FULL PAPER

Three New *Lycopodium* Alkaloids from *Phlegmariurus fargesii*

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Three new *Lycopodium* alkaloids, fargesiines A – C (**1** – **3**, resp.), along with a known compound (**4**), were isolated from the whole plants of *Phlegmariurus fargesii*. Their structures were elucidated on the basis of spectroscopic data and chemical correlations. Compounds **1** – **4** were tested in an assay for acetylcholinesterase (AChE) inhibitory activity.

Keywords: *Phlegmariurus fargesii*, *Lycopodium* alkaloids, Fargesiines A – C.

Introduction

The *Lycopodium* alkaloids are quinolizine, or pyridine and α-pyridone type alkaloids, isolated from the plants of family Lycopodiaceae and Huperziaceae [1][2]. Some of them, such as huperzines A and B, exhibit potent acetylcholinesterase (AChE) inhibitory activities, attracting great interests from synthetic, biogenetic, and biological points of view [3 – 5]. *Phlegmariurus fargesii* (Huperziaceae) is distributed in southern China and traditionally used to treat contusion, strain, and swelling. Recently, we isolated a new *Lycopodium* alkaloid, lycopodine *N*-oxide, from *P. fargesii* [6]. In the continuing research on this plant, three new *Lycopodium* alkaloids, named fargesiines A – C (**1** – **3**), together with a known compound, lycognidine (**4**), were obtained. Herein, we reported the isolation and structure elucidation of these alkaloids.

Results and Discussion

The crude alkaloid fraction obtained from the whole plants of *P. fargesii* was subjected to repeated column chromatography (CC) on silica gel and preparative HPLC yielded three new *Lycopodium* alkaloids (**1** – **3**), together with a known compound (**4**) (*Fig. 1*).

Fargesiine A (**1**) was isolated as yellowish solid and had a molecular formula of $C_{26}H_{37}NO_5$ as determined by positive-ion-mode HR-ESI-MS (*m/z* 444.2743 ($[M + H]^+$)). The ^{1}H-NMR spectrum (*Table*) exhibited signals of a Me group (δ(H) 0.95 (*d*, *J* = 6.3)), a MeO group (δ(H) 3.84 (*s*)), and three aromatic H-atoms (δ(H) 6.64 (*dd*, *J* = 8.0, 1.8), 6.72 (*d*, *J* = 8.0), and 6.78 (*d*, *J* = 1.8)). The ^{13}C-NMR data (*Table*) and HSQC spectrum indicated the presence of 26 C-atoms including five quaternary carbons, nine CH, ten CH_2, and two Me groups. The above data revealed that the structure of **1** was similar to those of lycoposerramine O [7], and the major difference was that lycoposerramine O had one more AcO group than **1**. The planar structure of **1** was elucidated by extensive analyses of ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra (*Fig. 2*). The ^{1}H,^{1}H-COSY spectrum revealed the presence of three fragments. The linkages of these partial structures were deduced by HMBC spectrum. Especially, the HMBC H–C(5)/C(17) indicated that the dihydroferulic acid ester moiety was positioned at C(5). In addition, the MeO at C(22) was confirmed by the HMBC MeO/C(22). The relative configuration of **1** was deduced by a ROESY experiment (*Fig. 2*), and

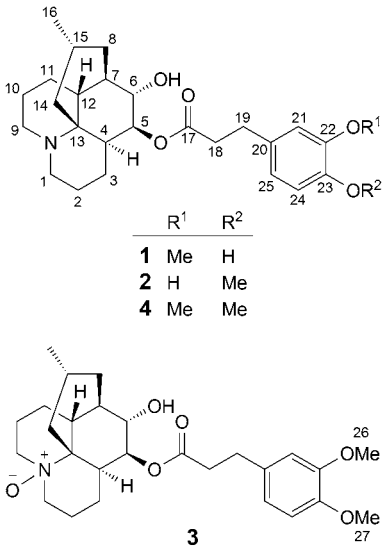

Fig. 1. Chemical structures of compounds **1** – **4**, isolated from *Phlegmariurus fargesii*

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Table. ^{1}H- and ^{13}C-NMR Data of Compounds **1** – **3**. At 500 (^{1}H) and 125 MHz (^{13}C), in CD_3OD (**1** and **2**) or $CDCl_3$ (**3**) (δ in ppm, J in Hz)

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	3.61 (*td*, *J* = 13.9, 4.0), 2.90 – 2.94 (*m*)	48.4	3.61 (*td*, *J* = 13.9, 4.0), 2.92	48.7	3.61 (*td*, *J* = 13.9, 4.3), 3.17 (*d*, *J* = 10.0)	63.8
2	2.02, 1.75[a])	19.8	2.01, 1.72[a])	19.7	1.82 – 1.88 (*m*), 1.77 (*d*, *J* = 14.8)	22.3
3	1.33 – 1.42 (*m*), 1.30	21.5	1.34 – 1.44 (*m*), 1.28	21.3	1.55 – 1.65 (*m*), 1.33 (*d*, *J* = 13.2)	20.3
4	2.93[a])	30.0	2.93[a])	30.0	2.90 – 2.97 (*m*)	32.6
5	5.07 (*d*, *J* = 6.5)	75.2	5.06 (*d*, *J* = 6.5)	75.1	5.02 (*d*, *J* = 7.1)	76.1
6	3.45 (*s*)	75.1	3.45 (*s*)	75.0	3.48 (*s*)	75.3
7	2.01[a])	43.9	2.00[a])	43.7	2.00 (*s*)	43.2
8	1.60 (*dd*, *J* = 12.6, 5.0), 1.20 – 1.26 (*m*)	39.8	1.65, 1.20 – 1.27 (*m*)	39.7	1.48 – 1.54, 1.20 – 1.28 (*2 m*)	39.3
9	3.80 (*td*, *J* = 13.9, 3.5), 3.00 (*d*, *J* = 12.8)	48.4	3.80 (*td*, *J* = 13.9, 4.0), 3.00 (*d*, *J* = 13.1)	48.7	4.02 (*td*, *J* = 13.7, 3.3), 3.17 (*d*, *J* = 10.9)	59.7
10	1.92 – 1.98 (*m*), 1.71	25.2	1.92 – 1.99 (*m*), 1.68	25.0	2.42 – 2.50, 1.60 – 1.66 (2 *m*)	20.5
11	2.22 – 2.32 (*m*), 1.53 (br. *d*, *J* = 14.2)	25.0	2.22 – 2.32 (*m*), 1.53 (br. *d*, *J* = 14.2)	24.9	2.06 – 2.16 (*m*), 1.48 (br. *d*, *J* = 14.0)	24.2
12	1.65[a])	45.5	1.66[a])	45.4	2.32 (*d*, *J* = 13.5)	37.4
13	–	65.1	–	64.9	–	74.4
14	2.63, 1.02 (*t*, *J* = 12.4)	40.8	2.64, 1.00 (*t*, *J* = 12.3)	40.7	2.39 (*d*, *J* = 13.6), 1.94 (*dd*, *J* = 13.6, 6.0)	32.1
15	2.45 – 2.55 (*m*)	25.7	2.48 – 2.58 (*m*)	25.6	2.20 – 2.30 (*m*)	24.6
16	0.95 (*d*, *J* = 6.3)	24.1	0.97 (*d*, *J* = 6.4)	23.9	0.93 (*d*, *J* = 6.3)	23.9
17	–	173.7	–	173.4	–	172.4
18	2.62 – 2.68 (*m*, 2 H)	37.7	2.60 – 2.70 (*m*, 2 H)	37.3	2.61 – 2.66 (*m*, 2 H)	36.5
19	2.85 – 2.90 (*m*, 2 H)	32.0	2.80 – 2.87 (*m*, 2 H)	31.6	2.90 (*t*, *J* = 7.5, 2 H)	30.8
20	–	133.2	–	147.7	–	132.7
21	6.78 (*d*, *J* = 1.8)	113.7	6.67 (*d*, *J* = 2.1)	116.6	6.70 (br. *s*)	112.1
22	–	149.2	–	134.5	–	149.3
23	–	146.4	–	147.7	–	148.0
24	6.72 (*d*, *J* = 8.0)	116.5	6.84 (*d*, *J* = 8.1)	113.0	6.79 (*d*, *J* = 8.0)	111.7
25	6.64 (*dd*, *J* = 8.0, 1.8)	122.1	6.63 (*dd*, *J* = 8.1, 2.1)	120.6	6.71[a])	120.3
26	3.84 (*s*, 3 H)	56.7	3.82 (*s*, 3 H)	56.6	3.86 (*s*, 3 H)	56.2[b])
27	–	–	–	–	3.85 (*s*, 3 H)	56.1[b])

[a]) Overlapped signals are reported without designated multiplicity. [b]) Assignment may be reversed.

the coupling constant of H–C(5) (*d*, *J* = 6.5) indicated that H–C(5) was α-oriented. Hydrolysis of **1** with KOH gave a compound (**5**) (*Fig. 2*), whose spectroscopic data and optical rotation were identical with those of deacetyllycoclavine [6][8]. Therefore, the structure of **1** was determined as shown in *Fig. 1*, and named fargesiine A.

Fargesiine B (**2**) was obtained as yellowish solid. Its molecular formula, $C_{26}H_{37}NO_5$, was established by positive-ion-mode HR-ESI-MS (*m/z* 444.2746 ($[M + H]^+$)). In the ^{1}H-NMR spectrum (*Table*), the signals of a Me group (δ(H) 0.97 (*d*, *J* = 6.4)), a MeO group (δ(H) 3.82 (*s*)), and three aromatic H-atoms (δ(H) 6.63 (*dd*, *J* = 8.1, 2.1), 6.67 (*d*, *J* = 2.1), and 6.84 (*d*, *J* = 8.1)) were readily discerned. The ^{13}C-NMR and HSQC spectra exhibited 26 C-atom resonances including five quaternary carbons, nine CH, ten CH_2, and two Me groups (*Table*). These evidences indicated that the structure of **2** was nearly identical with that of **1**. Detailed interpretation of 2D-NMR spectra (*Fig. 3*) revealed that the structural difference between **1** and **2** was the replacement of the dihydroferulic acid ester moiety in **1** by a dihydroisoferulic acid ester moiety in **2**, which was confirmed by the HMBC MeO/C(23). Thus, the structure of fargesiine B (**2**) was determined as shown in *Fig. 1*.

Fargesiine C (**3**) was obtained as yellowish solid. Its molecular formula was determined to be $C_{27}H_{39}NO_6$ by positive-ion-mode HR-ESI-MS (*m/z* 474.2849 ($[M + H]^+$)). The ^{1}H- and ^{13}C-NMR data (*Table*) were very similar to those of lycognidine (**4**) [9], although the ^{13}C-NMR signals of C(1) (δ(C) 63.8), C(9) (δ(C) 59.7), and C(13) (δ(C) 74.4) were shifted downfield compared with those of **4**. These observations led us to assume that **3** was the *N*-oxide derivative of **4**. Further in-depth analysis of ^{1}H,^{1}H-COSY, HSQC, HMBC, and ROESY spectra enabled us to establish the structure of **3** (*Fig. 1*). Oxidation of **4** with *m*-chloroperbenzoic acid (*m*-CPBA) afforded an *N*-oxide derivative (*Scheme*), whose spectroscopic data and optical rotation were identical with those of **3**. Thus, **3** was elucidated to be lycognidine *N*-oxide, and named fargesiine C.

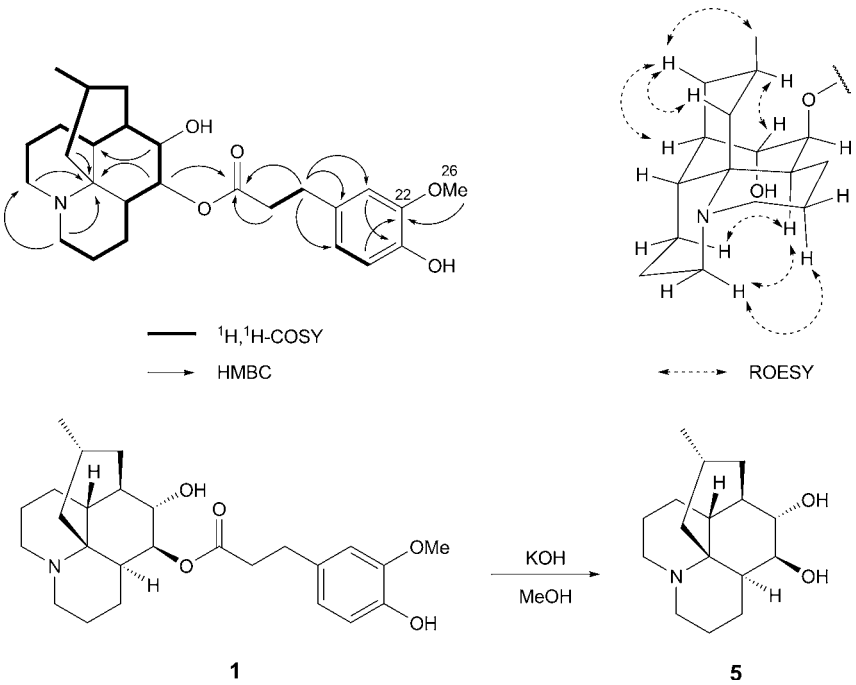

Fig. 2. Selected 2D-NMR correlations of **1**, and chemical correlation of **1** and deacetyllycoclavine (**5**)

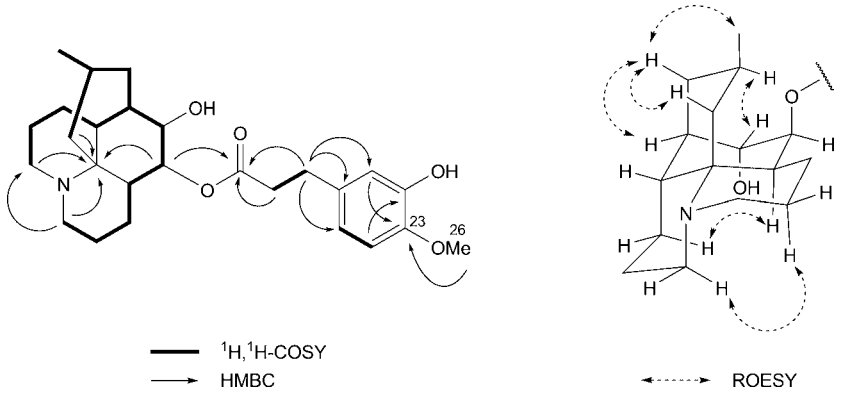

Fig. 3. Selected 2D-NMR correlations of **2**

The AChE inhibitory activities of compounds **1** – **4** were evaluated by a modified *Ellman* method [10]. However, all of them were found to be inactive (IC_{50} > 200 μM).

Supporting Information

NMR Spectra of compounds **1** – **3** are available online.

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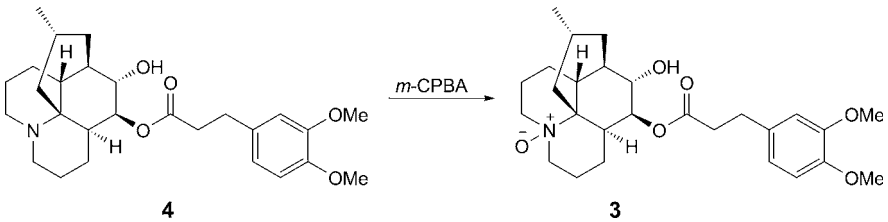
Experimental Part

General

Column chromatography: silica gel (SiO_2, 300 – 400 mesh; *Qingdao Marine Chemical Group Co.*, Qingdao, P. R. China). Prep. HPLC: *Shimadzu LC-6AD* pump; *Shimadzu SPD-20A* detector; *Shim pack ODS* column (5 μm, 250 mm × 20 mm i.d.); t_R in min. Optical rotations: *JASCO P-1020* polarimeter. IR Spectra: *Shimadzu FTIR-8400S* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AVANCE III 500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-ESI-MS: *Agilent 6520B Q-TOF* mass spectrometer; in m/z.

Scheme. Chemical correlation of **3** and lycognidine (**4**)



4 **3**

Plant Material

The whole plants of *P. fargesii* were collected in Guangxi Zhuang Autonomous Region, P. R. China, in June 2011. The plant was identified by one of the authors (*K. P.*). A voucher specimen was deposited with the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation

The air-dried whole plants of *P. fargesii* (1 kg) were extracted with MeOH three times at room temperature. The residue of the MeOH extract was dissolved in 3% tartaric acid and the soln. filtered and then extracted with AcOEt. The aq. layer was adjusted to pH 9 with Na_2CO_3, and extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated to give a residue (1.5 g), which was subjected to CC (SiO_2, $CHCl_3$/MeOH 1:0 → 0:1): *Frs. A – F. Fr. C* (80 mg) was subjected to repeated CC (SiO_2, $CHCl_3$/MeOH 5:1): **4** (15 mg). *Fr. D* (110 mg) was subjected to CC (SiO_2, $CHCl_3$ sat. with $NH_3{\cdot}H_2O$/MeOH 1:0 → 0:1): *Fr. D1 – Fr. D4. Fr. D2* was purified by prep. HPLC (MeCN/0.1%TFA 12:88; 8 ml/min; 280 nm): **1** (8.5 mg; t_R 27) and **2** (4.0 mg; t_R 30). *Fr. D3* was subjected to repeated CC (SiO_2, $CHCl_3$ sat. with $NH_3{\cdot}H_2O$/MeOH 20:1): **3** (7.0 mg).

Fargesiine A (= **(5*β*,6*α*,13*α*,15*R*)-6-Hydroxy-15-methyllycopodan-5-yl 3-(4-Hydroxy-3-methoxyphenyl)propanoate**; **1**): Yellowish solid. $[\alpha]_D^{24} = -22.8$ (c = 0.20, MeOH). IR (KBr): 3450, 1733. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (pos.): 444 ($[M + H]^+$). HR-ESI-MS: 444.2743 ($[M + H]^+$, $C_{26}H_{38}NO_5^+$; calc. 444.2744).

Fargesiine B (= **(5*α*,6*α*,15*R*)-6-Hydroxy-15-methyllycopodan-5-yl 3-(3-hydroxy-4-methoxyphenyl)propanoate**; **2**): Yellowish solid. $[\alpha]_D^{24} = -18.2$ (c = 0.10, MeOH). IR (KBr): 3450, 1731. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (pos.): 444 ($[M + H]^+$). HR-ESI-MS: 444.2746 ($[M + H]^+$, $C_{26}H_{38}NO_5^+$; calc. 444.2744).

Fargesiine C (= **(1*S*,8a*R*,9*R*,11*R*,12a*R*,13*S*,14*S*)-13-Hydroxy-11-methyl-5-oxidododecahydro-1,9-ethanopyrido[2,1-*j*]quinolin-14-yl 3-(3,4-dimethoxyphenyl)propanoate**; **3**): Yellowish solid. $[\alpha]_D^{24} = +6.0$ (c = 0.10, $CHCl_3$). IR (KBr): 3420, 1735. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS (pos.): 474 ($[M + H]^+$). HR-ESI-MS: 474.2849 ($[M + H]^+$, $C_{27}H_{40}NO_6^+$; calc. 474.2850).

Hydrolysis of **1**

To a stirred soln. of **1** (5.0 mg) in MeOH (0.3 ml) was added aq. 1M KOH (0.3 ml). The mixture was stirred at room temperature for 18 h, and then concentrated under reduced pressure. The residue was subjected to CC (SiO_2, $CHCl_3$ sat. with $NH_3{\cdot}H_2O$/MeOH 20:1) to give compound **5** (2.1 mg). Spectroscopic data and optical rotation obtained were identical with those of deacetyllycoclavine [6][8].

m-*CPBA Oxidation of* **4**

To a stirred soln. of **4** (4.0 mg) in dry CH_2Cl_2 (0.5 ml) was added *m*-CPBA (85%, 4.0 mg). After 2.5 h at 0 °C, the mixture was directly subjected to CC (SiO_2, $CHCl_3$ sat. with $NH_3{\cdot}H_2O$/MeOH 20:1) to afford semisynthetic **3** (3.2 mg). Spectroscopic data and optical rotation were in agreement with those of natural **3**.

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