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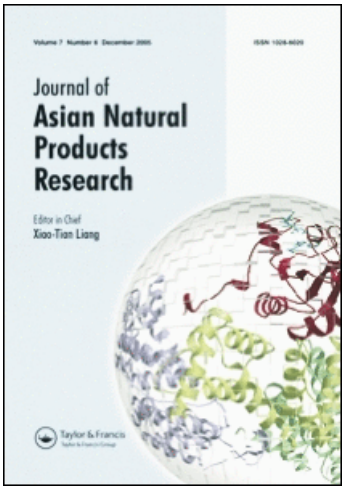

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H. -f. Pi[a]; H. -l. Ruan[a]; Y. -h. Zhan[a]; J. -z. Wu[a]
[a] Faculty of Pharmaceutical Sciences, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China

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Two new steroidal alkaloids from bulbs of *Fritillaria lichuanensis*

H.-F. PI, H.-L. RUAN, Y.-H. ZHAN and J.-Z. WU*

Faculty of Pharmaceutical Sciences, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430030, China

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Two new steroidal alkaloids, lichuanine (**1**) and lichuanisinine (**2**), were isolated from the bulbs of *Fritillaria Lichuanensis* P. Li *et* C.P. Yang. Their structures were determined to be (20*S*,25*R*)-5α, 14α-cevanine-3β, 6β-diol (**1**) and (20*S*,25*S*)-5α,14α-cevanine-3β,6β-diol-*N*-oxide (**2**) by means of spectral analysis and chemical evidence.

Keywords: *Fritillaria Lichuanensis*; Liliaceae; Steroidal alkaloid; Lichuanine; Lichuanisinine

1. Introduction

Fritillaria Lichuanensis P. Li *et* C.P. Yang is a new *Fritillaria* species growing in the northwest district of Hubei Province, China. In our studies on the chemical constituents of this plant, two new C-nor-D-homosteroidal alkaloids, lichuanine (**1**) and lichuanisinine (**2**), were isolated from the bulbs of *Fritillaria lichuanensis*. This paper describes the isolation and structural elucidation of the two new alkaloids.

2. Results and discussion

Compound **1** (figure 1) appeared as white feathery needles (EtOAc-Me_2CO), mp 159-160°C. HREI-MS showed a $[M]^+$ ion peak at *m/z* 415.3453, corresponding to the molecular formula $C_{27}H_{45}NO_2$. EI-MS revealed the fragment ions at *m/z* 415 (100%), 400$[M - 15]^+$, 386$[M - 29]^+$, 358 $[M - 57]^+$, 344$[M - 71]^+$, 215, 718, 164, 111 (base peak), 98 which were typical cleavage ions of 5α,14 α-cevanine alkaloid lacking the hydroxyl group at C-20 [1,5,7]. The IR spectrum of **1** showed the presence of hydroxyl group at 3416 cm^{-1}, 1036 cm^{-1} and *trans*-quinolizidine moiety at 2750 cm^{-1}, so the junction of E and F rings is *trans*. The ^1H NMR spectrum of **1** exhibited a singlet at δ 1.02 (3H, s), indicating a C-19 angular

*Corresponding author. E-mail: ywjz@mails.tjmu.edu.cn

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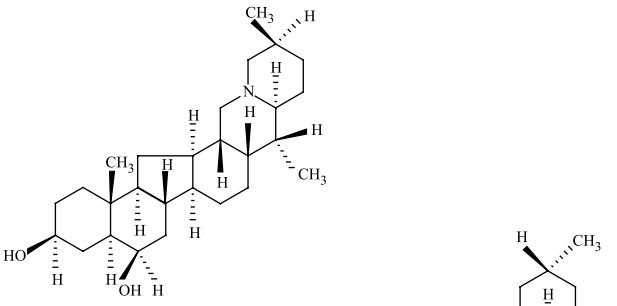
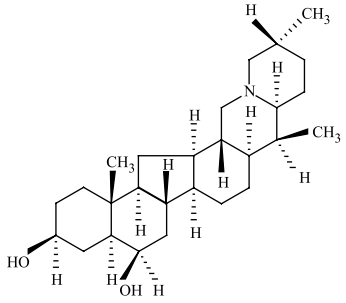


hupehenine



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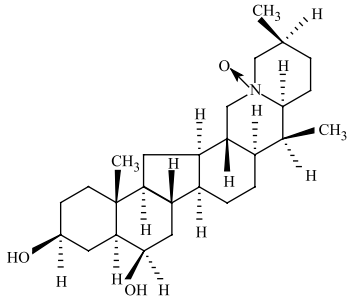

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Figure 1. The structures of hupehenine, **1** and **2**.

methyl group of a steroidal ring system with a hydroxyl group at C-6, two doublets at δ 0.83 (3H, d, $J = 6.6$ Hz) and 0.89 (3H, d, $J = 7.2$ Hz) corresponding to secondary methyl groups at C-25 and C-20, and two methine protons on the carbon bearing a hydroxyl group at δ 3.66 and 3.86 [7]. The ^{13}C NMR spectrum of **1** showed 27 carbon signals, including three methyls, 11 methylenes, 12 methines and one quaternary carbon on the basis of DEPT experiment. The assignment of the ^{13}C NMR signals (table 1) was made with the aid of ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY spectroscopy [2,3]. In the ^{1}H NMR spectrum of **1**, a tertiary methyl signal (H_3-19) was shifted downfield because of the presence of β-axial hydroxyl group at C-6; the secondary methyl signal at δ 0.83 showed the presence of an α-equatorial methyl group at C-25; the signal at δ 0.89, ascribable to the methyl group at C-20, further supported the absence of hydroxyl group at C-20. A multiplet centred at δ 3.66 ($W_{1/2} = 24$ Hz) was associated with the α-hydrogen at C-3 bearing a hydroxyl group, another multiplet centred at δ 3.86 ($W_{1/2} = 8$ Hz) was associated with α-hydrogen at C-6 bearing a hydroxyl group according to their half-height width [3]. In the ^{13}C NMR spectrum of **1**, the chemical shift of C-21 was at δ 8.3, which was similar to that of C-21 (δ 8.6) in shinonomenine (a known alkaloid isolated from *Veratrum qrandiflorum* Loesen) [8], more upfield than that of hupehenine (a known alkaloid isolated from *F. hupehensis*) [2,6] (table 1),

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Table 1. ^{13}C NMR spectral data of **1** hupehenine and **2**.

No.	***1***	*Hupehenine*	***2***	*No.*	***1***	*Hupehenine*	***2***
1	39.5	39.3	38.1	15	24.5	28.7	25.1
2	31.4	31.2	31.3	16	26.8	17.6	26.4
3	71.9	71.7	72.7	17	31.2	41.4	49.6
4	34.7	34.5	35.7	18	62.2	59.0	60.2
5	47.8	48.0	48.7	19	14.7	15.7	15.2
6	73.2	73.0	73.7	20	38.4	38.7	39.5
7	39.6	39.4	39.6	21	8.3	14.7	8.3
8	37.6	36.6	36.7	22	66.9	62.2	66.9
9	57.4	57.0	58.8	23	30.2	24.7	32.0
10	35.5	35.4	37.8	24	33.5	30.0	40.7
11	25.4	30.8	27.5	25	31.7	28.4	32.6
12	39.4	39.1	40.6	26	65.1	61.4	68.4
13	38.6	39.0	40.0	27	19.6	18.3	15.6
14	40.7	41.2	42.2				

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caused by the steric effect of a lone pair on N atom to the β-axial methyl group at C-20. Comparing of the chemical shifts of C-16, C-18, C-22 of **1** with that of hupehenine, the signals of **1** were shifted downfield, indicating of the configuration of H-17 was α-axial orientation in **1** different from hupehenine, so D/E has a *trans* juncture in **1**. In the NOESY spectrum of **1**, the proton signal of H_3-21 correlates with H-13 (δ 1.52) H-18(δ 1.72) and H-23(δ 1.38), but no NOESY correlation between H-17 and H_3-21 was observed, which supported the orientation of the methyl group at C-20 as β, H-17 as α and D/E *trans* juncture. Thus, the structure of compound **1**, named lichuanine, was established as (20*S*,25*R*)-5α,14α-cevanine-3β,6 β-diol.

Compound **2** (figure 1) was a white powder, mp 148-151°C. HREI-MS showed a $[M]^+$ ion at *m/z* 431.3408, corresponding to the molecular formula $C_{27}H_{45}NO_3$. The EI-MS revealed the fragment ions at *m/z* 431 (M^+100%), 416$[M - 15]^+$, 359$[416 - 57]^+$, 345$[416 - 71]^+$, 180$[164 + 16]^+$, 127$[111 + 16]^+$, 98. which were a cleavage ions of 5α,14α-cevanine alkaloid, the fragment peaks at *m/z* 180, 127 indicated the presence of an oxygen in E ring or F ring of **2**, different from other cevanine alkaloids. The IR spectrum of **2** showed the presence of hydroxyl group at 3433 cm^{-1}. The ^1H NMR spectrum of **2** exhibited a singlet at δ 0.99 (3H, s), indicative of a C-19 angular methyl group of a steroidal ring system with β-hydroxyl group at C-6. Two doublets at δ 0.92 (3H, d, $J = 7$ Hz) and 0.96 (3H, d, $J = 7$ Hz) corresponded to secondary methyl groups at C-25 and C-20, by comparison with **1**, the secondary methyl signal at δ 0.92 was shifted downfield, which suggested the presence of a β-axial methyl group at C-25 [5]. Two methine protons on carbon bearing a hydroxyl group atδ 3.54 ($W_{1/2} = 24$ Hz) and 3.77 ($W_{1/2} = 8$ Hz) were associated with two α-hydrogens at C-3 and C-6, respectively. In the ^{13}C NMR spectrum of **2**, the signal at δ 8.3 showed the presence of a β-axial methyl group at C-20. The ^{13}C NMR spectrum of **2** showed 27 carbon signals, including three methyl carbons, 11 methylene carbons, 12 methine carbons and one quaternary carbon on the basis of DEPT experiment, which were identical with compound **1**. This observation, coupled with only two hydroxyl groups in ^1H NMR spectrum of **2**, and the observation of the fragment ions at *m/z* $[164 + 16]^+$, $[111 + 16]^+$ in the mass spectrum, suggested that compound **2** is an *N*-oxide [6], so there was no *trans*-quinolizidine moiety at 2750 cm^{-1} In IR spectrum of **2**. The assignment of the ^{13}C NMR signals (table 1) was made with the aid of $^1H-^1H$ COSY, HMQC, HMBC and NOESY experiments. The signals of C-16, C-18, and C-22 were shifted downfield because of the D/E *trans* junction and the

induction effect of N^+[3,4]. There was no the NOE cross-peak between H_3-21 and H-22, suggesting the configuration of H-22 was of α-axial orientation, so E/F is a *trans* junction. From the evidence described above, the structure of compound **2**, named lichuanisinine, was established as (20*S*,25*S*)5α,14α-cevanine-3β,6β-diol-*N*-oxide.

3. Experimental

3.1 General experimental procedures

Melting points were determined on X4 apparatus and are uncorrected. IR spectra were recorded on an Mi-colet 306 FT-IR spectrometer. EI-mass spectra were measured on a JEOL JMS-DX-300 mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were run on a GE-omega 600 spectrometer (600 Hz and 150 Hz, respectively). TLC was performed on silica gel (Qingdao Ocean Chemical Inc., China) using Dragendorff's reagent for detection. Column chromatography was carried out on silica gel (100–200 mesh).

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3.2 Plant material

The bulbs of *Fritillaria lichuanensis* P. Li *et* C.P. Yang were collected and identified by Detai Peng, Lichuan Institute of Chinese Materia Medica, China. A voucher specimen was 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 ethanol extract was dissolved in 2% HCl. The acidic fraction was basified with ammonia to pH $>$ 11, and the liberated alkaloids were then taken into chloroform to give a total alkaloid fraction (32 g). The total alkaloids were further fractionated by column chromatography on silica gel with petroleum–Me_2CO–Et_2NH containing increasing contents of Me_2CO to yield lichuanine (**1**) and lichuanisinine (**2**).

3.3.1 Compound 1. (Lichuanine), white feathery needles (EtOAc-Me_2CO); mp 159–160°C. HR-MS *m/z*: 415.3453 $[M]^+$, (calcd for $C_{27}H_{45}NO_2$, 415.3450); IR ν_{max} (KBr) cm^{-1}: 3416, 1036, 2930, 2870, 1468–1435, 2750. EI-MS *m/z*: 415 (M^+100%), 400$[M-15]^+$, 386$[M-29]^+$, 358$[M-57]^+$, 218, 178, 164, 111, 98. ^{1}H NMR ($CDCl_3$): δ 1.02 (3H, s, H_3-19), 0.83 (3H, d, $J = 6.6$ Hz, H_3-27), 0.89 (3H, d, $J = 7.2$ Hz, H_3-21), 3.66 (1H, m, $W_{1/2} = 24$ Hz, H-3), 3.86 (1H, m, $W_{1/2} = 8$ Hz, H-6). ^{13}C NMR: see table 1.

3.3.2 Compound 2. (Lichuanisinine), white powder; mp 148-151°C. HR-MS *m/z*: 431.3408 $[M]^+$, (calcd for $C_{27}H_{45}NO_3$; 431.3444). IR ν_{max} (KBr) cm^{-1}: 3433, 1063, 2930, 2870, 1468-1435. EI-MS *m/z*: 431 (M^+100%), 416[M-15]$^+$, 359$[416-57]^+$, 345$[416-71]^+$, 180$[164+16]^+$, 127$[111+16]^+$. ^{1}H NMR (CD_3OD) δ: 0.99 (3H, s, H_3-19), 0.92 (3H, d, $J = 7$ Hz, H_3-27), 0.96 (3H, d, $J = 7$ Hz, H_3-21), 3.54 (1H, m, $W_{1/2} = 24$ Hz, H-3), 3.77(1H, m, $W_{1/2} = 8$ Hz, H-6). ^{13}C NMR: see table 1.

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