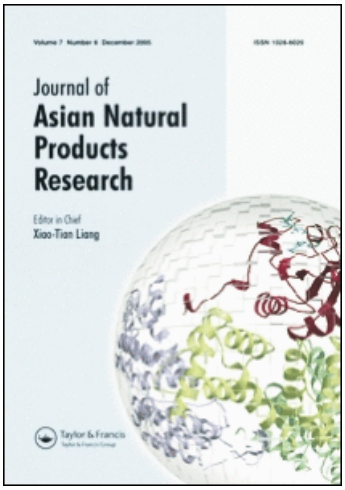

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Steroidal alkaloids from bulbs of *Fritillaria lichuanensis*

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A new steroidal alkaloid, hupehenizioiside (**1**), together with four known steroidal alkaloids hupehenizine (**2**), hupehenirine (**3**), peiminine (**4**) and hupeheninoside (**5**), were isolated and identified from the bulbs of *Fritillaria lichuanensis*. The structure of hupehenizioiside (**1**) was determined to be (20*R*,25*S*)-5α,14α,17β-cevanine-6-oxo-3β-*O*-β-D-glucoside by spectral analysis and chemical evidence. Compounds (**2**)–(**5**) were isolated from *Fritillaria lichuanensis* for the first time.

Keywords: *Fritillaria lichuanensis*; Liliaceae; Steroidal alkaloid; Hupehenizioiside

1. Introduction

Fritillaria lichuanensis P. Li *et* C.P. Yang is a new *Fritillaria* species growing in the northwest district of Hubei province, China. With regard to the non-basic chemical constituents of the bulbs, we have reported the presence of cholest-5-en-3-oxyl hexadecanoate, octadecanoic acid, palmitic acid, *ent*-kauran-16β,17-diol, β-sitosterol and β-daucosterol [1]. From the basic fraction, we reported two new *C-nor-D-homo* steroidal alkaloids lichuanine and lichuanisinine [2]. In the course of our continuing studies on the plant, a new steroidal alkaloid, hupehenizioiside (**1**), was isolated from the bulbs of *Fritillaria lichuanensis* together with four known steroidal alkaloids hupehenizine (**2**), hupehenirine (**3**), peiminine (**4**) and hupeheninoside (**5**) (figure 1). This paper describes the isolation and structural elucidation of these alkaloids.

2. Results and discussion

Compound **1** was isolated as white feathery needles ($CHCl_3$–MeOH), mp 258–260°C. HR-MS showed the molecular ion peak at *m/z* 576.3906 $[M + 1]^+$, corresponding to the formula $C_{33}H_{53}NO_7$. In the FAB-MS the fragments at *m/z* 576 $[M + H]^+$, 574 $[M - H]^+$

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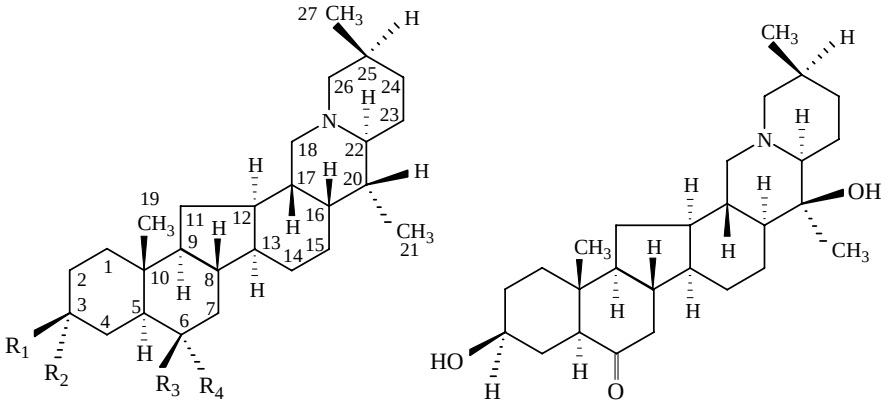

	R_1	R_2	R_3	R_4
(1)	glc	H	=O	
(2)	OH	H	=O	
(3)	=O		OH	H
(5)	glc	H	OH	H

(4)

Figure 1. Structures of **1**, **2**, **3**, **4** and **5**.

were observed. The IR spectrum of **1** showed the presence of hydroxyl group (3430 cm^{-1}, 1042 cm^{-1}), a carbonyl group (1699 cm^{-1}), and *trans*-quinolizidine moiety (2760 cm^{-1}), indicating the E and F rings junction is *trans* [3]. The ^{1}H NMR spectrum of **1** exhibited a singlet signal at δ 0.72 (3H), which was assigned to angular methyl group at C-19 of the steroidal alkaloid having the carbonyl group at C-6, the tertiary methyl signal (H_3-19) was shifted upfield by carbonyl group at C-6. The secondary methyl signal at δ 0.83 (3H, d, $J = 7.2$ Hz) showed the presence of an α-equatorial methyl group at C-20; another secondary methyl signal at δ 1.09 (3H, d, $J = 6.6$ Hz) showed the presence of a β-axial methyl group at C-25 [4]. A methine proton on carbon bearing a hydroxyl group at δ 3.84 (1H, m, $W_{1/2} = 24$ Hz, H-3) was shifted downfield in comparison with that of **2** due to the glycosylation shift [5]. The ^{13}C NMR spectrum of **1** showed 33 carbon signals, including three methyls, 12 methylenes, 16 methines, one quaternary carbon, and a carbonyl carbon on the basis of a DEPT experiment. The assignment of the ^{13}C NMR signals (table 1) was made by 2D NMR spectroscopy. The ^{13}C NMR signals of **1** are similar to **2** except for C-3 (δ 76.3).

Table 1. ^{13}C NMR spectral data of **1**.

No.	***1***	*No.*	***1***	*No.*	***1***
1	37.5	12	38.4	23	24.7
2	30.3	13	39.5	24	30.3
3	76.3	14	40.9	25	28.4
4	30.1	15	26.8	26	59.1
5	56.4	16	17.1	27	18.2
6	211.8	17	46.8	1′	100.7
7	46.9	18	56.5	2′	73.2
8	39.5	19	12.7	3′	78.0
9	56.5	20	35.6	4′	69.5
10	38.4	21	15.6	5′	75.5
11	28.5	22	62.0	6′	61.5

The glycosylation shift shows that the sugar moiety is attached to C-3; the anomeric configuration of the sugar moiety was assigned as β-glucopyranose from the coupling constant of $J = 7.8$ Hz and the characteristic signals at δ 100.7 (C-1), 73.2 (C-2), 78.0 (C-3), 69.5 (C-4), 75.5 (C-5) and 61.5 (C-6).

Acid hydrolysis of **1** with 1 M HCl gave hupehenizine and D-glucose. From the above evidence, the structure of compound **1**, named hupehenizioside, was determined to be (20*R*,25*S*)-5α,14α,17β-cevanine-6-oxo-3β-*O*-β-D-glucoside.

3. Experimental

3.1 General experimental procedures

The melting points were determined on an X4 apparatus and are uncorrected. The IR spectra were recorded on a Mi-colet 306 FT-IR spectrometer. The MS spectra were measured on a JEOL JMS-DX-300 mass spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were run on a GE-omega 600 spectrometer (600 and 150 Hz, respectively) by using DMSO-d_6 as the solvent. The TLC was performed on silica gel (Qingdao Marine Chemical Inc., China) by using Dragendoff's reagent for detection. The column chromatography was carried out on a silica gel column (100–200 mesh).

3.2 Plant material

The plants of *Fritillaria lichuanensis* were collected and identified by De-Tai Peng, Lichuan Institute of Chinese Materia Medica, China. A voucher specimen is deposited in the Lichuan Institute of Chinese Materia Medica.

3.3 Extraction and isolation

The powdered crude bulbs (7 kg) of *F. lichuanensis* were extracted with 95% EtOH. The EtOH extract was dissolved in 2% HCl. The acidic solvent was basified with ammonia to pH $>$ 11, followed by chloroform extraction to give the crude total alkaloid (32 g), which was further fractionated by column chromatography on silica gel with petroleum/Me_2CO/Et_2NH containing increasing contents of Me_2CO to yield hupehenizioside (**1**), hupehenizine (**2**), hupehenirine (**3**), peiminine (**4**) and hupeheninoside (**5**).

3.3.1 Compound 1. White feathery needles ($CHCl_3$–MeOH), mp 258–260°C. HR-MS *m*/*z*: 576.3906 $[M + 1]^+$ (calcd for $C_{33}H_{53}NO_7$, 575.7858); IR ν_{max} (KBr) cm^{-1}: 3430, 1042, 2930, 2870, 1468–1435, 2760. FAB-MS *m*/*z* 576 $[M + H]^+$, 574 $[M - H]^+$. ^{1}H NMR (MeOD): δ 0.72 (3H, s, H_3-19), 1.09 (3H, d, $J = 6.6$ Hz, H_3-27), 0.83 (3H, d, $J = 7.2$ Hz, H_3-21), 3.84 (1H, m, $W_{1/2} = 24$ Hz, H-3). ^{13}C NMR (MeOD): see table 1. A solution of (**1**) (5 mg) in 0.05 N HCl in MeOH was heated at 80°C for 2 h. The reaction mixture was basified with ammonia to pH $>$ 11, and then extracted with $CHCl_3$. From the $CHCl_3$ layer and the reaction solvent, hupehenizine and D-glucose were detected by TLC, by direct comparison with authentic samples.

3.3.2 Compounds 2–5. The structures of compounds **2**–**5** were deduced from their MS, ^{1}H NMR, and ^{13}C NMR spectra. A comparison of their physical and spectral data (mp, IR, EIMS, ^{1}H NMR, ^{13}C NMR) with the reported literature values [6,7] as well as a direct comparison with authentic samples proved their structures to be hupehenizine, hupehenirine, peiminine and hupeheninoside.

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