Cernuosides A and B, Two Sucrase Inhibitors from *Pulsatilla cernua*

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Two oleanane-type oligoglycosides, cernuosides A (**1**) and B (**2**), were isolated from the roots of *Pulsatilla cernua* (Ranunculaceae). Structure elucidation was accomplished by 1D and 2D NMR (DQF−COSY, TOCSY, HMQC, HMBC, and ROESY) methods, FABMS, and hydrolysis. Both compounds showed moderate activity against sucrase.

As part of our interest in glycosidic compounds from Ranunculaceae plants,[1,2] we have isolated two triterpene glycosides, trivially named cernuosides A (**1**) and B (**2**), from the roots of *Pulsatilla cernua* (Thunb.) Bercht. et Opiz. *P. cernua* is used in traditional Chinese medicine for antitumor, antidiabetic, antiamoebic, and antibacterial purposes,[3] and triterpene glycosides have been previously reported from this plant.[4]

A MeOH extract of the roots was partitioned between *n*-BuOH and H_2O after defatting with *n*-hexane and $CHCl_3$. The *n*-BuOH layer was chromatographed on D101 resin, and the fractions that showed inhibition of rat intestinal sucrase were subjected to further separation on an ODS column to yield **1** and **2**.

FABMS of cernuoside A (**1**) showed an $[M + Na]^+$ ion at *m/z* 1551, consistent with a molecular formula of $C_{71}H_{116}O_{35}$. Analysis of the ^1H and ^{13}C NMR spectra revealed that **1** was a bisdesmosidic heptasaccharide saponin bearing sugar chains at the C-3 and C-28 positions of a triterpene aglycon. Acid hydrolysis of **1** afforded oleanolic acid, together with glucose, arabinose, and rhamnose, all of which were identified by comparison with authentic samples on TLC. Upon alkaline hydrolysis, **1** yielded a prosapogenin, together with rhamnose and glucose. The prosapogenin was subsequently identified as oleanolic acid 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)[*β*-D-glucopyranosyl(1→4)]-α-L-arabinopyranoside by comparison of the NMR data and physical properties with literature values.[5,6]

The most significant feature of the ^1H NMR spectrum of **1** was the presence of seven singlets together with two doublets (due to rhamnose residues) of methyl groups. Although most of the sugar proton signals overlapped partially or completely at *δ* 3.80−4.90, the signals for anomeric protons of the monosaccharide residues were well-resolved. It was, therefore, possible to identify the spin-systems associated with the seven individual monosaccharides by a TOCSY experiment with the aid of a DQF−COSY spectrum. All proton signals of the sugar moieties were assigned as shown in Table 1. Following the assignment of proton signals, the resonances of the attached carbons were established by an HMQC experiment (Table 2). The sugar sequences of the oligosaccharide chains as

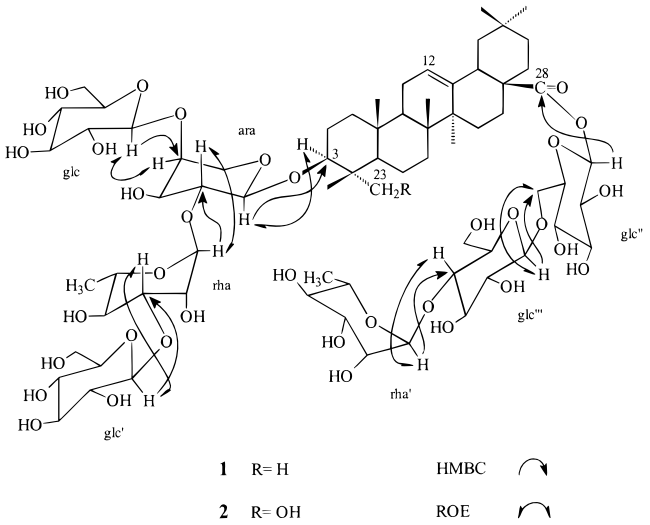

Figure 1. The structures and key correlations of HMBC and ROE of saponins **1** and **2**.

well as the glycosidic sites were subsequently determined by HMBC and ROESY data (Figure 1). Finally, the stereochemistry of the anomeric protons was deduced from a comparison of the ^{13}C chemical shifts and the anomeric $^1H-^1H$ coupling constants with those of reference sugars. These data allowed us to establish the relative sterochemistry as α-arabinopyranose, α-rhamnopyranose, and *β*-glucopyranose. Thus, cernuoside A (**1**) was determined to be a new heptasaccharide saponin with a structure of 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)[*β*-D-glucopyranosyl(1→4)]-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside.

Cernuoside B (**2**) showed a quasimolecular ion at *m/z* 1567 in the FABMS spectrum, corresponding to $[M (C_{71}H_{116}O_{36}) + Na]^+$. Acid hydrolysis of **2** afforded glucose, arabinose, and rhamnose. Analysis of the spectroscopic characteristics indicated that **2** was structurally similar to **1**, with the exception that **2** possessed hederagenin as the aglycon. In addition, it was noted that the chemical shifts of C-1, -3, and -5 of arabinose were somewhat different from the corresponding signals in **1**, which could be due to the influence of the hydroxyl group on C-23 of hederagenin.[7] The ^1H and ^{13}C signals of **2** (Tables 1 and 2) were assigned by a combination of DQF−COSY, TOCSY, and HMQC analyses. The sugar sequences and linkage sites of the saccharide chains were determined on the basis of HMBC and ROESY results (Figure 1), and the anomeric

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Table 1. ^{1}H NMR Data of **1** and **2**[a,b]

proton	**1**	**2**	proton	**1**	**2**
(a) C-3			(b) C-28		
ara 1	4.80 d (5.5)	5.05 d (5.5)	glc″ 1	6.20 d (8.0)	6.22 d (8.0)
2	4.49	4.51	2	4.13	4.13
3	4.21	4.03	3	4.25	4.24
4	4.29	4.25	4	4.30	4.32
5a	3.79	3.67	5	4.08	4.10
5b	4.29	4.23	6a	4.32	4.31
glc 1	5.15 d (7.7)	5.12 d (7.8)	6b	4.64	4.64
2	4.03	4.02	glc‴ 1	4.96 d (8.0)	4.97 d (8.0)
3	3.99	3.99	2	3.93	3.93
4	4.93	4.95	3	4.15	4.11
5	4.20	4.19	4	4.33	4.33
6a	4.22	4.21	5	3.61	3.61
6b	4.51	4.51	6a	4.06	4.05
rha 1	6.10 br s	6.18 br s	6b	4.17	4.17
2	4.94	4.94	rha′ 1	5.77 br s	5.80 br s
3	4.73	4.77	2	4.66	4.66
4	4.45	4.45	3	4.53	4.53
5	4.58	4.67	4	4.32	4.32
6	1.51 d (5.9)	1.52 d (5.9)	5	4.89	4.88
glc′ 1	5.37	5.38	6	1.71 d (5.4)	1.68 d (6.4)
2	4.11	4.11			
3	4.24	4.24			
4	4.27	4.27			
5	3.87	3.89			
6a	4.40	4.43			
6b	4.50	4.50			

[a] Recorded in pyridine-d_5. Assignments were established by interpretation of the DQF−COSY, HMQC, HMBC, and ROESY spectra. [b] J values (in Hz) in parentheses. Overlapped signals are reported without designating multiplicity.

Table 2. ^{13}C NMR Data of **1** and **2**[a]

	carbon	**1**	**2**		carbon	**1**	**2**
	1	38.9	40.0	glc	1	104.9	104.9
	2	26.6	26.0		2	74.5	75.1
	3	**88.6**[b]	**81.1**[b]		3	78.4	78.4
	4	39.6	43.5		4	71.4	71.4
	5	56.0	48.1		5	78.2	78.2
	6	18.5	18.1		6	62.3	62.3
	7	33.1	32.5	rha	1	101.5	101.3
	8	39.9	39.9		2	71.7	71.7
	9	48.0	47.5		3	**83.4**[b]	**83.4**[b]
	10	37.0	36.8		4	72.9	72.9
	11	23.7	23.8		5	69.6	69.7
	12	123.0	122.9		6	18.5	18.5
	13	144.1	144.0	glc′	1	106.5	106.6
	14	42.1	42.0		2	75.3	75.3
	15	28.2	28.3		3	76.6	76.5
	16	23.3	23.3		4	69.3	69.6
	17	47.0	46.9		5	76.6	76.6
	18	41.6	41.6		6	61.8	61.8
	19	46.2	46.1	*C-28*			
	20	30.7	30.7	glc″	1	95.6	95.6
	21	34.0	33.9		2	73.9	73.9
	22	32.5	32.7		3	78.7	78.7
	23	28.3	63.9		4	70.8	70.8
	24	17.2	14.1		5	78.0	78.0
	25	15.6	16.1		6	**69.2**[b]	**69.1**[b]
	26	17.4	17.5	glc‴	1	104.8	104.8
	27	26.1	26.3		2	74.7	74.7
	28	**176.5**[b]	**176.4**[b]		3	76.5	76.5
	29	33.1	33.0		4	**78.2**[b]	**78.2**[b]
	30	23.8	23.6		5	77.1	77.1
C-3					6	61.2	61.2
ara	1	105.3	104.8	rha′	1	102.7	102.7
	2	**75.4**	**75.4**[b]		2	72.5	72.5
	3	73.9	75.1		3	72.7	72.7
	4	**81.1**[b]	**81.1**[b]		4	73.9	74.0
	5	65.7	66.3		5	70.3	70.2
					6	18.4	18.4

[a] Recorded in pyridine-d_5. Assignments were established by interpretation of the ^{13}C DEPT, HMQC, and HMBC spectra. [b] Values given in **boldface** indicate the glycosidic positions of the aglycon.

configuration of each individual monosaccharide was deduced from the coupling constant of the anomeric proton, as in the case of **1**. Hence, the structure of cernuoside B (**2**) was established as 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)[*β*-D-glucopyranosyl(1→4)]-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside.

Cernuosides A and B displayed moderate inhibitory activity against the intestinal sucrase of rats, with IC_{50} values of 59.5 and 45.8 μm, respectively. They probably contribute to the ethnomedical use of the plant as an antidiabetic drug.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 16 PC FT−IR spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using a sodium lamp operating at 589 nm in MeOH. ^{1}H (400 or 500 MHz), ^{13}C (100 or 125 MHz), and 2D NMR spectra were recorded on JEOL JNM-EX 400 or Varian Unity INOVA-500 spectrometers. FABMS spectra were determined on a Finnigan MATTSQ7000 spectrometer. Column chromatography was carried out on D101 resin (60−80 mesh) and ODS gel (10−40 μm). TLC was conducted on Si gel 60 F_{254} and RP-18 F_{254} S plates (Merck).

Plant Material. The roots of *P. cernua* (Thunb.) Bercht. et Opiz. were collected in April of 1993, in the Jilin Province of China and authenticated by Dr. Xian-Min Cui. A voucher specimen has been deposited in the Herbarium of China Pharmaceutical University.

Extraction and Isolation. Air-dried and ground roots of *P. cernua* (200 g) were extracted with MeOH (800 mL × 3, 2.5 h each) at boiling temperature. The extract was concentrated, defatted with cyclohexane and $CHCl_3$, and partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was dried under reduced pressure and subjected to column chromatography on D101 resin (ca. 500 g) using EtOH−H_2O as eluent. The fractionation was guided by the inhibitory activity against rat intestinal sucrase. The active fraction, eluted with 70% EtOH, was subjected to a reversed-phase C_{18} column (ca. 100 g, 10−40 μm) eluted with MeOH−H_2O (15:85−70:30) to yield **1** (160 mg) and **2** (120 mg).

Acid Hydrolysis of 1 and 2. Each sample (10 mg) was refluxed with 10% HCl in 50% EtOH (5 mL) for 4 h. The solution was diluted with water and neutralized with Ag_2CO_3.

The neutralized solution was extracted with $CHCl_3$ and purified on a Sephadex LH-20 column using MeOH as eluent to yield the aglycon. The aglycon was identified by comparison with an authentic sample and literature values. The neutral hydrolysate revealed the presence of glucose, arabinose, and rhamnose by HPTLC (*n*-BuOH−HOAc−H_2O, 4:1:1) when compared with authentic samples.

Alkaline Hydrolysis of 1 and 2. The saponin (20 mg) was dissolved into 1.0 M KOH (10 mL) and warmed at 50 °C for 18 h. The reaction mixture was handled as previously described[2] to afford the prosapogenin, which was identified by comparison NMR data with authentic samples. The water layer was further hydrolyzed with 10% HCl and analyzed by HPTLC to reveal the presence of glucose and rhamnose.

Cernuoside A (1): white powder; mp 225−228 °C; $[\alpha]^{20}_D$ −24.1° (*c* 0.22, MeOH); IR ν_{max} 3410 (OH), 2940, 1734 (COOR), 1636 (C=C), 1386, 1074 (C−O−C), 578 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.86 (3H, s CH_3), 0.87 (6H, s, 2 × CH_3), 1.06 (3H, s CH_3), 1.13 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.27 (3H, s, CH_3), 3.25 (1H, dd, J = 3.5, 12.0 Hz, H-3α), 5.40 (1H, br s, H-12); ^1H NMR data of the saccharide residues, see Table 1; ^{13}C NMR (125 MHz, pyridine-d_5), see Table 2; FABMS *m*/*z* 1551, 1529, 1379, 1071.

Cernuoside B (2): white powder; mp 230−233 °C; $[\alpha]^{20}_D$ −26.6° (*c* 0.25, MeOH); IR ν_{max} 3412 (OH), 2940, 1734 (COOR), 1635 (C=C), 1387, 1075 (C−O−C), 580 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.84 (3H, s, CH_3), 0.85 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.15 (3H, s, CH_3), 3.87 (1H, overlapped, H-23a), 4.25 (1H, overlapped, H-3α), 4.63 (1H, overlapped, H-23b), 5.41 (1H, br s, H-12); ^1H NMR data of the saccharide residues, see Table 1; ^{13}C NMR (125 MHz, pyridine-d_5), see Table 2; FABMS *m*/*z* 1547, 1567.

Bioassay. Crude preparations of sucrase from the small intestine of rats were prepared from intestinal mucosa according to the procedures of Kesseler.[8] The reaction mixture consisted of crude enzyme solution (0.3 mL), sucrose (30 mM), and test sample (**1**, **2**) in 0.1 M potassium phosphate buffer (pH 6.3) (0.7 mL). After incubation for 30 min at 37 °C, the reaction was stopped by adding 2.0 mL of 2M Tris-HCl buffer (pH 7.0). The amount of liberated glucose was determined by the glucose-oxidase method using a commercial test kit (Jianchan Biological Products Co., Nanjing, China). Acarbose was used as a positive control in the experiment.

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Supporting Information Available: DQF−COSY, TOCSY, and other spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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