Isopalhinine A, a Unique Pentacyclic Lycopodium Alkaloid from Palhinhaea cernua

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ABSTRACT

A new pentacyclic (5/6/6/6/7) Lycopodium alkaloid named isopalhinine A (1), which possesses a sterically congested architecture built with a tricyclo[4.3.1.03,7]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 selective, and reversible acetylcholinesterase (AChE) inhibitors. 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.

Keywords: Lycopodium, Isopalhinine A, Palhinhaea cernua, Huperzine A

1. Introduction

2. Results and Discussion

3. Experimental Section

4. Conclusion

5. Acknowledgments

6. References

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Isopalhinine A (1) was obtained as colorless columnar crystals (from CH$_3$OH). Its molecular formula was deduced as C$_{18}$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 $^1$H 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. However, interestingly, it possesses a unique linkage of N–C5 as evidenced by the HMBC correlations from H7 and H216 to C5 and H28 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.

In the $^1$H–$^1$H COSY spectrum, correlations of H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2$/H$_2}$/
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/H8β, H16β/H14β, and H8α/H14α were clearly apparent, which supported the presence of a cage-like motif of a tricyclo[4.3.1.0³,⁷]decane (isotwistane). The key correlations of H7/H10β and H11β 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 (strong) and H3/H16α (weak) were also observed. Based on the observations, thus, the relative configuration of 1 was established as 3S*, 4S*, 5S*, 7R*, 12S*, 15R*.

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. The DFT calculated value of (3S,4S,5S,7R,12S,15R)-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 3S, 4S, 5S, 7R, 12S, 15R.

Palhinine B (2) was obtained as colorless diamond-shaped crystals (from CH3OH/H2O, 20:1). Its molecular formula, C17H25NO3, was elucidated based on the [M + H]+ ion peak at m/z 292.1914 (calcld 292.1912) in the HRESIMS. In the 1H NMR spectrum (Table S1, Supporting Information (SI)), a singlet N-methyl proton at δH 2.17 (3H, s, H17) and an oxymethine proton at δH 4.09 (1H, m, H2) were clearly apparent. The 13C NMR and DEPT spectra exhibited 17 carbon signals due to a N-methyl (δC 47.3, C17), eight methylenes, three methines (including an oxymethine at δC 71.7), and four quaternary carbons (including two carbonyl groups at δC 210.9 and 219.5). The above data revealed that palhinine B (2) shares the same skeleton as that of palhinines A (4) and B (2) were both isolated in the present study, the absolute configuration of 2 was established as 2R, 4R, 7S, 12S, 15R.5

Palhinine C (3) showed the same molecular formula, C17H23NO3, as that of 2 by analysis of the HRESIMS. In the 1H–1H COSY spectrum, an oxymethine proton at δH 3.89 (1H, td, J = 10.2, 4.2 Hz, H2) showed correlations with H21 and H23, which indicated the position of the hydroxyl group located at C2. However, the different 1H and 13C NMR chemical shifts of C2 in CDCl3 (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 H14β and H16β (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

[Diagram of Scheme 1]

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),6 a *Lycopodium* alkaloid that is common in

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the genus of *Lycopodium*. 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. Intermediate 8 might exist in either a carbinolamine form (8) or an amino ketone form (9). Eolation of 9 accompanied by an SNi intramolecular substitution reaction between C4 and C16 will accomplish the key intermediate 10. Intermediate 10 might exist in either a carbinolamine form (10) or an amino ketone form (11). Enolation of 11 accompanied by an SNi intramolecular substitution reaction between C4 and C16 will accomplish the key intermediate 12. Intermediate 12 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 μM. 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 μM, 25 μM, and 64 μg/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[4.3.1.03,7]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 fawcettine backbone having such a N–C5 bond, which is most likely due to the inversion of the stereocenter at C4. 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. 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.