A new alkaloid from *Fritillaria ussuriensis* Maxim

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**A B S T R A C T**

Pingbeimunone A (1), a new compound, together with the known ussuriedine (2), benzof[7,8]fluoren[2,1-b]quinolizine cevane-3,6,16,20-tetrol (3), ebeiedinone (4), pingbeimine C (5) and verticine (6) were isolated from *Fritillaria ussuriensis*. The structure was elucidated on the basis of spectral analysis (IR, NMR and MS spectroscopy). In addition, their AChE inhibitory activities were also tested.

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1. Introduction

*Bulbus Fritillariae*, Beimu in Chinese, derived from the bulbs of several *Fritillaria* species (family Liliaceae) (such as *F. ussuriensis*, *F. puqiensis*, *F. hupehensis*, *F. cirrhosa* and *F. ebeiensis*), has been used as an antitussive and expectorant herb in traditional Chinese medicine for more than 2000 years [1]. In the 2005 edition of Chinese Pharmacopoeia, *F. ussuriensis* Maxim. (Ping-Beimu) was officially recorded. A number of alkaloids have been previously reported from this plant [2–4]. Steroidal alkaloids were established to be the major biologically active components in *F. ussuriensis* [5]. It is known that the alkaloids have been used in relieving cough, removing the phlegm and relieving asthma. Lin et al. reported that steroidal alkaloids (i.e., N-demethylpuqietine, hupheninoside, yibeinoside and chuanbeinone with IC₅₀ values of 6.4, 16.9, 6.5 and 7.7 μM, respectively) display AChE inhibitory activities from five *Fritillariae* species [6]. In the course of our search for naturally occurring alkaloids from *F. ussuriensis* Maxim, a new alkaloid together with five known alkaloids were found. Here we report on the structure elucidation of the new alkaloid 1 and acetylcholinesterase (AChE) inhibition test results for all six compounds (Fig. 1).

2. Materials and methods

2.1. General

Optical rotations were obtained on a Perkin-Elmer 341 Polarimeter. Melting points were measured on a Koller apparatus and were uncorrected. IR spectra were taken on a Nicolet 170SX FT-IR instrument. EIMS and HRESIMS were measured on HP 5988A GC/MS instrument and Bruker APEXII. NMR spectra were recorded with a Varian Mercury-400BB NMR spectrometer. Silica gel 200–300 mesh for column chromatography and silica GF254 for TLC were supplied by the Qingdao Marine Chemical Inc., China. Acetylcholinesterase (EC3.1.1.7, Sigma product No C2888), Acetylthiocholine iodide (ATCI), 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB), Huperzine A were purchased from Sigma (St Louis, MO, USA). MCI-GEL CHP 20P (75–150 μm) were from Mitsubishi Chemical Holdings Corp. Bovine serum albumin was from Roche (Shanghai, China) and all other organic solvents were analytical grade.

2.2. Plant materials

The bulbs of *F. ussuriensis* Maxim were purchased from Huanghe Herb Market in Lanzhou and identified by Associate Professor Lin Yang who majored in plant classification, School of Life Science and Engineering,
Lanzhou University of Technology, Lanzhou, China. A voucher specimen (No. 100428) is deposited at the School of Life Science and Engineering, Lanzhou University of Technology.

2.3. Extraction and isolation

The bulbs of F. ussuriensis Maxim (4.5 kg) were powdered and extracted by 0.5% H2SO4 aqueous solution (23 L×2). The

Table 1

<table>
<thead>
<tr>
<th>Position</th>
<th>δH (dd, J)</th>
<th>δC (DEPT)</th>
<th>Position</th>
<th>δH (d, J)</th>
<th>δC (DEPT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1α</td>
<td>1.26</td>
<td>44.77 (CH2)</td>
<td>14</td>
<td>-</td>
<td>143.74 (C)</td>
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<tr>
<td>1β</td>
<td>2.10</td>
<td>6.91 (d, J=8.0 Hz)</td>
<td>15</td>
<td>6.91 (d, J=8.0 Hz)</td>
<td>120.21 (CH)</td>
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<tr>
<td>2</td>
<td>3.65 (m)</td>
<td>71.41 (CH)</td>
<td>16</td>
<td>7.13 (d, J=8.0 Hz)</td>
<td>126.72 (CH)</td>
</tr>
<tr>
<td>3</td>
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<td>75.74 (CH)</td>
<td>17</td>
<td>-</td>
<td>141.91 (C)</td>
</tr>
<tr>
<td>4α</td>
<td>1.80 (m)</td>
<td>34.69 (CH2)</td>
<td>18</td>
<td>2.35 (s)</td>
<td>15.97 (CH3)</td>
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<tr>
<td>4β</td>
<td>1.86 (m)</td>
<td>71.78 (CH)</td>
<td>19</td>
<td>3.02 (s)</td>
<td>24.02 (CH3)</td>
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<td>5</td>
<td>2.30 (dd, J=13.2, 3.2 Hz)</td>
<td>60.20 (CH)</td>
<td>20</td>
<td>3.20 (m)</td>
<td>37.34 (CH)</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>214.70 (C)</td>
<td>21</td>
<td>2.57 (d, J=7.2 Hz)</td>
<td>22.21 (CH3)</td>
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<tr>
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<td>42.62 (CH2)</td>
<td>22</td>
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<td>67.94 (CH)</td>
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<tr>
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<td>3.16 (m)</td>
<td>23</td>
<td>3.16 (m)</td>
<td>71.78 (CH)</td>
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<td>45.18 (CH)</td>
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<td>1.99 (m)</td>
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<td>10</td>
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<td>2.30 (d, J=7.2 Hz)</td>
<td>32.51 (CH)</td>
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<tr>
<td>11α</td>
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<td>30.51 (CH2)</td>
<td>26α</td>
<td>2.86 (dd, J=10.8, 3.8 Hz)</td>
<td>54.58 (CH2)</td>
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<td>2.06 (dd, J=11.2, 10.8 Hz)</td>
<td>26β</td>
<td>2.06 (dd, J=11.2, 10.8 Hz)</td>
<td>-</td>
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<td>12</td>
<td>-</td>
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<td>0.83 (d, J=6.8 Hz)</td>
<td>19.13 (CH3)</td>
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<tr>
<td>13</td>
<td>-</td>
<td>134.22 (C)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

* NMR data were obtained in CD3OD solutions. Assignments were aided by a combination of 1H–1H COSY, HMQC, HMBC and NOESY experiments.
acid water solution was basified to pH = 11 with NaOH (4 M) and then extracted with chloroform (46 L × 2) to obtain the total alkaloid (4.5 g). The alkaloidal extract was subjected to column chromatography over a MCI-GEL using a gradient solvent system MeOH-H2O (50%, 60%, 70%, 80%, 90%) (V/V) to afford seven fractions (Fr. 1 to Fr. 7). Fr. 2 was subjected to silica gel column chromatography eluting with EtOAc-MeOH (40:1) (V/V) to yield compounds 1 (25 mg) and 5 (180 mg). Fr. 3 was subjected to silica gel column chromatography eluting with EtOAc-MeOH (40:1) (V/V) to yield compound 2 (34 mg). Fr. 4 was recrystallized with methanol to yield compound 3 (48 mg). Fr. 5 was subjected to silica gel column chromatography eluting with petroleum ether-acetone (3:1) (V/V) to yield compound 6 (39 mg). Fr. 6 was subjected to silica gel column chromatography eluting with petroleum ether-EtOAc (1:1) (V/V) to yield compound 4 (42 mg).

Pingbeimunone A (1): White amorphous powder, mp 155.1–157.5 °C; [α]20 D −3° (c 1.0, MeOH); IR (KBr): νmax: 3240–3497, 1706 cm−1; HRESIMS m/z: 442.2945 [M+H]+ (calcd for C27H40NO4, 442.2953); For 1H and 13C NMR spectral data see Table 1.

2.4. Bioassay procedures for AChE inhibition

The six compounds were tested for AChE inhibiting activity by Ellman’s method in 96-well microplates [7,8]. Briefly, 140 μL of 0.1 M sodium phosphate buffer (pH = 8.0), 20 μL sample solution and 15 μL enzyme solution were mixed and
incubated at 4 °C for 20 min. 10 μL of 0.01 M DTNB was added and the reaction was then started by adding 10 μL of 0.075 M ATCI. After incubating the reaction solution at 37 °C for 20 min, the optical densities were measured in a 96-well plate reader at 405 nm immediately. A blank positive control was set up by adding 20 μL Huperzine A (0.100 μg/mL in phosphate buffered saline) instead of 20 μL sample solution. Blanks were set up by adding 20 μL buffer solutions instead of 20 μL sample solution. Experiment control was set up by adding 15 μL buffer solutions instead of 15 μL enzyme solution in order to deduct sample background. All reactions were carried out thrice. The inhibition rate (%) was calculated by the following equation:

\[
\text{Inhibition}^\% = \frac{(\text{Blank} - \text{Blank positive control}) - (\text{Experiment} - \text{Experiment control})}{(\text{Blank} - \text{Blank positive control})} \times 100\%.
\]

3. Results and discussion

Compound 1, an amorphous powder, was shown to have the molecular formula C_{27}H_{39}NO_{4} by HRESIMS (m/z 442.2945 [M + H]^+) and \(^{13}\)C NMR spectrometry (Table 1).
Its IR spectrum displayed a broad hydroxyl and amine group absorption at $\nu_{\max}=3240-3497$ cm$^{-1}$ and a carbonyl band at 1706 cm$^{-1}$. Analysis of the $^1$H, $^{13}$C NMR and DEPT data (Table 1) and HMOC spectra of 1 revealed the presence of nine sp$^3$ methines, six sp$^3$ methylenes, one sp$^3$ quaternary carbons, six aromatic carbons (two sp$^2$ methines and four sp$^2$ quaternary carbons, $\delta$H 6.91 (d, J = 8.0 Hz), 7.13 (d, J = 8.0 Hz); $\delta$C 143.16, 134.22, 143.74, 120.21, 126.72, 141.91), one carbonyl carbon ($\delta$C 143.16) and four methyl groups. Among them, three sp$^3$ methines ($\delta$H 3.65 (m), 3.35 (m), 3.16 (m); $\delta$C 71.41, 75.74, 71.78) were ascribed to those bearing an oxygen atom, one sp$^3$ methine ($\delta$H 2.47 (dd, J = 11.9, 7.0 Hz); $\delta$C 67.94) and one sp$^3$ methylene ($\delta$H 2.86 (dd, J = 10.8, 3.8 Hz), 2.06 (dd, J = 11.2, 10.8 Hz); $\delta$C 54.58) were ascribed to those bearing a nitrogen atom. DEPT spectrometry showed 1 had 35 hydrogen atoms at least, while molecular formula of 1 was $C_{27}H_{36}NO_4$, as implied 1 had four active hydrogen atoms. These active hydrogen atoms can only be assigned to three hydroxyl and one amine groups. The $^1$H NMR and $^{13}$C NMR data of 1 closely related to those of known veratramine derivatives [9], which suggest 1 have same skeleton with veratramine. All $^1$H and $^{13}$C NMR signals were assigned by detailed interpretations of 2D-NMR experiments, including $^1$H, $^1$H-COSY, HMOC, HMBC and NOESY (Figs. 2, 3). The HMBC cross-peaks of H-4 with C-2; H-2 with C-10; H-3 with C-5; H-1 with C-3; H-20 with C-23; H-4, H-5, H-7, H-8 with C-6 showed that the three –OH groups were attached to C-2, C-3, C-23, respectively and carbonyl carbon was localized at C-6. The relative stereochemistry of 1 was elucidated by NOESY correlations and by comparison of chemical shifts with known veratramine derivatives. The NMR data of C-20-C-27 in 1 were almost identical with those of veratramine derivatives, which suggested relative stereochemistry of 1 at C-20-C-27 position was identical to (23R)-12, 13, 14, 15, 16, 17-hexadehydro-23-hydroxy-5α-veratranin-3-one [9]. This conclusion was further confirmed by observed NOESY correlations of H-20 to H-18, H-22; H-22 to H-18, H-24β, H-26β; H-23 to H-24α, H-24β to CH$_2$-27; H-24ç to H-25; H-25 to H-26ç and CH$_3$-27 to H-26β. Observed correlations of H-19 to H-1β, H-8; H-1β to H-2; H-1α to H-3, H-5; H-3 to H-5; H-5 to H-9 in NOESY spectra indicated 2-OH, 5-H, 9-H should be $\alpha$ orientation and 3-OH, 8-H should be $\beta$ orientation. Accordingly, compound 1 is a new alkaloid and named as pingbeimuone A.

Possible biogenetic pathway for pingbeimuone A was described in Scheme 1. The double bond of Veratramine 7, a natural product isolated from Psidium guajava which belongs to the Liliaceae family [10] and its biosynthesis is well-studied [11,12], was converted into ketone 8 by oxidation, followed by elimination of the alcohol to afford the corresponding alkene 9, 9 was subjected to an epoxidation followed by ring opening then afforded compound 1.

The five known compounds were identified as ussurine-dine (2) [2], benzo[7,8]fluoreno[2,1-b]quinolizine cevane-3,6,16,20-tetrol (3) [3], ebeiedine (4) [13], pingbeimuone C (5) [4], verticine (6) [14] by comparing their physical data (mp, MS, $^1$H and $^{13}$C NMR) with those reported in the literature.

The AChE inhibitory activities in vitro of compounds 1–6 were tested. Unfortunately none of these compounds showed appreciable AChE inhibitory activity at a concentration of 100 $\mu$g/mL (Table 2).

### Conflicts of interest

The authors declare that there are no conflicts of interest.

### Acknowledgments

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### References


