Hupermine A, a novel C_{16}N_{2}-type Lycopodium alkaloid from *Huperzia phlegmaria*

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**Abstract**

A novel C$_{16}$N$_{2}$-type Lycopodium alkaloid consisting of a quinolizidine with a 6-dimethylaminohexyl side chain, hupermine A (1), was isolated from the club moss of *Huperzia phlegmaria*, and the structure and relative stereochemistry were elucidated on the basis of spectroscopic data.

Lycopodium and *Huperzia* species are a well-known rich source of unique heterocyclic alkaloids of C$_{11}$N, C$_{16}$N, C$_{16}$N$_2$, C$_{22}$N$_2$, and C$_{27}$N$_3$ types and have attracted great interest from biogenetic\textsuperscript{1,2} and biological\textsuperscript{3} points of view. These unique skeletons have also been challenging targets for total synthesis.\textsuperscript{4} Among them, huperzine A, isolated from Chinese club moss *Lycopodium serratum* is a highly specific and potent inhibitor of acetylcholinesterase (AChE).\textsuperscript{3} The inherent inhibition of AChE has prompted the pursuit of the total synthesis\textsuperscript{5} and SAR\textsuperscript{6} studies of huperzine A. Recently, we isolated new types of alkaloids such as lycobeline A\textsuperscript{7} from *Huperzia goebelii*, lycotetrastine A\textsuperscript{8} from *Huperzia tetrasticha*, huperminone A\textsuperscript{9} from *H. phlegmaria*, lycocinidine A\textsuperscript{10} from *Lycopodium chinense* and lycoparin A\textsuperscript{11} from *L. casuarinoides*. During our continuing search for biogenetically interesting intermediates and new alkaloids with a novel skeleton from Lycopodium and *Huperzia* species, hupermine A (1), a novel alkaloid consisting of a quinolizidine with a 6-dimethylaminohexyl side chain was isolated from the club moss of *Huperzia phlegmaria* (L.) Rothm. In this Letter, we describe the isolation and structure elucidation of 1.

The club moss of *H. phlegmaria* (500 g) collected in Malaysia was extracted with MeOH (1.5 L $\times$ 3) at rt, and the extract (47 g) was partitioned between EtOAc and 3% aq tartaric acid. The water-soluble fraction was adjusted to pH 9 with saturated Na$_2$CO$_3$ and was extracted with CHCl$_3$. The CHCl$_3$-soluble fraction (730 mg) was subjected to an amino SiO$_2$ column (Hexane/EtOAc, 1:0 $\rightarrow$ 0:1, CHCl$_3$/MeOH, 1:0 $\rightarrow$ 0:1) and a SiO$_2$ column (CHCl$_3$/MeOH/TFA, 1:0:0 $\rightarrow$ 0:1:0.1) to afford hupermine A (1, 5.8 mg, 0.0012%) and huperzine A (80.5 mg, 0.015%).

**Table 1**

<table>
<thead>
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<th>Position</th>
<th>$\delta_H$</th>
<th>$\delta_C$</th>
<th>Position</th>
<th>$\delta_H$</th>
<th>$\delta_C$</th>
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<td>60.0</td>
<td>10a</td>
<td>1.13 (1H, m)</td>
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<tr>
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<td>27.8</td>
<td>10b</td>
<td>1.54 (1H, m)</td>
<td>25.9</td>
</tr>
<tr>
<td>3</td>
<td>1.30 (2H, m)</td>
<td>27.5</td>
<td>11a</td>
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<td>25.0</td>
<td>12a</td>
<td>1.90 (1H, m)</td>
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<tr>
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<td>12b</td>
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<tr>
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<td>32.8</td>
<td>19</td>
<td>3.32 (1H, br d, 13.6)</td>
<td>32.8</td>
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</table>

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Hupermine A\textsuperscript{12} \(\{\text{EI}^2\Delta^2 + 39 (c 1.0, \text{MeOH})\}\) showed a pseudomolecular ion peak at \(m/z\ 281 (M+H)^+\) in the ESIMS. The molecular formula was established to be \(C_{18}H_{36}N_{2}\) by the HRESITOFMS \(m/z\ 281.2960 (M+H)^+, \Delta +0.3\ \text{mmu}\). Its \(^{13}\text{C}\) NMR spectrum (Table 1) revealed eighteen carbon signals due to three \(sp^3\) methines, twelve \(sp^3\) methylenes, and three methyl groups. Among them, two \(sp^3\) methines (\(\delta_C\ 57.8; \delta_H\ 3.07,\) and \(\delta_C\ 50.6; \delta_H\ 2.91),\) two \(sp^3\) methylenes (\(\delta_C\ 49.2; \delta_H\ 2.68\) and \(3.32,\) and \(\delta_C\ 60.0; \delta_H\ 2.23\) and \(2.23))\) and two methyl groups (\(\delta_C\ 45.6; \delta_H\ 2.21 \times 2\)) were ascribed to those bearing a nitrogen atom.

The connectivity of almost all hydrocarbons was deduced from a detailed analysis of the \(^1\text{H}–^1\text{H}\) COSY spectrum shown in Figure 1. The presence of a quinolizidine ring was indicated by the HMBC correlations of H-9b/C-11 and C-13, and H-9a/C-7. Furthermore, the HMBC correlation of N-Me protons (\(\delta_H\ 2.21)\)/C-1 (\(\delta_C\ 60.0))\) established the connection among C-1, C-17, and C-18 through a nitrogen atom. Thus, the gross structure of hupermine A was elucidated to be 1, possessing a quinolizidine ring system and a dimethylaminohexyl group at C-7.

The relative stereochemistry of 1 was elucidated by the NOESY correlations and the \(^3\text{J}_{\text{H-H}}\) coupling constants (Fig. 2). The NOESY correlation of H-7/H-12b indicated the \(\text{cis}\)-junction of the quinolizidine ring, \(\alpha\)-orientation of H-7, and \(\beta\)-orientation of H-13. The coupling pattern of H-8a (ddd, 12.7, 12.7, 12.7) suggested the orientation of H-15 to be \(\alpha\).

A plausible biogenetic pathway of hupermine A (1) was proposed as shown in Figure 3. Hupermine A might be generated from a quinolizidine unit (A) and a \(\Delta^1\)-piperideine unit through C\textsubscript{16}N\textsubscript{2}-type cermizine D\textsuperscript{13} followed by cleavage of C-5\textsubscript{A}N bond.

On the basis of biogenetic considerations, the absolute stereochemistry of 1 was presumed to have the same as those of cermizine D.

Hupermine A (1) showed weak cell growth inhibitory activity against HL-60 cells (IC\textsubscript{50} 39 \(\mu\text{M}\)).\textsuperscript{14} Apart from cytotoxicity assessment, 1 was found to be inactive in inhibiting inducible nitric oxide synthase (iNOS) activity of murine monocyctic cell line, RAW246.7 cells.\textsuperscript{15} The anti-lipid droplet accumulation activity of 1 on murine pre-adipocyte cell lines, MC3T3-G2/PA6 cells;\textsuperscript{16} and anti-melanin deposition activity on murine melanoma cell line, B16-F10 cells were also negative.\textsuperscript{17} Presently, 1 is being assessed for further biological activities.

Acknowledgments

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Figure 1. Selected 2D NMR correlations for hupermine A (1).

Figure 2. Selected NOESY correlations for hupermine A (1).

Figure 3. Plausible biosynthetic pathway of 1.
References and notes


12. *Hupermine A* (1): colorless amorphous solid; δ H 3.93 (c 1.0, MeOH); IR (Zn–Se) v max 2929, 2856, and 1456 cm⁻¹; ESIMS m/z 281 (M+H)⁺; HRESMS m/z 281.2960 (M+H; calcd for C18H37N2, 281.2957).


