Pergamon

Tetrahedron Letters 41 (2000) 5733–5736

TETRAHEDRON LETTERS

Huperzine P, a novel Lycopodium alkaloid from *Huperzia serrata*

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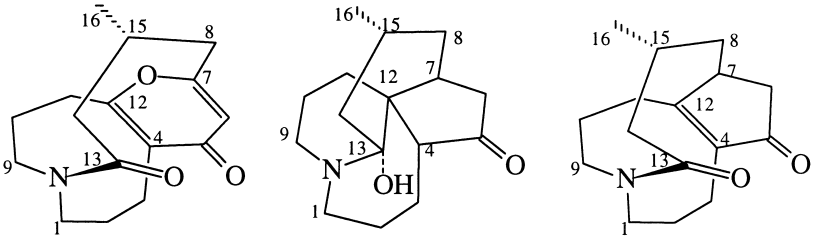
Received 10 April 2000; accepted 2 June 2000

Abstract

Huperzine P (**1**), a new irregular fawcettimine-type Lycopodium alkaloid, was isolated from *Huperzia serrata* and the absolute stereostructure was established by 2D NMR and X-ray crystallographic analysis.

Keywords: natural products; huperzine P; Lycopodium alkaloid; *Huperzia serrata*.

Previous investigations[1–5] have shown that *Huperzia serrata* (equivalent to *Lycopodium serrata*, a Chinese folk medicinal plant) contains many alkaloids including huperzine A, which was reported to increase efficiency for learning and memory in animals and shows promise in the treatment of Alzheimer's disease and myasthenia gravis. These interesting results led us to examine the mother liquors from a large-scale isolation of huperzine A (10 kg of dry whole plants), which after purification by repeated chromatography over silica gel, gave a novel skeletal Lycopodium alkaloid, huperzine P (**1**,17 mg), and two known compounds, fawcettimine (**2**, 86 mg) [6] and phlegmariunine B (**3**, 750 mg).[7,8] The absolute stereostructure of **1** is reported in this paper.



1 **2** **3**

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0040-4039/00/$ - see front matter
PII: S0040-4039(00)00893-5

Huperzine P (**1**)[9] has the molecular formula $C_{16}H_{21}NO_3$ (*m/z* 275.15310) deduced from the high-resolution MS spectrum. The IR spectrum showed the presence of a lactam group (ν_{max} 1685.5) and a conjugated carbonyl group (ν_{max} 1614.2, ν_{max} 1598.7). The ^{13}C NMR (Table 1) and DEPT spectra showed one methyl group, eight methylene groups, one methine group, a lactam carbon, a conjugated carbonyl carbon, an sp^2 methine group and three sp^2 quarternary carbons. The HMQC and $^1H-^1H$ COSY spectra indicated the presence of two fragments: $-CH_2CH(CH_3)CH_2-$ and $-CH_2CH_2CH_2N(CO-)CH_2CH_2CH_2-$ (Fig. 1). The HMBC spectrum indicated the long-range correlations between C-4 and H-2, H-3, H-6 and H-11, between C-5 and H-3 and H-6, between C-7 and H-6 and H-8, between C-12 and H-3, H-10 and H-11, and between C-13 and H-1,

Table 1
^1H and ^{13}C NMR (400 MHz) for **1**[a]

Position	^1H (J in Hz)[b]	^{13}C[c]	Position	^1H (J in Hz)[b]	^{13}C[c]
1 α	2.64 ddd (14.3, 8.2, 3.3)	47.89 (t)	9 α	3.75 ddd (15.3, 5.6, 4.8)	46.81 (t)
β	4.09 ddd (14.3, 8.3, 3.5)		β	3.17 ddd (15.4, 10.0, 5.4)	
2 α	1.88 dddd (15.1, 8.4, 5.1, 3.3)	23.88 (t)	10α	2.01[d]	23.80 (t)
β	2.01[d]		β	2.46[d]	
3 α	3.22 ddd (14.1, 8.5, 5.6)	21.28 (t)	11 α	2.61 dt (15.0, 5.7)	28.81 (t)
β	2.38 dt (14.6, 5.6)		β	2.99 ddd (15.0, 10.0, 5.0)	
4	--------	120.76 (s)	12	-------	163.21 (s)
5	--------	180.55 (s)	13	-------	170.96 (s)
6	6.02 s	115.04 (d)	14 *endo*	1.97 dd (14.8, 1.7)	39.30 (t)
7	---------	166.73 (s)	*exo*	2.50 dd (15.1, 10.1)	
8 *endo*	2.74 dd (13.0, 5.3)	41.06 (t)	15 *endo*	2.82 m	35.27 (d)
exo	1.99 dd (12.8, 10.1)		16	1.14 d (6.8)	23.20 (q)

[a] Assignments were made by ^1H-^1H COSY,HMQC and HMBC data.
[b] Solution in $CDCl_3$ referenced to $CHCl_3$ at •7.27;
[c] Solution in $CDCl_3$ referenced to $CHCl_3$ at •77.23;
[d] Overlapping signals

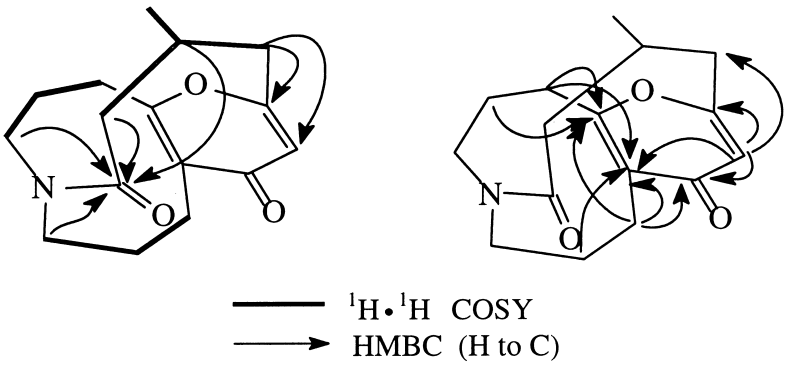

Figure 1. $^1H-^1H$ COSY and important HMBC correlations of **1**

H-9 and H-14. Thus, the relative stereostructure of **1** was proved as depicted. In order to determine the absolute stereostructure of **1**, we conducted the X-ray crystallographic analysis of **1**. [10] Fig. 2 shows the X-ray structure of **1**, and the absolute stereostructure was established as shown in **1**. Huperzine P (**1**) possessing a novel skeleton, an oxygen atom inserted in C_7-C_{12}, is the first example in Lycopodium alkaloid.

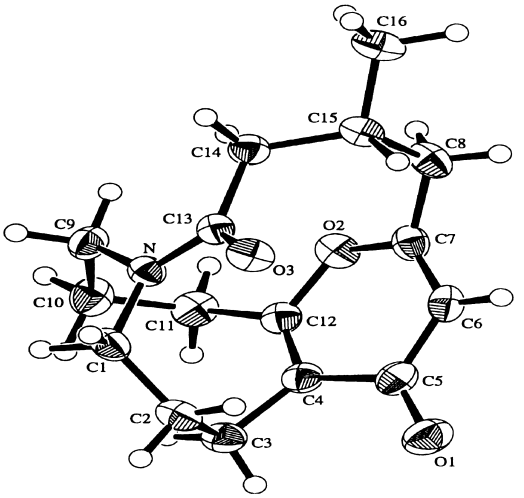

Figure 2. X-Ray structure of **1**

Bioactivity tests in vitro showed that the inhibition of acetylcholinesterase (AChE) activity induced by compounds **1**, **2** and **3** were less pronounced than huperzine A [the concentration (μ mol/l) of inhibitor and inhibition rate (%) of AChE were estimated to be 90/7, 95/23, 96/28, 0.082/50, respectively.]

Acknowledgements

The authors would like to thank Prof. Xi-Can. Tang of this institute for bioactivity tests.

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8. Compound **3** was implied to probably be an artifact of phlegmariunine A upon basic treatment in Ref. 7. During this study, we did not find this to be the case, so proving **3** to be a natural product.

9. Compound **1**: colorless prisms, mp 320.5–321.5°C, $[\alpha]_D^{25}$ 2.501° (*c* 1.0, $CHCl_3$); HREIMS *m*/*z* 275.15310 [M^+] (calcd $C_{16}H_{21}NO_3$: 275.15213); IR (film) ν_{max} cm^{-1}: 1658.5 (lactam group), 1614.2, 1598.7 (conjugated carbonyl group), 1444.4 (double bonds), 1415.5, 1161.0, 887.1; EIMS: *m*/*z* (rel.int.): 275 [M^+] (97), 260 (22), 246 (9), 232 (11), 205 (22), 188 (21), 176 (14), 150 (100), 122 (47).
10. The crystal data for **1** are as follows: data were acquired with a Rigaku AFC7R diffractometer Mo-Kα radiation ($\lambda = 0.71069$ Å), graphite monochromated, orthorhombic, $C_{16}H_{21}NO_3$ (MW:275.35), space group $P2_12_12$ with $a = 8.734(2)$ Å, $b = 20.330(2)$ Å, $c = 7.613(2)$ Å, $V = 1351.8(4)$ $Å^3$, $Z = 4$, and $D_{calc} = 1.353$g cm^{-3}. The final *R* value was 0.045 for 1822 reflection.