Isolation of a new lycodine alkaloid from *Lycopodium japonicum*

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A new lycodine alkaloid, N-methylhydroxypropyllycodine (1), was isolated from the club moss *Lycopodium japonicum* Thunb, together with five known compounds, N-methyllycodine (2), huperzine (3), β-obscurine (4), α-obscurine (5) and des-N-methyl-α-obscurine (6). Their structures were elucidated by spectroscopic analyses, including 2D NMR techniques.

**Keywords:** *Lycopodium japonicum*; Lycopodiaceae; lycodine alkaloid

1. Introduction

*Lycopodium japonicum* Thunb (Lycopodiaceae) is a traditional Chinese medicinal plant for the treatment of sprains, strains and myasthenia (Zhang & Zhang 2004; Chinese Pharmacopoeia Commission 2010). Previous investigations have shown that this plant is a rich source of lycodine alkaloids possessing diverse structures of unprecedented skeletons (Sun et al. 2008; Wang, Liu, et al. 2012; Wang, Zhang, et al. 2012). As a part of our research of structurally unique and biologically active compounds from medicinal plants of Yunnan, China, we have isolated and identified a new lycodine alkaloid, N-methylhydroxypropyllycodine (1), as well as five known compounds, N-methyllycodine (2), huperzine (3), β-obscurine (4), α-obscurine (5) and des-N-methyl-α-obscurine (6) from L. japonicum. Our procedures and findings are reported in this article (Figure 1).

2. Results and discussion

Compound 1 was obtained as a colourless gum. The molecular formula of 1 was determined to be C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O by HR-EI-MS ([M] <sup>+</sup> 314.2364), implying seven degrees of unsaturation. The IR
The 1H and 13C NMR and DEPT spectra of 1 displayed three methyls, seven sp3 CH2, four sp3 and two sp2 CH, three sp2 and one sp3 quaternary C-atoms. The 13C NMR spectra [δ 157.2 (s), 157.1 (s), 135.7 (s), 135.1 (d), 121.5 (d)] showed that 1 had a trisubstituted pyridine ring. 1H–1H COSY and HSQC analyses indicated the existence of four fragments, including an isolated 2-hydroxy-propyl group. Careful inspection in the 1H and 13C NMR spectra revealed that 1 was similar to hydroxypropyllycodine (7) previously isolated from Lycopodium obscurum (Ayer & Kasitu 1989). Compound 1 differs from 7 only in the additional N-Me group instead of an N–H bond, which was further confirmed by HMBC correlations from H-17 (δ 2.65) to C-9 (δ 50.5) and C-13 (δ 59.4). Therefore, the structure of 1 was elucidated to be N-methylhydroxypropyllycodine.

By comparison of the obtained spectroscopic data with those reported in the literature, the chemical structures of known compounds were determined as N-methyllycodine (2) (Nakashima et al. 1975), huperzinine (3) (Yin et al. 2006), β-obscurine (4) (Castillo et al. 1976), α-obscurine (5) (Nakashima et al. 1975) and des-N-methyl-α-obscurine (6) (Liu & Wang 2012).

3. Experimental

3.1. Apparatus and reagents

Optical rotations were determined on a Horiba SEAP-300 spectropolarimeter (Horiba International Corporation, Kyoto, Japan). NMR spectra were recorded on a Bruker DRX-AV-500 spectrometer (Bruker BioSpin Group, Rheinstetten, Germany) at 500 MHz for 1H and 125 MHz for 13C using standard pulse sequence programs. All chemical shifts were recorded with respect to TMS as an internal standard. MS was obtained on a VG Auto Spec-3000 spectrometer (VG PRIMA, Birmingham, England). IR was measured on a Perkin-Elmer 241 polarimeter (PerkinElmer, Boston, MA, USA). Column chromatography was carried out on silica gel H (10–40 μm, Qingdao Haiyang Chemical Factory, Qingdao, China) and RP-18 (40–75 μm, Fuji Chemical Industrial Co., Ltd, Tochigi, Japan). TLC was performed on silica gel GF254 (Yantai Jiangyou Silica Gel Co., Ltd, Yantai, China). Solvents were of industrial purity and distilled prior to use.

3.2. Plant material

The whole plants of L. japonicum were collected from Pingbian County of Yunnan Province, China in August, 2011 and identified by Prof. Shugang Lu, School of Life Science, Yunnan University, Kunming, China, where a voucher specimen (No. 1108018) has been deposited.
3.3. Extraction and isolation

The air-dried powdered whole plants of *L. japonicum* (5.5 kg) were extracted with MeOH (25 L × 4) at room temperature. The MeOH extract was partitioned between EtOAc and 1% aq. H$_2$SO$_4$, and the acidic aqueous phase was basified with aq. Na$_2$CO$_3$ to pH 10 and the alkaloids were extracted with CHCl$_3$. The crude alkaloids (10 g) were subjected to silica gel chromatography eluting with gradient CHCl$_3$:MeOH (15:1 → 5:1) to provide fractions A–C. Fr. A (2.2 g) was further isolated on RP-18 column eluting with 80% aq. MeOH, and then purified on silica gel column with CHCl$_3$:MeOH (5:1) to afford 1 (30 mg), 2 (15 mg), 3 (45 mg) and 4 (20 mg). Fr. B (1.7 g) was subjected to RP-18 chromatography eluting with 50% aq. MeOH, and then isolated on silica gel column with CHCl$_3$:MeOH (5:1), to yield 5 (40 mg) and 6 (30 mg).

3.3.1. N-methylhydroxypropyllycodine (1)

Colourless gum; [α]$_D^{27}$ = 1.70 (c = 0.0053, MeOH); EI-MS: 314 [M]$^+$, HR-EI-MS m/z: 314.2364 [M]$^+$ (calcd for C$_{20}$H$_{30}$N$_2$O: 314.2358); IR (KBr): 3441, 2924, 2866, 1631, 1571, 1409, 1115, 1085; $^1$H NMR (CDCl$_3$, 500 MHz): δ 7.99 (1H, d, $J$ = 8.0 Hz, H-3), 6.95 (1H, d, $J$ = 8.0 Hz, H-2), 4.23 (1H, m, H-19), 2.70 (1H, m, Ha-9), 2.65 (3H, s, H-17), 2.64 (1H, m, Hb-6), 2.59 (1H, m, Hb-9), 2.08 (1H, m, H-7), 1.94 (1H, m, H-12), 1.83 (1H, m, Ha-10), 1.74 (1H, m, Ha-8), 1.53 (1H, m, Ha-11), 1.43 (2H, d, $J$ = 11.5 Hz, H-14), 1.30 (3H, d, $J$ = 6.0 Hz, H-20), 1.27 (1H, m, Hb-11), 1.26 (1H, m, Hb-8), 1.17 (1H, m, H-15), 1.14 (1H, m, Hb-10), 0.81 (3H, d, $J$ = 6.5 Hz, H-16); $^{13}$C NMR (CDCl$_3$, 125 MHz): δ157.2 (s, C-1), 157.1 (s, C-5), 135.7 (s, C-4), 135.3 (d, C-3), 121.5 (d, C-2), 67.2 (d, C-19), 59.4 (s, C-13), 50.5 (t, C-9), 48.1 (t, C-14), 44.1 (t, C-18), 43.8 (t, C-8), 36.4 (q, C-17), 35.2 (t, C-6), 34.5 (d, C-12), 33.8 (d, C-7), 26.9 (t, C-11), 26.5 (d, C-15), 23.1 (q, C-20), 22.4 (q, C-16), 19.6 (t, C-10).

4. Conclusion

In our study, six lycodine alkaloids were isolated from the club moss *L. japonicum* Thunb. N-methylhydroxypropyllycodine (1) was a new alkaloid with 2-hydroxy-propyl and N-Me groups.

Supplementary material

Supplementary material relating to this article is available online.

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