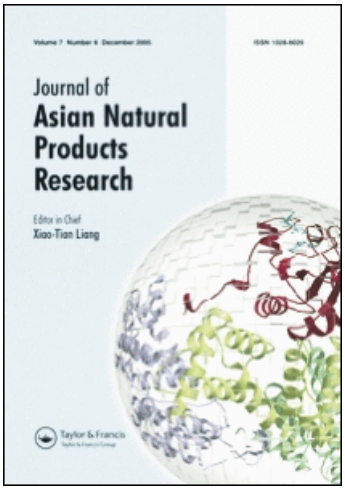

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THREE NEW PHLEGMARIURINE B TYPE *LYCOPODIUM* ALKALOIDS FROM *HUPERZIA SERRATA*

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Phlegmariurine B (**1**), a known alkaloid, along with three new analogous compounds, 2α-hydroxyphlegmariurine B (**2**), 2-oxoyphlegmariurine B (**3**) and 11-oxophlegmariurine B (**4**), were isolated from the $CHCl_3$ fraction of total alkaloids of whole plant of the Chinese medicinal herb *Huperzia serrata*. Their structures were elucidated by spectral analysis.

Keywords: *Huperzia serrata*; *Lycopodium* alkaloids; 2α-Hydroxyphlegmariurine B; 2-Oxoyphlegmariurine B; 11-Oxophlegmariurine B

INTRODUCTION

Phlegmariurine B (**1**), isolated successively from *Phlegmariurus fordii* (Baker). Ching [1] and *Huperzia serrata* (Thunb.) Trev [2] of *Lycopodium* plant, was a unique structural type as *seco*- fawcettimine between C12 and C13 among *Lycopodium* alkaloids [3]. As a continuation of chemical constituents studies on *Huperzia serrata* [4–6], we reexamined the $CHCl_3$ extract of the total alkaloids of this plant, obtained **1** and three new analogues, 2α-hydroxyphlegmariurine B (**2**), 2-oxophlegmariurine B (**3**) and 11-oxophlegmariurine B (**4**). This paper describes the structure elucidation of those compounds (Scheme 1).

RESULTS AND DISCUSSION

Compound **1** was identified as phlegmariurine B based on analyses of EIMS, IR, ^1H, ^{13}C and DEPT NMR spectra [2]. Its relative configuration was defined as 7α, 15α *via* chemical conversion from phlegmariurine A (**5**) [1], whose configuration was determined by X-ray analysis [7]. The complete assignments of ^1H- and ^{13}C-NMR signals (Tables I and II) were established by using HMQC, DEPT and ^1H-^1H COSY spectra.

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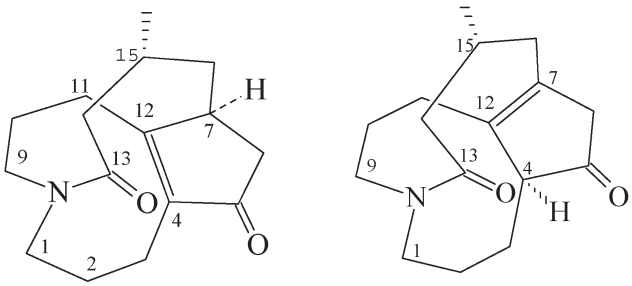

1
2: 2α-OH
3: 2-OXO
4: 11-OXO

5

SCHEME 1 Structures of **1**–**5**.

The molecular formula $C_{16}H_{23}NO_3$ for compound **2** was deduced from its HR-EIMS spectrum. In the IR spectrum, a band at 3361.4 exhibited the presence of a hydroxyl group, the others resembled those of **1**. Comparison the ^{13}C-NMR data (Table II) with those of **1**, the most important difference was the presence of an oxygen-bearing methine signal at δ 60.72 and the absence of a methylene at δ 19.73 in the former, which suggested **2** to be 2-hydroxylated phlegmariurine B. The orientation of 2-OH was assigned as α by the analyses of the ^{1}H-NMR data (Table I) combining with the study of molecular model. Its ^{1}H-NMR spectrum displayed the proton coupling constants $J_{1\alpha,2}$ was 9.9 Hz. According to *Karplus* Equation ($J = 4.22 - 0.5\cos\alpha + 4.2\cos^2\alpha$), the dihedral angle between H-2 and H-1α was more close to 180° rather than 0° or 60°. Furthermore, from the viewpoint of biogenesis, **2** was a possible derivative from **1**, and **1** was a cage structure, the hydroxyl group of **2** might be obtained only from the *exo* (α) orientation. Therefore, compound **2** was assigned as 2α-hydroxyphlegmariurine B.

Compound **3**, $C_{16}H_{21}NO_3$ (HR-EIMS found: M^+ 275.1542, calcd: 275.1521), obtained as white amorphous powder, showed a positive effect on *Dragendorff*'s reagent. Its ^{13}C-NMR spectrum (Table II) showed a carbonyl carbon signal at δ 204.59, and two methylene carbons signals at δ 58.58 and 38.27, the others were nearly overlapped with those of C4–C16 of **1**. The ^{1}H-NMR spectrum (Table I) indicated two pairs of AB model protons at δ 3.18 and δ 5.14 (1-CH_2, $J = 15.7$ Hz), and δ 3.29 and δ 3.69 (3-CH_2, $J = 17.4$ Hz), respectively, which confirmed **3** to be 2-oxophlegmariurine B.

Compound **4** was isolated as colorless prisms. The molecular formula was established as $C_{16}H_{21}NO_3$ by the HR-EIMS spectrum. In the ^{13}C-NMR spectrum (Table II), a conjugated carbonyl carbon signal at δ 199.96 replaced the C-11 signal at δ 28.91 in **1**, which suggested **4** to be 11-oxophlegmariurine B. Additionally, in contrast to those of **1**, the differences of $\delta_{C\text{-}4}$ and $\delta_{C\text{-}12}$ and decrease of $\delta_{C\text{-}7}$ of **4** were consistent with the conjugated effect and γ-gauche effect from 11-carbonyl group, respectively. Hence, **4** was formulated as 11-oxophlegmariurine B.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on a *Fisher-Johns* melting point apparatus and are uncorrected. CD spectra were taken on a *Jasco J*-715 spectropolarimeter in $CHCl_3$. IR spectra

TABLE I ^{1}H-NMR data of compounds **1**–**4** (400 MHz, $CDCl_3$)

Position	**1**	**2**	**3**	**4**
1α	2.81 ddd (14.1, 11.4, 4.7)	2.66 dd (13.3, 9.9)	3.18 d (15.7)	2.90 ddd (14.2, 12.5, 4.6)
β	4.05 dt (14.1, 2.5)	4.07 dd (13.3, 5.6)	5.14 d (15.7)	4.19 dd (14.2, 4.7)
2α	1.41 dtt (14.0, 4.7, 2.4)	–	–	1.59 dtt (13.7, 6.0, 1.5)
β	2.44*	4.60 tt (9.9, 5.3)	–	2.49*
3α	2.44*	2.68 dd (13.2, 9.8)	3.29 d (17.4)	2.82 td (14.2, 1.5)
β	2.62*	2.73 dd (13.2, 5.0)	3.69 d (17.4)	2.49*
6α	2.35 dd (19.0, 7.1)	2.37 dd (19.3, 7.1)	2.45*	2.49 dd (19.8, 6.9)
β	2.20 dd (19.0, 2.2)	2.20 dd (19.1, 1.9)	2.45*	2.28 dd (19.8, 2.5)
7	2.75 br.s	2.76 br.s	2.88 br.s	3.47 br.s
8 *endo*	1.76 dd (15.0, 4.6)	1.77 dd (14.9, 4.6)	1.93*	1.67 ddd (14.9, 4.2, 1.5)
exo	1.90 dd (15.1, 7.7)	1.90 dd (15.0, 11.2)	1.93*	1.79 ddd (15.0, 11.2, 3.8)
9α	3.18 dt (15.3, 3.7)	3.27 dt (15.1, 3.0)	3.46 dt (15.5, 4.5)	3.31 ddd (15.4, 6.6, 1.9)
β	3.87ddd (15.2, 11.5, 3.7)	3.89 ddd (15.5, 12.5, 3.0)	4.07 ddd (15.2, 11.0, 4.0)	4.05 ddd (15.3, 13.5, 3.3)
10α	2.73*	2.82 qt (13.6, 4.1)	2.44*	3.59 td (13.5, 6.6)
β	1.92*	1.91*	2.11 dt (15.7, 4.6)	2.82 ddd (13.8, 3.3, 2.0)
11α	2.64*	2.64 td (14.1, 3.7)	2.78*	–
β	2.73*	2.74 ddd (14.0, 5.0, 3.4)	2.78*	–
14 *endo*	1.86 d (14.8)	1.84 d (14.7)	1.94 d (13.8)	1.86 d (15.2)
exo	2.43 dd (14.6, 8.5)	2.40 dd (14.7, 8.7)	2.46*	1.97 dd (15.2, 7.8)
15 *endo*	2.12 m	2.11 m	2.46 m	2.10 m
16	1.07 d (7.0)	1.05 d (7.0)	1.09 d (6.4)	1.03 d (7.0)

*Overlapping signals.

TABLE II ^{13}C-NMR data of compounds **1**–**4** (100 MHz, $CDCl_3$)

Position	**1**	**2**	**3**	**4**
1	51.12 t	56.52 t	58.58 t	51.73 t
2	19.73 t	60.72 d	204.59 s	19.68 t
3	23.36 t	32.36 t	38.27 t	24.05 t
4	141.69 s	138.78 s	136.06 s	149.73 s
5	207.35 s	207.59 s	205.76 s	208.30 s
6	38.49 t	38.27 t	37.67 t	38.18 t
7	41.15 d	41.32 d	41.54 d	36.20 d
8	40.95 t	40.86 t	39.98 t	40.32 t
9	50.98 t	51.50 t	49.76 t	48.26 t
10	25.77 t	25.49 t	26.10 t	44.69 t
11	28.91 t	28.81 t	28.27 t	199.96 s
12	171.65 s	174.22 s	174.38 s	162.63 s
13	173.30 s	173.94 s	175.66 s	173.91 s
14	40.75 t	40.30 t	39.09 t	40.71 t
15	27.11 d	27.24 d	27.83 d	26.44 d
16	26.59 q	26.42 q	25.41 q	26.09 q

were recorded on a *Nicolet Magna* 750 *FTIR* (KBr) spectrophotometer. EIMS and HREIMS data were obtained with *MAT*-95 and *MAT*-711 mass spectrometer. NMR spectra were recorded on a *Bruker AM*-400 instrument with CD_3Cl as solution and residual $CHCl_3$ peak (δ_H: 7.26; δ_C: 77.10) as reference with chemical shift δ in 1 and J in Hz. Optical rotations were measured using a *P.E.* 241 *MC* polarimeter in $CHCl_3$. Silica gel (200–300, 400 mesh) was used for column chromatography (CC) and precoated plates of silica gel ($HSGF_{254}$) for TLC.

Plant Material

Fresh whole plant of *Huperzia serrata* (Thunb) Trev. (Huperziaceae) was collected in Zhejiang Province of China and identified by Dr Xiao-Qiang Ma. Voucher sample (No. 97-63) was deposited in the Herbarium of this institute.

Extraction and Isolation

The air-dried whole plants (10 kg) were powdered and extracted with 1% aqueous tartaric acid at room temperature and the concentrated acidic extract basified to pH9 with concentrated NH_3 solvents, then extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated to obtain the $CHCl_3$ fraction of total alkaloid extract, which was chromatographed over silica gel (1 kg) column with gradient eluants ($CHCl_3$, 1000 ml; 1–4% methanol in $CHCl_3$, each 1000 ml) to afford fr. 1–5. Fr. 3 (2.0 g) was chromatographed on silica gel column eluting with EtOAc/acetone (2:1, 1200 ml), collected every 100 ml volume, gained 3 fr.s: fr. 3.1–3.4, fr. 3.5–3.7, and fr. 3.8–3.12. Vaporized solvent under reduced pressure, and dissolved with 10 ml acetone, respectively. Fr. 3.5–3.7 yielded crude crystals, recrystallized twice with acetone resulting in phlegmariurine B (**1**, 750 mg); the concentrated mother liquid (235 mg) was subjected to silica gel (20 g) CC with $CHCl_3/CH_3OH$ (20:1, 480 ml), collected with 10 ml tubes and detected using TLC (silica gel $HSGF_{254}$, $CHCl_3/CH_3OH$ 15:1, iodine vapor for detection); yielding **2** (13 mg, R_f: 0.42,), **3** (3 mg, R_f: 0.51), and **4** (15 mg, R_f: 0.62).

Phlegmariurine B (**1**): Colorless prisms from acetone, 750 mg, mp 110–111°C; CD (c 3.9×10^{-4}, $CHCl_3$): $[\theta]_{255} - 5.8 \times 10^4$; IR (KBr) ν_{max} cm^{-1}: 3363.3, 2921.7, 1687.4,

1643.2, 1623.8, 1457.9, 1417.4, 1234.2, 1091.5, 817.7; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$) see Tables I and II; EIMS (*m/z*): 261 (M^+, 100), 246 (20), 233 (23), 218 (22), 190 (30), 176 (30), 150 (55).

2α-Hydroxyphlegmariurine B (**2**): Colorless prisms from acetone, 13 mg, mp 240–241°C; $[\alpha]_D^{25}$: −0.17 (*c* 0.17, $CHCl_3$); CD (*c* 4.3×10^{-4}, $CHCl_3$): $[\theta]_{226} - 4.4 \times 10^4$; IR (KBr) ν_{max} cm^{-1}: 3361.4, 2915.9, 1691.3, 1641.2, 1604.5, 1465.7, 1039.5; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR(100 MHz, $CDCl_3$) see Tables I and II; EIMS (*m/z*): 277 (M^+, 65), 258 (8), 259 (17), 249 (94), 234 (100), 220 (35), 206 (39), 192 (30), 178 (42), 164 (37), 150 (28); HR-EIMS: 277.1687 (M^+), calcd for $C_{16}H_{23}NO_3$, 277.1678.

2-Oxo-phlegmariurine B (**3**): White amorphous powder, 3 mg, $[\alpha]_D^{25}$: −2.22 (*c* 0.033, $CHCl_3$); CD (*c* 2.2×10^{-4}, $CHCl_3$): $[\theta]_{231} + 1.6 \times 10^5$, $[\theta]_{259} - 1.3 \times 10^5$; IR (KBr) ν_{max} cm^{-1}: 3434.7, 2925.5, 2850.3, 1731.8, 1668.2, 1456.0, 1053.0; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR(100 MHz, $CDCl_3$) see Tables I and II; EIMS (*m/z*): 275 (M^+, 64), 248 (9), 232 (9), 219 (16), 204 (14), 192 (14), 183 (18), 176 (21), 170 (24), 154 (78), 150 (42), 123 (22); HR-EIMS: 275.1542 (M^+), calcd for $C_{16}H_{21}NO_3$, 275.1521.

11-Oxo-phlegmariurine B (**4**): Colorless prisms from acetone-petroleum ester, 15 mg, mp 233–235°C; $[\alpha]_D^{25}$: −5.08 (*c* 0.35, $CHCl_3$); CD (*c* 3.7×10^{-4}, $CHCl_3$): $[\theta]_{250} + 8.2 \times 10^4$, $[\theta]_{291} - 8.9 \times 10^4$; IR (KBr) ν_{max} cm^{-1}: 3382.6, 2950.6, 2912.0, 1702.9, 1664.3, 1623.8, 1469.5, 1432.9, 1375.0 1207.2, 1180.2, 1151.3, 1085.7, 954.6; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$) see Tables I and II; EIMS (*m/z*): 275 (M^+, 33), 247 (4), 232 (4), 220 (18), 219 (100), 163 (88), 154 (22), 148 (22), 121 (20); HR-EIMS: 275.1520 (M^+), calcd for $C_{16}H_{21}NO_3$, 275.1521.

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