Pulsatilloside C from the Roots of *Pulsatilla chinensis*

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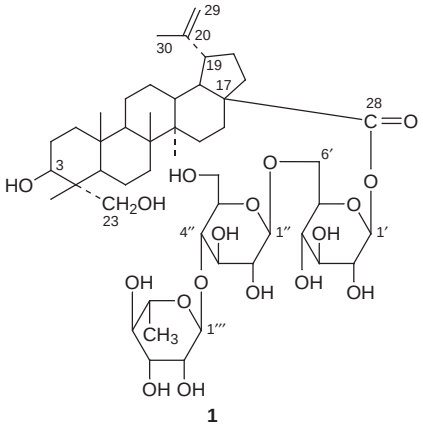
A new lupane-type triterpene saponin, pulsatilloside C (**1**), was isolated from the roots of *Pulsatilla chinensis*. Its structure was established to be 3β,23-dihydroxylup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

The roots of *Pulsatilla chinensis* (Bunge) Regel (Ranunculaceae) are used in traditional Chinese medicine to treat amoebic diseases, vaginal trichromoniasis, and bacterial infections.[1] Previously, we reported on the isolation and structure determination of the major lupane-type saponin, 3β,23-dihydroxylup-20(29)-en-28-oic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, and some minor constituents such as 23-hydroxybetulinic acid, pulsatillic acid, pulsatilloside A, and pulsatilloside B from the roots of *Pulsatilla chinensis*[2,3] and on the cytotoxic activities of pulsatillic acid against P-388 murine leukemia, Lewis lung carcinoma, and human large-cell lung carcinoma cells.[3] Further investigation of the plant extract has now led to the isolation of pulsatilloside C (**1**), a new triterpene ester glycoside whose structure was determined by 1D and 2D NMR (^{13}C NMR, ^{1}H NMR, DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, and ROESY) methods, field desorption MS, and hydrolysis.

The MeOH extract of the roots was defatted with *n*-hexane and $CHCl_3$ before partitioning between *n*-BuOH and H_2O. The *n*-BuOH layer was chromatographed on a Sephadex LH-20 column to give several saponin-containing fractions. Column chromatography of one fraction on Si gel yielded a new lupane-type triterpene glycoside (**1**).



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Compound **1** was obtained as an amorphous powder, mp 182−185 °C. The field desorption MS of **1** showed a quasi-molecular ion $[M + Na]^+$ at *m*/*z* 965, consistent with a molecular formula of $C_{48}H_{78}O_{18}$. Upon acid hydrolysis, **1** yielded 23-hydroxybetulinic acid (by direct comparison with an authentic sample) and the sugars glucose and rhamnose. The ^{1}H and ^{13}C NMR spectra of **1** clearly showed the presence of a triterpene bearing an olefinic group and an ester carbonyl group. The DEPT spectrum of **1** revealed signals for 6 methyls, 14 methylenes, 21 methines, and 7 quaternary carbons. The NMR data also suggested the presence of three sugar residues, clearly indicated by three anomeric carbon signals at δ 95.3, 102.7, and 105.0, and three anomeric proton signals at δ 6.29 (d, J = 8 Hz), 4.96 (d, J = 8 Hz), and 5.78 (br s). These data indicated that the sugar chain was composed of two β-glucose and one α-rhamnose residues, and their absolute configurations were assumed to be D and L, respectively.

A comparison of the ^{13}C NMR data of **1** with those of 23-hydroxybetulinic acid[4] indicated ester glycosylation shifts at C-28 (−5.34 ppm) and C-17 (+0.43 ppm). Thus, the carboxyl at C-17 was glycosylated, and compound **1** was determined to be a monodesmosidic ester glycoside. Further evidence to support this conclusion was obtained when compound **1** was shown to be hydrolyzable in both acidic and alkaline conditions.

HMQC, HMBC, and ROESY spectra of **1** allowed the assignments of all proton and carbon signals (Table 1) as well as the sequence of the trisaccharide chain. Thus, in the HMBC spectrum of **1**, a cross peak between the anomeric proton (H-1′) of the inner glucose unit at δ 6.29 and the C-28 carboxylic carbon at δ 175.1 was displayed. The spectrum also exhibited a correlation between the methylene carbon signal at δ 69.4 due to C-6′ of the inner glucose unit and the anomeric proton signal at δ 4.96 due to H-1″ of the central glucose unit. It was concluded that the inner glucose is bonded to the carboxyl group of the aglycon, whereas the central glucose must be linked to the C-6′ hydroxyl of the former glucose residue. Moreover, the HMBC spectrum revealed a correlation cross peak between the terminal rhamnose anomeric proton (δ 5.78, H-1‴) and C-4″ (δ 78.4) of the central glucose. Thus, pulsatilloside C (**1**) was established to be 3β,23-dihydroxylup-20(29)-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Experimental Section

General Experimental Procedures. Optical rotation was measured in MeOH on a Perkin−Elmer 241MC

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

C	δ_C	δ_H (J in Hz)	C	δ_C	δ_H (J in Hz)
1	39.17	0.90, 1.59	glucose 1′	95.28	6.29 d (8.0)
2	27.81	1.83, 1.84	(inner) 2′	73.94	4.18 dd (8.0, 9.2)
3	73.33	3.66 dd (10.4, 5.0)	3′	78.64	4.21
4	43.04		4′	70.77	4.31
5	48.68	0.68	5′	77.99	4.09
6	18.56	1.40	6′	69.38	4.31, 4.70 br d
7	34.38	1.37			
8	41.22		glucose 1″	105.03	4.96 d (8.0)
9	51.00	1.39	(central) 2″	75.29	3.96 t
10	36.93		3″	76.46	4.12
11	21.20	1.15, 1.39	4″	78.44	4.39
12	26.11	1.18, 1.84	5″	77.12	3.66 d (10.4)
13	38.41	2.60	6″	61.34	4.10, 4.19
14	42.84				
15	30.89	1.19, 1.72	rhamnose 1‴	102.68	5.78 br s
16	32.33	1.42, 2.60	(terminal) 2‴	72.56	4.68
17	57.03		3‴	72.74	4.56 br d
18	47.48	1.70	4‴	74.04	4.30
19	49.84	3.37	5‴	70.35	4.95
20	150.92		6‴	18.56	1.65 d (6.0)
21	30.21	1.45, 2.18			
22	37.38	1.47, 2.16			
23	67.63	3.40 d (9.3), 3.59 d (9.3)			
24	13.01	0.87 s			
25	16.92	0.94 s			
26	16.49	0.99 s			
27	14.97	1.33 s			
28	175.06				
29	110.16	4.68 br s, 4.82 br s			
30	19.50	1.69 s			

[a] Measured in pyridine-d_5 at 500 MHz. Assignments were made with the aid of HMQC, HMBC, and ROESY spectra. Overlapped signals are reported without designating multiplicity.

automatic recording polarimeter. Field desorption MS was recorded on a MAT-711 spectrometer. NMR spectra (400 and 500 MHz) were recorded on a JEOL JNM-GX400 or a GE Omega-500 NMR spectrometer in C_5D_5N.

Plant Material. The roots of *P. chinensis* were collected in Anhui Province, People's Republic of China, in March 1992. The plant material was identified by Dr. Xian-Min Cui, and a voucher specimen (no. 920082) has been deposited at the herbarium of the China Pharmaceutical University, Nanjing.

Extraction and Isolation. The air-dried roots of the plant (850 g) were extracted in boiling MeOH. After filtration, excess solvent was removed under reduced pressure to give a residue (120 g) that was defatted with *n*-hexane (3 × 500 mL) and $CHCl_3$ (3 × 500 mL), followed by partitioning between *n*-BuOH and H_2O. The *n*-BuOH layers were combined, concentrated, and dissolved in a small amount of MeOH. The solution was then added to Et_2O and centrifuged to give a saponin fraction (15.5 g). A portion of the precipitate (13.0 g) was separated into six fractions on a Sephadex LH-20 column using MeOH as the eluent. The third fraction (2.2 g) was subjected to Si gel (150 g, 200−400 mesh) column chromatography using $CHCl_3$−CH_3OH−H_2O (75:25:2) as eluent to afford **1** (60 mg, 0.008%).

Pulsatilloside C (1): amorphous powder (MeOH); mp 182−185 °C; $[\alpha]^{25}_D$ −8.3° (*c* 0.522, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; field desorption MS, *m*/*z* 965 $[M + Na]^+$.

Acid Hydrolysis of 1. The saponin (20 mg in 10 mL of MeOH) was refluxed in 10 mL of 2N HCl for 3.5 h; H_2O was added to the reaction mixture, and this was extracted with $CHCl_3$ (3 × 20 mL). The $CHCl_3$ extract was purified on a Sephadex LH-20 column eluted with MeOH to afford a crop of 23-hydroxybetulinic acid (6 mg),[3] which was identified by NMR and IR by comparison with an authentic sample. The aqueous layer of the hydrolysate was neutralized with Ag_2CO_3, and the neutral hydrolysate revealed the presence of glucose and rhamnose on high-performance TLC when compared with authentic samples.

Alkaline Hydrolysis of 1. The saponin (10 mg) was refluxed in 5 M NH_4OH in 50% EtOH (20 mL) for 6 h. The reaction mixture was extracted with EtOAc (3 × 20 mL). The EtOAc layer was evaporated to give a residue that was chromatographed on Sephadex LH-20 using MeOH to give 23-hydroxybetulinic acid identified by TLC, IR, and mmp with an authentic sample.

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