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A new *Lycopodium* alkaloid from *Phlegmariurus fargesii*

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[ABSTRACT]

AIM: To investigate the chemical constituents from the whole plants of *Phlegmariurus fargesii*.

METHOD: Compounds were isolated by repeated silica gel column chromatography. Their structures were elucidated by spectroscopic methods and chemical correlation. The acetylcholinesterase (AChE) inhibitory activity of the isolated compounds was evaluated.

RESULTS: A new *Lycopodium* alkaloid, lycopodine *N*-oxide (**1**), along with lycopodine (**2**), 8,15-dehydrolycopodine (**3**), 6*α*-hydroxylycopodine (**4**), deacetyllycoclavine (**5**), *N*-methylhuperzine B (**6**), lycodine (**7**), and phlegmarine (**8**), was isolated.

CONCLUSION: Compound **1** is a new *Lycopodium* alkaloid, and compound **3** was obtained from nature for the first time. Other alkaloids are isolated from this plant for the first time.

[KEY WORDS] *Phlegmariurus fargesii*; *Lycopodium* alkaloids; Lycopodine *N*-oxide

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Introduction

The *Lycopodium* alkaloids are quinolizine, or pyridine and α-pyridone type alkaloids, isolated from *Lycopodium* (sensu lato) [1-3]. Some of them exhibit potent acetylcholinesterase (AChE) inhibitory activities, attracting great interest from synthetic, biogenetic, and biological points of view [4]. Ayer separated the *Lycopodium* alkaloids into four structural classes: lycopodine class, lycodine class, fawcettimine class, and a miscellaneous group [5]. As for the lycopodine class, there are an increasing number of reports on their *N*-oxide derivatives. Interestingly, as illustrated by miyoshianine A and C [6-7], most *N*-oxide derivatives of the lycopodine class alkaloid also bear a hydroxyl group at C-12, and the biosynthetic implication of this phenomenon remains unknown. *Phlegmariurus fargesii* (Herter) Ching is distributed in Southern China, and is traditionally used to treat contusion, strain, and swelling. The chemical constituents of this plant have not been reported so far. As a part of an investigation on the alkaloids of *Lycopodium* and its related genera, the crude base fraction of *P. fargesii* collected in Guangxi Zhuang Autonomous Region, China was examined. As a result, a new alkaloid, lycopodine *N*-oxide (**1**), along with lycopodine (**2**), 8,15-dehydrolycopodine (**3**), 6*α*-hydroxylycopodine (**4**), deacetyllycoclavine (**5**), *N*-methylhuperzine B (**6**), lycodine (**7**), and phlegmarine (**8**), was isolated. The isolation and structure elucidation of the above alkaloids are reported.

Results and Discussion

The MeOH extract of the whole plants of *P. fargesii* was partitioned between EtOAc and 3% tartaric acid. The water soluble material was adjusted to pH 10 and exhaustively extracted with $CHCl_3$. The $CHCl_3$ extract was subjected to repeated column chromatography on silica gel to afford compounds **1** – **8** (Fig. 1).

Compound **1** was obtained as a colorless solid and exhibited a pseudo-molecular ion peak at *m/z* 264 $[M + 1]^+$ in the ESI-MS, and its molecular formula was revealed as $C_{16}H_{25}NO_2$ by HR-ESI-MS at *m/z* 264.1963 $[M + 1]^+$ (Calcd.

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264.195 8). The ^{13}C NMR spectrum (Table 1) showed signals due to four sp^3 methines, nine sp^3 methylenes, one secondary methyl group, and two quaternary carbons. Among them, two methylenes (δ_C 62.9 and 59.0) and one quaternary carbon (δ_C 75.6) could be assigned as bearing a nitrogen atom. Comparison of ^{1}H and ^{13}C NMR data of **1** with those of lycopodine

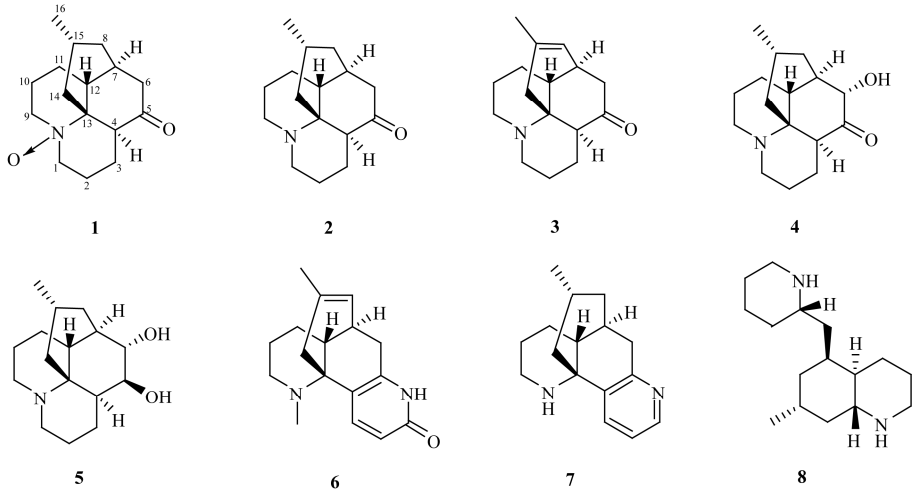

Fig. 1 Structures of compounds 1–8

Table 1 ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data for compound 1 in $CDCl_3$ (*J* in Hz)

Position	δ_H (mult., Hz)	δ_C
1	3.72 (td, *J* = 13.9, 4.5)	62.9 t
	3.43 [a]	
2	1.93 (m)	21.3 t
	1.84 (m)	
3	2.22 (m)	17.3 t
	1.68 (m)	
4	2.97 (dd, *J* = 12.2, 3.0)	48.4 d
5		207.4 s
6	2.58 (dd, *J* = 16.7, 6.4)	41.8 t
	2.30 (d, *J* = 15.0)	
7	2.25 (m)	36.1 d
8	1.68 (m)	41.2 t
	1.37 (td, *J* = 12.7, 3.5)	
9	4.00 (td, *J* = 12.9, 3.1)	59.0 t
	3.43 [a]	
10	2.72 (m)	19.8 t
	1.94 (m)	
11	1.88 (m)	23.2 t
	1.68 (m)	
12	2.65 (br d, *J* = 13.2)	37.3 d
13		75.6 s
14	2.25 (m)	34.8 t
	2.11 (dd, *J* = 13.4, 4.1)	
15	1.53 (m)	25.6 d
16	0.93 (d, *J* = 6.2)	22.6 q

[a] Overlapped signals

(**2**), which was also isolated in this study, revealed that **1** had a similar structure to **2**. The molecular formula of **1** was larger than that of **2** by one oxygen atom. Further, the ^{13}C NMR signals of C-1, C-9, and C-13 (δ 62.9, 59.0, and 75.6, respectively) in **1** were shifted to lower field compared to C-1, C-9, and C-13 (δ 47.2, 47.8, and 59.6, respectively) of **2** [8-9], implying that **1** was the *N*-oxide derivative of **2**. Detailed analyses of the ^{1}H-^{1}H COSY, HSQC, and HMBC spectra confirmed the planar structure of **1** (Fig. 2). The relative configuration of **1** was deduced by a ROESY experiment, which showed ROESY correlations between H-2 with H-9, between H-4 with H-11, and between H-12 with H-8 and H-14 (Fig. 2). In addition, oxidation of **2** with *m*-chloroperbenzoic acid (*m*-CPBA) afforded the *N*-oxide derivative whose spectroscopic data and $[\alpha]_D$ value were identical with those of **1** (Fig. 3). Therefore, **1** was elucidated to be lycopodine *N*-oxide.

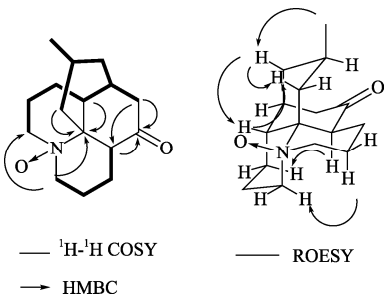

Fig. 2 Key 2D NMR correlations of 1

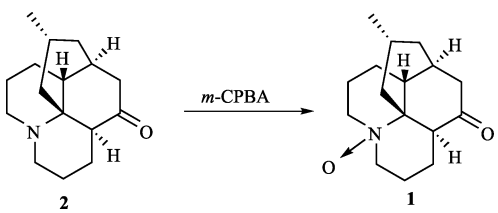

Fig. 3 Chemical correlation of 1 and 2

After *N*-oxidehuperzine E [10] and lycopladine E [11], compound **1** is the third *N*-oxide derivative of a lycopodine-class alkaloid without being hydroxylated at C-12. Known alkaloids were identified as lycopodine (**2**) [8], 8,15-dehydrolycopodine (**3**) [12], 6*α*-hydroxylycopodine (**4**) [13], deacetyllycoclavine (**5**) [14], *N*-methylhuperzine B (**6**) [15], lycodine (**7**) [9], and phlegmarine (**8**) [16-17] by comparing their physical and spectroscopic data with those reported.

The acetylcholinesterase (AChE) inhibitory activity of compounds **1** – **8** was determined. Compound **6** inhibited AChE at IC_{50} = 19.6 μmol·L^{-1} (tacrine as positive control, IC_{50} = 0.2 μmol·L^{-1}), whereas the other alkaloids did not show such activity (IC_{50} > 200 μmol·L^{-1}).

Experimental

General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were measured on a Bruker Tensor-27 spectrophotometer. NMR spectra were recorded on Bruker ACF-500 spectrometer using standard Bruker pulse programs with TMS as an internal standard. Mass spectra were obtained on an Agilent Micro Q-TOF mass spectrometer. Silica gel (Qingdao Marine Chemical Factory, China) was used for column chromatography. Silica gel GF_{254} plates (Qingdao Marine Chemical Factory, China) were used for thin-layer chromatography and spots were visualized by spraying with Dragendorff's reagent.

Plant material

The whole plants of *P. fargesii* were collected in Guangxi Zhuang Autonomous Region, China, in June 2011. The botanical identification was made by one of the authors, Dr. LUO Jian-Guang. A voucher specimen was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and isolation

The air-dried whole plant of *P. fargesii* (1.0 kg) was extracted with MeOH at room temperature. The MeOH extract was partitioned between EtOAc and 3% tartaric acid. The aqueous layer was adjusted to pH 10 with satd. Na_2CO_3 and partitioned with $CHCl_3$. The $CHCl_3$ extract was concentrated to give a residue (1.5 g), which was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (1 : 0→0 : 1) to give six fractions (Fr. A-Fr. F). Fr. A was repeatedly separated over silica gel columns with $CHCl_3$–MeOH (1 : 0→0 : 1) to afford compounds **1** (2.5 mg), **2** (9.0 mg), **3** (2.2 mg), **4** (2.0 mg), **6** (3.0 mg), and **7** (2.8 mg). Fr. F was subjected to repeated silica gel chromatography ($CHCl_3$ sat. with NH_4OH– MeOH, 1 : 0→0 : 1) to give compounds **5** (20.0 mg) and **8** (1.5 mg).

Lycopodine *N*-oxide (1). Colorless solid; $[\alpha]_D^{27}$ –21.0 (*c* = 0.10, $CHCl_3$); IR (KBr) υ_{max} 3 443, 2 924, 1 704, 1 638, and 1 400 cm^{-1}; ^{1}H and ^{13}C NMR data: see Table 1. Positive ESI-MS *m/z* 264 $[M + H]^+$; HR-ESI-MS *m/z* 264.196 3 $[M + H]^+$ (Calcd. for $C_{16}H_{26}NO_2$: 264.195 8).

Lycopodine (2) Colorless solid; $[\alpha]_D^{23}$ –29.7 (*c* = 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) :δ 3.44 (1H, td, *J* = 14.0, 4.0 Hz), 3.20 (1H, td, *J* = 12.0, 3.0 Hz), 2.85 (1H, dd, *J* = 12.0, 2.5 Hz), 2.59 (1H, td, *J* = 15.0, 6.0 Hz), 2.54 (1H, dd, J = 12.5, 4.0 Hz), 2.28 (1H, d, *J* = 18.0 Hz), 2.06-2.16 (2H, m), 1.40-2.00 (11H, m), 1.34 (1H, td, *J* = 13.0, 4.0 Hz), 1.10–1.30 (2H, m), and 0.88 (3H, d, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 212.6, 60.5, 47.2, 46.6, 44.3, 43.3, 42.7, 42.2, 42.1, 36.6, 25.4, 25.2, 24.6, 19.0, and 18.6; Positive ESI-MS *m/z* 248 $[M + H]^+$.

8,15-Dehydrolycopodine (3) Colorless solid; $[\alpha]_D^{23}$ –22.0 (*c* = 0.10, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz): δ 5.48 (1H, d, *J* = 5.5 Hz), 3.41 (1H, td, *J* = 12.5, 2.5 Hz), 3.21-3.30 (2H, m), 2.73-2.80 (2H, m), 2.70 (1H, d, *J* = 12.5 Hz), 2.61 (1H, dd, *J* = 14.5, 4.5 Hz), 2.27 (1H, br s), 1.66-2.20 (10H, m), 1.63 (3H, s), and 1.45 (1H, br d, *J* = 14.0 Hz); ^{13}C NMR (CD_3OD, 125 MHz): δ 213.0, 133.5, 127.3, 62.9, 48.0, 47.9, 44.8, 43.2, 42.9, 40.8, 38.8, 26.2, 25.1, 22.8, 19.8, and 19.0; Positive ESI-MS *m/z* 246 $[M + H]^+$.

6*α*-Hydroxylycopodine (4) Colorless solid; $[\alpha]_D^{23}$ –18.0 (*c* = 0.15, $CHCl_3$); ^{1}H NMR (CD_3OD, 500MHz): δ 3.70 (1H, s), 3.34 (1H, td, *J* = 14.0, 3.5 Hz), 3.25 (1H, t, *J* = 12.0 Hz), 2.63 (1H, dd, *J* = 14.0, 8.0 Hz), 2.58 (1H, br d, *J* = 12.0 Hz), 2.47 (1H, dd, *J* = 15.5, 8.0 Hz), 2.35 (1H, m), 1.68 (1H, br d, *J* = 12.0 Hz), 1.53–1.65 (5H, m), 1.43 (1H, m), 1.24-1.36 (3H, m), 0.88 (1H, t, *J* = 13.0 Hz), and 0.85 (3H, d, *J* = 5.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 207.0, 79.2, 62.3, 47.8, 46.3, 44.3, 43.6, 40.8, 40.0, 35.8, 27.5, 27.5, 27.4, 23.5, 20.5, and 19.7; Positive ESI-MS *m/z* 264 $[M + H]^+$.

Deacetyllycoclavine (5) Yellowish solid; $[\alpha]_D^{23}$ –31.4 (*c* = 0.21, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500MHz): δ 3.85 (1H, d, *J* = 4.5 Hz), 3.73 (1H, s), 3.42 (1H, td, *J* = 14.0, 3.5 Hz), 3.17 (1H, td, *J* = 12.0, 2.5 Hz), 2.56–2.65 (3H, m), 2.52 (1H, dd, *J* = 14.5, 4.5 Hz), 2.00 (1H, m), 1.90 (1H, dd, *J* = 13.5, 4.0 Hz), 1.77-1.87 (2H, m), 1.66-1.76 (3H, m), 1.44-1.57 (3H, m), 1.33-1.42 (2H, m), 1.23 (1H, m), 0.85 (3H, d, *J* = 6.0 Hz), and 0.79 (1H, t, *J* = 14.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 78.8, 75.1, 55.3, 47.6, 47.4, 45.1, 44.3, 43.1, 40.6, 29.4, 27.2, 26.9, 24.4, 24.2, 23.3, and 20.6; Positive ESI-MS *m/z* 266 $[M + H]^+$.

***N*-Methylhuperzine B (6)** Yellowish solid; $[\alpha]_D^{23}$ –37.1 (*c* = 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500MHz): δ 7.86 (1H, br s), 6.42 (1H, d, *J* = 9.5 Hz), 5.40 (1H, d, *J* = 5.5 Hz), 2.90 (1H, dd, *J* = 19.0, 5.5 Hz), 2.66 (3H, s), 2.50-2.63 (3H, m), 2.30-2.40 (2H, m), 1.60-2.00 (4H, m), 1.60 (3H, s), and 1.20-1.40 (3H, m); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.3, 142.9, 141.7, 132.8, 125.2, 117.9, 117.9, 50.9, 46.5, 43.3, 37.9, 34.6, 33.0, 29.9, 26.0, 23.3, and 20.4; Positive ESI-MS *m/z* 271 $[M + H]^+$.

Lycodine (7) Colorless solid; $[\alpha]_D^{23}$ –7.3 (*c* = 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500MHz): δ 8.46 (1H, d, *J* = 4.5 Hz), 8.18 (1H, d, *J* = 8.5 Hz), 7.23 (1H, dd, *J* = 7.5, 4.5 Hz), 3.20 (1H, dd, *J* = 19.0, 7.0 Hz), 3.13 (1H, d, *J* = 11.5 Hz), 2.76 (1H, d, *J* = 19.0 Hz), 2.64 (1H, td, *J* = 14.0, 3.5 Hz), 2.20 (1H, dd, *J* = 8.5, 3.5 Hz), 2.07 (1H, d, *J* = 12.5 Hz), 1.93-2.04 (2H, m), 1.75–1.86 (2H, m), 1.60–1.70 (3H, m), 1.43 (1H, td,

J = 15.0, 3.5 Hz), 1.28 (1H, m), 0.88 (1H, m), and 0.81 (3H, d, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 158.5, 148.2, 133.9, 131.0, 122.3, 59.8, 48.9, 43.2, 42.6, 41.0, 35.2, 33.5, 32.2, 26.1, 25.3, and 21.9; Positive ESI-MS *m/z* 243 $[M + H]^+$.

Phlegmarine (8) Pale yellowish oil; $[\alpha]_D^{23}$ –24.0 (*c* = 0.10, $CHCl_3$); ^{1}H NMR (CD_3OD, 500MHz): δ 3.35-3.40 (2H, m), 3.20 (1H, m), 2.98–3.05 (2H, m), 2.85 (1H, t, *J* = 9.0 Hz), 2.15 (1H, d, *J* = 9.5 Hz), 1.84-2.03 (8H, m), 1.55-1.80 (4H, m), 1.46(1H, m), 1.40 (1H, m), 1.10-1.32 (4H, m), 1.00 (3H, d, *J* = 6.5 Hz), and 0.75 (1H, q, *J* = 12.5 Hz); ^{13}C NMR (CD_3OD, 125 MHz): δ 61.2, 56.0, 46.2, 45.7, 44.7, 41.2, 39.2, 38.4, 37.8, 31.4, 31.3, 27.4, 23.8, 23.5, 23.3, and 22.3; Positive ESI-MS *m/z* 251 $[M + H]^+$.

*Chemical correlation of **1** and **2***

To a stirred solution of **2** (3.0 mg) in dry CH_2Cl_2 (0.5 mL) was added *m*-CPBA (85%, 3.0 mg). After 2 h at 0 °C, the reaction mixture was subjected to aluminum oxide column chromatography ($CHCl_3$–MeOH, 1 : 0→0 : 1) to afford semi-synthetic **1** (1.4 mg). All of the spectroscopic data and $[\alpha]_D$ value were in agreement with those of natural **1**.

Determination of AChE inhibitory activitiy

Determination of AChE inhibitory activity was carried out according to the Ellman method [18].

References

[1] Ma X, Gang DR. The *Lycopodium* alkaloids [J]. *Nat Prod Rep,* 2004, **21** (6): 752-772.

[2] Ayer WA, Trifonov LS. *The Alkaloids* [M]. Academic Press, 1994, **45**: 233-236

[3] Hirasawa Y, Kobayashi Ji, Morita H. The *Lycopodium* alkaloids [J]. *Heterocycles,* 2009, **77** (2): 679-729.

[4] Liu JS, Zhu YL, Yu CM, *et al*. The structures of huperzine A and B, two new alkaloids exhibiting marked anticholinesterase activity [J]. *Can J Chem,* 1986, **64** (4): 837-839.

[5] Ayer WA. The *Lycopodium* alkaloids [J]. *Nat Prod Rep,* 1991, **8** (5): 455-463.

[6] Tong XT, Tan CH, Ma XQ, *et al*. Miyoshianines A and B, two new *Lycopodium* alkaloids from *Huperzia miyoshiana* [J]. *Planta Med*, 2003, **69** (6): 576-579.

[7] Sun Y, Yan J, Meng H, *et al.* A new alkaloid from *Lycopodium japonicum* Thunb [J]. *Helv Chim Acta,* 2008, **91** (11): 2107-2109.

[8] Yang H, Carter RG, Zakharov LN. Enantioselective total synthesis of lycopodine [J]. *J Am Chem Soc,* 2008, **130** (29): 9238-9239.

[9] Nakashima TT, Singer PP, Browne LM, *et al*. Carbon-13 nuclear magnetic resonance studies of some *Lycopodium* alkaloids [J]. *Can J Chem,* 1975, **53** (13): 1936-1942.

[10] Wang HB, Tan CH, Tan JJ, *et al*. Two new *N*-oxide *Lycopodium* alkaloids from *Huperzia serrata* [J]. *Nat Prod Res*, 2009, **23** (15): 1363-1366.

[11] Kubota T, Yahata H, Ishiuchi Ki, *et al*. Lycopladine E, a new $C_{16}N_1$-type alkaloid from *Lycopodium complanatum* [J]. *Heterocycles,* 2007, **74** (1): 843-848.

[12] Ayer WA, Piers K. Transannular hydride shifts. An application to the synthesis of *Lycopodium* alkaloids [J]. *Chem Comm (London),* 1965, (21): 541-542.

[13] Lee SS, Chen CH. Proton- and carbon-13-NMR spectral analyses of alkaloid L20, a *Lycopodium* alkaloid [J]. *Zhonghua Yaoxue Zazhi,* 1993, **45** (3): 237-242.

[14] Munoz OM, Castillo M, San Feliciano A. High-resolution NMR studies of paniculine and related *Lycopodium* compounds [J]. *J Nat Prod,* 1990, **53** (1): 200-203.

[15] Hu P, Gross ML, Yuan SQ, *et al*. Mass spectrometric differentiation of huperzinine, *N*,*N*-dimethylhuperzine A and N-methylhuperzine B [J]. *Org Mass Spec,* 1992, **27** (2): 99-104.

[16] Nyembo L, Goffin A, Hootelé C, *et al*. Phlegmarine, a likely key intermediate in the biosynthesis of the *Lycopodium* alkaloids [J]. *Can J Chem,* 1978, **56** (6): 851-856.

[17] Wolfe BH, Libby AH, Al-awar RS, *et al*. Asymmetric synthesis of all the known phlegmarine alkaloids [J]. *J Org Chem*, 2010, **75** (24): 8564-8570.

[18] Ellman GL, Courtney KD, Andres Jr V, *et al*. A new and rapid colorimetric determination of acetylcholinesterase activity [J]. *Biochem Pharmacol*,1961, **7** (2): 88-95.

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