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Palhinine A, a Novel Alkaloid from *Palhinhaea cernua*

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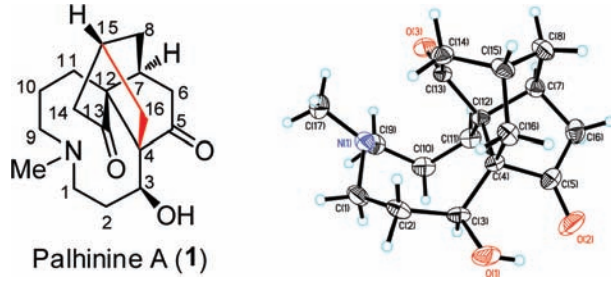
ABSTRACT



Palhinine A, a novel $C_{16}N$-type *Lycopodium* alkaloid with a unique 5/6/6/9 tetracyclic ring system, was isolated from the whole plant of *Palhinhaea cernua* L. (Lycopodiaceae). Its structure was elucidated by spectroscopic methods, and the absolute configuration was determined by single-crystal X-ray diffraction analysis using the Flack parameter. Palhinine A is reported as the first example of *Lycopodium* alkaloids of which C-16 is fused to a new ring through a C-16−C-4 lingkage.

The *Lycopodium* alkaloids are quinolizine or pyridine and α-pyridone type alkaloids from the genus *Lycopodium* (*sensu lato*), which have unique heterocyclic skeletons of $C_{11}N$, $C_{15}N_2$, $C_{16}N$, $C_{16}N_2$, $C_{22}N_2$, and $C_{27}N_3$.[1] Some of these alkaloids inhibit acetylcholinesterase (AChE), such as the well-known huperzine A (HupA).[2] Many of these compounds provide a challenge for total synthesis.[3] *Palhinhaea cernua* L., with synonyms of *Lycopodium cernuum* L., *Lepidotis cernua* (L.) P. Beauv., or *Lycopodiella cernua* (L.) Pic. Ser. in both *Flora of North America* and *Flora of China*,[4] is a

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member of the Lycopodiaceae family. The species is traditionally used to heal contusions, scald, and rheumatism by the Chinese people and is broadly distributed in Eastern and Southern China.[5] Its chemical constituents have not been heavily investigated, and only a few *Lycopodium* alkaloids such as lycocernuine have been isolated and reported.[5] In our research of alkaloids from ferns species,[6] palhinine A (**1**), a novel C_{16}N-type alkaloids with an unique 5/6/6/9 tetracyclic ring system, was isolated from the whole plant of *P. cernua*. The isolation and structure elucidation of **1** is reported here.

The whole plant of *P. cernua*, a climbing fern, was collected in Liping County of Guizhou Province, People's Republic of China and identified by Dr. Guang-Wan Hu at the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. SZ09048) was deposited in the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany. The air-dried and powdered sample (16.5 kg) was extracted with 70% MeOH three times (4, 4, and 3 h). The extracts were partitioned between EtOAc and 1% HCl/H_2O. Water-soluble materials, adjusted to pH 10 with 17% NaOH/H_2O solution, were extracted with $CHCl_3$, producing an alkaloid-rich extract (82.0 g). The latter was subjected to a silica gel column ($CHCl_3$/MeOH, 1:0 to 0:1) to produce fractions I−VIII. Fraction II (17.0 g) was further separated using a RP-18 column (MeOH/H_2O, 50:50), a Sephadex LH-20 column (MeOH), and preparative TLC (petroleum ether/Me_2CO/NH_3•H_2O, 80:10:1). The resulting fraction was then crystallized from Me_2CO/H_2O (10:1) to produce compound **1** (24.0 mg, 0.00014% yield).[7]

Palhinine A (**1**) was obtained as colorless blocks (mp 102−103 °C). Its molecular formula, $C_{17}H_{25}NO_3$, was established on the basis of high-resolution ESI-MS for the $[M + H]^+$ ion at *m*/*z* 292.1911 (calcd 292.1912), indicating six degrees of unsaturation. The IR spectrum showed absorptions for hydroxy and carbonyl groups respectively at 3480, 1719, and 1686 cm^{-1}. In the ^1H NMR spectrum (Table 1), a singlet of one methyl group at δ_H 1.93 ppm and a doublet of one tertiary methyl group at δ_H 4.43 ppm (J = 9.6 Hz) were shown. The ^{13}C NMR and DEPT spectra of **1** (Table 1) revealed 17 carbon signals due to two keto carbonyls (δ_C 205.3 and 222.6 ppm), two sp^3 quaternary carbons (δ_C 56.5 and 59.1 ppm), three sp^3 methines, nine sp^3 methylenes, and one *N*-methyl (δ_C 44.1 ppm). The doublet signal at δ_H 4.43 ppm was ascribed to C-3 (δ_C 73.9 ppm) through analysis of the HSQC spectrum. The structure of **1** should be a C_{16}N-type *Lycopodium* alkaloid on the basis of above-mentioned data, but a doublet signal of 16-CH_3 like others[1] disappeared in ^1H NMR spectrum. Since all of these functional groups accounted for two degrees of unsaturation, the remaining four degrees of unsaturation suggested to be contributed by a tetracyclic ring system in the structure of **1**.

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1** in $CDCl_3$ (δ in ppm, J in Hz)

	δ_H	δ_C
1a	2.64 (1H, m)	52.9 (t)
1b	2.43 (1H, m)	
2a	2.04 (1H, m)	33.8 (t)
2b	1.48 (1H, m)	
3	4.43 (1H, d, 9.6)	73.9 (d)
4		59.1 (s)
5		222.6 (s)
6a	2.65 (1H, m)	43.2 (t)
6b	2.23 (1H, d, 19.6)	
7	2.14 (1H, dd, 9.0, 9.0)	43.6 (d)
8a	1.94 (1H, m)	35.8 (t)
8b	1.41 (1H, ddd, 13.6, 3.8, 2.7)	
9a	2.42 (1H, m)	54.7 (t)
9b	2.07 (1H, m)	
10	1.54 (2H, m)	25.0 (t)
11β	2.31 (1H, m)	27.8 (t)
11α	1.22 (1H, ddd, 14.8, 3.7, 3.7)	
12		56.5 (s)
13		205.3 (s)
14a	2.49 (1H, m)	45.5 (t)
14b	2.28 (1H, m)	
15	2.36 (1H, m)	29.9 (d)
16a	2.44 (1H, m)	31.4 (t)
16b	1.64 (1H, ddd, 15.4, 2.7, 2.7)	
N-CH_3	1.93 (3H, s)	44.1 (q)
3-OH	4.59 (1H, br s)	

The $^1H-^1H$ 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. On the basis of the existence of fragments **a** and **b**, and the HMBC cross-peaks (Figure 1) of *N*-CH_3/C-1 and C-9, H_2-2/C-4, H-11α/C-12, and H-11β/C-4, a 1-azacyclononane ring (ring **A**) was confirmed in **1**. A cyclopentanone unit (ring **B**) and a cyclohexanone unit (ring **C**) were validated by the presence of fragment **c** and the HMBC correlations from H-3 and H-7 to C-5, H-6b and H-8b to C-12, H-7 to C-11, and H-11α to C-13. Rings A−C in **1** coincide with those in fawcettine-type *Lycopodium* alkaloids such as lycoposerramine T, *N*-methyllycoposerramine T, and *N*-formyllycoposerramine T.[8] The HMBC correlations from H-3 to C-16 and H-16b to C-5 showed the connection of C-4, and C-12

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(7) Palhinine A (**1**): colorless blocks; mp. 102−103 °C (from Me_2CO/H_2O, 10:1); $[\alpha]^{25.6}_D$ −9.6 (*c* 0.45, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (1.89) nm; IR (KBr) ν_{max} 3480, 1719, 1686, 1384, 1105 and 1048 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1. ESIMS *m*/*z* 292 $[M + H]^+$; HRESIMS *m*/*z* 292.1911 $[M + H]^+$ ($C_{17}H_{26}NO_3$ calcd 292.1912).

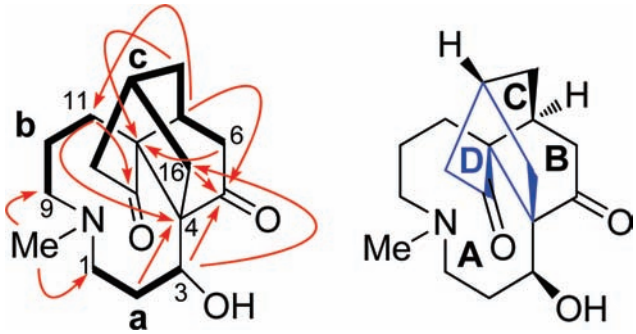

Figure 1. Selected ^{1}H−^{1}H COSY (bold) and HMBC (arrows) correlations of **1**.

to C-16 to form a new six-membered ring (ring **D**). A cage structure of bicyclo[2.2.2]octanone is constructed by rings C and D. The basic planar structure of **1** was herein determined.

The relative configuration of partial chiral centers in **1** was elucidated by the ROESY spectrum of **1** (Figure 2). The

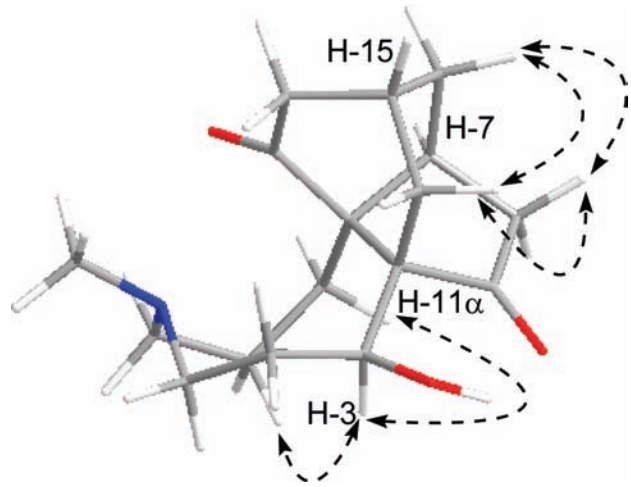

Figure 2. Key ROESY correlations and relative configuration for **1**.

ROESY correlations of H-3/H-11α indicated that both of two protons possessed the same orientation, which was arbitrarily assigned as α-orientation. The orientation of H-7 and H-15 could not be determined by the ROESY spectrum because the chemical shifts of some protons such as H-11β (δ_H 2.31 ppm) and H-14b (δ_H 2.28 ppm) are very close to each other.

The crystals of **1** were obtained from a mixed solution of acetone/water (10:1), and single-crystal X-ray crystallographic analysis (Figure 3)[9] was performed. The result established the absolute configuration of **1** as 3*S*,4*S*,

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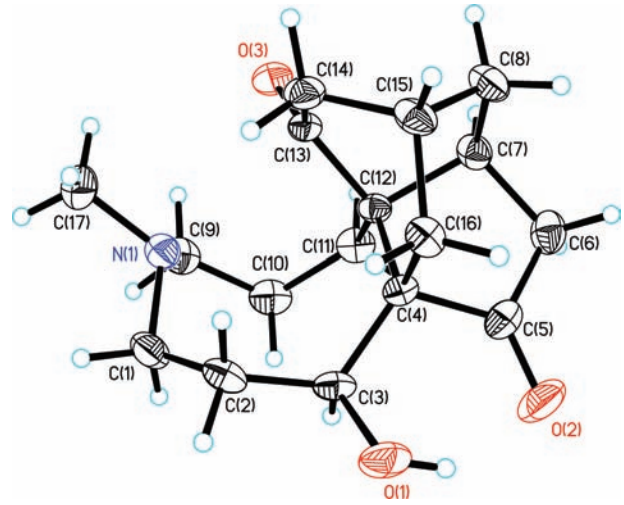

Figure 3. X-ray crystal structure of **1** (ORTEP drawing).

7*S*,12*S*,15*R* in light of the Flack parameter[10] of 0.2(2), using anomalous dispersion with copper radiation.[11]

Pelletierine was recognized as the biosynthetic precursor of the *Lycopodium* alkaloids. This results in a methyl group (C-16) that is always present in the molecular structure. Sometimes the methyl group is oxidized to a hydroxymethyl or carboxyl group.[1] Therefore, palhinine A represents the first example of a *Lycopodium* alkaloid in which the C-16 is fused to a ring through formation of a carbon−carbon bond between C-16 and C-4. However, the biogenetic pathway for **1** is remains unclear.

Biological tests *in vitro* showed that compound **1** was inactive ($IC_{50} > 200$ μM) against acetylcholinesterase using the improved Ellman's method (tacrine as positive control, $IC_{50} = 0.20$ μM),[12] butyrylcholinesterase by the improved Ellman's method (tetraisopropylpyrophosphoramide as posi-

(9) Crystal data for palhinine A (**1**): $C_{17}H_{25}NO_3$, $M = 291.38$ (excludes the solvent), colorless blocks, size 0.25 × 0.18 × 0.12 mm^3, orthorhombic, space group *P*212121; $a = 9.1204$ (4) Å, $b = 11.6853$ (4) Å, $c = 14.1079$ (6) Å, $\alpha = \beta = \gamma = 90.00°$, $V = 1503.54$ (11) Å^3, $T = 296(2)$ K, $Z = 4$, $\rho_{calcd} = 1.287$ g m^{-3}, μ(Cu Kα) = 0.7 mm^{-1}, $F(000) = 632$, 5148 reflections in $h(-9/10)$, $k(-13/12)$, $l(-13/15)$, measured in the range $4.91° \le \theta \le 67.06°$, completeness $\theta_{max} = 93.6\%$, 2459 independent reflections, $R_{int} = 0.0128$, 2397 reflections with $|F|^2 \ge 2\sigma|F|^2$, 193 parameters, 0 restraints, GOF = 1.039. Final R indices: $R_1 = 0.0318$, w$R_2 = 0.0906$. *R* indices (all data): $R_1 = 0.0327$, w$R_2 = 0.0918$. Flack parameter 0.2(2), largest difference peak and hole = 0.14 and −0.136 e Å^{-3}. The intensity data for **1** were collected on a Bruker SMART APEX-II diffractometer using graphite-monochromated Cu Kα radiation. The structure of **1** was solved by direct methods (SHELXS97), expanded using difference Founier techniques, and refined by the program and full-matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at calculated positions. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 782667). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

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tive control, $IC_{50} = 1.35\ \mu M$),[12a,13] and human chronic myelogenous leukemia K562 cells by the MTT method (adriamycin as positive control, $IC_{50} = 0.96\ \mu M$).[14]

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Supporting Information Available: Experimental procedures, spectroscopic data for compound **1**, and X-ray crystallographic data of compound **1** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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