Puqienine F, a Novel Veratramine Alkaloid from the Bulbs of *Fritillaria puqiensis*

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Received November 10, 2005; accepted December 27, 2005

A novel veratramine alkaloid, called puqienine F (1) and possessing a 12,16-epoxy ring, was isolated from the bulbs of *Fritillaria puqiensis* G. D. Yu *et* G. Y. Chen (Liliaceae). The structure was elucidated by extensive spectral and X-ray crystallographic analyses.

Key words *Fritillaria puqiensis*; Liliaceae; veratramine alkaloid; puqienine F; X-ray

Fritillaria L., a large genus of the Liliaceae family, includes about 130 species worldwide[1] which have been found to be rich sources of new and bioactive steroidal alkaloids.[2] Many species of *Fritillaria* have traditionally been used as herbal remedies in Japanese, Turkish, Pakistani and Southeast Asian folk medicines.[3,4] In China, the bulbs of many *Fritillaria* species ("Beimu" in Chinese) have been used as antitussive and expectorant herbs in traditional Chinese medicine for more than 2000 years, and nine species, including *Fritillaria thunbergii*, *F. cirrhosa*, *F. unibracteata*, *F. przewalskii*, *F. delavayi*, *F. ussuriensis*, *F. walujewii*, *F. pallidiflora* and *F. hupehensis*, were recorded in the Year 2005 edition of China Pharmacopoeia as the plant sources for herbal Beimu.[5] At the same time, the bulbs of *F. puqiensis* G. D. Yu *et* G. Y. Chen, native to Hubei Province of China, are expected to be an important substitute for Beimu due to their antitussive activity.[6,7] In our search for biologically interesting alkaloids from this plant, we previously isolated two veratramine alkaloids (puqienine A and B) and three secosolanidine alkaloids (*N*-demethylpuqietinone, puqietinonoside, and puqietinone) from its dried bulbs.[8] Our recent investigation resulted in the isolation of a new veratramine alkaloid designated puqienine F (**1**). This paper describes the isolation and structure elucidation of **1**.

Results and Discussion

Puqienine F (**1**), obtained as colorless columnar crystals from MeOH, gave a positive reaction to Dragendorff reagent. Its molecular formula was determined to be $C_{28}H_{45}NO_5$ by high resolution electrospray ionization mass spectroscopy (HR-ESI-MS) with seven degrees of unsaturation. The IR spectrum displayed absorption bands for hydroxyl (3402 cm^{-1}) and carbonyl (1689 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** (Table 1) showed two tertiary methyl signals at δ 0.71 (3H, s) and 1.59 (3H, s), two secondary methyl signals at δ 0.67 (3H, d, J=6.6 Hz) and 1.30 (3H, d, J=6.9 Hz), and an *N*-methyl signal at δ 2.32 (3H, s). These characteristic data suggested that **1** was a veratramine-type alkaloid.[9] Two typical proton resonances at δ 3.70 (1H, br, m, H-3α) and 4.09 (1H, br, m, H-23α) showed the existence of 3β and 23β hydroxyl group, respectively. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra (Table 1) disclosed the presence of a carbonyl carbon, two oxygenated quaternary carbons, three oxy-methines, and the absence of olefinic carbons. Considering that six of the seven degrees of unsaturation had been accounted for, **1** was inferred to possess an epoxide ring. Apart from the above signals, the relevant proton and carbon resonances (Table 1) were completely assigned based on ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) experiments. A comparison of the NMR data with

Table 1. ^{1}H- and ^{13}C-NMR Data and HMBC Correlations of **1**

Position	^{13}C[a]	^{1}H[b]	HMBC[c]
1	37.2 t	α 1.08, m	2, 10, 19
		β 1.39, m	2, 3
2	31.5 t	α 1.90, m	
		β 1.57, m	1, 3
3	70.3 d	3.70, br m	
4	31.3 t	α 2.22, m	2, 3, 5, 10
		β 1.74, m	2, 3, 5, 10
5	56.4 d	2.08, dd, 12.1, 2.6	3, 4, 6, 9, 10, 19
6	210.0 s		
7	45.8 t	α 2.13, m	6, 8, 9
		β 2.49, m	5, 6, 8, 9
8	46.8 d	1.92, m, overlap	6, 9, 11
9	58.5 d	1.94, m, overlap	5, 7, 8, 10, 11
10	38.6 s		
11	25.6 t	α 2.10, m	9, 10, 12, 13
		β 1.69, m	9, 10, 12
12	98.9 s		
13	80.9 s		
14	47.6 d	2.89, ddd, 16.4, 10.8, 5.6	7, 8
15	38.7 t	α 1.80, m	8, 12, 17
		β 1.52, m	14, 16, 17
16	80.2 d	4.77, ddd, 20.1, 12.3, 7.5	12, 13, 14, 20
17	57.0 d	2.32, dd, 7.5, 2.9	13, 15, 18, 20, 21, 22
18	21.8 q	1.59, s (3H)	12, 13, 17
19	12.5 q	0.71, s (3H)	1, 5, 9, 10
20	31.5 d	2.51, m	17, 21, 22, 23
21	12.5 q	1.30, d, 6.9 (3H)	17, 20, 22
22	70.1 d	1.87, dd, 11.4, 3.2	20, 21, 28
23	66.8 d	4.09, br m	
24	43.0 t	α 1.05, m	25, 27
		β 1.93, m	22
25	23.5 d	2.24, br m	27
26	65.7 t	α 1.82, m	22, 24, 25, 27, 28
		β 2.76, ddd, 11.8, 6.7, 3.5	22, 24, 25, 28
27	19.5 q	0.67, d, 6.6 (3H)	24, 25, 26
28	41.4 q	2.32, s (3H)	22, 26

a) 125 MHz for ^{13}C. *b*) 500 MHz for ^{1}H in C_5D_5N, chemical shifts and coupling constant are given in ppm and Hz, respectively. *c*) Carbons correlated with the proton. The ^{1}H and ^{13}C chemical shifts were assigned by a combination of DEPT, HMQC and HMBC experiments.

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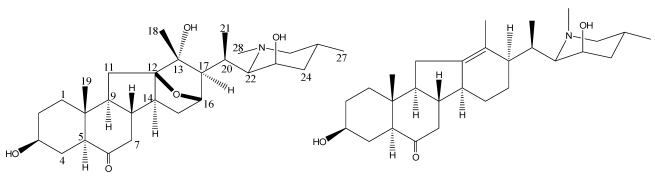
those of the known veratramine alkaloid, puqienine B (**2**),[8] revealed that **1** was similar to **2** except for some notable differences at the ring *D*. Firstly, the olefinic quaternary carbons (C-12 and C-13) of **2** corresponded to two oxygenated quaternary carbons (δ_C 98.9 and 80.9, respectively) in **1**; secondly, the H-16 and C-16 resonances of **1** were found to be significantly deshielded [δ_H 4.77 (1H, m), δ_C 80.2] compared to the corresponding resonances of **2**. Therefore, two possible linkages, *i.e.*, an epoxy bridge *via* C-12 or C-13 linked at C-16, were surmised for **1**. The resultant epoxy linkage between C-12 and C-16 was determined by an HMBC correlation between H-16 and C-12 in the HMBC spectrum (Fig. 1). Thus, a hydroxyl group was unequivocally located at C-13.

The configurational assignments were established through the nuclear Overhauser effect spectroscopy (NOESY) spectrum as shown in Fig. 2. The key correlations of H-1/H-3, H-3/H-5, H-4β/Me-19, Me-19/H-8, H-9/H-5, H-9/H-14 proved to be *A*/*B trans*, *B*/*C trans*, 3β-OH, 14α-H. NOEs were also observed between Me-19 and Me-18, and between H-14 and H-17, implying both the C-13 methyl group and the C-17 side chain were in β-orientation. Similarly, the substituents in the piperidine part of the molecule were confirmed as 22α-H, 23β-OH and 25α-Me. Finally, combined with the single crystal X-ray diffraction (Fig. 3), **1** was elucidated as (20*R**,22*R**)-12β,16β-epoxy-5,6,12,13-tetrahydro-3β,13α,23β-trihydroxy-*N*-methyl-5α-veratraman-6-one, and was termed puqienine F.



1 puqienine B

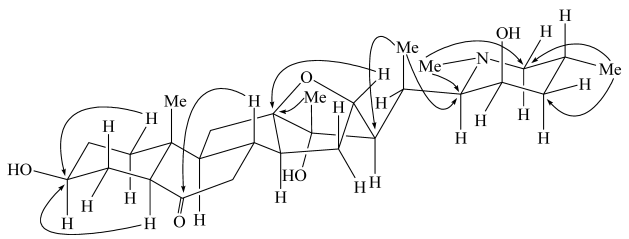


Fig. 1. Significant HMBC Correlations of **1**

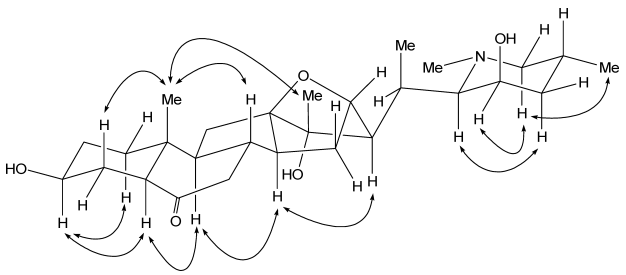


Fig. 2. Key NOESY Correlations of **1**

Experimental

General Experimental Procedures The melting point was measured on an X4 micro-melting point apparatus and was uncorrected. Optical rotation was determined in MeOH on a PE-241 MC digital polarimeter at 20 °C. IR spectra were recorded in KBr disks on a Nicolet Impact 410 spectrophotometer. NMR spectra were obtained in C_5D_5N containing TMS as an internal standard on a Bruker Am-500 NMR spectrometer. HR-ESI-MS spectra were performed on an ESI-TOF mass spectrometer (Agilent, U.S.A.). Column chromatography was performed with silica gel (200—300 mesh, Qingdao Marine Chemical Factory, China). TLC was conducted on silica gel G plates (0.25 mm thick, Qingdao Marine Chemical Factory). X-Ray crystallographic data collection for compound **1** was carried out on a Nonius CAD4/PC single-crystal X-ray diffractometer.

Plant Material The bulbs of *F. puqiensis* were collected from Puqi County, Hubei Province of China, in May 2000, and authenticated by one of the authors (P. Li). A voucher specimen (No. 255830) was deposited at the Herbarium of the Department of Pharmacognosy, China Pharmaceutical University.

Extraction and Purification The dried ground bulbs (10 kg) of *F. puqiensis* were extracted with 70% EtOH, and the extract was partitioned between ether and 2% HCl. Water-soluble materials, after being adjusted at pH 9.2 with NH_4OH, were partitioned with $CHCl_3$. The $CHCl_3$ extract was subjected to a silica gel column using $CHCl_3$–MeOH of increasing polarity as eluant to obtain five fractions, A—E. Fraction E was further separated by repeated column chromatography and eluted with $CHCl_3$–MeOH–diethylamine (100 : 7 : 5) to afford **1** (20 mg).

Compound **1**: Colorless columnar crystals (from MeOH), mp 216—218 °C; $[\alpha]_D^{20}$ −65.5° (*c*=0.1, MeOH); positive HR-ESI-MS *m/z*: 476.3374 $[M+H]^+$ (Calcd for $C_{28}H_{46}NO_5$, 476.3371); IR (KBr) ν_{max}: 3402, 2936, 1689, 1066 cm^{-1}; ^{1}H-NMR (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz): see Table 1.

Single-Crystal X-Ray Structure Determination Crystals of **1**, crystallized from MeOH, belong to the monoclinic space group $P2_1$. Crystal data: $C_{28}H_{45}NO_5\cdot(H_2O)_2$, a=7.7700(16) Å, b=21.022(4) Å, c=8.6210(17) Å, V=1365.9(5) Å^3, Z=2, D_{calc}=1.234 Mg/m^3, λ=0.71073 Å, μ(Mo$K\alpha$)=0.087 mm^{-1}, F(000)=552, T=293(2) K. A colorless columnar with dimensions of 0.4×0.3×0.3 mm was used for X-ray measurements on a Nonius CAD4/PC single crystal X-ray diffractometer. Data collection yielded 2962 reflections, 2759 were independent. All non-hydrogen atoms were identified by difference Fourier maps, and hydrogen atoms were yielded theoretically. Full-matrix least-squares refinement on F2 led to a final $R[I>2\sigma(I)]$, R(all), and GOF of 0.0555, 0.0749 and 1.184. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.593 and −0.593 eÅ^{-3}, respectively. Crystallographic data (excluding structure factors) for com-

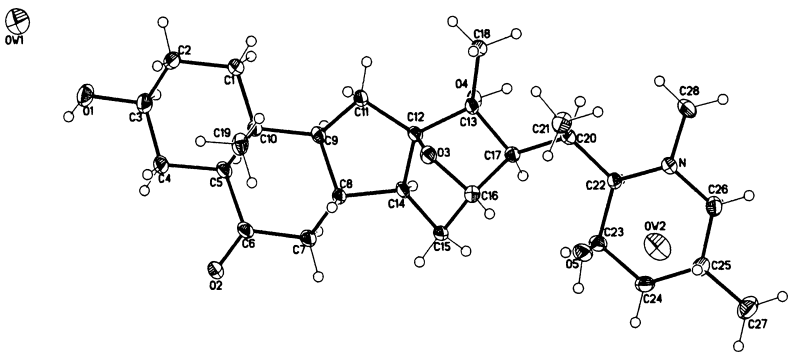


Fig. 3. X-Ray Structure of **1** with 30% Probability Ellipsoids

pound **1** have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 268685. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, U.K. (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgements We are grateful for financial support from the cultivation fund of the key scientific and technical innovation project, Ministry of Education of China (No. 2004-295) and the National Natural Science Foundation of China (No. 30070886).

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