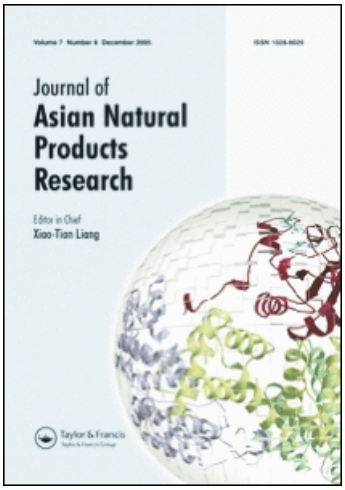

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Lycopodium alkaloids from *Huperzia serrata*

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A new lycopodane-type *Lycopodium* alkaloid, 6α-hydroxy-5,15-oxide-lycopodane (**1**), and seven known alkaloids were isolated from the whole plants of *Huperzia serrata*. Their structures were elucidated by means of spectroscopic methods. 12-Deoxyhuperzine O (**2**) was reported as a naturally occurring alkaloid for the first time, and showed an antagonist effect on the *N*-methyl-D-aspartate receptor with an IC_{50} value of 0.92 μM.

Keywords: *Huperzia serrata*; *Lycopodium* alkaloids; 6α-hydroxy-5,15-oxide-lycopodane; 12-deoxyhuperzine O; [^{3}H]MK-801 binding; NMDA receptor

1. Introduction

Huperzia serrata (Thunb.) Trev. (=*Lycopodium serratum* Thunb.) is a traditional Chinese herbal medicine used to treat strain, swelling, and schizophrenia [1]. Its chemical constituents have been extensively investigated in the last decades since the discovery of huperzine A – a potential acetylcholinesterase inhibitor [2,3]. In the previous studies, we isolated a number of new *Lycopodium* alkaloids named as huperzine series from the title plant [3–5]. Further investigation of another collection of this plant resulted in the isolation of a new lycopodane-type *Lycopodium* alkaloid: 6α-hydroxy-5,15-oxide-lycopodane (**1**) and seven known alkaloids: 12-deoxyhuperzine O (**2**) [6], huperzines E and F [4,7], phlegmariurine B [8], *N*-methyl-huperzine B [2], huperzinine [9], and 8-deoxy-13-dehydroserratinine [10] (Figure 1). Compound **2** was reported as a naturally occurring alkaloid for the first time, and showed potent inhibitory effect on the *N*-methyl-D-aspartate (NMDA) receptor with an IC_{50} value of 0.92 μM.

2. Results and discussion

Compound **1** was obtained as colorless prisms, its HR-EI-MS showing a molecular ion peak at *m/z* 263.1873, in agreement with the molecular formula $C_{16}H_{25}NO_2$. The IR absorption at 3430 cm^{-1} and EI-MS fragment ion peak at *m/z* 246 ($[M - 17]^+$) revealed the presence of a hydroxyl. The ^{13}C NMR spectrum (Table 1) of **1** showed 16 carbon signals (1 × Me, 8 × CH_2, 5 × CH, and 2 × C). Among them, two methylenes at δ 47.9 and 47.6, and the sp^3 quaternary carbon at δ 54.4 were ascribed to those neighboring to the N-atom, which is diagnostic of lycopodane-type *Lycopodium* alkaloids. Without carbonyl and double bond signals in the ^{13}C NMR spectrum, the unsaturated degree deduced from the molecular formula indicated **1** to be a

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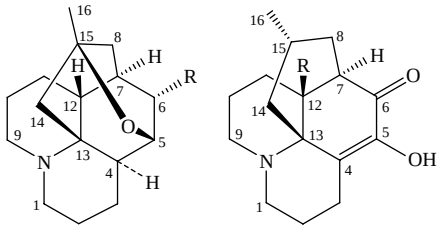


1 R = OH
3 R = H

2 R = H
4 R = OH

Figure 1. Structures of **1**–**4**.

pentacyclic alkaloid. The above evidences reminded us that **1** might be a hydroxylated derivative of 5,15-oxidolycopodane (**3**) [11]. Compared to the ^{13}C NMR spectral data of **1** with those of **3**, the most important difference was a hydroxyl-bearing methine (δ_C 71.9) instead of the methylene (δ_C 31.0, C-6) of **3**, demonstrating a 6-hydroxylated analog of **3**. Besides, these minor chemical shift changes, such as C-5, C-7, and C-4 ($\Delta\delta = +3.5, +6.3$, and -6.0 ppm, respectively, ongoing from **1** to **3**), were coincident with the inductive effect or γ-gauche effect caused by the axial hydroxyl on C-6, which indicated **1** to be a 6α-hydroxy-5,15-oxidelycopodane. This conclusion was further confirmed by HMBC (Figure 2) cross-peaks of H-6/C-5 and C-7, and NOESY (Figure 2) correlation of H-6 and H_{endo}-8.

Compound **2** had the molecular formula $C_{16}H_{23}NO_2$ established by the molecular ion peak at *m/z* 261.1729 in the HR-EI-MS spectrum. Its EI-MS spectrum showed diagnostic fragment ion peaks at *m/z* 218 ($[M - 43]^+$), 204 ($[M - 57]^+$, base peak), 190 ($[M - 71]^+$), and 176 ($[M - 85]^+$), indicating a lycopodane-type *Lycopodium* alkaloid [12]. The ^{13}C NMR spectrum of **2** displayed 16 carbon signals, comprising one methyl, eight methylenes, three methines,

Table 1. ^1H and ^{13}C NMR spectral data of **1** and **2** (400 and 100 MHz, in $CDCl_3$).

	1		**2**	
Site	^{13}C	^1H	^{13}C	^1H
1	47.9 (t)	3.38 td (13.7, 3.0) 2.57 br d (13.7)	46.4 (t)	3.50 td (13.7, 3.9) 2.66 dt (13.7, 3.1)
2	18.8 (t)	1.32 br d (13.0) 2.02 qt (13.0, 4.4)	16.7 (t)	2.09 qt (12.8, 3.1) 1.35 m
3	24.2 (t)	2.07 qd (13.0, 4.3) 1.49[a]	20.7 (t)	2.95 dd (16.8, 5.5) 2.14 ddd (16.8, 11.7, 6.7)
4	25.4 (d)	2.50 dd (12.2, 4.6)	128.8 (s)	
5	77.8 (d)	3.57 t (1.8)	144.7 (s)	
6	71.9 (d)	3.98 br s	196.3 (s)	
7	40.2 (d)	2.05 br s	48.8 (d)	2.50 dt (4.7, 2.4)
8	43.5 (t)	1.68[a] 1.65 dd (12.7, 4.0)	36.6 (t)	1.69 dd (13.0, 4.2) 1.24 td (13.0, 4.9)
9	47.6 (t)	3.21 td (12.3, 2.3) 2.54 br d (12.3)	48.9 (t)	2.98 td (11.6, 4.2) 2.58 dt (11.6, 2.3)
10	27.0 (t)	1.67[a] 1.50[a]	25.6 (t)	1.65 m (2H)
11	27.0 (t)	1.48[a] 2.17 qd (13.7, 3.2)	26.7 (t)	1.39 m (2H)
12	46.7 (d)	1.62 br d (12.2)	47.4 (d)	1.70[a]
13	54.4 (s)		58.6 (s)	
14	42.3 (t)	2.69 dd (12.5, 1.8) 1.07 d (12.5)	40.1 (t)	2.41 dd (12.6, 4.2) 1.00 t-like (12.6)
15	72.0 (s)		26.3 (d)	1.39 m
16	28.3 (q)	1.18 s	21.6 (q)	0.90 d (6.5)

Note: [a]Overlapped signals.

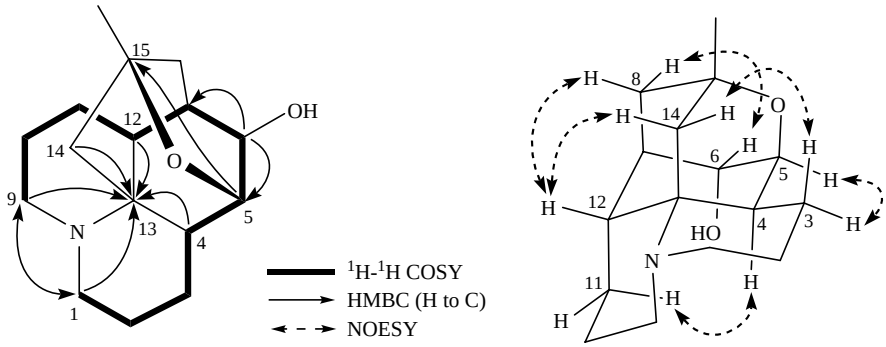
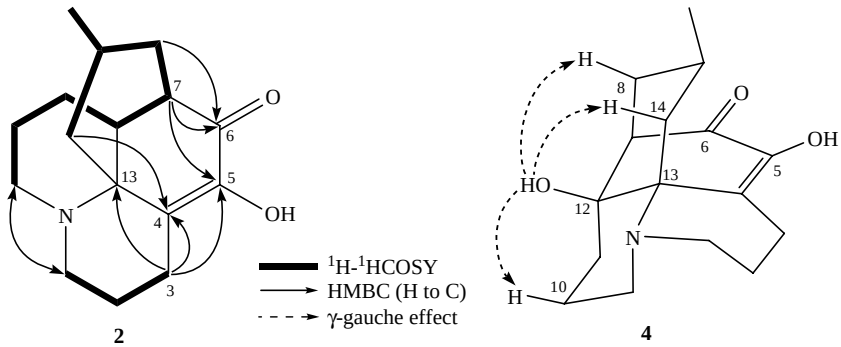


Figure 2. Selected 2D NMR correlations of **1**.

and one sp^3 and three sp^2 quaternary carbons. Among them, three sp^2 quaternary carbons (δ 128.8, 144.7, and 196.3) were ascribed to an enolic diketone function, similar to those of huperzine O (**4**) [13]. The main difference between the ^{13}C NMR spectral data of **2** and **4** was that **2** lacked the hydroxyl-bearing quaternary carbon (C-12 of **4**) and appeared as a methine (δ 40.1), suggesting **2** to be a 12-dehydroxylated analog of **4**. According to the theories of the inductive effect and γ-gauche effect, dehydroxylation at C-12 of **4** is expected to decrease the chemical shifts of these neighboring carbons (C-11, C-13, and C-7, observed $\Delta\delta = -3.5, -3.9$, and -5.6 ppm, respectively) and increase the chemical shifts of the carbons with γ-axial proton (C-8, C-10, and C-14, observed $\Delta\delta = +4.6, +5.5, +7.3$ ppm, Figure 3), but have a little effect on the remaining C-atoms. The above data, thus, led to the assignment of **2** to be a 12-deoxyhuperzine O. This conclusion was also confirmed by the 2D NMR experiments (Figure 3).

Ayer and Law [6] reported the structure of **2** as a reaction product when lycoclavine was treated with NaOH or lycopodine was oxidated by SeO_2, which suggested that those natural enolic diketone lycopodane-type *Lycopodium* alkaloids, including **2**, huperzines E, F, and O, might be biogenetically derived from lycopodine.

An assay of the antagonist effect of the eight alkaloids on [^{3}H]MK-801 binding in the rat cerebral cortex was performed. The results showed that these enolic diketone alkaloids had potent antagonist effect on the NMDA receptor in the rat cerebral cortex, the IC_{50} values of **2**, huperzines E and F being 0.92 ± 0.25, 29.1 ± 8.6, and



2 **4**

Figure 3. Significant 2D NMR correlations of **2** and γ-gauche effect illustration of **4**.

89.1 ± 10.5 μM, respectively, while the other alkaloids showing less or no activity.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Fisher–Johns melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. The IR spectra were recorded on a Nicolet Magna 750 FT-IR spectrometer. The NMR spectra were taken on a Bruker AV-400 spectrometer using TMS as an internal standard. EI-MS and HR-EI-MS spectra were obtained on a MAT-95 mass spectrometer. Silica gel (200–300 mesh) or silica gel H (Qingdao Haiyang, Co., Qingdao, China) were used for column chromatography (CC), and silica gel $HSGF_{254}$ (Yantai Jiangyou Guijiao Kaifa Co., Yantai, China) was used for TLC.

3.2 Plant material

The dried whole plants of *H. serrata* (Huperziaceae) were collected in Xuefeng Mountain, Hunan Province, China, in July 2008. The plant was identified by Dr Ji Huang of the Shanghai Institute of Materia Medica. Voucher sample (No. 20080705) is deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried whole plants of *H. serrata* (5 kg) were extracted with 95% EtOH, and the extract was dissolved in 1% HCl solution, and then partitioned with $CHCl_3$. The aqueous layer was adjusted to pH 10 with ammonia solution, and then partitioned again with $CHCl_3$. The $CHCl_3$ layer was isolated through silica gel CC eluted with petroleum ether–$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$–MeOH (10:1, 5:1), and finally MeOH. The $CHCl_3$ fraction was concentrated to remove the solvent and dissolved with hot Me_2CO, after cooling, yielding mixture needle solids. The mixtures were isolated by silica gel CC with $CHCl_3$–MeOH (25:1) to afford **2** (52 mg), huperzines E (310 mg) and F (248 mg). The remaining mother liquid was purified by CC of silica gel with gradient petroleum ether–Me_2CO as the eluant to yield eight subfractions: C1–C8. 8-Deoxy-13-dehydroserratinine (45 mg), **1** (38 mg), and phlegmariurine B (860 mg) were obtained as prisms from C2, C3, and C6, respectively. The remaining residue of C3 was isolated by silica gel CC ($CHCl_3$–Me_2CO, 10:1) to get huperzinine (18 mg) and *N*-methyl-huperzine B (35 mg) as prisms.

*3.3.1 6α-Hydroxy-5,15-oxide-lycopodane (**1**)*

Colorless prisms, mp: 192–194°C; $[\alpha]_D^{20}$ −59 ($c = 0.52$, $CHCl_3$). IR (KBr) ν_{max}: 3430, 2900, 1452, and 1087 cm^{-1}. ^1H and ^{13}C NMR spectral data: see Table 1; EI-MS *m/z* 263 ($[M]^+$), 246, 220, 206, 190 (100), and 162; HR-EI-MS: *m/z* 263.1873 $[M]^+$ (calcd for $C_{16}H_{25}NO_2$, 263.1885).

*3.3.2 12-Deoxyhuperzine O (**2**)*

Yellowish needles, mp: 185–186°C; $[\alpha]_D^{20}$ −38 ($c = 0.32$, $CHCl_3$). IR (KBr) ν_{max}: 3240, 2900, 1662, 1639, 1454, 1381, 1348, 1194, 1095, 1072, 902, 793, and 710 cm^{-1}; ^1H and ^{13}C NMR spectral data: see Table 1; EI-MS: *m/z* 261 ($[M]^+$), 218, 205, 204 (100), and 176. HR-EI-MS: *m/z* 261.1729 $[M]^+$ (calcd for $C_{16}H_{23}NO_2$, 261.1729).

3.3.3 [^{3}H]MK-801 Binding assay

Synaptic membrane from the cerebral cortex of Sprague–Dawley rats was prepared as prescribed in the literature [14]. Membranes 100 ml (50–300 μg protein), [^{3}H]MK-801 (DuPont NEN, Boston, MA, USA; final concentration 3 nM) 100 μl, and samples 100 μl (0.001–1 μM) were mixed at 23°C in the presence of L-glutamate 100 μl (10 μM),

and added with Tris-HEPES buffer (Tris 4.5, HEPES 5 mM, pH 7.4) to a final volume of 1 ml. Following incubation for 1 h at 23°C, binding was terminated by filtration using Whatman GF/B filters and a Brandel M-24 cell harvester. Radioactivity was measured using a Beckman LS 6000LL liquid scintillometer. All individual assays were carried out in replicates of three. The IC_{50} value was calculated using the computer software 'GraphPad InPlot'.

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