Notes

Huperzine R, a Novel 15-Carbon *Lycopodium* Alkaloid from *Huperzia serrata*

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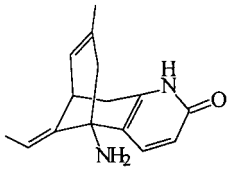
Huperzine R (**2**), a novel 15-carbon *Lycopodium* alkaloid, was isolated from the whole plant of *Huperzia serrata*, and the relative configuration was established using spectroscopic and X-ray crystallographic techniques.

Lycopodium species have long been studied, and many alkaloids have been reported thus far from this genus. Most of the compounds reported have a common formula of $C_{16}N$.[1] The discovery that huperzine A (**1**), a *Lycopodium* alkaloid isolated from *Huperzia serrata* (Thunb.) Trev. (Huperziaceae), was a potent acetylcholinesterase (AChE) inhibitor[2,3] interested many chemists and pharmacologists all over the world. As part of our continuing interest in this species,[4] we have examined the $CHCl_3$ extract of a basic residue of the dry whole plant of *H. serrata*, which after purification by repeated column chromatography over silica gel afforded huperzine R (**2**), a novel *Lycopodium* alkaloid possessing a $C_{15}N$ skeleton. In the present paper, we report on the isolation and structural elucidation of **2**.



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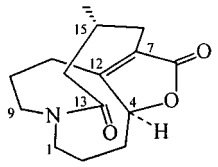

2

Huperzine R (**2**) showed a positive effect with Dragendorff's reagent and was attributed the molecular formula $C_{15}H_{21}NO_3$ from the HREIMS, in which the M^+ appeared at *m/z* 263.1499 (calcd for $C_{15}H_{21}NO_3$, 263.1521). The IR spectrum showed the presence of a lactam group (1675 cm^{-1}) and an α,β-unsaturated conjugated lactone group (1736, 1622, and 1454 cm^{-1}). The EIMS exhibited a fragmentation pattern quite different from those reported for previously published *Lycopodium* alkaloids.[5] In the ^{13}C NMR spectrum (Table 1), 15 carbon signals were observed, which were resolved into one methyl, eight methylenes, two methines, and four quaternary carbons through DEPT experiments. The $^1H-^1H$ COSY and HMQC spectra (Figure 1) indicated the presence of three isolated segments: (i) $-CH_2CH_2CH_2-$, (ii) $-CH_2CH_2CH_2CH<$, and (iii) $-CH_2CH(CH_3)CH_2-$. Three sp^2 quaternary carbons [δ 126.5, 163.4, and 173.3 (or 172.3)] and a methine (δ_C 81.7 and δ_H 4.79) implied the presence of a conjugated lactone five-numbered ring (Figure 1), which suggested the connection of segment ii through the methine (CH-4). The HMBC spectrum (Figure 1) enabled linkages to be established among the three segments via a lactam group (δ_C 172.28) and via the double bonds of the lactone ring. Therefore, the planar structure of huperzine R was determined as **2**, in which an oxygen atom replaced the usual C-5 of the other *Lycopodium* alkaloids.

Table 1. ^1H and ^{13}C NMR Assignments for **2**[a]

position	^1H (*J* in Hz)	^{13}C
1α	2.40 td (14.0, 1.3)	49.3 t
β	3.94 dt (14.0, 3.1)	
2α	2.21 qd (13.8, 3.0)	17.8 t
β	1.34 m	
3α	1.91 t (14.2)	31.7 t
β	2.53 m	
4	4.79 br d (3.8)	81.7 d
6		173.3 s
7		126.5 s
8α	2.01 t (12.1)	32.4 t
β	2.41 dd (12.1, 3.2)	
9α	3.10 br d (14.2)	50.1 t
β	4.02 td (14.2, 3.2)	
10α	2.15 qd (13.9, 3.0)	25.1 t
β	1.89 br d (13.9)	
11α	2.41 dd (14.0, 1.3)	26.1 t
β	2.91 td (14.0, 4.1)	
12		162.4 s
13		172.3 s
14 *endo*	1.99 dd (12.9, 3.0)	39.9 t
exo	2.56 dd (12.9, 11.2)	
15	2.48 m	29.0 d
16	1.10 d (6.2)	23.4 q

[a] Run in$CDCl_3$. δ values referenced to $CHCl_3$ residual peaks at δ_H 7.26 and δ_C 77.3, respectively.

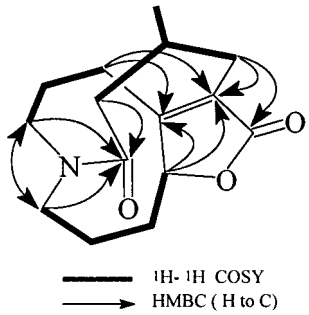

Figure 1. $^1H-^1H$ COSY correlations and important HMBC correlations of **2**.

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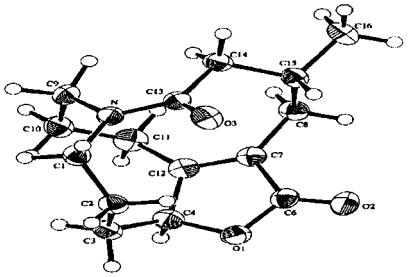

Figure 2. ORTEP view of the X-ray molecular structure of **2**.

To establish unambiguously the structure and relative configuration of **2**, an X-ray crystallographic analysis was conducted on huperzine R. Figure 2 shows an ORTEP drawing of **2**. Thus, the relative stereochemistry of huperzine R was confirmed. Huperzine R (**2**) possesses a novel $C_{15}N$ skeleton and is the first such example among the *Lycopodium* alkaloids.

An evaluation of activity on AChE in vitro was tested as previously described,[3] which showed that the inhibition on AChE activity induced by **2** was less pronounced than huperzine A (**1**) [the concentration (μmol) of inhibitor and inhibition rate (%) of AChE were estimated to be 95/27 and 0.082/50, respectively].

Experimental Section

General Experimental Procedures. The melting point was determined on a Fisher-Johns melting point apparatus and is uncorrected. The optical rotation was measured using a Perkin-Elmer 241 MC polarimeter in $CHCl_3$. The IR spectrum (KBr) was recorded on a Nicolet Magna 750 FTIR spectrophotometer. The NMR spectra were recorded on a Bruker AM-400 instrument. EIMS and HREIMS data were obtained with MAT-95 and MAT-711 mass spectrometers. Silica gel (200−300, 400 mesh, Qindao Haiyang Chemical Group Co., Qindao, People's Republic of China) was used for column chromatography, and precoated plates of silica gel ($HSGF_{254}$) were used for TLC. Single-crystal X-ray diffraction measurement was made with a Rigaku AFC7R diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71069 Å) and a 12 kW rotating anode generator, with SHELXTL PLUS used for structure solution and refinement.

Plant Material. Fresh whole plants of *Huperzia serrata* (Thunb) Trev. (Huperziaceae) were collected in Zhejiang Province, People's Republic of China, in August 1997 and identified by X.-Q.M. A voucher specimen (No. 97-63) was deposited in the Herbarium of our institute.

Extraction and Isolation. The total crude alkaloids (103 g) from 10 kg of *H. serrata* were obtained as previously described[4] and were chromatographed over silica gel (1 kg) with gradient eluents ($CHCl_3$, 1000 mL; 1−4% methanol in $CHCl_3$, each 1500 mL) to afford fractions 1−5. Fraction 2 (1.4 g) was chromatographed on a silica gel column eluting with EtOAc−acetone (2:1, 1000 mL), collecting 50 mL aliquots, to afford four fractions: 2.1−2.3, 2.4−2.5, 2.6−2.15, and 2.16−2.20. Fraction 2.4−2.5 (63 mg) was subjected to silica gel (10 g) column chromatography with $CHCl_3$−actone (2:1, 120 mL), collected in 5 mL aliquots and detected using TLC (silica gel $HSGF_{254}$, $CHCl_3$−actone, 2:1, iodine vapor for detection), yielding **2** (17 mg, R_f 0.38).

Huperzine R (2): colorless prisms (petroleum ether−actone), mp 189−191 °C, $[\alpha]^{25}_D$ −0.115° (*c* 0.417, $CHCl_3$); IR (KBr) ν_{max} 2941, 1736, 1675, 1622, 1454, 1369, 1118, 989, 864 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* 263 [M^+] (40), 261 (23), 246 (5), 233 (7), 218 (8), 194 (38), 176 (11), 154 (47), 139 (100), 138 (91), 122 (38), 84 (26), 70 (42); HREIMS *m*/*z* 263.1499 [M^+] (calcd for $C_{15}H_{21}NO_3$, 263.1521).

X-ray crystal structure analysis of 2: $C_{15}H_{21}NO_3$ (MW 263.34), space group $P2_1$ (#4) with a = 8.500(1) Å, b =7.7250-(9) Å, c =10.650(1) Å, β = 106.21(1)°, V = 671.5 (1) Å^3, Z = 2, and D(calcd) =1.302 g cm^{-3}. The final R value was 0.031 for 1518 reflections.

Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as deposition No. CCDC-165270. Copies of data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: + 44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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