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Steroidal alkaloids from the bulbs of *Fritillaria delavayi* Franch. (Liliaceae)

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Chemotaxonomy

1. Subject and source

The genus *Fritillaria* belongs to family Liliaceae, which includes about 130 species worldwide (Wang and Tang, 1980) and occurs mainly in the temperate regions of the northern hemisphere (Tsukamoto, 1989). The bulbs of many *Fritillaria* species ('*Beimu*' in Chinese) have been used as antitussive, anti-asthmatic and expectorant agents in traditional Chinese medicine for more than 2000 years (Shang and Liu, 1995). *Fritillaria delavayi* Franch. is widely distributed in Southwestern China. The bulbs of *F. delavayi*, '*Chuanbeimu*' in Chinese, were collected in July 2006 from Ganzi county, Sichuan province, China. The botanical identification was carried out by one of the authors (Prof. Shi-Lin Chen). A voucher specimen (LB-06-07) has been deposited in the Herbarium of Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College.

2. Previous work

Previous phytochemical investigations on *F. delavayi* led to isolation of six isosteroidal alkaloids and one steroidal alkaloid which were identified as delavine, delavinone, imperialine, chuanbeinone, delafrinone, delafrine, and (22*R*,25*S*)-solanid-5-enine-3β,5α,6β-triol, respectively (Kaneko et al., 1985, 1986, 1988).

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3. Present study

In our search for biologically active alkaloids from the Liliaceae plant, an unusual 6,12,22-triketo verazine-type steroidal alkaloid with a pyrrolidine side chain, (20*S*,25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-ene-6,12,22-trione **1**, named delavidine, together with six known isosteroidal alkaloids, chuanbeinone **2** (Kaneko et al., 1986), imperialine **3**, delavinone **6** (Kaneko et al., 1985), peimine **4**, peimisine **5**, and peiminine **7** (Zhang et al., 1998) were isolated from the methanol extract of the bulbs of *F. delavayi* (Fig. 1).

The air-dried bulbs of *F. delavayi* (14.7 kg) were powdered and refluxed three times with MeOH (25 L). The combined MeOH extract was evaporated under reduced pressure to afford crude extract (560 g). Two litres of water was added, and then extracted with petroleum ether, dichloromethane (pH 9–11) and *n*-butanol successively. The dichloromethane extract (pH 9–11, 20 g) was loaded on a silica gel column and eluted with petroleum ether/acetone/triethylamine gradient with increasing the ratio of acetone to yield 89 fractions. From fractions 11, 17, 48 and 50, precipitates were obtained and recrystallized with acetone for several times to give compound **2** (23 mg), **3** (39 mg), **4** (52 mg) and **5** (30 mg), respectively. Fractions 12–16 were combined and rechromatographed on flash silica gel by using petroleum ether/acetone/triethylamine = 3:1:0.1 as eluent to yield compound **1** (8 mg), **6** (18 mg) and **7** (26 mg).

Compound **1** was obtained as an amorphous powder from MeOH extracts of *F. delavayi* bulbs, gave a positive reaction to Dragendorff reagent. Its molecular formula was determined as $C_{27}H_{39}NO_4$ by HRESIMS at *m/z* 442.2950 $[M+H]^+$ (calc. for $C_{27}H_{40}NO_4$, 442.2952). The IR spectrum indicated absorption bands for hydroxyl (3470 cm^{-1}), carbonyl (1714 cm^{-1}) and α, β-unsaturated carbonyl (1699 cm^{-1}) groups. The ^1H NMR spectrum of **1** showed the presence of two tertiary methyl signals at δ 1.03 (3H, s, H-18) and 0.79 (3H, s, H-19), two secondary methyl signals at δ 1.04 (3H, d, $J = 6.8$ Hz, H-21) and 0.98 (3H, d, $J = 6.8$ Hz, H-27), and a multiplet signal centered at δ 3.53, which may be assigned to the α-hydrogen adjacent to the 3β-hydroxyl group. The ^{13}C NMR and DEPT spectrum of **1** showed the presence of total 27 carbon signals, including four methyls, nine methylenes, eight methines and six quaternary carbons (three for carbonyl carbons at δ 203.1, 209.1 and 212.6), which suggested the occurrence of a steroidal skeleton. Five of total 27 carbons were assigned to a pyrrolidine group with methyl substitute. The carbon signal at δ 173.9 was due to the carbon of an azomethine group (Kusano et al., 1976). Two aliphatic carbon resonated at δ 70.3 and 69.9 indicated the presence of oxygenated or nitrogen-linked carbons. Further ^1H–^1H COSY, HMQC, and HMBC experiments completely assigned the ^1H NMR and ^{13}C NMR spectra of **1** (Table 1). From the above data, compound **1** was strongly suggested to be a verazine-type steroidal alkaloid with a hydroxyl located at C-3, a pyrrolidine side chain, and three keto groups. Comparison of NMR data of compound **1** with those of (25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-ene-6,22-dione (Ori et al., 1992) suggested that all the ^{13}C NMR chemical shifts of two compounds were very similar except for those of rings C and D. Firstly, the carbon signals for C-11 and C-13 were shifted downfield dramatically to δ 38.4 and 56.9, respectively (see Table 1), which were caused by the presence of the carbonyl carbon at C-12 (δ 212.6). Secondly, the C-17 signal in **1** was shifted upfield to δ 42.9 (see Table 1) due to a strong shielding effect of the carbonyl carbon at C-12. This large upfield shift (*ca.* 10 ppm) often appeared when the γ-carbon atom was eclipsed or nearly eclipsed to the keto group (e.g., C-17 in 12-cholestanone) (Eggert and Djerassi, 1973). The position of carbonyl carbon was further confirmed to be located at C-12 through an HMBC experiment, which showed distinct long-range correlations between H-11 (δ 2.50, 2.25), H-14 (δ 1.45), H-17 (δ 2.60), H-18 (δ 1.03) and C-12 (δ 212.6) (Fig. 2), respectively. Thus, the structure of **1** was presumed to be (25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-ene-6,12,22-trione. The relative configurations for ring junctions and chiral center were further confirmed through the nuclear Overhauser effect spectroscopy (NOESY) spectrum as shown in Fig. 2. Briefly, in the NOESY spectrum the correlation between H-16α and H-17 indicated that the side chain was β oriented. The key correlations of H-2α/H-3, H-3/H-5, H-4β/Me-19,

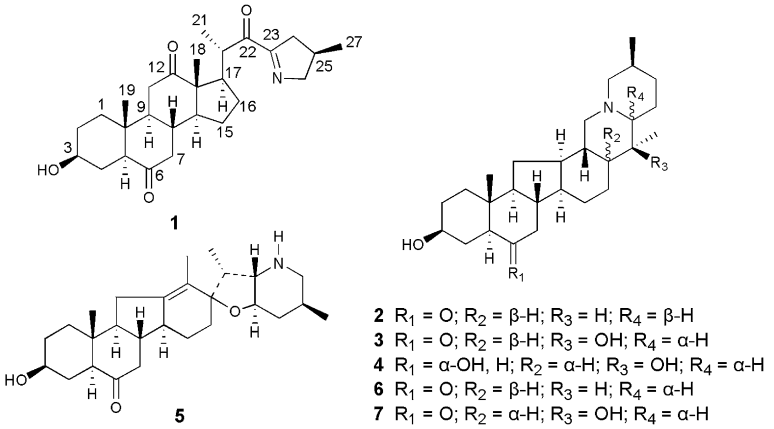

Fig. 1. The chemical structures of compounds **1**–**7** from *F. delavayi*.