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Diphaladine A，扁枝石松中一个新的石松生物碱*

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摘要：从扁枝石松（*Diphasiastrum complanatum*）的95%乙醇提取物中分离得到1个新的石松生物碱，经波谱技术鉴定其结构，命名为diphaladine A（**1**）。同时还分离得到其它9个已知石松生物碱类化合物，其中obscurumine A（**2**），L20，lycoposerramine-K，des-N-methyl-β-obscurine，des-N-methyl-α-obscurine，lycoflexion，和phlegmariurine B等7个化合物均为首次从该植物中分离得到。

关键词：扁枝石松；石松生物碱；diphaladine A
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Diphaladine A, a New Lycopodium Alkaloid from *Diphasiastrum complanatum* (Lycopodiaceae)

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Abstract: A new Lycopodium alkaloid, diphaladine A (**1**), was isolated from the 95% EtOH extract of *Diphasiastrum complanatum* together with other nine known alkaloids. Their structures were elucidated on the basis of extensive spectroscopic analysis. To the best of our knowledge, obscurumine A (**2**), L20, lycoposerramine-K, des-N-methyl-β-obscurine, des-N-methyl-α-obscurine, lycoflexion, and phlegmariurine B were all isolated from this plant for the first time.

Key words: *Diphasiastrum complanatum*; Lycopodium alkaloids; diphaladine A

Lycopodium alkaloids are a group of natural products with unique heterocyclic ring systems, which have attracted great interest from biogenetic (Ayer and Trifonov, 1994), synthetic (Cassayer *et al.*, 2002; Williams *et al.*, 1994), and biological (Liu *et al.*, 1986) points of view. Among them, huperzine A was reported to be a potent acetylcholinesterase (ACHE) inhibitor and have the ability to increase the efficiency of learning and memory in animals (Liu *et al.*, 1986). *Diphasiastrum complanatum* Holub belongs to the family Lycopodiaceae and is widely distributed in Yunnan, Sichuan, and Guizhou Provinces, and used as a traditional Chinese herbal medicine for the treatment of arthritic pain, quadriplegia, and contusion (Jiangsu Institute of Botany, 1990). In previous phytochemical investigations, several Lycopodium alkaloids have been reported from this plant (Ishiuchi *et al.*, 2006; Kubota *et al.*, 2007). As a part of our systematic research

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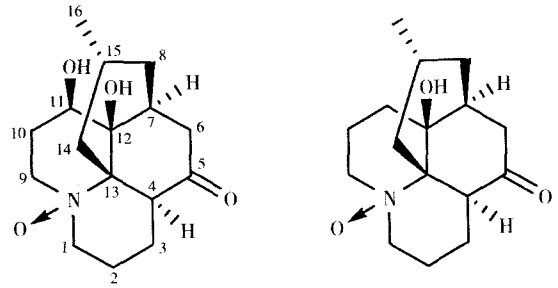
works on ferns (Li *et al.*, 2006; 2007), the chemical constituents of *D. complanatum* was studied and a new Lycopodium alkaloid, as well as nine known alkaloids were obtained. In this paper, we reported the isolation and structural elucidation of diphaladine A (Fig. 1).



1 **2**

Fig. 1 Structures of compounds **1** and **2**

Results and Discussion

Compound **1** was obtained as colorless oil. Its molecular formula, $C_{16}H_{25}NO_4$, was deduced on the basis of HR-ESI-MS ($[M+H]^+$ at *m/z* 296.1862; calcd. 296.1861). The IR absorptions at 1705 and 3175–3485 cm^{-1} indicated the presence of ketone and hydroxyl groups, respectively. In the EI-MS, the fragment peak ion at *m/z* 279 ($[M-16]^+$), due to the loss of one O-atom, suggested the existence of a N-oxide function. The ^1H and ^{13}C NMR spectra (Table 1) of **1** showed 16 carbon resonances due to a ketone carbonyl (δ_C 209.3), two quaternary carbons, four methines, eight methylenes, and one methyl group (δ_C 22.3, δ_H 0.94). Among them, two methylenes (δ_C 63.0, δ_H 3.03 and 3.61; δ_C 55.7, δ_H 3.03 and 4.41) and one quaternary carbon (δ_C 73.5) can be ascribable to bearing a nitrogen oxide function (Kashiwaba *et al.*, 1998), whereas one quaternary carbon (δ_C 72.0) and one methine (δ_C 72.2, δ_H 4.24) were ascribed as oxygen-bearing atoms. Considering the characteristic NMR data discussed above, along with the knowledge about the structural types of Lycopodium alkaloids isolated from *D. complanatum* and related plants previously (Kubota *et al.*, 2007; Takayama *et al.*, 2003), compound **1** can be ascribed to be a lycopodine-type Lycopodium alkaloid.

Table 1 ^1H and ^{13}C NMR data for compounds **1** and **2** in $CDCl_3$

No	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1α	3.03 (overlapped)	63.0 (t)	2.92 (1H, dd, 4.8, 13.3)	64.2 (t)
1β	3.61 (overlapped)		3.62 (1H, dt, 5.0, 13.5)	
2α	1.91 (1H, m)	21.3 (t)	1.91 (1H, m)	22.0 (t)
2β	1.95 (1H, m)		1.98 (1H, m)	
3α	2.12 (1H, m)	17.4 (t)	2.12 (1H, br d, 14.3)	18.7 (t)
3β	1.65 (1H, m)		1.69 (1H, m)	
4	3.87 (1H, dd, 2.2, 10.0)	48.5 (d)	3.30 (1H, m)	49.0 (d)
5		209.3 (s)		209.2 (s)
6α	3.61 (overlapped)	44.9 (t)	3.66 (1H, m)	44.8 (t)
6β	2.31 (1H, br d 13.6)		2.36 (1H, dd, 1.7, 17.1)	
7	2.23 (1H, m)	40.3 (d)	2.77 (1H, m)	42.5 (d)
8*exo*	2.07 (1H, dt, 2.8, 10.4)	36.8 (t)	2.01 (1H, m)	36.4 (t)
8*endo*	1.27 (1H, br d, 10.4)		1.37 (1H, br d, 12.4)	
9α	4.41 (1H, dt, 2.2, 10.2)	55.7 (t)	4.31 (1H, dt, 3.7, 13.0)	60.7 (t)
9β	3.03 (overlapped)		2.98 (1H, dd, 4.7, 12.8)	
10α	3.20 (1H, m)	26.4 (t)	2.79 (1H, m)	17.4 (t)
10β	1.79 (1H, br d, 11.8)		1.81 (1H, br d, 6.7)	
11α	4.24 (1H, s)	72.2 (d)	1.53 (1H, br d, 13.5)	30.3 (t)
11β			2.48 (1H, dt, 4.6, 13.7)	
12		72.0 (s)		74.6 (s)
13		73.5 (s)		73.1 (s)
14*exo*	2.51 (1H, t, 10.4)	30.1 (t)	2.52 (1H, t, 12.2)	30.6 (t)
14*endo*	1.98 (1H, m)		2.08 (1H, dd, 4.5, 13.4)	
15	1.56 (1H, m)	24.6 (d)	1.49 (1H, m)	25.9 (d)
16	0.94 (3H, d, 6.2)	22.3 (q)	0.97 (3H, d, 6.2)	22.8 (q)

A careful comparison of the ^{1}H and ^{13}C NMR data of **1** with those of obscurumine A (**2**) (Table 1) indicated that the two compounds were very similar to each other except for the presence of an O-bearing methine (δ_C 72.2) in **1** instead of the methylene group at C-11 (δ_C 30.3) in **2**. In addition, the downfield chemical shift of C-10 from δ_C 17.4 in **2** to δ_C 26.4 in **1**, and the upfield chemical shift of C-9 from δ_C 60.7 in **2** to δ_C 55.7 in **1**, also indicated that the C-11 of **1** was substituted by a hydroxyl group, which can be confirmed by the HMBC correlations between H-11 and C-7, C-9, and C-12. (Fig. 2). Furthermore, the HMBC correlations from H-1 to C-9 and C-13, from H-3, H-7, and H-14 to C-13, from H-3, H-6 to C-5, and from H-14 to C-4 (Fig. 2) also confirmed the structure.

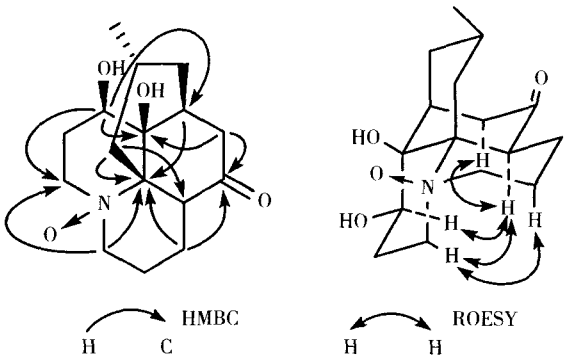


Fig. 2 Key HMBC and ROESY correlations of compound **1**

The relative stereochemistry of **1** was elucidated by ROESY experiment (Fig. 2). The ROESY cross-peaks of H-9α with H-2α and H-4 suggested the quinolizine partial structure was cis-oriented (Tan and Zhu, 2004; Tong *et al.*, 2003; Ortega *et al.*, 2004)). In addition, the ROESY correlations of H-4 with H-6α and H-11 suggested the hydroxyl group at C-11 was β-oriented. Thus, the structure of compound **1** was elucidated and named as diphaladine A.

The known alkaloids were obscurumine A (Morita *et al.*, 2005), lycopodine (Yuan *et al.*, 1995), L20 (Takayama *et al.*, 2003), lycoposerramine-K (Takayama *et al.*, 2003), lycodine (Nakashima *et al.*, 1975), des-N-methyl-β-obscurine (Ayer and Kasitu, 1989), des-N-methyl-α-obscurine (Ayer and Kasitu, 1989), lycoflexion (Takayama *et al.*, 2002), and phlegmariurine B (Tan *et al.*, 2002). The structures were determined by comparison of the spectroscopic data with those reported in the literature.

Experimental

General experimental procedures Optical rotations were recorded on a SEPA-300 polarimeter. IR spectra were determined on a Bio-Rad FTS-135 spectrophotometer with KBr pelets. The MS spectra were performed on a VG Autospec-3000 spectrometer. 1D NMR and 2D NMR were recorded on a Bruker AV-400 or DRX-500 Spectrometer with TMS as internal standard. Column chromatography was performed over silica gel (200-300 mesh, 10-40 μm, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden).

Plant Material The whole plant of *D. complanatum* was collected from Lvchun country, Yunnan Province, P. R. China, in June 2007, and was identified by Prof. Cheng Xiao. A voucher specimen (No. 20070603) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The dried and powdered plant materials of *D. complanatum* (10 kg) were extracted three times with 95% EtOH. After removal of solvent in a vacuum, the crude extract was dissolved in H_2O to form a suspension, which was adjusted with 5% HCl to pH 2, and then extracted with EtOAc. The aqueous phase was adjusted to pH 10 with $NH_3 \cdot H_2O$ and extracted with $CHCl_3$ to give crude alkaloids (4.8 g). The crude alkaloids were subjected to a silica gel column, using petroleum ether-acetone (from 1:0 to 0:1) as eluent, to obtain four fractions (Fr.1-Fr.4). Fr.2 was further separated using Sephadex LH-20 eluted with $CHCl_3$-MeOH (1:1) and repeated column chromatography with petroleum ether-EtOAc-Et_2NH (50:1:0.01) to yield lycopodine (20 mg) and lycodine (2.8 mg); Fr.3 was subjected to column chromatography over silica gel with $CHCl_3$-MeOH (from 100:1 to 10:1), followed by purification over Sephadex LH-20 eluted with $CHCl_3$-MeOH (1:1) to obtain L20 (50 mg), lycoposerramine-K (5.5 mg), des-N-methyl-α-obscurine (30 mg), and lycoflexion (70 mg); Fr.4 was subjected to repeated column chromatography over silica gel with $CHCl_3$-MeOH (from 30:1 to 5:1) and petroleum ether-acetone-Et_2NH (10:1:0.01), followed by Sephadex LH-20 column chromatography with MeOH to afford compound **1** (5 mg), des-N-methyl-β-obscurine (35 mg), obscurumine A (3 mg), and phlegmariurine B (9 mg).

Diphaladine A: colorless oil, $C_{16}H_{25}NO_4$; $[\alpha]_D^{23.3}$ = + 11.9 (c = 0.12, $CHCl_3$); IR (KBr): 3421, 3215, 2956,

2872, 1705, 1631, 1456, 1317, 1057, 987, 753 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHZ, $CDCl_3$) data see table 1; EI-MS *m/z* (%): 295 $[M]^+$ (5), 279 (20), 262 (100), 259 (24), 242 (69), 149 (44), 148 (33), 55 (24); HR-ESI-MS: 296.1862 $[M + H]^+$ (calcd. for $C_{16}H_{26}NO_4$, 296.1861).

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