A Pair of New C-21 Steroidal Glycoside Epimers from the Roots of *Cynanchum paniculatum*

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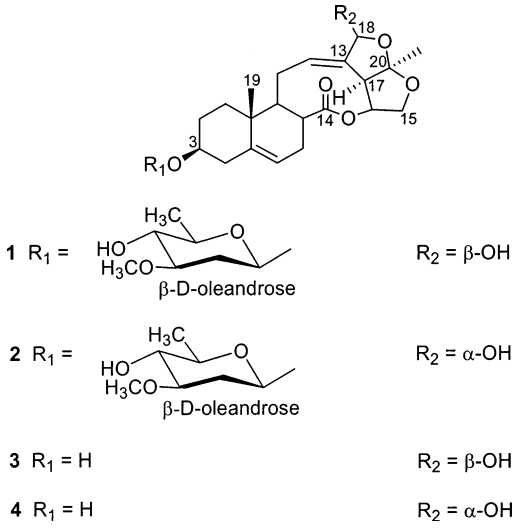
Two new C-21 steroidal glycoside epimers, paniculatumosides A and B (**1** and **2**), based on a 13,14:14,-15-disecopregnane-type skeleton, with neocynapanogenin B (**3**) and neocynapanogenin C (**4**) as two new aglycons, were isolated from the roots of *Cynanchum paniculatum*. Their structures were determined by spectral data interpretation.

The traditional Chinese medicine "Xu-Chang-Qing", which is the dried root of *Cynanchum paniculatum* Bunge (Asclepiadaceae), is a vivacious herb broadly distributed in the region of the Yangzi River, People's Republic of China. It relieves rheumatic pain and colds, reduces eczema, stops pains and tickles, and shows other curative effects.[1] It has been used as a gynecological medicine in Guizhou Province and as a contraceptive in Guangxi Province.[2] *C. paniculatum* is known to contain paenol[3] and 13,14/14,15-disecopregnane glycosides.[4,5] The latter are known to be mainly distributed in the Chinese medicines "Bai Qian", the roots of *C. glaucescens*,[6−9] and "Bai Wei", the roots of *C. atratum*.[2−10] Since "Xu-Chang-Qing" is different from both "Bai Qian" and "Bai Wei" in its therapeutic effects, a sample of this species, collected in Ningjin County, Shandong Province, was subjected to phytochemical investigation. A pair of new epimeric glycosides, paniculatumosides A and B (**1** and **2**), based on two new aglycons, named neocynapanogenins B and C (**3** and **4**), were isolated in this study. Herein, we present the structure elucidation of **1** and **2**.



Paniculatumoside A (**1**), a white powder, $[\alpha]^{26}_{D}$ −70.7° (*c* 2.9, $CHCl_3$), showed positive Lieberman−Burchard and Keller−Kiliani reactions, suggesting the presence of a pregnane aglycon and 2,6-dideoxy hexose sugar units.[13,14] The positive HRFABMS gave a $[M - 1]^-$ peak at *m*/*z* 519.2614, corresponding to the molecular formula $C_{28}H_{39}O_9$ [M − H] (calcd 519.2594). Inspection of its NMR spectral (proton, carbon, DEPT, HMQC, HMBC) data showed that compound **1** contains one anomeric carbon at δ 98.3 (C-1′) that correlated to the proton signal at δ 4.85 (dd, J = 8.2, 1.5 Hz), indicating the presence of one sugar unit in the β-form. The assignments of the NMR data for the aglycon moiety were readily recognized on the basis of 2D NMR experiments (HMQC and HMBC), in accordance with those of neocynapanogenin A,[5] except for those of C-6, C-7, C-8, C-12, C-13, and C-18 (see Table 1). The chemical shift changes of C-6, C-7, and C-8 were due to the hydroxyl having been removed from C-7. The chemical shift of C-18 was shifted from 167.4 to 108.3 ppm and indicated that C-18 was reduced from a lactone to hemiacetal carbon; therefore, the aglycon was determined to be a C-18 reduced product of neocynapanogenin A. A NOE experiment showed that H-18 and H-17, H-21 were correlated, indicating that H-18, H-17, and H-21 are on the same side of the ring. The configuration of the hydroxyl at C-18 was assigned as β. The remaining part of the NMR data of **1**, attributed to the fragment $C_7H_{12}O_3$, must belong to the saccharide moiety. The ^{13}C NMR data indicated the sugar to be D-oleandrose. Therefore, compound **1** was determined to be neocynapanogenin B 3-*O*-β-D-oleandroside.

PaniculatumosideB (**2**) was obtained as a white powder, $[\alpha]^{26}_{D}$ +3.2° (*c* 3.16, $CHCl_3$). The positive HRFABMS gave a $[M - 1]^-$ peak at *m*/*z* 519.2593, corresponding to the molecular formula $C_{28}H_{39}O_9$ (calcd, 519.2594). Inspection of the NMR spectral (proton, carbon, DEPT, HMQC, HMBC) data showed that compound **2** was very similar to **1**, with the differences between **1** and **2** being the chemical shifts of C-12, C-13, C-18, and C-20 (see Table 2). A NOE experiment showed no correlation between H-18 and H-17, indicating that H-18 and H-17 are in different orientations. Since H-17 is in the α-form, and H-18 is in the β-form, the configuration of the hydroxyl at C-18 was assigned the α-form. In addition, as both H-21 and H-18 showed no correlation in the NOE experiment, this also confirmed the hydroxyl at C-18 as α. The remaining part of the NMR spectrum of **2**, attributed to the fragment $C_7H_{12}O_3$, must belong to the saccharide moiety. The ^{13}C NMR data indicated the sugar as D-oleandroside. Therefore, compound

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for **1** in C_5D_5N

carbon	HMQC ^{13}C	HMQC ^{1}H[a]	HMBC[b]
1	37.2 (t)	1.63 (m), 2.49 (m)	C-5, C-10, C-19
2	29.7 (t)	1.26 (m), 2.50 (m)	C-1, C-3
3	77.0 (d)	3.78 (m)	C-1′
4	39.1 (t)	2.61 (m), 2.25 (m)	C-2, C-3, C-5, C-6, C-10
5	140.3 (s)		
6	120.1 (d)	5.37 (br s)	C-4, C-7, C-8, C-10
7	30.4(d)	2.25 (dd), 2.50 (m)	C-5, C-6, C-9, C-14
8	41.4 (d)	2.49 (m)	C-6, C-7, C9, C-10, C-14
9	52.1 (d)	2.00 (m)	C-8, C-14
10	37.8 (s)		
11	30.3 (t)	2.28 (m), 1.63 (m)	C-9, C-10, C-13
12	133.2 (d)	5.52 (br d, 10.5)	C-16, C-17, C-18
13	139.4 (s)		
14	179.4 (s)		
15	70.5 (t)	4.16 (dd, 5.3, 10.2), 4.44 (dd, 7.7, 10.2)	C-16, C-17, C-20
16	78.0 (d)	5.75 (ddd, 5.4, 7.9, 8.5)	C-12, C-14, C-20
17	56.0 (d)	3.32 (d, 8.5)	C-12, C-13, C-15, C-16, C-18, C-20, C-21
18	107.3 (d)	6.38 (s)	C-12, C-13, C-17, C-20
19	19.6 (q)	0.95 (s, 3H)	C-1, C-5, C-9, C-10
20	115.1 (s)		
21′	24.3 (q)	1.71 (s, 3H)	C-17, C-20
β-D-oleandrose			
1′	98.3 (d)	4.86 (br d, 9.4)	C-3, C-2′, C-5′
2′	37.5 (t)	1.81 (m), 2.60 (m)	C-1′, C-3′
3′	81.7 (d)	3.49 (m)	C-2′, C-4′, MeO-C-3′
4′	76.5 (d)	3.51 (m)	C-3′, C-5′, C-6′, MeO-C-3′
5′	72.9 (d)	3.65 (m)	C-1′, C-4′, C-6′
6′	18.8 (q)	1.59 (d, 6.0, 3H)	C-5′
MeO-C3′	57.1 (q)	3.47 (s, 3H)	C-3′

[a] Coupling constants are presented in Hz, and unless otherwise indicated, all proton signals integrate to 1H. [b] Proton showing HMBC correlation to indicated carbon.

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for **2** in C_5D_5N

carbon	HMQC ^{13}C	HMQC ^{1}H[a]	HMBC[b]
1	37.3 (t)	1.53 (m), 2.65 (m)	C-10, C-11, C-19
2	30.3 (t)	2.45 (m), 1.5 (m)	C-1, C-3, C-4, C-10
3	77.1 (d)	3.78 (m)	C-1′
4	39.1 (t)	2.61 (m), 2.35 (m)	C-2, C-3, C-5, C-6, C-10
5	140.3 (s)		
6	120.2 (d)	5.39 (br s)	C-4, C-8, C-10
7	30.4 (t)	2.85 (dd), 2.5 (m)	C-5, C-6, C-8, C-9, C-14
8	41.5 (d)	2.51 (m)	C-7, C-9, C-10, C-14
9	52.2 (d)	2.00 (m)	C-7, C-8, C-10, C-11, C-21
10	37.8 (s)		
11	29.1 (t)	2.60 (m), 2.12 (m)	C-8, C-9, C-12, C-13
12	130.2 (d)	5.75 (br d, 70.5)	C-17
13	145.7 (s)		
14	179.5 (s)		
15	70.0 (t)	4.16 (dd, 2.4, 9.7), 4.37 (dd, 7.3, 9.7)	C-16, C-17, C-20
16	78.3 (d)	5.74 (m)	C-14, C-20
17	56.8 (d)	3.36 (d, 8.2)	C-20, C-21
18	98.7 (d)	6.30 (s)	C-12, C-17, C-20
19	19.7 (q)	0.97 (s, 3H)	C-1, C-5, C-9, C-10
20	113.6 (s)		
21	25.0 (q)	1.82 (s, 3H)	C-17, C-20
β-D-oleandrose			
1′	98.3 (d)	4.87 (br d, 9.8)	C-3, C-2′
2′	37.5 (t)	2.57 (m), 1.76 (m)	C-1′, C-3′
3′	81.7 (d)	3.49 (m)	C-2′, C-4′, MeO-C-3′
4′	76.5 (d)	3.49 (m)	C-3′, C-5′, C-6′
5′	72.9 (d)	3.63 (m)	C-1′, C-4′, C-6′
6′	18.8 (q)	1.59 (d, 5.8, 3H)	C-5′
MeO-C3′	57.0 (q)	3.46 (s, 3H)	C-3′

[a] Coupling constants are presented in Hz, and unless otherwise indicated, all proton signals integrate to 1H. [b] Proton showing HMBC correlation to indicated carbon.

2 was determined to be neocynapanogenin C 3-*O*-*β*-D-oleandroside.

Compounds **1** and **2** were tested for activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and exhibited very weak activity against one clinical strain with minimum inhibitory concentration (MIC) values of 179.7 and 148.6 *μ*g/mL, respectively. The MIC of a control substance (cephapirin) was 10.5 *μ*g/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. IR spectra were recorded on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra were recorded on Bruker AM-400 MHz and DRX-500 spectrometers with TMS as internal standard. MS data were recorded on a VG autospec-3000 spectrometer.

HPLC was measured on Agilent 1100 series, HPLC supports: XTerra Prep RP-18 column.

Plant Material. *Cynanchum paniculatum* roots cultivated in Shandong Province were obtained in December 2001 and identified by Dr. H. Peng, Department of Plant Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, People's Republic of China, where a voucher specimen is deposited (voucher specimen number 0307938).

Extraction and Isolation. The roots (70 kg) of *C. paniculatum* were extracted three times with 95% ethanol to afford 5.5 kg of crude extract. The crude extract was separated into the ethyl acetate and aqueous solution portions. The ethyl acetate part (2.5 kg) was subjected to column chromatography eluted with $CHCl_3$ and $CHCl_3$−MeOH over Si gel to obtain nine fractions. Fraction 2 was separated by repeated column chromatography over Si gel (petroleum ether−EtOAc (4:1, 3:1, 2:1, 1:1), $CHCl_3$−MeOH (19:1, 9:1)), reversed-phase C_8 Si gel eluted with MeOH−H_2O (6:4), and reversed-phase C_{18} Si gel eluted with Me_2CO−H_2O (1:1), to afford 210 mg of a mixture of **1** and **2**. This mixture was purified by HPLC (isolation conditions: stationary phase, XTerra Prep RP-18 column; mobile phase, Me_2CO−H_2O (4.5:5.5); flow rate, 3 mL/min; t_{R1} = 8.6 min′, t_{R2} = 7.7 min′) to afford 110 mg of paniculatumoside A (**1**) and 28 mg of paniculatumoside B (**2**).

Paniculatumoside A (1): white powder, $[\alpha]^{26}_D$ −70.7° (*c* 2.9, $CHCl_3$); IR (KBr) ν_{max} 3460, 2937, 1722, 1283, 1133, 1070 cm^{-1}; ^1H and ^{13}C NMR (C_5H_5N), see Table 1; HRFABMS *m*/*z* 519.2593 $[M - 1]^-$ (calcd for $C_{28}H_{39}O_9$ 519.2594).

Paniculatumoside B (2): white powder, $[\alpha]^{26}_D$ +3.2° (*c* 3.16,$CHCl_3$); IR (KBr) ν_{max} 3460, 2937, 1722, 1283, 1133, 1070 cm^{-1}; ^1H and ^{13}C NMR (C_5H_5N), see Table 2; HRFABMS *m*/*z* 519.2614 $[M - 1]^-$ (calcd for $C_{28}H_{39}O_9$ 519.2594).

Antibacterial Assay. Strains of methicillin-resistant *Staphylococcus aureus* (MRSA) were obtained from Tokushima University Hospital as clinical isolates. After culturing all strains on Mueller-Hinton agar (Difco, Detroit, MI) at 37 °C for 24 h, the cells were resuspended in Mueller-Hinton broth (Difco) to give 10^8 colony-forming units/mL; the suspended cells were then incubated as described above. MIC values were determined using Mueller-Hinton agar according to the method described by the Japanese Society for Antimicrobial Chemotherapy (1981).[15] Cell suspensions (1×10^6 colony-forming units/mL) of MRSA were read after 20 h incubation at 37 °C.

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