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PUQIEDINONE, A NOVEL 5α-CEVANINE ALKALOID FROM THE BULBS OF *FRITILLARIA PUQIENSIS*, AN ANTITUSSIVE TRADITIONAL CHINESE MEDICINE

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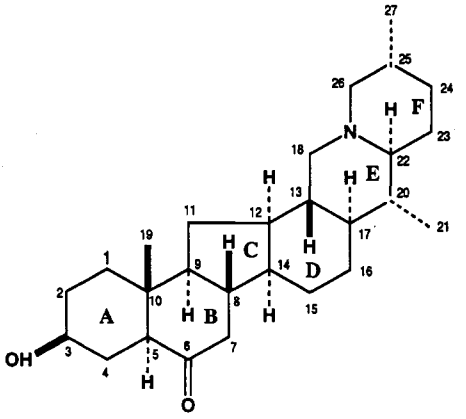
ABSTRACT.—A novel 5α-cevanine alkaloid, puqiedinone [**1**], was isolated from the bulbs of *Fritillaria puqiensis*, a traditional Chinese medicine used widely for its antitussive and expectorant properties. The structure of **1** was assigned as (20*R*,22*S*,25*R*)-20-deoxy-5α-cevanine-3β-ol-6-one based on spectral analysis and comparison with the structures of related known compounds. Puqietinone [**2**] was also identified from the same herbal plant and the structure was revised as (22*R*,25*S*)-*N*-methyl-22,26-epiminocholest-3β-ol-6-one based on spectroscopic and X-ray crystallographic methods.

"Beimu," an antitussive and expectorant traditional Chinese medicine, has been used widely for centuries in mainland China and other Chinese communities worldwide. It is derived from several *Fritillaria* species. *Fritillaria puqiensis* G.D. Yu et G.Y. Chen (Liliaceae) was discovered as a new species in 1985, and it has been used therapeutically in Hubei Province, People's Republic of China (1). We have investigated the active constituents in this herbal medicine and two major alkaloids have been isolated and characterized. In this paper, the characterization of a novel alkaloid, puqiedinone [**1**], is reported. Puqietinone [**2**] has been isolated previously from the same plant and its structure was assigned on the basis of preliminary spectroscopic data (2). However, in the present study, the configurations of the C-25 methyl group and C-22 in **2** were revised as 25*S* and 22*R*, respectively, by further spectroscopic analysis and X-ray crystallographic study. Both alkaloids have been tested and shown to possess antitussive (3) and antitumor activities. The antitumor properties of the bioactive constituents of *F. puqiensis* will be reported separately (4).



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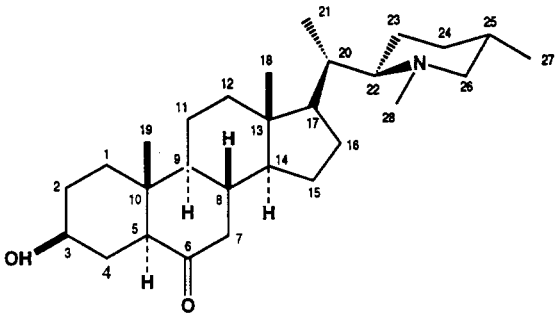

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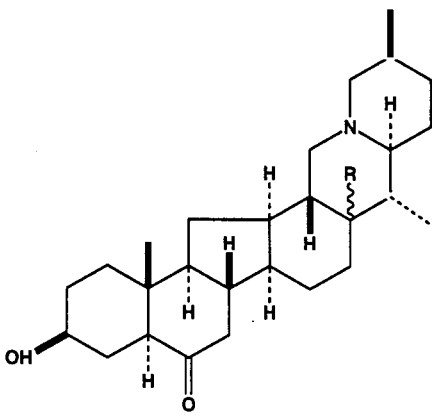
RESULTS AND DISCUSSION

As described in the Experimental, puqiedinone [**1**] was isolated from the crude alkaloid extract of *F. puqiensis* bulbs by Si gel cc. It was crystallized from C_6H_{12}-EtOH-$(C_2H_5)_2NH$ as colorless needles. The empirical formula of **1**, $C_{27}H_{43}NO_2$, mol wt 413.3281 (calcd 413.3286), was determined by hrms. The ir spectrum of **1** showed intense absorptions at 3525, 3280 (OH), 1690 (C=O), and 2750 cm^{-1} (*trans*-quinolizidine). The eims of **1** exhibited a molecular ion at *m/z* 413 (M^+, 36%) and diagnostic fragment ions at *m/z* 398 (8%), 112 (35%), 111 (100%), and 98 (9%). The base peak at *m/z* 111 and the eims fragmentation pattern suggested that **1** is an isosteroidal alkaloid (5).

The ^{1}H-nmr spectrum of **1** showed a singlet signal at δ 0.73, which was assigned to the C-10 tertiary methyl protons. The two doublet signals at δ 0.84 (3H, d, J=9.2 Hz) and 0.86 (3H, d, J=9.2 Hz) corresponded to the C-20 and C-25 secondary methyl protons, respectively. The signal of Me-25 was shifted upfield to δ 0.86, compared with that of the β-axial Me-25 (δ 1.08) in ebeiedinone [**3**], a known 5α-cevanine alkaloid isolated from *F. ebeiensis* var. *purpurea* G.D. Yu et P. Li (5). This upfield shift suggested an α-equatorial orientation with a shielding effect from the β-axial lone-pair electrons on the nitrogen atom (6). In fact, if both the methyl group and the lone pair of electrons on the nitrogen atom have axial orientations on the same side of the rings, and are either α-axial and α-axial or β-axial and β-axial, the chemical shifts of the methyl protons would be shifted downfield due to the anisotropic effect caused by the lone-pair electrons on the nitrogen atom (5–7). For example, ebeiedinone (5), ebeiedine (5), delavinone (7),



3 R=αH
4 R=βH

and delavine (7) are 5α-cevanine alkaloids with a β-axial lone-pair of electrons on the nitrogen, and the signals for the β-axial Me-25 in these alkaloids range from δ 1.08–1.09. In addition, chuanbeinone (6) has an α-axial lone-pair of electrons on the nitrogen and an α-axial Me-20 in its structure with a chemical shift of δ 0.98. In the presence of such a shielding effect, the signals for the methyl protons would appear relatively upfield. For instance, the signal for the α-equatorial Me-20 in ebeiedinone (5), ebeiedine (5), delavinone (7), and delavine (7) occurred at ca. δ 0.83. The signals for the α-equatorial Me-25 in ebeienine [β-axial lone-pair of electrons on the nitrogen (5)] and β-equatorial Me-25 in chuanbeinone [α-axial lone-pair of electrons on the nitrogen (6)] were δ 0.86 and δ 0.84, respectively. Based on the above ^{1}H-nmr data, the two methyl groups in **1** were inferred as having α-equatorial orientations, and the (22*S*)- and (25*R*)-configuration. In addition, the doublet signal for Me-20 confirmed the absence of a hydroxyl group at C-20 in **1** similar to those of 20-deoxy-5α-cevanine alkaloids (5–8). In the ^{1}H-^{1}H NOESY spectrum of **1**, two strong cross-peaks between H-22 (δ 1.33) and Me-27 (δ 0.86), and between H-17 (δ 0.63) and H-22 (δ 1.33) and Me-21 (δ 0.84), further indicated the α-orientation of these two methyl groups.

The ^{13}C-nmr spectrum of **1** revealed resonances for all 27 carbons as shown in Table 1. The multiplicities of each carbon signal were determined by DEPT experiments,

TABLE 1. ^{13}C-Nmr Data for Puqiedinone [**1**], Ebeiedinone [**3**] (5), and Delavinone [**4**] (7).

Carbon	Multiplicity[a] (DEPT)	Chemical shift (δ ppm)			^{1}H/^{13}C connectivity[b] **1** (δ ppm)
		1	**3**	**4**	
1	CH_2	36.9	37.0	37.6	H-1βe (1.58), H-1αa (1.55)
2	CH_2	30.4[d]	30.5	30.6	H-2βa (1.81), H-2αe (1.84)
3	CH	70.9	70.8	70.9	H-3αa (3.57)
4	CH_2	30.2[d]	30.1	30.3	H-4βa (1.47), H-4αe (1.47)
5	CH	56.7	56.8	56.7	H-5αa (2.15)
6	C=O	211.3	211.3	211.1	—
7	CH_2	46.0	46.1	47.0	H-7βe (2.49), H-7αa (1.84)
8	CH	40.1[c]	40.3	39.7	H-8βa (1.67)
9	CH	56.7	56.7	56.7	H-9αa (1.68)
10	C	38.4	38.4	38.3	—
11	CH_2	30.1[d]	30.1	30.0	H-11βa (1.80), H-11αe (1.83)
12	CH	41.2	41.4	39.3	H-12αa (0.96)
13	CH	40.9[c]	40.4	39.5	H-13βa (1.82)
14	CH	44.3	44.3	41.0	H-14αa (1.69)
15	CH_2	25.3	25.3	26.8	H-15βa (1.68), H-15αe (1.72)
16	CH_2	24.5	25.1	17.1	H-16βe (1.66), H-16αa (1.66)
17	CH	46.4	46.4	46.9	H-17αa (0.63)
18	CH_2	64.6	62.1	59.3	H-18βe (2.78), H-18αa (2.78)
19	CH_3	12.8	12.8	12.7	3H-19 (0.73)
20	CH	44.8	43.7	35.7	H-20βa (1.17)
21	CH_3	14.8	14.7	15.6	3H-21 (0.84)
22	CH	68.3	69.0	62.4	H-22αa (1.33)
23	CH_2	33.4	24.6	24.9	H-23βa (1.73), H-23αe (1.77)
24	CH_2	28.8	28.9	30.4	H-24βe (1.89), H-24αa (1.89)
25	CH	30.7	28.3	28.4	H-25βa (1.69)
26	CH_2	61.2	61.7	61.8	H-26βe (2.76), H-26αa (2.76)
27	CH_3	19.6	18.3	18.3	3H-27 (0.86)

[a]Determined by a DEPT experiment.

[b]Data for compound **1** only; heteronuclear correlation deduced from a ^{1}H-^{13}C COSY experiment.

[c,d]Assignments interchangeable.

while C/H connectivities were measured by the ^{1}H-^{13}C COSY nmr technique. The signal at δ 70.9 was assigned to the oxygen-bearing C-3 methine, and the resonance at δ 211.2 corresponded to a carbonyl carbon. The chemical shifts of C-18 and C-22 (carbons attached to the nitrogen atom), and C-16 appeared at δ 61.20, 68.32, and 24.47, respectively. The resonances of these three carbons were shifted downfield compared with those of D/E *cis*-cevanine alkaloids, such as delavinone [**4**] (δ 59.3, C-18; δ 62.4, C-22; δ 17.1, C-16) and delavine (δ 59.2, C-18; δ 62.5, C-22; δ 17.7, C-16) (7). However, they were consistent with those of D/E *trans*-cevanine alkaloids, as exemplified by compound **3** (δ 62.1, C-18; δ 69.0, C-22; δ 25.5, C-16) (5), ebeiedine (δ 61.8, C-18; δ 69.0, C-22; δ 25.6, C-16) (5), and shinonominine (δ 62.6, C-18; δ 68.0, C-22; δ 24.9, C-16) (10). This observation in **1** indicated the absence of any interaction between the C-16–C-17 bond and the two hydrogens at C-22 and C-18 in a D/E trans configuration (Figure 1) (7).

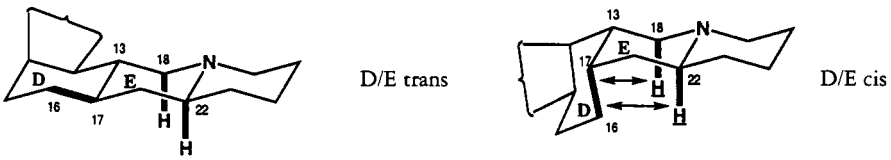


FIGURE 1. D/E trans and D/E cis ring fusions of 5α-cevanine alkaloids.

By comparison with the ^{13}C-nmr spectra of the model compounds **3** and **4**, the signals for C-23 and C-25 in **1** were shifted downfield to δ 33.4 and 30.7, respectively (see Table 1). This phenomenon is due to a deshielding γ-gauche interaction between C-23 and C-25 (Figure 2) (9). In addition, compared with the signals of Me-25 in **3** and **4** (both at δ 18.3), Me-25 in **1** also exhibited a downfield chemical shift to δ 19.6, due to the lack of a shielding γ-gauche interaction between C-23 and the β-axial C-25 methyl group in both **3** and **4** (Figure 2). The observation of such downfield shifts caused by the different γ-gauche interactions further confirmed the α-equatorial orientation of the C-25 methyl functional group in **1**. The resonances of all other carbon atoms were in good agreement with those of **3** and **4**, although some of the assigned signals for certain carbon atoms may be interchangeable as shown in Table 1.

Based on the above data, this new alkaloid, puqiedinone [**1**], was determined to be

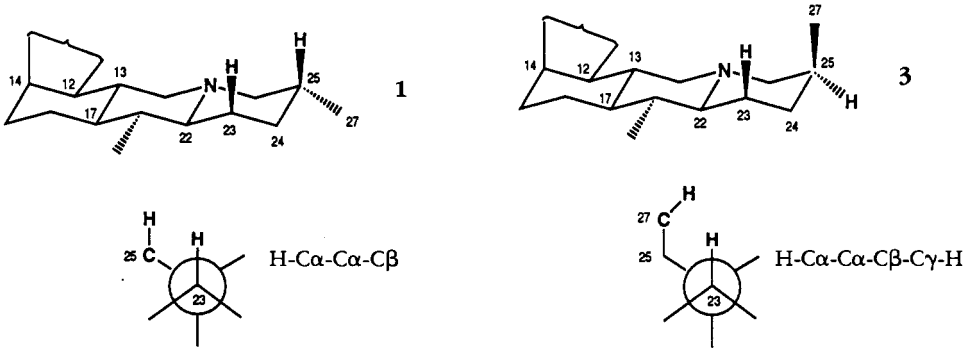


FIGURE 2. γ-Gauche interactions in **1** and **3**.

(20*R*,22*S*,25*R*)-20-deoxy-5α-cevanine-3β-ol-6-one. The ring fusions of **1** are A/B trans, B/C trans, C/D cis, D/E trans, and E/F trans. The six-membered rings A, B, E, and F are in the chair conformation, whereas ring D is in a boat conformation. The configurations at the chiral centers are OH-3 β-equatorial, Me-10 β-axial, Me-20 α-equatorial, Me-25 α-equatorial, and the lone pair of the nitrogen is β-axial.

Puqietinone [**2**], also isolated from *F. puqiensis*, differs from puqiedinone [**1**] in having a steroidal skeleton rather than an isosteroidal skeleton. The stereochemical assignments for C-22 and Me-25 were determined previously based solely on the ^{13}C-nmr data and by comparison with data of a known compound (2). However, in order to elucidate unequivocally the stereochemistry of **2**, we carried out further investigations, including an X-ray crystallographic study. The structure of puqietinone [**2**], as confirmed by X-ray crystallography, is shown in Figure 3. The X-ray crystallographic data revealed that the previous assignments of 22*S* and an α-equatorial 25*R*-methyl group were incorrect (2) and in fact, the configurations should be 22*R* and α-equatorial 25*S*. In **2**, all rings are in the chair conformation and the ring fusions are A/B trans, B/C trans, and C/D trans. The configurations at the chiral centers are OH-3 β-equatorial, Me-10 β-axial, Me-18 β axial, *N*-Me α-equatorial, and Me-25 α-equatorial. The structure was therefore definitively elucidated as (22*R*,25*S*)-*N*-methyl-22,26-epiminocholest-3β-ol-6-one.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All solvents except Et_2O (analytical grade) were distilled prior to use. Analytical tlc was carried out on glass sheets pre-coated (0.2 mm) with Si gel $60F_{254}$ (E. Merck, Darmstadt, Germany). Compounds were detected by spraying with Dragendorff's reagent. Flash cc was performed with Merck Si gel 60 (40–60 μm). Mps were recorded on a Griffin melting-point apparatus and are uncorrected. Hrms were recorded on a VG 70-70 high-resolution mass spectrometer. Lrms were recorded on a FT-ICR mass spectrometer. Ir spectra were recorded on a Nicolet spectrometer. ^{1}H-Nmr spectra were performed on an ARX-500 superconducting high-resolution Ft-nmr spectrometer (500 MHz) with residual $CHCl_3$ in $CDCl_3$ employed as the internal standard (assigned as 7.27 ppm downfield from TMS). ^{13}C-Nmr spectra were measured on the same spectrometer used for ^{1}H nmr with $CHCl_3$ or $CDCl_3$ employed as internal standard (assigned as 77.00 ppm downfield from TMS). Chemical shift assignments were based on a comparison with both calculated values and those of related known compounds.

PLANT MATERIAL.—The bulbs of *Fritillaria puqiensis* were collected by P. Li, Department of Pharmacognosy, China Pharmaceutical University, in Hubei Province, People's Republic of China, in June 1989. Voucher specimens are kept at the Herbarium of China Pharmaceutical University, Nanjing, People's Republic of China.

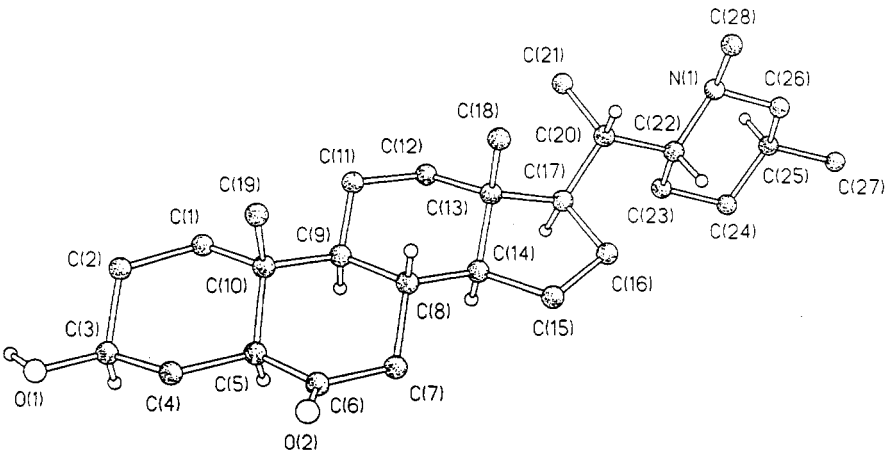


FIGURE 3. Perspective drawing of **2** by X-ray crystallography.

EXTRACTION AND ISOLATION.—The dried, ground bulbs (5 kg) were extracted with 50% aqueous $(CH_3)_2CO$ three times. Solvent was removed under reduced pressure and the resultant extracts passed through a Diaion HPA-25 column (anionic-exchanger, 30–70 μm, 50 g, Supelco, Bellefonte, PA) eluting first with H_2O, followed by 50% aqueous MeOH and then MeOH. The MeOH fractions were combined and evaporated to dryness. The residues were reconstituted using 0.5 M HCl in MeOH, alkalinized with NH_4OH and extracted with Et_2O. The combined organic extracts were then extracted with 5% aqueous tartaric acid. The aqueous extracts were basified and further extracted with $CHCl_3$. The organic extracts were concentrated and separated by flash cc using a gradient of *n*-C_6H_{12}-EtOH-$(CH_3CH_2)_2NH$ (from 110:1:1 to 34:1:1). Alkaloid **1** was obtained from a (54:1:1) eluted fraction, and alkaloid **2** from a (52:1:1) eluted fraction.

Puqiedinone [**1**].—Crystallization from *n*-C_6H_{12}-EtOH-$(CH_3CH_2)_2NH$ gave colorless needles of puqiedinone [**1**], mp 205–208°; $[\alpha]^{20}D$ −62.3° (*c*=0.2, $CHCl_3$); ir (KBr) ν max 3525, 3280, 2750, 1690 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 3.57 (1H, m, $W_{1/2}$=21 Hz, H-3αa), 2.78 (2H, m, H_2-18), 2.76 (2H, m, H_2-26), 2.49 (1H, dd, *J*=4.0 and 10.0 Hz, H-7βe), 2.15 (1H, dd, *J*=2.4 and 10.0 Hz, H-5αa), 0.86 (3H, d, *J*=9.2 Hz, Me-25), 0.84 (3H, d, *J*=9.2 Hz, Me-20), 0.73 (3H, s, Me-10), 0.63 (1H, m, H-17αa), 1.96–0.95 (26H, m, assignments for these protons are listed in Table 1); ^{13}C-nmr data, see Table 1; hrms *m/z* calcd for $C_{27}H_{43}NO_2$ 413.3286, found 413.3281; eims *m/z* 413 (36), 398 (8), 357 (9), 139 (6), 112 (35), 111 (100), 98 (9).

Puqietinone [**2**].—Mp 240–245°, $[\alpha]^{20}D$ +29.4° (*c*=0.64, $CHCl_3$); ^1H-nmr, ^{13}C-nmr, and eims data were reported previously (2); hrms *m/z* calcd for $C_{28}H_{47}NO_2$ 429.3609, found 429.3609.

X-RAY CRYSTALLOGRAPHIC ANALYSIS OF **2**[1].—A crystal of puqietinone [**2**] was found to belong to the orthorhombic system with space group $P2_12_12_1$, and the cell dimensions were determined as *a*=5.983(1)Å, *b*=10.453(2)Å, *c*=41.320(8)Å; *V*=2583.8(13)Å^3, *Z*=4, *d*(calcd)=1.105 g cm^{-3}, crystal dimensions 0.35×0.35×0.20 mm^3. X-ray crystallographic three-dimensional intensity data of **2** were collected on a Rigaku AFC7R diffractometer and 3441 independent reflections were measured by the ω-2θ scan technique over a scan range of (1.0+0.35tanθ)° using graphite monochromatized MoK_α radiation (λ=0.71073 Å). The residual fractors were *R*=0.085 for 1375 observed reflections with $|F_o| \geq 4\sigma(|F_o|)$. Computations were performed using the SHELXTL-PC program package.

ACKNOWLEDGMENTS

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[1]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.