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# Isopalhinine A, a Unique Pentacyclic *Lycopodium* Alkaloid from *Palhinhaea cernua*

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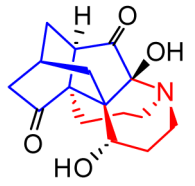
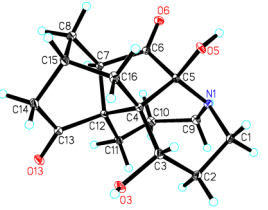
## ABSTRACT




isopalhinine A (**1**)




**A new pentacyclic (5/6/6/6/7) *Lycopodium* alkaloid named isopalhinine A (1), which possesses a sterically congested architecture built with a tricyclo[4.3.1.0$^{3,7}$]decane (isotwistane) moiety and a 1-azabicyclo[4.3.1]decane moiety, and palhinines B (2) and C (3) were isolated from *Palhinhaea cernua*. The structure and absolute configuration of 1 were elucidated by a combination of NMR spectra, optical rotation calculation, and X-ray diffraction experiment. A possible biogenetic pathway was also proposed.**

The *Lycopodium* alkaloids are a family of structurally diverse natural products from the genus *Lycopodium* (Lycopodiaceae).[1] The discovery of huperzine A, a potent, selective, and reversible acetylcholinesterase (AChE) inhibitor, has spurred the discovery of numerous structurally diverse and complex new *Lycopodium* alkaloids which have proven to be challenging targets for total synthesis.[1,2]

*Palhinhaea cernua* L. (syn.: *Lycopodium cernuum* L.), belonging to the family Lycopodiaceae, is a traditional Chinese herbal medicine in the treatment of contusions, scald, and rheumatism.[3] Previously, we reported a *Lycopodium* alkaloid named lycopalhine A (**5**) which has an intriguing hexacyclic (5/5/5/6/6/6) ring system formed by linkages of C16−C6 and C9−N2′ (Figure 1).[4] In our continued research aimed at discovering structurally interesting and bioactive *Lycopodium* alkaloids,[2a,b,4] isopalhinine A (**1**), palhinines B (**2**) and C (**3**), together with a known compound palhinine A (**4**),[5] were isolated from the plant. Among them, isopalhinine A (**1**) is a novel pentacyclic (5/6/6/6/7) *Lycopodium* alkaloid that possesses a sterically congested architecture built with a tricyclo[4.3.1.0$^{3,7}$]decane (isotwistane) moiety and a 1-azabicyclo[4.3.1]decane moiety. The functionalized bridged isotwistane system was formed by a unique linkage of C16−C4. Moreover, different from all of the reported naturally occurring fawcettimine-type *Lycopodium* alkaloids, isopalhinine A (**1**) has a 1-azabicyclo[4.3.1]decane moiety through a unique N−C5 bond. The formation of unique C16−C4 and N−C5 bonds in isopalhinine A (**1**) makes it one of the most sterically congested and structurally complex *Lycopodium* alkaloids.[1]

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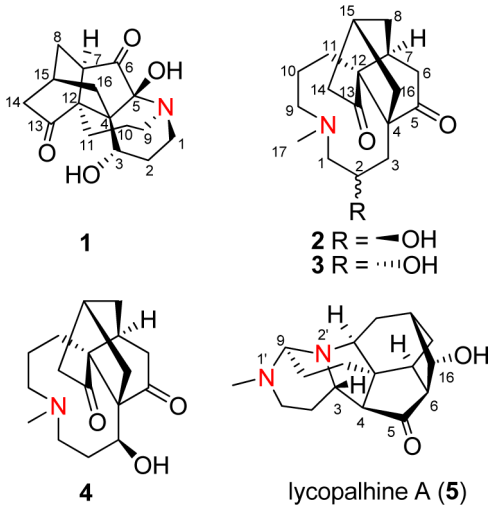



**Figure 1.** Chemical structures of isopalhinine A (**1**); palhinines A (**4**), B (**2**), and C (**3**); and lycopalhine A (**5**).

Isopalhinine A (**1**) was obtained as colorless columnar crystals (from $CH_3OH$). Its molecular formula was deduced as $C_{16}H_{21}NO_4$ on the basis of the $[M]^+$ ion peak at *m*/*z* 291.1465 (calcd 291.1471) in the HREIMS. In the $^1H$ NMR spectrum, an oxymethine proton at $\delta_H$ 3.70 was clearly shown (Table 1). The $^{13}C$ NMR spectrum exhibited 16 carbon signals (Table 1), which were classified from HSQC and HMBC data as eight methylenes, three methines (including an oxymethine at $\delta_C$ 74.9), two keto carbonyls ($\delta_C$ 216.1 and 220.6), a carbinolamine carbon ($\delta_C$ 91.1), and two quaternary carbons ($\delta_C$ 51.9 and 54.4). The characteristic chemical shift at $\delta_C$ 51.9 is typical for the quaternary carbon C12, which is present in most fawcettimine-type *Lycopodium* alkaloids.[1] In addition, on the basis of the missing characteristic doublet methyl signal for $C\underline{H}_3$16 in the $^1H$ NMR spectrum and the appearance of one more quaternary carbon at C4 ($\delta_C$ 54.4) in the $^{13}C$ NMR spectrum, isopalhinine A (**1**) was deduced as a fawcettimine-type *Lycopodium* alkaloid with a fused C16−C4 bond.[5]

In the $^1H-^1H$ COSY spectrum, the cross peaks of H7/$H_2$8/$H_2$14/H15/$H_2$16 suggested the presence of spin system **a** (Figure 2). The fragment **a** together with the HMBC correlations from H8$\beta$ ($\delta_H$ 1.74) and H7 ($\delta_H$ 2.47) to C12 and H14$\beta$ ($\delta_H$ 2.49) to C13 ($\delta_C$ 216.1) and C12 indicated the existence of a cyclohexanone ring (ring A). The HMBC correlations from H3 to C16 ($\delta_C$ 37.6) and H16$\beta$ ($\delta_H$ 1.84) to C4, C5 ($\delta_C$ 91.1), and C12 indicated that the linkage of C16−C4 and the presence of a cyclohexanone ring (ring B). Furthermore, the carbinolamine carbon ($\delta_C$ 91.1) and the carbonyl carbon ($\delta_C$ 220.6) were located at C5 and C6, respectively, which built a bridge between C4 and C7 as evidenced by the HMBC correlations from H7 and $H_2$16 to C5 and $H_2$8 and H7 to C6. Then, a cyclopentanone ring (ring C) was constructed. These data, finally, led to the assignment of a 5-hydroxy-tricyclo[4.3.1.$0^{3,7}$]decan-4,8-dione moiety.

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

| no. | $\delta_H$ | $\delta_C$ |
|---|---|---|
| 1α | 2.63, ddd (14.4, 4.8, 1.8) | 48.9, $CH_2$ |
| 1$\beta$ | 3.35, overlapped | |
| 2α | 1.91, m | 30.2, $CH_2$ |
| 2$\beta$ | 1.57, m | |
| 3 | 3.70, dd (12.0, 6.6) | 74.9, CH |
| 4 | | 54.4, Cq |
| 5 | | 91.1, Cq |
| 6 | | 220.6, Cq |
| 7 | 2.47, dd (12.0, 1.2) | 51.4, CH |
| 8α | 1.93, m | 33.1, $CH_2$ |
| 8$\beta$ | 1.74, br d (13.8) | |
| 9α | 2.68, dt (11.4, 3.0) | 50.3, $CH_2$ |
| 9$\beta$ | 3.35, overlapped | |
| 10α | 1.84, m | 24.4, $CH_2$ |
| 10$\beta$ | 1.49, m | |
| 11α | 2.20, ddd (16.2, 4.8, 4.2) | 28.1, $CH_2$ |
| 11$\beta$ | 2.15, overlapped | |
| 12 | | 51.9, Cq |
| 13 | | 216.1, Cq |
| 14α | 2.15, overlapped | 46.5, $CH_2$ |
| 14$\beta$ | 2.49, dt (18.6, 3.0) | |
| 15 | 2.15, overlapped | 27.4, CH |
| 16α | 2.59, dt (14.4, 3.6) | 37.6, $CH_2$ |
| 16$\beta$ | 1.84, dt (14.4, 2.4) | |

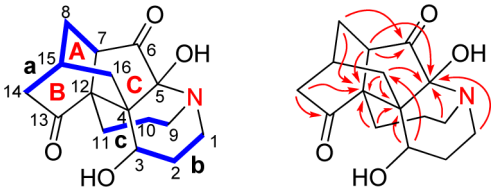



**Figure 2.** $^1H-^1H$ COSY (bold) and key HMBC (arrows) correlations of **1**.

In the $^1H-^1H$ COSY spectrum, correlations of $H_2$1/$H_2$2/H3 and $H_2$9/$H_2$10/$H_2$11 suggested the presence of spin systems **b** and **c** (Figure 2), respectively. The HMBC correlations from H1α ($\delta_H$ 2.63) to C9 ($\delta_C$ 50.3) and H9α ($\delta_H$ 2.68) to C1 ($\delta_C$ 48.9) indicated the connection of C1 and C9 through a nitrogen atom. Key HMBC networks from H11α ($\delta_H$ 2.20) to C12 and C4, as well as H3 to C4 and C12, were observed. Thus, it could be deduced that units **a** and **b** were connected to C12 and C4, which then formed a 1-azacyclononane ring. A carbon signal of $\delta_C$ 91.1 was observed in the $^{13}C$ NMR spectrum which suggested that it was a carbinolamine form of fawcettimine-type *Lycopodium* alkaloid.[1] However, interestingly, it possesses a unique linkage of N−C5 as evidenced by the HMBC correlations of H1α, H9α, and $H_2$16 with C5, which is totally unlike those reported for the carbinolamine form of fawcettimine-type *Lycopodium* alkaloids with a N−C13 bond. Therefore, the planar structure of **1** was

established as a pentacyclic fawcettimine-type *Lycopodium* alkaloid formed by unique linkages of C16−C4 and N−C5 bonds (Figure 2).

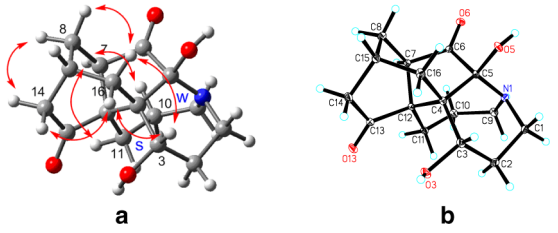



**Figure 3.** Key ROESY (double arrows, a) correlations and X-ray crystallographic structure (b) of **1**.

The relative configuration of **1** was determined by a ROESY experiment (Figure 3). The correlations of H16α/H8$\beta$, H16$\beta$/H14$\beta$, and H8α/H14α were clearly apparent, which supported the presence of a cage-like motif of a tricyclo[4.3.1.$0^{3,7}$]decane (isotwistane). The key correlations of H7/H10$\beta$ and H11$\beta$ indicated that these protons were cofacial and the 1-azabicyclo[4.3.1]decane moiety was located underneath ring C as shown in Figure 3. This deduction was further confirmed by an X-ray diffraction experiment using molybdenum radiation (Figure 3). Additionally, the correlations of H3/H16$\beta$ (strong) and H3/H16α (weak) were also observed. Based on the observations, thus, the relative configuration of **1** was established as 3*S**, 4*S**, 5*S**, 7*R**, 12*S**, 15*R**.

The absolute configuration of **1** was determined by the comparison of experimental and density functional theory (DFT) calculated optical rotation (OR) values. The OR was calculated at the B3LYP/6-311++G(2d,p) level of theory in methanol using the PCM solvent continuum model.[6] The DFT calculated value of (3*S*,4*S*,5*S*,7*R*,12*S*,15*R*)-**1** was +147.2, which was close to the experimental value of +124.0 in methanol. Thus, the absolute configuration of **1** was established as 3*S*, 4*S*, 5*S*, 7*R*, 12*S*, 15*R*.

Palhinine B (**2**) was obtained as colorless diamond-shaped crystals (from $CH_3OH/H_2O$, 20:1). Its molecular formula, $C_{17}H_{25}NO_3$, was elucidated based on the $[M + H]^+$ ion peak at *m*/*z* 292.1914 (calcd 292.1912) in the HRESIMS. In the $^1$H NMR spectrum (Table S1, Supporting Information (SI)), a singlet *N*-methyl proton at $\delta_H$ 2.17 (3H, s, H17) and an oxymethine proton at $\delta_H$ 4.09 (1H, m, H2) were clearly apparent. The $^{13}$C NMR and DEPT spectra exhibited 17 carbon signals due to a *N*-methyl ($\delta_C$ 47.3, C17), eight methylenes, three methines (including an oxymethine at $\delta_C$ 71.7), and four quaternary carbons (including two carbonyl groups at $\delta_C$ 210.9 and 219.5). The above data revealed that palhinine B (**2**) shares the same skeleton as that of palhinine A (**4**). The only difference between them was the position of the hydroxyl group, which was established from the COSY cross peaks of $H_2$1/H2/$H_2$3. The relative configuration of **2** was elucidated by an X-ray diffraction experiment using molybdenum radiation (Figure 4). Furthermore, based on the biosynthesis point of view and the fact that palhinines A (**4**) and B (**2**) were both isolated in the present study, the absolute configuration of **2** was established as 2*R*, 4*R*, 7*S*, 12*S*, 15*R*.[5]

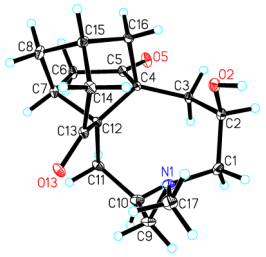
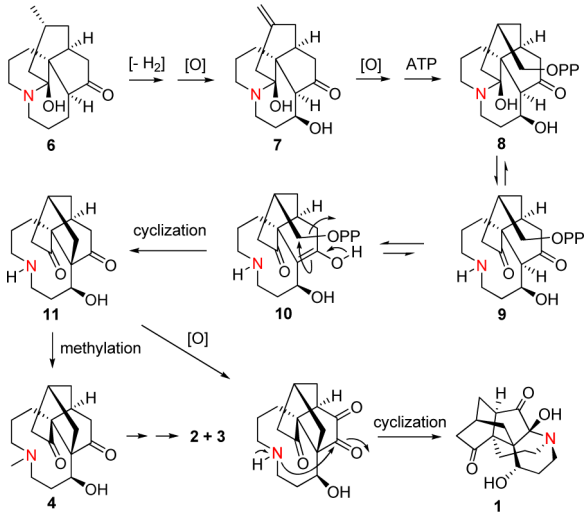


**Figure 4.** X-ray crystallographic structure of **2**.

Palhinine C (**3**) showed the same molecular formula, $C_{17}H_{25}NO_3$, as that of **2** by analysis of the HRESIMS. In the $^1$H−$^1$H COSY spectrum, an oxymethine proton at $\delta_H$ 3.89 (1H, td, $J$ = 10.2, 4.2 Hz, H2) showed correlations with $H_2$1 and $H_2$3, which indicated the position of the hydroxyl group located at C2. However, the different $^1$H and $^{13}$C NMR chemical shifts of C2 in $CDCl_3$ (Table S1, SI) suggested that the opposite configuration of the hydroxyl group between **2** and **3**. This deduction was further supported by the ROESY correlations of H2 with H14$\beta$ and H16$\beta$ (Figure S27, SI). Detailed 2D NMR data (SI) analysis indicated that the other parts of **3** were the same as those of **2**. Thus, the structure of **3** was established as a C2 epimer of **2**.

**Scheme 1.** Plausible Biogenetic Pathway of **1**−**4**




Based on the additional isolation of isopalhinine A (**1**) as well as palhinines B (**2**) and C (**3**), we could propose a possible biogenetic pathway as shown in Scheme 1. The biogenetic origin of **1**−**4** could plausibly be traced back to fawcettimine (**6**),[7] a *Lycopodium* alkaloid that is common in

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the genus of *Lycopodium*.[1] In brief, **6** underwent dehydrogenation and oxidation steps to produce intermediate **7**, which was followed by another oxidation step and adding a good leaving group such as diphosphate to produce intermediate **8**.[8] Intermediate **8** might exist in either a carbinolamine form (**8**) or an amino ketone form (**9**).[1] Enolation of **9** accompanied by an $S_Ni$ intramolecular substitution reaction between C4 and C16 will accomplish the key intermediate **11**.[8] Intermediate **11** underwent a methylation to get **4**, which could further convert to **2** and **3**. Moreover, **1** might be generated from oxidation and cyclization steps of **11**.

The new compounds (**1**−**3**) were evaluated for AChE and butyrylcholinesterase (BChE) inhibitory activities, but none of them showed obvious activities at a concentration of 50 $\mu$M. Moreover, due to small amounts obtained of **2** and **3**, only **1** and **4** were further evaluated for cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines, inhibitory activity against nitric oxide production in LPS-activated RAW264.7 macrophages, and antifungal activity against *Candida albicans* at concentrations of 40 $\mu$M, 25 $\mu$M, and 64 $\mu$g/mL, respectively. Unfortunately, neither of them exhibited obvious activities.

In conclusion, we have characterized a novel caged, rigid, and sterically congested *Lycopodium* alkaloid named isopalhinine A (**1**) that possesses a fused pentacyclic (5/6/6/6/7) ring system comprising a tricyclo[4.3.1.$0^{3,7}$]decane (isotwistane) moiety and a 1-azabicyclo[4.3.1]decane moiety, together with palhinines B (**2**) and C (**3**) from *P. cernua*. It is the first time that we discovered a naturally occurring *Lycopodium* alkaloid derived from the fawcettimine backbone having such a N−C5 bond, which is most likely due to the inversion of the stereocenter at C4.[9] In addition, it should be noted that two groups have completed the synthesis of the core isotwistane framework since the discovery of palhinine A (**4**) in 2010.[10] However, the total synthesis to construct the functionalized tetracyclic (5/6/6/9) ring system of **4** has not been reported so far. We hope that the discovery of **1**−**3** and the proposed biogenetic pathway could shed more light on the future total synthesis of this unique type of C16 fused *Lycopodium* alkaloid.

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**Supporting Information Available.** 1D and 2D NMR, and HRMS spectra of **1**−**3**, cif files of **1** and **2**, and the experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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