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Huperzine H, a New Lycopodium Alkaloid from *Huperzia serrata*

Wen Yun GAO, Yi Ming LI, Bao De WANG, Da Yuan ZHU*

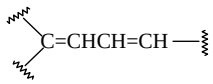
State Key Laboratory of New Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031

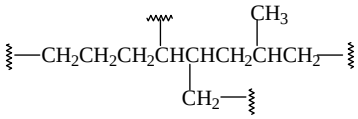
Abstract: A new lycopodium alkaloid with a novel skeleton was isolated from *Huperzia serrata*. Its structure was determined on the basis of spectral evidences.

Keywords: *Huperzia serrata* (Thunb.) Trev.; huperzine H; isolation; structure.

Huperzia serrata (Thunb.) Trev. is one of the most commonly used traditional Chinese herbal medicines for the treatment of contusions, strains, swellings and schirophremas[1] *etc*.. Many lycopodium alkaloids have been isolated[2, 3] from it including huperzine A, a compound which has shown potential effects on Alzheime disease. In our investigations, three new lycopodium alkaloids were obtained and reported[4,5]. This paper deals with the identification of huperzine H— a new lycopodium alkaloid with a novel skeleton.

Huperzine H is colorless needles with $[\alpha]_D$ -216 (*c* 0.02, $CHCl_3$), mp. 168-170 °C. It gave positive reaction with $KBiI_4$ indicating that the compound was an alkaloid. Its IR spectrum showed the absorption bands of carbonyl group (1710.5cm$^{-1)}$, α, β-unsaturated carbonyl group (1685.3cm$^{-1)}$ and double bonds (1547.6cm$^{-1)}$. The HREIMS of it showed that its molecular composition was $C_{16}H_{21}NO_2$. The EIMS possessed a molecular ion at m/z 259 (34%) and a prominent peak at m/z 231 (44%) [M-28], but no peak at [M-57] or [M-71] and this evidence indicated that the compound did not belong to lycodine group or lycopodine group[2]. The ^{1}H-NMR of huperzine H (**Table 1**) showed that it possessed 21 protons including three olefinic signals at δ 6.99 (1H, *dd*, *J*=4.1, 2.1), 6.77 (1H, *dd*, *J*=4.4, 2.1) and 6.18 (1H, *dd*, *J*=4.4, 4.1) and a methyl signal at δ 0.96 (3H, *d*, *J*=7.2). The ^{13}C-NMR of this compound (**Table 1)** showed 16 signals including two carbonyl groups (δ 209.55 and 191.76), four olefinic carbons [δ 135.84 (*s*), 129.31 (*d*), 118.36 (*d*) and 109.53 (*d*)] and 10 saturated carbons (CH×3, CH_2×6 and CH_3×1). The HMQC and ^{1}H- ^{1}H COSY of huperzine H showed that it consisted of the segments given below:





And this two segments accompanied by two carbonyl groups and a nitrogen atom can be connected as below by the HMBC of the compound. The HMBC results see **Figure 1**. Thus, the structure of huperzine H is characterized (see **Figure 2**). It is a new lycopodium alkaloid with a novel skeleton.

Figure 1. HMBC of huperzine H

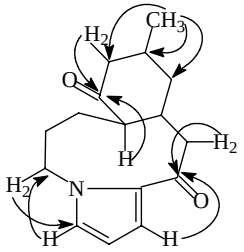


Figure 2. Structure of huperzine H

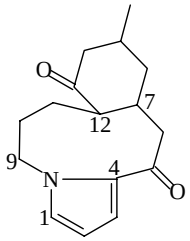


Table 1. NMR data of huperzine H

Site	δ_H (multi., Hz)	H-H COSY	HMQC (δ_C, DEPT)
1	6.77 (1H, *dd*, 4.4, 2.1)	2,3	129.31 (*d*)
2	6.18 (1H, *dd*, 4.1, 4.1)	1,3	109.53 (*d*)
3	6.99 (1H, *dd*, 4.1, 2.1)	1,2	118.88 (*d*)
4			135.84 (*s*)
5			191.76 (*s*)
6	a. 3.02 (1H， *dd*, 12.5, 9.5)	6b, 7	47.42 (*t*)
	b. 2.66 (1H， *brd*, 12.5)	6a	
7	2.36 (1H， *m*)	6a, 8b, 12	41.33 (*d*)
8	a. 1.88 (1H, *dd*, 12.11, 4.2)	7, 15,	39.38 (*t*)
	b. 1.82 (1H, *m*)		
9	a. 4.46 (1H, *brt*, 13.4)	9b, 10a	48.63 (*t*)
	b. 4.21 (1H, *dm*, 13.4)	9a, 10b	
10	a. 1.79 (1H, *m*)	9a, 10b, 11	32.18 (*t*)
	b. 1.56 (1H, *m*)	9b, 10a, 11	
11	a. 2.76 (1H, *dd*, 16.0, 3.4)	10a, 11b, 12	21.70 (*t*)
	b. 1.04 (1H, *brd*, 16.0)	10b, 11a, 12	
12	2.05 (1H, *brd*, 12.3)	7, 11a, 11b	55.74 (*d*)
13			209.55 (*s*)
14	a. 2.48 (1H, *dd*, 10.6, 5.9)	14b, 15	47.79 (*t*)
	b. 2.22 (1H, *d*, 10.6)	14a, 15	
15	2.42 (1H, *m*)	8, 14, 16	30.21 (*d*)
16	0.96 (3H, *d*, 7.2)	15	19.03 (*q*)

Experimental

General Procedures

Optical rotation was determined on a JASCO DIP-181 polarimeter. IR spectrum was recorded on a Perkin-Elmer 599B spectrophotometer. 1D and 2D-NMR were recorded with a Brucker AM-400 NMR spectrometer in $CDCl_3$. MS were obtained with a MAT-711 and a MAT-95 mass spectrometers. Column chromatography was carried out on silica gel (200-300 mesh) and neutral Al_2O_3 (200-300 mesh). Precoated plates of silica gel ($HSGF_{254)}$ and neutral Al_2O_3 were used for detection.

Plant Material

The whole plants of *Huperzai serrata* were collected at Xianju, Zhejiang province in August, 1997 and were identified by Dr. Xiaoqiang Ma of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen has been deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences (No. 97-36).

Extraction and Isolation

About 50 kg of dry plant was extracted with 1% HCl for 5 times. The combined HCl extracts were concentrated under vaccum to about 2 L and alkalized with concentrated ammonia water to pH 9~10. The alkalized solution was then extracted repeatedly with CHCl3 (5 × 1 L) until no alkaloids detectable in water layer. After CHCl3 was removed under vaccum, the procedure described above was repeated once more and about 5 kg of crude alkaloids were obtained which were submitted to silica gel columns [(20 × 80 cm) × 5] and eluted with CHCl3-acetone from 10 : 1 to 1 : 1, then with methanol. Concentration of the methanol fraction under vaccum gave a mixture about 50 g in weight which was chromatographed repeatedly on neutral Al2O3 (CHCl3-acetone) and silica gel (CHCl3-CH3OH-NH4OH) columns (3 × 50 cm) to give huperzine H (7 mg).

Huperzine H was obtained as colorless prism, mp. 168~170 oC, C16H21NO2; [α]D= -216 (c 0.02, CHCl3); IR ν^{KBr} cm^{-1}: 1710.5 (C=O), 1685.3 (C=O), 1547.6 (C=C), 1123.6; 1D and 2D-NMR data see Table 1; HREIMS (m/z): found 259.1564 (C16H21NO2, calcd 259.1567), 231.1620 (C15H21NO, 231.1618), 203.1663 (C14H21N, 203.1669); EIMS (m/z): 259 [M+] (34), 231 (44), 203 (16), 162 (16), 133 (18), 120 (31), 94 (31),81 (100).

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