(16)H_3]-C(14)H_2$, and (c) $C(9)H_2-C(10)H_2-C(11)H_2$ (Figure 1). In the HMBC spectrum (Figure S5, SI), correlations from H_2-1 to C-9 and C-13 indicated that C-1, C-9, and C-13 were connected through a nitrogen atom. HMBC correlations from H-1b to one olefinic carbon C-3 (δ_C 133.0), from H_2-2 to both of the olefinic carbons C-3 (δ_C 133.0) and C-4 (δ_C 143.1), and from H_2-6 to the keto carbon C-5 not only implied a linkage between C-2 and C-3 but also placed the double bond at C-3−C-4 to conjugate with the carbonyl group at C-5. The new carbon linkage between C-3 and C-13 was deduced from the HMBC correlations from H_2-2 to C-13 and from H-14a to C-3. Based on the above-mentioned connections, a tetrahydropyrrole unit (ring A) was constructed. HMBC correlations from H_2-6, H-7 to the keto carbon C-5, from H_2-10 to C-12, and from H-7 to C-11, C-12, and C-13, together with the α,β-unsaturated keto moiety (C3−C4−C5) deduced above, led to the characterization of an α,β-unsaturated cycloheptanone ring (ring B). A piperidine ring (ring C) was validated by spin system (c), together with the HMBC cross-peaks of H_2-10/C-12 and H-11b/C-13. Additionally, HMBC correlations from H-15 to C-13 and from H_2-14 to C-12 and C-13, which could establish the C-12−C-13−C-14 connections, combined with spin system (b) and the C-7−C-12 linkage constructed in ring B, revealed the presence of another ring in the structure: a cyclohexane ring substituted with a methyl group (ring D), which shared the C-7−C-12−C-13 unit with ring B. Thus, the tetracyclic ring system of this novel alkaloid was established, which consisted of a tetrahydropyrrole ring (C-1/C-2/C-3/C-13/N, ring A), an α,β-unsaturated cycloheptanone ring (C-3/C-4/C-5/C-6/C-7/C-12/C-13, ring B), a piperidine ring (C-9/C-10/C-11/C-12/C-13/N, ring C), and a cyclohexane ring (C-7/C-8/C-15(C-16)/C-14/C-13/C-12, ring D). The hydroxyl groups at C-4 and C-12 were inferred from the molecular formula and the chemical shifts of these two quaternary carbons. To further confirm the presence of the hydroxyl groups, the ^1H NMR spectrum of **1** was also acquired in acetone-d_6, which showed two exchangeable proton signals, one at 3.30 ppm and the other at 6.95 ppm, assigned as 12-OH and 4-OH, respectively. Meanwhile, the CIGARD HMBC spectrum exhibited ^4J correlations from H-2a to C-5 and from H-14a to C-4, which could unambiguously verify the planar structure of **1** (Figures S9−S13, SI). Therefore, the planar structure of **1** was elucidated as shown in Figure 1. The 5/7/6/6 tetracyclic skeleton is reported to be the first example of *Lycopodium* alkaloids with a new carbon linkage, C3−C13.

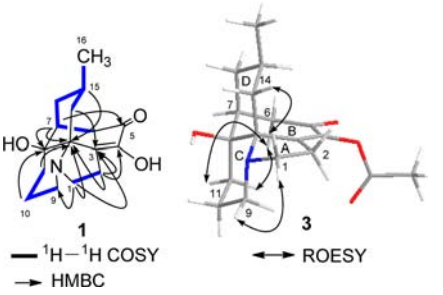


Figure 1. Selected 2D NMR correlations of **1** and **3**.

Initially, the relative stereochemistry of **1** was elucidated by a combination of $^3J_{H-H}$ couplings and ROESY correlations (Figure 1). However, some key proton signals in the ^1H NMR spectrum were overlapped or ambiguous, and the ROESY correlations revealed too much noise, which together made it difficult to determine the relative stereochemistry without further study. To overcome these difficulties, **1** was acetylated to yield **3**, a monoacetylate of the enol group at C-4 of **1**. Detailed analysis of 1D/2D NMR, MS, UV, and IR spectra (Figures S14−S18, S21−S23, and S26, SI) of **3** led to the characterization of its structure (Table 1). 1D−TOCSY experiments (Figure S19, SI) further confirmed the presence of the three isolated spin systems (a−c) mentioned above. The ^1H NMR spectrum of **3** revealed proton signals with adequate resolution due to the shielding and deshielding effects of the substituted acetyl group. H-15 was determined to be axially oriented based on the large coupling constant between H-15 and axial H-14a [dd, 12.8 ($^3J_{H14a-H14b}$), 12.8 ($^3J_{H14a-H15}$) Hz)] and the small coupling constant between H-15 and equatorial H-14b [dd, 12.8 ($^3J_{H14b-H14a}$), 3.2 ($^3J_{H14b-H15}$) Hz]. Similarly, H-7 was identified to adopt an equatorial orientation by the small coupling constant between H-7 and H-6a [dd, 16.8 ($^3J_{H6a-H6b}$), 4.0 ($^3J_{H6a-H7}$) Hz] and the small coupling constant between H-7 and H-6b [dd, 16.8 ($^3J_{H6b-H6a}$), 4.0 ($^3J_{H6b-H7}$) Hz]. The coupling constants of H_2-8 [H-8a (axial-H), ddd, 13.6 ($^3J_{H8a-H8b}$), 13.6 ($^3J_{H8a-H15}$), 5.6 ($^3J_{H8a-H7}$) Hz; H-8b (equatorial-H), br dd, 13.6 ($^3J_{H8a-H8b}$), 4.0 ($^3J_{H8a-H7}$ or $^3J_{H8a-H15}$) Hz] further confirmed that H-15 and H-7 adopt axial and equatorial orientations, respectively. Difference NOE experiments were also performed to determine the relative configuration of this new molecule (Figure S20, SI). When irradiating H-14b (equatorial-H), NOE enhancements of H-1a and H_3-16 were observed. Meanwhile, the irradiation of H-1b led to the enhancement of H-9a. When H-6a was irradiated, H-11a, H-11b, and H-7 showed NOE enhancements. Rings A and B share a pseudoplane in projection view as a result of the α,β-unsaturated keto moiety. Therefore, rings C and D were located on the opposite faces of this pseudoplane through bond C12−C13 based on the above-mentioned NOE

enhancements. In other words, rings C and D were *trans* fused, from which the relative configurations of C-12 and C-13 were determined. Thus, the relative configuration of **1** was established as shown in Figure 1.

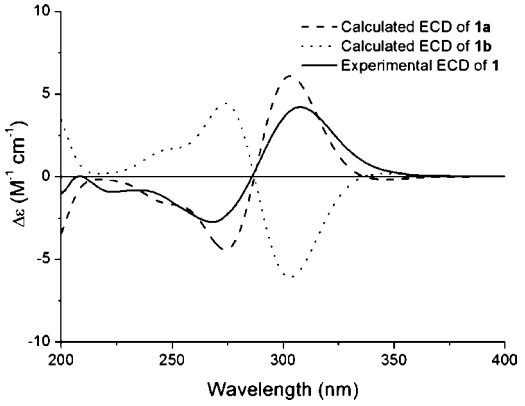


Figure 2. Comparison of the experimental and calculated ECD spectra of **1**.

The absolute configuration of **1** was elucidated by a calculated ECD method and $Rh_2(OCOCF_3)_4$-induced CD measurements, because a single crystal suitable for X-ray crystallography was not obtained. According to the established relative configuration of **1**, the possible absolute configurations of **1** were proposed to be (7*S*, 12*S*, 13*S*, 15*R*) (**1a**) or (7*R*, 12*R*, 13*R*, 15*S*) (**1b**). The calculated ECD spectrum of **1a** displayed a CD curve similar to the experimental spectrum of **1** (Figure 2). Meanwhile, the assigned configuration was also supported by CD measurements using $Rh_2(OCOCF_3)_4$ as an auxiliary chromophore.[4] According to the bulkiness rule, a positive Cotton effect around 350 nm (E band) in the CD spectrum of the in situ formed Rh-complex of **1** with the inherent CD spectrum subtracted (see Figure S30 in the SI) indicated the 12*S* configuration for **1**.[5] Therefore, the absolute configuration of **1** was determined to be 7*S*, 12*S*, 13*S*, 15*R*, $\Delta^{3,4}$(*E*).

Lycojaponicumin E (**2**) was obtained as colorless crystals [mp 220−222 °C (dec.)]. Its molecular formula was established to be $C_{17}H_{23}NO_3$ by HRESIMS at *m*/*z* 290.1752 $[M + H]^+$ (calcd 290.1751, $C_{17}H_{24}NO_3$), with 7 degrees of unsaturation. The ^{13}C NMR and DEPT spectra (Figures S33 and S34, SI) revealed 17 carbon signals attributed to two keto carbonyls (δ_C 210.0, 203.8), one tetrasubstituted double bond (δ_C 149.9, 145.0), two sp^3 quaternary carbons (δ_C 61.7, 54.0), one sp^3 methine (δ_C 27.4), nine methylenes including three aminomethylenes (δ_C 57.8, 57.6, 52.7, 48.9, 34.0, 29.3, 28.4, 26.1, 20.1), and one methyl (δ_C 21.7). These structural units accounted for 3 of the required 7 unsaturation degrees, thus indicating the presence of four rings (rings A−D) in the structure.

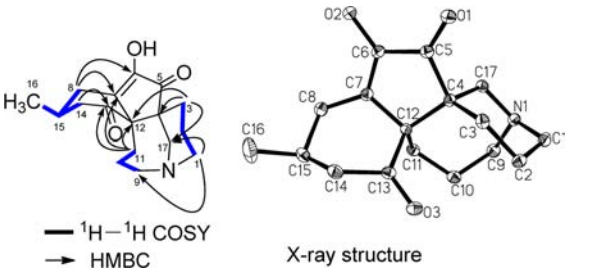


Figure 3. 2D NMR correlations and X-ray structure of **2**.

$^1H-^1H$ COSY and HSQC analysis (Figures S35 and S36, SI) revealed the presence of three isolated spin systems: (a) $C(1)H_2-C(2)H_2-C(3)H_2$, (b) $C(8)H_2-C(15)H[C(16)H_3]-C(14)H_2$, and (c) $C(9)H_2-C(10)H_2-C(11)H_2$ (Figure 3). In the HMBC spectrum (Figure S37, SI), cross peaks from protons H_2-1 of one aminomethylene to C-9/C-17 indicated that these three methylenes were connected through a nitrogen atom. HMBC correlations from H-3a to C-4, C-12, and C-17, and from H_2-11 to C-12, together with spin systems (a) and (c), led to the characterization of a piperidine ring (ring A) and an azepane ring (ring C). The double bond was deduced to be at C6−C7 based on HMBC correlations from H_2-8 to the olefinic carbons C-6 and C-7, thus conjugated with the carbonyl group at C-5 to form an α,β-unsaturated cyclopentanone ring (ring B). There was a hydroxyl group substituted at C-6 corresponding to the molecular formula as well as IR and NMR data. A cyclohexanone ring (ring D) was constructed based on the unit (b) and HMBC correlations from H_2-11 to C-7 and C-13 and from H_2-14 to C-12 and C-13. Therefore, the planar structure of **2** was established as shown in Figure 3. In the ROESY spectrum (Figure S38, SI), the cross peak of H-8b/H-11a revealed the relative configuration of C-12. However, the ROESY spectrum could not provide sufficient information to elucidate the relative stereochemistry of other chiral centers because some key proton signals overlapped. Fortunately, a suitable single crystal was obtained for X-ray diffraction,[6] the results of which established the relative configurations as well as the absolute configurations of each chiral center (4*S*, 12*S*, 15*S*) using the anomalous scattering of Cu Kα radiation. In contrast with our expectation, the cyclohexanone ring (ring D) demonstrated a boat

(4) Jadwiga, F.; Wojciech, J. S. *Tetrahedron: Asymmetry* **1999**, *10*, 1507–1520.

(5) Because the CD spectrum of the Rh complex of **1** was measured with the inherent CD spectrum subtracted, the positive cotton effect (307.5 nm) in the CD spectrum of **1** does not interfere with the induced positive Cotton effect (345.5 nm). According to the literature,[4] the UV−vis absorption of the Rh-complex with tertiary alcohols in the range 578−605 nm in chloroform, accompanied with the blue color of the Rh-complex solutions, indicates axial ligation through the hydroxyl oxygen, and the pink-colored solution of the Rh-complex together with absorption at 541 nm could identify the donor atom as the nitrogen. In our experiment, the absorption centered at 605 nm in the UV−vis spectrum as well as the blue color of the Rh-complex of **1** allowed us to determine that the Rh preferably complexed to the hydroxyl oxygen rather than the nitrogen atom.

(6) CCDC 848857 (**2**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif.

Table 1. ^{1}H and ^{13}C NMR Data of **1** and **3** (δ in ppm, J in Hz)

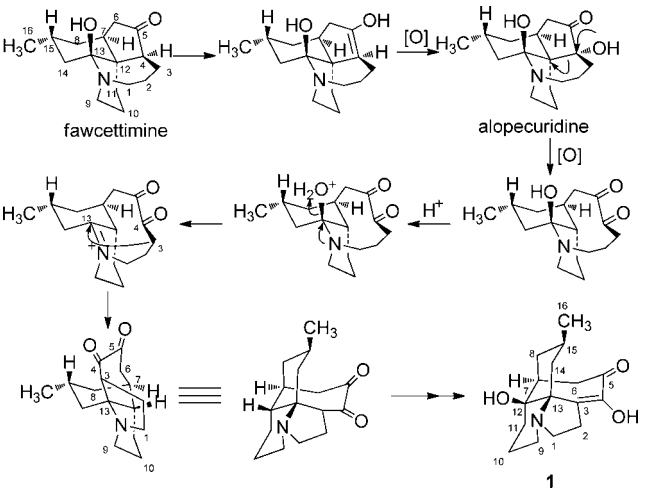
	1[a] ($CDCl_3$)		**3**[b] ($CDCl_3$)	
no.	δ_H	δ_C	δ_H	δ_C
1a	3.25, ddd (12.5, 8.5, 8.5)	49.3	3.75, ddd (12.0, 9.6, 9.6)	48.3
1b	2.76[c]		3.20, m	
2a	3.10, ddd (19.5, 8.5, 8.5)	29.1	2.93-3.10, 2H, m	26.6
2b	2.67, br dd (19.5, 8.5)			
3		133.0		128.8
4		143.1		142.8
5		195.8		194.7
6a	2.83, dd (18.0, 5.0)	42.6	2.88, dd (16.8, 4.0)	43.7
6b	2.75[c]		2.73, dd (16.8, 4.0)	
7	2.09, m	41.1	2.26[c]	42.6
8a	1.98, ddd (13.0, 13.0, 5.5)	36.0	2.08, ddd (13.6, 13.6, 5.6)	34.2
8b	1.35[c]		1.48, dd (13.6, 4.0)	
9a	2.86, m		3.57, br dd (12.8, 3.2)	
9b	2.44, ddd (13.0, 13.0, 3.5)	51.1	2.81, ddd (12.8, 12.8, 3.2)	51.3
10a	2.16, ddddd (13.0, 13.0, 13.0, 3.5, 3.5)	20.4	2.67, m	18.1
10b	1.58, m		1.78[c]	
11a	1.77, m	32.0	2.12, m	30.3
11b	1.50, m		1.78[c]	
12		70.7		69.6
13		73.8		77.8
14a	1.74, m	37.7	2.37, dd (12.8, 12.8)	35.2
14b	1.37[c]		1.52, dd (12.8, 3.2)	
15	1.40[c]	25.1	1.59, m	25.3
16	0.89, 3H, d (6.0)	21.5	0.97, 3H, d (6.4)	20.2
OAc			2.26[c], 3H	21.1
				168.3

[a] Data were recorded at 500 MHz for proton and 125 MHz for carbon. [b] Data were recorded at 800 MHz for proton and 200 MHz for carbon. [c] Overlapping signals.

conformation to keep the 16β-CH_3 in the quasi-equatorial position. Moreover, X-ray experiments also showed that the OH at C-6 could establish intermolecular hydrogen bonding interactions with the nitrogen atom in another molecule and not intramolecular interactions with the carbonyl group at C-5, which might provide a reasonable explanation for why the enol moiety could exist stably. Therefore, following lycojaponicumin B,[3] this is the second example of fawcettimine-type alkaloids possessing a boat conformation for the D ring with a β-oriented methyl group at C-15.

The plausible biogenetic pathway of lycojaponicumin D (**1**) was proposed in Scheme 1. As shown, fawcettimine[7] underwent a series of oxidation, protonation, and dehydration to form an iminium ion. The subsequent attack of the α-carbonyl carbon atom (C-3) on the iminium ion carbon (C-13) forges a new link between C-3 and C-13 and then forms the novel tetracyclic skeleton. The tetracyclic intermediate underwent hydroxylation and keto−enol tautomerism to afford **1**.

Scheme 1. Plausible Biogenetic Pathway of **1**



An *in vitro* assay showed that lycojaponicumin D (**1**) was slightly active against lipopolysaccharide (LPS)-induced pro-inflammatory factors in BV2 macrophages with IC_{50} = 48.61 μM (curcumin as positive control, IC_{50} = 3.12 μM).[8]

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Supporting Information Available. Experimental procedures, 1D/2D NMR, MS, UV, IR spectra for **1**−**3**, experimental and calculated ECD spectra for **1** and **3**, and X-ray crystallographic data of **2** in CIF format. 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.