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TWO NEW STEROIDAL ALKALOIDS FROM *FRITILLARIA USSURIENSIS*

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ABSTRACT.—Two new steroidal alkaloids, pingbeinine [**1**] and pingbeininosine [**2**], have been isolated from the leaves of *Fritillaria ussuriensis* and their structures have been determined based on the spectral and chemical data.

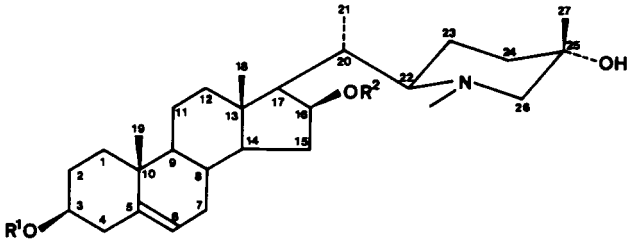
We previously reported three new steroidal alkaloids together with several known compounds from the bulbs and leaves of *Fritillaria ussuriensis* Maxim (Liliaceae) (1), which was widely used in Chinese medicine (2) to treat lung ailments and swollen throat. In a continuing study of the leaves of the same species, the present paper describes the isolation and structural elucidation of two new steroidal alkaloids named pingbeinine [**1**] and pingbeininoside [**2**].

Pingbeinine [**1**], mp 223–235°, $[\alpha]_D$ −32.8° ($c = 0.09$, MeOH), gave a positive digitonin test, which is characteristic of a genuine steroid with a 3β-OH (3). The steroid was analyzed for $C_{28}H_{47}NO_3$ by the $[M-1]^+$ peak of the high resolution eims spectrum (Δ 0.0 mmu). The uv spectrum exhibited only end absorption, and the ir spectrum showed the presence of a 3β-hydroxy-Δ^5 moiety (3348 and 1052 cm^{-1}) (4) and the absence of any carbonyl groups. The three oxygen functionalities were shown from the chemical shifts of the ^{13}C-nmr spectrum (δ 68.5, 71.6, and 72.4) to be all due to hydroxyl groups. Because ^1H- and ^{13}C-nmr spectra revealed that the molecule contained only one double bond (a one-proton multiplet centered at δ 5.34 and two carbon signals at δ 121.6 and 141.4, respectively), **1** should be a pentacyclic compound. The remaining ring was readily apparent from the high resolution eims spectrum. The base peak at *m/z* 128.1064, which corresponds to $C_7H_{14}NO$ (Δ −0.1 mmu), was assigned to the charged fragment arising from the cleavage between C-20 and C-22 (5). The partial structure was confirmed from the ^1H-nmr spectrum. Two three-proton singlets at δ 1.31 and 2.35 were assigned to C(OH)Me and NMe, respectively. In addition, a small W coupling was observed between a one-proton doublet (δ 2.75, $J = 11.0$ Hz) and a multiplet (δ 1.78), which were assigned to an equatorial proton next to the nitrogen and an equatorial proton adjacent to the quaternary carbon, respectively. The location of the third hydroxyl group was determined with the aid of the ^{13}C-nmr spectrum. First, carbon signals were assigned by comparing the chemical shifts of **1** with those of veracintine [**3**] (6). The chemical shifts of the carbons from 1 to 14 and of the two angular methyls were practically the same as those of veracintine, but the signals ascribable to C-15, C-16, and C-17 (δ 35.7, 72.4, and 60.6, respectively) were markedly shifted downfield from the corresponding carbon signals of veracintine (11.6, 44.0, and 7.0 ppm). The downfield shift demonstrated that the hydroxyl group was located at C-16 and was of β configuration, because in the case of 16α-OH the value of the C-16 chemical shift should be in the 75–77 range regardless of the configuration at C-22 (7). In addition, a multiplet centered at δ 4.50, whose width at half height was 17.7 Hz, was assigned to H-16α upon comparison with published data (5,8). This signal shifted downfield to δ 5.30 on acetylation. The configuration at C-22 was determined based on the

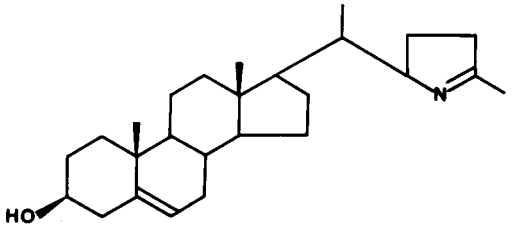
^{13}C-nmr spectrum. Compared to muldamine (7), which is a structurally related alkaloid with the 22*S* configuration, the signals for C-20, C-21, and C-22 in **1** were markedly shifted (− 9.0, 8.3, and 13.7 ppm, respectively), suggesting that **1** should have the 22*R* configuration. The stereochemistry at C-25 was determined by nOe difference spectral studies. Enhancements of the H-23 axial, H-24 equatorial, and H-26 equatorial protons (δ 1.72, 1.78, and 2.73, respectively) were observed on irradiation of the C-25 methyl resonance (δ 1.31), suggesting that the methyl should have a β-axial configuration and that the configuration is *S*. It is also noteworthy that H-20 (δ 2.48) exhibited an nOe upon irradiation at the NMe resonance (δ 2.35). The nOe observation confirmed the 22*R* configuration discussed earlier, because H-20 was not coupled with H-22, probably due to the proximity of the dihedral angle to 90°. Consequently, **1** was determined to be (22*R*,25*S*)-*N*-methylepiminocholest-5-ene-3β,16β,25-triol.

Pingbeininoside [**2**], mp 244–246°, [α]D −4.6° (*c* = 0.164, MeOH), showed a molecular ion peak at *m/z* 607 in the field desorption mass spectrum. The corresponding molecular formula of $C_{34}H_{57}NO_8$ suggested that **2** might be a glycoside of **1**. The eims spectrum strongly supported that assumption. Fragment peaks at *m/z* 163, 146, 127, 73, and 57 were ascribed to the sugar moiety, while peaks at *m/z* 445, 430, 427, 172, 128, 112, 110, 97, 84, and 70 were parallel with those of **1**. On hydrolysis with a mineral acid, **2** afforded **1** and D-glucose. In the ^{13}C-nmr spectrum of **2**, the chemical shifts for all carbons of the aglycone part corresponded closely to those for **1** except those for C-2, C-3, and C-4. The chemical shifts for C-2 and C-4 were shifted upfield; on the other hand the chemical shift for C-3 was markedly shifted downfield (−1.35, −3.04, and 7.95 ppm, respectively), indicating that the site of attachment of glucosyl group was at C-3. The configuration of the sugar moiety was shown to be β on the basis of the coupling constant of the anomeric proton (δ 5.08, *J* = 7.5 Hz). Thus **2** was established to be 3β-D-glucosylpingbeinine.

A pharmacological study of **1** and **2** is in progress and will be published elsewhere.



1 $R^1=R^2=H$
4 $R^1=R^2=Ac$
2 $R^1=glc$, $R^2=H$



EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on an X_4 type micro melting point apparatus and are uncorrected. Ir spectra were recorded on a Shimadzu IR-450 spectrometer. ^{1}H- and ^{13}C-nmr spectra were obtained on a TNM-400 spectrometer in $CDCl_3$, $CDCl_3/CD_3OD$, or C_5D_5N solution with TMS as an internal standard. NOe difference measurements were obtained on **1**. Eims, hreims, and fdms were recorded on an MAT-711 spectrometer. Si gel (200–300 mesh) from Qingdao Chemical Plant, China was used for cc. The following solvent systems were employed for Si gel tlc: solvent 1, $CHCl_3$-MeOH-H_2O (10:3:0.5); solvent 2, $CHCl_3$-Me_2CO-MeOH-H_2O (10:12:8:4); solvent 3, $CHCl_3$-MeOH (9:1). Each spot on the tlc was detected by spraying with Dragendorff's reagent.

PLANT MATERIAL.—The aerial parts were collected in June from plants cultivated in Liu-he Prefecture, Jilin Province, China. A voucher specimen is deposited in the herbarium of the Pharmacognosy Department of the Academy of Traditional Chinese and Materia Medica of Jinlin Province, China.

ISOLATION OF PINGBEININE [**1**] AND PINGBEININOSIDE [**2**].—Part of the isolation procedures was performed as described previously (1). Fraction 2 was further subjected to chromatography on Si gel with Et_2O-Et_2NH (7:1) to afford **1** (60 mg). Fraction 4 was repeatedly subjected to chromatography on Si gel with $CHCl_3$-MeOH-Et_2NH (4:2.5:1) to yield **2** (20 mg). Compound **1**: $C_{28}H_{47}NO_3$; colorless needles; mp 223–235° (recrystallized from MeOH); $[\alpha]_D$ −32.8° (c = 0.09, MeOH); ir λ max (KBr) 3348, 2960, 2855, 2790, 1456, 1380, 1189, 1130, 1052, 950, 932, 920 cm^{-1}; hreims *m/z* $[M-1]^+$ 444.3444 (calcd 444.3444 for $C_{28}H_{46}NO_3$), 128.1064 (calcd 128.1074 for $C_7H_{14}NO$); ^{1}H nmr ($CDCl_3/CD_3OD$) δ 0.95 (3H, s, H-18), 1.03 (3H, s, H-19), 1.10 (3H, d, J = 7.3 Hz, H-21), 1.31 (3H, s, H-27), 1.72 (1H, m, Hax-23), 1.78 (1H, m, Heq-26), 2.05 (1H, d, J = 11.0 Hz, Hax-26), 2.35 (3H, s, NMe), 2.48 (1H, m, H-20), 2.73 (1H, d, J = 11.0 Hz, Heq-26), 3.45 (1H, m, H-3), 4.50 (1H, m, $W_{1/2}$ = 17.7 Hz, H-16), 5.34 (1H, m, H-6); ^{13}C nmr ($CDCl_3$) δ 13.7 (q, C-18), 19.6 (q, C-19), 21.3 (q, C-21), 21.4 (t, C-11), 23.7 (t, C-23), 25.0 (q, C-27), 30.3 (d, C-20), 31.5 (t, C-2), 32.0 (d, C-8), 32.2 (t, C-7), 35.7 (t, C-15), 36.9 (s, C-10), 37.7 (t, C-1), 38.0 (t, C-24), 41.0 (t, C-12), 42.2 (t, C-4), 43.4 (s, C-13), 44.6 (q, NMe), 50.5 (d, C-9), 54.5 (d, C-14), 60.6 (d, C-17), 68.5 (s, C-25), 68.5 (t, C-26), 71.6 (d, C-3), 72.4 (d, C-16), 73.0 (d, C-22), 121.6 (d, C-6), 141.4 (s, C-5). Compound **2**: $C_{34}H_{57}NO_8$; colorless needles; mp 244–246° (recrystallized from MeOH); $[\alpha]_D$ −4.57° (c = 0.164, MeOH); ir λ max (KBr) 3357, 2930, 1635, 1450, 1374, 1063, 930, 890 cm^{-1}; fdms *m/z* $[M]^+$ 609, 445; eims *m/z* 445, 430, 427, 172, 170, 163, 156, 128 (base peak), 127, 112, 110, 85, 84, 73, 70, 57; ^{1}H nmr (C_5D_5N) δ 0.93 (3H, s, H-18), 1.08 (3H, s, H-19), 1.09 (3H, d, J = 6.4 Hz, H-21), 1.30 (3H, s, H-27), 2.63 (3H, s, NMe), 5.08 (1H, d, J = 7.5 Hz, H-1′), 5.56 (1H, m, H-6); ^{13}C nmr ($CDCl_3/CD_3OD$) δ 13.7 (q, C-18), 19.6 (q, C-19), 21.3 (q, C-21), 21.4 (t, C-11), 23.7 (t, C-23), 24.9 (q, C-27), 29.1 (t, C-2), 30.2 (d, C-20), 32.1 (d, C-8), 32.3 (t, C-7), 35.8 (t, C-15), 36.9 (s, C-10), 37.7 (t, C-1), 38.1 (t, C-24), 38.1 (t, C-4), 41.0 (t, C-12), 43.5 (s, C-13), 44.6 (q, NMe), 50.5 (d, C-9), 54.6 (d, C-14), 60.7 (d, C-17), 62.7 (t, C-6′), 68.4 (s, C-25), 68.5 (t, C-26), 71.6 (d, C-4′), 72.4 (d, C-16), 73.0 (d, C-22), 75.2 (d, C-2′), 78.0 (d, C-3*), 78.2 (d, C-3′*), 78.4 (d, C-5′*), 102.5 (d, C-1′), 121.6 (d, C-6), 141.4 (s, C-5). Assignments are interchangeable between carbons with asterisks.

ACETYLATION OF **1**.—Ac_2O (1 ml) was added to a solution of **1** (30 mg) in pyridine (2 ml) and allowed to stand at room temperature for 5 h. The reaction mixture was worked up in the usual manner to yield diacetylpingbeinine [**4**] (25 mg): $C_{32}H_{51}NO_7$; white powder; mp 153–156° (reprecipitated from $CHCl_3$/MeOH); eims *m/z* $[M]^+$ 529, 513, 172, 128 (base peak), 110, 84, 63; ^{1}H nmr ($CDCl_3$) δ 0.97 (3H, s, H-18), 1.02 (3H, s, H-19), 1.08 (3H, d, J = 6.4 Hz, H-21), 1.25 (3H, s, H-27), 2.02 (6H, s, Ac × 2), 4.58 (1H, m, H-3), 5.30 (1H, m, H-16), 5.34 (1H, m, H-6).

HYDROLYSIS OF **2**.—A solution of **2** (30 mg) in 2% H_2SO_4 (5 ml) was heated on an H_2O bath for 5 h. On cooling, the precipitates were filtered, washed with H_2O, and dried. The residue was chromatographed on a Si gel column with $CHCl_3$-MeOH (8:2) to give **1** (12 mg). D-Glucose was detected in the filtrate by paper partition chromatography and glc.

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