

Alkaloids from *Fritillaria Hupehensis*

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Phytochemical investigation of the bulbs of *Fritillaria hupehensis* resulted in the isolation and structural elucidation of a new highly conjugated alkaloid of veratramine type identified as 3 $\beta$ ,23 $\beta$ -dihydroxy-7,12(14)-dien-5 $\alpha$ -veratramin-6-one (**1**), together with two known alkaloids, ebeinine (**2**) and zhebeinine (**3**), which were isolated for the first time from *F. hupehensis*. The structures of alkaloids **1**—**3** were established by extensive 1D and 2D NMR spectral analyses.

**Keywords** *Fritillaria hupehensis*, alkaloid, veratramine group

## Introduction

*Fritillaria hupehensis* Hsiao et K.C. Hsia is a well known antitussive traditional Chinese medicine used for a long time, especially in the folk of Hubei province. Its bulbs have been recorded in the Pharmacopoeia of the People's Republic of China named Hubeibeimu.<sup>1</sup> The pharmacological studies indicate that the bulbs have good antitussive, expectorant and antiasthmatic activities.<sup>2,3</sup> Some C-nor-D-homo steroidal alkaloids have been hitherto reported with regards to the chemical constituents of *F. hupehensis*.<sup>4-12</sup> This paper describes the isolation of three trace alkaloids, including a new highly conjugated alkaloid of veratramine type and two known alkaloids, ebeinine and zhebeinine, which were isolated for the first time from *F. hupehensis*. The structure of the new trace alkaloid was elucidated on the basis of extensive spectral analyses (<sup>1</sup>H NMR, <sup>13</sup>C NMR, DEPT, <sup>1</sup>H-<sup>1</sup>H COSY, HSQC, HMBC and NOESY).

## Experimental

### Methods

Melting points were obtained on an XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were recorded with a Perkin-Elmer model 341 spectropolarimeter. UV spectra were measured with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. IR spectra were measured on a Shimadzu IR-460 spectrophotometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker AM-400D NMR spectrometer with TMS as an internal standard. FABMS and HREIMS were determined on a VG Auto spec-3000

spectrometer. Silica gel H (160—200 mesh, Qingdao Marine Chemical Group Co., Qingdao, China) was used for column chromatography and silica gel GF<sub>254</sub> (Qingdao Marine Chemical Group Co.) for TLC. Spots were visualized by spraying with Dragendorff's reagent.

### Plant material

The bulbs of *F. hupehensis* were collected from Enshi county, Hubei province, People's Republic of China, in April 2004, and identified by WU Ji-Zhou (Faculty of Pharmaceutical Sciences, Tongji Medical College). Voucher specimens (JZ-0401) of the plant were deposited with the Herbarium of Faculty of Pharmaceutical Sciences, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China.

### Extraction and isolation

The powdered bulbs (100 kg) of *Fritillaria hupehensis* were extracted with 95% EtOH under reflux (3 × 300 L) for three times (2, 1 and 1 h, respectively). After concentration of the combined extracts under vacuum, the residue was dissolved in 2% HCl solution. The acidic solution was basified with ammonia to pH above 11, so alkaloids were deposited and further dissolved in chloroform. The produced total alkaloids were 1120 g after the removal of chloroform. The alkaloids were further fractionated by repeated column chromatography of silica gel to obtain alkaloids **1** (7 mg), **2** (8 mg) and **3** (6 mg), using petroleum-Me<sub>2</sub>CO-Et<sub>2</sub>NH as elution solvent.

**1**: yellow powder (MeOH), m.p. 132.0—133.1 °C, [ $\alpha$ ]<sub>D</sub><sup>20</sup> -0.006 (c 0.001, CHCl<sub>3</sub>); UV (MeOH)  $\lambda$ <sub>max</sub> (log

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Received September 28, 2006; revised March 12, 2007; accepted July 8, 2007.

Project supported by the National Natural Science Foundation of China (No. 30572313) and Great Foundation on Society Development of China, Hubei Provincial Science & Technology Department (No. 2003AA302B08).

$\epsilon$ ): 242 (4.28), 348 (3.56) nm; IR (KBr)  $\nu$ : 3392, 2924, 1608, 1458, 1377  $\text{cm}^{-1}$ ; FAB-MS  $m/z$ : 428  $[\text{M}+1]^+$ ; HR-EI-MS calcd for  $\text{C}_{27}\text{H}_{41}\text{NO}_3$ : 427.3086, found 427.3090. The data of  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz) are listed in Table 1.

**2**: colorless raphide crystals (EtOAc), m.p. 99.7—101.2  $^\circ\text{C}$ ,  $[\alpha]_{\text{D}}^{20} -61.2$  ( $c$  0.12,  $\text{CHCl}_3$ ); IR (KBr)  $\nu$ : 3494, 2936, 2873, 2750, 1700, 1468, 1435, 1030  $\text{cm}^{-1}$ ; FAB-MS  $m/z$ : 414  $[\text{M}+1]^+$ ; HR-EI-MS calcd for

$\text{C}_{27}\text{H}_{43}\text{NO}_2$ : 413.3294, found 413.3289. The data of  $^1\text{H}$  and  $^{13}\text{C}$  NMR are listed in Table 2.

**3**: colorless raphide crystals (EtOAc), m.p. 189.1—190.0  $^\circ\text{C}$ ,  $[\alpha]_{\text{D}}^{20} -61.2$  ( $c$  0.11,  $\text{CHCl}_3$ ); IR (KBr)  $\nu$ : 3410, 2930, 2870, 2760, 1700, 1465, 1435  $\text{cm}^{-1}$ ; FAB-MS  $m/z$ : 414  $[\text{M}+1]^+$ ; HR-EI-MS calcd for  $\text{C}_{27}\text{H}_{43}\text{NO}_2$ : 413.3294, found 413.3290. The data of  $^1\text{H}$  and  $^{13}\text{C}$  NMR are shown in Table 2.

**Table 1** NMR data of compound **1** ( $\text{CDCl}_3$ , 400 MHz)

No.	$\delta_{\text{C}}$	$\delta_{\text{H}}$	HMBC	$^1\text{H}$ - $^1\text{H}$ COSY	NOESY
1 $\alpha$	37.6	1.30—1.38 (m)	C-2, 3, 10, 19	H-1 $\beta$ , 2 $\alpha$	H-2 $\alpha$ , 3, 5, 9
1 $\beta$		1.57—1.61 (m) <sup>a</sup>	C-2, 3, 5, 10	H-1 $\alpha$ , 2 $\beta$	H-2 $\beta$ , 19
2 $\alpha$	31.5	2.04—2.12 (m) <sup>a</sup>	C-1, 3, 4, 10	H-1 $\alpha$ , 2 $\beta$ , 3	H-1 $\alpha$ , 3
2 $\beta$		2.25—2.31 (m) <sup>a</sup>	C-1, 3, 4, 10	H-1 $\beta$ , 2 $\alpha$	H-1 $\beta$
3	70.7	3.88—3.94 (m)	C-1, 2, 4, 5	H-2 $\alpha$ , 4 $\alpha$ , 4 $\beta$	H-1 $\alpha$ , 2 $\alpha$ , 4 $\alpha$ , 5
4 $\alpha$	31.9	2.84—2.92 (m) <sup>a</sup>	C-2, 3, 5, 6, 10	H-3, 4 $\beta$ , 5	H-3, 4 $\beta$ , 5
4 $\beta$		1.67—1.77 (m)	C-3	H-3, 4 $\alpha$ , 5	—
5	54.9	2.24—2.32 (dd, $J=2.7, 8.8$ Hz)	C-4, 6, 7, 10, 19	H-4 $\alpha$ , 4 $\beta$	H-1 $\alpha$ , 3, 4 $\alpha$ , 9
6	199.7		—	—	—
7	113.3	5.93 (s)	C-5, 6, 8, 9, 14	H-9	H-15 $\alpha$
8	171.5		—	—	—
9	53.7	2.74 (br s)	C-8, 10, 11, 14, 19	H-7, 11 $\alpha$	H-1 $\alpha$ , 5
10	40.4		—	—	—
11 $\alpha$	33.8	2.55 (br s)	C-9, 12, 14	H-9, 11 $\beta$	H-18, 19
11 $\beta$		2.04 (br s) <sup>a</sup>	C-9, 12	H-11 $\alpha$	—
12	163.4		—	—	—
13	35.2	2.87—2.97 (m)	—	—	—
14	135.4		—	—	—
15 $\alpha$	22.9	2.21—2.29 (m) <sup>a</sup>	C-8, 12, 14, 16, 17	H-15 $\beta$ , 16 $\alpha$	H-16 $\alpha$
15 $\beta$		1.10—1.16 (m) <sup>a</sup>	C-14, 16, 17	H-15 $\alpha$ , 16 $\beta$	H-16 $\beta$
16 $\alpha$	23.2	2.07—2.11 (m)	C-13, 15, 17	H-15 $\alpha$ , 16 $\beta$ , 17	H-15 $\alpha$ , 17
16 $\beta$		1.60—1.66 (m) <sup>a</sup>	C-13, 15, 17	H-15 $\beta$ , 16 $\alpha$	H-15 $\beta$
17	40.0	2.33—2.39 (m)	C-13, 15, 16, 18, 20, 21, 22	H-16 $\alpha$ , 22	H-16 $\alpha$ , 20, 23
18	13.4	1.13 (d, $J=7.3$ Hz)	C-12, 13, 17	—	H-11 $\alpha$ , 20
19	12.3	0.62 (s)	C-1, 5, 9, 10	—	H-1, 11 $\alpha$
20	37.8	2.11—2.19 (m)	C-13, 16, 17, 21, 22	H-21	H-17, 18, 21, 22
21	14.6	1.29 (d, $J=7.3$ Hz)	C-17, 20, 22	H-20	H-20
22	70.4	2.52 (dd, $J=2.1, 7.4$ Hz)	C-20, 21, 23, 24, 26	H-17, 23	H-20, 21, 24 $\alpha$
23	68.7	3.75—3.83 (m)	C-20, 22, 24, 25	H-22, 24 $\alpha$ , 24 $\beta$	H-17
24 $\alpha$	45.2	2.20—2.26 (m) <sup>a</sup>	C-22, 23, 25, 26, 27	H-23, 24 $\beta$ , 25	H-24 $\beta$
24 $\beta$		1.31—1.37 (m)	C-23, 25	H-23, 24 $\alpha$	H-24 $\alpha$
25	32.3	1.61—1.67 (m) <sup>a</sup>	C-23, 24, 26, 27	H-24 $\alpha$ , 26 $\alpha$ , 26 $\beta$ , 27	H-26 $\alpha$ , 27
26 $\alpha$	55.1	3.04 (dd, $J=2.4, 8.2$ Hz)	C-22, 24, 25, 27	H-25, 26 $\beta$	H-25, 26 $\beta$
26 $\beta$		2.17—2.23 (m) <sup>a</sup>	C-22, 24, 25	H-25, 26 $\alpha$	H-26 $\alpha$ , 27
27	19.3	0.73 (d, $J=8.2$ Hz)	C-24, 25, 26	H-25	H-25, 26 $\beta$

<sup>a</sup> Overlapped.

**Table 2**  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts of alkaloids **2** and **3** ( $\text{CDCl}_3$ , 400 MHz)

Position	<b>2</b>		<b>3</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1 $\alpha$	1.20—1.30 (m)	38.3	1.20—1.30 (m)	37.0
1 $\beta$	1.56—1.64 (m)		1.57—1.65 (m)	
2 $\alpha$	1.16—1.22 (m)	30.3	1.07—1.15 (m)	30.1
2 $\beta$	1.77—1.85 (m)		1.72—1.84 (m)	
3	3.53—3.57 (m)	70.8	3.52—3.60 (m)	71.0
4 $\alpha$	1.40—1.48 (m)	30.0	1.41—1.49 (m)	30.5
4 $\beta$	1.85—1.95 (m)		1.92—1.98 (m)	
5	2.08—2.18 (m)	56.6	2.09—2.15 (m)	56.8
6		211.0		211.2
7 $\alpha$	1.94—2.00 (m)	46.0	1.94—2.02 (m)	46.0
7 $\beta$	2.41—2.49 (m)		2.45—2.53 (m)	
8	1.77—1.85 (m)	43.7	1.77—1.85 (m)	41.2
9	1.55—1.65 (m)	56.7	1.60—1.66 (m)	56.8
10		38.3		38.4
11	0.97—1.07 (m)	30.0	1.06—1.12 (m)	30.2
12	1.68—1.74 (m)	41.3	1.70—1.76 (m)	40.2
13	1.07—1.17 (m)	40.3	1.11—1.19 (m)	44.8
14	1.58—1.64 (m)	44.3	1.63—1.71 (m)	44.4
15 $\alpha$	0.77—0.87 (m)	25.2	0.78—0.88 (m)	25.3
15 $\beta$	1.80—1.86 (m)		1.72—1.82 (m)	
16	1.55—1.61 (m)	25.1	1.57—1.61 (m)	24.5
17	0.57—0.65 (m)	46.3	0.58—0.64 (m)	46.4
18 $\alpha$	1.61—1.69 (m)	61.6	1.62—1.66 (m)	61.2
18 $\beta$	2.71—2.79 (m)		2.70—2.80 (m)	
19	0.73 (s)	12.7	0.72 (s)	12.8
20	1.01—1.05 (m)	36.9	0.95—1.01 (m)	41.1
21	0.80 (d, $J=7.8$ Hz)	14.6	0.82 (d, $J=7.8$ Hz)	14.9
22	1.31—1.41 (m)	69.0	1.37—1.43 (m)	68.4
23 $\alpha$	1.02—1.10 (m)	24.5	1.03—1.11 (m)	28.8
23 $\beta$	1.88—1.98 (m)		1.87—1.95 (m)	
24 $\alpha$	1.75—1.85 (m)	28.8	1.78—1.88 (m)	33.5
24 $\beta$	2.09—2.13 (m)		2.15—2.23 (m)	
25	1.80—1.86 (m)	28.2	1.81—1.87 (m)	30.8
26 $\alpha$	1.61—1.69 (m)	62.0	1.62—1.70 (m)	64.9
26 $\beta$	2.58—2.64 (m)		2.61—2.69 (m)	
27	1.05 (d, $J=7.8$ Hz)	18.2	0.84 (d, $J=7.6$ Hz)	19.7

## Results and discussion

The total alkaloids of the powdered bulbs of *Fritillaria hupehensis* were separated and purified by repeated chromatography, allowing the isolation of a new trace alkaloid and two trace known alkaloids which were isolated from *F. hupehensis* for the first time. The

chemical structure of the new compound was characterized as follows.

Compound **1** (yellow powder) was assigned a molecular formula of  $\text{C}_{27}\text{H}_{41}\text{NO}_3$  elucidated by HREIMS, which was consistent with the data from the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. The IR spectrum exhibited strong absorptions due to hydroxy ( $3392\text{ cm}^{-1}$ ) and carbonyl ( $1608$

$\text{cm}^{-1}$ ) functional groups. The UV spectrum [ $\lambda_{\text{max}}(\log \epsilon)$ : 242 (4.28), 348 (3.56)] indicated the presence of an  $\alpha,\beta,\gamma,\delta$ -unsaturated ketone group.<sup>13,14</sup> The NMR spectra of **1** indicated the presence of five quaternary carbons (including one ketonic carbon and three olefinic carbons), ten tertiary carbons (including two oxygenated carbons and one olefinic carbon), eight secondary carbons and four primary carbons. Analysis of NMR spectra of **1**, combined with the correlation of biosynthesis between natural products in the same plant, led to the conclusion that **1** was a C-nor-D-homo steroidal alkaloid of veratramine group<sup>13,14</sup>. The HMBC experiment revealed the veratramine-7,12(14)-dien-6-one fragment because of the correlations of H(4 $\alpha$ )/C(6), H(5)/C(6), H(7)/C(6), H(5)/C(7), H(9)/C(8), H(9)/C(14), H(11 $\alpha,\beta$ )/C(12), H(11 $\alpha$ )/C(14), H(15 $\alpha,\beta$ )/C(14) and H(15 $\alpha$ )/C(12). The absence of H(17)/H(23) COSY correlation and C(17)/H(23), H(17)/C(23) HMBC correlations suggested the 17,23-seco group.

The relative configurations of the substituents were revealed by the analysis of the ROESY spectrum of **1**, and correlations of H(3)/H(5), H(5)/H(9), H(13)/H(17), H(17)/H(20), H(20)/H(22) and H(23)/H(25) could be clearly observed from the spectrum, suggesting that the protons at C(3), C(5), C(9), C(13), C(17), C(20), C(22), C(23) and C(25) possess  $\alpha,\alpha,\alpha,\beta,\beta,\beta,\beta,\alpha$  and  $\alpha$  orientations, respectively. Thus, **1** was elucidated as 3 $\beta$ ,23 $\beta$ -dihydroxy-7,12(14)-dien-5 $\alpha$ -veratramin-6-one (Figure 1).

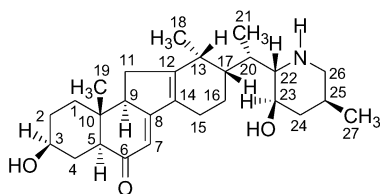


Figure 1 The structure of compound **1**.

Only incomplete  $^1\text{H}$  NMR data of ebeinine (**2**) and zhebeirine (**3**) were reported in references,<sup>15,16</sup> and detailed 1D and 2D NMR data were also described and assigned in this paper. Their FAB-MS showed  $[\text{M}+1]^+$  peaks at  $m/z=414$  altogether, corresponding to the molecular formula  $\text{C}_{27}\text{H}_{43}\text{NO}_2$  based on their HREIMS. Their IR spectra ( $2750\text{ cm}^{-1}$ ) suggested typical signals for *trans*-quinolizidine skeleton. They were identified to be ebeinine and zhebeirine by direct comparison with the authentic samples, respectively. The detailed  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts were assigned by DEPT, HSQC, HMBC and NOESY experiments<sup>17</sup>, and they were isomers on the configuration of 25-carbon.

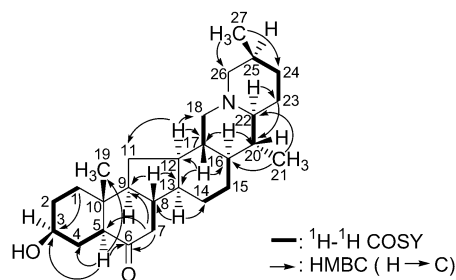


Figure 2 The  $^1\text{H}$ - $^1\text{H}$  COSY and key HMBC correlations of compound **2**.

## Acknowledgements

We gratefully acknowledge Dr. Xiao Wei-Lie (Kunming Institute of Botany, Chinese Academy of Sciences, Kunming) for the technical support with 2D NMR measurements.

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(E0609283 ZHAO, C. H.; ZHENG, G. C.)