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Casuarines A and B, *Lycopodium* alkaloids from *Lycopodium casuarinoides*

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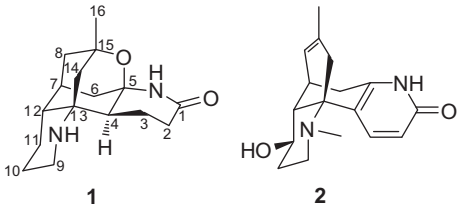
ABSTRACT

A phytochemical study on *Lycopodium casuarinoides* has led to the isolation of two new $C_{16}N_2$-type *Lycopodium* alkaloids, casuarine A (**1**), a cage-like structure featured with a fused 6/6/6/6/6 pentacyclic ring system, and casuarine B (**2**). Their structures were elucidated based on the spectroscopic data and further confirmed by X-ray analysis. In vitro acetylcholinesterase (AChE) inhibitory activity assay showed that compound **2** exhibited moderate anti-AChE activity with IC_{50} 46.40 μM.

Lycopodium alkaloids, possessing a complex heterocyclic ring system and wideranging biological properties, have attracted great interest from biogenetic, synthetic, and biological perspectives.[1,2] In particular, huperzine A (hup A), a $C_{16}N_2$-type alkaloid isolated from the Chinese folk medicinal herb *Qian Ceng Ta* (*Huperzia serrata*),[3,4] has been shown to be a highly potent, specific, and reversible inhibitor of acetylcholinesterase (AChE).[5–7] Until now, more than 300 *Lycopodium* alkaloids were reported.[1,8] Among them, most of the *Lycopodium* alkaloids possessing AChE inhibitory activity belong to lycodine class, such as, huperzine A, huperzine B,[4] and *N*-methylhuperzine B.[9] In our continuing efforts to search for structurally interesting and bioactive *Lycopodium* alkaloids, two new $C_{16}N_2$-type alkaloids, casuarine A (**1**), a cage-like structure featured with a fused 6/6/6/6/6 pentacyclic ring system, and casuarine B (**2**), were isolated from the club moss *Lycopodium casuarinoides*. Compound **2** exhibited moderate AChE inhibitory activity with IC_{50} 46.40 μM. Herein, we report the isolation, structure elucidation, and the anti-AChE activity of **1** and **2** .



1 **2**

The club moss *L. casuarinoides* (7 kg) collected in Guangxi province of China was extracted with 70% EtOH/H_2O (24 h × 3), and the EtOH extract was partitioned between EtOAc and 10% HCl/H_2O. Water-soluble materials, after being adjusted at pH 10 with saturated Na_2CO_3, were then partitioned with $CHCl_3$. $CHCl_3$-soluble materials (29 g) were subjected to reversed-phase MPLC (RP-18) (MeOH/H_2O, 10%→95%), a silica gel column ($CHCl_3$/MeOH/H_2O, 90:10:1→50:50:1 and then petroleum ether/acetone/diethylamine, 50:40:1→10:80:1), and a Sephadex LH-20 column (MeOH) to afford casuarines A (**1**, 15 mg, 0.0002%) and B (**2**, 22 mg, 0.0003%), together with the known $C_{16}N_2$-type alkaloids, huperzinine (1605 mg, 0.023%),[10] *N*-demethylhuperzinine (8 mg, 0.0001%), huperzine B (15 mg, 0.0002%), and huperzine D (30 mg, 0.0004%).[11]

Casuarines A[12] {$[\alpha]_D^{13.2}$ + 8.9 (*c* 1.0, MeOH)} was obtained as a colorless crystal and its molecular formula was established to be $C_{16}H_{24}N_2O_2$ by HREIMS at *m*/*z* 276.1831 $[M]^+$ (calcd 276.1838), indicating six degrees of unsaturation. IR absorptions implied the presence of amino (3431 cm^{-1}) and carbonyl (1667 cm^{-1}) functionalities. Analysis of the ^1H and ^{13}C NMR spectra of **1** (Table 1) revealed 16 carbon signals due to four quaternary carbons, three tertiary carbons, eight methylenes, and one methyl group. Among them, one sp^2 quaternary carbon was attributable to the amide group (δ_C 171.9), and two sp^3 quaternary carbons (δ_C 82.6 and 74.3) were attributed to those attached to an oxygen atom.

The gross structure of **1** was elucidated by analysis of 2D NMR data (Fig. 1). The ^1H–^1H COSY cross-peaks of **1** disclosed the presence of two structural fragments, **a** (C-2–C-4) and **b** (C-9–C-12 and C-6–C-8). According to the HMBC cross-peaks of H-2 (δ_H 2.56 and

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Table 1
^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of **1** and **2** (δ in ppm, J in Hz)

No.	**1**[a]		**2**[b]	
	δ_H (mult, J, Hz)	δ_C (mult)	δ_H (mult, J, Hz)	δ_C (mult)
1		171.9 s		165.7 s
2a	2.56 (dd, 17.9, 5.0)	31.8 t	6.37 (d, 9.5)	118.5 d
2b	2.31 (m)			
3a	1.91 (ddd, 17.9, 12.7, 5.0)	15.7 t	7.89 (d, 9.5)	142.6 d
3b	1.68 (m)			
4	2.24 (dd, 13.2, 2.8)	35.9 d		122.1 d
5		82.6 s		144.7 s
6a	1.86 (br d, 10.6)	36.3 t	2.93 (dd, 17.1, 5.2)	30.0 t
6b	1.53[c]		2.32 (d, 17.1)	
7	2.01 (m)	33.6 d	2.97 (m)	30.8 d
8a	1.65 (m)	41.8 t	5.49 (d, 5.2)	125.5 d
8b	1.53[c]			
9a	2.83 (m)	39.1 t	2.72 (m)	50.3 t
9b	2.78 (m)		2.61 (m)	
10a	1.77 (m)	25.9 t	1.54 (m)	29.9 t
10b	1.66 (m)		1.78 (m)	
11a	1.74[c]	24.2 t	3.42 (td, 10.7, 5.1)	68.5 d
11b	1.52 (m)			
12	1.74[c]	45.8 d	1.87 (dd, 10.7, 3.4)	41.5 d
13		53.7 s		59.9 s
14a	1.71 (d, 12.8)	43.5 t	2.67[c]	44.0 t
14b	1.24 (d, 12.8)		1.80 (m)	
15		74.3 s		133.9 s
16	1.18 (3H, s)	28.2 q	1.57 (3H, s)	23.1 q
N-CH_3			2.67[c]	37.8 q

[a] Recorded in $CDCl_3$.
[b] Recorded in CD_3OD.
[c] Overlapping signals.

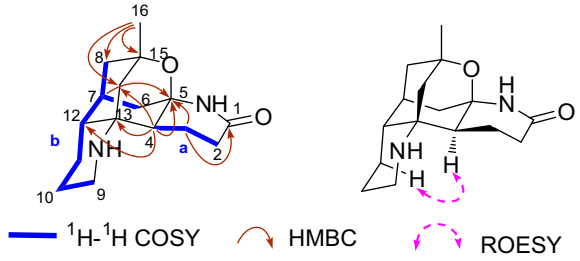


Figure 1. Selected 2D NMR correlations for casuarine A (**1**).

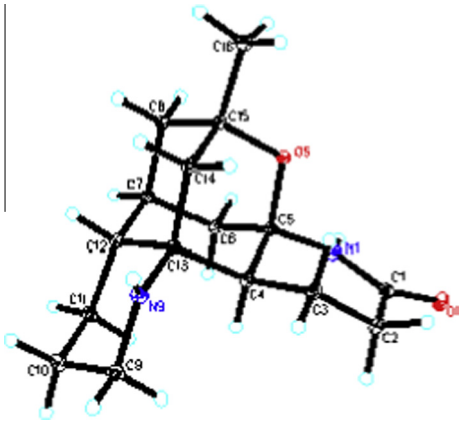
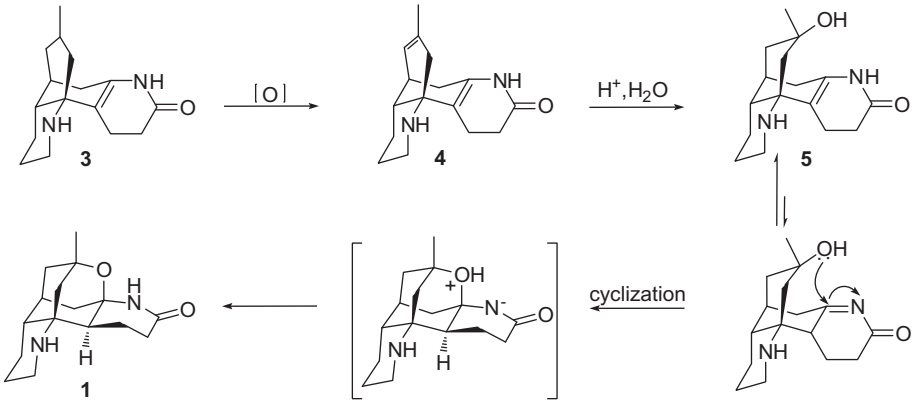


Figure 2. X-ray structure of casuarine A (**1**).

2.31) to C-1 (δ_C 171.9), H-3 (δ_H 1.91 and 1.68) to C-1 (δ_C 171.9) and C-5 (δ_C 82.6), and H-4 (δ_H 2.24) to C-5, the partially structured C-1–C-5 was constructed. An HMBC cross-peak of H-7 (δ_H 2.01) to C-5 indicated the connectivity of C-5 and C-6. Connectivity of C-9 (δ_C 39.1) and C-13 (δ_C 53.7) through a nitrogen atom was revealed by HMBC cross-peak for H-9 (δ_H 2.83, 2.78) to C-13. HMBC cross-peaks of H-4 with C-12 (δ_C 45.8), C-13, and C-14 (δ_C 43.5) suggested the connectivities of C-4, C-12, and C-14 via C-13. Connectivities of C-8, C-14, and C-16 through C-15 were elucidated by HMBC correlations from H-16 (δ_H 1.18) to C-8 (δ_C 41.8), C-14, and C-15 (δ_C 74.3). Finally, the connectivities of C-1 with C-5 through a nitrogen atom and C-5 with C-15 through an oxygen atom can be deduced from the molecular formula and confirmed by the special ^{13}C NMR data of C-5 and C-15 as well as the HMBC correlations from H-16 (δ_H 1.18) to C-5. Thus, the gross structure of casuarine A was elucidated to be **1** which possessed a cage-like structure featured with a fused 6/6/6/6/6 pentacyclic ring system that was never found in lycodine-type alkaloids (Fig. 1).

The relative configuration of **1** was established by ROESY spectrum (Fig. 1). The correlation of H-4 with H-11a indicated H-4 was α-oriented. Due to the overlaps of H-6b/H-8b and H-11a/H-12 and the structure novelty, a single X-ray diffraction study of compound **1** was made which validated the planar structure and established the relative configuration of **1** (Fig. 2).[13]

Casuarine B[14]{$[\alpha]_D^{24.2}$ −32.3 (*c* 0.12, $CHCl_3$)}, a colorless amorphous powder, has a molecular formula $C_{17}H_{22}N_2O_2$ as established by HREIMS at *m*/*z* 286.1676 $[M]^+$ (calcd 286.1681), suggesting eight degrees of unsaturation. IR absorptions implied the existence of amino (3419 cm^{-1}) and carbonyl (1653 cm^{-1}) groups. The UV absorption at 230 and 311 nm indicated the presence of the pyridine ketone ring. Analysis of the 1D and 2D NMR spectra revealed the existence of 17 carbons due to one carbonyl carbon (δ_C 165.7), three sp^2 quaternary carbons (δ_C 122.1, 133.9, and 144.7), one sp^3 quatemary (δ_C 59.9), three sp^2 methines (δ_C 118.5, 142.6, and 125.5), one sp^2 methine (δ_H 3.42; δ_C 68.5) that attached to an oxygen atom, four sp^3 methylenes, one methyl (δ_H 1.57; δ_C 23.1), and one *N*-methyl (δ_H 2.67; δ_C 37.8). Based on above data, the structure of **2** was concluded to be a lycodine-type alkaloid which was



Scheme 1. Plausible biogenetic pathway of **1**.

resembled to those of *N*-methylhuperzine B.[10,15] The only difference was that **2** had one hydroxy group which was connected to C-11 as deduced from the HMBC correlations for H-7 (δ_H 2.97), H-9a (δ_H 2.72), H-9b (δ_H 2.61), and H-12 (δ_H 1.87) to C-11 (δ_C 68.5). The ROESY spectrum of **2** showed cross-peaks of H-12/H-10b, H-12/H-14a, and H-11/H-6a that indicated H-12 was β-oriented and H-11 was α-oriented.[15,16] Thus, the structure of casuarine B (**2**) was elucidated.

A plausible biogenetic pathway of casuarine A (**1**) was proposed as shown in Scheme 1. Biogenetically, **1** may be derived from des-*N*-methyl-α-obscurine (**3**). As shown, **3** underwent an oxidation to produce intermediate **4**, followed then by an addition in the acidic conditions to afford intermediate **5**. Intermediate **5** further underwent an important tautomerism and a cyclization to yield **1**.

Casuarines A (**1**) and B (**2**) were tested for AChE inhibitory activities using the Ellman method reported previously (huperzine A as positive control, IC_{50} = 0.03 μM).[17] Finally, casuarine B showed a moderate AChE inhibitory activity (IC_{50} = 46.40 μM), while casuarine A showed no activity.

Acknowledgments

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References and notes

1. For reviews of the Lycopodium alkaloids, see: (a) Hirasawa, Y.; Kobayashi, J.; Morita, H. *Heterocycles* **2009**, *77*, 679; (b) Kobayashi, J.; Morita, H. In *The Alkaloids; Cordell*, G.A., Ed.; Academic Press: New York, 2005; Vol. 61, p 1.; (c) Ma, X.; Gang, D. R. *Nat. Prod. Rep.* **2004**, *21*, 752–772.
2. For selected examples, see: (a) Hirasawa, Y.; Kato, Y.; Wong, C. P.; Uchiyama, N.; Goda, Y.; Hadi, A. H. A.; Morita, H. *Tetrahedron Lett.* **2013**, *54*, 1593–1595; (b) Zhao, F. W.; Sun, Q. Y.; Yang, F. M.; Luo, J. F.; Hu, G. W.; Liu, F.; Wang, Y. H.; Long, C. L. *J. Braz. Chem. Soc.* **2012**, *23*, 349–354; (c) Wittayalai, S.; Sathalalai, S.; Thorroad, S.; Worawittayanon, P.; Ruchirawat, S.; Thasana, N. *Phytochemistry* **2012**, *76*, 117–123; (d) Dong, L. B.; Yang, J.; He, J.; Luo, H. R.; Wu, X. D.; Deng, X.; Peng, L. Y.; Cheng, X.; Zhao, Q. S. *Chem. Commun.* **2012**; (e) Zhao, F. W.; Sun, Q. Y.; Yang, F. M.; Hu, G. W.; Luo, J. F.; Tang, G. H.; Wang, Y. H.; Long, C. L. *Org. Lett.* **2010**, *12*, 3922–3925; (f) He, J.; Chen, X. Q.; Li, M. M.; Zhao, Y.; Xu, G.; Cheng, X.; Peng, L. Y.; Xie, M. J.; Zheng, Y. T.; Wang, Y. P.; Zhao, Q. S. *Org. Lett.* **2009**, *11*, 1397–1400.
3. Liu, J. S.; Zhu, Y. L.; Yu, C. M.; Zhou, Y. Z.; Han, Y. Y.; Wu, F. W.; Qi, B. F. *Can. J. Chem.* **1986**, *64*, 837–839.
4. Liu, J. S.; Yu, C. M.; Zhou, Y. Z.; Han, Y. Y.; Qi, B. F.; Zhu, Y. L. *Acta Chim. Sin.* **1986**, *44*, 1035–1040.
5. Kozikowski, A. P.; Tückmantel, W. *Acc. Chem. Res.* **1999**, *32*, 641–650.
6. Bai, D. L.; Tang, X. C.; He, X. C. *Curr. Med. Chem.* **2000**, *7*, 355–374.
7. Tan, C. H.; Zhu, D. Y. *Chin. J. Nat. Med.* **2003**, *1*, 1–7.
8. Chen, Y. G.; Liu, Y. J.; Jiang, J. H.; Liu, B. *J. Yunnan Norm. Univ.* **2010**, *30*, 12–24.
9. Shen, Y. C.; Chen, C. H. *J. Nat. Prod.* **1994**, *57*, 824–826.
10. Liu, J. S.; Yu, C. M.; Zhou, Y. Z.; Han, Y. Y.; Qi, B. F.; Zhu, Y. L. *Acta Chem. Sin.* **1986**, *44*, 1035–1040.
11. Liu, J. S.; Huang, M. F. *Phytochemistry* **1994**, *37*, 1759–1761.
12. *Casuarine A* (***1***): colorless crystals (acetone); mp 169–170 °C; $[\alpha]_D^{13.2}$ + 8.9 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε): 201 (2.90) nm. IR (KBr) ν_{max} 3431, 2927 and 1667 cm^{-1}. ^1H and ^{13}C NMR (Table 1). EIMS *m*/*z* 276 $[M]^+$; HREIMS *m*/*z* 276.1831 $([M]^+$ calcd for $C_{16}H_{24}N_2O_2$, 276.1838).
13. *Crystal data for Casuarine A* (***1***): $C_{16}H_{24}N_2O_2$, *M* = 276.37, orthorhombic, *a* = 7.8469(7) Å, *b* = 12.7423(11) Å, *c* = 13.6732(12) Å, α = 90.00° β = 90.00°, γ = 90.00°, *V* = 1367.1(2) $Å^3$, *T* = 100(2) K, space group *P*212121, *Z* = 4, μ (MoKα) = 0.089 mm^{-1}, crystal dimensions 0.60 × 0.20 × 0.18 mm was used for measurement on a Bruker APEX DUO diffractometer using graphitemonochromated Mo Kα radiation. The total number of reflections measured was 14,632, of which 3885, were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0397, wR_2 = 0.1050. Crystallographic data for the structure of 1 have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 930550). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.
14. *Casuarine B* (***2***): colorless amorphous powder; $[\alpha]_D^{24.2}$ − 32.3 (*c* 0.12, $CHCl_3$). UV ($CHCl_3$) λ_{max}(log ε): 230 (3.58) nm. IR (KBr) ν_{max} 3419 and 1653 cm^{-1}. ESIMS *m*/*z* 287 $(M+H)^+$; ^1H and ^{13}C NMR (Table 1); HREIMS *m*/*z* 286.1676 $[M]^+$ (calcd for $C_{17}H_{22}N_2O_2$, 286.1681).
15. Kobayashi, J. I.; Hirasawa, Y.; Yoshida, N.; Morita, H. *J. Org. Chem.* **2001**, *66*, 5901–5904.
16. Choo, C. Y.; Hirasawa, Y.; Karimata, C.; Koyama, K.; Sekiguchi, M.; Kobayashi, J. I.; Morita, H. *Bioorg. Med. Chem.* **2007**, *15*, 1703–1707.
17. Ellman, G. L.; Courtney, K. D.; Anders, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88–90.