A new alkaloid and arbutin from the whole plant of *Huperzia serrata*

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A new *Lycopodium* alkaloid together with a phenolic glycoside—arbutin, were isolated from the whole plant of *Huperzia serrata*. The new compound was assigned the trivial name of huperzine I. The structures of these two compounds were determined on the basis of spectral evidence.

Keywords *Huperzia serrata*, Lycopodiaceae, isolation, structural elucidation, alkaloid, phenolic glucoside

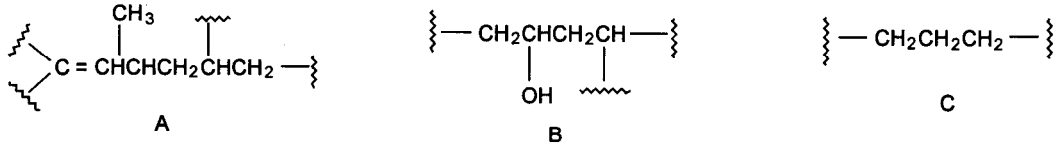
Introduction

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] Up to now, many Lycopodium alkaloids have been isolated and identified from this plant including huperzine A, a compound with strong bioactivities, especially the antiChEI activity and memory-enhancing effects.[2,3] In our further studies of this plant, some new *Lycopodium* alkaloids were obtained and reported.[4,5] This paper deals with the isolation and structural elucidation of a new *Lycopodium* alkaloid—huperzine I (**1**) and a known compound—arbutin.[6]

Results and discussion

The crude alkaloids of the whole plant of *H. serrata* were obtained according to a standard method.[7] Si gel chromatography of the crude alkaloids and elution with $CHCl_3$-acetone followed by MeOH afforded two alkaloid-rich fractions. Repeated chromatography of the MeOH fraction over neutral Al_2O_3 and Si gel afforded a novel Lycopodium alkaloid (**1**) which was assigned the trivial name of huperzine I, and a phenolic glycoside, arbutin.

Huperzine I (**1**) was obtained as colorless needles, $[\alpha]_D + 141.1°$ (*c* 0.05, CH_3OH). It had the molecular formula $C_{16}H_{23}NO_2$ as deduced from its HREIMS and NMR data. The IR spectrum of **1** showed the presence of a hydroxyl group (3330 cm^{-1}) and a carbonyl group (1730 cm^{-1}). The EIMS showed that a molecular ion was at *m/z* 261 and a base peak at *m/z* 192. The ^1H NMR (Table 1) exhibited an olefinic proton as a doublet at δ 5.66, one oxygenated methine as a broad doublet at δ 3.93 and a methyl doublet at δ 1.04. The ^{13}C NMR (Table 1) showed 16 carbon signals including a carbonyl at δ 216.6, two olefinic carbons at δ 145.4(s) and 126.4(d), an oxygenated carbon at δ 70.5 (d) and a quaternary carbon at δ 46.8. The other 11 signals all appeared upfield (CH × 3, CH_2 × 7, CH_3 × 1). Moreover, the ^1H-^1H COSY and HMQC of this compound showed that it possessed the segments given below:



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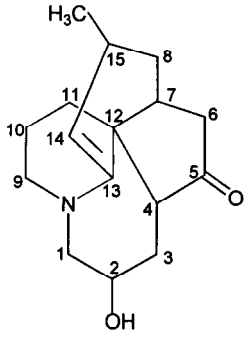
and these three segments accompanied by the carbonyl group, the quaternary carbon and the nitrogen atom should be connected as shown in Fig. 1 by the selected HMBC correlations (Fig. 1) of the compound. Therefore, the structure of huperzine I was assigned as **1**. The orientation of the hydroxyl group attached to C-2 was not determined.



1

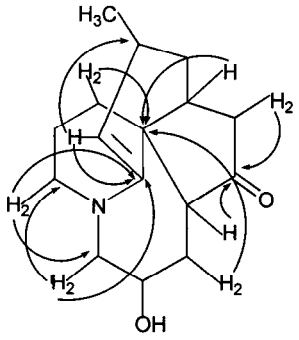


Fig.1 Selected HMBC correlation for **1**.

Arbutin was isolated as colorless powder, mp 195—197℃. It gave negative reaction with $KBiI_4$ but a dark blue coloration with ethanolic $FeCl_3$ reagent which indicated that it was a phenolic compound. The IR spectrum of this compound showed the absorption bands of hydroxyl and aromatic groups. The EIMS showed a molecular ion peak at m/z 272 and a base peak at m/z 110. Its ^1H NMR (in CD_3COCD_3) indicated the presence of aliphatic protons at δ 3.07—5.03 (7H) including a signal at δ 4.74(1H, d, J = 8 Hz) assignable to the anomeric proton of a β-glucosyl moiety. Two signals at δ 6.91(2H, d, J = 8 Hz) and δ 6.70(2H, d, J = 8 Hz) were assigned to four aromatic protons. The physical property and spectroscopic data of this compound were identical with the reported data of arbutin, namely 1, 4-dihydroxy-1-β-*D*-glucopyranoside.[6] Thus, the structure of this phenolic glucoside was elucidated and this conclusion was further confirmed by its ^{13}C NMR data. This is the first report of the type of compound from a plant in the genus *Huperzia*.

Table 1 NMR data of **1** in $CDCl_3$

Site	δ_H(multi. J in Hz)	^1H-^1H COSY	δ_C
1a	3.25(dd, 15, 3)	2	65.2(t)
1b	3.08(d, 15)	2,3	
2	3.93(brd, 6)	1, 3a	70.5(d)
3a	2.29(brd, 13)	2, 4	35.2(t)
3b	1.62(d, 13)	4	
4	2.63(d, 12)	3a, 3b	48.1(d)
5			216.6(s)
6a	2.77(dd, 16, 8)	7	44.5(t)
6b	2.04(brd, 16)		
7	2.16(m)	6a, 8b	28.0(d)
8a	1.35(brd, 13)	14, 15	34.1(t)
8b	1.22(ddd, 13, 13, 5)	7, 15	
9	3.01(m)	10a, 10b	52.7(t)
10a	2.40(m)	9, 11b	21.5(t)
10b	1.38(brd, 13)	9, 11a	
11a	2.00(brs)	10b	39.2(t)
11b	1.54(ddd, 13, 13, 4)	10a	
12			46.8(s)
13			145.4(s)
14	5.66(d, 6)	8a, 15, 16	126.4(d)
15	2.26(m)	8a, 8b, 14, 16	36.9(d)
16	1.04(d, 7)	15	20.8(q)

Experimental

Optical rotations were determined on a JASCO DIP-181 polarimeter. Circular Dispersion was taken on a JASCO 500A polarimeter. IR spectra were recorded on a Perkin-Elmer 599B spectrophotometer. MS were ob-

tained on a MAT-711 and a MAT-95 mass spectrometers. 1D and 2D-NMR were recorded with a Brucker AM-400 NMR spectrometer in $CDCl_3$.

The whole plant of *H. serrata* was collected at Xianju, Zhejiang province in August 1997 and was identified by Dr. Ma Xiaoqiang of Shanghai Institute of Materia Medica, Chinese Academy of Sciences (SIMM, CAS). A voucher specimen was deposited at the herbarium of SIMM, CAS (No. 97—36).

About 50 kg of dry plants were extracted with 1% HCl for 5 times. The combined extracts were concentrated under reduced pressure to about 2 L and alkalized with concentrated ammonia water to pH 9—10. The alkalized solution was then extracted with $CHCl_3$ until no alkaloid was detectable (5 × 1 L). After $CHCl_3$ was removed under reduced pressure, the procedure described above was repeated once more and about 5 kg of crude alkaloids were obtained which were submitted to silica gel columns and eluted with $CHCl_3$-acetone from 10:1 to 1:1, then with methanol. Concentration of the methanol fraction under reduced pressure gave a mixture about 50 g in weight which was chromatographed repeatedly on neutral Al_2O_3 ($CHCl_3$-acetone = 20:1 to 8:1) and silica gel ($CHCl_3$-CH_3OH-NH_4OH = 8:1:0.1 to 4:1:0.1) to give **1** (15 mg), and arbutin (20 mg).

Huperzine I (**1**) Colorless needles ($CHCl_3$ + acetone), mp 154—156℃, $[\alpha]_D^{20}$ = +141.1° (*c* 0.03, CH_3OH). ν_{max}^{KBr}: 3330(OH), 2910(CH), 1730 (C=O), 1415, 1099, 1072(C-O) cm^{-1}. ^1H and ^{13}C NMR data, see Table 1. EIMS *m/z* (%): 261[M^+] (45), 246(15), 218(7), 192(100), 164(34), 148 (19); HREIMS *m/z*: 261.1713 (calcd. for $C_{16}H_{23}NO_2$, 261.1722), 246.1479 ($C_{15}H_{20}NO_2$, 246.1488), 192.1390 ($C_{12}H_{18}NO$, 192.1394).

Arbutin is white powder, mp 195—197℃. ν_{max}^{KBr}: 3410(OH), 2889(CH), 2840(CH), 1512, 1220, 1221, 1067, 1014(C—O), 833 cm^{-1}. δ_C (400 MHz, CD_3COCD_3): 6.91(2H, d, *J* = 8 Hz), 6.70(2H, d, *J* = 8 Hz), 4.74(1H, d, *J* = 8 Hz), 3.84(1H, dd, *J* = 11, 1 Hz), 3.67(1H, dd, *J* = 9, 5 Hz), 3.31—3.57(4H, m); δ_C (400 MHz, CD_3COCD_3): 153.5(C-1), 152.0(C-4), 119.0(CH × 2, C-2 and C-6), 116.3(CH × 2, C-3 and C-5), 103.2(C-1′), 77.8(C-3′), 77.6(C-5′), 74.6(C-2′), 71.3(C-4′), 62.6(C-6′). EIMS *m/z* (%): 272[M^+](2), 162[M − 110]$^+$ (12), 110(100).

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